Abstract Book

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Anatomy I

Anatomical landmarks of sphenoidal sinus endoscopical surgery

ESC-ID 583
Author Popescu A, Margineanu C, Stoleriu MG, Antohe I
Country Romania
University "Gr.T.Popa", University of Medicine and Pharmacy
Department Department of Anatomy

Introduction: The recent progresses of endoscopical surgery of the nasal fossae and the parasnasal pneumatised cavities impose reevaluation of local anatomy in order to establish precise landmarks of meatal regions and sinusal ostia.

Aim: Our study proposes to determine the topographical relations of the sphenoidal sinus with the main surrounding neuroarterial structures and to establish their endosinusal landmarks.

Material and methods: The anatomical material studies consists of 20 dry skulls from Anatomical Institute collection. The sinusal lumen opened by both coronal and parasagittal sections was observed by means of Zeiss surgical microscope and the images were recorded on microscope’s Sony video line or on Sony F 717 digital camera. The extrasinusal observations were focused on left and right carotic sulci, sphenoidal lingula and middle and anterior clinoideal processes. The endosinusal exploration was performed by direct light, by transillumination and by endoscopical examination passing the thin 3.5 mm flexible Storz endoscope through the sphenoideal sinus orifice. Fifty 2D CT skull images from non neurological patients were also examined in order to establish the correspondence between anatomical and imagistic data.

Results and conclusions: Our study pointed out the extreme variability of the sphenoidal sinus and allowed us to establish five degrees of sinusal pneumatisation. According to the extension of pneumatization, on inferior sinusal wall we have determined the progressive endoluminal prominence of the round, pterygoid and pterygopalatine canals. On the lateral walls there are demonstrated two carotic swellings, one inferior of the infracavernous part and one superior of the paraclinoid part of internal carotic artery superolaterally the optic canal may protrude the sinusal cavity. The incidence of this anatomical landmarks variations, studied comparatively on dry skulls and CT images confirmed the concordance between anatomical and imagistical data.

Anatomofunctional study of Glaserian fissure

ESC-ID 587
Author Stoleriu MG, Antohe I, Margineanu C
Country Romania
University "Gr.T.Popa", University of Medicine and Pharmacy
Department Department of Anatomy

Introduction: The petrous part of the temporal bone represented a continuous attraction for the anatomists during centuries. During Renaissance, for the first time Vesalius affirmed the unity of temporal bone and named maleus and incus. Gian Filippo Ingrassia and Gabrielle Fallopio separately, discovered and described the stapes. Realdo Colombo and Bartholomeo Eustachio described internal acoustic meatus and acoustic and facial nerves. In 1670 the Swiss anatomist Johann Heinrich Glaser described the petrotympanic fissure.

Aim: The goal of our study is to precise the mode of Glaserian fissure formation, to point out the functional interrelations between the tympanal bone, petrous part and squama of the temporal bone at middle part of skull exobasis and to describe the anatomical details that sustain the existence of an anterior hillus of the middle ear.

Material and methods: The anatomical material studied consists of 20 dry skulls from the collection of Iaşi Institute of Anatomy. The glenoid cavity, antero-lateral surface of tympanal bone and Glasser fissure were observed by means of surgical Zeiss microscope and the mesosomical images were recorded on digital Sony camera. In order to expose the inferior processus of tegmen tympani, the superior edge of tympanal bone as well as the squama neighbour to the fissure to be described were sculptured and thoroughly chiseled using a fine dental drill. The anatomical study was completed by morphological examination of 20 CT slices of the skull base.

Results and discussions: Our study allowed to determine the variation of exocranial tympanic tegmen surface, the mode of Glasser fissure formation, the presence of sutureal bones into the fissure, the relation of the upper border of tympanic bone and their interrelletion with sphenoidal spine, the tubal process of tympanic bone and osseous part of auditory tube. Incidental absence of processus inferioris tegminis tympani was also demonstrated. The functional organization of tegmen tympani includes an horizontal part that forms the roof of tubo-tympanic cavity and a vertical part procident through petrosquamous space. It articulates with the upper border of tympanic bone forming a schindylesis-like suture. The functional organization of this inferior processus that closes lateraly the isthmus of osseous auditory tube and, superiorly, the iter chordae anterius canal is also detailed. The study is completed by morphometrical assement of processus inferior tegminis tympani. Clinical considerations include the relations with parotid gland, the possible involvement of chorda tympani nerve and direct communication of the middle ear with latero-pharyngeal spaces, source of infections propagation.

Anatomical and morphometrical study of the pterygoid canal

ESC-ID 593
Author Antohe I, Margineanu C, Stoleriu MG
Country Romania
University "Gr.T.Popa", University of Medicine and Pharmacy
Department Department of Anatomy

Aim: Our study proposes to actualize the classical data concerning the macro and mesosomical anatomy of the pterygoid canal. Its traject and orifices were comparatively investigated on dry skulls and on CT images obtained from non-neurological patients. Morphological study was completed with morphometrical assesment of the pterygoid canal on both osteological preparates and CT images. Material and methods. The anatomical material studied consist of: • 100 dry skulls (200 pterygoid canals); • 100 CT images of the cephalic extremity of non-neurological patients; • The CT images of 10 intact dry skulls.
Anatomical methods: Seriated frontal section of the skull base between foramen lacerum and pterygopalatine fossae were performed by means of Gillis manual saw or of a thin blade electrical saw. The mesoscopy dissection of the pterygoid canals was performed by means of Zeiss surgical microscope. Using a dental drill (fine round head – 15000 rotations/min.,) we have sculptured the external compact wall of the canal in the spongy bone of pterygospheiodal junction.

Imagistical methods: The images of anatomical specimens were calibrated with a millimetric strip and recorded on the Sony video line of surgical microscope or on Sony F717 digital camera. The CT examination was performed on a Phillips Aura Computer Tomograph in the department of Neuroradiology and Neuroimagistics of the University Hospital. The dry skulls were examined by direct acquisition of coronal section perpendicular on the orbitomeatal plane. Axial and coronal 1mm 2D sections of the cephalic extremity were practised at the all 100 patients.

Results and conclusion: Our study allowed us to describe new anatomical features. Thus the openings of the canal were named aperture (posterior, quadrangular and anterior, estuary shaped) canalis pterygoidei. The trajectory of pterygoid canal was divided into crus anterior and crus posterior and the value of the in between angle was determined. The examination of CT images confirmed the existence of the described segments of pterygoid canal and its lateral angulation. From topographical view point, the isthmus corresponds to posterior or extension of sphenoidal sinus. The morphology of the pterygoid canal is strongly influenced by sinusual pneumatisation. When the size of the sinus increases, crus posterior becomes shorter and the crus anterior, longer, may be situat-ed infrasinual or transsinual, transversing the sinus cavity included into a messo-like osseous septum. The same details were displayed on CT images and the comparative morphometry was performed demonstrating the qualitative and quanti-tative concordance of mesoscopy data on dry skulls and CT images on living patients.

Relations of the initial segment of the oculomotor nerve and adjacent arteries

ESC-ID 83
Author Trandafilovic M, Cvetkovic M
Country Serbia
University University of Nis
Department Institute of Anatomy

Introduction: The relationship between the initial part of the oculomotor nerve and posterior cerebral and superior cerebellar arteries is well understood, but there is still insufficient data about details of this relationships.

Aim: The aim of work was to examine relationships of the initial segment of the oculomotor nerve with two cerebral arteries – posterior cerebral artery and superior cerebellar artery, in foetal and adult period.

Material and methods: The examination was performed on 35 human brains (26 adults and 9 fetuses). Arteries in foetal brain were perfused with Micropaque and examined by photo film. Adult cases were examined during the forensic autopsy and then basis of the brain with vessels were photographed. Neurovascular relationship between oculomotor nerve, posterior cerebral and superior cerebellar artery is described and noted in scheme and photo film.

Results: The close relationship of initial segment of the oculomotor nerve with basilar artery in 31.4%, with superior cerebellar artery in 37.1%, with P1 and P2 segments of posterior cerebral artery in 80% and 68.6%, consequently, with posterior communicating artery in 42.9% of cases was noted. The root of the nerve was appeared above P2 segment in 5.7% of cases and under the level of superior cerebellar artery in 2.9% of cases. Special neurovascular relationship between oculomotor nerve with two roots and adjacent arteries is noted in 2.9% of cases.

Conclusion: Neurovascular relationship between oculomotor nerve and adjacent arteries, as morphological characteristic on the ventral side of the brain trunk, have pathoanatomical meaning by existing of the compressive lesions, vascular penetrations, arteriovenous malformations and the arterial aneurysms.

Celiac artery aneurism: Case analysis and current investigation techniques

ESC-ID 679
Author Tamas-Szora A, Trifan A, Tamasy F, Suciu M, Tatar A, Todoran A
Country Romania
University University of Medicine and Pharmacy \ "Iuliu Hatieganu" , Cluj Napoca
Department General medicine

Introduction: Aneurysm of the celiac artery is an uncommon clinical problem; fewer than 180 cases have been reported in the world medical literature. This condition can be asymptomatic or, more frequently, produce only vague abdominal discomfort, therefore most of the cases are discovered either during rupture or incidentally during diagnostic imaging for other diseases. We present the particular aspects of a celiac artery aneurism, discovered on a human anatomy piece and a review of premorbid complications diagnosis.

Means and Method: The corpse belonged to a 45 year old male, conserved through formalisation (Formaldehyde 37 %), for one year. The aneurysm observed after proper abdominal wall and cavity preparation, was evaluated in situ (relative position, shape, size, consistency) and by histopathological means. Serial slides obtained after paraffin embedding were stained with Orcein, Masson’s Trichrome, Hematoxylin Eosin and observed under microscope (200x, 400x magnification).

Results: The Celiac Artery aneurism presented as an oval sacciform structure, 2.3 cm long and 1.5 cm in diameter. The dilation included the artery’s emergence point and covered the entire length of the arterial body. Normal branching of the artery was noted. Histopathological examination revealed a dissecting aneurysm; this diagnostic was sustained by intraparietal thrombosis present in both Orcein and Masson’s Trichrome staining.

Conclusion: The present case, because of it’s high lethal potential, underscores the importance of thorough celiac artery evaluation whenever the abdominal aorta is investigat-ed, or whenever a case of unexplained superior abdominal pain is manifested. Current studies indicate the high sensibl-ity and specificity of three-dimensional computed tomographic angiography, contrast-enhanced abdominal Computer Tomograph, and abdominal Magnetic Resonance Imaging.
Renal medulla anatomy based on virtual anatomical models of the human kidneys applying to the mini-invasive surgery planning

ESC-ID 691
Author Voroschukh R, Vdovichenko V, Shuba D
Country Ukraine
University Kharkiv State Medical University
Department General Surgery and Clinical Anatomy

Background and purpose: Kidney surgery becomes more sophisticated using several organ-preserving and minimally invasive techniques such as cryotherapy and radiofrequency ablation etc. [1, 2, 3]. Performing of such techniques is needed with detailed information of the human individual spatial and topographical renal anatomy. Up-to-date scanning technologies (CT, MRI, ultrasound etc.) can supply doctors with accurate high-resolution 2D visualization of the human renal topographic anatomy in different section planes. Modern computer-based volume rendering techniques makes possibilities to create virtual 3D models of the human renal anatomy based on these data [4]. In our study we describe anatomy of the human renal medulla pyramids based on anatomical cryosection, CT, MRI data with respect to their distribution in 3D space and in relation to other renal anatomical structures. Material and Methods: The research was based on anatomical material of 150 normal human kidneys after autopsy and 20 CT and MRI with gadolinium contrast enhancement of renal cortex or medulla of living humans without any detectable renal pathology. Anatomical cryosections images from the male and female Visible Human Dataset (VHD) were also studied [5]. The calycopelvic complexes on anatomical specimens were injected by gelatin. Anatomical cryosection technique was used to obtain serial cross-section images of the human kidneys. The system of topographic and volumetric analysis (Virtual Anatomist) based on proposed method of voxelized anatomical modeling of inner organs and virtual planning of surgical operation on them was developed and used for this study [6]. Advantages of this method in comparing with other existing ones [7] are saving of all the anatomical data from source 2D images in resulted 3D models and their editable property which allow doing virtually anatomical preparation or simulation of surgical procedures.

Results: The final results are available as interactive editable 3D models of the human kidneys, renal pyramids and calicopelvic complexes. Topography of renal medulla was greatly variable. In the study group, the volume of the kidney was 136.0 ± 4.0 cm³, the volume of renal cortex was 67.5 ± 12.5 cm³, the volume of renal medulla was 33.5 ± 6.25 cm³, kidney cross-sectional area on sagittal plane was 4114.2 ± 69.0 mm². Renal parenchyma measurements were all significantly larger in the left kidney that those in the right one (P <0.05). However, there was no significant difference by the sex. The renal pyramids were distributed in mostly cases in three parts of the kidney along its length. Study of skeleton topology, syntopy, virtual planning and guidance of fine-needle biopsy (FNB) of renal parenchyma and calicopelvic complex were performed on these 3D models obtained from serial images of CT/MRI and VHD cryosections.

Conclusion: New anatomic data of renal pyramids morphology and topographical distribution in 3D space were obtained. Proposed method of voxelized anatomical modeling and developed system of topographic and volumetric analysis are generally applicable to all 2D data obtained from CT, MRI and anatomical cryosection specimens. Virtual planning of organ-preserving kidney surgery and guiding of minimally invasive procedures can be performed based on 3D virtual models obtained from them. Topographic approach to the study of the human body and applying of several navigation systems to perform mini-invasive surgery could be also easy realized using this method in near future [8, 9]. In the near future, it could be the basis of new surgical and anatomical simulation tools.

The anatomy of the intra- and extra-pulmonary airways’ venous system of the human lung

ESC-ID 436
Author Ciocâlteu AM, Bordu SI, Lilea GC, Mandrina I
Country Romania
University Universitatea de Medicina si Farmacie Craiova
Department Anatomy

Introduction: One of the major factor affecting inflammatory pulmonary diseases is the bronchial system’s circulation. Recent studies have shown that the distinct separation made between the nutritional and the functional circulation should be regarded with at least some reserve. Aim In the present study we aim to describe the distribution of the branches of the pulmonary and of the bronchial veins servicing the airways of the human lung.

Materials and methods: The materials for the study comprised of 5 pairs of lungs from adults aged between 24 and 40. The technique used to highlight the pulmonary veins’ branches was the injection of colored gelatin, after first washing the pulmonary vascular system with distilled water. After fixation in a solution of 10% formaldehyde we used an operator microscope in order to expose the microcirculation of the intra- and extra-pulmonary airways through microdissection.

Results: The extrapolummary airways’ microcirculation The dissection of the pulmonary veins showed relatively large venous branches, originating from the pulmonary veins, that have an upwards trajectory on the main bronchi, and then are continued on the terminal part of the trachea. These form a vascular network on both the anterior and the posterior side of the airways. Furthermore, this network communicates directly, without the interposition of capillaries, with branches from the bronchial veins, which are much smaller in caliber. These pulmonary veins’ branches that are distributed to the extrapolummary airways have shown a constant distribution throughout the 5 pairs of lungs we have studied. 'The intrapulmonary airways’ microcirculation In what concerns the intrapulmonary airways, we have found venous branches which drain exclusively into the pulmonary veins, and which are distributed to the airways in a sectorial manner.

Conclusion: The branches of the pulmonary veins that are distributed to the extrapolummary airways have direct anastomoses with the bronchial veins, without the interposition of capillaries. The venous blood of the terminal part of the trachea and also of the main bronchi is drained mostly by the pulmonary veins and also by the bronchial veins, the latter having a much lesser importance. The venous blood of the intrapulmonary airways is drained exclusively by the pulmonary veins’ branches. These are distributed sectorially to consecutive, independent sectors of the airways.
Lymphangiogenesis in the fetal and early postnatal mouse heart

ESC-ID 965
Author Juszynski M, Ciszek B, Jablonska A, Ratajska A
Country Poland
University Medical University of Warsaw
Department Department of Pathological Anatomy

Heading: Until now not much has been known about the structure of lymphatic vessels in the heart. Moreover, the knowledge on lymphangiogenesis in fetal heart was negligible. The research on lymphangiogenesis became possible after the discovery of markers specific for lymphatic vessels. Aim: The examination of the development of lymphatic vessels in the mouse heart during embryonic and early postnatal life and the analysis of its course and location.

Method: The research was performed on normal mice hearts, fetal and mature, from 11 ED to 8 PD. The hearts were collected from fetuses and newborn mice, frozen, cut serially and stained with anti-LYVE antibody. Some of the hearts were doubly stained with anti-LYVE and anti-SMA antibodies. Other hearts were immersed in formaldehyde solution and stained with anti-LYVE antibody using the whole-mount method. Ink with gelatine or latex were injected subepicardially. The course and location of lymphatic vessels on the surface of the atrioventricular groove, on the posterior surface of intraventricular grooves, anterior and posterior, on the surface of intraventricular grooves, anterior and posterior, on the surface of the atrioventricular groove, on the posterior surface of the left atrium and left ventricle. The diameter of the vessels is significant, but the vessels lack lamina media. There are also many single and aggregated LYVE (+) cells apart from the vessels. Moreover, LYVE (+) cells form significant aggregations. Results: On ED 11 we found some diffused aggregations of cells with very week LYVE assay located among myocardial trabecules of both ventricles and in the atria, but their number was negligible. In the following developing stages LYVE(+) cells are located subepicardially forming primitive tubes. Subepicardial assay is dispersed, but present also in the atria. It is more sensitive in the ventriculo-atrial dorsal groove, where LYVE(+) cells form significant aggregations. LYVE(+) cells begin to form the web of vessels, which is not yet continuous at this stage. In some of the areas, the tubes form appendices, sprouting into myocardial wall. On ED 15 it is possible to distinguish the main lymphatic vessels: on the surface of intraventricular grooves, anterior and posterior, on the surface of the atrioventricular groove, on the posterior surface of the left atrium and left ventricle. The diameter of the vessels is significant, but the vessels lack lamina media. There are also many single and aggregated LYVE (+) cells apart from the vessels. Moreover, LYVE(+) cells form vessel web locally among the myocardial trabecules. In the postnatal hearts lymphatic vessels grow reaching myocardium and locating in the vicinity of the coronary arteries on the dorsal surface of the cone. A great number of the vessels is also present subepicardially. The course and location of lymphatic vessels in the mature mice hearts are similar.

Conclusion: The lymphatic vessels in the heart develop from the diffused buds, which mature by developing into a thin web and enlarging their diameter. The aggregations of the greatest lymphatic vessels appear on ED 15 and correspond with the vessels of the mature mouse heart.

Looking for a simple method of satellite cells differentiation toward osteoblasts in vitro

ESC-ID 750
Author Wlodkowska M, Wrobel E
Country Poland
University Medical University of Warsaw
Department Department of Biophysics and Human Physiology

Regenerative medicine of bone is still looking for new sources of autologous cell transplants. Particularly, the growing interest in cells obtained from so-called stem cell-niches can be observed. Among them, satellite cells, which take part in regeneration and growth of skeletal muscles, are believed to possess the ability to achieve osteogenic phenotype. Our aim was to verify if the medium used for terminal differentiation of human bone derived cells may be effective for such a purpose. The C2C12 cell line was used, which is commonly regarded as comparable to human satellite cells. C2C12 cells were cultured in two different culture media: standard medium (DMEM with antibiotic, L-glutamine, 10% FCS) and the same medium supplemented with vit C, D3 and dexamethasone. Cells were cultured for 25 days. Every two days, cell number, alkaline phosphate activity (ALP), total protein amount (BCA) and osteocalcin production was assessed. Cell morphology was observed and index of fusion was counted. The cell number increased slightly during the first culture period and then became stabilized. Cells in standard medium were found to fuse into long, slim tubes, whereas those in the stimulating medium cells observed in microscope created myosacks (multi-branched star shaped structures with abundant nuclei). Such behavior was reflected in the results of fusion index calculation. The BCA level was the same during the whole observation period in both populations. ALP activity in standard medium was undetectable whereas high ALP activity was found in stimulated culture as early as 5 day after the addition of the differentiating medium. The average osteocalcin concentration at all time points was equal to 0 in both populations. Singular osteocalcin positive measurements were found in the stimulated population. Vit C, D3 and dexamethasone enriched medium is commonly used for maturation of non-differentiated cells with osteogenic potential. In this experiment it was shown that such medium is able to at least seriously disturb the spontaneous myogenesis of inculture C2C12 cells. It seems however, that this is not enough to achieve the terminally differentiated osteoblast capacity. Furthermore, surprisingly high ALP activity in stimulating medium (which is normally undetectable in C2C12 culture) as well as osteocalcin traces, indicate that the medium used in this experiment may be a good basal medium for further research on C2C12 cells abilities toward osteogenic differentiation.
Anesthesiology and Reanimation I

Tight glycaemic control in the critically ill patients receiving parenteral nutrition (PN) in the intensive care unit (ICU).

ESC-ID 911
Author Tay BCW, Peter JD, Andrews MD
Country United Kingdom
University University of Edinburgh
Department College of Medicine and Veterinary Medicine

**Heading:** Ever since the Van den Berghe study was published in the New England Journal of Medicine in year 2001, considerable enthusiasm and significant resources has been allocated to achieving tight glycaemic control in critically ill patients in the Intensive Care Unit. We have a tight glycaemic control protocol for all critical care patients, including those on Parenteral Nutrition (PN) in the ICU. It is debatable whether we are over-feeding our patients with PN or to lack of large patient number trials and guidelines, but reliable caloric infusion is required to safely infuse insulin.

**Aim:** The aim of this study is to establish the association between tight glycaemic control and total parenteral nutrition. The focus will be put on answering whether patients with PN are more prone to be hyperglycaemic or hypoglycaemic; identification of risk factors in developing hyperglycaemia or hypoglycaemia in patients receiving PN. We also hope to suggest solutions that can improve the glycaemic control in patients with PN.

**Design:** Prospective, observational, cohort study with good external validity.

**Setting:** 15 bedded university intensive care unit.

**Patients:** Level II & III critical care patients.

**Method:** 64 patients received 2000kcal parenteral nutrition everyday were under tight glycaemic control by insulin infusion, plasma glucose levels were measured, separated into 4 levels: life-threatening hypoglycaemia (below 3.5 mmol/l), hypoglycaemia (3.5 – 4.3 mmol/l), normoglycaemia (4.4 – 7.0 mmol/l) and hyperglycaemia (higher than 7.0 mmol/l), and analysed in different risk-factor groups (Pre-existing diabetes mellitus, Catecholamines Infusion, Steroid therapy, Obesity, Elderly patient, Surgery or trauma).

**Results:** Patients received PN had a higher mean glucose levels: life-threatening hypoglycaemia (below 3.5 mmol/l), 4.69% (n = 30) of patients developed at least one episode of life-threatening hypoglycaemia (below 3.5 mmol/l), 46.9% (n = 30) of patients developed at least one episode of hypoglycaemia and 9.4% (n=6) of patients had at least one episode of life-threatening hypoglycaemia.

**Conclusion:** Parenteral Nutrition is a very important risk factor of hyperglycaemia and hypoglycaemia among critically patients under tight glycaemic control in ICU; particularly in patients who were diabetic, with glucocorticoids infusion or medical patients. The patients’ glucose level need to be monitored more closely than other general critical care patients and insulin level needs to be adjusted very cautiously. A new glycaemic control protocol, more resources, training and time are needed in order to achieve the ideal tight glycaemic control in the critically ill patients receiving PN in ICU.

Anesthesiological care evaluation in Vilnius University Emergency Hospital

ESC-ID 685
Author Rainys D, Sarunas J, Judickas S, Rucinskas A
Country Lithuania
University Vilnius University
Department Medicine Faculty

**Aim:** Anesthesiologists are now “perioperative physicians” supervising care in a variety of locations from preoperative evaluation clinics to intensive care units and pain clinics, and to operating room sites. The perioperative period is emotionally stressful for many patients who may have fears about surgery and anesthesia. The anesthetist can alleviate many of these fears and foster trust in managed medical care. The aim of our study was to evaluate patients’ opinion about anesthesiological care in Vilnius University Emergency Hospital.

**Methods:** An uncontrolled retrospective questionnaire inquiry of patients (n = 140) on the second day of postoperative period was performed in Vilnius University Emergency Hospital in 2006.

**Results:** 73.57% (n = 103) of patients were satisfied with the quality of postoperative analgesia and 26.34% (n = 37) – unsatisfied, according to the values of visual analogue scale (VAS) filled by themselves during postoperative period. Only 67.14% (n = 94) of respondents were acquainted with possible negative events of anesthesia. The pre and postoperative care of anesthetist was evaluated farther: „very well“ 55.7% (n = 78) and 51.42% (n = 72), „well“ 33.57% (n = 47) and 30% (n = 42), „satisfactory“ 7.85% (n = 11) and 8.57% (n = 12), „unsatisfactory“ 2.85% (n = 4) and 10% (n = 14) (p <0.05). 98.57% (n = 138) of patients were given analgetics well-timed after operation. The impression about anesthesia was named as „very well“ in 39.29% (n = 55) of respondents, „well“ – 49.29% (n = 69), „satisfactory“ – 11.42% (n = 16). 35% (n = 49) of patients felt „very well“, 42.86% (n = 60) - „well“, 19.28% (n = 27) - „satisfactory“ and 2.86% (n = 4) „unsatisfactory“ after anesthesia. 65% (n = 91) of entrants were satisfied with analgesia during the first postoperative day and 85.71% (n = 78) of them remain satisfied with the quality of analgesia during further days in hospital (p <0.05). Before the general anesthesia (55%, n = 77), the part of patients who felt „very anxious“ was 37.66% (n = 29), „little anxious“ – 37.66% (n = 29) and 24.67% (n = 19) „without anxiety“, but after regional anesthesia (n = 63, 45%) the numbers were different respectively – 11.11% (n = 7), 53.97% (n = 34) and 34.92% (n = 22) (p <0.001). Patients who were unsatisfied with postoperative pain management commonly were those, who believed that it must hurt after operation (86.48%, n = 32, p <0.05). 98.58% (n = 138) of patients would like to have the same method of anesthesia during other possible operations in future.

**Conclusion:** Three of four patients were satisfied with anesthesiological care in Vilnius University Emergency Hospital. Our trial results revealed the need for paying attention to pre-
operative anxiety treating, belief, that it must hurt after operation, denial, pain management on the first postoperative day, closer communication between anesthesiologist and patient, especially after surgery.

**Evaluation of dexamethazone epidural injection on headache of post spinal anesthesia**

**ESC-ID**: 966  
**Author**: Hosseinpour M, Asaei SH, Zare F  
**Country**: Iran  
**University**: Jahrom university of medical science  
**Department**: Motahari Hospital

**Introduction**: we know that about 15% of patients undergoing spinal anesthesia would develop with headache. In this study we want to investigate the effect of epidural injection of dexamethazone on the headache proceeding with spinal anesthesia.

**Material and method**: In this study we divided 400 patients into two groups 200 patients in group A after managing spinal anesthesia with 4 cc marcaine 0.5% we injected 8 mg dexamethazone which was diluted with 5 cc normal saline to the epidural area. In group B there were 200 patients that we injected only 5 cc normal saline as a control group and then we followed these two groups for one week for the headache.

**Results**: in group B in the first day after spinal anesthesia 15% of the patients developed with headache and after that the rest of week just 7% had headache. In the group A 10% of the patients developed with headache in the first day and 5% in the rest of the week.

**Conclusion**: It seems that injecting epidural dexamethazone decreases the rate of headache proceeding with spinal anesthesia. Key words: dexamethazone, headache, spinal anesthesia

**Effects of thoracic epidural anaesthesia on mesenteric leukocyte endothelium interaction and endothelial permeability in a rodent model of normotensive endotoxaemia.**

**ESC-ID**: 713  
**Author**: Enigk F, Elster A, Mousa SA, Habazettl H, Schafer M, Schaper J  
**Country**: Germany  
**University**: Charite Universitätsmedizin-Berlin  
**Department**: Physiology, Campus Benjamin Franklin

**Aim**: Endotoxaemia is associated with an activation of the sympathetic nervous system which goes along with elevated plasma catecholamine levels. Previous in vitro and in vivo studies have shown that catecholamines induce the expression of endothelial and leukocytic adhesion molecules resulting in enhanced leukocyte adhesion to endothelial cells. This key step of the inflammatory immune response may cause severe injury to the vascular endothelium and the underlying tissue. The aim of this study was to quantify the effects of splanchic sympathetic blockade by means of thoracic epidural anaesthesia (TEA) on the endotoxin-induced immune response and subsequent vascular injury.

**Method**: This controlled experimental study was conducted in male Sprague-Dawley rats weighing 300 ± 26, mean ± SD) g. Under general anaesthesia (ketamine/urethane) epidural catheterisation and vascular catheterisation for haemodynamic monitoring and drug as well as fluid application was performed. Rats were allocated to receive either epidural infusion of lidocaine 1% or normal saline (30 µl/h) paralleled by a continuous i.v. infusion of Escherichia coli lipopolysaccharide (LPS, serotype 0127:B8, 1.5 mg/kg per h) or normal saline. 3 groups were investigated: endotoxaemia control (LPS + TEA -, n = 11), TEA (LPS + TEA +, n = 10) and healthy control (LPS – TEA -, n = 11). After 5 h of endotoxaemia leukocyte-endothelium interaction was quantified in mesenteric venules by intravital videomicroscopy. Endothelial permeability was determined by wet-to-dry weight ratios of intestinal tissue specimens. Expression of gut endothelial adhesion molecules was assayed by immunohistochemistry.

**Results**: Mean arterial pressure did not differ among groups, while heart rate increased in those groups receiving LPS. LPS boosted the expression of endothelial adhesion molecules E selectin and ICAM-1. In addition, there was a significant, LPS-induced rise in the number of adherent and transmigrated leukocytes with an increase in gut wet-to-dry weight ratio. TEA significantly attenuated the expression of endothelial adhesion molecules and decreased leukocyte rolling by 71 [54/89] % (median [quartiles]) and firm adhesion by 66 [0/80] % (vs. LPS + TEA –, p < 0.05). Leukocyte extravasation was not altered by TEA. Furthermore, TEA lowered LPS-induced endothelial permeability by 19 [3/29] % vs. LPS + TEA – (p < 0.05).

**Conclusion**: Endotoxaemia induced an inflammatory response with capillary leakage. Thoracic epidural anaesthesia attenuated both phenomena. Inhibition of endothelial adhesion molecule expression due to splanchic sympathetic blockade may represent a molecular mechanism underlying this beneficial effect of TEA.

**Biochemistry I**

**The metabolic changes in adrenal cortex during the modelling of acute cholestasis**

**ESC-ID**: 279  
**Author**: Kiziukevich DL, Ambruskevich AV, Bogdanovich TI, Kusnetsov OE, Kiziukevich LS  
**Country**: Belarus  
**University**: Grodno State Medical University  
**Department**: Pediatrics

**Aim of investigation**: The complex evaluation of metabolic and functional disorders developing in the adrenal cortex 24 or 72 hrs after the start of acute subhepatic cholestasis.

**Material and methods**: 40 male mongrel white rats (body weight 190.0 ± 30.0 g) were studied. The treated animals were subdivided into 2 groups according to the time of obstructive jaundice. In 1st group (10 rats) the subhepatic cholestasis lasted 24 hrs and was induced by a common bile duct (CBD) ligation near the hepatic portae. In 2nd group (10 rats) the similar CBD ligation was performed for 72 hrs. Sham-operated rats (n = 20) with intact CBD served as the control for both groups. At the end of cholestatic treatment, the degree of cholestasis and the functional activity of adrenal cortex were estimated using the blood concentrations of
total bile acids and cortisol (the hormone of adrenal cortex) measured with a biochemical microanalyzer Archimed 2000 [1]. Succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities were determined using the histochemical methods in the cortical substance of fresh-frozen cryostatic adrenal slices [2]. The intracellular metabolism in the various adrenocortical zones was quantitatively estimated using the software Bioscan NT.

Results: The results have shown that 24 hrs after the start of experimental cholestasis the serum total bile acid concentration dramatically increased (by a factor of 15.6; P < 0.001). Simultaneously the cortisol concentration increased by 30% (P < 0.05), but the SDH and LDH activities in cryostatic slices of adrenal cortex (in glomerular, fascicular and reticular zones) fell down. 72 hrs after the start of subhepatic cholestasis the serum total bile acid concentrations rose even more (by a factor of 30.5 vs. the control; P < 0.001). Cortisol concentration increased by a factor of 2.8 vs. the control (P < 0.01). SDH and LDH activities in cryostatic slices of adrenal cortex (in glomerular, fascicular and reticular zones) were slightly and insignificantly higher than in control.

The metabolic and functional disorders in adrenal cortex during the first three days of experimental

ESC-ID 280
Author Amburskevich AV, Kiziukevich DL, Bogdanovich TI, Kusnetsov OE, Kiziukevich LS
Country Belarus
University Grodno State Medical University
Department General Medicine

Aim of investigation: The complex evaluation of metabolic and functional disorders developing in the adrenal cortex 24 or 72 hrs after the start of acute supraduodenal cholestasis.

Material and methods: 40 male mongrel white rats (body weight 190.0 ± 30.0 g) were studied. The treated animals were subdivided into 2 groups according to the time of obstructive jaundice. In 1st group (10 rats) the supraduodenal cholestasis lasted 24 hrs and was induced by a common bile duct (CBD) ligation near the duodenum. In 2nd group (10 rats) the similar CBD ligation was performed for 72 hrs. Sham-operated rats (n = 20) with intact CBD served as the control for both groups. At the end of cholestatic treatment, the degree of cholestasis and the functional activity of adrenal cortex were estimated using the blood concentrations of total bile acids and cortisol (the hormone of adrenal cortex) measured with a biochemical microanalyzer Archimed 2000 [1]. Succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activities were determined using the histochemical methods in the cortical substance of fresh-frozen cryostatic adrenal slices [2]. The intracellular metabolism in the various adrenocortical zones was quantitatively estimated using the software Bioscan NT. The results have shown that 24 hrs after the start of experimental supraduodenal cholestasis the serum total bile acid concentration dramatically increased (by a factor of 12.2; P < 0.001). Simultaneously the cortisol concentration increased by a factor of 2.2 vs. the control (P < 0.05), but the SDH and LDH activities in cryostatic slices of adrenal cortex (in glomerular, fascicular and reticular zones) had a trend to increase.

The ways of early detection of diabetic nephropathy

ESC-ID 305
Author Tkachenko A
Country Ukraine
University Kharkov State Medical University
Department Biochemistry

Introduction: Kidney’s lesion in diabetes mellitus is one of the main reasons for mortality of patients. The functional condition of kidney’s endothelium plays the key role in the nephropathy genesis and can be examined by the measurement of the levels of NO and endothelin. The direct correlation between conditions of the endothelium and thrombocyte aggregation was recognized. That is why the purpose of our research was study of endothelium-derived factors and aggregative properties of thrombocytes on the initial stage of diabetic nephropathy.

Material and methods: 40 patients with diabetic nephropathy were examined. I-II stages of the nephropathy were diagnosed on the basis of the proteinuria, the level of glomerular filtration, the levels of blood creatinine and urea, the relative density of the urine. The level of endothelin-1 was measured by ELISA. The stable intermediates of NO content (S-nitrosothiol) and NO precursor - L-arginin were detected by chromatographic analysis. Aggregative properties of thrombocytes and their sensitivity to L-arginin in vitro were measured by Burn method. The activity of eNO-synthase was established spectrometrically.

Results: Every examined patient with the nephropathy had decreased level of L-arginine, S-nitrosothiol and eNO-synthase activity compared with patients suffered from diabetes mellitus without the nephropathy. The endothelin-1 content was increased in the main group, i.e. the presence of endothelial dysfunction was established. The patients with diabetic nephropathy had increased level of ADP-induced thrombocyte aggregation. It’s known the S-nitrosothiol inhibits thrombocyte aggregation and endothelin-1 activates it, i.e. the connection between increased level of thrombocyte aggregation and the dysfunction of endothelium is present. We studied ADP-induced thrombocyte aggregation under an influence of 1 IM of S-nitrosoglutathione. The influence ratio is higher in patients with nephropathy probably due to considerably increased level of free-radical processes in diabetic nephropathy. In such conditions the high concentrations of a peroxynitrite can be produced (the thrombocyte aggregation inducing agent). The incubation of the thrombocyte enriched plasma with 300 ìM of L-arginine led to inhibition of ADP-induced thrombocyte aggregation only in patients with diabetic nephropathy due to increased production of NO by thrombocytes! The direct correlation between eNO-synthase activity, the levels of the proteinuria and ADP-induced thrombocyte aggregation in presence of L-arginine was found.

Conclusion: The aggregative properties of thrombocyte in presence of L-arginine can be used for the early detection of diabetic nephropathy.
Biochemical markers of activity of pathology process in elderly patients with chronic pancreatitis

ESC-ID 50
Author Kendzerska T
Country Ukraine
University Bukovinian State Medical University
Department Internal Medicine and Clinical Pharmacology

In the pathogenesis of development of chronic pancreatitis (CP) with concomitant ischemic heart disease (IHD) the role of metabolic disorders is an important factor. So the aim of our research is studying of intensity of oxidative modification of proteins, activity of fibrinolytic and proteolytic (by level of azocasein, azocalbumin and azocol) systems in elderly patients with CP with concomitant IHD (exertional angina I-II FC). 62 patients with CP with concomitant IHD (from 50 to 85 years old, 64.4 ± 9.1) and 30 healthy persons had been examined. For discovering factors that independently describe status of metabolic changes and disorders in hemostasis in patients with CP with concomitant IHD factor analysis was performed (using Statistica for Windows, Release 5.5). In examination of oxidative modification of proteins reliable (p<0.05) raise of neutral aldehyde- and ketone-derivatives (2.42 ± 0.09 micromole/g of protein, in healthy 1.37 ± 0.08 micromole/g of protein) was found. Simultaneously level of alkali aldehyde- and ketone-derivatives practically did not change. Degradation of fibrinolytic activity, mostly due to enzymatic activity (0.51 ± 0.02 Å440 ml/h, 0.83 ± 0.04 Å440 ml/h in healthy, p<0.05), overall activity (respectively 0.83 ± 0.03 Å440 ml/h, 1.31 ± 0.08 Å440 ml/h, p<0.05) and less due to non enzymatic activity (respectively 0.40 ± 0.01 Å440 ml/h, 0.48 ± 0.04 Å440 ml/h, p<0.05) was found. The activation of both unlimited and limited proteolysis with maximal raise of intensity of proteolytic degradation of high-molecular proteins (4.55 ± 0.18 Å440 ml/h with normal value of 1.93 ± 0.11 Å440 ml/h, p<0.05) was found. From all studied biochemical parameters most important as a prognostic factor in elderly patients with CP with concomitant IHD are those factors that are connected with fibrinolytic and proteolytic (degradation of high-molecular proteins) activities of blood plasma. The dynamics of changes of contents of oxidative modification of proteins products, fibrinolytic and proteolytic activity in blood plasma in patients with CP with concomitant IHD can be used for determination of activity of pathological process and serve as a base for differential treatment for such patients.

The small GTPase RhoA links the Kaposi sarcoma-associated herpes virus-G protein coupled receptor to heme oxygenase-1 expression and tumorigenesis

ESC-ID 72
Author Martín MJ, Tanos T, García AB, Coso O, Marinissen MJ
Country Spain
University Universidad Autonoma de Madrid
Department Biochemistry

Heme oxygenase-1 (HO-1), the inducible enzyme that metabolizes the heme group, is highly expressed in human Kaposi Sarcoma lesions (KS). Its expression is upregulated by the G protein-coupled receptor from the Kaposi sarcoma-associated herpes virus (vGPCR). HO-1 mediates both vGPCR-induced tumorigenesis and vascular endothelial growth factor (VEGF) expression, but the molecular mechanisms that link the viral receptor to HO-1 remain unidentified. Here we show that vGPCR induces HO-1 expression and transformation through the G?13 subunit of heterotrimeric G proteins and the small GTPase RhoA. Uncoupling signal transmission from G?13 to RhoA or inhibiting RhoA by the dominant negative mutant RhoAN19 or the C3 toxin, impairs vGPCR-induced ho-1 promoter activity. NIH-3T3 cells expressing constitutively activated G?13 (G?13QL) or RhoA (RhoAQL) implanted in the dorsal flank of nude mice develop tumors, which display elevated levels of HO-1 and VEGF-A as well as spindle-shaped cells characteristic of vGPCR-derived tumors and human KS lesions. Strikingly, targeted knocked-down expression of RhoA by shRNA results in reduced vGPCR-induced HO-1 expression, VEGF-A secretion, cellular transformation and tumor-forming potential. Similarly, shRNA knocked-down expression of HO-1 or inhibition of HO-1 activity by chronic administration of the HO-1 inhibitor Sn protoporphyrin (SnPP) to mice bearing RhoAQL-induced tumors significantly reduced tumor growth by nearly 70%. Our study shows that vGPCR induces HO-1 expression through a G?13/RhoA-dependent signalling pathway and uncovers a role for HO-1 as a potential therapeutic target in tumors where RhoA expression or activity is upregulated.

The effects of ursodeoxycholic acids on oxidative stress level and DNase activity in hepatic tissue

ESC-ID 82
Author Jovic M, Jovic M, Sokolovic D
Country Serbia
University University of Nis
Department Biochemistry

Introduction: Accumulation of toxic hydrophobic bile acids(BA) during cholestasis can activate Fas death receptors and increase production of reactive oxygen species(ROS) simultaneously inducing apoptosis of hepatocytes. The major beneficial effects of treatment with ursodeoxycholic acid (UDCA) are protection against cytotoxicity of BA, stimulation of hepatobiliary secretion, antioxidative activity and blocking the apoptotic signal. The aim of study: The aim of the present study was to evaluate the effect of UDCA on oxidative stress and DNA fragmentation in liver tissue of bile duct-ligated(BDL) rats.

Methods: Four groups of Wistar rats were investigated: I-control (sham operated), II-rats treated with UDCA (25mg/kg b.w. i.g.), III-rats with common bile duct-ligation (BDL), IV-cholestatic rats treated with UDCA. The animals were killed after 9 days.

Results: Increased plasma activity of GGT, AF(cholestatic markers) and concentration of BA in BDL rats was registered, while oral administration of UDCA induce decreasing of activity and concentration of all mentioned parameters (p <0.001). Lipid peroxidation-MDA and oxidative modification of proteins-carbonyl group content are significantly increased in BDL group (p <0.05). The apoptotic effect in cholestasis is probably triggered by the increase activation of alkaline- DNase and acid-DNase (p <0.001). UDCA was found to be protective in liver of BDL rats. It decreases MDA...
levels (p <0.01); moderately decrease carbonyl group; shows effect on terminal apoptotic reaction, because it decreasing alkaline-DNase and acid-DNase activity (p<0.05) compared with cholestatic rats.

Conclusion: These results support a novel effects of UDCA in the control of endonuclease activity and therefore in the DNA stability and cell apoptosis, beside established role in reducing oxidative damage and maintaining of antioxidant defenses in cholestatic liver.

The expression level of de-novo DNA methyltransferase DNMT3B isoforms in patients with gastric cancer

ESC-ID 961
Author Kruszyna L
Country Poland
University Karol Marcinkowski University of Medical Sciences in Poznan
Department Department of Biochemistry and Mol. Biology

Introduction: Gastric cancer is the second most common cause of cancer death in the world. DNA methylation is a major epigenetic modification in human cancers through the silencing of some tumor suppressor genes. Methylation occurs at the C-5 position of cytosine in the context of the CpG dinucleotides. In mammals this is performed by a family of DNA methyltransferases (DNMTs) that include maintenance DNMT1 and de novo DNMT3A and DNMT3B. Primary transcript of DNMT3B is alternatively spliced into five distinct isoforms and DNMT3B4 splice variant functions as a negative regulator of DNA methylation.

Aim: The aim of this study was to determine the expression level of all DNMT3B isoforms and to investigate the relation between the elevated mRNA levels of DNMT3B isoforms and the clinicopathologic characteristics.

Material and Methods: 27 patients with gastric cancer were investigated in this study. All these patients had undergone curative surgical resections. Samples of primary gastric cancer and matched noncancerous normal tissues were surgically obtained from each patient and immediately snap-frozen in liquid nitrogen. The total RNA was isolated from the fresh-frozen tissues, purified and reversibly transcribed. Quantitative analysis of DNMT3B splice variants were performed by real-time quantitative PCR (RQ-PCR).

Results: The RQ-PCR analysis revealed that the mRNA levels of DNMT3B isoforms were elevated in tumor tissue in comparison with control normal tissue. We first analyzed all DNMT3B splice variants in 5 patients. We observed increased level of DNMT3B2 and DNMT3B3 isoforms in tumor tissue in comparison with control tissue.

Conclusion: DNMT3B is a de novo methyltransferase and plays a crucial role in carcinogenesis. It is overexpressed in many cancer types. To this time the level of DNMT3B splice variants was not investigated in gastric cancer. Elevated mRNA expression of DNMT3B may be an independent prognostic factor in gastric cancer and further analysis in whole group of patients is necessary.

The search of amyloidogenic determinant of human alpha-lactalbumin

ESC-ID 200
Author Egorov VV
Country Russia
University Medical University
Department Molecular Virology and Genetic Engineering

Amyloidoses are the group of conformational diseases which concerned with formation of toxic oligomers during fibrillogensis. Study of molecular basis of toxic oligomers formation process can lead to development of new drugs against amyloidoses. We use human alpha-lactalbumin as a model of disease-causing fibrillogenic proteins because of cytotoxicity of it's oligomeric forms. During the analysis of aminoacid sequences of six amyloidogenic peptides (from lysozyme of T4 phage, transthyretin, amylin, prion protein, insuline and amyloid-beta peptide) it was found that all of them contain mirror symmetry motifs. Such patterns was founded in primary structure of human alpha-lactalbumin. Onó of these patterns is situated near the beta-domain of this protein. With the help of transmissive electronic and atomic force microscopy it was shown that peptide identical to the mirror symmetry motif (35-51 residues) of human alpha-lactalbumin (GYDQTAVENNESTYG) can form abnormal (amyloid-like) fibrils. In further investigation it was shown that similar peptide contains 5 aminoacid substitutions in non-symmetrical hydrophobic core (GYDTQTVVNNHHTDYG) can form abnormal fibrils too (1). We showed that during fibrillogensis two molecules of latter peptide can interact with one molecule of alpha-lactalbumin. Formation of these complexes did not prevent fibrillogensis but changed stechiometry of toxic prefibrillar oligomers. Designing of peptide inhibitors which can change structure of prefibrillar oligomers can be a new strategy for amyloidosis prevention.

Biochemical and morphofunctional hepatic aspects in experimental induced diabetes treated with natural polyphenols

ESC-ID 215
Author Tesliariu O
Country Romania
University University of Medicine and Pharmacy "Gr.T.Popa"
Department Physiopathology

Introduction: Polyphenols are wide variety of compounds that occur in fruits and vegetables, wine, tea, cocoa products. They are supposed radical scavangers, but recent data indicate that polyphenols can stimulate antioxidant transcription and detoxification defense systems through antioxidant responsive elements (AREs).

Aim: In this study we investigated the effects of some natural polyphenols obtained from black grape's kernel, in streptozotocin induced diabetes. Material and method: We used 4 groups of Wistar rats: Group C – control; Group D – experimental induced diabetes with streptozotocin, 60 mg/kgbw, i.p, in a single dose; Group P – received a natural polyphenols solution, 0.028 g/kgbw, p.o., at 2 days interval, for 16 weeks; Group D+P – received polyphenolic extract, in the same dose, for 16 weeks, after the induced diabetes. We investigated antioxidant activity of catalase (CAT), glutathione peroxidase...
Characterization of structural domains in the Type III restriction endonuclease EcoP15I by limited proteolysis, mass spectrometry and insertional mutagenesis

ESC-ID 765
Author Wagenführ K, Pieper S, Mackeldanz P, Linscheid M, Krüger DH, Reuter M
Country Germany
University Charité Universitätsmedizin Berlin
Department Institute of Virology

The Type III restriction endonuclease EcoP15I forms a heterooligomeric enzyme complex that consists of two modification (Mod) subunits and two restriction (Res) subunits. It cuts DNA with two inversely oriented recognition sites (5' CAGCAG). Structural data on Type III restriction enzymes in general are lacking because of their remarkable size of more than 400 kDa and the laborious and low-yield protein purification procedures. We took advantage of the EcoP15I-overexpressing vector pQEP15 and affinity chromatography to generate a quantity of EcoP15I that is high enough for comprehensive proteolytic digestion studies and analyses of the proteolytic fragments by mass spectrometry. We show here that in the presence of specific DNA the entire Mod subunit is protected from trypsin digestion, whereas in the absence of DNA stable protein domains of the Mod subunit were not detected. In contrast, the Res subunit is comprised of two trypsin-resistant domains of approximately 77-79 kDa and 27-29 kDa, respectively. The cofactor ATP and the presence of DNA, either specific or unspecific, are important stabilizers of the Res subunit. The large N-terminal domain of Res contains numerous functional motifs that are predicted to be involved in ATP-binding and hydrolysis and/or DNA translocation. The small C-terminal domain harbours the catalytic center. Based on our data, we conclude that both structural Res domains are connected by a flexible linker region that spans 23 amino acid residues. To confirm this conclusion, we investigated several EcoP15I enzyme mutants obtained by insertional mutagenesis in and around the predicted linker region within the Res subunit. All mutants tolerated the genetic manipulation and did not display loss of function or alteration of the DNA cleavage position. In contrast, deletion of the linker region destroyed the catalytic activity of EcoP15I.

Dermatology I

The frequency of various symptoms among patients with atopic dermatitis

ESC-ID 517
Author Ryduchowska M
Country Poland
University Medical University
Department Medical

Background: Atopic dermatitis (AD) is the chronic and recurrent illness related to the epidermis and proper skin, being characterized by very intensive itch, typical location, the characteristic skin changes and coexistence of different atopic diseases at the patient or in his family. Diagnosis of atopic dermatitis depends on clinical features because no definitive diagnostic test exists. In 1980 Hanifin and Rajka published major and minor criteria for AD.

Aim and objective: The aim of our work was the estimation of the frequency of the occurrence of individual clinical criteria according to the Hanifin and Rajka at the patients with atopic dermatitis, which were patients of Clinical Dermatology, Venerology and Allergology Medical University of Gdańsk in years 2002-2006. Subjects and methods: In 168 patients of Clinical Dermatology, Venerology and Allergology Medical University of Gdańsk with atopic dermatitis the occurrence of the major and minor features isolated by Hanifin and Rajka was analysed (65 sex manful and 103 sex female, in age from 1 to 49 years). Every patient was subjected the subjective and objective investigation under the angle of large and small clinical criteria according to Hanifina and Rajki. Additionally, the blood was taken every ill in the aim of the sign of the level IgE.

Results: The occurrence the large criteria confirmed the decided majority of studied: pruritus (93%), chronic/relapsing dermatitis (96%), dermatitis in classical distribution (91%) and personal or family history of atopy (84%). The frequency of the occurrence of small features introduced as follows: keratosis pilaris (73%), ichthyosis vulgaris (7%), skin reactions from ingested food (73%), intolerance of wool (53,5%), cheilitis (49%), nipple eczema (14%), hand/foot eczema (58%), orbital darkening (64%), early age of onset (80%), white dermatographism (40%), Dennie-Morgan infraorbital fold (39%), anterior neck folds (15,5%), itch when sweating (66%), tendency toward cutaneous infections (40%), facial erythema (52%), emotional factors (55%), periocular accentuation (38%), pityriasis alba (16%), cataract (2%), keratoconus (0,5%), different oculistic disorders (5%).
elevated serum IgE (78%), 53% of studied patients had positive skin tests with various allergens.

Conclusion: The most often we observed all large criteria, keratosis pilaris, early age of onset, elevated serum IgE, skin reactions from ingested food, itch when sweating and orbital darkening.

Neurophysiologic mechanisms of itch in patients with atopic dermatitis: pilot research of long-latency evoked potentials

ESC-ID 11
Author Toropina MM, Lvov AN, Ivanov OL
Country Russia
University Moscow Medical Setchenov Academy
Department Dermatology

The aim Pruritus or itch is defined as a subjective sensation that provokes a desire to scratch. Its mechanisms are still not fully understood, though it is closely related to pain and is thought to be conducted along unmyelinated C-fibers. The aim of this investigation was to study the central mechanisms of itch by the obtaining of brain answers to the selective C-fibers stimulation in patients with atopic dermatitis (AD).

Subjects and methods: Long-latency evoked potentials (LLEP) were registed by means of Contact-Heat Evoked Potential Stimulator (CHEPS) in 13 AD – patients (12 women and 1 man with average age 26.9 ± 10.0 years) and 12 healthy volunteers (11 women and 1 man with average age 27.1 ± 7.0 years). Short heat impulses (t = 54&###186;C) were applied to ventral surface of the forearm near the elbow flexion and to check skin on the face. All patients were in exacerbation stage with SCORAD index 39-78, on average 54 points. Forearm flexion surface demonstrated most lesions just as itch intensity in all AD patients while the cheek area was free of eruptions in 3 patients, slightly or moderately affected in 8 and considerably affected in 2 patients. Icht intensity varied from 1.5 to 8.5, on average 5.5 points according to visual analog scale.

Results: In control group we obtained LLEP in form of negative-positive complex with latency of the most prominent positive peak 436 ± 32 ms after forearm stimulation and 379 ± 21 ms after cheek stimulation. The conduction velocity of afferent impulse was calculated by means of measurement of the distance between stimulation areas and latency difference of LLEP. It corresponded to conduction speed along C-fibers namely 1m/s. LLEP with the same configuration were acquired in AD patients however larger variations of amplitudes and latencies were observed. When compared with normal data a significant latency prolongation of LLEP to cheek stimulation in AD patients 409 ± 38 ms (δ <0.05) and not marked prolongation of LLEP to forearm stimulation 451 ± 29 ms (δ = 0.1) were revealed. Averaged LLEP-waveform from the cheek area in AD group was significantly lower in amplitude as compared to control averaged LLEP, though statistical amplitude data did not differ apparently due to large sampling dispersion.

Discussion and conclusion: The acquired data show the disturbances in C-fibers afferentation in AD patients. The most significant changes were observed while stimulating the skin regions with minimal lesions and itch (cheek area), whereas LLEP to the stimulation of affected areas (forearm) tended towards normal parameters. We hypothesized that AD patients have an initial C-fibers insufficiency which contributes to itch appearance. Constant intensive scratching stimulates afferent fibers and produces compensatory effect. The research should be continued in order to confirm the obtained data.

The clinical usage of uridine for the treatment of the hand-foot syndrome (palmar-plantar erythrodyssaesethaesthesia) and mucositis induced by 5-FU and its oral derivates (Capecitabine)

ESC-ID 818
Author Pestka A, Partyka D
Country Germany
University University of Medical Science in Poznan
Department Department of Pharmaceutical Technology

Aim: The hand-foot syndrome (HFS) is an erythematous skin lesion of the palma and planta of the hand and feet is most often caused by cytostatic chemotherapy. It is a side effect of 5-FU and its oral derivates that results when a small amount of drug leaks out of the smallest blood vessels in the palms of the hands and soles of the feet. The amount of drug in the capillaries of the hands and feet increases due to the friction and subsequent heat that is generated in those extremities. As a result, more drug may leak out of capillaries in these areas. The main goal of this study is to confirm the effectiveness of uridine in comparison to placebo.

Methods: Studies are taking place with 10 patients with colon cancer and breast cancer whom are treated with 5-FU in the oncologic hospita in Poznan Poland. All patients were administered capecitabine and intravenousalsby 5-FU

Results: 1 am in the process of confirming the effects of uridine , and the results will be available in a month.

Conclusion: The medical usage of uridine-ointment is confirmed in comparison to the placebo.

Ethnic variation of hair follicle size and follicular penetration of topically applied substances

ESC-ID 841
Author Luther N, Teichmann A, Lademann J
Country Germany
University Charité Universitätsmedizin Berlin
Department Department of Dermatology

Ethnic variability in skin function and structure has been the subject of many investigations. Recently it has been shown that ethnic differences may exist. However, the published results are often contradictory. The skin barrier is one of the most important skin functions and represents a challenge in medical treatment and cosmetic care. Hair follicles offer holes in this skin barrier and therefore are well suitable to contribute to the skin penetration process. As a result of this, their racial variability has to be considered. The aim of this study was to compare the follicle size and their distribution on the scalp and calf and to investigate the amount of topically applied substances penetrated into the hair follicles and into stratum corneum in three ethnic groups: Africans, Caucasians and Asians. Sodium fluoresceine was applied on
the demarcated skin area of 4 x 12 cm² on the calf of 18 male volunteers (6 Africans, 6 Asians, 6 Caucasians). Differential stripping, a combined method of tape stripping and skin surface biopsies, was applied to investigate both, the amount of sodium fluoresceine penetrated into the stratum corneum and the hair follicles at different time points (30 min., 24 h, 96 h). Cyanacrylate skin surface biopsies were removed from untreated skin to obtain follicular casts for morphological analysis. Moreover, the following skin parameters were investigated: transepidermal water loss, pH, corneocyte variability and sebum production. The differences in volume and surface of the terminal hair follicle on the scalp between Caucasians, Asians and Africans were statistically significant. The surface for Caucasians was (0.42 ± 0.12 mm²), for Africans (0.3 ± 0.05 cm²) and for Asians (0.31 ± 0.06 mm²), the difference was statistically significant (p = 0.047). The volume for Caucasians was (0.017 ± 0.007 mm³), for Africans (0.009 ± 0.002 mm³) and for Asians (0.009 ± 0.003 cm³), the difference was statistically significant (p = 0.040). The hair shaft diameter on the scalp for Asians was (78 ± 10 µm), for Caucasians (67 ± 9 µm) and for African (62 ± 4 µm), the difference was statistically significant (p = 0.009). The follicular penetration after 30 min for Caucasians was (7.03 ± 1.8 Units) and for Africans (5.05 ± 1.9 Units), the difference was borderline significant (p = 0.078). The follicular penetration after 24 hrs for Caucasians was (1.05 ± 0.7 Units) and for Asians (2.14 ± 0.9 Units), the difference was statistically significant (p = 0.03). No statistical difference was found for the investigated skin parameters between Asians, Caucasians and Africans. The results of this study show, that there are ethnic differences in the hair follicle reservoir and the hair size on the scalp. The ethnic variability in the skin penetration needs to be taken into consideration to adapt therapy and cosmetic care.

Does the presence of the identified antinuclear antibodies in patients with DLE confirm SLE?

ESC-ID 607
Author Fabisiak A, Ciesielska K
Country Poland
University Medical University of Warsaw
Department Dermatology

Lupus Erythematosus (LE) is a connective tissue disease. There are two main types of LE: systemic (SLE) and cutaneous (CLE). The main representative of CLE is DLE (discoid LE). SLE involves both skin and organs, while DLE is only limited to the skin. Antinuclear antibodies (ANA) are present in sera of 95% of patients with SLE. Antibodies to double-stranded DNA (ds-DNA) and anti-Sm antibodies are highly specific markers for SLE. In patients with DLE, ANA are found in 30% of cases and these antibodies are usually not specific. The aim of our study was to analyse if the presence of the antinuclear antibodies in patients with DLE, especially specific antibodies like anti-Ro, anti-La, anti-RNP, leads to an appearance of systemic symptoms or it is a risk factor of conversion of the DLE to the SLE type. 212 patients with DLE from the Department of Dermatology of Warsaw Medical University were included in this study. DLE was diagnosed on the basis of patients&amp;#8217; typical clinical features of DLE. In some cases the diagnosis was confirmed by histopathologic examination of skin lesions. All patients underwent routine clinical examination and labatory tests. ANA, deteted by indirect immunofluorescence (IF), were present in 111 patients of which in 77 patients, ANA were identified by double immunodiffusion (DID) on agarose gel. Systemic symptoms such as arthritis and arthralgia, hematological disorders, renal disorders (which are the main criteria for classification of SLE) and Raynaud&amp;#8217;s phenomenon, alopecia, elevated ERSs, muscles pain, lymphadenopathy (additional criteria for classification of SLE) were present in 35/77 (45%) patients with identified ANA, 13/34 (38%) with non-identified ANA and in 28/101 (27%) ANA negative patients. Conversion from DLE to SLE was observed in 7/212 (3,3%). ANA positive patients. Among them in 6 patients antinuclear antibodies were identified (Ro, La, RNP). Five of those patients had symptoms that fulfilled 4 of the ARC (American College of Rheumatology) criteria, and 2 patients-3 of the criteria. All of these patients presented symptoms which were additional criteria for classification of SLE. Results show that the presence of ANA in patients with DLE is the risk factor of development the systemic symptoms. Therefore these patients should be under observation with close attention.

The role of TNF alpha in a murine model of atopic dermatitis

ESC-ID 881
Author Hoser D, Ernst D, Dahten A, Lugor EO, Worm M
Country Germany
University FU Berlin
Department Biology

Atopic dermatitis (AD) is a chronic inflammatory skin disease. It is characterized by dryness, pruritus and inflammation of the skin and is often associated with elevated serum IgE levels. In AD inflammatory cytokines like TNFalpha are released and TNFalpha levels in tissue and serum of patients with AD were reported to be significantly increased. Still the use of anti-TNFalpha therapies is not established and until know failed to show consistent efficacy. Both, cases with amelioration or temporarly improvement of AD but also cases with new onset of AD during anti-TNF therapy have been reported. Here we examined the role of TNFalpha using TNF-knockout mice (TNF-/-) in a murine model of AD. Dermatitis was induced by sensitization of mice with ovalbumin (OVA)/alum, followed by epicutanous application of OVA/alum. Skin biopsies were analyzed immunohistochemically. Furthermore OVA specific IgE, IgG1 and total IgE were measured by ELISA. Cytokine levels of spleen cultures, stimulated with OVA/CD28 or PMA/ionomycin, were detected using multiplex analysis. The local immune response was not altered in TNF-/- mice as indicated by the thickness of the epidermis and the infiltrates of CD4+ and CD8+ cells. However we observed a profound reduction of the systemic immune response in the TNF-/- mice. These results suggest that TNFalpha mediated pathways are important for the sensitization phase of the allergic immune response, however for the local response multiple factors are relevant.
Follicular penetration – in vitro testing of rigid and flexible liposomes

ESC-ID 129
Author Trauer S, Richter H, Bütemeyer R, Lademann J, Linscheid M
Country Germany
University Humboldt- University
Department Chemistry

Aim: The follicular penetration of two different liposomal formulations (rigid and flexible) was compared. The critical parameters occlusion, body area and humidity were examined.

Method: The Franz Diffusion Cell (FD-C) is an in vitro method often used for skin absorption tests. In this study, a new in vitro method was developed to mimic the dermal application of formulations with this test system. After donor application, human full thickness skin fixed in the FD-C was massaged for 3 minutes (female subjects, plastic surgery). The liposome membranes were labelled with Lissamine Rhodamine B and the core contained carboxyfluoresceine, allowing the analysis of the penetration pathway using Confocal Laser Scanning Microscopy. The recovery rate was determined for each skin layer (donor, heat separated epidermis, dermis and receptor).

Results: If the humidity was 75% (Organisation for Economic Co-operation and Development Test guideline (OECD TG) 428, skin absorption), it was not possible to detect differences between occlusion and no occlusion. If the skin is observed as an entity, no body area differences were detectable, while significant differences were found between the skin layers. If the humidity was varied, a significant influence for the liposomal formulation was detectable.

Conclusion: The influence of the liposomal formulations depends on humidity (summer vs. winter situations in living quarters) as well as the different body areas in the deeper skin layers (as the follicle act as reservoirs for liposomal dermal applications). The follicular pathway was influenced indirectly by the humidity and the properties of the examined liposomes.

Follicular penetration of FA-PLGA nanoparticles into the excised skin of animals relevant for veterinary use

ESC-ID 171
Author Knorr F, Lademann J, Richter H, Schanzer S, Teichmann A
Country Germany
University Charité Universitätsmedizin Berlin
Department Department of Dermatology

Aim: The aim of this study was the investigation of the penetration behaviour of nanoparticles into the hair follicles of the skin of several animal species relevant for veterinary practitioners and medical research. The penetration into the hair follicles of excised cow, pig, dog and mouse skin was studied.

Method: After application of several Natrosol hydrogel formulations containing fluorescent PLGA nanoparticles onto fresh skin samples, skin biopsies were taken and shock frozen. The frozen biopsies were cut using a dermatome in such a manner that vertical slices through the hair follicles were obtained. The histological samples were then examined in a fluorescent microscope, and the penetration depth of the nanoparticles was measured. The experimental parameters skin sample, nanoparticle size, penetration time and application with or without massage were varied.

Results: The PLGA particles penetrated into the hair follicles in a size-dependent and time-dependent manner. The application of massage increased penetration depth into the hair follicles. Differences could be observed between the different species.

Conclusion: The hair follicles represent a long-time reservoir for topically applied nanoparticles. PLGA nanoparticles penetrate deeply into the hair follicles, and are detectable there for the course of several days. In light of these results, PLGA nanoparticles can be seen as promising tools for drug delivery to the hair follicle.

Dermatitis artefacta, case reports

ESC-ID 720
Author Bartus IB, Fekete GL, Fekete JE
Country Romania
University University of Medicine and Pharmacy
Department Medicine

Objective: Dermatitis artefacta is a rare and difficult condition for diagnosis and treatment, with the highest incidence of onset in a late adolescence to early adult life.

Materials and methods: We report three clinical cases of young people with multiple skin lesions, unconfounded to known dermatoses, and localised on easily reached parts of the skin. Most patients are young women who have a personality disorder, borderline features are common and the patient's denial of psychological distress.

Results: This disease is more often at women, the report being from 3:1 to 20:1, adolescence and older persons.

Conclusion: Characteristic is the localisation, strange aspect and the absence of other clinical signs. The anamnesis is subjective, most of the time patients lie, suffering of psychical manifestation. Few of them recognise autogression. This is why the disease can be confirmed after a psychological examination. Treatment includes a long term monitorisation, psychotherapeutic and psychiatric treatment.

Epidemiology/Social Medicine/Public Health I

Epidemiological evaluation of occupational injuries in aluminium smelting industry in Montenegro

ESC-ID 13
Author Scepovic M, Yukadinovic T, Kezunovic LC
Country Montenegro
University University of Montenegro
Department Department of Occupational Medicine

Introduction: Traumatic occupational injuries have enormous human, social and financial importance considering
them as an important cause of lost working hours and production losses, and a cause of physical and psychological trauma for injured workers as well. Injuries are the leading cause of absenteeism, invalidity and mortality among workers. Furthermore, they bring about significant economic damage for the society.

The aim of the study: to determine the injury incidence rate, the frequency and severity rate, distribution of occupational injuries by the: working experience, weekday, month of the year, working hours and shift; to estimate the most frequent type of occurred injuries, as well as relation of occupational injuries and injured workers' workplaces; to identify the most common source of injury and to classify occupational injuries according to their localization.

Method: retrospective descriptive epidemiological study included 537 male workers employed in Aluminium smelting industry - Electrolysis factory in Podgorica, in the year 2005. Relevant data were obtained using „Occupational injuries Register 1-109” for the year 2005, from the Department of Safety and Protection at Work in Aluminium smelting industry in Podgorica, Montenegro. Standard statistical methods of data analysis were used in this study.

Results: the occupational injury incidence rate was 6.89 injuries per 100 workers, the frequency rate was 48.21 injuries per 1 000 000 man-hours worked, the severity rate was 1.5 working days lost per 1 000 man-hours worked. Average age of injured workers was 36.30 ± 7.66 years and the occupation- al accidental work-place injuries were the most frequent in the age group of 30–34 years. Average age of length of work in service among injured workers was 8.16 ± 4.81 years. The number of injuries was highest on Monday (24.33%), in February (21.62%) and December (13.50%), during the first shift (61.11%) and in the seventh working hour (21.62%). Potroom workers and technicians for machines maintenance were the most commonly injured (59.99%). The most frequent source of injury were liquid metal and electrolyte (32.44%), causing combustions of upper and lower extremities in the largest number of cases (70.27%).

Conclusion: results of this paper could have unassuming contribution to the process of control and prevention of occupational injuries. It has been shown, considering extremely high injury incidence rate and unacceptably high frequency rate, that concerted efforts have to be pointed at improvement in conditions of working environment, and also at introducing continued programmes for workers’ education about safe work at their workplaces.

Physical growth in schoolchildren living in Babol, north of Iran: Is the United States CDC 2000 reference

ESC-ID 27
Author Montazer M, Hoseinzadeh F, Rezapour Z
Country Iran
University Babol University of Medical Sciences
Department Midwifery-Nursing

Introduction: To date, there is no national reference for height, weight and body mass index (BMI) indices in Iranian children. Since the US CDC 2000 growth reference is widely used in Iran, the assessment of its sufficiency is essential.

Methods: The sample consisted of 528 girls and 575 boys aged between 5 and 18 years during 2005. Samples were collected by a multistage sampling method. First, stratification was conducted based on the possible difference in socioeconomic status between children living in urban and suburban areas, then, each school was assumed to be a cluster. All participants were established to be healthy and their height, weight and BMI evaluated. The data were transformed to Standard Deviation Scores (SDS) based on the US CDC 2000 parameters, then mean SDS within and through age groups, and between genders, were tested using t-test. SPSS 14.0 was used for data analysis.

Results: The mean SDS (SD) for all ages for height, weight and BMI were 0.2(1.00), 0.1(1.01) and -0.01(1.16), for boys, and 0.2(1.04), 0.29(1.09) and 0.25(1.13), for girls. These mean SDS were all significantly different from zero, except for boys BMI (p = 0.000). The mean SDS for weight and BMI, but not height, were significantly different between two genders. Height SDS decreased by increasing age. Weight and BMI SDS did not differ by age. The growth pattern was somehow similar between two genders.

Discussion: Although the growth indices in the studied population were statistically different from the US reference, but these differences were all smaller than half standard percentile; thus, they were not clinically significant. Regarding the standard deviation scores and the growth patterns seen, the US reference seems sufficient for growth monitoring, except for height, in Babol school age children.

Study on the impact of special working conditions, namely change in environmental conditions, change of diet, long working hours and job stress on development of diabetes and/or hypertension

ESC-ID 541
Author Omer MS, Eltahir OE, El Kheir DY, Homeida MM
Country Sudan
University University of Medical Sciences and Technology
Department Medicine and Epidemiology

Introduction: Over the past four decades diabetes and hypertension emerged as important medical problems in the developing areas of the world, including Africa. In Sudan, a country lying in the centre of Africa, changes in eating habits and environmental conditions have been reported to contribute to this great increase. Diabetes is a clinical syndrome characterized by hyperglycemia, due to absolute or relative deficiency of insulin. This affects the metabolism of carbohydrates, proteins and fats, causing significant disturbance of water & electrolyte homeostasis. According to the WHO, in 2004 more than 150 million people worldwide suffered from diabetes mellitus. This figure is expected to double by the year 2025. On the other hand, hypertension is defined as systolic blood pressure equal to or more than 140 mmHg and/or diastolic blood pressure equal to or more than 90 mmHg. The American Hypertension Association has estimated 43.3 million adults with hypertension in 1991. Therefore, in this study, we aimed at finding the impact of diabetes and hypertension risk factors, including change in eating habits (increased sweet and high refined diet), environmental conditions (urbanization and sedentary work) and job stress on the prevalence of diabetes and/or hypertension in Sudan.
Materials and Methods: This descriptive cross-sectional community-based study was conducted in employees of three different 4-star hotels: The Meridian, Plaza, and Grand Holiday Villa. The sample included 240 study participants, and information was taken by face-to-face interview, the blood pressure and glucose levels were determined using the standard methods, with the results recorded in a redesigned questionnaire.

Results: The total sample prevalence of diabetes and hypertension was 13.5%, 30% respectively, divided by the three hotels; it was 15.2%, 12.1%, 13.2% for diabetes in employees of Meridian, Plaza and Grand Holiday Villa, respectively, and 28.2%, 31.1%, 30.7% for hypertension. The risk factors for both diabetes and hypertension were shown to be older age, positive family history, long working hours (more than 8 hours per day), job stress and diet changes.

Conclusion: The prevalence of diabetes and hypertension this high in the selected group supporting the hypothesis that there is strong relationship between the special studied risk factors and prevalence of diabetes and hypertension, so this special group of population might benefit if closer follow-up and education on their specific situation is done.

Parent’s knowledge and attitudes about vaccination of their children

ESC-ID 803
Author Steric M, Strbac M
Country Serbia
University University of Belgrade
Department Medical Faculty

Introduction: For the past few years, the importance of parents’ insufficient knowledge about vaccines, which can reduce the adequate turnout of children to vaccination has been more and more emphasized.

Aim: The aim of this cross-sectional study was to investigate parent’s knowledge and attitudes about vaccination of their children, in the population of Belgrade.

Methods and materials: The study comprised of 200 parents who visited one of five Public Health Services (randomly chosen), during the period 01-15.02.2007. The semistructured questionnaire about parent’s knowledge and attitudes about vaccination was filled in by them. Statistical analysis included q test and correlation analysis.

Results: The most children of investigated parents-184 (92%) received all compulsory vaccines for their age. The most frequent reasons of underimmunisation were acute child’s illness (44%), nonconscious parents (31%) and parent’s fear of side-effects (25%). Statistically significant correlation was observed between high education level and vaccination rates of their children (q = 0.481; p = 0.001 for mothers and q = 0.238; p = 0.017 for fathers). One hundred and sixty eight (84, 0%) parents thought that vaccines have positive health effects, 20 (10%) that vaccines not have any effects, and 12 (6%) that vaccines have negative health effects. Statistically significant correlation was registered between parents who have more than one child and the positive attitude about vaccination (q = 0.278; p = 0.005). The most of parents (69, 5%) information about immunization got from pediatricians, 37 (18, 5%) from scientific literatures and 20 (10, 0%) from mass media. The parents with high level of education frequently vaccinated their children against diseases which are not included in obligato-ry immunization schedule (q test = 0.520, p = 0.002).

Conclusion: In Belgrade population there is a high level of knowledge and positive impression of parents for immunization of their children, resulting in vaccination applied orderly.

Incidence of admission hyponatremia in adult ICU and its impact on morbidity and mortality

ESC-ID 551
Author Govarthanan R, Vetrivel R, Mani AK
Country India
University Madras Medical College, Tamilnadu
Department Department of Critical Care

Aim: To estimate the incidence of hyponatremia (Serum Na<130 mmol/L) based on the admission serum sodium values in adult patients admitted to a mixed medical-surgical Intensive Care Unit (ICU) in an urban hospital in South India and its impact on morbidity and mortality.

Method: Retrospective chart review of 267 patients with hyponatremia (Serum Na<130 mmol/L) out of 1938 patients admitted to the ICU between April 2005 to June 2007.

Setting: Tertiary care community hospital.

Result: One thousand nine hundred and thirty eight patients got admitted to ICU between April 2005 to June 2007. Two hundred and eighty seven patients (13.78%) were found to be hyponatremic with the serum sodium <130 mmol/L and forty two (31.1%) of the hyponatremic patients required mechanical ventilatory support. The results were comparable to a prior study done by Bennani et al[1]. The overall mortality among patients admitted to ICU was 8.1% (n = 156) but the mortality among hyponatremic patients was 15.7% (p <0.05) which was significantly higher suggesting that admission hyponatremia in ICU is an independent predictor of mortality (p <0.05 ,OR = 2.54) [2]. The mortality in the hyponatremic patients requiring mechanical ventilation was also significantly increased (p <0.05, OR = 22.7), especially in female patients (p<0.05). However age > 65 years, gender and presentation with seizures does not appear to be associated with increased mortality [3].

Conclusion: Admission hyponatremia in ICU is an independent predictor of mortality and the incidence of mortality is increased in patients requiring mechanical ventilation, especially in female patients. Age > 65 years, gender and presentation with seizures does not appear to be associated with increased mortality.

Breastfeeding, dietary patterns, overweight and obesity in Mexican preschool children

ESC-ID 336
Author Japenga EJ, Neufeld LM, Reijneveld SA, Gips CH
Country The Netherlands
University Rijks University Groningen
Department Medical Sciences

Introduction: In 2001, a prevalence of obesity of 28% was reported in school aged children in Mexico. The highest prevalence was found in children aged 5-6 years (39%). Since prevalence of obesity has increased dramatically, nearly 50%
in children and adolescents in the last 10 years in both urban and rural areas; this represents an urgent public health problem. Better understanding of factors influencing weight gain, such as diet and the role of breastfeeding in early life, could lead to better intervention methods to reverse the current obesity epidemic and prevent associated devastating health consequences.

Methods: Data was available from a cohort of children born during a randomized micronutrient supplementation trial (n = 917) and a cross-sectional follow-up of these same children when they were 4-7 years of age (n = 262). Information including dietary consumption was collected through questionnaires from birth until 24 months of age at several determined moments. Anthropometric measurements were done at these moments as well, and again during follow-up. Additional information on Socio Economic Status and the amount of television viewing was collected. A multiple linear regression model was then constructed to determine the association between Body Mass Index (BMI) (kg/m²) of the child at follow-up and BMI of the at age of two, BMI of the mother, sex, age, breastfeeding practices (divided in high vs. low intake), Socio Economic Status and the amount of TV viewing.

Results: The prevalence of the sum of overweight and obesity at follow-up was 11%. Breastfeeding practices at age of three months showed a positive association with BMI at follow-up (p < .05). The mothers reported daily consumption of typical Mexican foods like tortillas (89%) and frijoles beans (79%) in the daily nutrition of the children at age 2. 47.3% of the children still received breast milk at age 2. Breast milk at age 2 showed a significant negative association with BMI at age 2 (p < .05). The mothers reported daily consumption of typical Mexican foods like tortillas (89%) and frijoles beans (79%) in the daily nutrition of the children at age 2. 47.3% of the children still received breast milk at age 2. Breast milk at age 2 showed a significant negative association with BMI at age 2 (p < .05).

Conclusion: Children who had high breast milk consumption at 2 years of age had lower BMI at the same age than their counterparts who received little or no breast milk. Although that relationship did not persist in terms of BMI, possibly due to confounders.

Evaluation of peer education method in changing the level of knowledge and attitude toward ecstasy

ESC-ID 876
Author Vouchanka A
Country Belarus
University Gomel State Medical University
Department Department of Preventive Medicine

Urgency: Most of pupils have psychoemotional tension. Success of the education is reached by intensification of the mental activity children and teenager, conditioned by raised volume of the educational workload and deficit of academic time. It’s known that essential oils (EO) have an influence to central nervous system and influence to capacity to work and concentration of attention. There are a lot of terpenoids amongst component EO, which raise air antimicrobial action. Purpose: To study the influences EO lavenders, rosemary, spearmint pepper on mental capacity to work and concentration of attention. There are a lot of terpenoids amongst component EO, which raise air antimicrobial action. Material and methods: Estimation to capacity to work carried out with 21 children 5-6 years old by help of special figure tables. The volume of the work and its quality were valued. The factor to productivity was counted by formula \( Q = C \cdot C'(C+D), \) where \( c \) - a number of looking through lines, \( d \) - a number of mistakes. In 2 weeks experience was repeated and before its carrying out in auditorium was sprayed EO in quantity 1 ml to 1 m2. All participant of the experiment were organized olfactory and skin-allergic test. Control tests of the air in playing rooms were carried out by sedimentation method before moist cleaning.

The comparative estimation of the influence of the essential oils on factors of mental capacity to work and their antimicrobial action to air microorganisms

ESC-ID 876
Author Vouchanka A
Country Belarus
University Gomel State Medical University
Department Department of Preventive Medicine

Urgency: Most of pupils have psychoemotional tension. Success of the education is reached by intensification of the mental activity children and teenager, conditioned by raised volume of the educational workload and deficit of academic time. It’s known that essential oils (EO) have an influence to central nervous system and influence to capacity to work and concentration of attention. There are a lot of terpenoids amongst component EO, which raise air antimicrobial action. Purpose: To study the influences EO lavenders, rosemary, spearmint pepper on mental capacity to work and concentration of attention. There are a lot of terpenoids amongst component EO, which raise air antimicrobial action. Material and methods: Estimation to capacity to work carried out with 21 children 5-6 years old by help of special figure tables. The volume of the work and its quality were valued. The factor to productivity was counted by formula \( Q = C \cdot C'(C+D), \) where \( c \) - a number of looking through lines, \( d \) - a number of mistakes. In 2 weeks experience was repeated and before its carrying out in auditorium was sprayed EO in quantity 1 ml to 1 m2. All participant of the experiment were organized olfactory and skin-allergic test. Control tests of the air in playing rooms were carried out by sedimentation method before moist cleaning.
The results and discussion: After breathing of EO lavenders factor Q has formed 4.3 ± 0.9 that is 2.3 times above result of the control test (1.9 ± 0.3), differences are statistical significant, p <0.005. The volume of the performed work increased in 1.9 times and has formed 9.7 ± 0.5 after influence EO lavenders, p <0.005. The quality of the executed work greatly did not change. Amount mistakes in control test has formed 15 ± 1.9, after influence EO lavenders this factor became is 12.9 ± 1.1. After influence EO spearmint factor Q became above, than after influence EO lavenders, and has formed 5.7 ± 0.5. Amount looking through lines did not change (9.8 ± 0.7), amount mistakes fell before 8.1 ± 1.2 in contrast with factor at influence EO lavenders. After breathing EO rosemary factor Q has formed 8.1 ± 0.5, average amount looking through lines - 10.8 ± 0.6, amount mistakes- 4.3 ± 0.9 that realistically above result of the control test, p <0.005. For determination an antimicrobial action of EO were explored and is analyzed tests of the air. After 30-minute exposure EO lavenders concentration microorganism fell on 33%, in 60 minutes - on 67%. At estimation result of tests, executed in 5 hours after experiment is installed that EO does not possess the steadfast antimicrobial action, amount colony decreased on 47.7%. In case of using EO rosemary after 30-minute exposure amount colony fell on 48.2%, in hour on 77.3% in concerning to control result. In 5 hours amount microorganism midair remained approximately such (fell on 74.5%). The most denominated and the most stable antimicrobial effect was noted in case of using EO spearmint pepper.

So, change the factors bacterial polluted was following: 82.5% after 30-minute exposure, 92% in 1 hour, and 85.5% in 5 hours after spraying the emulsions EO spearmint pepper.

Findings: 1. The Factor Q and volume of the executed work increases, and amount mistakes decreases: checking - lavender - spearmint - rosemary. 2. Antimicrobial activity of EO and duration antimicrobial effect increases abreast: lavender-rosemary-spearmint. 3. EO rosemary, spearmint can be recommended as facility for sanation of the air of the sleeping rooms in 1 hour before appear in the dreams.

Modular, dedicated Internet portal as a tool enhancing activity of students scientific society

ESC-ID 877
Author Nedoszytko M, Karwacki G
Country Poland
University Medical University of Gdańsk
Department Pediatrics, Hematology, Oncology and Endocrinology

Students Scientific Society at Department of Pediatric, Hematology, Oncology and Endocrinology of the Medical University of Gdańsk has been founded in 2002 and embraces scientifically active students of Faculty of Medicine and Public Health. Currently the Society holds 72 members, becoming the largest student scientific group at the University. The work coordination and activities of this number of students requires introduction of methods of agile communications and data exchange between society members. In 2003 a web portal has been launched with the address www.bedepediatra.amg.gda.pl, meaning www.iwillbecomeapediatrician.amg.gda.pl. The aim of the portal was to enhance activities of the society and integration of its’ members. Since the foundation, site has undergone many modifications and development and currently presents a multi-module portal and a virtual place of everyday meetings. The aim of the work is to present an evolution of students scientific society internet website and extinguish specific elements that influenced its’ development and ameliorated the quality of work of the students society during the course of evolvement. The website contains a publicly accessed area, zone dedicated to the members and an administrative site. During first 3 months of the academic year 2006/2007, 3774 single logins (38/day) have been registered and 1780 (18/day) file downloads have been performed. Portal’s forum is respectively divided and moderated according to society’s scientific projects and holds 122 single subjects and 602 posts. Site’s interactive chat hold 9069 entries. In order to optimize the analysis and data interpretation in scientific projects, tools such as dedicated database clients are provided for society members. Calendar module allows synchronization of meetings and common scheduling. The circulation of data and information flow are enhanced by file exchange system and internal society communicator based on e-mail accounts. Each member manages his own webspace, which allows to present himself and communicate with other members. The whole portal is supervised using a vast, functional administrative site, which allows coordinators and administrators to update the site efficiently and control progress of scientific activities of society’s members.

The prevalence of childhood obesity in a sample of schoolchildren in community of Vozdovac

ESC-ID 131
Author Jovanovic N
Country Serbia
University University of Belgrade
Department School of Medicine

Introduction: Obesity of schoolchildren is in correlation with a lot of diseases. Aim: The aim of the study was to estimate the prevalence of childhood obesity in a sample of schoolchildren in community of Vozdovac (Belgrade), and to investigate the correlation between child obesity and presence of selected diseases.

Method: The study comprised of 854 pupils from 2 primary schools in the community of Vozdovac (Belgrade). The anthropometric data as well as the data about presence of selected diseases were obtained from medical records of regular health survey in 2006-2007. Classification of childhood obesity has been done according to WHO percentiles reference data for obesity and overweight in children. Statistical analysis included t-test and correlation analysis.

Results: In our sample of schoolchildren, the prevalence of obesity was 30.7%. The following diseases were present among pupils in the sample: spinal deformities 192 (22.5%), chest wall deformities 90 (10.5%), and foot deformities 226 (26.5%). The statistically significant correlation has been
registered between child obesity and presence of both spinal deformities (\(\bar{n} = 0.192; p = 0.001\)) and foot deformities (\(\bar{n} = 0.099; p = 0.049\)). The chest wall deformities was more frequent in the group of children with normal weight compare with obese group (\(-2 t = 0.206; p = 0.052\)). The presence of hypertension has been in correlation with childhood obesity (\(\bar{n} = 0.261; p = 0.001\)). The statistically significant correlation has also been registered between physical activity and the absence of childhood obesity (\(\bar{n} = -0.093; p = 0.055\)).

**Conclusion:** In our sample of schoolchildren, the high prevalence of obesity has been shown. Furthermore, the significant correlations between childhood obesity and presence of hypertension and deformities of spinal core and foot have also been observed.

**Health research: Knowledge and attitudes amongst Pakistani medical students**

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<th>ESC-ID</th>
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<tr>
<td>Author</td>
<td>Khan H, Haq Khawaja R, Fatmi Z</td>
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**Introduction:** Health research training is an important part of medical education.

**Methods:** It was a cross-sectional pilot study conducted among a group of Pakistani medical students at the Aga Khan University, Karachi. Through stratified random sampling, a pre-tested, structured and validated questionnaire was administered to 220 medical students. Knowledge and attitudes were recorded on a scale (graded in percentages). Descriptive statistics were performed for mean scores and proportions. Multiple linear regression model was used to test association of age and year of study with the knowledge and attitude. ANOVA and t-test were used to look for similar putative associations of type of high school, mode of study and gender. A p-value of <0.05 was considered as significant.

**Results of 197 students:** 122 (62.6%) were males and 73 (37.4%) females. Mean age of the study participants was 20.92 \(\pm\) 1.79 years. Mean scores of students were 49.0\% on knowledge scale and 53.7\% on attitude scale. Knowledge and attitudes improved significantly with increasing years of study in medical college. Increase in duration of study by one year increased the knowledge score by 4.1\% with a correlation coefficient (r) 0.30 and coefficient of determination (r2) 0.09 [p-value = 0.019]. Similarly, increase in duration of study by one year increased the attitude score by 6.67\% with a correlation coefficient (r) 0.45 and coefficient of determination (r2) 0.20 [p-value = 0.001].

**Conclusion:** Medical students demonstrate moderate level of knowledge and attitude towards health research. Intensive training in this regard is associated with significant improvement in knowledge and attitudes of students towards health research.

**From syringe to sex: Comparison of high-risk sexual behavior between HIV-positive and HIV-negative injection drug user in Karachi, Pakistan**

| ESC-ID | 659 |
| Author | Taj F, Aly Z, Tanwir M, Zafar AM, Sohani AA, Syed F, Tariq A, Fah |
| Country | Pakistan |
| University | Aga Khan University |

**Aim:** AIDS is a disease of global importance. (1) HIV/AIDS epidemics in Asia have been driven largely by the ‘transmission chain’ from injecting drug users (IDUs) to sex-workers. (2) Pakistan has approximately 0.1 million HIV-positives. (3) High-risk sexual behavior among IDUs contributes significantly towards HIV/AIDS spread. This study aims to compare high-risk sexual behavior between HIV-positives and HIV-negatives IDUs.

**Methods:** This was an observational, non-randomized, case-control study. We interviewed IDUs, sero-diagnosed by Sindh-AIDS-control program and registered with a harm reduction program in Karachi.

**Results:** Out of 140 IDUs, sixty were HIV-positive and eighty were HIV-negative. Mean age of sample was 34.18 years. All were male. Mean age of initiation of drugs was 16.9 years. Mean duration of intra-venous drug abuse was 2.58 years. 42.1\% were illiterate but 98.5\% had heard about AIDS. More HIV-positive (75\%) were unmarried compared to HIV-negative (57.5\%). IDUs living with family were less likely to be HIV-positive (OR: 0.35, p-value: 0.004) which may be protective. Most IDUs (80.6\%) has had sexual intercourse at least once. Among these, majority (81\%) was HIV-positive including alarming proportions of heterosexuals (75\%), bisexuals (18.8\%) and homosexuals (6.25\%). Those who used condoms during intercourse, in past, were more likely to be HIV-negatives (OR = 0.51). No HIV-positive was currently using condom. HIV-positives were more likely to had contact with sex-worker (OR: 1.69). HIV-positives (62.5\%) had a higher prevalence of STI compared to HIV-negatives (22.18\%) (OR: 1.81).

**Conclusion:** High-risk sexual behavior among HIV-positive IDUs is alarming. We strongly recommend aggressive actions, including safe-sex counseling and considering isolation of HIV-positive, to prevent impending HIV/AIDS epidemic in Pakistan.

**Intervention study to improve cognitive fitness: Motivational and volitional strategies for mental and physical exercise of elderly women**

| ESC-ID | 916 |
| Author | Evers A, Heuser I, Reischies FM, Dimeo FC, Schwarzer R |
| Country | Germany |
| University | FU Berlin |
| Department | Health Psychology |

**Background:** During the course of normal aging a consistent decline of cognitive processes occur. Meta-Analyses indicate that physical and mental activities have an influence on maintaining or even enhancing cognition in older adults (Colcombe & Kramer, 2003; Churchill et al., 2002).
Behavioural changes, like attending a computer- or fitness course require motivational and volitional strategies. Behavioural intentions can be predicted by social cognitive variables, like outcome-expectancies, self-efficacies and risk-perception (Bandura, 1997). Volitional strategies like planning and developing high recovery self-efficacy are essential to translate goals into action (Schwarzer, 1999; Sniehotta, F.F., Scholz, U., Schwarzer, R. 2006). Within a mental and physical intervention program for elderly women the effects of these interventions are analyzed on the background of compliance, focussing on the above-mentioned variables.

Central Research Questions: The project “Berlin stays fit” offers elderly women the opportunity to perform challenging activities for a period of six months. We examine whether elderly women can benefit from these intense physical or mental training programs in terms of cognitive function, whether physical and mental trainings differ in terms of cognitive benefits and which volitional strategies help bridging the intention-behaviour gap. Hypotheses: It is expected that intervention effects can only be measured in those women who attend courses regularly. To perform this new behaviour, volitional strategies are essential, e.g. using planning strategies and developing high self-efficacy. In detail, it is hypothesized that (1) a psychological planning intervention is positively associated with behaviour (attending classes); (2) a positive feedback paper, influencing self-efficacy is also positively associated with behaviour and (3) recovery self-efficacy measured in the volitional phase is a predictor of health behaviour.

Method: Healthy women aged 70 and older are randomly assigned to three different groups with a target n of 12 per group. It is expected to treat seven cohorts (total n = 252). One group will get the physical intervention (exercise group), the other group will get the mental intervention (computer course) and the third group is the control. Both interventions take place for six months in total, three times per week for 90 minutes each. Participating women are tested cognitively and physically before and after treatment. Both intervention groups get a postal planning intervention one month after having started the course, after four months they get a positive feedback paper to strengthen their self-efficacy. Social-cognitive variables are measured before, in-between and after the treatment.

Project Status: Since September 2006 five cohorts have started the project (n = 180 women). Another two cohorts are going to follow. Until October four cohorts will already have finished the intervention including all post measures. Due to the study design final data analyses regarding the outcome variables are expected after finishing the last cohort in 2008.

Comparison of the Se status of vegetarians versus omnivores in Germany

ESC-ID 424
Author Hoeflich J, Hollenbach B, Koehrle J, Schomburg L
Country Germany
University Charité Universitätsmedizin Berlin
Department Institut für Experimentelle Endokrinologie

Introduction: Selenium (Se) is an essential trace element for humans (1). The positive health effects of Se are caused by selenoproteins that contain Se in form of the 21st amino acid selenocysteine (2). The safe nutritional range of Se is narrow, and overdoses are toxic. Until now 25 different genes coding for a small number of human selenoproteins are identified (3). These proteins have fundamental physiological functions, e.g. protection from peroxides and reactive oxygen species (ROS) or metabolism of thyroid hormones. The two most important factors for adequate Se status are full supply of Se by nutrition and the correct distribution of Se within the body. Selenoprotein P (SePP) is responsible for Se storage and distribution. Deficits of Se – whether through malnutrition or lack of SePP – represents a risk factor for e.g. cancer, sepsis or cardiovascular diseases.

Hypothesis: In Europe, dietary Se is thought to be mainly supplied by red meat, eggs, fish and milk. Therefore certain dietary preferences, e.g. by vegetarians, vegans or people with certain food restrictions because of inborn or acquired diseases, might lead to Se deficiency that represent a health threat.

Materials and Methods: We compared the Se status from serum samples of healthy vegetarians and area-matched controls. A dietary questionnaire was used to analyze food, drink and lifestyle habits. Total Se was determined by a fluorescent method involving the formation of piazselenol after complexation with di-amino naphthalene (DAN), SePP concentrations were measured via a newly developed immunoassay employing two independent polyclonal sheep antibodies. Plasma glutathione peroxidase activity as a secreted selenoenzyme from kidney was measured by a coupled optical enzymatic test. Western blot comparison of representative vegetarians and controls were performed to compare the selenoprotein profiles synthesized. Ethical approval and written consent of each participant was obtained.

Results: By now, we were able to recruit 20 independent vegetarians and the same number of controls. We selected for similar body composition (BMI 18-25), age (18-40) and lifestyle. Serum Se concentrations were comparable in the samples. GPx activities were within the expected range and SePP concentrations were not significantly different among vegetarians and controls.

Conclusion: Our results shown that in contrary to expectations and current textbook knowledge (4), even in Europe the vegetarians display a comparable Se status and selenoprotein expression profile to omnivores. Since the European soil is poor in Se, it remains to be investigated from which sources the Se is derived in a purely vegetarian diet. Financial support from the DFG and Deutsche Krebshilfe is gratefully acknowledged.

“Scoop and run – but where? Transport time and trauma facilities in Karachi”

ESC-ID 439
Author Channa R, Jaffrani HA, Hasan T, Khan AJ, Razzak J
Country Pakistan
University Aga Khan University
Department Medical College

Objective: Time taken to reach an appropriate health care facility determines outcome among road traffic injury (RTI) patients. Rapid growth of cities in many urban areas in developing countries has outpaced the development of designated trauma centers leading to potential delays in getting to these
facilities. This study was conducted to determine the difference in time to reach a facility if patients are taken to a nearest (non trauma designated) emergency care facility (ECF) compared to a designated trauma center (TC). These emergency care facilities were also assessed for the availability of supplies and equipment required for “basic” trauma care. Methods: Karachi, the largest city of Pakistan has a population of over 14 million. Three government designated TCs and numerous smaller hospitals offer 24-hour emergency care. 5/18 towns with the highest number of RTIs were identified through medico-legal records. Time taken to reach the designated TC and the nearest ECF was measured. A questionnaire was used to determine the availability of supplies and equipment for basic trauma care. Results: Our study covered 30% of city’s population and 3.5% of total city area. All 3 TCs were located in these towns and were close to each other (5.0-10.5 km.). The transport timings to the ECFs averaged 4.3 (+ 2.4), compared to an average of 13 (+2.5) minutes for TCs (p<.001). Although most ECFs had the basic equipment for management of airway, O2, IV fluids etc, less had pain medications like Morphine (70%) and even fewer possessed a c-spine collar (45%). Conclusion: While transport time to the nearest ECF is shorter than that to the designated trauma center, many ECFs do not have the basic equipment to provide care for trauma victims. We recommend that efforts should be focused on improving trauma care at the ECF to ensure timely life saving interventions.

Sunlight exposure, a major preventive factor in the development of hypertension and other diseases

ESC-ID 958
Author Omipidan SA
Country Nigeria
University St. Luke School of Medicine-Libera
Department Medicine

Background: An independent observation study of the effects of sunshine on the reduction of blood pressure, carried out on the residents of Ibi-Araba, Lagos-Nigeria. Sunlight is known to convert cholesterol to vitamin D-3; insoluble unconjugated bilirubin (direct) to soluble, excretable conjugated bilirubin (indirect); induce sweating; and convert aldosterone and other steroids to less active or inactive metabolites. Additionally, sunlight affects the pineal gland and melatonin secretion through its network of receptors. Melatonin secretion is greater in the dark and it inhibits the secretion of lutinizing hormone. Consequently a decrease in melatonin secretion will cause a subsequent rise in sexual steroids, which are convertible to vitamin D-like compounds. Most of these effects of sunlight have the effect of reducing blood volume or reducing sodium levels in the blood and interstitium. Would sunlight exposure have a measurable effect on blood pressure by reduction in blood volume? If there were a measurable difference in blood pressure levels, would that difference be therapeutic? Methods: A brief history was taken from each of the 186 subjects and their blood pressure was measured. Results: The overall average blood pressure was found to be 135/79 mm Hg, and the overall average pulse of 71, with an average daily sunlight exposure of 4 hours and 4 minutes. The average blood pressure of people with 1 hour per day of sunlight exposure was 141/85 mm Hg. The average blood pressure for 2 hours per day exposure to sunlight was 145/83 mm Hg. The average blood pressure of people with 7 hours per day was 121/72 mm Hg. The nine-hour result was 121/77 mm Hg. Altogether; the statistical power for the Systolic data was 0.0021. The statistical power for the Diastolic data was <0.00001. Both values are considered highly statistically significant.

Conclusion: Sunlight exposure of three (3) hours per day or more is a major preventative factor in the development of hypertension in the general population. The optimum daily exposure to sunlight is seven (7) hours to prevent hypertension.

Prevalence and predictors of depression among an elderly population of Pakistan

ESC-ID 473
Author Ganatra HA, Zafar SN, Qidwai W, Rozi S
Country Pakistan
University The Aga Khan University
Department Medical College

Objective: To assess the magnitude and precipitators of the problem of depression in an elderly population of Pakistan. Method: A cross-sectional study was conducted using a convenience sample of 402 people aged 65 and above visiting the Community Health Center of the Aga Khan University, Karachi. Questionnaire based interviews were conducted for data collection and the 15-Item Geriatric Depression Scale was used to screen for depression. Univariate and multivariate logistic regression analyses were performed to identify factors associated with depression.

Results: Most of the subjects were married (76.4%) men (69.7%). The mean age was 70.57 years. The prevalence of depression was found to be 22.9% and multiple logistic regression analysis indicated that higher number of daily medications (p value = 0.03), total number of health problems (p value = 0.002), financial problems (p value <0.001), urinary incontinence (p value = 0.08) and inadequately fulfilled spiritual needs (p value = 0.067) significantly predicted depressive symptoms.

Conclusion: We have identified several risk factors for depression in the elderly which need to be taken into account by practicing family physicians and health care workers.

Euthanasia opinion amongst preclinical and clinical students of Marmara University, Medical Faculty

ESC-ID 227
Author Sanem Aslıhan A
Country Turkey
University Marmara University
Department Medical Faculty (English)

Aim: As far as a medical procedure, Euthanasia appears to be a noncomplex, straightforward concept. However, when we look deeper through scientific methods including Medicine, Jurisprudence, Philosophy, Sociology, Religion, and Ethics, Euthanasia is one of the most debated practices amongst doctors and the public. In this study, our aim was to compare and contrast preclinical and clinical students’ opinions about
euthanasia and observe if students’ opinions would change after meeting with patients, who are terminally ill with no chance of cure or treatment.

Methods: The research type chosen was cross-sectional. A survey was applied to 180 Marmara University Medical School students from first to sixth year. The survey was prepared by the help of scanning related litterateur. Survey contained 25 questions, 16 of which were related to four specific cases, that included different patient situations where people interviewed wanted euthanasia either for themselves or for their relatives. Data was then analyzed by using, Chi square test.

Results: The percentage of students who believe that Euthanasia should be legal was 35%. The percentage of students who believe that euthanasia violates Medical Ethics was 48.9%. None of methods of euthanasia was acceptable to 49.7% ± 11.3 of the students. The percentage of students who believe the decision to have euthanasia should be left up to the treating doctor was 37.8%, up to an ethical committee the patients was 71.7%, up to immediate family was 40%, up to the courts was 20.6%. 75.4% of the students who agree that euthanasia should be illegal, believe that because of the concerns of possible exploitation the students who agree that euthanasia should be illegal, the students who agree that because of the concerns of possible exploitation (X² = 30,062 p = 0.0001). 63.9% of students had sufficient knowledge about euthanasia- No significant relationship was found between class (i.e. preclinical vs clinical) and knowledge. Clinical students who would accept euthanasia for themselves, assuming that they are in a terminal illness situation with no hope were more than pre-clinical students (p<0.05). The students who do not approve any type of euthanasia (91.7% ± 1.3) stated that they would never practice euthanasia as a doctor (p <0.001).

Conclusion: Generally, euthanasia knowledge seems relatively sufficient regardless of whether they were pre-clinical or clinical. Half of students do not accept euthanasia as a legitimate practice, nor find it appropriate for the purposes of Medical Ethics. Most of the students think the patient should make euthanasia decision. In our research, students who want euthanasia for themselves if they were as a patient with no hope, were mostly clinical students. Consequently clinical students looked more positively to euthanasia than preclinical students. We thought that this was because of clinical students regularly see patients at hospitals on a daily basis and may be sympathizing with them. Lastly, we can also say that theoretical information does not conform to practical experiences. (Mentor: Assit. Prof. Gokhan Aykan, PhD, Social Sciences Institute of Beykent University, Istanbul/ Turkey)

Genetics I

Clostridium perfringens enterotoxin (CPE): a new tumor-specific suicide gene for cancer gene therapy

ESC-ID 771
Author Boelling O, Kohbelt D, Aumann J, Schlag PM, Walther W
Country Germany
University Charite Universitaetsmedizin Berlin
Department Robert-Roessle-Klinik, Surgery/Surg. Oncology

Aim: Clostridium perfringens enterotoxin (CPE) is a single polypeptide which is produced from bacterial strain type A and can cause food poisoning. Claudin 3 and claudin 4 were identified as receptors for CPE. It has been shown that CPE binds to claudin 4 with higher affinity than to claudin 3. Both claudins are transmembrane proteins found in tight junctions of epithelial cells and are involved in the regulation of the cell permeability. CPE binding to these receptors triggers formation of membrane pore complexes leading to rapid cell death. Importantly, claudin 3 and claudin 4 were shown to be highly overexpressed in numerous human cancers such as breast, ovarian, pancreatic and colon cancer. Thus, this overexpression can be used for the selective antitumor action of CPE treatment. The antitumoral activity of external application of recombinant bacterial CPE has been reported by several groups. The aim of this study was to use the CPE coding cDNA for gene therapy of claudin 3 and claudin 4 overexpressing cancer cells to achieve efficient and selective tumor cell killing.

Methods: First, the CPE cDNA was isolated from the total Clostridium perfringens bacterial DNA by PCR amplification using CPE-specific primers. The 990 bp CPE cDNA was modified by addition of the Kozak-sequence for eukaryotic expression and cloned into a eukaryotic expression vector pcDNA3.1. Different human cancer cell lines were tested for claudin 3 and claudin 4 expression by specific PCR. The colon carcinoma cell line HCT116 was found to express both receptors. Inversely, the melanoma cell line SK-Mel 28 does not posses CPE receptors. Both cell lines were used for the in vitro transfection experiments. CPE expression was proven by Western blotting and RT-PCR.

Results: 24 h after the transfection of the CPE-expressing vector into HCT116 cells cell death of about 50% of the cells was observed. By contrast, in SK-Mel 28 cells, which lack claudin 3 and claudin 4, no cytotoxic effect was found, which points to the selective CPE-cytotoxicity. CPE expression was shown by Western blotting and RT-PCR.

Conclusion: These results give first evidence that the CPE can be used for novel suicide gene therapy of tumors overexpressing claudin 3 and claudin 4 proteins and is leading to the rapid cell killing.
Gliomas are the most frequent malignant primary brain tumors. Astrocytomas, arising from astrocytic glia, comprise up to half of all intracranial tumors. They progressed through the stages of increased malignancy, associated with the accumulation of genetic abnormalities in different chromosomes and gene expression changes [1]. As a result of extensive studies of the molecular pathogenesis of cancer, several novel regulatory pathways and networks have been identified. Delineation of these pathways has revealed some unique events, marked by morphological and histological changes of cells, and the expression of genes and proteins that accompany oncogenic transformation. Thus, the cell-therapy changes during cancer development. By reading these changes accurately, we can improve the early detection and diagnosis of individual cancers.

Methods: Serial Analysis of Gene Expression (SAGE), Northern blot hybridization, RT-PCR, real-time RT-PCR.

Results: In an effort to identify the genes that might be used as molecular markers of glial tumors, we have here analyzed gene expression in GB and normal adult human brain (NB) by Serial Analysis of Gene Expression. Our results, demonstrate that about 180 genes are expressed at markedly higher or lower levels in glioblastomas compared with normal brain cells. The majority of genes with more than 5-fold changed activity in GB tumors are related only to a few different groups: genes encoding proteins involved in angiogenesis, extracellular matrix (ECM), immune response, drug-resistance, IGF-axis, and MAP-kinase cascade. To assess the reliability of expression patterns, we arbitrarily selected twelve differentially expressed transcripts and evaluated them by Northern blot analysis, when their expression level was sufficient, also allowing to detect alternative transcripts. If expression level of target genes was low for Northern blot analysis real-time RT-PCR methods was used. Expression patterns were usually reproducible between different samples. It is important to note, however, that there were differences in expression between individual tumors, with a few glioblastoma samples exhibiting either high or low amounts of individual transcripts. Such differences in gene expression undoubtedly contribute to the observed heterogeneity in the biological properties of cancers derived from the same organ.

Conclusion: This altered pattern of gene expression in both tumor and host cells, can be viewed as a molecular marker in the analysis of malignant progression of astrocytic tumors, and as possible clues for the mechanism of disease.

Introduction: In the field of gene therapy, it has been a great challenge to develop an ideal delivery vehicle (vector) that is compatible with biosafety, tissue-specific targeting and site-specific integration. rHIV is a HIV-derived retroviral vector capable of integrating the genetic cargo it carries into the host genome, enabling long-term expression of the transgene. Previous studies have shown that chromosomal integration-site selection of retroviruses in vivo is not a random process. But, the mechanisms for target site selection of rHIV integration in the human genome are poorly understood. With their pluripotency and extensive self-renewal capacity, hMSCs set a novel platform for gene therapy applications. Thus, this project aimed (a) to study the target site selection of rHIV integrating in human bone marrow-derived hMSCs and (b) to map out the integration sites constructing a comprehensive catalogue for future rHIV vector design, minimizing the risks of insertional mutagenesis which counterbalance the therapeutic benefits while optimizing the effectiveness of rHIV integration in hMSCs.

Methodology: Third generation rHIV carrying green fluorescent protein (GFP) were used to transduce bone marrow-derived hMSCs from donor. Successfully transduced cells expressing GFP was detected by ultraviolet light, then were lysed and the genomic DNA (gDNA) was purified using DNEasy kit (Qiagen). These gDNA were fragmented to various sizes with blunt end-generating restriction enzymes and ligated separately to an adaptor (Clontech Universal GenomeWalker kit). Segments containing 3’ end long terminal repeats from rHIV: hMSC chromosomal segment ligated to the adaptor were amplified by polymerase chain reaction (PCR). Second round of amplification was performed to generate more specific segments containing proviral-host cell DNA junctions. PCR products of different sizes (100-300 bp) containing the junctions were cloned into plasmid vectors and transformed into E. coli using heat shock process. After overnight culture, blue/ white screening was used to analyze the transformants containing the PCR inserts. Positive clones were randomly selected and inoculated overnight. Following cell lysis, the plasmid vectors were purified, fragmented and analyzed for PCR inserts using agarose gel electrophoresis. The purified positive clones of plasmid vectors were sent for sequencing of the proviral-host cell DNA junctions. The hMSC chromosomal DNA segments were then mapped to the human genome sequence draft using BLAT software. These segments represented different rHIV integration sites in hMSCs genome.

Results: Among 92 purified positive vector samples prepared, 35 possessed desirable PCR inserts (100-300 bp). From these samples, 12 characteristic integration sites had been analyzed which four located within gene coding regions, four near active genes (upstream and downstream) and four within non-coding regions in hMSCs chromosomal DNA.

Conclusion: In this study, integration of rHIV into hMSCs has shown a preference toward active gene regions, as demonstrated in other cell lines. However, at least 100 inte-
gested the degradation of some amyloid proteins might be presented as the factor providing the generation and propagation for understanding the site-specific integration mechanisms of rHIV in hMSCs and future design of rHIV vector.

The chaperone Hsp104 new function: changing the protein amount

Yeast Heat Shock Protein 104 (Hsp104) is known to decompose the protein aggregates generated either by stress influence or attended permanently. Thus, Hsp104 may be represented as the factor providing the generation and propagation of the yeast prion aggregates [1]. Recently it has been suggested that the degradation of some amyloid proteins might be assisted by Hsp104, providing thereby the aggregation of these proteins [2].

Aim: In this work, we have decided to investigate the Hsp104 influence on the aggregating mammalian prion protein (PrP) and beta-amyloid peptide (Aβ) and non-aggregating protein GFP (green fluorescent protein) in the yeast Saccharomyces cerevisiae cell to provide the natural environment for Hsp104.

Methods: The classical genetic approaches and the methods of molecular cloning have been used in our work with the S. cerevisiae. The Western-Blotting approach has been applied to analyze the levels of protein production. GFP has been fused with PrP and Aβ to visualize the Hsp104 influence under a fluorescent microscope. The Real-Time PCR approach has been employed for quantification of the mRNA production.

Results: We have constructed the plasmids containing hybrid PrP-GFP and Aβ-GFP genes under the control of inducible PCUP1 and constitutive PGPD promoters. Chimerical products of these genes were shown by Western-Blotting to aggregate in the yeast cell and aggregates were observed under a fluorescent microscope. Further, we expressed these hybrid constructions simultaneously with Hsp104 on the different levels of production employing the PCUP1 promoter. Suddenly, Western-Blotting hybridization has demonstrated that the particular protein amount is changed by Hsp104 and this influence depends on the promoter’s features. The PrP-GFP, Aβ-GFP and GFP genes expression under the PCUP1 promoter leads to the great encreasing of the particular proteins production. Vice versa, the employing of PGPD promoter leads to the delay of protein amount. Surprisingly, this effect is independent on proteins ability to aggregate. Furthermore, the quantity of mRNA was shown to remain constant. These results give us the opportunity to suppose the existing of Hsp104-mediated mechanism of the post-transcriptional protein production regulation. Using the Hsp104 overproduction simultaneously with PCUP1, we may additionally increase the protein production. Thus, we assumed the applied possibilities of this result for yeast biotechnology.

Conclusion: The Hsp104 overproduction leads to the transcriptional independent changing of the particular protein amount depending on the promoter’s features.

The newly identified metastasis associated gene OMCC is a master regulator of the Met signaling pathway

Aim: Colon Cancer is the second leading cancer disease in europe. 65% of the patients develop metastases and have a very poor prognosis. The main problem for the successful treatment is the evaluation of the primary tumor’s metastatic potential. Therefore cancer research focuses on the identification of reliable molecular markers for early prognosis of the patients clinical outcome. We identified the gene Over-expressed in Metastatic Colon Cancer OMCC by differential display RT-PCR of colon cancer tissues. OMCC cDNA was cloned in our lab. Sequence analysis revealed SH3 and PXXP protein-protein-interaction domains and tyrosine-phosphorylation sites. The aim of this project is the characterization of the molecular function of OMCC and the identification of potentially involved target genes.

Methods: OMCC transfectants were analyzed in proliferation, migration, and invasion assays. siRNA technology was used to prove gene specificity. Intracellular translocation of OMCC was shown by immunofluorescence and Western blotting of nuclear extracts. OMCC regulated target gene expression was tested in CAT reporter assays and verified by chromatin-immunoprecipitation. OMCC and Met expression in primary colon tumors were analyzed by quantitative real-time RT-PCR.

Results: OMCC transfectants, but not mutants thereof lacking the SH3 or PXXP domain, showed enhanced proliferative, migratory and invasive properties. All these biological effects could be significantly reduced by transfection of OMCC-specific siRNA. Since OMCC domain structure is typical for proteins involved in receptor tyrosine kinase (RTK) signaling, we treated OMCC clones with hepatocyte growth factor (HGF), ligand of the RTK Met. HGF treatment resulted in even higher growth rates, striking morphological changes in the scatter assay and nuclear accumulation of OMCC. Remarkably, a strong Met overexpression was observed in OMCC clones, that could be reverted by OMCC specific siRNA. To corroborate the hypothesis of an OMCC regulated Met expression we analyzed the Met promoter in reporter assays. CAT expression in OMCC transfectants was highly increased. We confirmed this by showing an OMCC interaction with the authentic Met-promoter sequence. OMCC expression levels in primary, not yet metastasized colon tumors were significantly higher in patients that later developed metastases. Interestingly, OMCC was even of greater prognostic value, than the established metastasis marker Met.

Conclusion: Here we demonstrate, that OMCC is a transcriptional regulator of the Met gene. The Met signaling path-
way is often deregulated in colon cancer, affecting many
important cellular mechanisms associated with metastasis.
OMCC has great potential as a prognostic marker and could
provide a powerful tool for the early identification of high
risk colon cancer patients.

The cohesion as a new aspect of human
hereditary diseases caused by disfunction of
transcriptional regulation

ESC-ID  608
Author    Rodionova NS, Markov AV, Smirnov AF
Country   Russia
University Saint-Petersburg State University
Department Department of Genetics and Breeding

The structure of chromatin depends on its activity and has
strong correlation with two DNA-binding protein complexes:
condensin and cohesin. Cohesin in a form of multisubunit
complex provides accurate sister chromatides segregation
during interphase. Recent studies have shown that the cohe-
sion complexes also affect gene expression and development.
The Cornelia de Lange (CdLS) and Roberts/SC phocomelia
(RBS/SC) genetic syndromes in humans are caused by muta-
tions affecting components of the cohesion complexes, also
some of chromosomes nondisjunctions may be caused by
cohesion disfunction. Drosophila mälanogaster is one of the
most convenient model for studying of cohesion on the inter-
phasic nucleus. Studies in Drosophila mälanogaster suggest
that effects on gene expression are most likely responsible for
developmental changes in CdLS. Effects on chromatides cohesion are evident in RBS/SC syndrome, and these enables
to propose that changes in expression of genes located in het-
erochromatin could contribute to the developmental prob-
lems. Immunostaining with polyclonal antibodies to proteins
DRAD21 and SA (subunits of cohesin complex) has shown the
presence of cohesion complexes at regions of less con-
densed chromatin (interbands) of the polytene chromosomes.
We have analyzed heat-induced puffs to visualize features of
cohesion complexes distribution before and after induction. It
was shown that for heat-induced puffs the rearrangement of
cohesion complexes is typical after puff formation. At the
same time puff formation at the region of exact band can lead
to appearance of cohesion complexes in this area and keeps
up to end of induction. Our data show that typical features of
cohesion complexes distribution are permanent for each puff.
In these regions cohesion complexes are spread uniformly
along the all puff, but positive correlation with interbands is
disturbed. We have shown the attraction of cohesion com-
xplexes to the regions of polytene chromosomes with increasing
transcriptional activity and its dissociation from deacti-
vated puffs after decrease of hypertranscription. So, we can
suggest that cohesion plays significant role in chromatin
structuring during transcription. Thus, our results may help to
shade light on cellular mechanisms of some human diseases
correlated with effects of cohesion-transcription linkage and
are one of the first steps to develop the therapy for them.

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Yeast-based test system for screening of human
beta-amyloid aggregation modulators

ESC-ID  209
Author    Lada AG, Saifitdinova AF, Rubel AA,
Galkin AP
Country   Russia
University Saint-Petersburg State University
Department Department of Genetics and Breeding

Aim of the research: A wealth of data indicates that
oligomerization of Abeta is crucial for pathogenesis of
Alzheimer disease. The goal of our study is to create a test
system for screening of anti-amyloidogenic agents based on a
powerful S. cerevisiae model.

Methods: Standard methods of molecular cloning were used.
Mutant A21P-A sequence was obtained by site-directed muta-
genesis. Methods of classical yeast genetics and biochemical
assays were applied. Semi-denaturating agarose gel elec-
trophoresis followed by Western blot hybridization and protein
immunostaining was used for analysis of A-SUP35MCp
oligomerization state.

Results: We had constructed A-40-SUP35MC hybrid gene
encoding protein in which human beta-amyloid peptide fused
with C-terminal part of yeast translation termination factor
eRF3 (SUP35). High production of A-SUP35Mc leads to
effective translation termination on premature stop codons.
Nevertheless, we have got one A-SUP35MC-expressing
cloned with high level of nonsense suppression. Preliminary
data obtained using semi-denaturing agarose gel elec-
trophoresis reveal existence of A-SUP35MCp oligomers in
total protein from this strain. Therefore, nonsense-suppres-
sion most likely generated by the A-SUP35MCp oligomeriza-
tion. Mutant peptide A21P-A is characterized by low aggre-
ger rate. Substitution of plasmid encoding A-SUP35MCp
in nonsense-suppression positive clone with plasmid bearing
mutant A21P-A sequence in hybrid gene A40-SUP35MC
increase translation termination efficiency. Elimination of
nonsense-suppression in this clone has been achieved by both
GuHCl treatment and HSP104 deletion. Replacement of the
plasmid encoding A-SUP35MCp in this clone with the plas-
mid encoding SUP35MC gene devoid of A sequence also
leads to nonsense-suppression elimination. However, reverse
plasmid substitution (SUP35MC for A-SUP35MC) restores
GuHCl-curable nonsense-suppression. This data indicate that
in the strain under investigation an unknown prion has been
induced. Designated as [ABA+] (Amyloid Beta Aggregase),
it demonstrates non-Mendelian inheritance in cross with the
isogenic [aba-] strain lack of prion acquired by GuHCl treat-
ment. However, monogenic segregation was observed when
[ABA+] strain was crossed with a non-isogenic [aba-] strain
obtained independently. We conclude that combined action of
[ABA+] and unknown chromosomal mutation cause A-
SUP35MCp-dependent nonsense-suppression. Hence our
[ABA+]-containing yeast strain allows visualizing the aggre-
gation status of A-SUP35MC fusion protein. We are going to
use this yeast-based test system for screening agents affecting
beta-amyloid oligomerization.

Conclusion: We have created unique yeast-based test-system
for large-scale screening of factors affecting human A
oligomerization.

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In silico search for the cryptic RSS in repetitive elements of mouse genome

ESC-ID 213
Author Gubsky AY, Zinkovsky VG
Country Ukraine
University Odessa National I. I. Mechnikov University
Department Microbiology

V(D)J recombination is a unique molecular mechanism which provide rearrangements of immunoglobulin (Ig) and T-cell receptors genes (TCR) in B- and T-cell precursors. However, when this system gets out of control the protein RAG1/2 can initiate DNA breaks outside Ig and TCR loci. As a result of such illegitimate V(D)J recombinase activity in human (HPRT, SCL, MTS1, BCL2) and mouse (Notch1, RIt1) genes can occur deletions that are frequently observed in B- and T-cell neoplasms [1, 2, 3]. In this case sites targets of RAG1/2 are cryptic recombination signals (cRSS) which are not structurally identical to recombination signal sequences (RSS) of Ig and TCR genes. Early, we showed that in the mouse genome (Build 35.1), there are about 5 mln of 12 bp and 23 bp spacer cRSS (12cRSS, 23cRSS) outside the Ig and TCR loci. Supposedly, 6724 from them have high recombination potential. Their hepatocytes and nonamers corresponded to CACAGTG and ACAAAAACC sequences or differed from them for 1-2 not functional nucleotides. 88 and 2799 of them are located in exons and introns of 2373 proteinencoding genes. With the use of 12/23-bp spacer rule we have found out that such cRSS can hypothetically participate in the formation of deletions of exons in 90 genes and in the formation of inversions in 103 genes. 31 genes can suffer from both type of damage [4]. At present, having matched coordinates of 6724 cRSS with coordinates of 9262721 known in the mouse genome repetitive elements (they have been taken from the NCBI), we observed that 3840 (57%), 733 (11%), 124 (2%), 34 (0.5%) 41 (0.6%) cRSS are the structural elements of non-LTR retrotransposons, endogenous retroviruses and LTR retrotransposons, simple repeats, DNA transposons and other repeats (not identified) respectively. Having researched the localization of such cRSS we observed that in 46 % cases the nucleotide sequences of motives are fully localized inside repeats and in 54 % cases are localized partly. In overall, the structure of cRSS localized in repeats considerably differs from the consensus sequences 12RSS and 23RSS of functional V, D, J segments of mouse Ig, TCR genes. Only 57 (2 %) 12cRSS and 101 (4 %) 23cRSS have high weight matrix coefficients (0.90-0.80 and 0.80-0.75 respectively). Our results are showing that 390 unique types of repeats participate in spreading of motives which can theoretically mediate of mouse genome instability when the system of V(D)J-recombination gets out of control.

C-terminal BRCT domain of microcephalin is involved in chromosome condensation

ESC-ID 233
Author Ghani Kakhi M, Walther D, Sperling K, Neitzel H
Country Germany
University Charité Universitätsmedizin Berlin
Department Chromosome Diagnostics

Aim: MCPH1, the gene underlying primary microcephaly and the premature chromosome condensation syndrome

Is the -174G/C polymorphism of the interleukin-6 gene associated with the metabolic syndrome and its features?

ESC-ID 210
Author Mielcarek M, Karasinska A, Cymbala A, Depczynska K, Burko M, Karpinski P, Slezak R
Country Poland
University Medical University
Department Clinical Genetics

Aims/hypotheses: Clinical features such as lipid abnormalities, impaired glucose and insulin homeostasis, excessive anthropometric measurements as well as obesity, type 2 diabetes mellitus (T2DM) or the metabolic syndrome (MS) were analyzed for association with the -174G/C polymorphism of the promoter region of IL6 gene. Numerous studies on this polymorphism from all over the world have yielded controversial results. The disparity in the outcomes may partly be related to inter-ethnic variations. The aim of our research was to investigate whether and how the IL6 gene promoter polymorphism was associated with traits of the MS and the MS as a whole in Polish Caucasian men.

Subjects/methods: The study involved 2 groups of men: 161 individuals with the MS and 75 healthy controls both in similar age and from the same environment. The MS was defined according to the International Diabetes Federation 2005 criteria for European men (waist circumference ≥94cm and 2 of 4 further factors: 1. triglyceride levels ≥1.7mmol/l or treatment for this abnormality, 2. HDL-cholesterol <1.03mmol/l or treatment for this abnormality, 3. blood pressure- systolic ≥130mmHg and/or diastolic ≥85mmHg or treatment for T2DM). Genomic DNA was extracted from blood leukocytes. Polymerase Chain Reaction was used to amplify the promoter region of IL6 gene. PCR products were digested with LweI restriction enzyme, electrophoresed on agarose gel and visualized under ultraviolet light.

Results: The -174G allele was more commonly observed among the MS patients (0.59 vs. 0.47; p = 0.04). There was no significant correlation between genotypes and the MS. In the MS group C-carriers presented lower percentage of adipose tissue (0.31 vs. 0.28; p = 0.03) and higher HDL cholesterol level (1.27 vs. 1.33; p = 0.04). In the control group GG homozygous carriers presented higher diastolic blood pressure (DBP), LDL cholesterol, cholesterol and percentage of adipose tissue when compared to CC homozygous persons (p <0.05). In the same group CC homozygous patients demonstrated lower values of DBP, hip circumference, body weight, cholesterol level than heterozygous men (p <0.05).

Conclusion: The present studies suggest that single-nucleotide polymorphism of the IL6 promoter region may be associated with several features of body composition, parameters of lipid homeostasis and the metabolic syndrome. In summary, the results showed that Polish Caucasian men with the G-allele are more prone to develop the metabolic syndrome whereas C-allele likely acts as a protective trait in studied population.
Fibroblasts for premature chromosome condensation in binucleate Mcph-/- and wild type cells for mitotic delay after insert in trapped cell line of BayGenomics. Mcph-/- mice clear G1 cells after cytokinesis block by cytochalasin B. No difference was seen between Mcph-/- and wild type DNA damage induced by x-ray or replication stress by hydroxyurea. There was no difference in mitotic delay and frequency of their DNA lesions. Fibroblast cultures from homozygotes of Mcph-/- mouse fibroblast culture is dependent on topoisomerase II checkpoint, we used Topo II inhibitor to assay mitotic delay as well as chromosome condensation. Wild type fibroblasts affected by DNA damage become delayed in the G2 phase of the cell cycle to allow for repair of their DNA lesions. Fibroblast cultures from homozygotes and wild type were treated with x-ray irradiation and hydroxyurea and evaluated for mitotic delay and frequency of prophase like cells.

Results: We established for the first time the mouse model of autosomal recessive primary microcephaly. Modified method of long range PCR with candidate primers helped us to localize insert in trapped cell line of BayGenomics. Mcph-/- mice fibroblast cultures showed high frequency of prophase like cells (PLCs) about 6-8% while it was only 0.2-0.5% in wild type cells indicating on premature chromosome condensation. BRCT domains are found in many proteins that participate in DNA damage checkpoint regulation, DNA repair, and cell cycle control. The aim of this study was the generation and characterization of the first mouse model of MCPH in which the C-terminal BRCT domain of the mouse Mcph1 gene is deleted.

Methods: The Mouse embryonic stem cell line RRO608 (BayGenomics) has an insertional gene trap in intron 12 of the Mcph1 gene, disrupting the second C-terminal BRCT domain of the protein microcephalin. RT-PCR reaction on RNA isolated from ES cell line confirmed the fusion transcript of Mcph1-vector. Gene trap insertions can occur anywhere within an intron. Although heterozygous animals can be easily identified by PCR from genomic DNA using primers in lacZ part of the vector, homozygous animals are more difficult to genotype by PCR without prior characterization of the insertion site. In the RRO608 ES line, the trap inserted into the 100 kb sized intron12 containing the Angp2 gene. The candidate primer approach by long range PCR was applied and vector-intron joint point was determine by sequencing. Fibroblast cultures were established from mice tails and grown in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and penicillin-streptomycin. Chromosome preparation was done by routine method and mitotic index and PCC phenotype was assayed under light microscope. To check whether PCC phenotype of Mcph-/- mouse fibroblast culture is dependent on topoisomerase II checkpoint, we used Topo II inhibitor to assay mitotic delay as well as chromosome condensation. Wild type fibroblasts affected by DNA damage become delayed in the G2 phase of the cell cycle to allow for repair of their DNA lesions. Fibroblast cultures from homozygotes and wild type were treated with x-ray irradiation and hydroxyurea and evaluated for mitotic delay and frequency of prophase like cells.

Results: We established for the first time the mouse model of autosomal recessive primary microcephaly. Modified method of long range PCR with candidate primers helped us to localize insert in trapped cell line of BayGenomics. Mcph-/- mice fibroblast cultures showed high frequency of prophase like cells (PLCs) about 6-8% while it was only 0.2-0.5% wild type cells indicating on premature chromosome condensation before mitosis in Mcph-/- cells. There was no difference between Mcph-/- and wild type cells for mitotic delay after DNA damage induced by x-ray or replication stress by hydroxyurea. No difference was seen between Mcph-/- and wild type fibroblasts for premature chromosome condensation in binuclear G1 cells after cytokinesis block by cytochalasin B.

Conclusion: The C-terminal BRCT domain of microcephalin is involved in the fundamental process of chromosome condensation but is not actively involved in DNA repair process. Ablation of C-terminal BRCT domain abrogates G2 chromosome condensation in mouse which probably relates to topoisomerase II dependent decatenation check point. Recent finding for distinctive features of decatenation check point in progenitor cells may describe mechanism of proliferation decrease of brain neuroprogenitor cells leading to microcephaly in MCPH.

Short-hairpin RNA-mediated stable knock-down of OMCC decreases the migration properties of human colon cancer cells

ESC-ID: 755
Author: Kelch I, Arlt F, Smith J, Fleuter C, Schlag PM, Stein U
Country: Germany
University: Charité Universitätsmedizin Berlin
Department: Robert-Roessle-Klinik, Max-Delbrück-Zentrum

Aim: RNA-Interference (RNAi) is a recently discovered process, which describes the posttranscriptional silencing of genes via short double stranded RNA in a sequence-specific manner. Nowadays it is a powerful tool in functional genomics and might also be suitable for gene therapeutic applications. Especially in the field of cancer research factors involved in the metastasis process are in the focus because of its great impact on the mortality rate of cancer patients. By comparison of non-metastasized and metastasized tumors an unknown gene was identified in our lab and named OMCC, Overexpressed in Metastatic Colon Cancer. Further tests revealed that OMCC is prognostic for colon cancer metastasis. Enhanced OMCC level led to liver metastasis in vivo. Reduced OMCC expression of colon cancer cells via short interfering (si)RNAs diminished cell motility effectively. Since siRNAs have to be applied in high dosages and reveal only short time effects we aim at the establishment of stable and long-term gene knock-down. Short hairpin (sh)RNAs, which can be processed into siRNAs inside the cell, offer an interesting approach but less is known about efficient design and optimal application.

Methods: Based on previously validated siRNA sequences we designed two OMCC-specific shRNAs and used a commercial RNAi plasmid for transfection of the human colon cancer cell line SW620. This cell line is characterised by high intrinsic OMCC expression and in vivo metastasis. Stable shRNA clones were identified by neomycin selection, PCR, and quantitative real-time PCR. To test motility properties of the transfectants we performed a Boyden chamber assay. We examined proliferation skills by growth curve analysis.

Results: After neomycin selection the shRNA-expressing plasmid was detected in four clones which all showed a reduced OMCC expression compared to wild type cells and scrambled shRNA transfectants. Two of these clones stably maintained the shRNA plasmid and therefore showed a reduced OMCC expression to approximately 20%. These stable shRNA transfectants exhibited a different phenotype and diminished proliferation properties compared to the control cells. Moreover the number of migrating cells was decreased according to their knock-down OMCC expression status. These data confirm previous transient experiments using siRNA oligonucleotides and reveal the efficiency of stable shRNA-mediated knock-down.

Conclusion: Here we show that our shRNAs effectively decrease endogenous OMCC expression and cell motility in human cancer cells which enables further studies in vitro and in vivo.
Heat inducibility of the human cytomegalovirus (CMV) immediate-early promoter: identification of a possible mechanism

ESC-ID 759
Author Kobelt D, Aumann J, Bölling O, Fichtner I, Lemm M, Stein U, Schlag PM, Walther W
Country Germany
University Humboldt Universitaet Berlin/ Charitè Universitatsmedizin Berlin
Department Max-Delbrueck-Centrum/ Robert-Roessle-Klinik

**Aim:** The human cytomegalovirus (CMV) immediate-early (IE) promoter controls the expression of the viral IE1-Gene and is widely used as inert control promoter. Although it was widely assumed, that the CMV-promoter is inert toward heat-induction, we and others suggest heat-inducibility of this promoter. Here we analyze in vitro and in vivo heat-inducibility and a possible mechanism of heat-stress induction of this promoter. These data may have impact for gene therapy experiments in combination with hyperthermia.

**Methods:** SW480 human colon carcinoma cells were stably transfected with pcDNA3.1-CMV-bCD, in which the bacterial cytosine deaminase (bCD) is controlled by the CMV-promoter. For in vitro experiments these cells were heated to 42°C for 2 hours using a temperature-controlled incubator. After hyperthermia, cells were collected at different time points using Trizol. Expression was examined with quantitative real-time RT-PCR and Western-blot. For in vivo experiments these clones were used to establish tumors in the food pad of NOD/SCID mice. After hyperthermia at 41.5°C for 1 hour, tumors were collected at different time points. The in vivo expression was also examined with quantitative real-time RT-PCR and Western-blot. Transcription factor binding sites within the CMV-promoter were identified using PROMO 3.0, and candidates related to stress were verified using EMSA. To further investigate the transcription factor binding, the respective binding site was mutated using PCR-based site directed mutagenesis.

**Results:** Here we show, that the CMV-promoter is inducible by heat stress in vitro and more importantly, in vivo. In vitro we detected a five-fold induction directly after hyperthermia. The maximum induction was about 25-fold compared to the control after 12 to 24 hours following heat-stress. Moreover, in vivo we found a three-fold induction directly after hyperthermia, which lasted for up to six hours. Using PROMO 3.0, several transcription factor binding sites were identified, including a binding site for NF-Y and YB-1. This site was further investigated, due to its relation to stress-mediated signaling. By contrast, the CMV-promoter does not have a heat shock factor (HSF1/2) binding site.

**Conclusion:** The human cytomegalovirus immediate-early promoter is not inert towards heat-stress, but mediates significant heat-inducibility. This induction is not mediated by HSFs. However, heat-induction could be mediated by the stress related transcription factors NF-Y or YB-1, pointing to a possible mechanism of heat-stress response.

Gynaecology I

Ovarian cycle disorders in hypothyroid women are due to the leak of calcitonin, not thyroxin and triiodothyronine only?

ESC-ID 543
Author Vira K
Country Ukraine
University National O O Bohomolets University
Department Institute of Pathology

Ovarian cycle disorders with luteal phase inferiority up to amenorrhea and infertility in women, stay even nowadays one of the actual gynecological as well as endocrinological problems. L-thyroxin monotherapy is a world standard in hypothyroidism correction but it does not provide a sufficient quality of life in hypothyroid women and some of them continue to have ovarian cycle disorders. Mechanisms of those hypothyroid ovarian cycle disorders are not studied enough and we supposed them to be related not only to thyroid hormones but also to calcium homeostasis disturbance due to the leak of calcitonin. Our study was provided on 30 female cycling Wistar rats. After total thyroidectomy, 10 of them received L-thyroxin (3 mkg/kg every day) per os, 10 rats received the same dose of L-thyroxin and injections of calcitonin (1 IU/kg every other day) and 10 rats stayed without any treatment. The control group consisted of 3 intact cycling Wistar rats. We studied experimental rats ovaria in different terms after thyroidectomy and control rats ovaria by transmission electron microscope. We studied also vaginal smears to detect the state of ovarian cycle and serum calcium and thyroxin level to control the calcitonin and L-thyroxin dose. We saw that the ultrastructural state of the corpus luteum in rats receiving calcitonin and L-thyroxin was much better then in those, receiving L-thyroxin only that may be the manifestation of the calcitonin influence on the ovarian cycle luteal phase normalization.

Women's sexual pain and its management

ESC-ID 319
Author Seitaridis S
Country Greece
University University of Athens
Department Faculty of Medicine

**Introduction:** Approximately 15% of women have chronic dyspareunia that is poorly understood, infrequently cured, often highly problematic, and distressing. Chronic dyspareunia is an urgent health issue.

**Aim:** To provide recommendations/guidelines concerning state-of-the-art knowledge for the assessment and management of women's sexual pain disorders.

**Methods:** An international consultation, in collaboration with the major sexual medicine associations, assembled over 200 multidisciplinary experts from 60 countries into 17 committees. One six-member committee focused on women's sexual pain disorders, developing recommendations over a 2-year period.

**Main outcome measure:** Expert opinion was based on grading of evidence-based medical literature, widespread internal committee discussion, public presentation, and debate.
Results: There is increasing evidence for the role of neuropathic pain mechanisms in the pathophysiology of sexual pain disorders. Empirical literature has demonstrated the comorbid presence of clinical psychopathology. With regard to the pathophysiologic role of the pelvic floor and sexual pain disorders, studies reveal that (i) differentiation between vaginismus and dyspareunia using clinical tools is difficult; (ii) vaginal spasms have not been identified; (iii) physical therapists can differentiate vaginismic women from matched controls based on muscle tone/strength differences; (iv) the traditional treatment of vaginismus with vaginal "dilatation" plus psycho-education, desensitization, and so forth is not evidence-based; (v) pelvic floor muscle tone/strength measures for women suffering from vulvar vestibulitis syndrome are intermediate between those of women with vaginismus and no-pain controls; and (vi) the pelvic floor musculature is indirectly innervated by the limbic system and highly reactive to emotional stimuli and states. Pelvic floor therapies for dyspareunia may be effective.

Conclusion: Recommendations include (i) revising the definitions of vaginismus and dyspareunia; (ii) integration of treatment approaches; (iii) validation of nonspecific treatment effects; (iv) controlled studies to test interventions; and (v) sexuality education to help prevent sexual pain.

The significance of early hysteroscopy in detection of uterine abnormalities in repeated implantation failure

ESC-ID 582
Author Shakeri MR
Country Iran
University Tehran University of Medical Science
Department SSRC

Object: in our study we attempt to determine the usefulness of hysteroscopy in women with repeated implantation failure (RIF)

Material and method: we studied 150 patients with normal transvaginal sonography and hysterosalpingography(HSG) whom had two unsuccessful IVF-ET attempts. all hysteroscopies were performed 1-6 month after the last failed IVF-ET cycle.our study was carried out in the hospital and with use of general anesthesia.

Results: after analyzing the hysteroscopy results we found out that 33 patients hysteroscopy showed an important unsuspected endouterine abnormality. Partial septum, adhesion, submucosal myoma and endometrial polyp were the most frequent findings.

Conclusion: endouterine abnormality diagnosed with the use of hysteroscopy(in 33 case) were significant but they were not revealed by either HSG or US. And leads us to conclude that diagnostic hysteroscopy could be an essential procedure before introducing a patient in to an IVF program.

The comparison of RCAS1 blood serum level in cystadenoma and cystadenocarcinoma

ESC-ID 838
Author Szul J, Kotlarz A
Country Poland
University Collegium Medicum of Jagiellonian University
Department Gynecology Oncology

Introduction: RCAS1 has been shown to be responsible for tumor cell escape from host immunological surveillance in such cancers as breast, esophageal, gastric, liver, lung, head and neck, uterine, cervical, endometrial, and ovarian cancers. RCAS1 can be also expressed in a soluble form, as has been demonstrated in blood serum derived from women with ovarian, endometrial, and head and neck cancers. A reverse correlation between the presence of soluble RCAS1 in blood serum and the number of peripheral blood cytotoxic lymphocytes has been shown. This protein expression has also been noted in non-neoplastic diseases such as immune-mediated diseases of liver and benign tumors. RCAS1 blood serum level might indicate the level of immune tolerance associated with tumor growth.

Methods: In our study, we considered serum samples obtained from 12 women. These blood serum samples were obtained from two groups of patients. The first group consisted of 6 patients in whom radical hysterectomy with lymphadenectomy was performed because of ovarian cancer. The second group included 6 patients in whom laparotomy or laparoscopy was performed because of benign ovarian tumor. The study group consisted of patients who were operated in 2006 in the Department of Gynecology, Obstetrics and Oncology, of the Jagiellonian University, Krakow, Poland. The blood serum level of RCAS1 was assessed using anti RCAS1 monoclonal antibody by ELISA Kit.

Results: Statistically significantly higher sRCAS1 was identified in patients with ovarian cystadenocarcinoma than in cystadenoma.

Conclusion: The tumor associated immune tolerance is also created by benign tumors, however its level increases significantly in malignant tumors.

Sexual arousal disorders in female medical students

ESC-ID 890
Author Karlinski M, Siuda M, Jarosinski M, Czapla K, Kabaa K, Rusinek A
Country Poland
University Medical University of Warsaw
Department 2nd Department of Obstetrics and Gynecology

Introduction: Female sexual arousal disorder is described as the persistent or recurrent inability to attain or maintain sufficient sexual excitement, expressed as a lack of subjective excitement or genital lubrication/swelling or other somatic response. Despite the fact, that this particular problem is often reported by a significant number of women of all age groups, it still receives inadequate attention and has not been sufficiently estimated.

Aim of the study: The aim of our study was to evaluate the
prevalence of sexual arousal disorders and their association with other factors in female medical students. 

**Material and methods:** The study involved 359 female Polish medical students from Warsaw, Cracow, Lublin and Bydgoszcz. The inclusion criteria of sexual initiation and internal coherence of given answers were matched by 287 females (mean age 23.3 ± 1.7 years, BMI 21.0 ± 2.6 kg, menarche at the age of 12.9 ± 1.35 years). To maximize credibility of acquired data, an anonymous online questionnaire was used. Sexual arousal disorder was defined as difficulties with attaining subjective or objective arousal before intercourse or attaining it only during the intercourse. For statistical significance U Mann-Whitney test and chi2 test were performed, using Statistica 7.1 PL software.

**Results:** Experience of any sexual arousal disorder was reported by 59.9% of the study group (subjective - 35.7%, objective - 42.2%, arousal only during the intercourse - 35.3%). Different subtypes tended (p<0.01) to coexist. Lower height, weight, earlier oral initiation, vaginismus, infrequent alcohol consumption, moderate or lower self-perceived sexual attraction and sexual needs proved to be more characteristic (p<0.05) for females with sexual arousal disorder. Women suffering from the disorder were more frequently (p<0.05) satisfied with their sexual life. 

**Conclusion:** The prevalence of sexual arousal disorders in female medical students reaches 60% and concerns a distinguishable group of women. However, sexual arousal disorder seem not to affect overall sexual satisfaction.

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Patients after Wertheim-Meigs operations due to colli uteri cancer—what have an influence on the duration of hospitalisation?

**ESC-ID** 639  
**Author** Sadowska A, Heleniak K  
**Country** Poland  
**University** Medical University of Gdansk  
**Department** Gynaecology

**Purpose:** The aim of study was to present the factors which may have an influence on the duration of the hospitalisation of patients operated with Wertheim-Meigs method. Clinical information were collected regarding the age at hysterectomy, blood transfusion during the operation, number of labours, carcinoma invasion in parametrium, postoperative complications, presence and kind of complaints before the procedure, premenopausal age or menopause, metastases to lymphatic nodes, operations, duration of the procedure, laborative results, BMI, stage. 

**Patients and methods:** A retrospective chart review of 125 patients operated at the Department of Gynecology of the Medical University of Gdansk was conducted (2004-2006). Median duration of the hospitalisation was 12 days (range 8-49). On the ground of clinical observations and postoperative standards of treatment in this procedure hospitalisation longer than 12 days was regarded as significantly prolonged. It was the reason for isolating a group of 40 patients whose duration of postoperative hospitalisation exceeded this period (group A). The comparative group was the other 85 patients whose period of hospitalisation was shorter than 12 (group B).

**Results:** The analysis of the chosen parametres revealed that the most significant differences were in comparing factors as follows: blood transfusion during the operation (A:52.5%, B:36%), number of labours>3 (A:67.5%, B:40%), carcinoma invasion in parametrium (A:35%, B:23%), postoperative complications (A:27.5%, B:9.5%). The group of factors where differences weren't significant includes: moderate age at hysterectomy (A:56, B:51), presence and kind of complaints before the procedure (A:82%, B:73%), menopause (A:65%, B:58%), metastases to lymphatic nodes (A:33%, B:28%), operations in anamnesis (A:41%, B:46%). Factors where differences weren't noticed were: moderate duration of the procedure (A:3.5h, B:3.5h), laborative results, BMI (A:25.88, B:25.06), stage.

**Conclusion:** The duration of the hospitalisation after Wertheim-Meigs method may be regulated by many factors. The more often blood transfusion during the operation, higher number of labours, carcinoma invasion in parametrium and postoperative complications were observed the longer the hospitalisation was. There were no significant proofs that duration of the procedure, laborative results, BMI and stage have any influence on duration of the hospitalisation after the procedure.

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Differences Between Mothers in Graz/Austria and Istanbul/Turkey

**ESC-ID** 387  
**Author** Karamustafaoğlu B, Akalin BE, Moertl MG  
**Country** Turkey  
**University** Istanbul University  
**Department** Istanbul Faculty of Medicine

**Introduction:** The aim was to profile and show some differences between mothers who recently gave birth in the department of obstetrics and gynecology of Medical University of Graz and Istanbul Faculty of Medicine and to find out the effect of being Turkish immigrant in Austria on several criteria.

**Methods:** This study is questionnaire-based. The questionnaire is composed of 9 multiple choice, 4 multiple response, 3 numeric, 2 open-ended, 1 yes-no, 1 likert scale questions about age, education level, profession, homeland, number of children, parity, gravidity, use of medication during pregnancy, smoking and alcohol use during pregnancy, number of prenatal visits, gestational age at birth, way of delivery, epidual analgesia during labor, work during and after pregnancy and breastfeeding. 62 mothers in Istanbul, 55 mothers in Graz were included in this study. The mothers are separated into three groups: mothers in Istanbul (Group 1), mothers in Graz except Turkish immigrants (Group 2) and Turkish immigrants in Graz (Group 3). Obtained data are statistically investigated with chi-square and unpaired t-test.

**Results:** There are 62 mothers in G1, 33 in G2 and 22 in G3. The means of the number of children are 1.76 ± 0.95 in G1, 1.88 ± 0.89 in G2 and 1.86 ± 1.04 in G3; means of the parity are 1.90 ± 1.16 in G1, 1.91 ± 0.95 in G2 and 2.05 ± 1.33 in G3. The means of the gravidity are 2.05 ± 1.14 in G2 and 2.27 ± 1.16 in G3; means of the child mortality are 0.15 ± 0.47 in G1, 0.03 ± 0.17 in G2 and 0.18 ± 0.5 in G3. The mean number of prenatal visits is in G1 12.67 ± 9.36, in G2 8.45 ± 4.48, in G3 10.09 ± 5.48. Number of mothers who smoked during pregnancy is 11 (18%) in G1, 5 (15%) in G2 and 6 (27%) in G3; who drank alcohol during pregnancy 2 (3%) in G1, 3 (9%) in G2 and 2 (10%) in G3. 60 mothers in G1 (97%), 26 mothers in G2 (75%) and 20 mothers in G3 (91%) used medication during pregnancy. 54 mothers in G1
(54%), 14 mothers in G2 (42%), 18 mothers in G3 (81%) used iron; 37 mothers in G1 (60%), 7 mothers in G2 (21%), 6 mothers in G3 (27%) used folic acid during pregnancy. In G1 6 mothers (10%), in G2 14 mothers (42%), in G3 4 mothers (18%) had epidural analgesia during labor. The mean gestational age at birth is in G1 36.87 ± 3.02; in G2 38.45 ± 7.18; in G3 36.14 ± 8.62. 55 mothers in G1 (90%), 17 mothers in G2 (61%), 15 mothers in G3 (71%) know that the duration of exclusive breastfeeding is 6 months.

Conclusion: These data show that during pregnancy, use of medication is more common in Istanbul than in the population of Graz except Turkish immigrants; folic acid use is lower in the whole population of Graz than in Istanbul; iron use is significantly higher in Turkish populations in Istanbul and Graz. The mothers in Istanbul are more conscious about exclusive breastfeeding than the population of Graz except Turkish immigrants. Epidural analgesia is more frequent in the population of Graz except Turkish immigrants than in Istanbul.

**The differences of RCAS1 endometrial expression between implantation window and menstruation**

ESC-ID 166
Author Wilczynska J, Stachura A, Mrugala A
Country Poland
University Jagiellonian University
Department Medicine

Introduction: Discreet ECM dissolution is necessary for the proper ovum implantation process as well as the interaction between immune cells and the endometrial cells seems to be important. RCAS1 expression is related with the development of selective suppression of activated immune cells within endometrium. The aim of the study was to evaluate ability of endometrial cells to suppress the cytotoxic immune response during implantation and menstruation.

Methods: RCAS1 expression was assessed by Western-blot method in endometrial tissue samples which were obtained from 15 patients. Tissue samples were classified according to the menstrual cycle phases, with division of the cycle into two phases: menstruation (8 cases), and implantation window (7 cases) ones.

Results: The statistically significantly higher RCAS1 expression was identified in endometrium during menstruation than during implantation window.

Conclusion: Discreet ECM dissolution during implantation window is accompanied by the growth of selective immune suppression and this process increases during menstruation.

**The quality of the placenta created in conception using assisted reproduction methods**

ESC-ID 175
Author Rokvic N
Country Serbia
University University of Novi Sad
Department Faculty of Medicine

Introduction: Because of the fact that between 16 and 18 percent of couples in Serbia are infertile, the methods of artificial reproduction are always being developed. But when manipulating with human preemnibional forms, their in vitro cultivation and later implantation, there are possibilities of damage to embryonic membranes and the following unsuccessful implantation. For the correct formation of the conceptus the adequate formation of embryonic membranes is necessary and because of that it is of great importance to identify the existence of any differences between the placentas of a naturally conceived child and one conceived through artificial insemination, the quality of those differences and their significance. Goal of this research was the microscopic examination of given samples from the placentas that have formed naturally and those conceived artificially, and the comparison of histological characteristics of these two groups.

**Material and methods:** The analysis was made with samples taken from 15 placentas from the Institute for gynecology and obstetrics of the clinical center of Novi Sad. The samples were taken in the delivery room and immediately placed in a fixative. Later, they were histological processed and colored using age and H&E methods. The samples were then microscopically analyzed and the characteristics used were fibrin deposits calculations and sincicial nodds.

**Results:** With the histological analysis it has been established that all placentas that were examined in this research show morphophysiological characteristics of mature placentas. The fibrin deposits and calculations can be found in both groups of placentas while the number of sincicial nods in placentas created by artificial insemination is slightly increased but that increase is statistically unimportant.

**Conclusion:** In the examined samples the difference between two groups of placentas is either minimal or non-existent.

**Treatment of menorrhagia with tranexamic and mfenamic acid Department of Obstetrics and Gynecology**

ESC-ID 238
Author Alikaj A, Gliozhrni O, Tena V, Kallfa E
Country Albania
University University of Tirana
Department Obstetric and Gynecology

Aim: To compare the efficacy and acceptability of mfenamic acid, and tranexamic acid for treating menorrhagia. A randomised controlled trial in 49 women with dysfunctional uterine bleeding.

Methods: Treatment for five days from day 1 of during three consecutive menstrual periods, 23 patients to take mfenamic acid 500 mg eight hourly, and 26 patients to take tranexamic acid 1 gr six hourly. Menstrual loss measured by the alkaline haematin method in three control menstrual periods and three menstrual periods during treatment; duration of bleeding; patients estimation of blood loss; sanitary towel usage; the occurrence of dysmenorrheal; and unwanted events.

Results: Mfenamic acid reduced blood loss by 20% (mean blood loss 186 ml before treatment, 148 ml during treatment) and tranexamic acid reduced blood loss by 54% (mean blood loss 148 ml before treatment, 75ml during treatment). Sanitary towel usage was significantly reduced in patients treated with mfenamic acid and tranexamic acid.

Conclusion: Tranexamic acid given during menstruation is safe and highly effective treatment for excessive bleeding. Patients with dysfunctional uterine bleeding should be offered medical treatment with tranexamic acid before a deci-
sion is made about surgery. Key words: menorrhagia mefenamic acid, tranexamic acid, dysfunctional uterine bleeding.

**Internal Medicine Cardiology I**

**Type 2 diabetic patients with microangiopathy have higher risk of cardiovascular disease estimated by UKPDS risk engine**

**Introduction:** Cardiovascular disease is major cause of premature death in most European populations [1]. Patients with type 2 Diabetes have 2-4 times increased risk of coronary heart disease and 2-5 times increased risk of stroke [2,3]. The United Kingdom Prospective Diabetes Study (UKPDS) introduce the first diabetes-specific cardiovascular disease estimator [2]. Risk Engine incorporates traditional cardiovascular risk factors and specific for diabetes (HbA1c value and time since diagnosis of diabetes). The aim of the study was to indicate other non-traditional factors for prediction of cardiovascular events in type 2 diabetes which may be useful to improve risk calculation.

**Material and method:** 172 patients with diagnosed type 2 diabetes, admitted to the Department of Internal Medicine and Diabetology between 1997 and 2006 were included in this study. Subjects who have previously undergone stroke or myocardial infarction (MI) were excluded from the analysis. Using UKPDS Risk Engine version 2 calculator risk for MI, fatal MI, stroke and fatal stroke were estimated. Patients were divided according to occurrence of microvascular complications of diabetes and cardiovascular risk was compared.

**Results:** 98 subjects with microangiopathy compared to patients without microangiopathy showed significantly higher risk for MI (0.44 ± 0.24 vs. 0.30 ± 0.20 p <0.001), fatal MI (0.38 ± 0.24 vs. 0.23 ± 0.19 p <0.001), stroke (0.36 ± 0.30 vs. 0.21 ± 0.25 p <0.001) and fatal stroke (0.059 ± 0.054 vs. 0.032 ± 0.042 p <0.001). Patients with retinopathy presented higher risk of MI (0.48 ± 0.23 vs. 0.30 ± 0.20 p <0.001), fatal MI (0.42 ± 0.23 vs. 0.23 ± 0.19 p <0.001), stroke (0.41 ± 0.30 vs. 0.20 ± 0.25 p <0.001) and fatal stroke (0.068 ± 0.053 vs. 0.031 ± 0.041 p <0.001). Patients with neuropathy demonstrated higher risk for MI (0.48 ± 0.26 vs. 0.36 ± 0.23 p <0.05) and fatal MI (0.41 ± 0.27 vs. 0.30 ± 0.22 p <0.05). Nephropathy was also a significant risk factor for MI (0.47 ± 0.27 vs. 0.34 ± 0.21 p <0.05) and fatal MI (0.40 ± 0.28 vs. 0.28 ± 0.20 p <0.05) and fatal stroke (0.059 ± 0.057 vs. 0.042 ± 0.048 p <0.05).

**Conclusion:** Microangiopathy is a strong risk factor of coronary heart disease and stroke in type 2 diabetes. The results suggest that inclusion of microvascular complications to diabetes specific cardiovascular risk estimators may improve discrimination and calibration of these tools.

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**The novel protein Cthrc1 is expressed in sites of collagen matrix deposition and inhibits TGF-ß sign**

**Introduction:** We identified Collagen Triple Helix Repeat Containing-1 (Cthrc1) as a novel gene induced in adventitial fibroblasts after arterial injury. Cthrc1 is a glycosylated 30kD protein which retains a signal sequence leading it into the extracellular space. In injured arteries Cthrc1 expression is associated with myofibroblasts. Furthermore, we demonstrated that Cthrc1 inhibits collagen matrix deposition in vitro.

**Aim:** To address the in vivo role of Cthrc1 we have performed a comprehensive expression analysis in developing mouse embryos and postnatal tissues. Moreover we established transgenic mouse lines that constitutively overexpress Cthrc1 including the arterial vessel wall and applied the carotid artery ligation model on transgenic (tg) and wildtype (wt) mice.

**Material and Methods:** Using in situ hybridization and immunohistochemistry we characterized the expression sites of Cthrc1 during embryonic development and in adult wt mice. Because of the obvious overlap of Cthrc1 expression and sites of collagen deposition we applied the mouse carotid artery ligation model to assess the in vivo effects of elevated Cthrc1 levels on vascular lesion formation in wt and tg mice. Collagen content was quantified as Sirius red stained area on sections of carotid arteries 2 weeks after ligation. TGF-ß has well-described profibrotic properties and downstream signaling of the activated TGF-ß receptor I kinase involves phosphorylation of Smad2 and Smad3. To examine the mechanism leading to the reduction in collagen we examined the levels of phosphorylated Smad2 and Smad3 (pSmad2/3) using immunohistochemistry. Staining was quantified by image analysis (ImageJ; NIH; n = 4 per group).

**Results:** Cthrc1 is widely expressed during mouse embryonic development. Abundant expression is detected in the developing skeleton. Bones from adults showed expression of Cthrc1 only in the bone matrix and periostium. Cthrc1 is typically expressed at epithelial-mesenchymal interfaces that include the epidermis and dermis. Cthrc1 tg carotid arteries demonstrated an approximate 50% reduction in Sirius red staining in the adventitia 2 weeks after ligation (p = 0.015). Immunoreactivity for pSmad2/3 was present in endothelium and adventitia of normal carotid arteries from wt and tg animals. In ligated arteries, staining for pSmad2/3 was increased and pronounced in neointimal smooth muscle cells of wt vessels while staining was reduced in tg arteries. Quantification of the percentage of the cross-sectional vessel wall area immunoreactive for pSmad2/3 was significantly reduced in Cthrc1 tg mice (p = 0.0034).

**Conclusion:** Cthrc1 expression overlaps considerably with those reported for interstitial collagens and TGF-ß family members. Furthermore we showed a significant reduction of fibrillar collagens in Cthrc1 overexpressing arteries following carotid artery ligation. Our data indicate that Cthrc1 is able to regulate collagen matrix deposition in the arterial wall by inhibiting TGF-ß signaling. As a negative regulator of colla-
Platelets functional activity and their ability to internalize viruses at patients with dilated card
gen matrix deposition and TGF-β signaling. Cthrc1 may have therapeutic value in antifibrotic treatment strategies.

**Introduction:** Platelets are multifunctional blood corpuscles and are involved in many pathophysiological processes. Besides their well-known role in thrombosis and haemostasis, they take part in vessel constriction and repair, transport of various substances, tumor growth, metastasis and killing, inflammation and host defense. They can directly occupy and internalize bacteria and viruses and so protect the organism.

It was noticed, that patients with cardiovascular diseases often have attendant virus infection. Sometimes such infection can be the reason of the disease. The aim of this study is evaluation of platelet’s functional activity and determination of intraplatelet cytomegalovirus (CMV) in the patients with dilated cardiomyopathy.

**Materials and methods:** 22 patients with dilated cardiomyopathy were included in the study. The average age was 45.9 ± 11.9 years (19 - 65). 8 patients had the illness developed after proven virus infection. The control group included 8 healthy volunteers. Platelet aggregation was estimated with LTA, especially at patients with old inflammation process. Patients with increased level of CMV IgG and IgM were indicated with fluorescence of only one virus late protein – gB that proved the absence of active replication process.

**Results:** Only 2 patients had platelets normal results. In patients with dilated cardiomyopathy platelets had increased ability to form small units (spontaneous aggregation) that indicated their activation. In 11 patients were increased MPV that was connected with high risk of thrombosis. In 16 patients were increased platelets aggregation (LPA) were assessed by scanning electron microscopy, mean platelets volume (MPV) – with thrombocytecrit method. The CMV presence was investigated with immunofluorescence method. We used 3 immunolabels to different virus proteins – late protein gB, early protein p65, and super early protein p72.

**Conclusion:** In this study, use of the dopamine agonists pergolide and cabergoline was associated with an increased risk of newly diagnosed cardiac-valve regurgitation.

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**Dopamine agonists and the risk of cardiac-valve regurgitation**

**Background:** Case reports and echocardiographic studies suggest that the ergot-derived dopamine agonists pergolide and cabergoline, used in the treatment of Parkinson’s disease and the restless legs syndrome, may increase the risk of cardiac-valve regurgitation.

**Methods:** We used data from the United Kingdom General Practice Research Database to identify a population-based cohort comprising 11,417 subjects 40 to 80 years of age who were prescribed antiparkinsonian drugs between 1988 and 2005. We conducted a nested case–control analysis within this cohort in which each patient with newly diagnosed cardiac-valve regurgitation was matched with up to 25 control subjects from the cohort, according to age, sex, and year of entry into the cohort. Incidence-rate ratios for cardiac-valve regurgitation with the use of different dopamine agonists were estimated by conditional logistic-regression analysis.

**Results:** Of 31 case patients with newly diagnosed cardiac-valve regurgitation, 6 were currently exposed to pergolide, 6 were currently exposed to cabergoline, and 19 had not been exposed to any dopamine agonist within the previous year. The rate of cardiac-valve regurgitation was increased with current use of pergolide (incidence-rate ratio, 7.1; 95% confidence interval [CI], 2.3 to 22.3) and cabergoline (incidence-rate ratio, 4.9; 95% CI, 1.5 to 15.6), but not with current use of other dopamine agonists.

**Conclusion:** In this study, use of the dopamine agonists pergolide and cabergoline was associated with an increased risk of newly diagnosed cardiac-valve regurgitation.

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**Determining factors of cardiac involvement in HIV immunosuppressed patients**

**Introduction:** The cardiac involvement in HIV patients is about 5-7% in HIV adults, but less studied in children. The heart can be affected by HIV directly; evidence of HIV-1 RNA in myocardial cells, detection of HIV p17, p24, gp 120, gp 160 viral proteins from myocytes. Other factors who can determine cardiac disturbances are: opportunistic/infectious factors, toxicity of ARV drugs on myocytes, whole involvement of immune system, malnutrition and lack of selenium. Cardiac involvement can be in some cases asymptomatic or can be identified a lot of particular aspects such as: cardiomyopathies, left ventricular dysfunction, myocarditis, pericarditis, endocarditis, pulmonary hypertension, systemic hypertension. Objectives: to analyze clinical, immune, metabolic fac-
tors which contribute to cardiac involvement in HIV patients; description of cardiac manifestations; evolution and prognostic of HIV infection in patients with cardiac involvement. *Material and methods*: there were studied 183 consecutive HIV/SIDA patients (ages 11 - 17, mean age: 13 ± 0.5 years) who were admitted in Infectious Disease Clinic Targu Mures, Romania between 2004 January 1 - 2005 March 1. From this cohort we studied a number of 33 (18.5%) children and teenagers with cardiac involvement. Evaluation was clinical (stage of disease, other diseases, HAART therapy) and immune (LCD4+ count). Cardiac staging was assessed by: clinic examination, radiological procedures, EKG, echocardiography and serum levels of cholesterol (total and fractions) and triglycerides.

*Results*: the average of pts with cardiac involvement in our study was 18.5%, in advanced stages of disease and after long HAART therapy (16 pts had CD4+ < 200, 12 pts had CD4+ between 200-499). Cardiac disorders were: asymptomatic disturbances of ST - T segment (27.3% of all pts), rhythm disorders (27.3% of all pts), pulmonary hypertension with advanced stage of disease, severe immune depression, diac involvement and worsening the prognosis of HIV/SIDA pts. Cardiac involvement was observed often in patients with advanced immunosuppression. The most severe cardiac aspects were associated with: tuberculosis, HIV encephalopathy, toxoplasmosis, recurrent pneumonias or bronchopneumonia. Changes in lipid metabolism were insignificant.

*Conclusion*: in HIV immunosuppressed patients cardiac involvement is a certain fact and its appearance is related with advanced stage of disease, severe immune depression, opportunistic infection.

**The importance of the ankle/brachial index in the follow-up of superficial femoral artery occlusions**

*ESC-ID* 347
*Author* Serban R, Tilea I, Scridon A, Cozlea L, Tatar CM, Tilea IA, Cozlea DL
*Country* Romania
*University* University of Medicine and Pharmacy
*Department* Cardiology

*Internal Medicine Objective*: The evaluation of the importance of determining the ankle/brachial index in the follow-up of superficial femoral artery occlusion patients treated by PTA.

*Methods*: We evaluated a number of 28 patients (22 males, 6 females) which were followed prospectively for 6 months after the peripheral transluminal angioplasty (PTA) of the superficial femoral artery. All cases have been evaluated anamnestically, clinically and by Doppler CW immediately after the angioplasty, respectively after 1, 3 and 6 months. The cases which presented signs of reocclusion were referred to angiography. The reobstruction was defined as reocclusion or as restenosis exceeding 70% of the vessel diameter. Patency rates were calculated separately by clinical criteria (using Fontaine/ Baker -Rutheford criteria, the presence/ absence of pulses) and by Doppler CW criteria.

*Results*: The pulse modifications (a. dorsalis pedis / tibialis posterior ) distal to the treated vessel segment had a sensitivity of 82%, respectively a specificity of 84% in identifying angiographically significant a reocclusion or stenosis. The sensibility of the ankle/brachial index was 72 respectively 64% with a specificity of 82% to 100% for values between 0.10 - 0.15. Six months after the PTA, angiographically determined vascular permeability was 76% ± 4.5%, while the suggestive ABI values for this were 76% ± 4.5%. A significant decrease of the IPS by 0.15 indicates a significant reocclusion or restenosis.

*Conclusion*: The usage of only clinical criteria significantly overestimates vascular patency because more than half of all vascular reocclusions are asymptotically.

**Influence of arterial hypertension on left ventricular hypertrophy in patients with metabolic syndrome**

*ESC-ID* 99
*Author* Cvijanovic D, Ivanovic B, Novakovic M, Radiojic S, Nastasovic T, Martic Z
*Country* Serbia
*University* University in Belgrade, School of Medicine
*Department* Department for Internal Medicine

*Introduction*: Metabolic syndrome (MS) is a complex disease which emerges on myocardium, as well as hypertension, which is a part of it.

*Aim*: finding out if the hypertension (AHA) alone is the main cause of myocardial hypertrophy, or it is MS on the whole.

*Method*: MS, defined both by the 2001 National Cholesterol Educational Program Adult Treatment Panel III definition, and the American Heart Association/ National Heart, Lung and Blood Institute revision as presence of three (or more) within 5 criteria (central obesity, raised triglycerides, reduced HDL cholesterol, raised blood pressure, raised fasting glucose). LVmass (ASE) = 0.8 (1.04 ([LVIDD + PWTD + IVSTD] 3 - [LVIDD])) + 0.6g was used. LVmass was indexed by body surface area partition (LVmass/BSA), values ≥131 g/m2 for men and ≥100 g/m2 for women was recognized as hypertrophy. Medical records for 134 pts (64 male,72 female) with MS, average age 52A ± 7.8 yrs were examinated and compared with 44 (20 male, 24 female) subjects with maximal 1 criteria for MS (AHA excluded), average age 47A ± 4 yrs. We used descriptive statistics, t test, Chi-square, and Fisher's tests for data analyzes.

*Results*: Subjects from control group had LVmass = 125.84 Â ± 47.05g (p<0.01) Pts with MS who filled up AHA as one of their criteria for MS (73% pts) were divided into group according to the level of AHA. Pts with 1st grade of AHA (20.89% pts) had statistically significant higher values of LVmass = 194.71 Â ± 47.05g (p<0.01). Pts with MS who filled up AHA as one of their criteria for MS (73% pts) were divided into group according to the level of AHA. Pts with 1st grade of AHA (20.89% pts) had statistically significant higher values of LVmass = 194.71 Â ± 47.05g (p<0.01). same as pts with 2nd grade AHA - 228.31 Â ± 63.44, (p<0.01), and 3rd grade AHA - 249.5 Â ± 66.85 (p<0.01) than control group. Pts with MS without AHA had elevated LVmass/BSA in only one case, pts with 1st grade AHA in 50%, 2nd grade AHA - 59%, 3rd grade AHA - 67%. Increasing LVmass/BSA values between control group and pts with MS without AHA weren't stat. significant (p>0.025), increasing LVmass/BSA pts with MS without AHA and pts with 2nd grade AHA was statistically borderline significant (p = 0.05). Increasing LVmass/BSA values between pts with MS without AHA and pts with 3rd grade AHA were statistically significant (p <0.01).
Impairing endothelial function. Furthermore, endothelial dysfunction is associated with cardiovascular disease (CVD) risk factors, particularly advancing age, higher systolic blood pressure, BMI, and recent cigarette smoking. In addition, serum cholesterol, impaired glucose tolerance, and diabetes have been inversely associated with FMD. Risk factors contribute to the development of atherosclerosis in part by impairing endothelial function. Furthermore, endothelial dysfunction identifies patients at risk for future CVD events. Advantages: The technique used to measure FMD is safe, non-invasive and reproducible. Also, the results are available immediately. It has an increased speed of measurement.

Flow mediated dilation as a parameter of endothelial dysfunction

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<tr>
<th>ESC-ID</th>
<th>359</th>
</tr>
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<tbody>
<tr>
<td>Author</td>
<td>Kinarivala M, Mehta M</td>
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**Introduction:** The vascular endothelium is a single layer of cells lining all of the blood vessels in the body, and over the past three decades it has emerged as a key player in vascular growth, vasoregulation and vasoprotection. During this period of discovery it was found that the endothelium is essential for vasodilatation in response to increases in blood flow-associated shear stress. This phenomenon has since been termed endothelium-dependent flow-mediated vasodilatation (FMD) and now forms an important branch of endothelial research. FMD is a measurement of the ability of the artery to relax and expand to accommodate increased blood flow. Technique: Endothelium-dependent vasodilatation. A sphygmomanometric cuff is placed either above the antecubital fossa or on the forearm. A baseline rest image is acquired, and blood flow is estimated by time-averaging the pulsed Doppler velocity signal obtained from a midartery sample volume. Thereafter, arterial occlusion is created by cuff inflation to supra-systolic pressure for a standardised time. This causes ischemia and consequent dilation of downstream resistance vessels via autoregulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. The longitudinal image of the artery is recorded continuously from 30 s before to 2 min after cuff deflation. A midartery pulsed Doppler signal is obtained upon immediate cuff release and no later than 15 s after cuff deflation to assess hyperemic velocity. Endothelium independent vasodilatation: An exogenous NO donor is used to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilation, reflecting vascular smooth muscle function. Clinical implications: Endothelial function, as measured by FMD, has both environmental and genetic determinants. Decreased FMD% is associated with cardiovascular disease (CVD) risk factors, particularly advancing age, higher systolic blood pressure, BMI, and recent cigarette smoking. In addition, serum cholesterol, impaired glucose tolerance, and diabetes have been inversely associated with FMD. Risk factors contribute to the development of atherosclerosis in part by impairing endothelial function. Furthermore, endothelial dysfunction identifies patients at risk for future CVD events. Advantages: The technique used to measure FMD is safe, non-invasive and reproducible. Also, the results are available immediately. It has an increased speed of measurement.

**Characterization of coronary atherosclerotic morphology in patients with stable angina and acute phase of acute coronary syndromes without ST segment elevation virtual histology study**

**Background:** Rupture of vulnerable atherosclerotic plaques is the cause of most acute coronary syndromes (ACS). Postmortem studies which compared stable coronary lesions and atherosclerotic plaques in patients who have died because of ACS indicated a high lipid-core content as one of major determinants of the plaque vulnerability. Virtual Histology (VH) is a novel diagnostic method which uses spectral analysis of IVUS radio frequency data to construct tissue maps that classify plaque into 4 major components (fibrous, fibro-fatty, necrotic core and dense calcium) and offers in vivo opportunity to assess plaque morphology.

**Aim:** Knowing that histologic studies suggest that plaque composition plays a central role in the pathogenesis and clinical manifestations of ischemic heart disease, our primary goal was to assess the potential relations of plaque composition determined by IVUS-VH in patients with stable angina and subjects in an acute phase ACS without ST segment elevation.

**Material and Methods:** In observational study, we examined 40 patients who undergo preintervention IVUS examination. Tissue maps were reconstructed from radio frequency data using IVUS-VH software.

**Results:** We have analysed 53 lesions in 40 patients. Stable angina was diagnosed in 24 patients (29 lesions) while acute phase ACS without ST elevation was diagnosed in 16 patients (24 lesions). There were no differences in demographics and the occurrence of atherosclerosis risk factors between the groups. We have observed no significant differences in the location and the angiographic lesion characteristics between studied groups. In patients in acute phase ACS without ST segment elevation IVUS-VH examination showed significantly larger area and percent of the necrotic core at the site of minimal lumen area (1.84 ± 0.90 mm2 vs. 0.96 ± 0.69 mm2; p < 0.001 and 22.28 ± 8.90% vs. 15.00 ± 10.19%; p < 0.01) and a larger mean and percent of the necrotic core volume in the entire lesion (20.94 ± 15.79 mm3 vs. 11.54 ± 14.15 mm3; p < 0.05 and 20.02 ± 7.52% vs. 14.99 ± 7.90%; p < 0.05) comparing to stable angina subjects.

**Conclusion:** In the present study, IVUS-VH detected necrotic core was significantly larger in atherosclerotic lesions in patients in acute phase ACS without ST elevation comparing to the stable angina subjects and it might be considered a marker of plaque vulnerability. IVUS-VH itself may play an important role in the detection of vulnerable plaque.
High sensitivity C–reactive protein as a tool to assess the efficacy of thrombolysis in acute myocardial infarction

ESC-ID 393
Author Devaraj P, Jeyakumar AC
Country India
University Dr. M.G.R. Medical University
Department Internal Medicine

Aims: To assess and correlate the efficacy of thrombolysis in ST Elevation Myocardial Infarction (Acute Transmural Myocardial Infarction) using plasma levels of High Sensitivity C-Reactive protein (HsCRP). To determine whether HsCRP correlates positively with mortality during hospital stay.

Methods: This was a prospective cohort study done on 72 patients with Acute ST Elevation Myocardial Infarction (STEMI). All the patients who fulfilled the inclusion criteria for initiating thrombolysis were included. 79 Patients were initially selected for the study, of which 7 were excuded because of the adverse effects they developed due to IV Streptokinase as thrombolytic therapy and hence prematurely stopped in them.

On admission, venous blood sample was collected before Thrombolysis. HsCRP was measured by ELISA and the reference range was set as 0.0 - 0.8 µg/dL or 0.0 - 8 mg/L. The thrombolysis was done with IV Streptokinase 1.5 lakh units in 100 ml 0.9 % normal saline over 1 hour. Aspirin and Heparin were also given. The data was prospectively collected in cardiac care unit and intermediate care unit for 1 week and cardiac death was the pre-specified primary end point. The HsCRP values were divided into 3 tertiles with I < 2mg/L, II = 2-4mg/L, and III > 4mg/L.

Results: The mean value of HsCRP in the study cohort was 3.866 (± 1.791 mg/L). Of the total of 72 patients, 16(22.2%) had values < 2mg/L, 33(45.8%) had values 2-4 mg/L and 23(31.9%) had > 4mg/L. The range of HsCRP was 1.510 to 7.915 mg/L. Among the 72 patients, 47(65.27) were successfully thrombolysed, 25(34.72%) had failed thrombolysis. The association between age and HsCRP was also tested with one-way ANOVA and Levene's T test. Pearson-chi-square (PCS) = 0.880 [p = 0.644], hence not significant (NS). Gender and HsCRP showed a PCS = 2.654 with p = 0.265 (NS). Diabetes mellitus (DM) AND HsCRP had PCS = 0.298 and p = 0.358 (NS). Smoking and HsCRP had PCS = 0.303 and p = 0.859 (NS). Body mass index (BMI) and HsCRP showed no significant association with a 'p' value of 0.520 (NS). Time delay from onset of chest pain to administration of thrombolysis and HsCRP showed no significant association [p = 0.974 (NS)]. Creatine kinase - MB fraction (CK-MB) and HsCRP didnot show any significant association [p = 0.335 (NS)]. Cholesterol and HsCRP had no significant relationship [p = 0.762 (NS)]. There was a significant association between failure of thrombolysis and the third tertile of HsCRP (with value >4mg/L). Mean value of HsCRP in successfully thrombolysed patients is 2.934 (± 1.038) mg/L. Mean value of HsCRP in failed thrombolysis patients is 5.617 ± 1.59 mg/L (by Levene's T test). There was a significant association between mortality rate and third tertile of HsCRP. There was a very significant association between mortality and third tertile of HsCRP with 'p' value <0.001(high significance). No significant relation was seen between hypertension and HsCRP [p = 0.268 (NS)]. Patients with Anterior Wall Myocardial Infarction (AWMI) showed significant association with high values of HsCRP [p ≤ 0.05]. There was no significant association between effective thrombolysis and the baseline variables (age and gender), and other variables like DM, smoking, BMI, time delay, CK-MB, cholesterol, hypertension. But there is a very high significant association between mortality and ineffective thrombolysis [p < 0.0001 (HS)].

Conclusion: High serum levels of HsCRP on admission in patients with STEMI was significantly associated with inadequate response to thrombolytic therapy with standard dose of Streptokinase as assessed by electrocardiographic criteria. High serum levels of HsCRP on admission in patients with Acute STEMI was associated with increased short-term mortality rate at one week. There is no significant association between HsCRP levels on admission and time delay and CK-MB levels on admission and the probability that high levels of HsCRP on admission had been induced from larger preceding myocardial necrosis seems unlikely. As AWMI had higher levels of HsCRP compared to other infarcts, HsCRP may be a useful non-invasive tool in future to spell out the outcome of thrombolysis and prognosis in patients with AWMI.

Role of visceral fat area determined by bioelectric impedance in selection of high-risk patients with the Metabolic Syndrome

ESC-ID 663
Author Latheef KK, Rudraraju KP, Mudunuru PV, Dragan S, Maria SU
Country Romania
University U.M.F. Timisoara
Department General Medicine

Background: It has been shown that the accumulation of visceral fat is associated with the criteria of the metabolic syndrome more closely than with the BMI itself or the amount of subcutaneous fat. Computed tomography (CT) scan can be used for the assessment of visceral fat area (VFA). However, the method is not cost-effective and/or radiation exposure is problematic; thus, it is often unsuitable for screening large groups of individuals. Objective: The purpose of this study is to correlate BMI, visceral fat area as determined by bioelectric impedance on an InBody 720 analyser and the criteria of the metabolic syndrome and to assess associations between: gender, age, lipid profile and body composition analysis made by bioelectric impedance.

Preliminary results: Sixty patients, mean age 54.25 ± 11.08, 30M, 30F have been included, BMI = 35.65 ± 9.96 kg/m2, SBP was 145.83 ± 20.25 mmHg, DBP was 88.91 ± 10.85 mmHg, TGL = 185.97 ± 86.58 mg/dl, HDL = 49.57 ± 12.26 mg/dl, glycemia = 101.66 ± 33.9 mg/dl, waist circumference = 111.28 ± 12.14 cm. The parameters determined by bioelectric impedance were: mean %body fat %BF = 40.8 ± 8.1, mean VFA = 179.22 ± 52.15 cm2. There were 83.1 % patients with BMI>30 are presenting with 91.2% which correlated well with %BF > 25 in 89.1% cases and visceral fat area VF >120.8 in 91.1% of cases.

Conclusion: There is a need for a simple and noninvasive method to assess visceral fat accumulation. The bioelectrical impedance analysis (BIA) method, which is based on the electric resistance difference between the fat and components of other organs, meets this need.

Funding: Grant CEEX 10/2005 funded by the Romanian Ministry of Research, program BIOTECH.
Infectious endocarditis during pregnancy: Report of a case

A 31-year-old woman, 28 weeks pregnant presented with a 1-day left medium quadrant pain, turbid urine, nausea, vomiting. The patient had a history of acute rheumatismal arthritis and mitral rheumatismal insufficiency. Trans-thoracic echocardiography showed vegetations on the aortic valve and on the posterior mitral valve. The repeated hemocultures on different environments were negative (the patient has received antibiotics treatment from the first hospital week). Abdominal echography suggested a spleen infarct, confirmed by Doppler exam. Under treatment with Ceftriaxon 3g/day the clinical symptoms and the inflammatory syndrome reduced. Echocardiographically there is a significant reduction of the vegetations, and in the hospital week 35 the patient has Caesarean operation, without intra and post surgical incidents, with a good evolution of the patient and accounting for 10% of maternal deaths.

Method: A 31-year-old woman, 28 weeks pregnant presented with a 1-day left medium quadrant pain, turbid urine, nausea, vomiting. The patient had a history of acute rheumatismal arthritis and mitral rheumatismal insufficiency. The gynecological exam reveals a normal state of pregnancy. Vital signs at admission included a temperature of 36.8°C, a pulse rate of 112 beats/min, a blood pressure of 100/60 mm Hg. Physical examination was significant for a 3/6 systolic murmur and a diastolic murmur, and articular pain in the right shank. Trans-thoracic echocardiography showed vegetations on the aortic valve and on the posterior mitral valve. The repeated hemocultures on different environments were negative (the patient has received antibiotics treatment from the first hospital week). Abdominal echography suggested a spleen infarct, confirmed by Doppler exam. Under treatment with Ceftriaxon 3g/day the clinical symptoms and the inflammatory syndrome reduced. Echocardiographically there is a significant reduction of the vegetations, and in the hospital week 35 the patient has Caesarean operation, without intra and post surgical incidents, with a good evolution of the patient and the fetus (masculine gender, weight 2300g).

Conclusion: Active aortic endocarditis is a serious, life-threatening condition, and surgical treatment is usually delayed until the infection is eliminated. Aortic valve replacement in the presence of active infection leads to a high risk of reinfection. The case characteristics are the unknown entering gate, the repeated negative hemocultures lead to wide range antibiotics treatment initiation and the clinical aspect without fever or other suggestive signs, which slowed establishing the diagnosis and initiating the treatment.

Positive effects of nicotine in diabetic angiopathies

Aim: To demonstrate the decrease of Neuropathies, Retinopathies and Vascularopathies in Diabetic patients, through the neo-vascularization effect of Nicotine (Nicotine patches)

Material and Methods: 30 patients (males) have been included in research with Diabetes Mellitus (Type I and Type II) hospitalized in the 6th City hospital of Simferopol from the year 2003 to 2006. In 30 patients we hypothesized that activation of nAChRs with nicotine would accelerate wound healing in diabetic foot wound full-thickness (0.8 cm) dorsum part of the foot and that is why we stick nicotine patches in the close proximity of the wound. At the same time In diabetic animals an nicotine agonist (epibatidine, 10-10 mol/L) or antagonist (hexamethonium, 10-4 mol/L) of nAChRs as well as the positive control basic fibroblast growth factor (bFGF, 25 µg/kg) were also tested.

Results: Nicotine significantly accelerated wound healing as assessed by closure rate and histological score. The effects of nicotine were equal to bFGF and were mimicked by epibatidine and blocked by hexamethonium. Histomorphometry revealed increased neovascularization in patients treated with nicotine. Furthermore, capillary-like sprouting from vascular explants was significantly enhanced by nicotine.

Conclusion: Nicotine agonist-induced stimulation of nAChRs decreases retinopathies, neuropathies and other micro-vascularopathies in diabetic patients by promoting angiogenesis.

Gopten® and some heart parameters modulation within experimental atherosclerosis in normotensive rats

Aim: The study investigates the effect of tranodolapril (Gopten®, Knoll,Germany) on left ventricular mass(LVM) and thickness(LVT) as the values of aa.coronariae smooth muscle cells nuclei and Kernogan’s index in hyperlipidemic normotensive rats.

Material and methods: The duration of the experiment is 90 days, carried out with normotensive male, sexually matured, age matched Wistar-Kyoto rats(n = 20 in group). Groups: 1-on standard diet, 2-on atherogenic diet 2%cholesterol and 3% cacao oil), 3-on atherogenic diet and tranodolapril within all the experiment, 4- on atherogenic diet and tranodolapril from 30th to 90th day. Systolic Blood Pressure (SBP) was measured non-invasively by the indirect tail-cuff method every ten days within the 3 months experiment, calculated as the average of three separate measurements at each session. The morphometry includes measurement of LVM and LVT on 30th and 90th day. The mean total aa.coronariae smooth muscle nuclei area (µm2)-MTSMCA, vessel wall thickness (µm)-VWT and outer diameter (µm)-VVOD and their ratio-index of Kernogan are carried out with a compute system for image cytometry automatic analysis Quantimed-600S in Leitz DMBRE, Leika microscope. Total cholesterol (TC) and triglycerides (TG) plasma levels are determined enzymatic spectrophotometrically and calibrated on the base of Boehringer Manheim Diagnostics. Data are expressed as mean ± SEM. P value of <0.05 is taken for statistical significance. SPSS-Version 8.0 for Windows is used.
Results: SBP values in the groups didn’t change significantly within the experiment (109 ± 3.1mmHg in groups 3 and 4 vs/ 107 ± 5.3mmHg in group1). LVM and LVT are reduced significantly in group3 in comparison with group2. LVM and LVT differed (p<0.05) in groups 3 and 4. At the end of the experiment MTSMCA is of highest value in group2, while in group3 they are the lowest. Ratio anterior-posterior diameter to left ventricular thickness in group 2 is 2.225 ± 0.35mm compared to 4.45 ± 0.5mm in group2 and 4.66 ± 0.6mm in group 1. VWT and Kernogan’s index are the highest in group 2, while in group3 they are the lowest.

Conclusion: Trandalopril, probably affecting local rennin-angiotensin systems, may benefit heart protection within hypercholesterolemia when applied from the beginning of the experiment and thus suggest its therapeutic role in reducing the risk of atherosclerosis beyond BP control.

Cardiomyocytes release TF-positive MP in response to TNF-α: Increase of the extracellular thrombogenicity by MP diffusion through endothelium

Aims: Tissue Factor (TF) is constitutively expressed within the myocardium especially by cardiomyocytes (CM). Myocardial inflammation is associated with an altered TF expression and increased local and systemic thrombogenicity. We investigated the transcriptional regulation of CM TF expression under inflammatory conditions. In a second step, TF-bearing microparticle (MP) generation from CM and diffusion through an endothelial monolayer was evaluated.

Methods: Murine CM (HL-1) were stimulated with TNF-α (10ng/mL) and/or pre-incubated with specific inhibitor for JNK, p38, ERK1/2 and NFκB. The TF expression was quantified by real-time PCR, western blot and TF activity assay. The release of MP from CM was assessed by FACS. A trans-well chamber system for co-cultivation of endothelial cells and CM was used to determine whether CM-derived thrombogenic MP would diffuse through an endothelial monolayer upon stimulation with TNF-α.

Results: TNF-α induced the TF expression in CM (mRNA: 3.45 ± 0.65-fold; protein: 3.26 ± 0.65-fold; TF activity: 2.40 ± 0.30-fold; P <0.005 vs. control). The inhibition of JNK (10µM SP600125) reduced the constitutive TF expression to 64.69 ± 0.07% (mRNA) and to 36.17 ± 0.05% (protein) of the control levels (P<0.001). JNK inhibition also reduced the TF induction after TNF-α stimulation to 18.75 ± 0.65% (mRNA) and 10.78 ± 0.66% (protein) of TNF-α stimulated controls (P <0.001). Increased amounts of thrombogenic MP were released in response to TNF-α (2.47 ± 0.15-fold; P <0.01). The inhibition of JNK led to an increase in the release of MP from CM (4.88 ± 0.63-fold; P <0.01), but not of MP-associated TF activity. MP generation was associated with an increased Rho activity, TF-positive MP from CM diffused upon stimulation with TNF-α through an endothelial monolayer upon stimulation with TNF-α and thus increased its thrombogenicity.

Conclusion: Cardiomyocytic TF expression and MP release was regulated through JNK. The permeability of an endothelial monolayer for MP of cardiomyocytic origin was increased after TNF-α stimulation. Thus, myocardial inflammation is associated with TF upregulation and release of TF-bearing MP from cardiomyocytes, contributing to an increased thrombogenicity in the heart.
Full-length and soluble Tissue Factor protects Cardiomyocytes against TNF-α induced Apoptosis

ESC-ID 948
Author Boltzen U, Antoniak S, Fechner H, Poller W, Schultheiss HP, Rauch U
Country Germany
University Charité Universitätsmedizin Berlin, Campus Benjamin Franklin
Department Charité Centrum II, Cardiology

Aim: Tissue Factor (TF) is constitutively expressed in the myocardial muscle. Cellular TF can be found in the heart as membrane bound (ITF) and as soluble (asTF) isoform. Recently, we showed myocardial TF expression to be altered in patients with dilated cardiomyopathy. We now questioned whether TF protects cardiomyocytes against the deteriorate effects of inflammatory cytokines.

Methods: In vitro experiments were performed to assess the role of cardiomyocytic TF beside the hemostasis. TF isoforms were over-expressed in a HL-1 cardiomyocyte cell line or down-regulated by inducible siRNA expression system. TNF-alpha induced cell death (Annexin-V/PI staining) and proliferation behavior (CSFE staining) were assessed dependent on the TF expression.

Results: TNF-alpha stimulation induced apoptosis in native and control cells. The ITF over-expressing cells showed an increased cell survival and decreased apoptosis rate compared to the controls (Annexin-V binding after 17h: 31.1 ± 2.6% vs. 50.0 ± 5.5% for TNF-alpha 50ng/mL, P <0.05; and 34.2 ± 2.6% vs. 55.2 ± 6.7% for TNF-alpha 100ng/mL, P<0.01). Over-expression of asTF completely inhibited the apoptosis rate (Annexin-V binding: 15.6 ± 2.6% vs. 29.3 ± 3.7% for TNF-alpha 50ng/mL, P<0.01; and 18.3 ± 3.6% vs. 35.3 ± 5.3% for TNF-alpha 100ng/mL, P<0.05). The siRNA-mediated knock-down of TF resulted in an increased apoptosis rate at high TNF-alpha concentrations (Annexin-V binding: 61.74 ± 1.84% vs. 54.44 ± 2.49% for TNF-alpha 50ng/mL, P<0.05; and 66.61 ± 2.41% vs. 57.37 ± 1.16% for TNF-alpha 100ng/mL, P<0.01). In addition, the proliferation rate of the TF over-expressing cells was increased up to 40%, whereas the TF knock-down resulted in an up to 30% reduction as compared to the respective controls (P<0.05).

Conclusion: In summary, increased myocardial TF expression protects cardiomyocytes against TNF-alpha induced apoptosis, thus contributing the maintaining of myocardial integrity.

Assessment of perfusion in non-infarct related segments in the settings of anterior acute myocardial infarction coronary blood flow

ESC-ID 182
Author Pracon R, Kaczmarska E, Warminski G
Country Poland
University Medical University of Warsaw
Department 2nd Faculty of Medicine

Aim: In the setting of acute myocardial infarction coronary blood flow may be slower also in non infarct related arteries. It may be hypothesized that this phenomenon impacts myocardium perfusion beyond infarct zone. The aim of our study was to assess viability and perfusion in non-infarcted myocardium using sestamibi G-SPECT imaging.

ST–segment resolution shows myocardial tissue reperfusion after PPCI for STEMI and is a strong predictor of outcome. Our aim was to estimate whether failure of ST– segment resolution can be predicted by clinical, electrographic and coronaryographic data available on the admission. We carried out a prospective study of 38 patients who underwent PPCI at Vilnius University Hospital Santarí_kiš Klinikos (VUH SK) in December 2006 and February 2007. Demographic and clinical characteristics were recorded. Electrocardiographic evaluation included ST- segment elevation, grade of ischemia and the resolution of ST segment elevation. ECG was analysed at the admission and within 24 hours after PPCI. Angiographic findings before PPCI were also analysed. Time from the onset of the STEMI pain till PPCI was calculated. The Chi test was used to compare dichotomous variables, and student t test was applied for continuous variables. Three fac-

Predictors of failure of ST - segment resolution after primary percutaneous coronary intervention

ESC-ID 184
Author Karvelyte N, Strazinskas M
Country Lithuania
University Vilnius University
Department Faculty of Medicine

ST–segment resolution shows myocardial tissue reperfusion after PPCI for STEMI and is a strong predictor of outcome. Our aim was to estimate whether failure of ST– segment resolution can be predicted by clinical, electrographic and coronaryographic data available on the admission. We carried out a prospective study of 38 patients who underwent PPCI at Vilnius University Hospital Santarí_kiš Klinikos (VUH SK) in December 2006 and February 2007. Demographic and clinical characteristics were recorded. Electrocardiographic evaluation included ST- segment elevation, grade of ischemia and the resolution of ST segment elevation. ECG was analysed at the admission and within 24 hours after PPCI. Angiographic findings before PPCI were also analysed. Time from the onset of the STEMI pain till PPCI was calculated. The Chi test was used to compare dichotomous variables, and student t test was applied for continuous variables. Three fac-
tors were found during our study, which had statistically important influence on the failed resolution of ST segment after PPCI: the third degree ischemia in ECG at the admission (p = 0.037), stenosis found in the trunk of left coronary artery (p = 0.031) and occlusion of one coronary artery along with stenosis of two other coronary arteries (triple vessel disease) (p = 0.031). Many factors were found which had no statistically significant impact on the resolution of ST segment after PPCI: age of patient (p = 0.823), sex (p = 0.875), time from the onset of the pain of STEMI till the restoration of blood flow during PPCI (p = 0.698), intensity of pain during myocardial infarction (p = 0.529), previous cardiac chest pain (p = 0.743), myocardial infarction in anamnesis (p = 0.964), myocardial infarctions in family (p = 0.44), primary arterial hypertension (p = 0.97), diabetes mellitus (p = 0.67), increased blood cholesterol (p = 0.189), overweight (p = 0.917), patients describing themselves as nervous persons (p = 0.646), smoking (p = 0.071), occlusion of right coronary artery (p = 0.943), occlusion of left anterior descending artery (p = 0.12), occlusion of left circumflex artery (p = 0.088), balanced coronary perfusion type (p = 0.964), right coronary perfusion type (p = 0.35), left coronary perfusion type (p = 0.088), collars between heart arteries (p = 0.182), left ventricle ejection fraction (p = 0.698). Failure of ST - segment resolution after PPCI for STEMI after PPCI is predicted by the degree of ischemia, stenosis found in the left main coronary artery and the number of diseased vessels.

Treatment with eplerenone ameliorates left ventricular dilatation and dysfunction in a CVB-3 induced murine myocarditis model

ESC-ID 952
Country Germany
University Charité, Universitätsmedizin Berlin
Department Campus Benjamin-Franklin, Department of cardiology

Introduction: Cardiotropic viruses are the predominant cause of myocarditis with consecutive development of a dilated cardiomyopathy (DCM) in a relevant proportion of patients. Left ventricular (LV) dilatation and dysfunction is essentially attributed to myocardial inflammation linked to an increased extracellular matrix protein production and an imbalance of the collagen degrading matrix metalloproteinases and their tissue inhibitors (MMP/TIMP System). Recently it has been shown in two different cell culture models that inhibition of the renin-angiotensin-aldosterone system (RAAS) decreases collagen synthesis and MMP transcription[1,2]. Therefore we hypothesized that inhibition of the RAAS has a beneficial effect on myocardial remodeling in DCM. Aim of this study was to investigate the effects of aldosterone receptor antagonism by eplerenone in a murine model of persisting coxsackievirus type 3 (CVB-3) induced myocarditis.

Methods: SWR/J mice were infected with 5x104 PFU CVB-3 (Nancy strain) and treated either with eplerenone (200 mg/kg bodyweight per day) or placebo since day 1 post infection. Sham-infected mice were used as controls (n = 10 per group). The LV function and volume were evaluated by conductance catheter after 8 days and collagen I and III transcription were measured by real time PCR. Total collagen content was quantified by picosirius red staining and MMP-9 protein content was measured by immunohistochemistry.

Results: Untreated CVB-3 infected mice displayed a reduced LV function with a significantly impaired +dp/dt(max) and -dp/dt(min) (2-fold and 1.6-fold respectively, p<0.05 vs. control) whereas eplerenone treated mice showed no significant change of LV function compared to control mice. Left ventricular enddiastolic volume was increased by 21 % in untreated CVB-3 infected mice (p=0.05 vs. control) while eplerenone treatment prevented LV dilatation. Real time PCR revealed a significantly increased collagen I and III transcription in infected mice (2.6-fold, 2.5-fold respectively, p<0.05 vs. control) which was normalized in eplerenone treated animals. Total cardiac collagen content in CVB-3 infected mice was increased (1.9-fold, p=0.01 vs. control) while treatment led to normalized values. MMP-9 protein content was significantly increased in infected mice (2.3-fold vs. control, p<0.01) and normalized in eplerenone treated animals.

Conclusion: Treatment with eplerenone in a murine CVB-3 induced myocarditis model ameliorates cardiac fibrosis, prevents LV dilatation and leads to an improved LV function by modulation of collagen transcription and MMP expression.

The prevalence of hypertension and its relation to obesity in a large cohort of 18-year-old high school students

ESC-ID 698
Author Grybo’s A, Artyszuk L, Dziliwski P
Country Poland
University Medical University of Warsaw
Department Department of internal medicine

Objective: To establish the prevalence of hypertension (HTN) and its relation to obesity in a large cohort of 18-year-old high school students in Warsaw. Design and Methods: The study included all senior year students in 12 high schools (n = 2217, M/F 1108/1109). In all subjects, blood pressure was measured 4 times (twice on two occasions, 1 month apart) in schools by trained nurses and the mean of all measurements was used to establish the diagnosis of HTN (SBP 140 mmHg and/or DBP 90 mmHg) or normotension (NT). Weight, height, waist circumference (WC) and BMI were measured in all subjects and BMI was calculated. Abdominal obesity (AO) was diagnosed according to the IDF criteria, while normal weight, overweight and obesity categories were based on BMI according to the WHO criteria.

Results: HTN was diagnosed in 184 (8.3%) subjects and was much more prevalent in males than in females (14.7% vs. 1.9%, p <0.0001). Overall, mean SBP was 120 ± 18 mmHg (range 89-191), DBP was 71 ± 9 mmHg (range 50-103), WC was 78 ± 10 cm (range 52-148), and BMI was 22.2 ± 5.3 kg/m² (range 14.9-44.8). Compared to NT group, subjects with HTN had greater WC (87 ± 11 vs. 77 ± 9 cm, p <0.0001) and BMI (25.1 ± 4.4 vs. 21.8 ± 3.0 kg/m², p <0.0001) and in subjects with HTN compared to the NT group, subjects with HTN had greater WC (87 ± 11 vs. 77 ± 9 cm, p <0.0001) and BMI (25.1 ± 4.4 vs. 21.8 ± 3.0 kg/m², p <0.0001). Males had higher SBP/DBP (125/73 ± 18/9 vs. 114/69 ± 15/8 mmHg, p<0.0001), WC (82 ± 10 vs. 75 ± 10 cm, p <0.0001) and BMI (22.8 ± 3.4 vs. 21.4 ± 3.0 kg/m², p <0.0001) than females. AO was diagnosed in 405 subjects (18.3%) and was much more prevalent in females than in males (26.3% vs. 10.2%, p<0.0001) and in subjects with HTN compared to the NT group.
group (28.8% vs. 17.3%, p <0.0001). The prevalence of AO in subjects with HTN was lower in males than in females (35.8% vs. 42.8 %, p <0.0001). In contrast, AO was much more prevalent in NT males than in NT females (29.9% vs 4.0%, p <0.0001). HTN was more prevalent in subjects with AO compared to those without AO (13.1% vs 7.2 %, p <0.0001). This difference in HTN rate was much larger between males with and without AO (61.1% vs 9.4%, p <0.0001), while HT rates in females with AO or without AO were much smaller (3.1% vs. 1.5%, p <0.0001). The prevalence of obesity/overweight was 2.6/12.5% overall, 3.8/15.2% in males, 1.4/9.6% in females, 13/31% in subjects with HTN, 14/30% in males with HTN, and 5/38% in females with HTN. In all obese/overweight groups, HTN was more prevalent than in non-obese/overweight subjects (24.2% vs. 5.5% overall, 34.3% vs. 10.1% in males, 7.4% vs. 0.2% in females, all p <0.0001). Correlations between SBP/DBP and WC or BMI were significant and similar, both overall and in males and females (r = 0.24 to 0.39 for SBP and r = 0.16 to 0.25 for DBP, all p <0.0001).

### Conclusion

1. Using adult diagnostic criteria, HTN was found in 8.3% of 18-year-old students. 2. HTN in adolescents is clearly related to both BMI and abdominal obesity as measured by WC.

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**Validation of the OMRON HEM-907 device for blood pressure measurement in hemodialysis patients with end-stage renal disease**

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<th>ESC-ID</th>
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<tbody>
<tr>
<td>Author</td>
<td>Kostyra K, Staszkow M, Shebani Z, Czarkowski M</td>
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<td>Country</td>
<td>Poland</td>
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<tr>
<td>University</td>
<td>Medical University of Warsaw</td>
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<tr>
<td>Department</td>
<td>Internal Medicine and Endocrinology</td>
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**Introduction:** The increasing availability of oscillometric blood pressure (BP) measuring devices has led to a significant increase in their use in clinical conditions. Prior to marketing, they should be validated according to the International Protocol for Validation of Blood Pressure Measuring Devices in Adults (IP), created by the European Society of Hypertension (ESH). Validation of new devices is carried out at rest only. IP protocol does not include specific groups of patients, when the accuracy of the oscillometric method could be limited. In hemodialysis patients with end-stage renal disease (HP) periodic changes in blood volume may disturb accuracy of oscillometric BP measuring devises. **Aim:** The aim of the study was to validate the accuracy of the oscillometric device for BP measurement (OMRON HEM-907) in HP according to the IP.

**Materials and methods:** In a group of 33 HP (15 F and 18 M) aged 54 ± 15yrs 99 BP measurements were performed simultaneously by two trained observers using mercury sphygmomanometers alternately with the HEM-907 devise. The differences between the BP values were calculated for each measure.

**Results:** The mean BP of study group was 152 ± 32/82 ± 18 mmHg. HEM – 907 passed the first phase of the IP. For the second phase, readings for the device differed by less than 5 mmHg for 72 of the systolic BP (SBP) readings and 65 of the diastolic BP (DBP) readings; by less than 10 mmHg for 89 of SBP and 83 of DBP readings; and by less than 15 mmHg of 98 SBP and DBP readings. There were 24 patients with 2 of 3 differences for SBP readings but only 21 with differences for DBP readings less than 5 mmHg (recommended value - RV<3); and 2 patients with no differences for SBP readings less than 5 mmHg but 7 – for DBP readings ( RV<3).

**Conclusion:** The OMRON HEM – 907 did not pass the II phase of IP by ESH when validated in the group of HP. Even if an oscillometric device fulfills validation criteria of IP, it does not render its accuracy in all clinical situations.

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**A comparison of clamidia pneumonia antibody serum levels in patients with acute myocardial infarction and control group**

| ESC-ID | 960 |
| Author | Adalat H, Shahram H, Akbar M, Marzich R, Peyman H, Arezu N |
| Country | Iran |
| University | Ardabil University of Medical Sciences |
| Department | Cardiology |

**Introduction:** Atherosclerosis is the major underlying cause of coronary artery disease including acute myocardial infarction (AMI), and AMI is one of the most common causes of human mortality in the world today. Many factors may have roles in generation or accentuation of atherosclerosis and recently it has been found that among the numerous agents resulting to atherosclerosis, infective agents can have a special role. In this survey, we studied the anti-clamydia pneumonia antibody serum levels in 60 patients with AMI and compared it with control group.

**Materials and methods:** This study was done as case-control manner in a 6 month period in Ardabil Bouali Hospital and the serum level of anti-clamydia pneumonia antibody(IgG) was detected by immunoassay method in 60 patients with AMI admitted in CCU section. The same test was done in control group that were 60 cases and approximately similar in gender and age with AMI group. The cases of control group were selected from other sections of hospital with regard to not having known heart disease.

**Results:** In total, 60 patient with AMI and 60 cases as control group were studied in this survey that 80% of each group were male and 20% were female and the age range in each group was 35-80 years. 63% of AMI group had anterior, the rest had inferior AMI. IgG serum titer in all of the patients of case and control groups was positive and above 5 unit/ml.

**Conclusion:** As mentioned above, there was no significant statistical difference between anti-clamydia pneumonia IgG serum titer in case and control groups.

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**Dynamics of left ventricular mass during long term use of bisoprolol and lerkanidipine in patients with essential hypertension**

| ESC-ID | 194 |
| Author | Shorikova D, Taoshun V, Shorikov E |
| Country | Ukraine |
| University | Bukovinian State Medical University |
| Department | Cardiology |

**Introduction:** Arterial hypertension determines distinct adaptive left ventricular geometric responses. Left ventricular
Determinants of QT interval duration and QT variability in cardiac transplant recipients

Aim: In general population, QT interval and QT variability are affected mainly by changes in ventricular repolarization and autonomic nervous system. Due to unique electrophysiology of the denervated heart, we sought to analyze the parameters that determine QT interval and QT variability in cardiac transplant recipients.

Methods: We involved 9 heart transplant patients who underwent implantation of a pacemaker that allows for intramyocardial ventricular evoked response (VER) measurement in a prospective study. We simultaneously made 3 consecutive 5-minute high resolution 12 lead ECG (HRECG) and VER measurements with the patients 1) having intrinsic heart rate, 2) having paced heart rate, and 3) having the heart paced from the opposite electrode. To determine the influence of ventricular electrophysiological properties we used the length of paced QT interval and VER measurement, whereas to determine the influence of autonomic nervous system we used the markers of heart rate variability. We analyzed digitalized HRECG data by using ECG Segment Analyzer, and VER measurements were analyzed by the laboratory in Graz, Austria. The data was then statistically analyzed by using the Pearson correlation coefficient.

Results: The length of QT interval on HRECG correlates with VERV index ($r = -0.51$, $P=0.03$), but not with heart rate variability ($r = 0.14$, $P=0.55$). On the contrary, we found a significant association with QT variability and heart rate variability parameters ($r = 0.83$, $P<0.001$), but no correlation of QT variability with VERV index ($r = 0.02$, $P=0.94$).

Conclusion: QT interval in cardiac transplant recipients appears to be a valid reflection of ventricular electrophysiological properties, while QT variability is influenced mainly by the changes in autonomic nervous tone. Therefore, by measuring QT interval and QT variability we can gain separate insight in ventricular electrophysiology and autonomic reinnervation of the transplanted allograft.
**Introduction:** The metabolic syndrome, associated with low high density lipoprotein (HDL) cholesterol (C) levels, and an increased risk for cardiovascular disorders, occurs at an alarming frequency in Western societies. The aim of the study was to evaluate whether an increase of HDL-C by human apolipoprotein (apo A-I) gene transfer (GT) could improve left ventricular (LV) dysfunction in an experimental model of the metabolic syndrome.

**Methods:** Intravenous GT was performed with 3 x 10¹² particles/kg of the E1E3E4-deleted vector Ad.hapoA-I, expressing human apo A-I, or of Ad.Null, containing no expression cassette, in 9 weeks old obese ZSF1 rats. Age-matched lean ZSF1 rats injected with the same dose of Ad.Null were used as controls. LV function was analyzed via a 2.0 F Millar-Tip catheter under basal and methoxamine-induced stress conditions at the day of sacrifice, 120 days after GT. AMP, ADP and ATP levels in LV were analyzed by HPLC. Phosphorylated (p) and total (tot.) 5’-AMP-activated protein kinase (AMPK) protein levels of the LV were analyzed by Western Blot. LV mRNA levels of peroxisome proliferator activated receptor gamma coactivator (PGC)-1 were determined by real-time RT-PCR.

**Results:** Ad.hapoA-I GT induced sustained expression of human apo A-I for 120 days with peak levels of 253 mg/dl at day 12 and was associated with a 1.5-fold increase of HDL-C at day 56 (p <0.05). Under basal conditions, no differences in LV systolic function were found between the groups. However, obese Ad.Null rats established an increased LV end diastolic pressure (LVEDP), which is a marker for diastolic dysfunction. Under both basal conditions and methoxamine-induced stress, LVEDP was significantly improved in the Ad.hapoA-I compared to the Ad.Null obese rats. ADP/ATP and AMP/ATP ratios were 1.9-fold and 3.7-fold lower in obese Ad.hapoA-I compared to obese Ad.Null rats, respectively (p <0.005). These findings were associated with a 1.8-fold (p <0.0001) increased ratio of p-AMPK/AMPK and 4.1-fold (p <0.005) higher PGC-1 LV mRNA expression in Ad.hapoA-I compared to Ad.Null obese rats.

**Conclusion:** Induced HDL-C levels by human apo A-I GT improve the energy metabolism in the diabetic heart via a p-AMPK-mediated pathway, leading to an ameliorated diastolic function.

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**Novel endothelial-protective effect of HDL: Down-regulation of Toll-like receptor 4**

**Introduction:** Endothelium-derived Toll-like receptor (TLR) 4 is known to be the key molecule in lipopolysaccharide (LPS)-induced neutrophil sequestration into lungs. The aim of our study was to investigate whether the endothelial-protective effects of HDL also include regulation of Toll-like receptor (TLR) 4. Therefore, we analyzed the effect of HDL supplementation on TLR4 expression in human aortic endothelial cells (HAEC) and the effect of increased HDL following adenoviral apo A-I (Ad.AI) gene transfer (GT) on lung TLR4 expression in an experimental model of LPS-induced endotoxic shock.

**Methods:** HDL (50 Âµg/ml) was supplemented to HAEC in the presence/absence of LPS (100 ng/ml). Surface-TLR4 expression on HAEC was analyzed by FACS. C57BL/6 mice were i.v. injected with 5 x 10¹⁰ total particles of Ad.AI or with Ad.Null. Fourteen days hereafter, mice were i.p. injected with LPS (80 mg/kg) or with saline and 20 hours afterwards sacrificed. For survival studies, endotoxic shock was induced in 25 mice per group. Lung myeloperoxidase activity (MPO), as a marker of neutrophil infiltration was analysed. Lung TLR4-mRNA expression was determined by real-time PCR, plasma interferon-gamma (INF-Gamma) levels by ELISA.

**Results:** HDL supplementation significantly reduced TLR4 expression on HAEC. Ad.AI resulted in human apo AI expression levels of 80 ± 10 mg/dl at day 14. By 24 hours after LPS injection, 81% of LPS-treated Ad.Null and saline mice died, in contrast to 50% of Ad.AI LPS-treated mice. This was associated with a 1.6- and 1.7-fold (p <0.05) lower lung/body weight ratio in Ad.AI compared to Ad.Null and saline LPS-injected mice. LPS-induced lung TLR4 mRNA expression was 2.0-fold (p <0.05) reduced after human apo AI-GT compared to LPS controls. Moreover, human apo AI-GT significantly reduced neutrophil infiltration in LPS-treated mice, as indicated by 1.88 Â± “fold (p <0.05) and 1.9 Â± “fold (p <0.05) reduced MPO activity compared to controls, respectively. Plasma INF-Gamma-levels were 2.8-fold and 2.2-fold (p <0.05) lower versus LPS-treated Ad.AI and saline mice, respectively.

**Conclusion:** The HDL-mediated reduction in lung TLR4 expression contributes for the reduced neutrophil infiltration and may subsequently account for improved mortality rate.
Anti-oxidative, anti-inflammatory and endothelial-protective effects of high density lipoprotein cholesterol induced by human apolipoprotein A-I gene transfer improve left ventricular function in an experimental model of diabetes mellitus

**Methods:** Diabetes mellitus was induced by a single injection of streptozotocin (STZ; 70 mg/kg, i.p.) in 8 weeks old Sprague Dawley (SD) rats. Intravenous GT with 3 x 10^12 particles of the E1E3E4-deleted vector Ad.hapoA-I, expressing human apo A-I, or of Ad.Null, containing no expression cassette, was performed 5 days after STZ-injection. Age-matched non-diabetic SD rats injected with the same dose of Ad.Null were used as controls.

**Results:** Six weeks after apo A-I GT, HDL-cholesterol levels were increased by 1.6-fold (p <0.001) compared to diabetic controls injected with the Ad.Null vector (STZ-Ad.Null). Apo A-I GT improved contractility parameters. This was associated with a decrease of cardiac oxidative stress, as evidenced by a 1.5-fold (p <0.05) reduced activated phosphorylation state of the stress-induced p38 MAPK compared to STZ-Ad.Null controls and an induction of the antioxidant enzyme, SOD. Specifically, apo A-I GT increased diabetes-downregulated SOD-2 by 1.2-fold (p <0.05) and normalized ec-SOD expression to levels found in non-diabetic controls, without affecting SOD-1 expression. Apo A-I GT resulted in a significant decrease of diabetes mellitus-induced left ventricular ICAM-1, VCAM-1 and TNF-alpha mRNA expression to levels not significantly different from non-diabetic Ad.Null controls. In addition, 3.0–fold (p <0.005) lower damaged endothelial cells and basement membranes were found in STZ-Ad.hapoA-I compared to STZ-Ad.Null rats, as assessed by electron microscopy. The apo A-I GT-mediated protection was associated with a 1.6-fold (p <0.0005) and 1.6–fold (p <0.005) induction of diabetes-downregulated phospho to total Akt and eNOS ratio, respectively.

**Conclusion:** Apo A-I GT reduced the development of STZ-induced diabetic cardiomyopathy.

Long term observation of ACS patients without significant stenoses in coronary arteries

**Introduction:** Patients with chest pain and normal coronary angiograms have a favourable long-term prognosis with an incidence of myocardial infarction and cardiovascular death similar to general population. However, despite the reassurance of normal angiography, such patients often remain symptomatic during follow-up and may be subject to hospital readmission with symptoms and electrocardiographic changes suggestive of ACS (Acute Coronary Syndrome). Long term prognosis and necessary pharmacotherapy are still controversial issues in these patients.

**Aim:** The purpose of this research was to evaluate long term follow-up of patients admitted to hospital with the diagnosis of ACS who did not have any critical stenoses in coronary angiography.

**Methods:** We reviewed data from 108 patients admitted to the Department of Interventional Cardiology, University Hospital in Krakow, Poland with ACS diagnosis without any critical changes in angiograms from July 2004 to June 2006. We assessed baseline demographics and past medical history and occurrence of MACE in-hospital and during follow-up period.

**Results:** This group included 52 men (48%), mean age was 58.5 ± 13.5 years. There were 13% of patients with diabetes mellitus, 72% had arterial hypertension, 52% had dyslipidemia, 6.5% present myocardial infarction in history. The patients were divided into two groups: troponin positive ACS (50%) and troponin negative ACS (50%). During the follow up period of 17 months there were 9.3% deaths (7.4% due to cardiovascular reasons). Myocardial infarction occurred in 5.6% of patients. Altogether during the study period 20.4% patients developed cardiovascular symptoms leading to hospital readmission.

**Conclusion:** Patients with diagnosis of ACS and without critical narrowing of coronary arteries are often readmitted to hospital within follow-up period. Substantial number of them develop myocardial infarction and 1 in 10 dies. Maybe because of that they should have a constant cardiologic control.

Evaluation of clinical and diagnostic value of VEGF - level in benign and malignant pleural effusions

**Objective:** Being a potential inducer of endothelial hyper-permeability, vascular endothelial growth factor (VEGF) has been shown to play an important role in pleural and peritoneal effusions formation, allowing proteins to diffuse from blood into the effusions. Cancer cells are most likely to be the
main sources of the VEGF production in malignant effusions. It is suggested that there is another origin (for example, infiltrating inflammatory cells) of the VEGF accumulation and another mechanisms (hypoxia, cytokines, infection agents) of the stimulation of VEGF expression in benign effusions.

**Purpose:** The purpose of the present study was to evaluate VEGF level in a series of 20 malignant pleural effusions from cancer patients with breast (n = 16), ovarian (n = 3) and uterus (n = 1) carcinomas and in a series of 20 benign effusions from patients with liver cirrhosis (n = 3), ischemic cardiomyopathy (n = 2), pancreatitis (n = 2), pneumonia (n = 4), heart failure (n = 6), kidney transplantation (n = 1), hyperthyroidism (n = 1) and COPD (n = 1). We studied also the clinical value of VEGF levels within the group of patients with malignant effusions. Our main goals were: A) to find out if there is a correlation of VEGF level with FIGO stage and tumor grade; B) to define, which of the laboratory parameters are of diagnostic and pathogenetic importance for the VEGF production and effusion formation.

**Methods:** Each effusion fluid probe was obtained by puncture for clinical reasons. The analyses have been performed in samples from a series of 40 effusions collected from June 2003 to April 2006. The VEGF levels were determined by the Quantikine Human VEGF ELISA. Data are expressed as pg/ml.

**Results:** Malignant effusions were distinguished from benign ones by higher VEGF level (median VEGF level in malignant effusions was 1144.19 pg/ml, in benign ones – 98,885 pg/ml). The mean VEGF values were accordingly 803.666 pg/ml in malignant effusions and 247.055 pg/ml in benign ones). There was no significant association of VEGF level and FIGO stage or tumor grade among the patients within one tumor type. This is probably due to the small sample size for each cancer type. No correlation could be found between VEGF amount and trombocyte, leucocyte, erytrocyte counts, thromboplastin time and incomplete thromboplastin time.

**Conclusion:** VEGF levels may be useful in discriminating malignant effusions from benign ones. Based on the fact that the VEGF levels are higher in malignant effusions than in benign ones, it may be confirmed that these conditions have different pathogenetic origin. VEGF level in malignant effusions doesn’t depend on the trombocyte, leucocyte, erytrocyte counts, thromboplastin time and incomplete thromboplastin time. It may be supposed, that these factors are unlikely to play significant pathogenetic role in VEGF production and accumulation in malignant effusions.

**Risk factors influencing the 5-year-outcome of patients after acute myocardial infarction with STEMI**

**ESC-ID**

471

**Author**

Maciorkowska D, Olszewska M

**Country**

Poland

**University**

Medical University of Białystok

**Department**

Department of Cardiology

Every year about 100, 000 people in Poland suffer from acute myocardial infarction. Despite significant improvement in the therapy of STEMI the remote mortality remains high. The knowledge which factors affect long term prognosis would be of great help to doctors taking care of patients after STEMI.

**Aim:** To analyse which risk factors influence the 5-year-outcome of patients with myocardial infarction with ST-segment elevation (STEMI), treated by primary percutaneous coronary intervention (pPCI) and who survived the first 30 days after STEMI.

**Methods:** Our registry comprised unselected patients with STEMI, treated with pPCI. The primary end point during the 5-year-follow-up was death from any cause. STEMI was diagnosed based on: chest pain, ECG changes (ST-elevation >1mm in at least 2 leads) and rise in myocardial necrosis markers. A Chi-square test, Student t test or Mann-Whitney test, Kaplan-Meier survival analysis with Cox test and Cox proportional hazards model were used for statistical analysis.

A p value of < 0.05 was considered to indicate statistical significance.

**Results:** From 505 patients 32 (6.3%) died within the first 30 days after MI. 473 patients (24.7% women) were included in our study (mean age 53.4 ± 11.14 years). TIMI 3 flow after pPCI in infarct related artery was obtained in 95.77% (N = 453). The mortality between 31st and 365th day was 4% (N = 19) and the 5-year mortality 15.6% (N = 74). In single-vari ate Cox model analysis the factors which significantly affected survival were: mean age (1.056), weight (0.965), heart rate (1.018), BMI index ( 0.903), TIMI Risk Score ( 1.33), Killip class (1.42), ejection fraction (0.961), number of arteries with significant stenoses (1.448), creatinin clearance ( 0.98). History of previous MI (p = 0.027), TIMI 3 flow after pPCI (p = 0.038), diabetes mellitus (p = 0.0082) and previous angina pectoris (p = 0.00581) also significantly affected prognosis as assessed in analysis.

**Conclusion:** The most important risk factors among patients, who survived the first 30 days after MI are age and TIMI risk score. Furthermore weight, ejection fraction, BMI index, creatinin clearance and fibrinogen concentration in plasma significantly affected survival.

**Estimation of nitric oxide synthesis and metabolism in patients with arterial hypertension with obesity**

**ESC-ID**

245

**Author**

Demydenko G, Gerasimchuk N

**Country**

Ukraine

**University**

State Medical University

**Department**

Internal Medicine

The purpose: to estimate the nitric oxide synthesis and metabolism on the basis of determination of eNOS and iNOS activity and the level of S-nitrosotiol in patients with arterial hypertension with obesity.

**Material and methods:** 83 patients with arterial hypertension and obesity and 20 practically healthy persons were examined. The concentrations of eNOS and iNOS were determined by biochemical method, and S-nitrosotiol in plasma by fluorometric method.

**Results:** It was revealed, that the level of S-nitrosotiol was statistically higher, than in control ones. The highest level was in hypertensive group with 2 and 3 stage of obesity (0.45 ± 0.11 and 0.45 ± 0.04 µMol/L). Normal âNOS activity was found in hypertensive patients with obesity of 1 st. 0.769 ± 0.119 pmol/min * µg protein, the increased NO synthesis was revealed in hypertensive patients with normal body mass and overweight (0.821 ± 0.145, 0.820 ± 0.138 pmol/min * µg protein). The decrease of eNOS activity was found in hypertensive patients with obesity of 2 and 3 st.(0.737 ± 0.111 è 0.683 ± 0.145 pmol/min * µg protein). The most highest acti-
The hypothyroidism was performed by means of the transplantation of a plant with hormone level was increased on 35 percent and 49.6. Combined culture of TOC with AOC or pituitary, after allotransplantation led to 34.3 percent T4 level rise. At combined transplantation of TOC and AOC resulted in 56.6 percent T4 level increase compared with thyroidectomized rats. It was shown that combined transplantation provided normalization of some metabolic factor under hypothyroidism compensations. All grafts were active in normalization of plasma glucose level after glucose tolerant test. The transplantation of thyroid monografts has not affected rats plasma cholesterol level. Whereas combined transplantation of thyroid with adrenal or pituitary explants decreased cholesterol level comparing with thyroidectomized rats. We defined the tendency of rise in the albumin level after combined transplantation with an adrenal. The graft histology on the 30th day after operation was shown that combined auto-, allo- and xenografts of the native thyroid and adrenal fragments differed from thyroid monografts by maximum acceptance of follicular structure with areas of the regenerations. In addition, the combined cultures of TOC with AOC and pituitary promoted the acceptance of typical follicular structure of the thyroid graft in comparison with separately cultured tissues. So, according to our results, we proved the positive effect of combine TOC and AOC transplantation which realized in normalization T4 and TSH and metabolic processes in hypothyroid rats. Therefore the combined cultured of two types of endocrine tissues are one of the effective ways of the graft processing for improvements his functional parameters. Combined transplantation of thyroid and adrenal tissues is an alternative way for primer hypothyroidism curing.

Internal Medicine
Endocrinology I

Result of experimental hypothyroidism correction by combined transplantation of organ cultures

ESC-ID 559
Author Bilyavskaya SB, Legach EI, Bozhok GA, Bondarenko TP
Country Ukraine
University Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences
Department Neuro-Humoral Regulation

Cell or tissue transplantation is often used in the treatment of endocrine pathology. Graft acceptance still has been remained a problem because of the immunological rejection reaction. It is assumed that combined transplantation of two types of endocrine tissues has some local immune, trophic and signal-regulatory effects on the main graft [Agrawal AK, Shukla S et al., 2004; Davalli AM, Scaglia L et al., 1999; De Fazio SR, Goozzi JJ, 1994]. The investigation is devoted to study the influence of auto-, allo- and xenotransplantation of hypertensive tissues with native and cultured thyroid tissue combined with adrenal and pituitary explants on rat experimental hypothyroidism. The organ cultures were obtained by the method [Turchin IS et al., 1990]. The hypothyroidism was performed by means of the retrograde thyroidectomy [Legach EI, 2005]. Auto-, allo- and xenografts were transplanted under the kidney capsule in the following combinations: single thyroid organ culture (TOC) or native fragments, TOC or native fragments with adrenal tissue, TOC or native fragments with pituitary explants. T4 and TSH rat serum levels were measured by radioimmunoassay. It was shown that all thyroid grafts had an ability to produce T4 on the 30th day after transplantation. Transplantation of native TOC xenograft combined with adrenal organ culture (AOC) resulted in 56.6 percent T4 level increase compared with thyreoidecetomized rats. In the case of single TOC graft the transplantation led to 34.3 percent T4 level rise. At combined culture of TOC with AOC or pituitary, after allotransplantation the T4 level was increased on 35 percent and 49.6 percent correspondently compared with thyreoidecetomized rats, and 14.6 percent and 41.62 percent compared with transplantation of separately cultured tissues. The plasma TSH level in all groups was in the feedback regulated by thyroxin level. It was shown that combined transplantation provided normalization of some metabolic factor under hypothyroidism compensations. All grafts were active in normalization of plasma glucose level after glucose tolerant test. The transplantation of thyroid monografts has not affected rats plasma cholesterol level. Whereas combined transplantation of thyroid with adrenal or pituitary explants decreased cholesterol level comparing with thyroidectomized rats. We defined the tendency of rise in the albumin level after combined transplantation with an adrenal. The graft histology on the 30th day after operation was shown that combined auto-, allo- and xenografts of the native thyroid and adrenal fragments differed from thyroid monografts by maximum acceptance of follicular structure with areas of the regenerations. In addition, the combined cultures of TOC with AOC and pituitary promoted the acceptance of typical follicular structure of the thyroid graft in comparison with separately cultured tissues. So, according to our results, we proved the positive effect of combine TOC and AOC transplantation which realized in normalization T4 and TSH and metabolic processes in hypothyroid rats. Therefore the combined cultured of two types of endocrine tissues are one of the effective ways of the graft processing for improvements his functional parameters. Combined transplantation of thyroid and adrenal tissues is an alternative way for primer hypothyroidism curing.

Can a man with hypogonadotropic hypogonadism be a father?

ESC-ID 595
Author Markovska Z, Cedomir Dimitrovski C
Country Macedonia
University St. Cyril and Methodius
Department Medical Faculty

Aim: Hypogonadotropic hypogonadism (HH) is a deficiency of the gonadotrophins: Luteinizing hormone (LH), Follicle Stimulating Hormone (FSH). It can be caused by Hypothalamic Gonadotrophin Releasing hormone (GnRH) deficiency which results in a reduced gonad function. The teenage years can be a very traumatic time for undiagnosed and untreated gonad syndromes. Sufferers fail to experience the physical changes to there body and to go through puberty at all. Despite of absent of secondary sexual characteristics and infertility they wish to have children. The aim of this paper is to present the lifestyle and treatment of the men whit hypogonadotropic hypogonadism who want to be fathers.

Material and methods: In this study were included 16 males patients with (HH) aged 26-36 (mean 31,7 years).Four of them were with Kallman’s syndrome and 12 with Isolated Gonadotropin Deficiency (IGD). All men had low serum testosterone levels, testicular volume <5.0ccm, 3 concentrations, volume and zoospermia. Before the infertility treatment was started, all the patients were on testosterone replacement therapy to induce puberty artificially for many years (4-15 years mean 10,2years). The infertility treatment with intramuscular injection Human Chorionic Gonadotrophin (HCG)/Human Menopausal Gonadotrophin (HMG) and subcutaneous GnRH battery - driven pump lasted 6-24month (mean 10.3). We compared the results of: serum testosterone, and gonadotrophins, semen analyses, measurements of testic-
nal volume and testicular biopsy before and during GnRH therapy.

Result: Whith testosterone replacement therapy which lasted for many years patients from Tanner’s stage 1 go to stage 4/5. So before the GnRH treatment was started, the patients were Tanner’s stage 4/5. During the treatment period of 6-24 month testosterone rose from 1.4nmol/l to 8.5 LH from 1, 5 i.u/l to 4, 6 i.u/l and FSH from 1, 4 i.u/l to 3.8 i.u/l. Testicular volume increased from 0.4ccm to 12.3ccm. The first spermatozoid appears 6 month (the earliest) and the latest at 24 month (mean 12 – 15 months) of the treatment. At the end of the study 11 of 16 patients become fathers.

Conclusion: Deficiency of hormone is healing easy and quick. The treatment of the infertility by stimulating the spermatogenesis whith (HCG) / (HMG) protocol of patients with (HH) is possible and sucessful. About 60% of patient’s wife become pregnant. So we can say that the men with hypogonadotrophic hypogonadism can be fathers with support of their medical team. Keywords: delayed puberty, induced puberty artificially, male infertility, fatherhood.

Immuinoisolation, culture and cryopreservation as methods for improvement of adrenal gland xenograft functioning

ESC-ID 441
Author Deng B. Dudetskaya GV, Alabedalkarim NM
Country Ukraine
University Institute for problems of Cryobiology and Cryomedicine, National Academy of Science
Department Neuro-Humoral Regulation

As compared the oral hormone substitution the transplantation of adrenal cortex tissue provides major advantages for the treatment of adrenal insufficiency. However, grafting of xenogeneic adrenal tissue fragments to adrenalectomized animals resulted in a limited survival time due to graft rejection. One strategy for prolongation of adrenal graft survival is to decrease the immunogenicity of graft by different ways: i) selective elimination of passenger immunocompetent cells from the graft through culture either cryopreservation or ii) limitation of donor-recipient immune cell interactions through immunoisolation. We compared an effectiveness of both methods applying them either separately or jointly. Porcine small intestine submucosa (SIS) prepared by Badylak S. F method was used as immunoisolating scaffold. Newborn piglet adrenal gland fragments were transplanted under kidney capsule to bilaterally adrenalectomized (A/E) rats in the following combinations: 1) 2 days organ culture (OC); 2) SIS encapsulated organ culture (OC&SIS); 3) cultured into SIS covering organ culture (SISOC); 4) fragments cryopreserved with slow cooling rate freezing (Cryo); 5) 2 days culture of cryopreserved fragments (CryoOC); 6) SIS encapsulated 2 days culture of cryopreserved fragments (Cryo&SIS); 7) 2 days culture into SIS covering of cryopreserved fragments (CryoSISOC). On 31st day after surgery rats were anesthetized with ether inhalation and blood was taken by cardiac puncture. Hydrocortisone and aldosterone blood levels were measured utilizing a commercial radioimmunoassay. Hematoxylin – eosin staining was performed for 7 mm paraffin-embedded sections of recipient’s kidneys with graft. After surgery 60 percent A/E rats died (median 21 days, range 4-26 days). Survival of adrenalectomized animals was significantly improved by grafting of xenogeneic adrenals. Transplantation of OC, OC&SIS, Cryo and CryoOC grafts resulted in rising of rat survival to 66 percents. Survival of rats bearing Cryo&SIS and CryoSISOC grafts amounted to 75 percents. All recipients with SISOC graft survived throughout the time experiment. It is known, that down-regulation of CYP21a hydroxase in rodent adrenals is responsible for high corticosterone and negligible cortisol levels in peripheral blood plasma of rats. We didn’t find the significant differences in the cortisol level between control and A/E rats (near 6-9 nM/ml), but aldosterone concentration declined from 37 to 1.06 pg/ml after adrenalectomy. Neither graft types fully compensated mineralocorticoid insufficiency of A/E rats, although recipients of SISOC and CryoSISOC grafts partial recovered the aldosterone level to 3 pg/ml. Significant increasing of cortisol level was displayed in plasma A/E rats with CryoOC and CryoSISOC grafts to 16.86 and 24.63 nM/ml, correspondingly. Histological examination illustrated that alone cryopreservation or culture of adrenal fragments did not improve the integrity of adrenal xenografts. Not preventing graft damage the SIS encapsulation of cryopreserved either non-frozen material provoked the reduction of cortisol level in recipients. However, when native or cryopreserved adrenal fragments were cultured into SIS capsule before transplantation, recipients demonstrated long time survival, keeping graft integrity and high cortisol plasma level.

Cryopreserved adrenal xenografts: Immune and hormonal aspects

ESC-ID 456
Author Ustichenko VD, Alabedalkarim NM
Country Ukraine
University Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Science of Ukraine
Department Neuro-Humoral Regulation

Wide clinical application of endocrine cell transplants is limited by relatively short time graft functioning in recipients. We investigated a possibility to improve the efficiency of porcine adrenocortical grafts by cryopreservation with different cooling rate of freezing. The success of transplantations was estimated by the ability to compensate adrenal insufficiency in unilateral adrenalectomized mice. Donor adrenal glands were taken from newborn piglets, minced on fragments and cryopreserved with 1 degree per min (slow) either 100-degree per min (fast) cooling rates with application of 10% dimexide. Before transplantation under kidney capsule cryopreserved adrenal fragments were cultured 48 hours at 24 degree. Immediately after adrenalectomy the following types of grafts were transplanted: fresh fragments (Nat), dimexite treated fresh fragments (DM), cryopreserved fragments – SlowCul and FastCul. On 30th day after surgery mice were sacrificed and blood plasma aliquots were taken for cortisol RIA measurement. Immune status of recipients was estimated through peritoneal macrophages ability to adhesion, phagocytosis and killing of St. aureus. Besides, hematoxylin–eosin staining was performed for paraffin-embedded sections of recipient’s kidneys with graft. We found that the transplantation of Nat graft led to raising plasma cortisol level but it was accompanied by the activation of recipients
PM as a sign of inflammation. In vitro data were confirmed by histology on the presence both rejected adrenal tissue and functioning adenocortical cells in graft area. According to (Wingenfeld C, 2002) the dimexide treatment stimulated angiogenesis and immune tolerance of murine osteochondral grafts. We found the high efficiency of DM graft in the compensation of hypocorticism similarly Nat grafts. Keeping phagocytic and adhesive activity the PM partial lost the ability to kill St. aureus in the case of grafting DM grafts. Unfortunately, the cryopreservation resulted in declining cortisol compensation of grafts, but PM of mice bearing cryopreserved grafts displayed the fall of killing ability. Besides, fast graft had some immunological advantages appearing in phagocytosis suppression and the normalization of adhesive ability. Culture of cryopreserved fragments improved their cortisol production at transplantation. Histology confirmed maintenance of integrity of these grafts closely Nat and DM grafts. However, positive effect of cryopreservation expressed as the inhibition of killing by PM was revealed only for FastCul grafts. Thus, our data showed that the efficiency of xenografts depends on the graft functional activity and the acuteness of immune response. We found before that the treatment of adenocysts by dimexide increased cortisol secretion in vitro. Early stages of necrosis or apoptosis in damaged during freezing adenocysts could stimulate cortisol secretion also. Probably, in first days after grafting the local release of glucocorticoid from transplanted adenocysts took place. It resulted in long-term immunological consequences, which have been exerted in the inability of recipient PM to kill phagocytic particles. Culture of cryopreserved grafts significantly improved hormone producing features of transplants and, apparently, kept immune advantages of cryopreserved grafts.

**Internal Medicine**

**Haematology and Oncology I**

**Alterations in distant oral mucosa and buccal epithelium in patients with OSCC and with epidemiological risk**

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<th>ESC-ID</th>
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<tr>
<td>Author</td>
<td>Osadcha O, Kirieieva S, Yurchenko N</td>
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<td>Taras Shevchenko National University</td>
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Oral squamous cell carcinoma (OSCC) with a relatively low survival rate has a tendency to expand in people population. The analytical epidemiology associates OSCC with long-term smoking tobacco, being alcohol, exposure to various carcinogens and genetic predisposition to carcinogens. One approach to overcoming this problem is non-invasive technology for mass screening and monitoring of individuals with risk of oral cancer. The aim of study is to perform comparative investigation of the state of epithelium in tumor-distant oral mucosa and buccal epithelium in patients with OSCC and in patients with risk of oral cancer. Biopsy specimens of tumor-distant mucosa, and buccal scrapes were taken from 16 patients with OSCC. Buccal scrapes of patients with risk of oral cancer were studied in 30 volunteers (smoking-drinking status) living in the 10 km area from Rivne NPS. Buccal scrapes from 10 healthy males (age 19 years, nonsmokers, nondrinkers) were used as controls. The state of epithelium in tumor-distant mucosa and buccal mucosa in patients with OSCC and with risk of oral cancer were analyzed taking into account the age, social-demographic parameters, smoking-drinking status, clinical stage, tumor stage, grade invasions, p53 status, proliferation index, apoptotic index, DNA content. p53 staining was performed with monoclonal anti-p53 antibody. The cells in S-phase were detected by monoclonal anti-BrdUrd-antibody. The cells in apoptosis detected by Hoechst 33342. DNA content detected by density Schiff’s Feulgen stain. Buccal scrapes were stained by Papanicolaou (Pap-test). Epitheliocytes were estimated by coloring of cytoplasm, by the dimension of nucleus, the structure of chromatin, DNA content and differentiation index (DI). The analysis of the tumor-distant mucosa epithelium and buccal epithelium has been revealed absence of normal epithelium in patients with OSCC. In tumor-distant mucosa detected progression of the histopathological phenotype from fields hyperplastic to dysplastic epithelium to Ca in situ with expression of p53. Pap-test in buccal epithelium from both patients with OSCC and patients with risk of oral cancer (43.33 %) detected similar disturbance in differentiation and maturation of epitheliocytes, presence of cells conglomerates and fields with predominant parabasal and intermediate cells and appearance of basal cells (2-10 %), significant decrease of DI and increase DNA content. Revealed similar cytology alterations in buccal epithelium may be used in serial cytological assay of exfoliated cells for screening and monitoring of individuals with risk of oral cancer. Individuals with revealed such alteration may be defined more correctly by molecular diagnostic. This technology is easy reproducible, economical and non-invasive.

**Evaluation of factors influencing colorectal cancer survival**

ESC-ID 278

Author Borys M, Knut K, Siepia M, Wierzbka K, Wozniacki P

Country Poland

University Medical University of Gdansk

Department Surgical Oncology

Colorectal cancer is one of the major health problems worldwide. It is the 4th most common malignant tumor with an estimated 1023152 new cases diagnosed in 2002, the most recent year for which international estimates are available. Colorectal cancer accounts to 10% of overall morbidity of malignant diseases worldwide – both in men (550465) and in women (472687). Morbidity of colorectal cancer has been increasing rapidly since 1975. It is estimated that more than half million deaths from colorectal cancer still occur worldwide annually. This is the 2nd most common cause of death from any malignant disease in men in the EU. There are essential differences in colorectal cancer survival among patients. This variation could be related to several factors including: stage of disease when it is discovered, treatment delivery, localization of primary lesion, presence of metastasis, and many others. The main objective of our study is to find and estimate factors that can influence survival in colorectal cancer. The research was performed on the group of 240 patients treated at Surgical Oncology Department in
MUG, Poland. There were 137 men and 103 women. Average age was 64.3 and it ranged from 31 to 87. Average observation period was 1512 days. During this time 135 people died. In this group average survival was 884 days. In our study group cancer was most commonly localized in rectum (28%) and sigmoid colon (27%). In TNM scale 45% of resected tumors were estimated as T3 and 33% as T4. In 110 cases cancer cells were found in resected regional lymph nodes. In 30 patients distant metastasis were found at the time of diagnosis. Usually disease was diagnosed at stages II/III (73%). Most commonly used surgery methods were right hemicolectomy and sigmoidectomy. Our study has revealed that patient’s survival is correlated mainly with 3 factors: staging (p = 0.013), grading (p = 0.02) and feature N (p = 0.039). Death was highly correlated with feature M (p <0.001), feature N (p <0.001) and staging (p <0.001). Research performed on this group has also revealed that localization of primary lesion was highly correlated with the depth of invasion of the intestinal wall (T) (p <0.001). Statistically the greatest invasion was found in ascending colon. Experience of the operating surgeon did not reach statistical significance for survival in the evaluated group. Concluding, if we want to extend the survival in colorectal cancer we must put more stress on earlier diagnosis. Stage of the disease at treatment delivery is the only factor influencing the survival that can be altered by the medical professionals. The only possible method to achieve it is screening. We have also revealed that cancers located in ascending colon were usually bigger than in other localizations. We think that this correlation comes from the fact that symptoms of these cancers most commonly are late and not specific enough to alarm the patient on time.

Breakthrough pain in cancer patients – Documentation system and clinical management

ESC-ID 830
Author Knut K, Gaertner J, Ostgathe C
Country Germany
University University of Cologne, Faculty of Medicine
Department Klinik und Poliklinik fur Palliative Medicine

Background: Breakthrough pain (BP) is commonly known as a factor exacerbating many cancer syndromes. Despite its importance, only few studies determined clinical management of BP. Moreover, any validated documentary tools has been published yet.

Aim: Aim of the study is to estimate the efficacy of documentation system targeting breakthrough pain and to compare profile of cancer pain patients receiving 3rd step opioid treatment in accordance to WHO guidelines.

Material and methods: The retrospective survey of random sample of cancer pain patients treated in our institution was performed. Based on the questionnaire which we had developed, data from routine inpatients &8821; documentation were assessed. Evaluated data included the efficacy and side effect profile of opioid medication, pain intensity and pathophysiology and type with profile of performed clinical alterations. Qualitative analysis (e.g. pain brief description) was added together to quantitative assessment, to find and identify deficiencies in the documentation system.

Results: Of 58 patients included, 29 L-Methadone patients and 17 patients with other opioids were analyzed (in 100% of included pts we could define BP). 375 episodes (EP) of BP were identified, median 2.4 EP/pts/day. The end-of-dose failure occurred in 28% of EP. We identified BP as being movement-related (MR) or not MR in 35% (n = 131) of the EP. Of these 131 EP, 58% were MR. The pain pathophysiology could be identified in 23% of EP (somatic 60%, visceral 27%, neuropathic 10% and mixed 3%). Pain intensity (NRS) was documented in 33% of the EP (n = 125). The average NRS at the beginning of the EP was 5.8 (range 2-10); it was never reported at the end of EP. Insufficiency in the first dose of rescue medication occurred in 16% of EP; the second dose was administered after an average of one hour (median 54 min, range 15-150). In 13% of the EP adverse effects related to rescue medication could be identified (drowsiness 55%, nausea 28%, tiredness 20%, myoclonus 2%). In 11% of the EP (29% one alteration, 52% two alterations, 19% three alterations), an alteration to accurate treatment was required to achieve sufficient pain management (dose escalation 27%, opioid switch 39%, change of application route 34%).

Conclusion: The alteration to the analgesic therapy and rescue medication was required in majority of BP patients. Though symptom assessment and documentation in our department follows international recommendations (MIDOS), routine documentation provided insufficient information to evaluate incidence of BP, or efficacy and safety of analgesic treatment of BP. Descriptive analysis of BP episodes demonstrates multidimensional nature of the phenomenon. Despite the significant impact of BP for the quality of life of pain patients, no validated documentation system to assess BP characteristics, or efficacy and safety of its treatment for neither clinical use nor for the conduction of interventional studies has been presented yet. Our working group considers the development of a standardized documentation system to improve the analgesic interventions targeting BP in the future. Supported by the Koeln Fortune Program / Faculty of Medicine, University of Cologne

LCIS/ALH without additional high-risk changes seen in mammmotomic biopsy

ESC-ID 323
Author Polom W, Polom K, Murawa D
Country Poland
University University of Medical Sciences Poznan
Department 1st Surgical Oncology Dept. WCO Poznan

Aim: Atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) are generally seen as risk factors for invasive ductal carcinoma or lobular carcinoma of the breast. The purpose of this study was to determine how to proceed clinically in the case of patients with diagnosis of primary LCIS or ALH by mammotomic biopsy.

Method: The materials assessed for LCIS or ALH without accompanying invasive changes came from 4236 mammalsotomic biopsies taken at the Department of Surgery of the Wielkopolska Oncology Centre in Poznan, Poland. Patients after core biopsy with a suspicion at mammography were treated surgically, while others are under the care of oncologists for observation. LCIS was diagnosed in 16 cases, while ALH was seen in 17.
Results: Of 9 patients treated surgically for LCIS, 2 women had invasive ductal carcinoma, 2 had lobular carcinoma, and 1 was shown to have ductal carcinoma in situ (DCIS). These patients radiologic studies indicated that 2 female patients had masses, 2 had radial scars, and one had microcalcifications. Seven (44%) patients with LCIS but no radiological findings suggestive of invasion were placed only under the care of an oncologist. Over a minimum period of 22 months, further changes were not indicated by radiologic studies in these women. Among 11 patients with ALH that underwent surgical treatment, post-operative follow-up showed that 4 had invasive ductal carcinoma and 1 had DCIS. Radiologic studies in these women demonstrated that 2 patients had masses, 1 had thickening, and two had microcalcifications. In the ALH group, 5 (29%) patients were not treated surgically but were under the care of an oncologist. Over a period of more than 2 years, no changes were noted in these patients. Conclusion: Invasive carcinoma or DCIS were determined in 31.25% patients who were diagnosed with LCIS on the basis of mammotomic biopsy, while 29.4% of the patients diagnosed with ALH proved to have invasive carcinoma or DCIS. Treatment by radical surgery seems to be necessary in those situations in which radiological studies indicate suspicious changes: masses or changes in tissue architecture.

Awareness and participation of polish and German society in colorectal screening. Comparative study

Introduction: Colorectal cancer (CRC) is the third most common type of cancer in men and women. The screening tests at the age above 50 are: a fecal occult blood test (FOBT) performed once a year, colonoscopy or sigmoidoscopy performed every 5-10 years.

Aim of the study: The survey was performed to assess the difference in knowledge and clinical approach to screening tests among polish and German patients.

Material and method: 45 patients with diagnosed CRC treated in Surgical Oncology Clinic of Medical University of Gdansk and 25 patients treated in the Clinic of Intern Medicine I in the University of Cologne and in the Clinic of Visceral Surgery in St. Hildegardis hospital in Cologne were enrolled in the comparative study. Clinical data were obtained from medical documentation and own questionnaire. Program Statistica 6.0 was used for statistical analysis.

Results: In the study group from Poland in 6 patients screening tests were performed before the admission (13.3%), 14 patients (31.11%) were aware how to detect CRC and 11 patients (24.4%) knew how to prevent CRC disease. Only 3 (6.6%) patients were aware of screening test. In the study group from Germany 8 patients underwent screening (32%), 12 patients (48%) were aware how to detect CRC and 11 patients (44%) knew how to prevent CRC disease. Only 4 (16%) patients were aware of screening tests. The difference in amount of screening tests performed in Poland and Germany is not statistically significant (p=0.0651). The difference in the awareness of screening tests has no statistical importance (p>0.05). Median time from the onset of the symptoms to the treatment was 97 days in polish group, in German it was 41 days. Median time from the diagnosis to the surgical treatment was 40 days in Poland and 11 days in Germany. In polish group, the most frequent screening test was colonoscopy 66% then FOBT 33% while in German the most frequent was FOBT 87.5% and then colonoscopy 25%.

Conclusion: The results demonstrate that in both groups of patients with CRC the awareness of screening is low. Although some patients in both groups were aware how to detect CRC and knew how to prevent CRC, this knowledge didn’t result in participation in screening tests program. However, the larger amount of people undergoing CRC screening, and the shorter time from the onset of the symptoms, and diagnosis to the treatment in Germany are the effects of the superiority of german health system. Finally, to achieve better results in participation in CRC screening more emphasis on educational programs is required.

Immune reconstitution in patients with autologous bone marrow transplantation and correlation with the multi pre-transplant chemotherapy

ESC-ID 362
Author Roy AK, Verma A, Singhal G, Imtiaz MA, Maheshwari S
Country Romania
University University of Medicine and Pharmacy Timisoara
Department Transplant

Introduction: Autologous bone marrow transplantation (ABMT) after high dose myeloablative chemotherapy remains one of the main treatment option for some diseases like poor prognosis lymphomas, some solid tumors as well as selected cases which lack a suitable matched donor. The ability of stem cells to reconstitute the blood elements varies from patient to patient due to various factors. Immune reconstitution is characterized by a recapitulation of the normal lymphoid ontogeny i.e. CD3+, CD19+ as well as CD3-CD56+CD16+ (NK cells). The reconstitution of immunologically competent cell population as well as the hematological reconstitution play a major role in the outcome of the ABMT.

Materials and Methods: We have followed prospectively the recovery of immune cells in 20 (n = 20) patients who underwent ABMT during 2001 to 2006, for Hodgkin Lymphoma-13, Non Hodgkin Lymphoma-2, Ewing Sarcoma-2, ALL-1, Neuroblastoma-1, and multiple myeloma-1 in the Bone Marrow Transplantation Center, Timisoara, Romania. The evolution was assessed at 30, 100, 180, 270, 365, 455, 575 and 760 days. Immunophenotyping was done from the peripheral blood with the help of flowcytometry. Correlation between treatment prior to ABMT and outcome of the immune reconstitution was also evaluated.

Results: Flowcytometry analysis of immune system revealed a severe global immune depression in the first 30 post transplant days with values of CD19+, CD3+ and CD4+ lymphocyte sub-populations remained <30% of the normal for the age, while the CD8+ and NK cells values reached upto 50%. In the following periods all the lymphocyte sub-populations showed a dramatic accentuation but still remaining lower than that of the age specific normal values. Dynamics of CD19+ and CD8+ cells are exceptions as CD19+ cells start-
ed to normalize by 3rd month while the CD8+ sub population presented with supra normal values in first 270 days in most of the patients, which normalized later in the follow up. This pattern of immune reconstitution in the studied group led to a constant reversal of the normal CD4+/CD8+ ratio.

Conclusion/Discussion: Immune reconstitution is found to be a continuous process through out the follow up. The global immune reconstitution in the studied group was poor and prolonged as compared to the results found in literature probably due to the intensive and prolonged treatment of our study group prior to ABMT. Implication: Knowledge of immune reconstruction may guide the clinicians in devising strategies to minimize the risk of post ABMT infections and complications.

Yersinia enterocolitica infection in the early period after matched sibling donor bone marrow transplantation – a case report

ESC-ID 625
Country Poland
University University of Medical Sciences in Poznan
Department Pediatric Oncology, Hematology and Transplantology

Introduction: In healthy persons Yersinia enterocolitica infections are infrequent, but responsible for 46% of all cases of clinical sepsis related to erythrocytes transfusion with fatality rate higher than 60%. After acute phase of enteritis Yersinia enterocolitica may persist for years in mucosal, sub-mucosal and lymphoid tissue and peripheral blood leukocytes from where can give a rise of symptomatic or cryptic bacteriemia. Recipients of allogeneic HSCT seem to be especially exposed to food-borne infections, reactivation of persisted endogenous infection, and transmission of infection with contaminated bone marrow or peripheral blood stem cells, and/or with unavoidable erythrocytes transfusions.

Case report: 11-year-old girl with AML in 2.CR underwent an allogeneic BMT from 13 year-old HLA matched brother. Patient was IgM (+) against HSV and EBV, IgG (+) against CMV, VZV and Toxoplasma. Donor was IgG (+) against HSV, EBV, CMV, VZV, Toxoplasma, and did not report any previous diseases. Conditioning for BMT consisted of busulfan and cyclophosphamide. For GvHD prevention CsA was given. On day “0” patient obtained 2.4x10^8 BM-NC/kg without any adverse reaction. However, due to infections observed short before BMT (sinusitis, bronchopneumonia) plus fever and elevated CRP observed during conditioning, antibiotics were given from day -7 to + 5. Cultures of transplant material were negative. Engraftment was achieved on day +15. From day +7 to +16 patient demonstrated septic fever and increasing CRP. However, all blood cultures were negative. Fever disappeared and CRP normalized after treatment with teicoplanin, piperacillin+tazobactam, TMP/SMZ, and liposomal amphotericin B, and patient was discharged on day +35 in good performance status. On day +85 despite of empirical therapy, worsening of patient’s general status with fever, bloody diarrhea with fragments of necrotic intestinal mucosa and increasing CRP occurred. Evaluation of blood revealed high, increasing titers of antibodies against Yersinia enterocolitica, which decreased during treatment with TMP/SMZ, cefepim, and aminoglycoside. On day +141 stool started to normalize. During investigation of patient’s family, high titers of IgG antibodies against Yersinia enterocolitica were found in donor of bone marrow, sister, and in father of patient. Patient left the hospital on day +183 in good general status without any complains, and with excellent hematological findings.

Conclusion: Serum antibody titers against Yersinia enterocolitica should be determined in recipients and donors of HSCT as well as in donors of blood products for HSCT recipients. Yersinia enterocolitica infection should be taken into consideration when making differential diagnosis of diarrhea in recipients of allogeneic HSCT.

Assay of sialic acid concentration in melanoma development dynamics

ESC-ID 662
Author Bobocea AC, Fertig TE, Mologhianu AA
Country Romania
University "Carol Davila" University of Medicine and Pharmacy, Bucharest
Department Faculty of Medicine, Department of Oncology

Background: Melanoma is mostly a fatal disease because of its rapid progression, outstanding aggressiveness and metastatic capacity. The incidence is continuously growing worldwide suggesting a public health problem. The lack of efficient treatment in advanced-stage melanoma stimulates the quest for more reliable markers to detect and stage melanoma. Sialic acid is an important factor that plays a key role in adhesion between cancer cells and endothelium, facilitating invasivity and metastatic potential.

Aim: The present research identifies variables that correlate lesional aspects and functional consequences to tumor progression and increased metastatic capacity at melanoma induced at mice. These laboratory study results complete pre-existent data and define models that can be used to predict human melanoma.

Method: C57BL/6 male mice specific pathogen-free at the ages of 8–12 weeks were used. Animal care and experimentation was carried out in accordance with general guidelines. Reference melanoma cell-lines B16, F1, F10 and transfected B16-F10, a highly metastatic subclone of B16-melanoma, were injected subcutaneously at mice to establish experimental tumor. Tumor growth was assessed by volume determination, assuming ellipsoidal growth. Survival time, survival comparison and tumor volume differences between groups were analyzed using statistical software. Cumulative curves were generated and compared to evaluate the potential impact.

Results: In preliminary experiments, mice receiving smaller numbers of tumor cells failed to exhibit lung metastasis. Three independent survival experiments were carried out comparing groups of 10 animals. After mice died, tumors and peripheral lymph nodes (inguinal, axillary, and brachial) were excised and analyzed counting the metastatic colonies. These results correlated with biochemical parameters such as sialic
acid. Sialic acid levels in the examined groups were significantly (p <0.001) raised. The survival analysis indicated that the elevated level predicts shorter survival. Histopathological studies also correlated with these above results. Highly metastatic B16-F10 melanoma cells, as compared with the low metastatic B16-F1 line, had higher level of sialic acid. Its expression in tumor cells associated increased invasive potential and contributed to metastasis. Sialic acid concentrations correlated with stage of disease, recurrence and degree of metastasis.

**Conclusion:** The data obtained in this study sustains that determination of sialic acid is useful for prognosis, therapy monitoring and prediction, rather than for early detection or screening. This marker has high specificity, appropriate sensitivity and proved to be significant prognostic factor and is well known to provide useful information to the clinicians. The assessment may be useful in evaluating clinical outcome at patients with melanoma undergoing targeted anti-cancer immunotherapy. New research may open perspectives for specific therapeutic targets of melanoma.

**P15 INK4B gene promoter methylation status in healthy population in Iran**

**ESC-ID** 409  
**Author** Yeganeh O, Ghaffari SH, Alimoghaddam K, Rostami S, Chahardovali B, Ghavamzadeh A  
**Country** Iran  
**University** University of Tehran  
**Department** Medicine

**Background:** In addition to deletion and point mutation, promoter region CpG island hypermethylation is one of the genes silencing ways. P15INK4B is a tumor suppressor gene that inhibits cyclin-dependent kinase (CDK) 4 and 6 in cell cycle. Recent studies have shown that P15INK4B gene is inactivated by 5’ CpG island methylation of the promoter region in some malignancies specially Leukemias, but there is no study in Iran to evaluate the methylation status of this gene’s promoter region in healthy population. The aim of this study was to evaluate methylation status of P15INK4B gene promoter in peripheral blood mononuclear cells (MNCs) of Iranian healthy population.

**Materials and Methods:** peripheral blood MNCs’ DNA was isolated from 20 healthy volunteers by salting out method. The samples were treated by sodium bisulphite and methylation status of the region was evaluated by Methylation Specific PCR (MS-PCR).indeed, the methylation status of P15INK4B promoter region in 5 Acute Promyeloblastic Leukemia (APL) patients was studied by this method as a pilot study.

**Results:** MS-PCR showed that none of the normal samples were treated by sodium bisulphite and methylation status of the region was evaluated by Methylation Specific PCR (MS-PCR).indeed, the methylation status of P15INK4B promoter region in 5 Acute Promyeloblastic Leukemia (APL) patients was studied by this method as a pilot study.

**Conclusion:** This study shows that the promoter region of P15INK4B gene of blood mononuclear cells from normal healthy individuals is not methylated and any methylation during malignancies is considered to be abnormal for this gene and may promote cancerogenesis. This is supported by our results on APL patients in the pilot study. Our next step is evaluation of this gene’s methylation status and its prognostic significance in APL and other leukemias.

**SNP-array-based detection of the minimally delineated region of chromosome 5q in patients with MDS**

**ESC-ID** 955  
**Author** Dunbar A, Gondek L, McDevitt M, Batista D, Jankowska A, Jiang Y, Maciejewski JP  
**Country** United States of America  
**University** Vanderbilt University  
**Department** Biology

**Introduction:** In myelodysplastic syndromes (MDS), chromosomal abnormalities have important clinical implications; however, defects can be found in only 50% of cases. We hypothesize that chromosomal lesions are present in a majority of MDS patients but remain undetectable due to the limited resolution of conventional metaphase cytogenetics (MC). Identification of cryptic defects on chromosome 5q using 250k single nucleotide polymorphism array (SNP-A) technology, together with its high resolution in regard to chromosomal topography may allow for a more exact delineation of commonly deleted regions (CDRs) and thus point towards pathogenic genes.

**Methods:** Using MC, we analyzed a cohort of 512 MDS patients and 51 healthy controls. Bone marrow DNA was available from 189 of the 512 individuals for SNP-A analysis. After SNP-A, positive results were validated by PCR-based microsatellite analysis, copy number determinations, and fluorescence in situ hybridization (FISH). The results of SNP-A karyotyping were also correlated with clinical parameters, including bone marrow morphology and routine MC.

**Results:** Using conventional metaphase karyotyping, 15% of the 512 patients showed a deletion of 5q (N = 76). These patients varied broadly by MDS sub-type. Because SNP-A allows for the detection of micro-deletions and copy-neutral loss of heterozygosity (LOH) (acquired uni-parental disomy), SNP-A was able to detect 5q defects in 27/189 patients as opposed to 20/189 patients as observed using MC. In addition, we also identified a new minimally deleted region on 5q that correlates highly with results published previously. This region harbors candidate tumor suppressors that could have implications in disease pathogenesis, most notably EGR1 and CTNNAl. In addition, we have identified other CDRs of interest on chromosome 5q that could also be involved in disease etiology.

**Discussion:** Identification of the minimally deleted region associated with del(5q) is a widely-reported topic in MDS research; however, newer techniques with higher resolution, such as the 250k SNP array, have not been applied. In doing so, we have found 27/189 patients with lesions on 5q—seven of which were previously undetectable by conventional MC. While the CDR defined here supports those previously reported, several other regions of interest on 5q have been identified. Detection of a CDR could have large therapeutic implications. Already, Lenalidomide, a novel immunomodulatory drug, has been shown to have a high response rate in MDS patients with 5q lesions, yet little is known about its mechanism of action. By isolating these minimally deleted regions, we can locate potential tumor suppressors that may be involved in disease etiology. To date, no inactivating mutations have been found on chromosome 5q; however, gene dosage effects or the presence of other non-synonomous SNPs or SNPs located in regulatory regions of gene promoters which predispose one to the disease cannot be ruled out.
Currently, we are performing genotyping analysis to identify such SNPs so that we may gain further insight into the pathophysiology of this complex disease.

**Molecular-biological features of homocysteine-induce drug resistance**

ESC-ID 703  
Author Mykytenko DO, Lukyanova NY, Chekhun VF  
Country Ukraine  
University Medical University  
Department Institute of Exp. Oncology

It is known that epigenetic aberrations, in particular altered methylation of DNA, play an important role in the acquirement of phenotype of drug resistance by malignant tumors. Homocysteine, an amino acid which elevated level has been registered in some cancers is known as a natural factor involved in altered process of methylation. It is shown that the cultivation of ovarian cancer cells of A2780 line and human breast cancer cells of MCF-7 line with homocysteine leads to their decreased sensitivity to cisplatine and doxorubicine. Immunophenotype patterns of A2780 cells cultivated with homocysteine are characterized by elevated activity of mdr-dependent, detoxication (GSTp), antiapoptotic (Bcl-2) systems, and these events are accompanied by hypermethylation of the genes – regulators of apoptosis (tp53, p73 and bcl-2), hypomethylation of promoters of the genes of drug resistance (mdr1, GSTp), and molecule of intercellular adhesion E-cadherin. The cultivation of MCF-7 cells with homocysteine results in increased activity of mdr-dependent, detoxication (GSTp) systems and decreased activity of antiapoptotic (Bcl-2) systems that are in correlation with the respective epigenetic aberrations - hypomethylation of promoters of the genes of drug resistance (mdr1, GSTp) and hypermethylation of the genes – regulators of apoptosis (tp53, p73 and bcl-2) and E-cadherin. So, an aberrated structure of DNA methylation is one of the primary events in initiation of signal cascades of drug resistance and is among leading mechanisms of regulation of activity of defense systems of ovarian cancer cells and human breast cancer cells upon elevated level of homocysteine in the system in vitro.

**Characterisation of apoptotic responses in a pituitary derived cell line**

ESC-ID 727  
Author Hasan Y, Farrell W, Shut S  
Country United Kingdom  
University University of Keele  
Department Institute for Science and Technology in Medicine

**Introduction:** Pituitary tumours are the most common intracranial neoplasms. They can be of significant morbidity both through mass effect and the inappropriate secretion of endogenous hormones. A novel pro-apoptotic mediator gene has been recently identified in pituitary cell lines through the technique of differential methylation and epigenetic silencing. The gene was named the Pituitary Tumour Apoptosis Gene (PTAG) for its role in the apoptosis pathway, and it has been shown to be underexpressed in most pituitary adenomas. Epigenetic mechanisms potentially play a central role in tumourogenesis through different mechanisms including methylation and histone modification. Methylation of the promoter region of PTAG leads to silencing of the gene, which was found to cause oncogenic transformation in 20% of pituitary adenomas. Other mechanisms than methylation are involved in other cases too. While genetic alteration is usually irreversible, epigenetic methylation is potentially reversible and provides the possibility for future treatment interventions.

**Aims:** The aims of this research are: To determine expression of PTAG at different time intervals post-exposure to the drug bromocriptine, which promotes apoptosis in pituitary cell lines. To study the level of promoter region activity and examine the number of apoptosis cells. To make a correlation between the level of PTAG expression, its promoter activity, and the number of apoptotic cells.

**Methods:** The rat pituitary tumour cell line, GH3 (Growth Hormone secreting), was used for this study. Cells were divided into two categories; one treated with bromocriptine while the other acted as untreated controls. Growth was stopped at: 30 minutes, 1hr, 2hrs, 4hrs, 8hrs, 12hrs, 24hrs, and 48hrs. All samples underwent RNA extraction, complementary DNA synthesis, and polymerase chain amplification (PCR). Specimens were examined for the presence of specific cDNA amplicons by electrophoresis, before employing quantitative Real Time PCR (qRT-PCR) to determine the level of PTAG expression. Pituitary tumour cell lines were transfected with a range of PTAG promoter-reporter (luciferase) constructs and co-transfected with a renilla construct to correct for transfection efficiency. Reporter activity was determined through chemiluminescence. The number of apoptotic cells was examined by Hoechst 3342 staining and microscopy.

**Results:** Since the project is still half-way through, results are still awaited and have not been finalised or analysed yet. Results will be ready for analysis by the end of July when the research period comes to an end. The enhanced understanding of gene regulation and expression and its correlation with the promoter activity may shed the light on future treatment options.

**Epstein-Barr virus presence in Hodgkin’s lymphoma cases and its relation to treatment response**

ESC-ID 493  
Author Nedeljkov A, Karan R, Nedeljkov R  
Country Serbia  
University University of Belgrade  
Department Hematology

**Aim:** The EBV-encoded latent membrane protein-1 (LMP-1) has been involved in neoplastic transformation in around 40% of classical Hodgkin’s lymphoma (HL) cases and this association is believed to be causal. Classical HL that contain the EBV genome may differ etiologically from EBV-negative HL tumors.

**Methods:** We have examined 96 cases of HL newly diagnosed in Institute of Hematology Clinical Center Belgrade. We used immunohistochemistry to detect LMP-1 in the Reed-Sternberg cells. The study was conducted at the Department of Pathology from 2001 to 2005.

**Results:** LMP-1 was detected in 44.8% of our cases: high
EBV association is found at the peak in older adults predominantly with mixed cellularity type. The EBV association was independently affected by histologic subtype (79.16% in mixed cellularity and 33.3% in nodular sclerosis), sex (74% in males and 31.25% in females), and age (77% in patients aged 40 years and older and 38% in patients younger than 40 years of age). Most patients presented with stage I (31.32%) or II (53.12%). The patients were treated with chemotherapy alone (54 pts), and with chemotherapy + radiotherapy (42 pts). 90% EBV-associated pts had achieved therapeutic response, with 80% CR and 20% PR. In the group of EBV-inflamed tissues.

Results: Mice systemically treated with OVA-alum show a rise of specific IgE antibodies after day 28. These IgE antibodies remain measurable in the serum. Additional OVA-aerosol treatment increases the specific IgE antibody titers and the number of IgE plasma cells in the spleen, showing comparable kinetics.

Conclusion: We could show that mucosal allergen challenge in sensitized mice boosts the specific IgE serum titers and also the number of systemically induced IgE plasma cells in the spleen. Allergen inhalation not only induces a local immune response, but also influences the systemic immune response.

Internal Medicine Immunology I

Mucosal allergen-challenge triggers the allergen-specific immune response and boosts IgE plasma cells

ESC-ID 770
Author Fokuhl V, Luger EO, Wegmann M, Achatz G, Renz H, Worm M, Radbruch A
Country Germany
University FU Berlin
Department Biology

Background: IgE antibodies play a major role in the pathogenesis of type I allergies like hay fever and allergic asthma. As the half-life of IgE antibodies is only 12 hours, IgE plasma cells constantly have to secrete large amounts of IgE to mount and maintain significant serum titers of allergen-specific IgE over long periods of time. It is not known, whether IgE-secreting plasma cells are short-lived products of a specific IgE over long periods of time. It is not known, whether IgE-secreting plasma cells are short-lived products of a chronic activation of B cells, or long-lived and capable of surviving for long time periods in the bone marrow or in inflamed tissues.

Methods: We analyzed the origin of allergen-specific plasma cells and the influence of repeated mucosal allergen challenge on the systemic immune response in an asthma mouse model. Female 4-6 weeks old BALB/c mice were sensitized with ovalbumin (OVA)-aerosol additionally every third day. To measure the impact of repeated aerosol treatment on the specific immune response, serum immunoglobulin titer (total and OVA-specific IgE) were measured by ELISA technology. Fluorescence microscopy of spleen cryosections was performed to localize IgE plasma cells and investigate their kinetics.

Results: Mice systemically treated with OVA-alum show a rise of specific IgE antibodies after day 28. These IgE antibodies remain measurable in the serum. Additional OVA-aerosol treatment increases the specific IgE antibody titers and the number of IgE plasma cells in the spleen, showing comparable kinetics.

Conclusion: We could show that mucosal allergen challenge in sensitized mice boosts the specific IgE serum titers and also the number of systemically induced IgE plasma cells in the spleen. Allergen inhalation not only induces a local immune response, but also influences the systemic immune response.

Frequency of allergy to fruit, vegetables and spices in patients with seasonal allergic rhinitis – study of 88 cases

ESC-ID 590
Author Ukleja N, Gawronksa-Ukleja E, Kuzminski A, Zbikowska-Gotz M, Bartuzi Z
Country Poland
University University of Mikolaj Kopernik in Torun
Department Collegium Medicum

Introduction: A common problem for doctors is coexistence of allergy for inhalatory allergens and fruit and vegetables. Many of patients also suffer from pathological symptoms after spiced food. The symptoms are gathered mainly with respiratory, but also digestive system (IgE-dependent mechanism). The reason can be cross-allergy for fruit, vegetables and spices or co-allergy.

The aim: The frequency of allergy to fruits, vegetables and spices in 88 patients with allergic rhinitis.

Material and methods: The research was carried out in 88 patients (46 F and 42 M) aged 9-62, treated in the Department and Clinic of Allergology, Immunology and Internal Medicine of Collegium Medicum, University of Mikolaj Kopernik in Torun because of seasonal allergic rhinitis. Every patient was interviewed for allergy history (using a questionnaire with full anonymity of patients), and had 1. Skin prick tests for 11 common inhalatory allergens and 15 food allergens using a set by Allergopharma 2. The level of total and specific IgE signified with an enzymatic method by Hycor Biomedical Technic EIA

Results: All of the patients suffered from seasonal allergic rhinitis. In 57 (64.8%) allergic family history was positive. Positive skin prick test for grass-pollen in 69 cases (78.4%), tree-pollen in 45 (51%), weed-pollen in 35 (40%); positive skin prick tests for fruit and vegetables in patients 28 (31.8%) – 19 patients for vegetables (21.6%) and 12 (13.6%) for fruits. Higher level of total IgE in 64 patients (72.7%); concentration of sIgE for inhalatory allergens was higher in 63 patients (grass-pollen – 45 (51%), tree-pollen – 35 (40%), weed-pollen – 12 (13.6%)); In 16 examined patients we also signified presence of sIgE for food allergens (vegetables – 11 (12.5%), fruit – 6 (6.8%)). At the same time a higher level of sIgE for inhalatory and food allergens appeared in 12 patients (13.6%) and simultaneous positive skin prick test for common inhalatory and food allergens in 28 patients (31.8%). Many patients in the interview complained of pathological symptoms after eating spices. In the group of 88 patients 12 (13.6%) had positive skin prick tests for spices (including curry, a very strong allergen) and 7(8%) of them had relevant sIgE level.

Conclusion: The results suggest necessity of diagnosing patients with seasonal allergic rhinitis for food allergy. 2. Although many patients complained of negative signs after eating spices, only 13.6% of them had positive skin prick tests for spices 3. An interesting problem would be to differ classical cross-reactivity from co-reactivity for food and inhalatory allergens, which would be possible with highly specific immunological methods.
A review of immunotherapy as a novel approach to cancer therapy

Most cancer patients are treated with some combination of surgery, radiation and chemotherapy. Radiation and chemotherapy have the disadvantage of destroying healthy as well as malignant cells and thus can cause severe side effects. Surgery can be used to remove the bulk of solid tumor but metastases and leukemias are unaffected. One long-held dream is that specificity of immune mechanisms could be harnessed against cancer cells. People with weakened immune systems (IS) are more likely to get cancer, but many people with normal immune systems (IS) still develop cancer. This is due to the defect in recognizing or the weakness of the IS in responding to cancer cells. Cancer cells themselves may also give off substances that keep the immune system from doing its job. The concept of cancer immunotherapy is to strengthen the body’s natural defense system to recognize and destroy the cancer. Strategies for the cancer immunotherapy can be divided into active and passive approaches. Active immunotherapy refers to immunization of the tumor-bearing host with materials designed to elicit an immune reaction capable of eliminating or retarding tumor growth. Active immunotherapy can be subdivided into specific or nonspecific immunization. Cancer vaccines, specific/nonspecific stimulants and adjuvant therapy are some examples of active immunotherapy. Passive immunotherapy refers to the creation of immune cells or immune components as in gene therapy or to transfer antibodies or immune cells from an outside source, as in cell therapy. The aim of this article is to explain these approaches which are based on distinct immunological mechanisms in detail and discuss about the novel approaches in this field. We will discuss about the clinical approaches and side effects as well.

Conclusion: Immunotherapy seems to offer great promise as a new dimension in cancer treatment.

Effect of shunt catheter on the systemic immune response: evaluation of neutrophil count, function, and rate of chemotaxis (Children’s Hospital Medical Center, Tehran University of Medical Sciences)

Objectives: 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: 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.

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.

Conclusion: 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. This article was published in J Neurosurg (4 Suppl Pediatrics) 106:288–291, 2007.

Long-lived plasma cells in lupus mice survive high-dose glucocorticoid and cyclophosphamide therapy

Background: Systemic lupus erythematosus (SLE) is characterised by polyclonal B-cell activation and autoantibody production by short- and long-lived plasma cells of the spleen, bone marrow and inflamed tissues (e.g. of the kidney), among other immune dysfunctions. By the age of 20 weeks, New Zealand Black/White mice (NZB/W) develop high autoantibody titres, lupus nephritis and proteinuria resembling those in human SLE. Nearly 35% of all splenic plasma cells (PCs) are long-lived plasma cells (LPCs) with half-lives of more than 6 months and survival independent of antigen presence or T-cell help. LPCs secrete huge quantities of antibodies and maintain plasma cell memory. Previously, we demonstrated that the cytotoxic drug cyclophosphamide depletes short-lived plasmablasts and plasma cells (SPCs), but not long-lived PCs. Autoreactive PCs are such resistant proliferating SPCs. During the third week, the mice received immunosuppressive doses of dexamethasone and/or cyclophosphamide. The PC populations in the spleen, bone marrow, and kidney were analysed by FACS and ELISPOT.

Methods: Lupus-prone NZB/W mice were fed bromodeoxyuridine (BrdU) in drinking water for 3 weeks to label proliferating SPCs. During the third week, the mice received immunosuppressive doses of dexamethasone and/or cyclophosphamide. The PC populations in the spleen, bone marrow and kidney were analysed by FACS and ELISPOT.

Results: All treatment protocols significantly depleted BrdU-positive SPCs in the spleen, but BrdU-negative LPCs remain unaffected. None of the protocols affect bone marrow PCs, which are mainly long-lived. In contrast to the spleen and bone marrow, the kidney contains more IgG than IgM autoantibody-secreting cells (ASC). A considerable proportion of renal IgG ASCs remained refractory to all treatments. Anti-
DNA ASCs were present in refractory compartments of the spleen, bone marrow and inflamed kidney.

**Conclusion:** The majority of plasma cells in the bone marrow are long-lived and refractory to glucocorticoids and/or cyclophosphamide. The spleen and inflamed kidney have different proportions of IgG and IgM secreting plasma cells, which are immunosuppressant-resistant LPCs. Consequently, effective methods for depletion of autoreactive, memory-providing long-lived plasma cells in patients with autoimmune diseases are still needed.

**Internal Medicine Infectious Diseases I**

The evolution in detecting treponema pallidum in blood bank of Oradea Romania between 1999 – 2006

**ESC-ID** 778  
**Author** Tiurbe AM, Burta LO, Maier C  
**Country** Romania  
**University** University of Medicine and Pharmacy Cluj Napoca  
**Department** General Medicine

**Introduction:** Our study proves the high security of transfusions by using some specific and sensitive methods for highlighting the infection with Treponema Pallidum.

**Materials and methods:** our study was due between 1999 – 2006. It presents the evolution of cases with antibodies anti Treponema Pallidum, at different categories of faithful donors, on different types of tests: VDRL, TPHA, ELISA. The Treponema pallidum test is compulsory for each donated blood unit; until 1999 VDRL test was used, in 1999 it was introduced the TPHA method; since 2003 this method was completed with ELISA method.

**Results:** until the introduction of TPHA method, there were detected a low number of positive results (1.95‰ in 1999); confirmation was due with additional tests (RBW and Kolmer). After the introduction of TPHA method, the number of positive cases significantly increased, with a peak in 2000 (9.52‰) and with a progressive decreasing along the studied period. Age distribution: in men (in sex distribution); between 31 – 50 years (age distribution); there are no significant differences between rural and urban samples.

**Discussion and conclusion:** by using more specific and sensitive methods for identification of the infection with Treponema pallidum, the number of positive blood donors increased, so that there were created better conditions for security transfusion therapy; the positive donors were excluded permanently from the blood donation.

**Hematologic manifestation of brucellosis in hospitalized patients**

**ESC-ID** 85  
**Author** Serajian E, Rahmani F, Pour Hasan A, Rikhtegar R, Pia H  
**Country** Iran  
**University** Tabriz University of Medical Sciences  
**Department** students reasearch department

**Background:** Brucellosis is an infectious disease primarily of domestic and wild animals caused by cocccobacilli of genus brucella. It constitutes a major health problem in many parts of the world, particularly in Mediterranean and Middle East. Brucellosis is a multisystem disease. Hematologic abnormalities including anemia, leucopenia, and thrombocytopenia can be encountered during the course of the disease. The aim of this survey is to study the hematologic manifestation in this group of patients.

**Method and Materials:** medical records of 50 hospitalized patients diagnosed as brucellosis were retrospectively studied over a 9-month period. Age, sex and hematologic manifestation were determined for each patient. Relation between the thrombocytopenia and the clinical signs, the grade of hematological complications and the titer of antibody, the presence of splenomegaly and the hematologic manifestation were investigated and the comparison between the hematologic changes on admission and at discharge were conducted.

**Results:** fifty patients aged 36.14 ± 21.56. 54% male and 46% female were enrolled. Hematologic manifestation comprised anemia (76%), thrombocytopenia (28%), leucopenia (20%) neutropenia (12%) and lymphopenia (8%). There was no significant relation between the thrombocytopenia and the clinical signs (p >0.05). The titer of antibody was significantly lower among the lymphocytopenics. The splenomegaly increased in count of the thrombocytopenia and disarrangement of the PT and PTT; likewise the rates of anemia and thrombocytopenia were significantly lower at discharge (p <0.05).

**Conclusion:** Our results are in conformity with other studies. The presence of some differences might be due to the lower number of the studied patient and inter-personal distinctions. The treatment causes a return a hematologic changes to the normal level after discharge.

The study of immunological properties of recombinant protein SCPB1 as potential vaccine component AGA

**ESC-ID** 128  
**Author** Duplik NV, Koroleva IV, Suvorov AN  
**Country** Russia  
**University** Saint-Petersburg State Technological Institute  
**Department** Fine Organic and Microbiological Synthesis

**Objectives:** Streptococcus agalactiae (group B streptococcus, GBS) is an important cause of sepsis and meningitis in newborns and pathology of pregnancy. It was well documented that streptococci group A, B and C (GAS, GBS and GGS) express on their surface C5a peptidase (C5a-ase). This enzyme related to serine proteases specifically cleaves the C5a complement component between His67 and Lys68. The
followed inactivation of C5a destroys the ability of C5a to act as an anaphylatoxin and a strong chemotactant. C5a-ase has also been demonstrated to bind fibronectin and participate in epithelium cell invasion. All this allowed regarding C5a-ase as a virulence factor of pathogenic bacteria.

**Aim:** Expression, purification of the recombinant protein SCPB1 and investigation of its immunological properties.

**Materials and methods:** For cloning pQE30-32 expression vectors (Qiagen, USA) were used. Male mice (16-18g) were immunized with 20 µg of SCPB1 together with Alum adjuvant subcutaneously two times with the interval of 4 weeks. Samples of immune sera were tested in ELISA every 10 days after the last immunization. For opsonophagocytosis peritoneal macrophages were infected with GBS strains (58/59 (Ia), 5/70 (lac), 60/59 (II), 7293 (III), 2/68 (VI), 5581 (III)). GBS were preliminary incubated with normal or immune sera, than incubated with macrophages for 15-30 min. Number of cell bound GBS was estimated under the microscope. Protection studies were accomplished in immune mice infected intraperitoneally with GBS strain (5/70) with 2.7x107 cells per mouse. Protection level was evaluated according to bacterial eradication from spleen.

**Results:** The N-terminal part of C5a-ase gene (scpB1) from GBS strain 090R (Ia) was generated by PCR. The amplified fragment 936 b.p. was cloned in E.coli using pQE expression vectors. SCPB1 (M.M. 43 kDa) was successfully expressed followed by purification with Ni sepharose. 24 mice and 2 rabbits were immunized with SCPB1 and immune sera were tested for the presence of anti SCPB1 antibodies during different periods of time by ELISA. The maximum titer of anti mouse SCPB1 IgG was found 1:5.1x103 and the maximum titer of anti rabbit SCPB1 IgG was found 1:106. Rabbit antiseraum was examined for its opsoning ability against six GBS strains of different serotypes employing mouse peritoneal macrophages. Infectivity indexes were increased more than twice in comparison with the control. That indicated significant opsonizing properties of anti rabbit SCPB1 antibodies. Recombinant protein SCPB1 was also investigated for its protective properties in vivo experiments. Following preliminary subcutaneous immunization with SCPB1 30 mice were injected intraperitoneally with GBS. The developing of infection was controlled by spleen testing. The results displayed obvious spleen clearance from GBS in contrast with control group in five hours.

**Conclusion:** 1. The N-terminal part of C5a-ase gene (scpB1) from GBS was cloned and recombinant protein SCPB1 (M.M. 43 kDa) was successfully expressed and purified. 2. The experiments on animal models demonstrated that SCPB1 possessed immunogenic and protective properties against GBS that allowed to propose SCPB1 as a one of vaccine components against GBS.

**Bloodstream infections in febrile children with various cancers: A 10-year experience at a single Iranian institution**

**ESC-ID** 933

**Author** Sadeghi M, Ramezanian E

**Country** Iran

**University** Iran University of Medical Science

**Department** Pediatrics

**Heading:** Pediatric patients with cancer can have fatal bacterial or fungal infections & antimicrobials have to be prescribed empirically in the presence of fever or other signs of infection.

**Aim:** knowing the causative agents & their changes with time is very helpful for their management.

**Method:** We reviewed the blood cultures of febrile children with cancer with or without neutropenia in Aliashgar children hospital,a referral Pediatric oncology center in Tehran,from 1997 to 2006.A comparison for the types and pattern of antimicrobial resistance between two period of 5 years was also done.

**Results:** 281 agents were isolated during this retrospective study . The most frequently isolated species or genus were coagulase negative Staphylococcus (22.8%), Staphylococcus aureus (14.2%), Escherichia coli (12.5%), Pseudomonas spp. (8.5%) , alpha hemolytic streptococci (6.8%), Klebsiella and Enterobacter spp. (each 5.3%), salmonella spp(4.6 %) and Candida spp. (2.5%). Overall,55% of isolates were gram positive.The rate of some gram negative organisms specially salmonella isolation has significantly decreased during previous 5 years;the reverse is true for some gram positive isolates except for enterococci.Between 40-50% of all isolates were resistant to various 3th generation Cephaloporins including ceftazidime ; resistance to Ceftriaxone has significantly increased during past 5 years. Resistance to aminoglycosides (except for Gentamicin ) Cefepime,Imipenem and Ciprofloxacin is still less than 20%.

**Conclusion:** Despite the similarity of most common types of isolates in this study comparing with other studies, the rate of resistance to 3th generation of cephalosporins is concerning,so that their monotherapy specially for acutely ill or septic patients can not be recommended anymore. Usage of newer modalities or adding an aminoglycoside from onset seems necessary in these circumstances.

**Ad vocating for HIV/AIDS in India:**

**Successes and challenges**

**ESC-ID** 936

**Author** Singh D

**Country** Ukraine

**University** Kharkiv State Medical University

**Department** Internal Medicine

India has the second largest population living with HIV/AIDS in the world. It is predicted that the current estimate of 5.1 million HIV positive people will grow to 20-25 million by 2010 and HIV/AIDS related deaths could slow economic growth by .86 percent up to 1.5 percent per annum. This paper examines the progress that is being made in fighting this epidemic by analyzing the efforts, experiences, and challenges faced by two Non-governmental Organizations (NGOs) that are located in two different parts of the country. We find that although the regions differ in culture and clientele, both NGOs face similar problems in combating the HIV/AIDS epidemic due to prevailing cultural norms, including those that prescribe gender roles within Indian society. Furthermore, we find that groups that are not traditionally seen as high risk to contract the disease, such as monogamous housewives who cannot demand safe sex practices from their husbands due to prevailing cultural norms, and female day laborers due to gender discrimination in the labor market and socioeconomic inequality in society, should in fact be considered high risk. In addition, some high risk groups are largely ignored within Indian society, such as the homosexual com-
munity, further inflaming the epidemic. Overall, cultural taboos related to sexual education in the public sector leaves everyone at a higher risk of contracting HIV/AIDS. Issues related to NGO accountability and donor funding also present new challenges.

**Chronic aseptic meningitis: A rare presentation of acute promyelocytic leukemia**

ESC-ID: 489  
Author: Taebi N, Farzadnia M, Amoeian S, Rahimi HR, Shakeri M  
Country: Iran  
University: Mashhad University of Medical Sciences  
Department: Pathology

**Introduction:** The occurrence of extramedullary disease (EMD) at presentation or at relapse has long been considered a rare event in Acute Promyelocytic Leukemia (APL). Our purpose from recent reports of EMD at presenting in APL have raised increasing concern about one of causes of chronic meningitis.

**Material and Method:** We describe a case of acute promyelocytic leukemia (APL) in 25 year old female patient was admitted in emam reza hospital, presenting with severe headache and vomiting and showed sings of meningeal irritation as well as papilledema. Cytocentrifuge examination of CSF showed an excess of promylocytes, but peripheral blood didn’t show any abnormal cell or blasts where as BMA show increase of promylocyte with multiple auer rod. After 2 weeks of aleukemic phase, this patient developed hematologic picture of APL confirmed with myeloperoxidase stain. She was diagnosed as having leukemic meningitis and after 3 week in spite of chemotherapy, she dead.

**Conclusion:** Leukemic relapse or first presentation of leukemia may be the etiology of aseptic meningitis, rarely central nervous system leukemia and leukemic meningitis are associated with normal bone marrow and sometimes patients at Aleukemic phase presents with meningitis. Therefore, we suggested that in patients with aseptic meningitis cerebrospinal fluid cytospine smear should be evaluated for neoplastic and leukemic cells.

**Internal Medicine Nephrology and Urology**

**Study of glycated peptides and aminoacids in urinary calculi**

ESC-ID: 8  
Author: Stanoeva K, Ivanova M  
Country: Bulgaria  
University: Medical University - Sofia  
Department: Department of Chemistry and Biochemistry

**Introduction:** Urinary calculi are a mixture of crystalline and organic components. The organic compound of urinary calculi contains glyco-peptides and glyco-amino acids. The glyco-peptides and glyco-amino acids are complexes between carbohydrates (glucose, fructose) and amino acid compound. They have absorption at 325 nm in the UV-spectrum.

Aim: The aim of this study was to examine the amino acids present in urinary calculi of Calcium oxalate (CaOx), of Uric acid (UrAc) and of Cystine (Cys).

**Materials and methods:** Experiments were performed on adult Wistar rats. It was developed an author's experimental model that provides surgically exclusion of intestinal segment being studied, easy instillation of GOBA solution via outer opening fistula, and in such way enables investigation of urinary calculi formation. The amino acids found at a highest rate in Calcium Oxalate (CaOx) calculi are Arg (21.62 nmol/ml), Gly (19.9 nmol/ml), Ser (17.37 nmol/ml), and in Uric Acid calculi – Asp (39.27 nmol/ml), Gly (37.39 nmol/ml), His (17.13 nmol/ml); and in Cystine calculi – Cys (774 nmol/ml), Lys (99.8 nmol/ml), Val (70.4 nmol/ml). There are no S-containing amino acids found in Calcium Oxalate and Uric acid calculi. The amino acid Gly is present in Calcium Oxalate, Uric Acid and Cystine calculi.

**Conclusion:** The examination of amino acids in the matrix of the urinary concrements shows different amino acids composition in the 3 types of urinary calculi which may have its importance in their formation. It is noticeable that the amino acid Gly is found in all 3 types of urinary stones. This may be related with the pathogenesis of urolithiasis.

**Reducing of mucus production after ileocystoplasty by optimization of neobladder epithelium cell composition**

ESC-ID: 783  
Author: Reznichenko A  
Country: Ukraine  
University: Dnipropetrovsk State Medical Academy  
Department: 1st Medical

Finding out an enteroplasty opened a new era in operative urology. Now orthotopic bladder substitution is the gold standard of urinary reconstruction after radical cystectomy. Ileocystoplasty has a favorable long-term outcome, but there are numerous complications in the early post-operating period. The first problem we face with right after the surgical procedure is excessive mucus production (EMP) of the neobladder. It causes great difficulties in neobladder emptying due to high urine viscosity and catheter obturation with mucus plugs, and might lead even to neobladder rupture due to mucus obstruction. A lot of studies aimed to resolve problems associated with EMP. Different pharmacological strategies were tried out. Found no effective solution studies in this field stopped. But the problem remains. As well as quality of patients’ life in the early post-operating period after ileocystoplasty remains to be less that satisfactory. Present research offers to look at the EMP problem from the other angle. As we know that morphological ground of the problem is activity of intestinal goblet cells it is suggested to modify neobladder epithelium cell composition towards decreasing of goblet cell number by induction of crypt stem cells directed differentiation with gamma-oxybutyric acid (GOBA).

**Aim:** To establish the fact of GOBA influence on intestinal epithelium morphology in vivo, to define and evaluate character of morphological changes, their expressiveness degree, reversibility, and possibility of clinical usage of these results. **Materials and methods:** Experiments were performed on adult Wistar rats. It was developed an author's experimental model that provides surgically exclusion of intestinal segment being studied, easy instillation of GOBA solution via outer opening fistula, and in such way enables investigation of the enteroplasty.
of GOBA local effects in environment mostly close to physiologi- cal. After surgery GOBA solution had been daily admin- istered. According to regimen and duration of GOBA input animals were randomly assigned to 4 test groups. After course of GOBA isolated intestinal segment were removed and underwent histological investigation.

Results: Histological analysis revealed marked decrease of goblet cells number. After 15 days of GOBA administration crypts contain practically no goblet cells. It was established that after 3 days of GOBA administration number of goblet cells tends to restore up to initial value, whereas a 15-day GOBA course leads to stable morphological changes.

Conclusion: Presented results give possibilities to consider that luminal administration of GOBA modifies interstitial epithelium cell composition towards decrease of goblet cells number in time-dependent manner. Decreasing of goblet cells number besides EMP problem might resolve metabolic complica- tions related to bladder substitutions since goblet cells produce bicarbonates as well. These results might have clinical implication and might being used in post-operating peri- od after ileocystoplasty with the purpose of reducing of mucus production and, correspondingly, improvement of patients’ quality of life.

Clinical and immunological aspects in patients with chronic bacterial prostatitis undergoing immunomodulatory therapy

| ESC-ID | 281 |
| Author | Bedrettinova DA, Kamalov AA, Efremov EA, Dorofeev SD, Melnik JI, Ochobotov DA |
| Country | Russia |
| University | Moscow State University |
| Department | Faculty of Basic Medicine |

CBP is a chronic inflammatory process linked with changes in the immune system, that requires immunomodulatory therapy. Interferons-alpha (IFN-alpha) can promote the differentiation and activity of host immune cells.

Aim: of this prospective, randomized, double-blind trial was to highlight the clinical and immunological aspects in patients with CBP and indicate effectiveness and safety of IFN-alpha-2b as addition to standard antibacterial therapy.

Methods: The subjects were 40 male patients (mean age 38.8 ± 9.05 years) with confirmed CBP. They were randomize d in two treatment arms with respect to efficiency of treatment (p <0.05). Before treatment concentrations of IFN in blood were below norm (IFN-alpha: 112.75 ± 5.6 ME and 114.64 ± 4.4 ME, IFN-gamma: 58.49 ± 3.84 ME and 61.73 ± 2.77 ME in I and II group respectively). After treatment statistically significant increase was observed in both groups, but most evident in I group than in II one (150.47 ± 4.9 vs. 132.01 ± 6.15 for IFN-alpha and 87.575 ± 2.79 vs. 73.515 ± 3.11 for IFN-gamma; p <0.05). Spontaneous NBT-test showed significant increase in I gr. (6.94 ± 0.59% vs. 13.03 ± 0.88%), that indicates compensation and reactivation of phagocytes. Stimulated NBT-test showed the same results in I gr. (19.31 ± 0.86% vs. 32.3 ± 2.63%), that means activation of capability of phago- cytes to complete phagocytosis in I gr. (p <0.05). Comparison of sIgA levels (40.54 ± 5 pg/ml in I gr. and 33.0 ± 3.0 pg/ml in control, p <0.05) indicates the enhancement of local immune response in group of interferonotherapy. Comparison of serum immunoglobulin’s levels shows the same tendency: statistically significant decrease of levels in I gr., that demonstrates normalization of immune parameters.

Conclusion: This study showed clinical benefit and safety of using IFN-alpha-2b in supp. in the management of CBP. Intensity of symptoms and inflammation decreases significant- ly, normalization of immunological aspects was approved. CBP should be treated by combination of etiotropic with immunomodulatory methods.

Are incidentally discovered findings on 4000 non-contrast enhanced CT for suspected ureteral colic clinically significant?

| ESC-ID | 568 |
| Author | Ather MH, Faizullah K, Achakzai I, Irani F, Siwani R |
| Country | Pakistan |
| University | Aga Khan University |
| Department | Medical College |

Objective: To study the clinical impact of incidentally dis- covered and alternate diagnoses on non-contrast enhanced CT (CT KUB) clinically significant.

Methods: In 4000 consecutive CT KUB scans performed from 2001 to mid 2005 for suspected reno-ureteral colic were identified through the radiology department data base. All official CT KUB reported by the radiologists were reviewed and radiological diagnoses of clinical entities not suspected otherwise were analyzed. All other relevant radiological, bio- chemical and serological investigations and per-operative findings were also noted. Data was analyzed on computerized statistical package SPSS™ version 14.0.

Results: Reno ureteral calculi were identified on 3120 examinations (78%) while 482 (12%) were unremarkable. An alternative or additional diagnosis was established or suggested on 398 examinations (9.95%). 74, 20, 7 and 1 patient had 2,3,4, and 5 additional findings. Overall there were 500 diagnoses besides stone and obstruction in 398 patients (1.26 findings/patient). Among patients with additional findings, 180 had concurrent Urolithiasis, of which (n = 89) renal and (n = 89) ureteral and 2 vesical calculi. There were 104 genitourinary and 66 non genitourinary tract diag- noses. The most common incidental finding was cholelithia- sis (14.3%), followed by simple renal cyst (9%) and appendicitis (5%).

Conclusion: A wide spectrum of significant, alternative and/or additional genitourinary and non genitourinary diag-
noses can be established or suggested CT KUB performed for suspected reno-ureteral colic. In this so far the largest reported series, the incidence of incidental and alternate diagnoses is about 1 in 10.

**Transplantvasculopathie in eNOS deficient mice**

**ESC-ID** 600  
**Author** Kampmann J  
**Country** Germany  
**University** Charité Universitätsmedizin Berlin  
**Department** Humanmedizin

**Background:** Allograft vascular dysfunction predisposes to arteriosclerosis and graft loss. Nitric oxide (NO) is upregulated in rejecting allografts and is protective against allograft arteriosclerosis in heart transplant mice by suppressing neointimal smooth muscle cell accumulation and inhibiting adhesion of platelets and leukocytes to the endothelium. However, the functional importance of endothelial nitric oxide synthase (eNOS) and inducible NOS (iNOS) in the rejecting allograft remains unclear. For this reason we were investigating neointimal proliferation and the role of NO synthases in chronic inflammation in a mouse model of eNOS deficient mice.

**Methods:** For studying the effects of eNOS deficiency in aortic allograft thoracic aorta of donor mice (BALB/c mice) was transplanted into two groups of recipients: a) C57BL/6J (Con; n = 6) and b) eNOS K.O. mice (eNOS K.O.; n = 6). After 4 weeks mice were sacrificed and the transplanted aorta was harvested. For immunohistochemistry the arteries were paraffin embedded or snap-frozen. Parameters of neointima formation, HE-staining, adhesion molecules, inflammatory cells in vascular wall and expression of NO synthases and cGMP were investigated by PCR and histomorphometric analysis.

**Results:** In eNOS KO mice the relative area percentage of proliferation marker α-actin positive smooth muscle cells were markedly decreased in intima by 87% (P < 0.01, compared with Con). Additionally the relative area percentage of α-actin in the media in eNOS KO mice was decreased by 87% (P > 0.05). Intima thickness of artery grafts in eNOS KO was reduced by about 42%, the area of intima plus media reduced by about 23%, lumen area increased by about 90%, and the intima/media ratio reduced by about 40% in HE staining compared with wild-type controls. Analysis of the adhesion molecules PECAM and VCAM revealed a reduced cell count in the intima, where positive cell staining for PECAM in the media was lowered by 65% (P<0.05) and for VCAM by 88% (P<0.05) compared to Con. In media PECAM cell count was decreased by 93% (P>0.05) compared to Con. Positive staining for VCAM in the media presented only one cell in Con and 0 in eNOS KO (P>0.05). In eNOS KO the cell count for the inflammatory cell parameter F4-80 was diminished in the intima of eNOS KO mice by 82% (p<0.05) compared to Con. In media the mean was 10 cells per artery in Con and 9.5 cells per artery in eNOS KO (P>0.5). For the lymphocyte marker CD8 cell count was derogated by 63% (p<0.01) in intima in eNOS KO compared to Con. In media however, there was no significant difference between eNOS KO and Con. cGMP as second messenger of NO showed a +6 fold higher relative area percentage of cGMP in eNOS KO mice (p<0.05) compared to Con in the intima. In the media there was a +3 fold higher relative area percentage per artery (p<0.01).

In eNOS KO expression of iNOS m-RNA was 82% higher than in Con.

**Conclusion:** In the eNOS Knock out model neointima formation, He, adhesion molecules and inflammatory cells in vascular wall were upregulated in Con, where as iNOS and cGMP were upregulated in eNOS K.O. The results suggest the development of neointima in grafts may partially originate from recipient. The increase of cGMP as second messenger of NO and the upregulation of iNOS as synthase suggest that eNOS deficiency may be protective in early development of transplant vasculopathy. The protective role of eNOS deficiency in the early development of vasculopathy may be due to the compensation of iNOS as reaction to the eNOS deficiency.

**Polyneuropathic changes in patients on chronic hemodialysis**

**ESC-ID** 604  
**Author** Jovanovic A, Djuric P, Jovanovic D  
**Country** Serbia  
**University** Belgrade University  
**Department** Medicine

**Introduction:** Uremic polyneuropathy is a common complication in patients with advanced chronic renal failure, patients with end-stage renal failure as well as in those on dialysis. The present study was aimed to analyze frequency and causes of polyneuropathy in patients on hemodialysis.

**Patients and methods:** Twenty nine patients (18 men), aged between 26 and 74 who were on chronic hemodialysis at the Institute of Urology and Nephrology in Belgrade, from 3 to 28 years, were analyzed. According to various renal diseases patients were divided into 3 groups: Group I: patients with glomerulonephritis (11); Group II: patients with tubulointerstitial nephritis (9); Group III: patients with other renal diseases (9). Biochemical parameters and dialysis adequacy were determined. Motor conduction velocity of peroneal and tibial nerves, and sensitive conduction velocity on sural nerve were measured.

**Results:** Polyneuropathy symptoms were found in more than 38% of patients. Mean values of all analyzed neurophysiological parameters except MCV and TL on peroneal nerve, were pathological. Significant correlation was found between peroneal MCV and F wave, and tibialis MCV and urea; tibialis TL and KT/V and hemoglobin concentrations; and suralis SCV and glycemia.

**Conclusion:** The worst values of neurophysiological parameters of the studied hemodialyzed patients were suralis SCV, peroneal and tibial F wave, and tibialis MCV and urea; tibialis TL and KT/V and hemoglobin concentrations; and suralis SCV and glycemia.
Short-term and long-term effects of delayed graft function (DGF) on graft survival in pediatric renal transplantation

ESC-ID  382
Author  Otukesh H, Hoseini R, Fereshtehnejad SM, Basiri A, Simforoosh N, Majid C
Country  Iran
University  Iran University of Medical Sciences
Department  Medicine

Introduction: Delayed graft function (DGF) generally refers to oliguria or the requirement for dialysis in the first week post-transplantation. It is the earliest and most frequent post transplantation complication that can occur. DGF is an extremely important post transplantation complication, because its occurrence has early and long term consequences for allograft survival. However, limited studies are performed about DGF and its complications in pediatric renal transplantation. Therefore, the aim of present study was to determine short-term and long-term effect of post-transplanted DGF on allograft outcome in kidney transplanted children.

Patients and methods: In this historical cohort study 230 children received transplants between 1985 and 2005 in Labafi Nejad hospital in Tehran, Iran were assessed with the mean follow-up time of 60.96 (SD = 40.46) months (range from 1 to 180 months). The children were divided in two groups: 183 children in group A (No DGF) and 47 patients in group B (DGF). Risk factors of DGF and the impact of DGF on renal function within the first year, long-term graft survival and post transplantation complications were analyzed and compared using Logistic regression model and Kaplan–Meier survival analysis.

Results: The incidence of graft failure at the end of follow-up period was significantly more common in DGF group (53.2% vs. 22.4%, P = 0.000). The mean survival time was 134.20 (SEM = 6.17) months in group A (No DGF) and 76.52 (SEM = 12.41) months in group B (DGF) (P = 0.000). The graft survival rate was 94.9%, 91.9%, 83.9%, 79.2% and 72% at 1, 3, 5, 7 and 8 years after transplantation in children without DGF versus 75.6%, 53.2%, 47.2%, 31.9% at 1, 3, 5 and 8 years after transplantation in patients with DGF. Dialysis before transplantation (P = 0.039), acute rejection (P = 0.000), immunosuppressive protocol without cecept (P = 0.000) and the presence of DGF (P = 0.000) were found as the significant risk factors for the occurrence of graft failure in future.

Conclusion: The results of our study showed that delayed graft function could remarkably and independently affect graft survival and worsen both short-term and long-term transplantation outcomes. This result is in contrast with studies that only believe in the effect of DGF on short-term graft survival. But in our study, when patients whose grafts had failed during the first year after transplantation were censored, still significant differences were noted in graft survival between patients with and without DGF. Thus, the prevention of DGF is one of the most important issues in graft survival improvement.

Comparison of the serum concentration of sodium and potassium in patients who died in intensive care

ESC-ID  395
Author  Javad SA, Mousavi FAJ, Fereshtehnejad SM, Shahab
Country  Iran
University  Iran University of Medical Sciences and Health Services
Department  Medical Student Research Committee

Introduction: Electrolyte monitoring of the patients in intensive care unit (ICU) is of great importance. On the other hand, the development of many electrolyte disturbances in the ICU can be prevented by attention to the usual intravenous fluids and nutrition. Hyponatremia and hyperkalemia are common electrolyte abnormalities in critically ill patients and seems to have important effect on patients survival. The incidence of Hyponatremia is unknown, their causes are multiple. The higher mortality especially in ICU is currently unexplained.

Objective: The aim of this article is to evaluate the prevalence of serum sodium and potassium disorders to assess their effects on mortality rate in ICU and to identify predictors of prognosis of patients in ICU.

Patients and methods: This retrospective case-control study was performed on 457 patients who were hospitalized in ICU of Rasoul-e-Akram hospital in Tehran, Iran for the period 2004-2006. They were divided in two groups: 239 patients who were died in ICU (case) and 218 patients who were discharged from ICU (control). Normal serum concentrations of sodium and potassium were assumed as 135-150 and 3.5-5.5 mEq/L. Data were analyzed using Chi2, Independent T-test, One Way ANOVA, Correlation and Receiver Operating Characteristic (ROC) curve.

Results: The mean serum sodium concentration in dead and discharged patients were 137.56 (SD = 8.56) and 137.17 (SD = 5.11) mEq/L, respectively. Whereas, hyponatremia was significantly more common in dead patients (39.7% vs. 28%, P <0.001). On the other hand, the mean serum potassium concentration in dead and discharged patients were 4.42(SD = 0.90) and 4.16 (SD = 0.59) mEq/L, respectively. Hyperkalemia was significantly more common in dead patients (9.2% vs. 0.9%, P<0.001). There was a significant negative correlation between serum sodium concentration and patient’s age (P = 0.029, R Spearman = - 0.123). In addition, ROC analysis showed that serum potassium concentration could potentially be a predictor of death in ICU patients (P = 0.003, Area Under Curve (AUC) = 0.581).

Conclusion: The result of our study show high prevalence of hyponatremia and hyperkalemia in dead ICU patients which is confirmed by some other studies too. Mortality of ICU patients is linked, in greater part, to organs dysfunction, but the severity of serum sodium and potassium concentrations remained significant predictors of mortality. Thus, correcting electrolyte disturbances in ICU patients is an important fact.
Introduction: Hepatic dysfunction is seen frequently after renal transplantation. The reported incidence depends on the criteria used to make the diagnosis and varies from 7% to 67%. Where as most of these enzymes abnormalities are of indeterminate clinical significance, a small percentage of patients progress to frank liver disease and death. The etiologies of liver dysfunction have been attributed to a verity of viral and/or pharmacological agents for which the evidence is circumstantial.

Material and Method: From October 1995 to October 2006, 382 patients received 476 allograft at the university of medicine and pharmacy „victor babes” Timisoara Romania 274 from cadaviric donors and 182 from living donors. 18 patients with clinically apparent jaundice there were 8 men and 9 females with the mean age of 36.0 and 32.6 years. 15 patients received one kidney transplant and 3 received two transplants. 9 patients received kidney graft from cadavic donor and 9 from the living related donors. Clinical presentation Most common symptoms in the group of 18 were • Fatigue (18.81%) • Anorexia (15.68%) • abdominal pain • ascites • Fever most common physical sign in 73% of the patients • Jaundice in 12 patients at the time of admission • Bleeding and ascetic were late manifestations • Palpable liver Pre-existing liver disease Of 18 patients only one patient had a history of pretransplantation liver disease. One patient had clinical hepatitis while on dialysis more then one year before transplantation. One more was HbsAg +ve before transplantation but without clinical evidence of hepatitis. Severe sepsis and liver failure appeared due to CMV infection. Serum bilirubin ranged from 1.4 -11.1 mg%. Men bilirubin level was 5.6 mg% in patients who subsequently died and 4.6 mg% who survived SGOT elevated level except one patient.

Conclusion: 90% of the patients had good renal function at the onset of jaundice and there was no disorientation on the renal function. Main cause of death following the transplantation is hepatitis. Hepatic malfunction appears 3rd leading cause of death in renal transplant. Number of viruses re associated with the malfunction of the liver, acute hepatitis and chronic active hepatitis.

Small molecule inhibition of TGF-beta signaling prevents renal tubular cell epithelial-to-mesenchymal transition and matrix protein production

Introduction: Epithelial-to-mesenchymal transition (EMT) is a major contributor to the pathogenesis of kidney fibrosis. EMT precedes tubular atrophy and tubulointerstitial matrix accumulation, i.e. tubulointerstitial renal fibrosis. Transforming growth factor beta-1 (TGF-beta1) has been identified as key inducer of EMT. Phosphorylation of Smad2/3 proteins is required for TGF-beta1-induced EMT. Thus, inhibition of Smad2/3 phosphorylation emerges as therapeutic target for attenuating EMT and renal fibrosis. In this study, we investigated the small molecule inhibitor LY2109761. This novel compound inhibits TGF-beta receptor types I and II kinases, and thereby Smad2/3 phosphorylation.

Methods: Growth-arrested and synchronized tubular epithelial cells from normal rat kidney (NRK-52E) were stimulated with TGF-beta1 (5ng/ml). Increasing doses of LY2109761 (10, 20 and 50 µmol/ml) were used to inhibit TGF-beta pathway. Decrease of Smad3 phosphorylation was estimated to proof effective TGF-beta receptor inhibition. Cellular expressions of fibronectin and plasminogen activator inhibitor type-1 (PAI-1) were analysed as indicators of matrix protein synthesis and inhibition of matrix protein degradation, respectively. Both are sensitive markers of TGF-beta action. EMT was assessed by the overexpression of alpha-smooth muscle actin indicating myofibroblastic phenotype.

Results: In cultured tubular epithelial cells, LY2109761 markedly reduced TGF-beta-stimulated epithelial-to-mesenchymal transition as indicated by prevention of alpha-smooth muscle actin overexpression. In a line, LY2109761 inhibited TGF-beta-induced Smad3 phosphorylation in time independent manner and to a zero-level (-530% decrease; p <0.005). TGF-beta-stimulated fibronectin overexpression was reduced by -185% (p <0.05) in the presence of LY2109761 in comparison to NRK-52E cells stimulated only with TGF-beta1. TGF-beta-induced PAI-1 overexpression was lowered by -210% (p <0.05) after treating with LY2109761, respectively.

Conclusion: The novel TGF-beta receptor inhibitor LY2109761 effectively prevents tubular epithelial cells transition to a mechenschymal phenotype involving inhibition of Smad3 phosphorylation, fibronectin and PAI-1 overexpression. The findings underscore that targeting the TGF-beta pathways is an important strategy in treating renal fibrosis.
and that LY2109761 is a prime candidate for the future treatment of progressive renal fibrosis and insufficiency.

**Relationship between renal dialysis and oral health status**

**ESC-ID**: 481  
**Author**: Rashidy D, Ghasemi M, Niknamfard, Rasidy A  
**Country**: Iran  
**University**: Shahed  
**Department**: Dentistry

**Background**: Previous studies on the severity of periodontal disease and dental caries in dialysis patients demonstrated different results. The aim of this study was to evaluate the effect of renal dialysis on periodontal status and DMF in patient referred to three hospitals in Tehran, Iran.

**Material and methods**: 120 volunteers comprising two groups of 60 each dialysis patients and healthy subjects participated in this study. The subjects in two groups were matched for their age, sex and plaque index. After the written consent, the general information and the PDI and DMF indices were evaluated in them. The data were evaluated by Mann-U-Whitney and chi square tests.

**Results**: The DMF was not statistically different in two groups (P>0.05). 68.3% of dialysis patients showed attachment loss, whereas this was present in 18.3% of healthy subjects. Gingivitis was noticed in 31.7% of dialysis patients and this was in 81.7% of healthy subjects and these differences were highly significant. (P<0.0001)

**Conclusion**: The observed higher rate of periodontal disease in dialysis patients, warrant us for higher level of consideration in this group and it is suggested that regular dental examination should be added to their usual treatment plan.

**Anaemic syndrome and changes of antioxidative activity in patients on haemodialysis**

**ESC-ID**: 741  
**Author**: Stanojevic J  
**Country**: Serbia  
**University**: University of Nis  
**Department**: Intern Medicine

**Introduction**: Patients in terminal stage of chronic renal failure on haemodialysis treatment have secondary anaemia and uremic intoxication which causes peroxidative process and results in developing of chronic oxidative stress(OS). Goal: The aim of study was to investigate influence of anaemic syndrome on antioxidative activity in patients on haemodialysis.

**Material and methods**: This study was done in Institute for Nephrology and haemodialysis in Nish. In this study was investigated population of 36 people in control group 18 (50%) women and 18 (50%) men ages 56.8 ± 10.9 and 85 patients in clinical group (34 women (40%) and 51 men (60%)), who were on chronic haemodialysis (HD). Clinic group was divided in two subgroup based on haemoglobin values in frist subgroup haemoglobin values were less than 8 g/dl; while in the second they were greater than 8 g/dl. In frist subgroup there were 40 patients, 38% women and 65% men, ages 56.8 ± 10; who spent on chronic haemodialis 83.4 ± 10.2 month. In second subgroup we had 45 patients, 46.6% women, 53.4% men, ages 61.2 ± 4.3, who spent on chronic haemodialysis 91.7 ± month. In all groups was analysed serum levels of catalase (CAT), one of the most important antioxidative enzyme system, measured in mcat/l; superoxide-dismutase (SOD) measured in U/l and glutathione peroxidase(GPh) measured in UgHb. We used spectrophotometric methods.

**Results**: This study shows that in clinical group in comparison with control group antioxidative activity is significantly lower. In patients in I group values of CAT and GPh are significantly lower in comparison with II group but for SOD statistic significance is not found. (for all three enzymes, I vs control group, p <0.01).

**Conclusion**: This study shows that in patients on chronic haemodialysis in comparison with those investigated control group exist significantly lower antioxidative activity.

**Internal Medicine Pulmology**

**The pathogenetical relationship between the diastolic function of right ventricular and secondary pulmonary hypertension**

**ESC-ID**: 292  
**Author**: Seredyuk V  
**Country**: Ukraine  
**University**: Ivano-Frankivsk State Medical University  
**Department**: Hospital Therapy No 1

**Purpose**: The pathogenetical relationship between the diastolic function of right ventricular (RV) and secondary pulmonary hypertension (PH) at patients with the chronic pulmonary heart (CPH).

**Methods**: Conventional echocardiography and Doppler-echocardiography of RV were performed in 107 patients (31 women, 76 men) with chronic heart failure (NHF) of the II-IV NYHA, middle ages (61.2 ± 7.5) of years, among them 63 patients with the CPH (basic group) and 44 of patients on arterial hypertension (control group). The isovolumic relaxation time (IVRT, s), peak early diastolic inflow velocity coincident with the E wave (Vmax E, m/s), peak late diastolic inflow velocity coincident with the A wave (Vmax A, m/s), and the ratio of peak early to late velocities (Vmax E/A, conditional units) and systolic pressure in a pulmonary artery (SPPA, mmHg) were measured.

**Results**: Found out more expressed violations of relaxation RV at heavy CPH at patients of basic group with a NHF III NYHA of index of IVRT made (0.72 ± 0.10) s, and with the NHF IV NYHA – (0.85 ± 0.11) s, while in a control group accordingly – (0.53 ± 0.07) s and (0.65 ± 0.08) s (p <0.05). Similar conformity to the law was observed and in relation to the Vmax A, when in a basic group progressive increase of this index was marked from Vmax A to the NHF IV NYHA with the parallel decrease of Vmax A in relation to similar indexes in a control group (p <0.05). In particular, in a basic group at NHF II NYHA the index of Vmax E/A made (0.94 ± 0.13), in a CHF III NYHA – (0.64 ± 0.09) and in a CHF IV NYHA is (0.53 ± 0.08) conditional units in a control group – according to (1.25 ± 0.10), (0.82 ± 0.06) and (0.69 ± 0.07) conditional units (p <0.05). During the leadthrough of cross-correlation analysis in patients on CPH from the NHF II-IV
NYHA it is found out strong cross-correlation intercommunications between the degree of PH from one side and indexes of RV metrical, by indexes, of the volumes of preloading on the right heart departments, RV indexes of IVRT, Vmax E, Vmax, Vmax E/A and level SPPA, from other side (p < 0.05) [1].

**Conclusion:** At a CPH in stage of decompensation diastolic dysfunction of RV characterized by increase of passive inflexibility, decrease of pliability of myocardium and increase of payment of right auricle in the process of the diastolic filling of RV as a result of PH, structurally-geometrical changes of RV and increase of level of preloading on the right heart departments.

**The treatment of obstructive sleep apnea syndrome with continuous positive airway pressure**

**ESC-ID** 554  
**Author** Gramada T, Boisteanu D, Vasiluta R  
**Country** Romania  
**University** University of Medicine and Pharmacy “Gr. T. Popa” Iasi  
**Department** General Medicine

**Aim:** This study presents the analysis of the treatment of obstructive sleep apnea syndrome (SAS) with continuous positive airway pressure (CPAP). 

**Material and method:** We studied 60 patients diagnosed with SAS by polysomnographic data in Clinic of Pulmonary Diseases Iasi, during 2006-2007. The treatment began by determining the efficient pressure for each patient. The treatment monitoring has been periodically made, initially monthly and later once every 3 months. The monitoring consisted of a brief anamnensis regarding symptoms, side effects, number of hours per night during which the CPAP had been used and a polygraphic recording.

**Results:** We noticed a strong correlation between the efficient pressure used for the treatment and the body mass index (BMI) (correlation index of 0.94). In addition, the results showed that the number of hours during which CPAP had been used was proportional with a decrease in the apnea and hipopnea index (correlation index of -0.74). Apart from this, in the case of 38 patients (63%) the systolic arterial tension showed that the number of hours during which CPAP had been used was proportional with a decrease in the apnea and hipopnea index (correlation index of -0.74). Apart from this, in the case of 38 patients (63%) the systolic arterial tension, morning tiredness, nictury, memory disorders, cephalalgia. From the 60 patients, 8 (13.3%) have abandoned the treatment with CPAP because of irritation of the nasal pyramid, vasomotor rhinitis, masque deterioration or claustrophobia.

**Discussion:** The amelioration of the symptoms of patients who have permanently and correctly used the CPAP device occurred from the first days of treatment. Consequently, this aspect has represented a motivation for them to continue the use of the CPAP device; they have also described an improvement of their professional and familial life.

**Conclusion:** CPAP is efficient in treating the obstructive sleep apnea syndrome, but we still need larger prospective studies for establishing the optimal duration of the treatment and the long term side effects. Apart from this, in the group we studied, we noticed no major complication and in case they appear, the treatment is stopped.

**Systemic use of the endolysin Cpl-1 rescues mice with fatal pneumococcal pneumonia**

**ESC-ID** 335  
**Author** Witzenrath M, Schmeck B, Doehn JM et al.  
**Country** Germany  
**University** Charité Universitätsmedizin Berlin  
**Department** Department of Internal Medicine / Infectious Diseases

**Rationale:** Community-acquired pneumonia (CAP) is a common disorder, associated with significant morbidity and mortality. S. pneumoniae is the predominant pathogen in CAP, and pneumococcal resistance to antimicrobial agents is increasing. The recently purified bacteriophage lytic enzyme Cpl-1 rapidly and specifically kills pneumococci.

**Objectives:** The aim of the study was to determine the therapeutic potential of Cpl-1 in severe pneumococcal pneumonia.

**Methods:** C57/B16 mice were transnasally infected with pneumococci and treated with Cpl-1 by intraperitoneal injections starting 24 h after infection.

**Measurements and main results:** All Cpl-1 treated mice survived fatal pneumonia. According to clinical appearance, decreased body weight, dyspnea, reduced dynamic lung compliance and PaO2/FiO2 ratio, as well as morphologic changes in the lungs, mice suffered from severe pneumonia at the onset of therapy. Cpl-1 distinctively reduced pulmonary bacterial counts, prevented bacteremia and systemic hypotension, and evoked rapid convalescence. The antimicrobial efficacy of Cpl-1 was comparable to amoxicillin for penicillin-susceptible pneumococci in vivo. Bacterial lysis by Cpl-1 did not cause significant inflammatory response in vivo, as determined by multiplex cytokine assay of lung and blood samples. In human epithelial cell cultures, lysed bacteria evoked less IL-8 secretion and cell death, as compared to viable bacteria.

**Conclusion:** The data suggest that Cpl-1 may provide a therapeutic perspective in pneumococcal pneumonia.

**Teenagers and smoking in western Romania**

**ESC-ID** 864  
**Author** Goda Rajendra S S Y, Dahiya M, Mihaicuta S, Kothari A, Gudagur J  
**Country** Romania  
**University** University of Medicine and Pharmacy, Timisoara  
**Department** Pneumology Clinic

**Introduction:** Cigarette smoking remains a major public health concern in adolescents despite all the campaigns to prevent smoke initiation and promote smoke cessation. Smoking is a problem of the modern world producing yearly victims among young adolescents. Smoking is a risk factor for many types of cancer, also cardio-vascular disease and pulmonary complications.

**Objectives and Aim:** Analysis of smoking habits and attitudes towards cigarettes, smoking frequently starts during childhood. We investigated a group of smoking teenagers from Western Romania, where cardiovascular and pulmonary pathology is predominant.

**Material and Method:** Target group: adolescents 16.6 ± 4 years. We applied an anonymous and detailed questionnaire (42 behavioral questions) about: - smoker/non-smoker - age...
of start of smoking - number of cigarette/day - daily/occasional smoker The comparative study included: Group A 2006 (our group): 450 students CAST(01) 2003: 348 students CAST(02) 2004: 400 students CAST(03) 2005: 528 students Results: Group A, of our time, a total of 450 adolescents participated in the program, 39.7% were male smokers. Comparing to previous years, the number of male and female smokers decreased, the peak point was CAST 02, when males were 53% and female was less then 50%. Average age to start smoking: 13.4 yrs. With ages between 10-19 (median age: 15.8yrs) were asked about their smoking habits. Smoking status differs throughout the years, as in CAST 01, adolescents revealed daily smokers of 36.9%, following year CAST 02, showed fast rise of daily smokers reaching 41.7%, in CAST 03, decreased until 37.2% and Group A, the percentage of daily smokers increased up to 43%. Non-smokers are more aware of the harmful effects of smoking, than smokers. Of the non-smokers in CAST 01 was the highest point 63.1% in the program; the following years it decreased reaching 47.9% non-smokers(GroupA). Among the non-smokers and the daily smokers statistical datas were registered, occasionally smokers and smokers of which the average rate of cigarettes smoked/day is higher then 10. Interpreting the grafical results, comparing to the other three studies, CAST 02 showed the highest of 26.6% of smokers who smoked more than 10/day.

Conclusion: Smoking habits start earlier than expected. Smoking is an important problem from childhood and in time it becomes a daily routine for 26.6% teenagers (CAST 02). Our data pointed out a high level of smoking and is comparable with other three studies done in our region. The number of smokers is slowly increasing in time (43% daily). In Romania, due to ignorance and lack of sanitary education, the establishment of an aggressive campaign against smoking, addressed to all of the population but especially to children, would represent a first step of a program which focuses on the health risks associated with tobacco use, prevention efforts, and treatment options.

Change of vital capacity (VC) after the resection of lung parenchym

Vital capacity (VC) is the maximum volume that an individual can expire after a single maximal inspiration. The VC is usually 3-6 litres, varying with age, gender and height and reduced in obstructive and restrictive defects. Goal: Is to analyze vital capacity changes after lung resection using retrospective study of clinical materials.

Material and methods: At the Institute of pulmonary diseases in Sremska Kamenica, from January the 1st year 1998 until the 31st December 2002, out of total 370 patients suffering from carcinoma bronchi 40 were selected. This survey observed lung function data directly before and after resection of lung parenchyma.

Results: Postoperative value of VC (average) was 2.7l for pulmectomy and 3.5l for lobectomy, consistent with operative extensiveness. The primary reason of postoperative com-
New lungs...New life?

ESC-ID: 898
Author: Koksa K, Kidzinska M, Knop M, Derus M
Country: Poland
University: Silesian Medical Academy
Department: Medical

Patient is qualified to lung transplantation in situation when pharmacological and surgical procedure id not succeed. Transplantation is the last possible way to save patients' lives. The aim of the research was to consider patients’ life quality, before and after lung transplantation. The scientific inquiry had contained six patients who had a successful lung transplantation during the years 2004-2006 One patient (K.A.) – 2 months after operation Three patients (P.A., R.P., K.K.) – 6 months after operation One patient (G.J.) – 12 months after operation One patient (B.E.) – 24 months after operation The reason of qualification to transplantation was extreme cardiac insufficiency in course of Idiopathic pulmonary fibrosis (4 patients) and emphysema (2 patients) To consider patients lives two tests were used: “The Saint George’s Respiratory Questionnaire” / SGRQ / - evaluate the quality of life of patients with chronic pulmonary disease “S-F Questionnaire” / - evaluate the quality of life of patients with chronic pulmonary disease “S-F 36” / - evaluate generally quality of life Patients were qualified in Patient were qualified in Cathedral and Institution of Lungs Diseases and Tuberculosis of Silesian Medical Academy The lung transplantation was made in Silesian Center Of Hearts Disease in Zabrze.

Conclusion: 1. Using Sf-36 and SGRQ is possible to analyse life quality in early period after transplantation. 2. The improvement of life quality and estimate of disease symptoms is best seen one year after transplantation. 3. The improvement is mainly seen in domains which are connected with physical health. However some patients did not feel improvement of psychological health.

Determination of the factors affecting duration of hospitalization in patients with chronic obstructive pulmonary disease

ESC-ID: 407
Author: Mousavi SAJ, Fereshtehnejad SM, Khalili N, Naghavi M, Yahy H
Country: Iran
University: Iran University of Medical Sciences and Health Services
Department: Medicine

Introduction: Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality, and is an important health economic problem. Since 1960, there has been an increase in mortality associated with COPD, especially in men. Acute exacerbations form a major component of the socioeconomic burden of COPD which mainly result in long-term hospitalization. Despite the high number of COPD-related hospitalizations, relatively little is known about the mortality rate and related determinants of patients hospitalized for this acute deterioration in the clinical course of COPD.

Objective: The aim of this study was to evaluate the factors affecting duration of hospitalization in Iranian patients with COPD.

Patients and methods: This prospective cross-sectional study was performed on 68 COPD patients who were hospitalized in Rasoul-e-Akram hospital in Tehran, Iran for the period 2005-2006. During hospitalization, patients’ chief complaint, symptoms and signs, results of physical examinations, spirometry, arterial blood gas (ABG) and ICU admission were recorded. Data were analyzed using Independent T-test, One Way ANOVA and Correlation tests.

Results: The patients were 41(60.3%) male and 27(39.7%) female with the mean age of 69.7(SD = 13.52) years. The mean duration of hospitalization was 11.82(SD = 5.49) days and 3(4.4%) patients were died. The family history of pulmonary disease (P = 0.018), habitual snoring (P = 0.031), mean baseline arterial PO2 (P = 0.010, R sp hearman = -0.361) were determined as factors affecting duration of hospitalization. On the other hand, other factors such as smoking (P = 0.992), patient’s gender (P = 0.735) and spirometric indexes did not significantly associate with duration of hospitalization.

Conclusion: The fact that people hospitalized with COPD have a subsequently increased risk of death compared with those not hospitalized suggests the former are an at-risk group and shows the importance of factors affecting duration of hospitalization. Our results show that more attention must be paid on habitual snoring and low arterial PO2 which may have potential effects on duration of hospitalization in COPD patients.

Asthmatic patients with chlamydia pneumoniae infection and their treatment

ESC-ID: 434
Author: Efremov B, Dobic D
Country: Macedonia
University: Ss. Cyril and Methodius University-Skopje
Department: Medical Faculty

Asthmatic Patients with Chlamydia Pneumoniae Infection and Their Treatment Heading: Asthma is a chronic inflammatory disease of the respiratory ways in which many cells take part, such as mast-cells, eosinophils an T-lymphocytes. In the sensitive individuals, the inflammation causes symptoms, which are associated with the commonly spread variable obstruction of the respiratory ways, which is often reversible, either spontaneous or either by treatment. The inflammation causes augmentation of the reactivity of the respiratory ways to different stimuli. It has been suggested that chronic Chlamydia pneumoniae infection could be a cause for adult onset of asthma. There are data to suggest that infectious organisms, particularly the atypical bacteria, Chlamydia pneumoniae may be involved in the asthma pathogenesis. It is not known whether this organism was allowed to persist after an infection, or was present prior to the development of asthma. The significance of atypical bacteria in the exacerbation asthma is still unclear.

Aim of the study: The purpose of this study was to determent whether anti-Chlamydial treatment with azithromycin will improve asthma symptoms and lung function in asthmatic patients.

Materials and methods: 30 patients (mean age 35.5 years) with moderate to moderately severe asthma were treated a median of 6 weeks with azithromycin 1000mg once weekly. All patients had Chlamydia pneumoniae infection detected by specific IgA 1:40 and specific IgG 1:256 against Chlamydia pneumoniae. Post treatment lung function and symptom score (cough, wheezing, dyspnea) were compared with baseline values.
**Results:** After 6 weeks of treatment with azithromycin there was significant reduction in symptom score (p < 0.01) and significant improvement in lung function FEV1 (p<0.01), Wilcoxon matched Pairs test.

**Conclusion:** Treatment with azithromycin significantly improved asthma symptoms and lung function indicating that Chlamydia pneumoniae may play an important role in enhancing the inflammatory processes in the lower airways. Awareness of inflammation as the main pathogenetic mechanism in asthma renewed the interest for the role of infection in the etiology.

**Chronic obstructive pulmonary disease in stage IIIB and IV lung cancer patients - incidence and implications**

**ESC-ID** 711

**Author** Jassem JM, Bobowicz M, Slominski JM, Jassem E

**Country** Poland

**University** Medical University of Gdansk

**Department** Allergology

**Introduction:** Lung cancer is one of the most common tumor and the main cause of cancer-related deaths in European countries. Chronic obstructive pulmonary disease (COPD) may coexist with lung cancer since both diseases are related to the same causative factor - cigarette smoking. Palliative care for lung cancer patients is often composed solely of the treatment for tumor symptoms, whereas some patients would benefit much more from the combined treatment including the treatment for COPD.

**Aim:** The aim of this study was to evaluate the incidence of COPD in advanced (pTNM = IIIB and IV) lung cancer patients and to assess the relation between the coexistence of these two diseases and clinical variables.

**Method:** The study was a retrospective analysis of the patients’ files from Department of Pneumonology and Department of Allergology, Medical University of Gdansk, Poland. The inclusion criteria were: non-small cell lung cancer with pTNM stage = IIIB or IV, spirometry test performed and relevant medical history. 115 patients (34 women and 81 men, aged from 40 to 80 years, mean: 64 years) were identified between 1999 and 2005 and included in the study. Computer software ‘Statistica 7’ (StatSoft 2006) was used for statistical analysis. Statistical significance was established at the level of p ≤ 0.05.

**Results:** COPD was diagnosed in 26 cases (22.6%), including 14 with mild or moderate and 12 with severe or very severe disease. COPD was significantly more frequent in squamous cell lung carcinoma in comparison to other subtypes (p = 0.0002). There was no tendency of coexistence of lung cancer and COPD in patients with higher exposure to cigarette smoke (p = 0.45).

**Conclusion:** Coexistence of squamous lung cancer and COPD is frequent. Therefore, it is important to consider the treatment for COPD in palliative care protocols for advanced lung cancer patients, especially in those exposed to cigarette smoke.

**Correlation between respiratory disturbance index (RDI) and systemic hypertension risk groups in patients having obstructive sleep apnea (OSA) and systemic hypertension (HTA)**

**ESC-ID** 717

**Author** Thanki CN, Thanki PN, Kundnani NR, Akruwala CY

**Country** Romania

**University** University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania

**Department** General Medicine

**Objective:** To evaluate the correlation between respiratory disturbance index (RDI) and groups of risk for systemic hypertension in patients with obstructive sleep apnea (OSA) and systemic hypertension (HTA).
Methods and method: 88 patients referred consecutively to the sleep laboratory for clinical suspicion of Sleep Apnea Syndrome. All patients completed a standardized questionnaire (Epworth Sleepiness Scale), a 30 minutes driving simulation test targeted to estimate the maintenance of wakefulness degree and an overnight polysomnography. Classification of systemic hypertension was done for adults over 18 according to American Society of Cardiology and WHO.

Result: There were Male 84.08%, female 15.92% and 54.5% with systemic hypertension. 6.25% group of risk A, 58.33% group of risk B, 35.41% group C. Respiratory disturbance index (RDI) - Group risk correlation r = 0.971994. RDI was significantly higher in patients with uncontrolled hypertension (blood pressure > or = 140 and/or 95 mmHg) than in those with controlled hypertension (31.8 ± 22.4 versus 24.3 ± 21.5; P < 0.01).

Conclusion: Sleepiness is the most common clinical symptom at patients with OSA and systemic hypertension. Systemic hypertension was found at 54.5% of OSA patients. There is a strong and direct correlation between RDI and groups of risk for hypertension. OSA can be a cause of uncontrolled hypertension.

Microbiology

Lung Klebsiella pneumoniae infection in anti-LPS protected and unprotected mice – Survival, bacterial load and histopathological differences

Aim: The purpose of this work was to examine the effect of anti-LPS monoclonal antibody activity in mice intranasally challenged with a lethal dose of Klebsiella pneumoniae (K. pneumoniae).

Method: In our experiments we used BALB/CN mice eight to twelve weeks old. The animals were separated in two groups. One group received 1 mg per animal of anti-LPS monoclonal antibodies and other received saline intraperitoneally 4 hours prior to infection. We used anti-LPS monoclonal antibodies that were previously shown to be highly specific for O1 LPS of K. pneumoniae. The animals were infected with a lethal dose of K. pneumoniae Caroli O1:K2 (2.6x10^2 CFU). The efficacy of monoclonal antibodies was determined by the survival study and by comparison of lungs colony counts and histopathological changes between two groups 24 and 48 hours after the infection.

Results: Our study has shown that pre-treatment of animals with anti-LPS monoclonal antibodies resulted in 33 % survival compared to unprotected animals that all died within 4 days. Besides the survival we also found different lung bacterial loads between two groups. Sections of the lungs obtained from the protected mice 24 and 48 hours after the infection showed lower degree of inflammation compared to unprotected mouse.

Conclusion: The anti-LPS monoclonal antibodies have shown to be protective in a lung model of K. pneumoniae infection. They reduced the bacterial loads in lung tissues of protected animals and histopathological changes compared to unprotected mice.

Role of CD44 variants in the pathogenesis of syphilitic placentalitis.

ESC-ID 341
Author Krabben G, Wainwright HC, Maske C, Timmer A, Gips CH
Country The Netherlands
University Rijksuniversiteit Groningen
Department Faculty of Medical Sciences

Introduction: The complex transmembrane glycoprotein CD44 is important in embryogenesis and implantation but also has a prominent role in inflammation and cell injury. A potential role of CD44 in syphilitic placentitis can be assumed. In the present study, we examined the expression patterns of the CD44 variants 2 and 7 in the human placenta infected with syphilis.

Methods: A retrospective study (n = 40) was performed using human placentas infected with syphilis and gestational age matched control placentas that were microscopically normal. Triple labeling fluorescent immunohistochemistry was used to show CD44 staining patterns of different cell types. Digital pictures of all cases were collected using fluorescent microscopy. Positive cells were counted and classified as villous, intervillous, endothelial, smooth muscle or trophoblast. Density of background, endothelial and smooth muscle staining was measured quantitatively. Statistical analysis of staining intensity was performed to compare infected placentas with controls.

Results: Histopathological examination of tissue sections showed characteristic features of the syphilitic triad of hypercellular villi, proliferative vascular changes and acute or chronic villitis. Higher CD44v2 expression was found of the syphilitic villous endothelium (p = 0.04). Positive CD44v7 staining in syphilitic placentas was found on endothelial cells (p = 0.002), on the smooth muscle cells surrounding the stemvilli vessels (p = 0.047) and on maternal inflammatory cells in the intervillous space (p = 0.012).

Conclusion: Upregulation of CD44v2 and CD44v7 has several effects. CD44 regulates recruitment and adhesion of T cells and macrophages to vessel walls and smooth muscle cells (SMCs). CD44v2 and CD44v7 upregulation in the villous endothelium may result in periarteritis and arteriitis. CD44v7 expression on SMCs, that change into collagen-producing myofibroblasts results in perivascular fibrosis. CD44v7 expressed on maternal macrophages may contribute to persistence of inflammation by inhibiting apoptosis of these cells.

This study provides new support for a role of CD44 in inflammatory diseases, e.g. in syphilitic placentitis.
Comparison of the virulence of different Legionella longbeachae strains in A/J mice

ESC-ID: 147
Author: Gobin I, Selenic K, Sarec M, Susa M, Doric M
Country: Croatia
University: University of Rijeka
Department: Department of Microbiology

Background: Legionella longbeachae is the most common cause of legionellosis in many parts of Australia, while in Europe and the United States, it rarely causes disease. Epidemiological studies have shown that gardening is a major risk factor in acquiring L. longbeachae infection. In previous studies the virulence of L. longbeachae strains were studied on guinea pigs animal model, and a remarkable variations in signs and disease outcome was shown. Wishing to overcome the inconsistency of the previous animal model we established a murine model of experimental legionellosis caused by L. longbeachae. We have shown that mice intratra-echally inoculated with 10^5 CFU L. longbeachae serogroup 1 (strain D4968) developed an acute disease and died within six days. In this study we were interested to explore if there was differences in virulence between L. longbeachae serogroup 1 strains.

Methods: The isolates of L. longbeachae serogroup 1 investigated in this study included: 2 clinical (A5H5 and A4C5) and 2 environmental (L6C9 and K8B9) Australian isolates as well as 3 clinical isolates from the United States (D4968, D4969, D4973). Inbread A/J mice 6 to 8 weeks old were intratra-echally inoculated with dose 10^5 CFU of bacteria. We followed mortality rate, intensity of bacterial multiplication and pathohistological changes in the lungs of infected mice 2, 24 and 72 hours post infection.

Results: Our previous studies have shown that 10^5 CFU of L. longbeachae serogroup 1 (strain D4968) was LD90. All tested strains of L. longbeachae serogroup 1 caused death of A/J mice within 7 days. Then we followed the replication of different L. longbeachae serogroup 1 strains in the lungs of infected A/J mice. There were no significant differences between all tested strains. During 72 hours post infection, bacteria rapidly replicated in the lungs of infected A/J mice, reaching the concentration of 10^8 and 10^9 CFU. All tested strains caused the same pathohistological changes in the lungs of A/J mice. These changes were in the form of multifocal brechchoepthymonia.

Conclusion: There were no significant differences in virulence of tested L. longbeachae serogroup 1 strains. Mice model seems to be a better experimental model to study the virulence of L. longbeachae infection.

Photodynamic therapy in the treatment of microbial infections

ESC-ID: 410
Author: Kiseleva ER, Titeev RA
Country: Russia
University: Sechenov Moscow Medical Academy
Department: Dentistry

Background: Photodynamic therapy (PDT) employs a non-toxic dye, termed a photosensitizer (PS), and low intensity light which, in the presence of oxygen, combine to produce cytotoxic species. The aim of this study is to investigate antimicrobial activity of cationic PS bacteriochlorin.

Methods: In our in vitro study we used bacteriochlorin (in concentrations 10, 20, and 40 mg/kg/l) and laser (830 nm) (irradiation doses 2, 4, 6, 8, and 10 J/cm2) on bacteria E. coli. In control test tubes we used bacteria affected either with laser irradiation or bacteriochlorin in different doses. Antimicrobial activity was estimated with the use of bioluminescence method.

Results: Bacteriochlorin in 40 mg/kg/l concentration can induce a >10 log decrease in the microbial population after irradiation around 4 J/cm2. The death-roll of bacteria increases consequentially with increase of irradiation dose and concentration level of bacteriochlorin. In control test tubes reduction of quantity of bacteria has not been observed.

Conclusion: Combination of bacteriochlorin with a corresponding source of radiation can be used for PDT of microorganisms.

Gram-negative bacteria exacerbate small intestinal immunopathology following peroral infection with toxoplasma gondii

ESC-ID: 700
Author: Heimesaat M, Fischer A, Fuchs D, Niebergall J, Goldenberg O, Jahn HK, Dunay IR, Hahn H
Country: Germany
University: Charité Universitätsmedizin Berlin
Department: Institute of Microbiology and Hygiene, CBF

Objectives: Commensal gut bacteria contribute to intestinal inflammation in inflammatory bowel diseases (IBD) and experimental colitis. Since ileitis models are scarce, the impact of gut flora on small intestinal inflammation has not been elucidated. Oral infection of susceptible mice with the parasite Toxoplasma (T.) gondii results in severe Th1-type small intestinal immunopathology characterized as pan-ileitis.

Methods: Gut flora changes during development of ileitis were identified by molecular and microbiological techniques. The contribution of gut bacteria to inflammation was determined by antimicrobial treatment and by the use of gnotobiotic mice.

Results: Ileitis was accompanied by a marked increase of the total bacterial load and a sharp decrease in bacterial diversity. Inflammatory changes were paralleled by a population shift from predominantly Gram-positive rods to Gram-negative bacteria identified as Escherichia coli and Bacteroides/Prevotella spp., respectively. Prophylactic and therapeutic antibacterial treatment with ciprofloxacin and/or metronidazole prevented or reduced small intestinal inflammation. Germfree mice generated by quintuple antimicrobial treatment and recolonized with total gut flora, E. coli or Bacteroides/Prevotella spp. developed small intestinal pathology after infection with T. gondii. Germfree mice or mice monoassociated with Lactobacillus johnsonii did not develop ileal inflammation.
Conclusion: Development of T. gondii-driven Th1-type immunopathology is accompanied by a shift from a highly diverse microflora to few Gram-negative species. The use of gnotobiotic mice will allow to study the impact of individual bacterial species on small intestinal immunopathology and to test the effect of gut flora modulation in IBD. Furthermore it may prove useful to test novel therapies based on modulation of gut flora.

In vitro antibacterial activity of 45 essential oils of Iran’ native plants

ESC-ID 482
Author Saghebdoust M, Azadbakht M, Fazli Bazaz BS
Country Iran
University Masandaran University
Department Pharmacognosy

Introduction: The antimicrobial properties of essential oils derived from many plants have been empirically recognized for centuries, but scientifically confirmed only recently. Practical uses of the activities have long been suggested in humans and animals, but only in the last years it has been reported that some essential oils are capable of inhibiting some bacteria and extending the shelf life of processed food. The aim of this study was to test a large number of essential oils against a diverse range of organisms comprising Gram positive and Gram negative bacteria. The purpose of this was to create directly comparable, quantitative, antibacterial data and to generate data for oils.

Materials and methods: Forthty five plants used in this test were collected in spring and summer of 2005 from different parts of Mazandaran, Iran then dried and were extracted by the hydrodistillation method using cleveenger apparatus. Micro organisms were obtained from the culture collection of Pasteur Centre for Pathology and Medical Research.

Aim: Antibacterial screening of 45 Iranian essential oils to determine the potential of these oils against selected bacterial species on small intestinal immunopathology and to test the effect of gut flora modulation in IBD. Furthermore it may prove useful to test novel therapies based on modulation of gut flora.

Methods: In a retrospective study, 43 patients with the final diagnosis of mitochondrial cytopathy were evaluated clinically and electrophysiologically (EMG and EEG). Clinical diagnoses were confirmed by the presence of ragged red fibres (RRF) in the muscle biopsies. In 33 cases genetic studies by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) were performed.

Results: 28 patients were diagnosed as mitochondrial myopathy, 9 suffered from progressive external ophthalmoplegia (PEO). There were 2 patients with proven mitochondrial encephalomyopathy, 3 with Kearns-Sayre syndrome and 1 patient with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). In the skeletal muscle biopsy, RRF, which are the most significant for these disorders, were present in the majority of cases (93%). In 23 cases the EEG investigation was performed, and abnormalities were observed in 10 of them. More than a half of all patients exhibited changes in EMG patterns. The presence of specific genetic mutations was confirmed in 5 cases. The mtDNA point mutation A3243G (2 cases) was related to mitochondrial myopathy, encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS). So-called “common deletion” 8468-13446 (4977 bp) (2 cases) and deletion about 4.5 kb (1 case) were identified in PEO and Kearns-Sayre syndrome, respectively.

Conclusion: The results of our study confirm remarkable clinical heterogeneity of mitochondrial cytopathies. Morphological evaluation of skeletal muscle remains the most informative study in the diagnosis of mitochondrial cytopathies. Final diagnosis in some cases can be confirmed by detection of the genetic defects. Genetic findings may have significant impact for new therapeutic approaches in the future [3].

Acknowledgements: We are grateful to Prof. A. M. Kamińska (Department of Neurology, Medical University of Warsaw) for skeletal muscle biopsy and to Dr. K. Tońśka (Institute of Genetic and Biotechnology, University of Warsaw) for identification of genetic mutations.

Neurology I

Mitochondrial cytopathies: clinical, morphological and genetic evaluation

ESC-ID 515
Author Kierdaszuk BM, Jamrozik Z
Country Poland
University Medical University of Warsaw
Department Department of Neurology

Mitochondrial cytopathies are heterogeneous disorders affecting multiple systems with particularly common involvement of the skeletal muscle and central nervous system. The variety of symptoms and signs requires biochemical, morphological and genetic evaluation [1]. The results of the genetic studies indicate that there is no direct correlation between genotype and phenotype in mitochondrial cytopathies [2].

Aim: Aim of the study was to define clinical features of mitochondrial cytopathies with relation to its genetic defects.

Methods: In a retrospective study, 43 patients with the final diagnosis of mitochondrial cytopathy were evaluated clinically and electrophysiologically (EMG and EEG). Clinical diagnoses were confirmed by the presence of ragged red fibres (RRF) in the muscle biopsies. In 33 cases genetic studies by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) were performed.

Results: 28 patients were diagnosed as mitochondrial myopathy, 9 suffered from progressive external ophthalmoplegia (PEO). There were 2 patients with proven mitochondrial encephalomyopathy, 3 with Kearns-Sayre syndrome and 1 patient with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). In the skeletal muscle biopsy, RRF, which are the most significant for these disorders, were present in the majority of cases (93%). In 23 cases the EEG investigation was performed, and abnormalities were observed in 10 of them. More than a half of all patients exhibited changes in EMG patterns. The presence of specific genetic mutations was confirmed in 5 cases. The mtDNA point mutation A3243G (2 cases) was related to mitochondrial myopathy, encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS). So-called “common deletion” 8468-13446 (4977 bp) (2 cases) and deletion about 4.5 kb (1 case) were identified in PEO and Kearns-Sayre syndrome, respectively.
Is plasticity related gene-1 involved in synaptogenesis?

Plasticity related gene-1 (PRG-1) is a novel member of the lipid phosphate phosphatase (LPP) superfamily and was identified by differential screening of a cDNA library constructed from lesioned hippocampus. Lipid phosphate phosphatases are ectoenzymes, involved in regulating phospholipid levels by dephosphorylation. It is known that signaling via extracellular phospholipids is important in the development and regeneration of the central nervous system (CNS) in the context of cell migration, mitogenesis and neurite retraction. We found that PRG-1 is selectively expressed in the brain and specifically in neurons. Overexpression of PRG-1 in neuronal cell lines, such as NIE-115 cells, renders the neurite resistant to LPA-induced collapse. Our interest centers on characterizing the role of PRG-1 in the CNS during development and after brain injuries. We firstly generated an antibody against a peptide sequence from the cytoplasmatic C-terminus of PRG-1. Our data by Real-time PCR analysis from hippocampus, as well as from neocortex mRNA transcripts, showed a linear increase of PRG-1 expression during development and the highest expression in early postnatal stages. Expression of PRG-1 was reduced in the adult brain. We also confirmed this finding by western blot analysis with the specific polyclonal antibody. Immunohistochemical staining of primary neurons showed the localization of PRG-1 in dendrites and dendritic spines. Our data suggest that PRG-1 plays a role during development and regeneration of the CNS. Further analyses will focus on the intracellular localization of PRG-1 in primary neurons, with a particular focus on colocalization studies using various synapse markers.

Cholinergic deficits after experimental traumatic brain injury

Aim: Traumatic brain injury (TBI) is one of the leading causes of death and disability mainly among young people and often results in significant cognitive impairments. Working memory deficits shown in animal models are believed to be connected with deficits of the cholinergic system, especially of the acetylcholine receptors (AChR). Previous findings from different animal models have shown declines in AChR receptor densities and changes in associated enzyme activities. The present study was conducted to evaluate these findings in one animal model with special attention regarding the time-course of posttraumatic events and critical brain regions. The identification of sensitive targets within the cholinergic system is a precondition for diagnostic neuroimaging in TBI-patients with Positron-Emission-Tomography (PET).

Methods: Three groups of male Sprague-Dawley rats (post-TBI survival time: 2 h, 24 h and 72 h) were anaesthetized and subjected to sham injury (control, n = 8) or controlled-cortical-impact injury (CCI) (n = 8) with 2 mm depth of impact at a velocity of 4 m/sec. Following surgery, animals were antagonized and returned to their homecage with free access to water and food. Following the designated time after injury, the rats were decapitated, brains were quickly removed and frozen in −35°C 2-methylbutan. To quantify the receptor density, 12 μm cryostat brain sections were incubated with 2 nM[3H]-epibatidine, a ligand for nicotinic acetylcholine receptors (nAChR), for 90 min or 8 nM [3H]-QNB, specific for muscarinic acetylcholine receptors (mAChR), for 120 minutes. Non-specific binding was determined in the presence of 300 μM nicotine or 1 μM atropine, respectively. All slides were preincubated with assay buffer (50 mM TRIS-HCl, pH 7.4, 120 mM NaCl, 5 mM KCl, 2.5 mM CaCl2, 1 mM MgCl2) for 15 minutes and experiments were carried out at room temperature. Additionally acetylcholinesterase (AChE) staining was performed using acetylcholiniodide.

Results: A significant decline in the receptor density of the nAChR in thalamus (72 h: TBI 16.2 ± 2.1 vs. sham 18.1 ± 1.8 fmol/mg protein and motor cortex (72h: TBI 7.92 ± 1.05 vs. sham 9.81 ± 1.02 fmol/mg protein) was observed after TBI. The mAChR show a similar, time-dependent reduction in these regions (thalamus 72 h: TBI 90.6 ± 4.9 vs. sham 100.7 ± 13.1 fmol/mg protein) and additionally in the corpus callosum (72h: TBI 33.2 ± 3.5 vs. sham 37.3 ± 4.1 fmol/mg protein) and basal forebrain (72h: TBI 268 ± 29. vs. sham 305 ± 42 fmol/mg protein). Histochemical staining indicates an increase in AChE activity (in terms of optical density) in the basal forebrain 2 h after TBI (120 % increase of optical density compared to control activity). A decreased enzyme activity was found in the hippocampus (85% of control activity).

Conclusion: Cholinergic receptor densities are significantly impaired after experimental TBI, especially in regions associated with attention. Considering the role of the AChR for cognition in the brain, it seems likely that the decline in receptor density contributes to attention and memory deficits as found in behaviourial experiments. We could also demonstrate that experimental TBI induces differences in acetylcholinesterase activity. Therefore it is possible that these changes are related to short-term impairments after traumatic brain injury.

Characteristics of TIA and its management in a tertiary care hospital in Pakistan

Aim: Transient ischemic attack is described as a brief episode of neurological dysfunction caused by focal brain ischemia, with clinical symptoms typically lasting less than an hour, without evidence of acute infarction (2). Recent studies depict TIA as a particularly unstable condition far from being benign (5,22). Risk of stroke is >10% in the first 90 days after TIA (5). The risk increases further with increasing frequency of TIA and concurrent hypertension (23). The
presentation, prognosis and intervention for TIA have not been studied in South-Asians. Studying its risk factors and natural history is important to discern which patient is at a high risk for recurrent TIA or stroke and needs early intervention (24).

Method: A retrospective chart review was done for 158 patients who were admitted with the diagnosis of TIA, as defined by ICD 9 code 435, from January 2003 to December 2005 in Aga Khan University Hospital, Karachi. The data was entered and analyzed in SPSS version 14.0.

Results: Among 158 patients 57.6% were male and 41.1% were female. The common presenting symptoms were motor (51.3%), speech impairment (43%), sensory (34.8%) and loss of balance/vertigo (29.1%). The median delay in presenting to the hospital was 4 hours. Those with motor symptoms were found to present earlier. The study showed that only 60.8% of all the patients presenting with TIA received any immediate treatment out of which 44.7% received aspirin. Radiological modalities were used in 91.1% of the patients. Classification of TIA showed the most common type to be undetermined and under-investigated (24.1%), followed by presumable cardio-embolic (20.3%), large artery atherosclerosis (18.4%), probable lacunar warning syndrome (15.8%) and lastly the truly undetermined type (1.3%). Of all the TIA patients 9.1% converted to stroke.

Conclusion: The natural history of TIA is comparable to international descriptions. A large percentage of patients are still not receiving initial treatment as described in available guidelines; even in a tertiary care hospital.

Effects of memory enhancers on stimulus induced sharp wave-ripples in the hippocampus of adult rat in vitro

ESC-ID 644
Author Liotta A, Behrens CJ, Heinemann U
Country Germany
University Charité Universitätsmedizin Berlin
Department Institute of Neurophysiology

Hippocampal sharp wave-ripple complexes have been observed in vivo during slow wave sleep (SWS) and behavioral immobility (1). They are thought to represent events in which half of their medium was replaced every 3 days (fasting period to eliminate fibroblasts). The culture medium was then replaced by DMEM plus 10% fetal calf serum (FCS). After 10 days Schwann cells labeled against s100 antigen which is specific for committed Schwann cells and then counterstained which Hoechst 33342. The purity of them was 99%. After transferring the purified Schwann cells to the 24-well plates, Irradiation applied daily for 3 days to each well. In our experiment, we have used a 632/8 nm wavelength, 125mW output power continuous wave laser. The output laser beam was directed at the center of the well and created a 25mm(2) spot on the well. Calculations were performed to assess the amount of power able to penetrate through the cell culture media, by determining the amount of power able to pass through the media. The plates were randomly placed in three treatment groups. The first group received 10 seconds of 632/8 nm laser light with a final power of 102 mW, for a final energy density of 4 J/cm². The second group received 2 minutes and 30 seconds of 102 mW, 632/8 nm laser light, for a final energy density of 60 J/cm². The final group was a control group that was treated identically to the other two groups, but wherein the laser was not turned on during treatments. The cells were kept in the dark at all times other than when exposed to laser light. At day 15 in vitro in each group we used Rat NGF Elisa Kit and Rat BDNF Elisa Kit. Also we used primer for NGF and primer for BDNF and cells underwent PCR to assess gene expression. In addition we used colorimetric MTT(dimethylthiazol tetrazolium bromide) kit and cell count for assessment of proliferation rate in cultured cells.

Results: Evaluation of cellular proliferation(colorimetric MTT assay) revealed that the average absorption for the 4 J/cm² group was 0.2173Â±0.01, 0.2045Â±0.01 for the 60 J/cm² group and 0.1507Â±0.01 for the 60 J/cm² group. No
significant difference between the treatment groups (4 J/cm² vs. 60 J/cm²) was found (P > 0.05). Irradiation increased the level of NGF three fold in the first group compared to the control group. NGF concentration was five fold in the second group compared to the control group. In the same way, In PCR we observed five fold increasing gene of NGF in first group and six fold in second group compared to the control group (P Value < 0.05) But about BDNF no differences in concentration and gene expression were monitored in the once irradiated as compared to none irradiated cells.

**Conclusion:** Our study showed that low level laser promotes proliferation rate, NGF secretion and gene expression of schwann cells in vitro but have no effect on BDNF secretion and gene expression. Laser can be used to enhance effect of schwann cell transplantation for neural tissue regeneration or can be used alone for that purposes.

**Febrile convulsions: the influence of positive family anamnesis on appearance time and relapse**

ESC-ID 403  
**Author** Saric S, Djurkovic J, Tomasevic M, Nikolic D  
**Country** Serbia  
**University** University of Belgrade  
**Department** General Medicine

**Heading:** Febrile convulsions (FC) are the appearance of epileptic seizures in the time of baby or childhood and they appear in age of 6 months to 5 years, without persuaded infection of the central nervous system or the other definite reason. The percentage of appearance is frequent in male children. The increased risk of occurrence of FC exist in twins and in families with positive anamnesis for FC on cousins in first and second generation (parents, brothers and sisters, aunts, uncles). Right pathogenetical mechanism of FC genesis is not well known, but there is important genetic condition to development.

**Aim:** The main goal of our research is to establish is there an influence of positive family anamnesis on febrile convulsions' appearance time and the correlation between relapse and positive family anamnesis.

**Material and methods:** Children, 611 of them, treated or controlled in 2006. year on the University Child Hospital in Belgrade are included in this research, on the basis on the ambulance data and the patients history of illnesses. The data processing was done with analytics and descriptive statistic and collected data is showed both with charts and graphics.

**Results:** The gain data show that FC are a little bit frequent in the male children. 184 of children have the positive family anamnesis. The majority of children have 1 seizure. The age of first attack is 22.65 month averagely, but average age of the first attack of children with positive family anamnesis is 19.15 months.

**Conclusion:** The first FC attack is averagely earlier on children with positive family anamnesis. Relapses of FC are occurred frequently in children with positive family anamnesis.
Blood-brain barrier disturbances following hyperthermia in rats with cortical dysplasia

ESC-ID  425  
Author  Akturk K, Gurses C, Kaya M, Kalayci R, Arican N, Ashihali B, Ekizo O  
Country  Turkey  
University  Istanbul  
Department  Faculty of Medicine

Introduction: Seizures disorders frequently occur early in life however the relationship between cortical dysplasia, febrile seizures, and epilepsy is unclear. The goal of the present study was to determine the role of hyperthermia on the integrity of blood-brain barrier (BBB) in rats with cortical dysplasia.

Materials and Methods: In 28 days old baby Sprague-Dawley rats used, BBB permeability was evaluated using sodium fluorescein (NaFlu) dye. Cortical dysplasia was induced by 145 cGy gamma irradiation to the pregnant rats on day E 17. To induce hyperthermia (39.5 ± 0.5°C), baby rats were exposed to an elevated ambient temperature for 30 minutes. Immunohistochemistry for occludin, glial fibrillar acidic protein (GFAP) and c-FOS, and western blot method was used for occludin protein. Electron microscopy was performed to evaluate structural properties of BBB. For this purpose, rats were perfusion fixed and the brains were removed and 1 mm cube tissue samples obtained from hippocampus. The samples were post-fixed in 1% osmium tetroxide for 1 h, then embedded in Epon. Ultra thin sections (70 nm) were stained with uranyl acetate and lead citrate and examined under JEOL 1011 transmission electron microscope.

Results: On the day of the experiment there was no mortality in hyperthermic animals. Blood pressure levels of rats with hyperthermia and rats with hyperthermia as well as cortical dysplasia increased significantly when compared to control values. Seizures the rats had were observed clinically; the longer the duration of hyperthermia was, the more seizures were seen. Both hyperthermia and cortical dysplasia did not change BBB permeability to NaFlu. Hyperthermia induced a generalized increase in BBB permeability to NaFlu in rats with cortical dysplasia and the highest increases (＞130% over control values) were recorded in diencephalon and cerebellum regions. Western blot analysis of brain capillaries showed that the expression of the transmembrane tight junction protein occludin was not changed in response to dysplasia and hyperthermia. Endothelium of BBB in control animals was observed to be ultrastructurally intact. No sign of ultrastructural damage was observed in microvessel endothelium by dysplasia. In cortical dysplasia plus hyperthermia, marked cellular injury edema in glial cells and perivascular vasogenic edema can be seen.

The Conclusion: These results indicate that immature rats are resistant to hyperthermic BBB disruption. However, hyperthermia may induce significant BBB permeability increase under cortical dysplastic conditions. We conclude that cortical dysplasia as an underlying pathology could increase the disruption of BBB integrity during hyperthermia.

Prior probability effects and their inter-hemispheric interactions in human prosaccades and antisaccades

ESC-ID  435  
Author  Gowani SA, Barton J, Fox C, Levin M  
Country  Pakistan  
University  The Aga Khan University  
Department  Medical College

Introduction: Knowledge about the prior probability of target location may be exploited by the ocular motor system to enhance the efficiency of saccadic programming. However, little is known about whether these effects vary with saccadic programming demands or about their spatial organization. Objectives Our first goal was to determine if the effect of prior probability differed significantly between prosaccades and antisaccades. Our second goal was to determine if prior probability effects differed between trials in which two targets were processed by the same cerebral hemisphere or by different hemispheres.

Methodology: We performed two experiments. In the first, we contrasted a low-probability condition (0.125) consisting of eight targets, with two high-probability conditions (0.50) consisting of two targets, one in which the targets spanned the horizontal meridian, and one in which the targets spanned the vertical meridian. In a second experiment we replicated this design and added a third high-probability condition, in which the two targets were confined to a single quadrant. Results: We found that the effects of increased prior probability on prosaccades were minimal, with at most a slight reduction in reaction time. Antisaccades showed a much larger reduction in reaction times and improved directional accuracy, with less reflexive prosaccade errors. Both the directional accuracy and spatial precision of antisaccades were better for targets that spanned the vertical meridian, indicating a between-hemisphere advantage.

Conclusion: We conclude, first, that prior probability effects are larger for the more attentionally demanding antisaccade task. Second, the effects of prior probability on the computation of antisaccade trajectory show an inter-hemispheric advantage, consistent with theories of hemispheric interactions in attentional processing, through either divided resource allocation or selective filtering by the corpus callosum.

The effect of ubiquitin on local immune cell infiltration after focal traumatic brain injury in rats

ESC-ID  437  
Author  Goelz L, Griebenow M, Casalis P, Woicechowsky C, Majetschak M, Thomale U  
Country  Germany  
University  Charité Universitätsmedizin Berlin  
Department  Department of Neurosurgery

Recently, our group was able to show the neuroprotective ability of ubiquitin after focal traumatic brain injury. Although, it has been hypothesized that ubiquitin influences capillary permeability and immune response, the exact mechanism of how ubiquitin exerts its positive effects needs to be further elucidated. Thus, we investigated the effect of ubiqui-
tin treatment on the invasion and activation of immune cells in the brain after Controlled Cortical Impact (CCI). Therefore, male Sprague Dawley rats (n = 27) were exposed to a moderate brain injury using the CCI model (1.5 mm penetration depth, 7 m/s impact velocity, 300 ms contact time).

The animals were randomized to either 1.5 mg/kg ubiquitin or placebo administration intravenously within 5 min after CCI. Gravimetric quantification of brain water content was measured after 24 hours and cerebral contusion volume was determined planimetrically using triphenyltetrazoliumchloride (TTC) staining after 7 days. The 24 hour samples were embedded in Tissue Tek and stored at –80°C, the 7 day samples were kept in Ethanol 96% and then embedded in paraffin. Cuts of 8-10 µm were used for immunohistochemical staining. Antibodies recognizing phagocytic cells - macrophages and reactive microglia- (ED1/CD68 and OX42/CD11b), activated microglia (Ox42/CD11b) and neutrophilic granulocytes (MPO/Myeloperoxidase) were used in frozen and paraffin samples. In addition, His48 was used to stain granulocytes in frozen sections and GFAP (glial fibrillary acidic protein) in paraffin samples to stain astrocytes. To this point, it is still a matter of debate whether or not ubiquitin directly influences immune response after CCI or exerts another mechanism of action. Correlation of invasion and activation of immune cells to the parameters of neuroprotectivity in the brain, such as lower cortical contusion volume and brain water content, will be performed.

**VEGF induced angiogenesis in long-term organotypic cultures of adult rat retina**

ESC-ID 476
Author Thein E, Freyer D, Rzeczinski S, Dinagl U
Country Germany
University Charité Universitätssmedizin Berlin
Department Neurology

We reported a model of longterm organotypic roller cultivation of adult rat retina (Ophthalmic Res. 2006;38:263-9). Retinas are cut into slices of approx. 1 mm² and cultured free-floating in glass flasks on a horizontal rotating roller drum (60 rpm). During culture the flat slices transform into ball-shaped tissue spheres (subsequently termed as retinal bodies, RB). Initially, the RBs contain an inner empty cavity. Within a few days the cavity entirely obliterated and fills with tissue. The system preserves retinal cytoarchitecture and cellular and neuronal interrelationships, including vascular structures, similar to in vivo conditions, which are amenable to neuroprotection studies (Neuropharmacol. 2007;52:1488-95). Neovascularization is the hallmark of proliferative diabetic retinopathy, this system may be suited to study mechanisms of neovascularization in the retina, as well as to test angio- or anti-angiogenic strategies. We have now characterized the dynamics of vascular structures during cultivation. Staining with von Willebrand factor (vWF) was used as vascular marker. vWF+ structures are found in RBs for at least 2 weeks in culture. During the first week, vWF+ vascular structures remain stable and can be found in all retinal layers. On day 14, vWF+ structures are less abundant, and occur only in the ganglion cell layer of the RB. We asked whether angiogenesis can be induced in this system by proangiogenic factors. We tested vascular endothelial growth factor (VEGF, from 0.25 to 25 ng/ml). Exposition to VEGF (Recomb. Mouse; Invitrogen, CA) started on day 2 in culture. RBs were fixed after 7 days, sectioned on a microscope, and stained for vWF by using a polyclonal rabbit anti-human primary antibody and LSAB 2 Systems from Dako (Hamburg, Germany). vWF+ vascular structures were evaluated by separately counting (a) longitudinal sections, and (b) cross sections of the vessels in randomly chosen sections from the center of the RB (n = 10 RBs of 2 different retinas in treated groups; n = 5 RBs of 1 retina in the untreated group). We observed an increase of the cross sections, expressed as sections / µm², in RBs treated with 0.25 ng/ml VEGF (9.1 / µm² ± 1.6, vs 6.3 / µm² ± 2.9 in controls; p<0.05). VEGF applied in dosages of 2.5 and 25 ng had no effect on the number of cross sections of vWF+ structures in the RBs. Concentrations of 250 ng/ml were toxic. We are presently investigating the effect of the hematopoietic cytokine, erythropoietin, on vascular structures in the cultured retinas. We conclude that organotypic cultures of retina can be used to study angiogenic factors in vitro.

**Correlation of lesion volume development using TTC and Nissl staining considering immune cell infiltration following days after focal brain injury**

ESC-ID 484
Author Faber P, Casalis P, Griebenow M, Woiciechowski Ch, Thomale UW
Country Germany
University Charité Universitätssmedizin Berlin
Department Neurosurgery

**Introduction:** Measurement of lesion volume in experimental traumatic brain injury (TBI) is important to determine the extent of secondary brain injury. However, there is still controversy about injury volume development at time points later than 72 hours, measured with energy dependent enzymatic staining (triphenyltetrazoliumchloride, TTC) which might be influenced by immune cell infiltration. In this context we correlated traumatic injury volume development after Controlled Cortical Impact (CCI) using TTC and H&E as well as cresyl violet (Nissl) staining considering the amount of immune cell infiltration by immunohistology (IHC).

**Methods:** prospective randomized experimental animal study - 24 Sprague Dawley rats were divided into 4 groups of different follow ups after focal cortical contusion: 48 hours, 72 hours, 5 and 7 days - Surgery was performed under isoflurane anesthesia and focal brain injury was applied to the intact dura mater with a penetration depth of 1.5 mm on left parieto-temporal cortex using the CCI model - At all time points, mentioned above, we compared TTC method with the H&E, Nissl staining as well as IHC with CD48+ sensitive antibody ED-1. - For TTC staining 1.2 mm sliced sections were prepared in order to examine injury volume by means of 2%TTC in 0.2M Phosphate Buffer pH 7.4 - For H&E, NISSL and IHC staining 10-30µm thick cryo-slices were performed from the same sections.

**Results:** The contusion volume after CCI increases over time. Correlation of different methods to determine lesion volume by TTC, HE and NISSL is still pending. The influence of immune cell infiltration determined by IHC on the contusion volume measurements still needs to be quantified. The successful achievement of this study will be to identify
Parkinson Disease Chronobiological approach with primate-MPTP model

Parkinson Disease (PD) is a multifactorial neurodegenerative disorder that is classically associated with the motor consequences of the nigro-striatal dopaminergic neuronal loss (tremor, rigidity, bradykinesia/akinesia). Initially thought to affect principally the dopaminergic structures, recent evidence suggest the involvement of other catecholaminergic systems in PD [1]. Moreover, neuronal degeneration affects other nervous regions such as the locus coeruleus, brainstem, thalamic and tegmental nuclei [2], structures involved, among other, in the regulation of sleep and circadian rhythms. It is now accepted that cognitive, neuropsychiatric, autonomic, sensitive and sleep/wakefulness disturbances are also a feature of PD. These alterations have been suggested to appear early in the disease [2], preceding the onset of the characteristic motor symptoms, but most of them have never been investigated systematically in appropriate protocols. The aim of our study was to test the hypothesis that alterations of sleep and circadian rhythms appear early in PD, before PD motor symptoms onset. Here we use macaque monkeys chronically treated with low doses (CLD) of the neurotoxin MPTP, a good behavioural and biological model to study the slow evolution of idiopathic PD [3,4]. Sleep structure and rest-activity cycles were recorded continuously before, during and after MPTP-treatment using actimetry (continuous motor activity recordings with infrared passive sensors). Actigraphy data revealed a clear increase of nocturnal motor activity and a reduction of daily activity in MPTP-treated monkeys, mimicking sleep fragmentation and excessive daytime sleepiness observed in PD patients. Those chronobiological alterations of the rest-activity cycle in CLD MPTP-treated monkeys preceded classical clinical signs classically used to diagnose PD. These results suggest that the CLD MPTP primate model can be used to study chronobiological alterations in PD. They also indicate that, in accordance with our hypothesis, an early onset of rest-activity cycle alterations exists in MPTP-treated monkeys, anticipating clinical symptoms. Therefore, chronobiological parameters could be very sensitive to reveal early pathological alterations, as early (but non entirely specific) PD markers. This study is a preliminary to further descriptions of modification of nocturnal sleep structure and evolution of brain activity in presymptomatic MPTP-treated monkeys.

Obstetrics I

Evaluation of antenatal care services in Federal Women Jail, Omdurman- Sudan 2006

ESC-ID 529
Author Dirar KM
Country Sudan
University University of Medical Sciences and Technology
Department Anatomy

Aim: This cross-sectional, facility based-study has been conducted in Omdurman Women Jail, to evaluate the antenatal care (ANC) services, reflected by the prevalence of obstetrical problems in the jail, compared to those in the outer community.

Methodology: All the inmates who were known to be pregnant at the study time (35 out of 591 inmates) were interviewed, and followed throughout their routine ANC general and obstetrical examinations. The obtained data were then analyzed and compared to those applied in the open community.

Results: 35 pregnant women were covered by this study, 20% were teenagers, and 80% between 20-35 years. They were all known to be pregnant before they go into jail. Concerning obstetrical history: primigravidae represented 11.4% and grand multiparae were 40%; hence both having obstetrical risk, they are going to give birth at Omdurman Maternity Hospital. Subjects usually don’t undergo an ultrasound unless strongly recommended by the unit’s doctor. 80% had no previous history of abortion, 11.4 % had had aborted once and 8.6% had history of repeated abortions. Causes of the abortions were not investigated, but the majorities were attributed to maternal febrile illnesses. Three different readings for the blood pressure were taken for each subject, and they all were within normal range. Haemoglobin estimations showed that 97.1% had variable degrees of anaemia; 40% had haemoglobin readings less than 10 g/dl, although they were on oral iron supplements.

Conclusion: Age representations, social and educational levels of the sample resemble to a great extent the national distributions; and hence results were representative for a homogeneous subpopulation. Pregnancy complications were found to be minimal and almost entirely absent; this is a good indicator of the Antenatal clinic performance within the facility; however, crucial follow-up methods (ultrasound) are not available, thus many important indicators for fetal wellbeing are missed.

Perinatal outcome in twins

ESC-ID 274
Author Aleksovska O, Aleksovska A, Zisovska E
Country : Macedonia
University "St. Cyril and Methodius" University, Medical Faculty
Department Clinic for Gynaecology and Obstetrics

Heading: Twins are siblings carried together in the womb and born at the same time. Similarities and differences between twins can be used to answer questions about the role
The assessment of clinical significance of inter-twin birth weight discordance. As risk of incidence of multiple pregnancies will be increased due to assisted reproduction and prenatal care improvements, the antenatal care practices have to be designed to ensure safe and closer to the term deliveries of the twin pregnancies. Twin studies are very important because they provide a unique approach to investigate the determinants of a disease or condition. However, the twin study method, in combination with other approaches, can be a powerful tool for revealing the causes of disease.

The influence of inter-twin growth discordance on foetal outcome

ESC-ID  790
Author  Labédzka M, Godziewicz T, Kierzkowski B, Ropacka M
Country  Poland
University  Poznan University of Medical Sciences
Department  Department of Perinatology and Gynaecology

Aim: The assessment of clinical significance of inter-twin birth weight discordance.

Material and Methods: Twin intra pair birth discordance analysis was conducted in a group of 357 patients in twin pregnancy admitted to ObGyn Hospital in Poznan in 1998-2006 year. Inter-twin growth discordance has been defined on the basis of birth weight differences, expressed as percentage of larger twin birth weight. The threshold for clinically significant discordance has been defined as 15% and above. The differences, in question, were arranged into four sub-groups: <15% (n = 105, 29%), 15-24,9% (n = 150, 42%), 25-34,9% (n = 60, 17%), & ≥35% (n = 42, 12%). Twin pregnancies were divided into 4 sub-groups according to inter-twin growth discordance. Twins with birth weight discordance below 15% formed the control group. The remaining sub-groups (&≥35%) have been compared with the control one. There were Shapiro-Wilk, Mann-Whitney U, Pearson Chi-square or Yates Chi-square tests used for statistical analysis.

Results: There were no statistically differences in mothers' age and gestational age at the delivery between the all study sub-groups and the control one. The parity had no meaning for the birth weight discordance. There was a large percentage of c-sections in the women with above 35% weight discordance between the babies in compare with the control one (97,56% vs. 78,64%, p = 0,01). In the above 35% weight discordance group the condition of the newborn (assessed with the Apgar score) was significantly worse. Foetal asphyxia has occurred significantly often in above 35% weight discordance group in compare with the control one (56,1% vs. 11,5%, p<0,0001 for smaller twin, 39% vs. 12,5%, p = 0,002 for larger twin). There were statistically significant differences in pH of umbilical vessels' blood of smaller twin between all study sub-groups and the control group. The gasometric parameters were statistically significant getting worse in conjunction with increasing of discordance. There were statistically significant differences in birth weight of smaller twin between all study sub-group and the control group. There were statistically significant differences in percentage of babies with birth weight below 1000 g and 1500 g between the group with discordance above 35% and the control one.

Conclusion: Inter-twin birth weight discordance correlates with abnormal foetal outcome. The degree of inter-twin birth weight discordance is crucial for foetal prognosis. As far as inter-twin discordance above 35% was concerned the most severe complications were observed. It was impossible to estimate twin-to-twin transfusion syndrome occurrence on the basis of collected data.

Postpartum outcome in premature parturition

ESC-ID  39
Author  Markovic M, Jelenkovic O
Country  Serbia
University  University of Nish
Department  Medicine

Introduction: Pregnancy is a very exceptional condition in generative age of women. Duration of pregnancy normally is 9 months long which is the same as 40 weeks or 280 days. Termination of pregnancy after 28 weeks of gestation is called parturition. Whether is pregnancy terminated before or after 28 weeks of gestation, parturition can be premature, on time or postponed, and infant can be premature, in term or postterm infant. Enormous part of premature infants are in danger of postpartum complications which are mostly caused by infections. Most frequent diseases among premature infants are RDS, ICH, perinatal asphyxia, hyperbilirubinaemia, hypoglycaemia and icterus. Extremely rarely only one disease is present, in most cases they are associated.
**Methods:** The aim of this study is to research postpartum outcome among premature infants through neonatal morbidity and perinatal mortality in correlation with weight of infant, AP and week of gestation.

**Material and method:** In this study we have prospectively collected data about postpartum outcome among premature infants at GAC in Nī from January till December in 2006 year. We examined 120 premature infants delivered from 24 to 36 weeks of gestation and their postpartum outcome in correlation with weight, AP and week of gestation.

**Results:** Among 120 premature infants, 10 of them were still-born, 8 had neonatal death, 97 got ill and only 5 of them had no complications. For statistically elaboration we used Student t-test for finding correlation between neonatal morbidity and perinatal mortality with weight of infant, AP and week of gestation. Comparison of morbidity and week of gestation showed that there was significant correlation (p = 0.023; p < 0.05). Hig values of morbidity are present from 33 to 36 weeks of gestation (t = 2.41; p = 0.02; p < 0.05). Between morbidity and AP was not found statistically significant correlation (p = 0.603; p > 0.05), but between mortality and AP was found statistically significant correlation (p = 0.012; p < 0.05). When we compared weight of infant we have not found statistically significant correlation, but in analysis with mortality we have found statistically significant correlation (p = 0.01; p < 0.05). Infant with birth weight lower than 1500 gr showed major mortality.

**Conclusion:** This study has shown us a very high values of morbidity in group from 33 to 36 weeks of gestation, and very high values of mortality in group from 24 to 28 week of gestation, birth weight less than 1500 gr and values of AP from 1 to 3.

**Ultraslow potentials as a predictor of severe preeclampsia**

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<td>Author</td>
<td>Popkov OV</td>
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<tr>
<td>Country</td>
<td>Ukraine</td>
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<td>University</td>
<td>Donetsk State Medical University</td>
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<td>Obstetrics and Gynecology</td>
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**Aim:** to disclose the role of ultraslow potentials as a diagnostic method for severe preeclampsia. To uncover their value as a predictor of its worsening. Background: Severe preeclampsia endows substantially maternal and perinatal morbidity and mortality. Opportune prediction permits to avoid complications. Ultraslow potentials of human brain as biochemical and neurophysiologic phenomenon allow to indicate the moment of emaciation and areactivity in patients with preeclampsia.

**Methods:** Group of 936 women (291 women throughout normal pregnancy and 645 women who had high risk of development of preeclampsia) was included [2]. Preeclampsia and subgroup of severe preeclampsia were defined according to generally accepted criteria [1]. The ultraslow potentials (USP) registration was conducted with high-resistance intensifier in leads forehead-thear. Terms of registration: 20 weeks of gestation, first signs of preeclampsia, third day of intensive care and first and fifth days after delivery. Diagnostically evident criteria: the initial level of USP, level of USP after functional test [4, 5].

**Results:** severe forms of preeclampsia occurred in 7.3% of study patients (n = 47). The initial levels of USP in patients with severe preeclampsia were lower for sure than in groups of women with mild forms and normal pregnancy (P < 0.001). Non-parametric analysis showed that women with severe preeclampsia manifested later had significantly less expressed USP reaction in respond of functional test (P < 0.01). In 15 women (32%) we registered USP even getting positive. According to early made investigations [3,5], clinical data and outcomes this kind of USP curve was recognized as inauspicious. Those patients also formed basic mass in a group with complications and high perinatal death rate. At the same time, those 15 patients formed the most part (88.2%) of women who had USP getting positive at the first registration in 20 weeks of gestation.

**Conclusion:** 1. The registration of ultraslow potentials can be used as a diagnostic instrument in women with preeclampsia.
2. The reaction of ultraslow potentials in respond of functional test is the most diagnostically evident criterion, which may be used as a predictive test.
3. Women who had prediatively unfavorable types of curve at the first time of registration had the highest risk of development of severe preeclampsia in the future.

**Association of C196T ITGB3 and I/D PLAT gene polymorphic variants with recurrent pregnancy loss**

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<tr>
<td>Author</td>
<td>Shevchenko KG, Malysheva OV</td>
</tr>
<tr>
<td>Country</td>
<td>Russia</td>
</tr>
<tr>
<td>University</td>
<td>Saint Petersburg State University</td>
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<td>Department</td>
<td>Biology and Soil Science</td>
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**Introduction:** Single nucleotide polymorphism C196T in ITGB3 gene and insertion/deletion (I/D) polymorphism of PLAT gene are the common risk factors reported to date for trombophilias, what can lead to spontaneous abortion (SA). The aim of this study was to find out whether these gene polymorphisms are associated with pregnancy loss or not.

**Methods:** Genotyping was performed by PCR-PLRF method in 192 women with idiopathic miscarriage before the 20th week of gestation, divided into three subgroups: 61 with one, 68 with two and 59 with three or more SA. 108 women with the live birth and without a history of pregnancy loss comprised the control group.

**Results:** No significant differences were observed between distributions of any studied genotypes both in group with miscarriage and control group. Therefore, the PLAT genotypes frequencies significantly differed in subgroups with the different numbers of SA. In the subgroup with the history of a single SA D/D genotype occurred with the frequency 18.0%, whereas in the subgroup with the history of two or more SA it was found in 35.5% of all cases (P = 0.0254; OR = 2.410). The frequency of the D/D genotype in group with 1 SA also significantly differed from the frequency in the control group C 34.3% (P = 0.0326; OR = 0.4222). The statistically significant differences of the PLAT genotypes frequencies between the groups with two or more SA and control group were not observed.

**Conclusion:** ITGB3 and PLAT gene polymorphisms are not associated with pregnancy loss themselves. However, in case of the history of SA, the D/D genotype of PLAT gene is associated with recurrent spontaneous abortions (fetal loss). Our results indicate that the genetic reasons for recurrent miscarriage and single SA may be different.
Vaginal misoprostol for medical evacuation of early pregnancy failure

Objective: To evaluate the efficacy of vaginal misoprostol in bringing about evacuation of uterus in early pregnancy failure.

Introduction: Early pregnancy failure (also known as missed abortion or blighted ovum) is a common problem in obstetrics and gynecology. Approximately 10% to 20% of clinically recognised pregnancies are diagnosed as non-viable in the first and second trimesters. Treatment of early pregnancy failure usually consists of waiting for spontaneous expulsion or surgical evacuation. Expectant management has the disadvantage of uncertainty as to the timing of expulsion. Surgical methods have attendant complications like hazards of anesthesia, cervical laceration with the possibility resulting in incompetent cervix, uterine perforation, hemorrhage, infection, Asherman’s syndrome etc.

Methods: A prospective observational clinical trial was carried out on 60 patients admitted with missed abortion or blighted ovum of less than 14 weeks gestation during the period from October 1999 to July 2001. Four hundred micrograms of misoprostol was placed into the posterior vaginal fornix and the patients were examined 24 hours later. If there was no response, i.e., no expulsion of products of conception the same dose was repeated after 24 hours. The primary outcome was complete expulsion of uterine contents within 24 hours of initial or repeat misoprostol. Side effects were also noted.

Results: Out of the 60 patients, 46 (76.7%) expelled their products completely with misoprostol, out of whom 43 (71.7%) expelled with single dose administration thus showing that the insertion of the second dose of misoprostol was not of much use in bringing about expulsion in cases who did not respond to the first dose. The mean insertion expulsion time was 9.7 ± 5.6 hours. Even in the failed group of 14 patients (23.3%), 10 (71.4%) showed favorable cervical changes, which made subsequent evacuation much easier without dilatation.

Conclusion: The use of 400 mg of vaginal misoprostol for early pregnancy failure resulted in complete expulsion of the products of conception in 71.7% of the patients after single dose and in 76.7% after two doses thus reducing the need for surgical treatment. When complete drug induced expulsion did not occur within 24 hours of the second dose of misoprostol, the cervical ripening property of misoprostol helped in performing surgical evacuation without dilatation.

Influence of a ureaplasmosis on current of pregnancy and its outcomes

Absence of a common opinion about a degree of the importance of ureaplasmas in development of pathological conditions of urogenital tract causes the big interest to the given infection, that, undoubtedly, is an actual problem of modern obstetrics. It can cause infectious-inflammatory processes in the organism of mother and fetus. Purpose: to define features current of pregnancy, labours, the early neonatal period and morphological changes of placenta at women with Ureaplasma infection.

Materials and methods: The retrospective analysis of labours of 60 women from Gomel Maternity Hospital. The basic group - 30 women with Ureaplasma infection, comparison - 30 women without such infection. At all patients the somatic, obstetrics-gynecologic, reproductive anamnasis and current of pregnancy, labours and postnatal period is analysed, current of early neonatal period at newborns and placentas are investigated. Ureaplasma infection has been diagnosed with the bacteriological method of research. Statistical data processing is carried out with the help of spreadsheets Excel of package Microsoft Office 2003, programs Statistics 6.0.

Results: Middle age of women in main and comparison groups authentically did not differ and has made 27.6 ± 5.9 and 26.6 ± 6.1 year accordingly. The pathology of excretory system revealed twice more often at patients with a ureaplasmosis (26.7 ± 8.1% against 13.3 ± 6.3% in comparison group). In the basic group statistically significantly is more often (\(p = 0.0076\)) one medical abortion 10 (33.3 ± 8.8%) against 1 (3.3 ± 3.3%) in the basic group. In the anamnesis at women of group of comparison, met relapsed nonspecific colpitis - 30.0 ± 8.4%. At half of women from the basic group pseudo-erosion colli uteri is revealed. Chronic salpingoophoritis with identical often met at women of both groups. In the anamnesis at women of group of comparison statistically significantly is more often (\(p = 0.0153\)), than in group of comparison, met relapsed nonspecific colpitis - 30.0 ± 8.4%. At half of women from the basic group pseudo-erosion colli uteri is revealed. Chronic salpingoophoritis with identical often met at women of both groups. In the anamnesis at women of group of comparison statistically significantly is more often (\(p = 0.0076\)) one medical abortion 10 (33.3 ± 8.8%) against 1 (3.3 ± 3.3%) in the basic group. In the anamnesis at women of group of comparison statistically significantly is more often (\(p = 0.0301\)) were menacing premature birth: 26.7 ± 8.2%. Statistically significantly is more often (\(p = 0.0314\)) at women with ureaplasmosis USD there was a pathology of amniotic fluid volume (20.0 ± 7.3%). Women of the basic group in 3 times had a weakness of patrimonial activity is more often (20.0 ± 7.3%). Congenital pneumonia was diagnosed at 10.0 ± 5.5% newborn from women of the main group. Statistically significantly is more often (\(p = 0.0301\)) at women of the basic group has been diagnosed hypoplasia of placenta-26.7 ± 8.1% cases. Placentae of women from basic group statistically significantly is more often (\(p = 0.0326\)) are observed petrifaction (36.7 ± 8.8%).

Conclusion: Ureaplasma urealiticum is found out in women with relapsed nonspecific colpitis. Pregnancy at women with a ureaplasmosis proceeds with menacing spontaneous abortion, and pathology of amniotic fluid volume. There are the
hypoplasia of placenta and petrifaction (as a possible outcome of a chronic inflammation) in the placentas of women with Ureaplasmosis.

Effects of nutrition’s carbohydrate content on the metabolism of pregnant women by means of nutritional diary, indirect calorimetry and blood glucose measurements in relation to their carbohydrate tolerance

Objective: Since recommendations for nutrition in pregnancy are varying widely (1) we assessed the effects of two diets differing in percentages of carbohydrates (CH). We measured resting energy expenditure (REE), respiratory quotient (RQ) and proportional use of macronutrients by indirect calorimetry (IC) as well as blood glucose. Women’s carbohydrate tolerance was determined by oral glucose tolerance test (oGTT) applying O’Sullivan’s criteria. (2)

Method: 52 pregnant women in gestational week 30 ± 3.1 were asked to eat low (<35%) and rich (>55%) in CH for a week each in a randomized order. Total energy intake (TEI) was recorded by nutritional diary. During two days blood glucose profiles were obtained by home glucose meters. IC was carried out after an overnight fast as well as oGTT. Res.: Considering all 52 study participants there was a difference in nutritional CH consumption of 39 and 49% in the low-CH and high-CH week respectively (p <0.001). TEI differed significantly as well (1809 vs. 2137 kcal/d, p <0.001). While total amounts of CH were lower in the low-CH week (169 vs. 256 g/d, p <0.001), consumption of fats (84 vs. 83 g/d) and proteins (88 vs. 85 g/d) didn’t differ. REE didn’t change (2029 vs. 2057 kcal/d) whereas IC revealed a lower RQ (0.75 vs. 0.80, p <0.001) and proportional use of CH (17.4 vs. 29.8%, p <0.001) in the low-CH week. The mean daily blood glucose was lower as well (91 vs. 97 mg/dl, p <0.001). Regarding the 18 women diagnosed with GDM differences between symptoms of antenatal maternal depression and anxiety as well as on scores on the EPDS in the third trimester of pregnancy versus BMI of neonates (r = 0.24, p = 0.03 and r = 0.22, p = 0.05, respectively). Woman who scored 12 or more on the EPDS in the third trimester of pregnancy as well as woman who scored 50 or more on the STAI in the third trimester of pregnancy delivered neonates with significantly higher BMI than woman who scored less than 12 on the EPDS and less than 50 on the STAI in the first, second and third trimesters of pregnancy. After these woman delivered a birth we analyzed 102 birth delivery medical case histories and obtained information about birthweight, height, Apgar scores of neonates and calculated Body Mass Index (BMI) of neonates.

Results: There was positive and significant correlations between scores on the EPDS in the third trimester of pregnancy as well as on scores on the STAI in the third trimester of pregnancy versus BMI of neonates (r = 0.24, p = 0.03 and r = 0.22, p = 0.05, respectively). Woman who scored 12 or more on the EPDS in the third trimester of pregnancy as well as woman who scored 50 or more on the STAI in the third trimester of pregnancy delivered neonates with significantly higher BMI than woman who scored less than 12 on the EPDS and less than 50 on the STAI in the third trimester of pregnancy (14.6 ± 1.5 vs. 13.2 ± 1.2, p = 0.013 and 14.0 ± 1.4 vs. 13.2 ± 1.2, p = 0.02, respectively).

Conclusion: Symptoms of antenatal maternal depression and anxiety are associated with higher BMI of neonates. Woman with clinically significant symptoms of depression and/or symptoms of anxiety have delivered neonates with significantly higher BMI when compared to woman without symptoms clinically significant symptoms of depression and/or symptoms of anxiety.
Cesarean section delivery trends now and ten years ago

ESC-ID  160
Author  Fazlic H, Matijevic R
Country  Croatia
University  University of Zagreb, Medical School
Department  Obstetrics

Surgical termination of pregnancy by cesarean section, the rates of which are increasing, carries greater risk for mother compared to the vaginal delivery, without fully evident benefit for the newborn. The aim of this paper was to observe the trends of frequency and main indications of pregnancy termination by cesarean today and ten years ago, and to estimate the influence of trends of this frequency on perinatal mortality and morbidity. The method of conducting this research was retrospective analysis of all the deliveries by cesarean in tertiary referral center in 2006 and ten years before, in 1996. The total number of deliveries in 1996 was 3116, out of which 352 (11.29%) were performed by cesarean, while in 2006 the total number of deliveries was 3079, out of which 701 (22.76%) were performed by cesarean. Perinatal mortality reduced from 14.2% in 1996 to 7.39% in 2006. In 1996 the most common cesarean section indication was fetal hypoxia 72/352 (20.17%), while in 2006 its rate dropped 119/701 (16.97%), becoming the third most common indication. Apgar score after 5 minutes was statistically well higher in 2006 (Mann-Whitney test, z = 13.5, p <0.001). In 2006 the most common indication was the fetopelvine disproportion 150/701 (21.39%), while in 1996 the third most common 56/352 (15.90%). The newborns delivered by cesarean in 2006 mainly weighed more than those delivered by cesarean in 1996 (weight median in disproportion group in 2006 was 3770 g, weight median in group without disproportion in the same year was 3250 g, weight median in disproportion group in 1996 was 3650 g, weight median in group without disproportion in the same year was 3000 g, Mann-Whitney test, z = 5.33, p <0.001). Comparing the birth weight in groups with disproportion as indication for cesarean and groups without this indication in both years, we discovered statistically great difference in birth weight in groups with disproportion (weight median in disproportion group in 2006 was 3770 g, weight median in disproportion group in 1996 was 3650 g, weight median in group without disproportion in 2006 was 3250 g, weight median in group without disproportion in 1996 was 3000 g, Mann-Whitney test, z = 5.33, p <0.001). Prior cesarean was the fourth most common indication in 1996, 59/352 (16.76%) as well as in 2006, 110/701 (15.69%). Vaginal deliveries after a prior cesarean in 2006, 55/2764 (1.98%) are somewhat less common than the same vaginal deliveries in 1996, 42/2378 (1.77%), while the number of cesarean after a prior vaginal delivery increased, 119/701 (16.97%) in 2006, 66/352 (18.75%) in 1996. Breech presentation as indication for the cesarean did not change much comparing the two years in question, 66/352 (18.5%) in 1996, 127/701 (18.11%) in 2006, while the number of newborns in breech presentation delivered vaginally decreased, 55/2764 (1.98%) in 1996 and 14/701 (0.59%) in 2006. The rate of gemini delivered by cesarean did not change significantly, 24/701 (3.42%) in 2006 and 11/352 (3.12%) in 2006. The frequency of pregnancy termination by cesarean increased significantly in 2006 in comparison with the year 1996. This increase possibly influenced the decrease of perinatal mortality between the two years in question, but this was not the only cause. The indications did not change greatly, except for termination of breech presentation pregnancies becoming more and more common, in accordance with current world guidelines.

Cesarean section - multidimensional analysis

ESC-ID  438
Author  Zalech-Rechnio A, Kuran J, Modzelewski J, Grêda M, Staniak M, Molga E, To P
Country  Poland
University  Medical University of Warsaw
Department  II Department of Obstetrics and Gynecology

Background: The cesarean section rate is steadily increasing but it is still an operation putting patients at risk of many complications.

Aim: To analyze the indications, circumstances and fetal and maternal outcome in elective and emergency cesarean sections.

Material and methods: This is a retrospective study conducted by examining 279 obstetrical records of patients with singleton pregnancy, delivered in the Second Department of Obstetrics and Gynecology, Medical University of Warsaw. Results: There were 53.8% emergency and 38.7% elective cesarean sections (cs). The remaining 7.5% were cs on patients with elective indications that had to be operated on in emergency circumstances. The main indication for emergency cs were fetal distress (50.8%), arrested labour (25.8%), placenta abruptio and hemorrhage (8.8%). Elective cs was performed because of: cephalopelvic disproportion (38%), previous operations on uterus (14.8%), ophthalmological indications (13%) and other maternal diseases (10.2%). There was no significant difference in estimated blood loss between emergency and elective cs (622.8 vs. 600 ml). It was, however, significantly higher in cs due to placenta abruptio and hemorrhage (1333.3 ml) and placenta previa (875ml). Fetal outcome was dependent mainly on gestational age at the time of operation and less on the indications themselves. It is worth pointing out, however, that 30.4% of elective cs was performed prior to 36gestational weeks (gw), while 96.3% of elective cs took place after completed 35 gw. When HELLP and eclampsia were the indications, the cs was conducted significantly earlier (32.8gw). Low (≤ 7) 5th minute Apgar score (10% of the whole group) correlated with following indications: fetal distress (57.1%), placenta abruptio and haemorrhage (17.9%), preterm labour (10.7%), HELLP and eclampsia (7.1%) and fetal anomalies (7.1%).

Conclusion: 1. The majority of performed cesarean sections were emergency procedures. 2. The main indications for cs were: fetal distress, arrested labour, cephalopelvic disproportion, placenta abruptio, HELLP and eclampsia. 3. Gestational age correlates highly with fetal outcome.
Pain during labour is considered to be more intense than most acute and chronic pain syndromes. 98% of parturients suffer pain of diverse severity, still the pain, suffered by each of them is different and not all of parturients want to take labour analgesia. Nowadays parturient’s request is an indication for labour analgesia while pain control is an important factor of satisfaction with labour. Aim of study: to evaluate the severity of labour pain and to find factors having influence on it.

Materials and methods: questionnaire inquiry was performed in January – March 2007 in the Clinic of Obstetrics and Gynaecology of Vilnius University. Inquiry consisted of 2 stages - before and after delivery. Inclusion criteria: full-term single pregnancy, absent regular uterine contractions, age of 18. Expected and experienced pain was evaluated using visual analogue scale (VAS), values are given in VAS points. There were analyzed only questionnaires filled by women who delivered naturally. Statistical analysis performed using SPSSv.15.0, tests used include independent sample T-test, paired sample T-test, ÷2, Pearson’s correlation.

Results: 119 questionnaires fulfilled the selection criteria (response rate 90.2%) 73 women whereof delivered naturally (61.3%). The mean age of parturients 27.8 ± 5.6 yrs. Their expected labour pain was 7.2 ± 1.9, experienced 8.1 ± 1.8 (p <0.05). Primiparas experienced the pain similar to their expected (7.4 ± 1.9, experienced 8.0 ± 1.8, p = 0.17), but women delivering repeatedly experienced worse pain (expected 7.0 ± 1.9, experienced 8.1 ± 1.8, p <0.05). Husband’s or boyfriend’s participation did not have any influence on pain intensity: labour pain with husband participating 7.9 ± 1.7, without 8.3 ± 1.9, p = 0.45. Women who attended special classes during pregnancy expected the pain of 7.1 ± 2.1 points but experienced 8.6 ± 1.6, p <0.05. Whomen who did not attend them expected 7.2 ± 1.9 and experienced 7.9 ± 1.9, p <0.05. There is no significant correlation between the intensity of experienced pain and knowledge about labour (r = 0.01, p = 0.9), between the intensity of experienced pain and readiness for delivery (r = 0.2, p = 0.1).

Conclusions: The pain experienced during labour (labour pain) is worse than expected; it does not depend on the knowledge about labour, readiness, participating family members.

Introduction: Monochorionic twin pregnancies complicated by severe twin-twin transfusion syndrome before 26 weeks of gestation are associated with high risks of fetal loss, perinatal death, and subsequent handicap in the survivors. The prognosis for untreated severe TTTS is dismal, with perinatal mortality rates of up to 90%. This condition can now be treated by either serial amnioreduction or endoscopic laser coagulation of the vascular anastomoses responsible for fetal transfusion. The Eurofoetus consortium compared the results of serial amnioreduction and laser coagulation and showed that 6-month survival rate of at least one of the twins was significantly higher in the laser group (76.4%) than in the amnioreduction group (51.4%). Twin reversed arterial perfusion sequence (TRAP) is the most extreme form of inter twin transfusion. The perfused twin is non-viable, while up to 50% of pump twins die perinatally, either from hydrops or prematurity. Objective of this study was to determine the strategy in following monochorionic twin pregnancies, and to work on the endoscopic operating technique in TTTS and TRAP sequence.

Methods: Between March 2005 and May 2007 we performed intrauterine surgery in 10 pregnant women before 26 weeks of gestation. Endoscopic laser coagulation of the vascular placental anastomoses was performed (in 6 cases) and bipolar cord coagulation of the abnormal twin (in 4 cases). The control group of 12 patients was made up retrospectively since 1998 to 2005. No treatment modalities were applied in this group. The groups were compared with the use of the t-test, proportions were compared with the use of the &#967;2 test.

Results: At inclusion, the groups were similar with respect to demographic, clinical, and ultrasonographic characteristics. In both groups, the majority of fetuses were classified as Quintero stage 3. Coagulation of the communicating vessels and cord occlusion was successful in all cases. The neonatal survival rate was higher in the 1st group than in the 2nd group (43.75% vs. 37.5%, p >0.5 ). The rate of intrauterine fetal death in the 1st group was lower than in the 2nd group (31.25% vs. 45.83%, p <0.5). Among the live-born infants, the median gestational age at delivery in the 1st group was older than in the 2nd group (36.4 ± 2.0 weeks vs. 32.1 ± 2.5 weeks, p >0.2), and the median birth weight was also higher in the 1st group than in the 2nd group (2530 ± 844 g vs. 1604 ± 523 g, p>0.5). All the survivors were developing normally at a median age of 12 months (range, 7 to 18).

Conclusion: Our preliminary experience suggests that: 1) Perinatal survival rate after endoscopic treatment of TTTS and TRAP sequence is higher than in untreated group. 2) Maximum prolonging of pregnancy is currently the best method in improving perinatal survival rates. 3) Endoscopic laser coagulation of the vascular placental anastomoses or bipolar cord coagulation can be effectively used in achieving this aim.
Artificial miscarriage and contraception at young and dispensary work for women health protection

ESC-ID 752
Author Bojan S, Sladjana M
Country Serbia
University Pristina University
Department General

Introduction: Frequency of spontaneous abortions is analyzed in period of adolescence on Gynecology Department, Hospital Center Kosovska Mitrovica during the period of nine years.

Aim: Aim of work is to show significance of Dispensary work for women protection in group-pedagogic work with adolescents, in purpose to reduce number of abortions, and unwanted pregnancies using contraception.

Material and methods: It is executed the analysis of number of artificial interrupted pregnancy at early age population before and after beginning of group work with adolescent.

Results: Results shows that group work with adolescents represents good model in reproductive health protection in young, it comes to reduced number of unwanted pregnancies and abortions, and improvement of health in global.

Ophthalmology I

3T3 feeder layer is essential for establishing the primary culture of cornea’s epithelium, a rabbit

ESC-ID 360
Author Jundzis M, Futyma M, Pokrywczywska M, Jundzis A, Jasinski M
Country Poland
University Collegium Medicum UMK in Bydgoszcz
Department Tissue Engineering Unit of the Medical Biology

Introduction: The methods of tissue engineering are being more frequently used in surgical reconstruction of the cornea. The aim of this work was to compare two methods of primary raising of rabbits’ cornea epithelium: at the feeder layer of 3T3 and without it’s.

Material and method: The feeder layer consisted of 3T3 fibroblasts cultivated in DMEM/ Ham’s F-12 (PAA) supplemented with 10% (PAA) fetal bovine serum with addition of antibiotics. Cell proliferation was stopped at 75% surface covering of the flask during 2 hours incubation with a $6 \times 10^5$ g/ml concentration of a mitomycin C (Kyowa) medium raised at a temperature of 37 degrees Celsius. 4 rabbits’ eyeballs were used in experiment. 1-2 mm2 tissue samples were taken from the area of cornea’s rim. The cornea's epithelium cells were isolated with a 0.05% Trypsin- and 0.91% EDTA- solution. The cornea's epithelium cells were cultivated at flask covered with feeder layer (METHOD I) and at bottles without a feeder layer (METHOD II). Epithelial cells were cultivated in DMEM/ Ham’s F-12 with 10% FBS with addition of EGF, insulin, hydrokortisone, trijodthyronine (Sigma) and antibiotics (PAA) at a temperature of 37 degrees Celsius at the 5% CO2 atmosphere. The cells were passaged when bottom of the flask was covered in 70-90%. Cells were stained with Pancytokeratine (Dako) to confirm epithelial character of the culture.

Results: 6 primary cultures were established. Cells in the culture without feeder layer were small and had a spindle-like elongated shape. No cell division figures were observed. The cultures after 1 week looked like cultures which mitotic potential was exhausted. The cells cultivated without a feeder layer could not have been passaged. Cells growth on feeder layer had an epithelial-like morphology. After 1 week 90% of the area was covered with cells. Culture established according the first method retained the morphology like epithelial after three passages.

Conclusion: A different morphological appearance of the cornea's epithelium cells was observed depending on the applied method. The results confirm the positive influence of the 3T3 feeder layer on raising the primary cultures of the rabbits’ cornea’s epithelium cells.

The immune system role in pathogenesis of glaucoma

ESC-ID 692
Author Narmin A
Country Azerbaijan
University Azerbaijan Medical University
Department Pediatrics

Glucoma is multi-dimensional disease with increasing intraocular pressure (IOP). In some patients with low IOP can develop glaucoma but some patients with high IOP don’t have glaucoma. That is why we propose that in the genesis of glaucoma immune system has a main role. The alteration can influence to this disease in 2 ways. First the damage can occur directly by antibodies and the second by the death of neurons. The second can occur because of high IOP, ischemia or some drugs which destroy nerve cells of eye. The aim of this study was to learn immunological status of patients with glaucoma.

Materials and Methods: In this study 37 patients with primary open angle glaucoma (POAG), normal pressure glaucoma (NPG) and 17 control patients were included.

Results: Immunodetection in patients with POAG and NPG show that level of cytokine IL-6, IL-10 were higher than in control group. Also we checked heat shock proteins in POAG and NPG groups and saw that autoantibodies to heat shock proteins were higher than in NPG than in POAG.

Conclusion: Received analysis detected high immune inflammation factors which could provide further information about possible autoimmune mechanisms in the pathogenesis of glaucoma.
**Orthopedics and Traumatology I**

**Feasibility of PET-guided intensity modulated radiation therapy (IMRT) with dose escalation in the treatment of head and neck squamous cell carcinoma**

**Background and purpose:** Head and neck cancer is the 5th most common malignancy in Flanders, affecting in 1953 males during 2000 and 2001. [1] Classically, treatment consisted of radical surgery and postoperative irradiation, whereas radical radiation was reserved for medically inoperable patients. The last decades much interest is being put in organ preservation combined modality treatment, in order to improve survival rates.[3] Nevertheless recent progress in treatment of head and neck malignancies, treatment is still far from optimal. Concerning radiation therapy, the limiting step is acute toxicity during treatment, with subsequent stop of the study. One patient died of toxicity was scored during treatment up to 1 month after treatment insights and/or chemotherapeutic agents might reduce acute toxicity without increasing local recurrence rates.

**Conclusion:** Feasibility of PET-guided intensity modulated radiation therapy (IMRT) with dose escalation in the treatment of head and neck squamous cell carcinoma.

**Methods:** 41 patients with proven cancer of the oropharynx, hypopharynx or larynx were included in the dose escalation trial; 23 in the 1st level and 18 in the 2nd level. Dose was escalated to 72.5 Gy in the 1st level and to 77.5 Gy in the 2nd level. Treatment consisted of two phases; phase I consisted of 10 fractions with dose escalation (2.52 in level 1, 3 Gy in level 2). Phase 2 consisted of 22 fractions of 2.16 Gy. Acute toxicity was scored during treatment up to 1 month after treatment, using the Common Toxicity Criteria v2.0. A grade 3 or higher toxicity was considered significant. We calculated incidence of significant dysphagia, mucositis and radiodermatitis. The overall, local recurrence-free and disease specific survival were calculated using SPSS 14.0

**Results:** Median treatment duration was 45 days. 92.6% of patients received their prescribed end dose. Dose escalations, mucositis or radiodermatitis were seen in 52.2%, 21.7 and 52.2% of patients in the 1st level respectively, and in 55.6, 55.6 and 27.8 in the 2nd level. Chemotherapy was thought to contribute significantly to acute toxicity. One patient died of toxicity during treatment, with subsequent stop of the study. One year local control rate was 82%; one year Kaplan-Meier overall survival was 70%.

**Conclusion:** Acute toxicity is high but tolerable. New treatment insights and/or chemotherapeutic agents might reduce acute toxicity without increasing local recurrence rates. Further research is mandatory to assess the clinical value of IMRT with dose escalation based on PET information.

**About biochemical changes in organic basis of bone tissue in patients with ankylosing spondylitis depending on stage of disease**

**ESC-ID** 575

**Author** Frolkina I, Magomedov S, Polulyakh M, Kuzub T

**Country** Ukraine

**University** Institute of Traumatology and Orthopedics

**Department** Biochemistry

Ankylosing spondylitis are characterized by pathochemical changes directly in connective tissue. Pathochemical processes which damage all the system of connective tissue in general are accompanied by great changes in collagen metabolism and glycosoaminoglycans (GAG). Most objective clinical indicators of metabolic processes in connective tissue are the following: hydroxyproline and glycosoaminoglycans. Aim of our study was to investigate metabolic lesion of collagen, glycosoaminoglycans and mineral metabolism in patients with ankylosing spondylitis having different activity of pathologic process.

**Methods:** Patients with ankylosing spondylitis of the 1st and 2nd stages of disease activity were examined. In patients' blood serum activity of collagenase, hydroxyproline fraction glycosoaminoglycans, alkaline phosphatase, calcium, phosphorus and creatinine were defined.

**Results:** Study of connective tissue metabolism showed higher content of collagenase in blood serum including free and protein connected hydroxyproline at all stages of disease. The content of glycosoaminoglycans in patients' blood serum was higher than norm in 4-6 times. Calcium was in norm limits at all stages of disease while phosphorus content increased and was at the same level independently of disease stage. Creatinine values were higher at the beginning of disease and decreased below the norm at the 2nd stage of disease.

**Conclusion:** Initial development of ankylosing spondylitis takes place against a background of metabolism lesion of basic components of organic basis of bone tissue. At later stages of pathologic process development mineral metabolism is damaged.

**Effects of a frame-vibration damping system in tennis racquets on lateral epicondyritis – A prospective, randomized controlled trial**

**ESC-ID** 592

**Author** Doerfler F, Soeller F, Zirngibl W, Jansson V

**Country** Germany

**University** Ludwig-Maximilians-University, Munich

**Department** Orthopaedic Surgery

**Background:** Racquet frame vibrations after tennis ball impact are widely considered to play an important role in lateral epicondyritis. However, up to now there is no clinical evidence that a reduction of them by damping systems results in an improvement of lateral epicondyritis.

**Aim:** To investigate if tennis players with lateral epicondyritis benefit from the use of racquets with a damping system.

**Methods:** 102 tennis players with lateral epicondyritis were enrolled in the trial and divided into 2 groups: 51 with acute lateral epicondyritis (duration of symptoms <3 months, group
1), 51 with chronic lateral epicondylitis (>3 months, group 2). With the damping system, consisting of piezoelectric fibers in the frame and a processor chip in the handle, either activated or deactivated, the otherwise identical tennis racquets were randomly assigned in a double-blind fashion. In each group 26 players received the racquet with the activated system (subgroup A) and 25 with the deactivated one (subgroup D). The players were instructed to solely use this trial racquet for 12 weeks according to their normal playing routine. Outcome measures at 0.6 and 12 weeks included pain on the VAS during play and in daily life activities, upon pressure to the point of maximum tenderness with the Dolrometer, as well as the "Mayo Clinic Elbow Performance Index" and the "Hospital for Special Surgery Total Elbow Scoring System (HSS2)".

**Results:** Among players with acute epicondylitis, at 12 weeks, group A had improved significantly in all outcome measures (each with p<0.001), whereas group D only had a significantly increased Mayo Index and HSS2 Score (p<0.01 and p<0.01, respectively). Group A had a significant higher improvement in all measures at any time when compared to group D. In group 2 with chronic epicondylitis, group A again had significant better results for all measures after 12 weeks (each with p<0.01). Group D improved in the Mayo Index Score and with the Dolometer (p<0.05 and p<0.001, respectively). When compared to each other, group A had significant better outcomes in all measures at 12 weeks. However, after 6 weeks no significant difference was found in 4 of the 5 outcome measures. Only on the VAS in daily life did group A perform better (p<0.01).

**Conclusion:** For the first time, a distinct improvement of lateral epicondylitis could be demonstrated for players using a racquet with damping system. These findings strongly suggest that racquet frame vibrations are a key factor in the pathophysiology of this entity. The conflicting results in group 2 may be explained as further signs that lateral epicondylitis is a self limiting disease.

**Pathology I**

**AgNOR parameters of chorionic villi lesions of different type**

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**Aim:** Our aim was to assess AgNOR parameters of chorionic villi in complete and partial hydatidiform moles using AgNOR staining method. This method reveals argyrophyllic proteins of the nucleolar organizer region (NOR), the number and size of which reflect cell proliferation activity.

**Methods:** We examined formalin-fixed and paraffin-embedded tissue sections from 10 complete and 10 partial hydatidiform moles using H&E and AgNOR method (Bio-Optica, Milan, Italy). In H&E stained slides we counted number of syncytiotrophoblast (ST), cytotrophoblast (CT), fibroblasts and Hofbauer cells per chorionic villus. In AgNOR-stained slides we counted AgNOR-positive and AgNOR-negative syncytiotrophoblast, cytotrophoblast and Hofbauer cells as well as AgNOR dots per nucleus.

**Results:** An average number of syncytiotrophoblast was 38.2 ± 1.8 in partial hydatidiform mole and 57.0 ± 2.1 in complete mole; an average number of cytotrophoblast, respectively, – 11.0 ± 0.7 and 18.7 ± 1.1; fibroblasts – 18.2 ± 0.8 and 24.2 ± 0.9; Hofbauer cells – 2.1 ± 0.2 and 3.7 ± 0.4. An estimation of AgNOR parameters in AgNOR-stained slides has revealed that AgNOR-positive syncytiotrophoblast was 13.1 ± 1.4 in partial mole and 13.5 ± 1.1 in complete mole, an average number of cytotrophoblast, respectively – 8.2 ± 0.7 and 7.5 ± 0.6, Hofbauer cells – 4.1 ± 0.3 and 5.5 ± 0.2. An average of AgNOR dots per nucleus in syncytiotrophoblast was 6.2 ± 0.3 in partial mole and 4.8 ± 0.4 in complete mole, in cytotrophoblast, respectively – 1.8 ± 0.2 and 3.8 ± 0.2, in Hofbauer cells – 4.3 ± 0.2 and 6.2 ± 0.5.

**Conclusions:** AgNOR parameters of syncytiotrophoblast, cytotrophoblast and Hofbauer cells in complete and partial hydatidiform moles were alike. Nevertheless, the different number of AgNOR dots per nucleus in each type of cells (higher in complete mole than in partial mole) suggests higher proliferation activity in complete mole. Thus, AgNOR method is simple and cost-effective staining method which can be used as additional staining in diagnostics of trophoblastic gestational diseases.

**Primary carcinoma of the endometrium: A histochemical analysis of mucins**

**Background:** In women genital tract epithelial mucins, known as glycoproteins, are localized in cervix, endometrium, tuba uterina and Bartolini glands. Mucins in endometrium, like mucins that are in epithelial layer in other organs, have a protective, antiinflammatory, autoregulatory, adsorptive and lubricant role.

**Aim:** Rare studies about the changes in mucosubstances during endometrial neoplastic transformation aimed our study toward histochemical examination of mucins in this type of neoplasm.

**Methods:** We used 95 biopsies of endometrial carcinomas gained after hysterectomy. Material was fixed in Bouin solution for 24 hours, routinely processed and embedded in paraffin. On section 5 micrometer thin, classical HE method for verification of pathohistological lesions and histochemical PAS-AB (pH 2.5) method, were applied.

**Results:** Histochemical analysis of fucomucins and sialomucins has shown that there was a direct proportion between amount of mucins secretion and degree of tumor differentiation. Also, beside low production of mucins in adenocarcinoma of the endometrii, there was some quality changes that had their manifestation like: 1)same time presence of neutral and acid mucins 2)secretion only acid mucins 3)secretion only fucomucins In good differentiated tumors was found a hyper secretion of neutral and hypo secretion of acid mucins. In adenocarcinoma G2 we have found hypo and no secretion of acid mucin, while there was neutral mucins in clue and focally presence. In low differentiated tumors was found no secretion of mucins, while in better differentiated spots of same tumor was find a preasens of neutral and acid mucins.
**Influence of immunomodulatory drugs on the nitric oxide level in experimental mild hepatitis**

**Material and Methods:** Liver injury was induced by the injecting Con-A intraperitoneally. After the injection 15 minutes later, the mice were divided into 3 groups: I group (15 mice) were given 0.2 ml of saline solution, II group (15 mice) – 0.25 mg/kg of Plaferon LB and III group (15 mice) – 4 mg/kg of Dexametazone. After 8, 24, 48 hours following the administration of Concavalin-A the materials were taken for its next examination. NO in the serum was measured by Griess reagent. The degree of the liver injury was assessed by the determination of serum alanin aminotransferase (ALT) activity.

**Results:** By the given results, NO level is elevated at 8 hours after Concavalin-A treatment without any further significant change till the end of the experiment. Treatment by PLB significantly decreases NO level at 24 hour compared to I and III groups. However, under the influence of PLB the synthesis of nitric oxide was intensified 48 hour later due to Con-A injection.

**Conclusion:** Our data indicate that Plaferon LB and not Dexametason support the oxidative status in injured liver in Concavalin-A induced hepatitis experimental model. So the results of Plaferon LB action show its protective role on liver tissues.

**Proteasomal activity of blood leucocytes and endothelial dysfunction in cholesterol atherosclerosis.**

Proteasomal proteolysis plays a significant role in cell adhesion molecules expression, functioning of intercellular signal systems, apoptosis of smooth muscle cells etc. which take part in endothelial disfunction and atherosclerosis. Energy deficiency, oxidative stress and accumulation of modified proteins can influence on proteasome activity. But changes in proteasomal proteolysis under different pathological conditions, particularly facilitating atherosclerosis, are still unknown. Objective: To study changes of proteasome activity in blood leucocytes during modeling atherosclerotic process.

**Methods:** The experiments were performed on 20 rabbits. We assessed proteasomal activity in animals with atherosclerotic changes caused by cholesterol diet. Trypsin-like (TL), chymotrypsin-like (CTL) and peptidyl-glutamyl peptide

**Lipomatous hypertrophy of the interatrial septum: Morphological analyses of the 18 autopsy cases**

**Background:** Lipomatous hypertrophy of the interatrial septum (LHIS) was first described at autopsy in 1964 and is defined as large deposits of adipose tissue in the interatrial septum. LHIS may represent an incidental autopsy findings or may be associated with atrial arrhythmias or sudden death.

**Conclusion:** According to the grade of tumor differentiation there was a reduction of epithelial mucins secretion.
Hydroxylase (PGPH) proteasome activities were studied in isolated blood leucocytes.

**Results:** TL proteasome activity in rabbit monocytes with atherogenic diet was increased 1.5 fold (P ≤ 0.45), CTL - 1.9 fold (P ≤ 0.05) and PGPH - 11.6 fold (P ≤ 0.0001). TL activity in lymphocytes decreased by 44.2% (P = 0.95), CTL and PGPH have no significant changes. In neutrophyls TL proteasome activity was increased by 34% (P = 0.4), and PGPH 1.8-fold (P = 0.031). CTL - have no significant changes. This changes correlate with pathomorphological features of endothelial cell in intima of aorta.

**Conclusion:** The data obtained shows the common character of proteasome activity changes in different types of blood leucocytes and pathogenesis of atherogenic process, include an endothelium dysfunction, and influence on proteasomal prolytes may have protective effect in this pathological processes.

**A study on histological, mucin histochemical and immunohistochemical features of Barrett's esophagus**

**ESC-ID** 455  
**Author** Mihajlovic D, Milovanovic J, Stojanovic P  
**Country** Serbia  
**University** Nis  
**Department** Medicine

**Introduction:** Barrett's esophagus refers to an acquired change characterized by the replacement of normal squamous epithelium of the lower part of the esophagus with columnar epithelium. It occurs in people with prolonged gastroesophageal reflux disease. Three types of epithelium can be found in Barrett's esophagus: columnar metaplasia (cardiac type), intestinal metaplasia, and fundic type. Intestinal metaplasia is significant in the sense that it can evolve, through intestinal displacement, into adenocarcinoma. Therefore, both an early discovery of Barrett's esophagus and the application of adequate therapy are important for the prevention of adenocarcinoma development. The aim of the work: an investigation of histological, mucin histochemical and immunohistochemical features of Barrett's esophagus.

**Materials and methods:** Endoscopically biopsies of thirty patients with Barrett's esophagus were studied. These biopsy specimens were fixed 10% formaldehyde, processed in histokinette, embedded in paraffin and cut on microtome. Paraffin sections of 4 micrometer thickness were stained with HE, PAS, HID-AB (pH = 2,5) and ABC, by the use of monoclonal antibody for MUC2, a specific marker for the intestinal metaplasia.

**Results:** Columnar metaplasia was present in 58.6% of the examined cases. Intestinal metaplasia was discovered in 24, 1% of the cases, whereas low-grade and high-grade displasia was present in the lowest percentage - 17.3%. The fundic epithelium type was not observed. Inside the columnar metaplasia area, the neutral mucin stain was purple-red by PAS reaction. In the intestinal metaplasia areas, the epithelial mucin was found in the fields of adenocarcinoma.

**Conclusion:** On the basis of the obtained results, the authors concluded the following: Barrett's esophagus was lined with columnar metaplasia and intestinal metaplasia of the incomplete type. Secretion of the epithelial mucins was a significant characteristic of columnar and intestinal metaplasia; hiposecretion and asecretion were the characteristics of adenocarcinoma.

**Pediatrics I**

**Do pacifiers reduce the risk of sudden infant death syndrome? A meta-analysis**

**ESC-ID** 42  
**Author** Hauck FR, Omojokun OO, Siadaty MS, Bonsa Fynn R  
**Country** Ukraine  
**University** Lugansk state medical university  
**Department** General medicine

**Objective:** Pacifier use has been reported to be associated with a reduced risk of sudden infant death syndrome (SIDS), but most countries around the world, including the United States, have been reluctant to recommend the use of pacifiers because of concerns about possible adverse effects. This meta-analysis was undertaken to quantify and evaluate the protective effect of pacifiers against SIDS and to make a recommendation on the use of pacifiers to prevent SIDS.

**Methods:** We searched the Medline database (January 1966 to May 2004) to collect data on pacifier use and its association with SIDS, morbidity, or other adverse effects. The search strategy included published articles in English with the Medical Subject Headings terms “sudden infant death syndrome” and “pacifier” and the keywords “dummy” and “soother.” Combining searches resulted in 384 abstracts, which were all read and evaluated for inclusion. For the meta-analysis, articles with data on the relationship between pacifier use and SIDS risk were limited to published original case-control studies, because no prospective observational reports were found; 9 articles met these criteria. Two independent reviewers evaluated each study on the basis of the 6 criteria developed by the American Academy of Pediatrics Task Force on Infant Positioning and SIDS; in cases of disagreement, a third reviewer evaluated the study, and a consensus opinion was reached. We developed a script to calculate the summary odds ratio (SOR) by using the reported ORs and respective confidence intervals (CI) to weight the ORs. We then pooled them together to compute the SOR. We performed the Breslow-Day test for homogeneity of ORs, Cochran-Mantel-Haenszel test for the null hypothesis of no effect (OR = 1), and the Mantel-Haenszel common OR estimate. The consistency of findings was evaluated and the overall potential benefits of pacifier use were weighed against the potential risks. Our recommendation is based on the taxonomy of the 5-point (A–E) scale adopted by the US Preventive Services Task Force.

**Results:** Seven studies were included in the meta-analysis. The SOR calculated for usual pacifier use (with univariate ORs) is 0.90 (95% confidence interval [CI]: 0.79-1.03) and 0.71 (95% CI: 0.59-0.85) with multivariate ORs. For pacifier use during last sleep, the SORs calculated using univariate and multivariate ORs are 0.47 (95% CI: 0.40-0.55) and 0.39 (95% CI: 0.31-0.50), respectively.

**Conclusion:** Published case-control studies demonstrate a significant reduced risk of SIDS with pacifier use, particularly when placed for sleep. Encouraging pacifier use is likely to be beneficial on a population-wide basis: 1 SIDS death could be prevented for every 2733 (95% CI: 2416-3334) infants who use a pacifier when placed for sleep (number needed to treat), based on the US SIDS rate and the last-sleep multivariate SOR resulting from this analysis. Therefore, we recommend that pacifiers be offered to infants as a potential method.
to reduce the risk of SIDS. The pacifier should be offered to the infant when being placed for all sleep episodes, including daytime naps and nighttime sleeps. This is a US Preventive Services Task Force level B strength of recommendation based on the consistency of findings and the likelihood that the beneficial effects will outweigh any potential negative effects. In consideration of potential adverse effects, we recommend pacifier use for infants up to 1 year of age, which includes the peak ages for SIDS risk and the period in which the infant's need for sucking is highest. For breastfed infants, pacifiers should be introduced after breastfeeding has been well established.

Online monitoring of thoracic air distribution by quadrant impedance measurement (QIM) in healthy and surfactant depleted newborn piglets

ESC-ID 571
Author Domby M, Blassnig N, Navarro-Psahas S, Löb V, Simbruner G, Rüdiger M, Burkhardt W
Country Austria
University Innsbruck
Department Pediatrics

Introduction: Mechanical ventilation should achieve a homogeneous air distribution in the lung. To evaluate pulmonary ventilation, thoracic X-ray is used, however, that method is not suitable for continuous bedside monitoring. Measurement of electrical impedance allows the estimation of the intrapulmonary air content. Electrical impedance tomography (EIT) was used in clinical studies to visualize pulmonary ventilation, however, EIT is not applicable for neonatal practice, since too many electrodes are required. The newly developed Quadrant Impedance Measurement (QIM) method uses fewer electrodes and allows the measurement of changes in impedance in four thoracic quadrants depending on tidal volume (Tidal Impedance: TI) and on the endexpiratory thoracic air volume (Residual Impedance: RI).

Hypothesis: The following two hypothesis are tested in an animal model: (1) Total Tidal Impedance correlates with the tidal volume in healthy and surfactant depleted lungs. (2) Total Residual Impedance is affected by the endexpiratory pressure (PEEP).

Methods: Newborn piglets were ventilated with volume control. Impedance was measured in four quadrants (right/left: cranial/caudal) and summed up resulting in Total TI and RI (presented as relative changes of impedance -). TI was measured for tidal volumes of 4, 6, 8 ml/kg (1st hypothesis) and RI at PEEP levels of 2, 4, 6 cm H2O (2nd hypothesis). Measurements were performed in healthy animals and after bronchoalveolar-lavage.

Results: 6 newborn piglets with mean weight of 2 (± 0.4 kg) were examined. In general the Total TI did correlate with the tidal volume (P <0.05, R2 = 0.963), however, TI was significantly lower after lavage. RI was affected by different PEEP-levels, but to a smaller extent after lavage

Discussion: QIM is a valid method to measure changes in tidal volume in thoracic quadrants in healthy and diseased lungs. RI seems to correlate with the functional residual capacity, however, further studies are required to validate that assumption. The study was supported by the DFG (SI 785/3).

Effects of perfluorocarbons on surfactant exocytosis in alveolar type II cells

ESC-ID 324
Author Hackspiel I, Hobi N, Haller T, Rüdiger M, Wemhöner A
Country Austria
University Medizinische Universität Innsbruck
Department Neonatologie

Background: The pulmonary surfactant covers the alveolar surface and – by reducing surface tension – prevents endexpiratory alveolar collapse. Disturbances of the surfactant system lead to alveolar collapse with subsequent respiratory insufficiency which requires mechanical ventilation. Pulmonary surfactant is produced and stored (as lamellar bodies – LB) in alveolar type II (ATII-) cells. Surfactant is exocytosed by fusion of LB with cell membranes [J Cell Biol. 2001; 155(2):279-89]. Liquid ventilation with perfluorocarbons (PFC) is a potentially lung protective ventilatory strategy for patients with a disturbed surfactant system. PFC dissolve respiratory gases and improve oxygenation by recruiting collapsed alveoli. To wean patients from liquid ventilation, properties of the endogenous surfactant system have to be restored. Effects of PFC on the pulmonary surfactant system are discussed controversially. Objective: To study the effect of PFC on exocytosis and surfactant storing in lamellar bodies (LBs) of isolated ATII-cells.

Methods: AT II-cells were isolated from rat lungs and cultured in DMEM for 48 hours in 96-well tissue culture plates. Cells were exposed to PFC (Perfluorodecalin) 10 vol% for 1-4 hours before measurement of exocytosis. Exocytosis was assessed by an established fluorescence assay which quantifies the fusion of LB with plasma membrane equitabie to exocytosis of its content [Wemhoeener J of Biomolecular Screening 11(3);2006]. After PFC-incubation cells were stimulated with ATP, PMA (phorbol 12-myristate 13-acetate) and both together during a 45 min kinetic protocol.

Results: Perfluorodecalin did not affect spontaneous surfactant secretion. PFC incubation for 1 hour did also not affect the exocytosis rate of ATP, PMA and ATP+PMA stimulated cells [PFC+ATP vs. ATP 31 ± 7 vs. 33 ± 1%; PFC+PMA vs. PMA 33 ± 3 vs. 31 ± 1%; PFC+ATP+PMA vs. ATP+PMA 39 ± 1 vs. 40 ± 2%]. In contrast, in cells that were incubated with PFC for 4 hours the stimulated exocytosis was significantly lower [PFC+ATP vs. ATP 18 ± 0.07 vs. 32 ± 0.08%; PFC+PMA vs. PMA 17 ± 1 vs. 30 ± 3%; PFC+ATP+PMA vs. ATP+PMA 18 ± 3 vs. 39 ± 3%*; *p <0.05]. PFC incubation did not affect cellular viability.

Conclusions: The results suggest that PFC-incubation for 4 hours inhibits the exocytosis of surfactant. It could be speculated that Perfluorodecalin (by incorporation into the cellular membrane) stabilizes the cellular membrane of ATII-cells and thus, prevents the fusion of LB with cell membrane. Further studies are required to test whether inhibition depends on lipid solubility of PFC. The study was supported by MFF Tirol and Tiroler Wissenschaftsfonds.
Reduced calorie and protein supply in children who have developed bronchopulmonary dysplasia?

Background: Despite many advances in neonatal care, up to 40% of the extremely prematurely born infants develop a chronic lung disease (Bronchopulmonary Dysplasia - BPD). Pathogenesis of BPD is multifactorial. Disturbance in lung development is an important factor. Beside intrauterine growth retardation, postnatal malnutrition could affect lung development [Semin Perinatol, 2006 Aug; 30 (4): 200-8].

Objectives: Preterm infants who develop BPD have a lower caloric and protein intake during the first 14 days than infants without BPD.

Designs/Methods: Retrospective study of premature infants with a gestational age of less than 31 weeks and a birth weight >1500 g, born between 08/04-12/06. Analysis of growth parameters, total, enteral and parenteral intake of calories, proteins and fluid during the first 14 days. Parameters were compared of infants who developed BPD (defined as additional oxygen dependency for at least 28 days) with infants without BPD.

Results: During the study period 97 premature infants where hospitalized, n = 26 with BPD (27 ± 1 weeks) and n = 71 without BPD (28 ± 1 weeks). The BPD-group had a lower birth weight than non-BPD infants (965 ± 255 g vs. 1170 ± 246 g; p < 0.01). At a age of 14 days BPD-infants were smaller (38.5 ± 2.6 vs. 36.8 ± 3.3 cm; p = 0.03) and had a lower weight (1191 ± 241 vs. 1015 ± 254 g; p < 0.01) than non-BPD infants, in the 36 gestational week the infants were smaller (42 ± 2 vs. 43 ± 2 cm; p = 0.02) and had a lower weight (1883 ± 286 vs. 2038 ± 286 g; p = 0.03). During the first two weeks cumulative total fluid intake was similar in both groups [1860 ml/kg (Interquartile Range 1813, 1904) vs. 1864 IQR (1792, 1929); p = 0.2], cumulative enteral fluid supply was significantly lower in the BPD group [456 ml/kg (IQR 744, 235) vs. 685 (IQR 987, 511); p < 0.01]. The cumulative enteral intake of calories [311 kcal/kg (IQR 557, 157) vs. 489 (IQR 728, 358); p = 0.01] and proteins [7 g/kg (IQR 3, 13) vs. 13 (IQR 7, 18); p = 0.01] was significantly lower in the BPD-group. The cumulative total intake of calories [1089 Kcal/kg (IQR 1190, 966) vs. 1154 (IQR 1221, 1081); p = 0.1] and proteins [220 g/kg (IQR 226, 191) vs. 216 (IQR 238, 199); p = 0.91] was similar in both groups. No differences regarding side effects of enteral feeding were observed (incidence of necrotizing enterocolitis was similar in both groups).

Conclusion: Preterm infants with BPD received less enteral feeding, which was well compensated by parenteral nutrient supply. Thus, the total amount of nutrients was equal in both groups. The data, however, suggest that a progressive increase in enteral feedings might be beneficial in preventing BPD. The study was supported by MFF Tirol and Tiroler Wissenschaftsfonds.

Intrathoracic budesonide improved the lung functions in meconium-instilled rabbits without influencing cardiovascular variables

Introduction and aim: Systemic corticosteroids diminish the lung edema, inflammation, airway hyperreactivity, and pulmonary vasoconstriction associated with meconium aspiration, but may have side effects on cardiovascular functions. Aim of this study was to evaluate effects of intratracheally administered corticosteroid budesonide on the lung functions as well as on the cardiovascular parameters of meconium-instilled rabbits.

Method: Conventionally (f. 30/min, inspiration time Ti 50%) air-ventilated adult rabbits intratracheally received 4 ml/kg of saline (Sal group, n = 5) or human meconium (25 mg/ml). From this moment, all animals were oxygen-ventilated. When respiratory failure developed, meconium-instilled rabbits intratracheally received budesonide (0.25 mg/kg, 0.5 ml/kg) by infusion effect of high-frequency jet ventilation (f. 300/min, Ti 20%) 0.5 and 2.5 h after meconium instillation (Mec+Bud group, n = 8) or were left without treatment (Mec group, n = 8). All animals were conventionally ventilated for additional 5 h after the first treatment dose. Respiratory and cardiovascular parameters, blood gases and white blood cells count (WBC) were measured in regular time intervals. Right lungs were used to determine lung edema by wet/dry weight ratio and oxidative damage to lipids and proteins in the lung homogenate by estimation of thiobarbituric acid-reactive substances, dityrosine and lysine-lipid peroxidation products. Left lungs were saline-lavaged and differential WBC was estimated microscopically in the lavage fluid sediment.

Results: Budesonide treatment significantly improved gas exchange and decreased right-to-left pulmonary shunts, central venous pressure and ventilatory pressures. Moreover, budesonide reduced meconium-induced lung edema formation, neutrophil count in the lavage fluid associated with higher counts of WBC and neutrophils in the blood, and diminished oxidative modifications of proteins and lipids in lung homogenate compared to non-treated Mec group (all P < 0.05, 0.01 or 0.001). No significant changes in blood pressure or heart rate were observed in budesonide-treated vs. non-treated group (P > 0.05).

Conclusion: Intratracheally administered corticosteroid budesonide effectively improved the lung functions and reduced inflammation induced by meconium instillation in rabbits with no significant side effects on cardiovascular variables. The study was supported by Grant of Agency for Science and Ministry of Education (VEGA) No. 1/2306/05.
Iron deficiency association with helicobacter pylori infection in children

ESC-ID 138
Author Okhovat S, Fatemi M, Haghani F, Farhangfar B
Country Iran
University Islamic Azad University Najafabad
Department Medicine

Background: Iron deficiency (ID) and iron deficiency anemia (IDA) are among the most prevalent diseases of childhood and can have irreversible effects on this group of age. Researchers and clinicians have reported several cases of IDA patients and coexistent helicobacter pylori (H.P) infection whose anemia had responded to H.P eradication treatment. Despite these case reports this association has not been fully investigated. Objective: The aim of this study is to answer the question whether Iron deficiency and Iron deficiency anemia are related to helicobacter pylori infection in children or not.

Design and Methods: This is a retrospective case control study. The participants were 42 girls and 33 boys, 2-14 years of age (mean ± SD; 4.9 ± 1.96). Cases had iron deficiency or iron deficiency anemia more than three months. Three pediatricians who were interested in this study referred these iron deficient or iron deficiency anemic patients to us during one-year period. The control cases were healthy children selected from the same pediatricians’ patients by simple random sampling. A total of 29 cases in ID group: (mean age ± SD; 5.6 ± 1.9), 21 cases in IDA group: (mean age ± SD; 3.9 ± 1.3), and 25 in control group, (mean age ± SD; 5.1 ± 2.2) were enrolled in this study to provide it with 75% power and 5% alpha error. Height, weight, family number, family income and parents educational level were recorded. Hematological values of complete blood count, serum iron, serum ferritin, total iron binding capacity and IgG anti H.P were measured. Stool examination for ova and parasites including giardia lamblia cyst and occult blood were performed.

Results: Iron deficient, iron deficiency anemic, and control groups were not significantly different in height, weight, family number, family income and father or mother educational level. IgG anti H.P positive rate was significantly higher in iron deficient group compared to control group, [48% vs. 16%; Pearson χ² (1 , N = 75 ) = 6.294, P = 0.012]. Difference between iron deficient anemic cases and controls for IgG anti H.P positive rate was not statistically significant, [33% vs. 16%; Pearson χ² (1 , N = 75 ) = 1.885, P = 0.17]. Among height, weight, age, and family number only height was statistically different between IgG anti H.P positive and IgG anti H.P negative patients, (Z = -3.6; p < 0.001).

Conclusion: This study showed that iron deficiency is associated with helicobacter pylori infection in children between 2-14 years of age. Also IgG H.P positive state is significantly associated with lower height in this group of age. Thus the clinician must be aware of these probable associations encountering iron deficient children. The study failed to show statistically significant association between iron deficiency anemia and helicobacter pylori infection. Larger sample size may be needed to reveal this probable association.

Genetic markers in the onset of ulcer disease in children

ESC-ID 150
Author Andriychuk D, Sorokman TV
Country Ukraine
University Bukovinian State Medical University
Department International Relations

The aim of our project was to investigate the genetical component of ulcer disease of the duodenum (UDD) in children following monogenic signs of inherited polymorphism.

Material and Methods: We investigated 47 children of the school age suffering from UDD (basical group), and 21 healthy child (comparative group). Children of basical group were divided into two subgroups depending on inheritance of UDD: 2̶children with inheritance – complicated UDD (70.6%), 22̶those with genetically “clean” families (29.4%). Genetical predisposition to UDD was evaluated by association with monogenic signs: antigens of blood groups of ÅA0, Rh, P, MN, Le (Luis) systems and group-specific component Gc. The role of exact marker in UDD appearance was estimated employing Wolff’s criterion (RR).

Results: Analysis of heritance polymorphism genetic markers distribution pointed on prevalence of blood group Å(²²) (57.9 ± 2.6% and 18.2 ± 1.3% correspondently, q = 10.65, δ -0.05, RR = 1.67), Rh(+) (q = 3.78, δ -0.05, RR = 1.13), Å(-) (76.5 ± 1.7% and 18.2 ± 1.5% correspondently, q = 5.78, δ -0.05, RR = 1.16), Le-âo+ (54.5 ± 2.4% and 27.3 ± 2.1% correspondently, q = 6.86, δ -0.05, RR = 1.25), Î (68.8 ± 2.1% and 26.4 ± 2.1% correspondently, q = 3.87, δ -0.05, RR = 1.24), Gc 2-2 (46.4 ± 1.4% vs. 14.3 ± 1.5%, RR = 1.31) in children suffering from UDD. Persons with blood groups Å(²²) (44.4 ± 2.3% and 11.7 ± 1.9% correspondently, q = 5.02, δ -0.05, RR = 0.46), Å(+) (81.92 ± 2.4% and 23.5 ± 2.5%, q = 7.70, δ -0.05, RR = 0.53), MN (55.5 ± 2.2% and 22.4 ± 2.2% correspondently, q = 3.94, δ -0.05, RR = 0.38), Le-â (54.6 ± 2.2% and 30.8 ± 2.3% correspondently, q = 4.79, δ -0.05, RR = 0.42), Gc 2-1 (62.9 ± 1.6% vs. 23.4 ± 1.4%, q = 5.85, δ -0.05, RR = 0.57) were prevalent in a comparative group.

Conclusion: Hence, comparative analysis of heritage polymorphism’s 6 systems markers demonstrated that UDD significantly more often develops in persons – who carry blood groups Å(²²), Å(+) and Åo-, Le-âo+, Î, group-specific component Gc 2-2, and significantly less often – in those with blood groups Å(²²)-, Å(+) and Le-âo-, Î, group-specific component Gc 2-1.

Moderate hypothermia and methylprednisolone suppress inflammatory response in human endothelial cells

ESC-ID 665
Author Rößler J, Diestel A, Schmitt K, Berger F
Country Germany
University Charité Universitätsmedizin Berlin
Department Reformstudiengang Medizin

Background: The mortality rate of children with congenital heart disease has been significantly reduced during the past decade due to substantial progress in diagnostics and cardiac surgery. However, inflammation and capillary leak syndrome are still common complications after corrective surgery. Hypothermia is a standard method for organ protection dur-
ing cardiac surgery in children. It decreases blood flow and the metabolic rate of oxygen in all organs. However, the morphological and biochemical mechanisms of hypothermia induced cell protection have not been clearly established. The aim of our studies was to investigate endothelial cell function after treatment with methylprednisolone under moderate hypothermia (32°C) in comparison to control cells (37°C).

Methods: Primary human endothelial cells (HUVEC) were isolated from normal human umbilical cord veins and kept under permanent moderate hypothermia (32°C) and normothermia (37°C) over a time curve of 48h. To mimic inflammatory response after Cardio Pulmonary Bypass cells were stimulated with 500U/ml TNF alpha. Furthermore the routinely used anti-inflammatory drug methylprednisolone was administered to the cells. Cell viability (Trypan blue exclusion and MTT-assay), IL-6 secretion (ELISA) and eNOS expression (immunocytochemistry), were investigated under different conditions of cooling and stimulation.

Results: Stimulated and unstimulated HUVEC kept under moderate hypothermia (32°C) as well as viable as control cells (37°C). IL-6 secretion was significantly reduced after 2h of 32°C in stimulated HUVEC. IL-6 release increased after 6h in activated HUVEC (32°C) compared to activated control cells (37°C). 1mM methylprednisolone minimises the IL-6 release in stimulated HUVEC and significantly decreases eNOS expression in TNF alpha stimulated HUVEC.

Conclusion: Moderate hypothermia and methylprednisolone could lead to less inflammation and cell activation and may probably reduce the risk of postoperative endothelial cell dysfunction depending on the time course of cooling.

Are asthmatic children predisposed to become obese?

ESC-ID 192
Author Szroniak A, Labedzka I
Country Poland
University Poznan University of Medical Sciences
Department Pediatric Pneumonol Allerg Clinic Immunol

In the last two decades there has been a significant increase in the prevalence of both asthma and obesity. Simultaneous increase of these two phenomena can suggest the causal nexus. This interdependence has been explained as evidence that asthma causes obesity due to a lack of physical activity and treatment with systemic corticosteroids among children with asthma. On the other hand many studies suggest that obesity induces changes in T cell function and may be partly involved in the pathophysiology of allergy and asthma. The purpose of the study was to verify the hypothesis of the greater risk of overweight and obesity in the group of children and adolescents with asthma.

Methods: Study group consisted of 100 children and adolescents: 35 girls and 65 boys with mild persistent, moderate persistent and severe persistent asthma (age: 3-18 years). All children were diagnosed with asthma at least 6 months before this study. The questionnaires have been completed according to the physical and spirometric examination, analysis of medical documentation and anamnesis. The details obtained by a questionnaire included: perinatal history, feeding, allergic history, treatment, frequency of symptoms, hospitalizations, physical activity, the family size, allergy in the family. Diagnoses of atopic background relied on the results of skin prick test. Healthy weight for height of each child was evaluated based on anthropometric measurements of stature and weight and body mass index (weight/height2) values referred to the standards for children and adolescents of Poznan district in the year of 2000. Overweight and obesity were defined as 1) as 90th and 97th BMI-percentile-for-age and gender and 2) according to age and sex specific cut off points of worldwide standards. All the individual heights and weights and BMI values were standardized on means and standard deviation according to sex and age categories.

Results: Asthmatic children more often did not reach values of body height according to the sex and age specific stature norms. There was found significantly higher frequency of under- and overweight and obesity in the group of asthmatic children at the background of Polish (q = 22.503; p = 0.00208) and worldwide standards (q = 26.653; p <0.000002). Except for the atopic dermatitis, statistical analysis of differences between BMI values in all combinations: with severity of asthma, treatment with inhaled corticosteroids, physical activity and others, produced insignificant results. The average z-score of BMI was significantly lower (F = 5.917; p = 0.01681) in relation to the presence than to the absence of atopic dermatitis.

Conclusion: Asthma may rise the risk of being overweight and can cause a delay of the physical growth process.

Developmental expression of GATA-6 in the human liver and its role in hepatic erythropoietin gene regulation

ESC-ID 970
Author Salmon H, Hussels CS, Thurisch BM, Knöpfle G, Dame C
Country Germany
University Charité Universitätmedizin Berlin
Department Dept. of Neonatology, CVK

The transcriptional factor GATA-6 is a zinc-finger protein crucially involved in liver development. Throughout fetal and early postnatal life the liver is the primary erythropoietin (Epo) production site while in adults Epo is mainly produced by the kidneys. This switch is supposed to be one factor contributing to the pathophysiology of the anemia of prematurity, which is associated with inadequately low Epo plasma concentrations. We have recently identified GATA-4 as a developmental-stage-specific activator of Epo gene expression in fetal hepatocytes. Since in many tissues GATA-4 and GATA-6 often share redundant functions, we aimed to investigate the developmental expression profile of GATA-6 in the human liver as well as its role in Epo gene regulation. By means of quantitative PCR, we could show that GATA-6 expression in the human liver decreases during development. Overexpression of human GATA-6 did not significantly alter Epo mRNA expression in human hepatoma-derived cells (HepG2), neither under normoxic nor hypoxic conditions. Co-transfections of human GATA-6 and FOG-2 (friend of GATA) constructs did not change Epo gene expression either. The previously demonstrated in vitro binding of GATA-6 to the minimal Epo promoter shown by EMSA (electrophotoretic mobility shift assay) could not be confirmed in vivo. By means of chromatin-immunoprecipitation (ChiP) we did not detect a binding of GATA-6 to the minimal Epo promoter in the fetal mouse liver (e11). In conclusion, as already shown for the murine liver, GATA-6 is developmentally down-regu-
lated in the human fetal liver. In contrast to GATA-4, GATA-6 is not involved in the transcriptional regulation of Epo gene expression. Future investigations aim to identify new GATA-6 target genes during liver development.

**Pharmacology and Toxicology I**

**Octreotide therapy of refractory ultra rapid opioid detoxification (UROD)-associated diarrhea: A randomized controlled trial**

ESC-ID: 835  
Author: Norouzi S, Aghazade S, Norouzi N  
Country: Iran  
University: Tehran University  
Department: Medicine

*Introduction:* Ultra Rapid Opioid Detoxification (UROD) is a new technique with the use of µ-opioid receptor antagonists to precipitate withdrawal and provides a rapid transition from opioid dependency to oral naltrexone therapy. Refractory Diarrhea (RD) is a side effect of using opioid antagonists by activating parasympathic system, potentially reducing the therapeutic benefit of treatment. RD isn’t stopped with conventional medicine therapy and has been shown that octreotide is effective in controlling RD in HIV patients. It has no interaction with conventional medicines which are used in UROD and doesn’t have any important side effects. The objective of this study is to investigate the relation between administering octreotide in UROD and reducing RD as one of the symptoms of withdrawal syndrome.  

*Materials and methods:* This study was a randomized controlled trial including 450 participant; 348 male and 102 female, with opiate dependency aged 20 to 49 years, volunteer for detoxification, had capability to have General Anesthesia (GA) that was one of our including criteria, study period was from Dec 2005 – Oct 2006 they were randomly allocated in two groups. Both groups were under prescription of routine conventional medicine, study group (G1) received an additional extra 2 dosages of octreotide (2.0.05 mg/person) by subcutaneously injection. Both group followed up during GA (4-5 hours) and 24 hours after recovery in the ward. And the incidence of RD in the two groups was followed up. The data was analyzed with SPSS11 and student T-test with cut points of significant that was set at 0.05. All the procedures were under supervision of Tehran University of Medical Students Ethical committee.  

RESAULTS: The incidence of RD significantly decreased in the G1 (8/315) comparing to the control group (G2:129/135) which the differences statistically significant (2.5% vs. 95.6%, p < 0.01)  

CONCLUSION: Octreotide therapy is potentially safe and effective for controlling RD caused by UROD.

**Effects of GABA agonists, GABA uptake inhibitor and glycine agonist on pharmacoresistant seizure like events in hippocampal slice cultures**

ESC-ID: 857  
Author: Wahab A, Albus K, Heinemann U  
Country: Germany  
University: Charité Universitätsmedizin Berlin  
Department: Institute of Neurophysiology

*Aim:* Standard antiepileptic drugs fail to block epileptiform activity induced by low magnesium or 4-aminopyridine in rat hippocampal slice cultures [1]. In order to investigate mechanism of antiepileptic drug-resistance we have started an investigation into its pharmacological properties and here report effects of gamma-aminobutyric acid (GABA) agonists, a GABA uptake inhibitor and a glycine receptor agonist.  

*Methods:* Hippocampal slice cultures prepared from 6 to 10-days old Wistar rats were continuously superfused with prewarmed (35 -36oC) and oxygenated (95%O2, 5%CO2) MEM. Single unit and population activities were recorded with tungsten microelectrodes and glass pipettes positioned in the pyramidal layer of areas CA3 and CA1 of hippocampus. The [K+]o concentration was measured with an ion-sensitive electrode in CA3. A primary afterdischarge was induced by electrical stimulation (0.1ms, 100Hz, 1s) with a pair of tungsten electrodes positioned in the hilus. The drugs tested were the GABAA receptor agonists muscimol (1-25 µM) and isoguvacine (10-100 µM), the GABAB agonists (±)-baclofen (25-200 µM), (+)-baclofen (25-200 µM) and (-)-baclofen (25-200 µM), and the GABA uptake inhibitor nipeptic acid (200 µM-2mM) and the glycine receptor agonist taurine (500 µM).

*Results:* Seizure like events consisting of tonic-clonic periods and/or tonic periods followed by clonic-like recurrent activity were reliably induced by omitting Mg2+ and increasing KCl to 5mM. Seizure like events were synchronous in CA1 and CA3 and were associated with rises in [K+]o in CA3. Isoguvacine (100 µM), muscimol (10 and 25 µM), and nipeptic acid (2 mM) completely blocked seizure like events. In contrast the various isoforms of baclofen and taurine failed to show complete inhibition against pharmacoresistant epileptiform activity. The primary afterdischarge was blocked by isoguvacine, muscimol and nipeptic acid and not by baclofen and taurine. Excitatory effects of the GABA agonists were not observed at any time in vitro (10 to 64 days). The effects of GABA agonists and nipeptic acid in hippocampal slice cultures are similar to those reported in combined entorhinal-hippocampal slices of adult rats [2].  

*Conclusion:* Our data support the idea that although the GABAergic system is functionally well developed in hippocampal slice cultures inhibitory mechanisms may be hampered by a low availability of the intrinsic transmitter GABA. It remains to be seen if and to what extent this contributes to pharmacoresistance of seizure like events against antiepileptic drugs.
Preventive action of ALA-1, an acetone fraction from Aconitum in acute seizure and kindling models in mice

ESC-ID 97
Author Perveen H, Jawed H, Zeeshan M, Shaheen F, Simjee SU, Adnan SH
Country Pakistan
University University of Karachi
Department Pharmacology

Like other diseases that have been cured by the medicines that are based on natural products, there are several plants used in folk medicine for curing the epilepsy. Keeping facts in mind such as the limitations of current antiepileptic drug therapy and prehistoric role of natural products based drug discovery, this study was carried out with an aim to investigate the anticonvulsant and anti-epileptogenic activity of the acetone fraction of ALA-1 extracted from Aconitum, an herb used in folk medicine as anti-inflammatory agents, sedatives and as anti-neuralgics. The anticonvulsant activity was tested using MEST and PTZ-induced seizure models in mice whereas, pentylenetetrazol (PTZ)-induced kindling was used to evaluate the anti-epileptogenic activity of ALA-1. The pharmacological kindling was induced by repeated subcutaneous injection of pentylenetetrazol in subthreshold doses (50 mg/kg). Repeated administration of PTZ produced a gradual increase in the seizure intensity, leading to the development of kindling (stage 0 – stage 5). Our results showed that the ALA-1 significantly abolished HLTE in MEST at the dose of 10 mg/kg and also retard the convulsion induced by PTZ and attenuated the severity of seizures. Thus our findings suggest that ALA-1 from Aconitum might contain an active anticonvulsant compound(s) which plays a protective role in MEST and PTZ-induced seizures and the development of PTZ-induced kindling in mice. Thus our results support additional studies to evaluate full therapeutic potential of ALA-1.

Diazepam relaxed ET-1 precontracted thoracic rat aorta in a concentration dependent manner

ESC-ID 363
Author Ziberna L, Groselj B, Berden J
Country Slovenia
University University of Ljubljana
Department Institute of Pharmacology

Aim: Diazepam is a benzodiazepine drug which pharmacological effects are mediated by binding to GABA(A) receptors in CNS but also acts on many peripheral tissues. The effect of diazepam on isolated thoracic rat aorta, precontracted by ET-1, was tested.

Method: Aortic rings (3-4 mm wide) were placed in organ baths, filled with oxygenated Krebs-Henseleit solution at 37 °C. After 60 min equilibration (2.0 g tension), rings were repeatedly contracted with 60 mM KCl until stable response. Endothelial function was assessed on precontracted rings by the degree of relaxation caused by 0.1 mM acetylcholine. Precontraction was induced by 0.1 microM noradrenaline. There were 3 experimental groups: intact aortic rings, endothelium denuded rings and rings with inhibited nitric oxide synthase (NOS) and cyclooxygenase (COX) by application of LNNA and indomethacine. Relaxation of preconstricted rings for more than 40 % in non-inhibited rings indicated intact endothelium. The rings precontracted by 10 nM endothelin (ET-1) followed by application of Apaurin (diazepam) in concentrations ranging from 1 microM to 80 microM.

Results: Diazepam applied to the aortic rings in concentrations of 1, 10, 20, 40, 60 and 80 microM relaxed precontracted rings on average by 26%, 42%, 55%, 71%, 81% and 91%, respectively. In higher concentrations complete relaxation was observed. Rings with inhibited NOS and COX relaxed rings on average by 13%, 25%, 37%, 40% and 42% by the application of 1, 10, 20, 40 and 60 microM diazepam, respectively. Maximum relaxation of the endothelium denuded rings was attained at 55% by 20 microM diazepam. Additional application of diazepam did not enhance relaxation.

Conclusion: Diazepam showed concentration dependent relaxation of ET-1 precontracted aortic rings. Mechanism for the relaxation is only partially dependent of nitric oxide synthase activity.

The influence of specific stimulator PPAR-Y receptors pioglitazone on rat mucous coat of colon

ESC-ID 400
Author Beregova T, Voronina O, Gryschuk V, Gurlenko T
Country Ukraine
University Taras Shevchenko Kyiv National University
Department Department of Pharmacoo-Physiology of Biologic

Most recent research concerning the extragastric effects of gastrin has been directed towards its effects on the colon. It has detected the property of gastrin to play a role in proliferation of the normal colon. Gastrin also stimulates the growth of some tumor cells in colon and the elevated plasma gastrin concentration are the risk factor not only for gastric cancer but also for colon. The link between chronic omeprazole administration, as a form of endogenous hypergastrinemia, and the development of colon carcinoma is controversial.

Aim: To investigate the effect of longterm omeprazole-caused hypergastrinemia in the rat colon, to study the effect of agonist of Peroxisome proliferator-activated receptors (PPAR-Y receptors) pioglitazone on histological structure of the colon.

Materials and methods: 90 male Wistar rats were divided into 9 groups. The first four groups of rats (control) treated with salt solution (0.2 ml) during 7, 14, 21 and 28 days, the next four groups were given proton pump inhibitor omeprazole (14 mg/kg) once daily by injection for 7, 14, 21 and 28 days. The ninth group simultaneously received injection of omeprazole and pioglitazone (10 mg/kg) during 28 days.

Tissue sections of the colon was fixed in 10% formalin, embedded in paraffin, stained with haematoxylin and eosin. For morphometric analysis width mucous, nuclei size of gland cells, size of gland cells and interior of colon crypt were estimated.

Results: During 7 and 14 days of treatment of omeprazole the insignificant changes in the research parameters were
observed. After 21 days of treatment by omeprazole a gradually rise of gland cells and mucous thickness take place. The treatment by omeprazole for 28 days lead to increase in size of gland cells for 29%, of width mucous for 35% and increase in values of interior of colon crypt for 70%. But nuclear area of glandular cells was decreased for 18%. After simultaneously treatment by omeprazole and pioglitazone a tendency to normalization was observed. A values of nuclear area of gland cells was certainly increased and differ from the control for 11%, other parameters such as size of glandular cells (0.4%) and a meaning of width mucous (18% respectively) are certainly become lower than at first group.

**Conclusion:** Our resulys demonstrated that longterm hypergastrinemia reduce to elevamation of colonocytes’ proliferation and decrease of differentiation. So, it is evidence of hyperplasia of epithelium with dysplasia indications. As a result of treatment by pioglitazone a partly return a values of colon mucous to normal level was observed. In other words, prohibition of the pioglitazone receptors with initial dysplasia indications which caused by omeprazole-induced path is able to hold up development further tumor transformation.

### The effects of agonist and antagonist adenosine receptors in the memory process

**ESC-ID** 913  
**Author** Costoiu LE, Paunescu H, Segarceanu A, Ghita I  
**Country** Romania  
**University** University of Medicine and Pharmacy Bucharest  
**Department** General Medicine

**Introduction:** The present study is an experimental research on possible effects of agonist and antagonist adenosine receptors in the memory process. Studies of experimental pharmacology were conducted on rats. The “Y” maze test was used, injecting either adenosine and theophylline intraperitoneally for a 10 days period. All three stages of the memory process were investigated: acquisition, consolidation and retrieval.

**Material and Methods:** The “Y” maze is a labyrinth divided in three segments. It is a simple two-trial recognition test to measure spatial recognition memory in rats. There are two visits through the “Y” maze. During the first visit one arm is isolated and it is the “unknown arm”. During the second visit the three arms are free to be explored. The acquisition process takes place before the first visit. During the first visit the consolidation process takes place and during the second visit the retrieval process takes place. Each visit lasts for 5 minutes. The research was conducted on 6 groups of rats. Group no.1 – injected with adenosine – 20 micrograms/kg body before the first visit through the “Y” maze; Group no.2 – injected with adenosine – 40 micrograms/kg body before the first visit through the maze; Group no.3 – injected with theophylline – 20 micrograms/kg body before the first visit; Group no.4 – injected with adenosine – 20 micrograms/kg body after the first visit through the maze; Group no.5 – injected with adenosine – 20 micrograms/kg body after the second visit through the maze; Group no.6 injected with infusion of saline (control group). The parameters used were: the average time spent in the “unknown arm” and the average number of visits in the “unknown arm”, compared with the control group.

**Results:** Small doses of adenosine injected before the acquisition proved an increase in the capacity to explore, doses injected after acquisition increased the capacity of memorizing after 6 days and doses injected after the second visit through the maze have statistically increased the retrieval process after 7 days compared with the control group. Small doses of theophylline have influenced the acquisition in a negative way, decreasing the average of time spent in the “unknown arm”.

**Conclusion:** Although the memory process is complex, the results show that by activating the adenosine receptors the capacity of memorizing increases; theophylline has an antagonic effect.

### Selective agonist of cannabinoid receptor 2 produces antitumor and antiangiogenic activity

**ESC-ID** 188  
**Author** Vidinsky B, Gal P, Vidobva Z, Pilatova M, Mojzis J  
**Country** Slovakia  
**University** Faculty of Medicine, P.J.Safarik University, Kosice  
**Department** Department of Pharmacology

Cannabinoids are the major active components of Cannabis sativa L. (Marijuana). Their derivatives exert a wide array of effects by activating the specific G protein-coupled CB1 and CB2 receptors (cannabinoid receptors). These receptors are normally engaged by a family of endogenous ligands – the endocannabinods. It is well known that the unwanted psychotropic effects of cannabinoids are mediated largely or entirely by CB1 receptors (1, 2, 4, 5). Therefore, selective targeting of CB2 receptors would be desirable to avoid side effects in clinical practice. The aim of our study was to show the effect of selective CB2 agonist on cancer and endothelial cells. As the selective CB2 agonist, JWH 133 (3-(1,1-Dimethylbutyl)-1-deoxy-Δ8-tetrahydrocannabinol) was used in all experiments. To determine to effects on cancer cells, following cell lines were selected: A 549 (Lung cancer), CEM, Jurkat (Acute leukemia), HeLa (Cervix cancer) and MCF-7(Breast cancer). For the assessment of the cytotoxic effect of tested agent, the thiazolyl blue (MTT) method was used. In order to ascertain the induction of cell death by JWH 133, DNA fragmentation, reflecting the endonuclease activity characteristic of apoptosis, was analyzed. Cell viability was examined using Colony formation assay (HeLa). To assess the effect of JWH 133 on angiogenesis, wound healing assay (cell migration) and activity of matrix metalloproteinase 2 (MMP-2) were studied in human umbilical vein endothelial cells (HUVECs). Our study showed that JWH 133 exerted significant cytotoxic effect and induced apoptosis at concentration of 100 mol/L (p <0.01). Different effects on cell survival were shown in HeLa cells (Colony formation assay). JWH 133 at the concentrations of 10 mol/L and 100 mol/L significantly inhibited the survival of cancer cells (p <0.01). However, at lower concentrations (1 mol/L, 100mol/L, 10 nmol/L) significantly stimulative effect and higher number of surviving cells compared to control were observed (p <0.01). Additionally, the migration of endothelial cells was completely and partially inhibited at concentrations of 100 mol/L as well as 10 mol/L and 1 mol/L, respectively. Finally, the inhibition of MMP-2 activity was recorded at the concentration of 100 mol/L. In conclusion, selective agonist of CB2 receptor showed antiproliferative effects at micromolar concentrations, in both cancer as well as endothelial...
High performance liquid chromatographic method for quantification of oseltamivir carboxilat acid in human serum using solid phase extraction: application to human pharmacokinetic studies

ESC-ID 449
Author Majnouini MB, Bahrami GH, Mohammadi B, Kiani A
Country Iran
University Kermanshah University of Medical Sciences
Department Pharmacology

**Heading and aim:** Oseltamivir phosphate is a pro-drug of anti influenza neuraminidase inhibitor and it has been developed for treatment of both A and B strain of the disease. The drug is rapidly metabolized to its active form oseltamivir carboxilate. A fast and sensitive isocratic High performance liquid chromatographic method for quantification of the carboxilate form of oseltamivir in human serum developed in this study.

**Method:** Sample preparation involved solid phase extraction of the drug and an Internal standard (vaniline). A mobile phase consisted of water containing trethylamine (2ml/L PH: 3 adjusted with phosphoric acid) and methanol (86:14v/v) was used and Chromatographic separation achieved using C18 analytical column. A UV detector set at 215 nm was applied for detection of the analytes. Result: Under the above conditions the drug and internal standard were eluted at retention times of 9.4 and 8.4 min respectively. The calibration curve was linear over the concentration rang of 0.05 Åg/ml to 3.2 Åg/ml and limit of quantification was 50ng/ml

**Discussion:** The method showed excellent selectivity, specificity, sensitivity, precision and accuracy and was applied in a bioequivalence study of two different oseltamivir phosphate preparations following single dose administration of the drug in 24 healthy volunteers.

**GN1, a synthetic analog of glucosamine, reduces the severity of collagen-induced arthritis in rats**

ESC-ID 204
Author Simjee SU, Awan SI, Anjum S, Jawed H
Country Pakistan
University University of Karachi
Department Pharmacology

The anti-arthritic effects of GN1 an analog of glucosamine was investigated on collagen induced arthritis (CIA) in SD rats. Arthritis was induced in rats by multiple intradermal injections of emulsion containing bovine type II collagen in IFA and challenged again with the same antigen preparation 7 days later. Increased hind paw swelling was significantly suppressed with no further noticeable retardation of body weight in the groups treated with glucosamine (P < 0.05) and its analog GN1 (P < 0.02) as compared to control arthritic rats. In contrast, the animals in the arthritic control group showed a gradual decrease in their body weight. The histopathological evaluation of isolated knee joints by grading system, classification of the stages in arthritic lesion development, revealed suppression of the inflammatory changes in the GN1 treated animals. In addition, both the proinflammatory markers C-reactive protein (CRP) and low-density lipoproteins (LDL) levels were also found to be significantly decreased in animals treated with GN1 (P < 0.03 for CRP and P < 0.05 for LDL). The arthritic animals receiving no other treatment or vehicle only showed a significantly elevated CRP and lipoproteins levels. These results suggest that GN1 may have anti-arthritic properties and also act as an anti-inflammatory agent.

**Prednisolone enhances quinolone-induced toxicity in human-derived tenocytes in vitro**

ESC-ID 500
Author Fagh A, Sendzik J, Webb J, Baumann-Wilschke I, Stahlmann R
Country Germany
University Charité Universitätmedizin Berlin, Campus Benjamin Franklin
Department Institute of Clinical Pharmacology and Toxicology

Clinical data suggest that glucocorticoids increase the risk of quinolone-induced tendon rupture. We conducted in vitro experiments with human-derived tenocytes to study the action of these drugs in tissue. Cells were incubated for 24 hours with (a) the fluoroquinolone levofloxacin at concentrations of 0, 0.3, 1, 3, 10, and 30 mg/l medium, (b) 100 µg prednisolone/l medium, and (c) levofloxacin at the same range of concentrations in combination with prednisolone. We analysed the early apoptotic response of the cells as indicated by annexin V staining by flow cytometry. In addition, we studied the apoptosis marker “activated caspase-3” (casp-3) in tenocytes incubated with 0, 3, 10, 30 mg levofloxacin/l medium for 1 to 4 days using immunoblotting. The annexin V staining showed a concentration-dependent increase of apoptosis in cells incubated with levofloxacin alone. We observed a significant difference to controls at concentrations of 1 mg/l and higher (control: 8.2 % ± 1.5 % positively stained cells, 1 mg/l: 13.2 % ± 2.7 %, p < 0.05, t-test). Tenocytes incubated...
with 100 µg prednisolone/l medium alone showed a significant increase of apoptotic cells as well. A combined incubation with levofloxacin and prednisolone resulted in an enhanced response revealing a significant difference already at a concentration of 0.3 mg/l levofloxacin. The immunoblotting of activated casp-3 as a key enzyme in the apoptotic pathway showed time- and concentration-dependency in comparison to the untreated control. A pronounced increase (approx. 10-fold) was seen after 10 mg levofloxacin/l medium on the fourth day of incubation. The evidence of these results with tenocytes in vitro suggests that programmed cell death plays an important role in the pathomechanism of this adverse reaction. The clinical observations that glucocorticoids enhance the quinolone-induced tendopathy are supported by our data.

### The effects of simvastatin on ischemia-reperfusion injury of sciatic nerve in adult rats

| ESC-ID | 762 |
| Author | Raza ML, Zeeshan M, Ahmad M, Shaheen F, Simjee SU |
| Country | Pakistan |
| University | HEJ JCCBS University of Karachi |
| Department | Pharmacology |

**Objective:** In the present study the DNS-II acetone fraction isolated from Delphinium nordhagenii is tested in kindling model induced by PTZ. Chemical kindling is produced by using subconvulsive dose (50mg/kg) of pentylenetetrazole in mice every 48 hrs for the 16 treatments (Mason and Cooper, 1972) the resultant seizures were classified as stage 0 to stage 5. The animals showing the score 4-5 consider being as kindled. Animals were divided in seven groups with 6 animals in each group. The saline (vehicle control), diazepam (7.5 mg/kg), phenytoin (20 mg/kg) and DNS II (60 mg/kg, 65 mg/kg and 75 mg/kg) were administered interaperitoneally to Group 1 Group II, Group III, Group IV, Groups V-VII respectively. However, PTZ was administered subcutaneously. The drugs and DNS II fraction were administered 30 minutes before pentylenetetrazole injection and were placed in separate cages.

**Results:** The kindling in the PTZ group were developed fully after the 13 treatment and the animals showed a gradual increment in the seizure score compare to the groups received the AED i.e. phenytoin group exhibited the score 3 and diazepam showed a twitches and jerks, while the groups receiving DNS II retarded the kindling acquisition completely.

**Conclusion:** The results shows that DNS II possesses powerful antiepileptogenic activity, further purification from this fraction is required to get the active molecule that may serve as new antiepileptic drug.

### Physiology I

**Dual effect of claudin-16 expression in MDCK cells**

| ESC-ID | 782 |
| Author | Pfaffenbach S, Richter JF, Amasheh S, Fromm M, Günzel D |
| Country | Germany |
| University | Charité Universitätsmedizin Berlin |
| Department | Institute of Clinical Physiology |

Tight junctions play a key role in paracellular ion transport, especially in the kidney. Claudin-16 (alternative name: para-cellin-1), a member of the claudin family, is a tight junction protein and localized in the thick ascending limb of Henle's loop. Its defect causes familial hypomagnesemia, hypercalciuria and nephrocalcinosis (FHHNC). This had been taken as indication that claudin-16 conveys paracellular Mg2+ and Ca2+ transport. However, it was demonstrated that claudin-16-mediated Mg2+ permeability alone is not large enough to explain the degree of Mg2+ loss occurring in FHHNC.

**Aim:** In order to elucidate this discrepancy we characterized the effects of claudin-16 on ion transport parameters when expressed in a kidney cell line.
Methods: High-resistance MDCK-C7 (Madin-Darby canine kidney) cells, stably transfected with human claudin-16, were grown to confluence on filter supports and used for electrophysiological experiments (Ussing chamber) and Ca2+ imaging (confocal laser scanning microscopy) employing fluo-4-AM. Vector-only transfected cells served as controls.

Results: Mg2+ flux data and biionic potential measurements demonstrated a significant, albeit small, increase in paracellular Mg2+ permeability of claudin-16-transfected cell layers. In addition, divergent cations were found to activate the basolateral Ca2+-sensing receptor (CaSR), inducing transcellular Cl- secretion which consequently alters the driving force for paracellular Mg2+ movement. This effect was greatly enhanced in claudin-16-expressing cells. To investigate intracellular pathways following CaSR activation in claudin-16-expressing and in control cells, intracellular Ca2+ concentrations ([Ca2+]) were imaged. Stimulation of CaSR by the application of 100 µM NiCl2 caused intracellular Ca2+ to oscillate, however, no differences between claudin-16 transfected and control cells were found with respect to amplitude and frequency of these oscillations.

Conclusion: It is concluded that claudin-16 expression in MDCK cells has a dual effect. It increases paracellular Mg2+ permeability and indirectly interacts with the signal caused by CaSR activation. This latter interaction is not due to a direct effect of claudin-16 on CaSR or on Ca2+ loading of the cells. As the observed increase in [Ca2+]i activates apical Ca2+-sensitive Cl- channels, claudin-16 may directly or indirectly affect these channels. Thus, lack of functional claudin-16 as in FHHNC will not only reduce Mg2+ permeability but may also alter the electric driving force for Mg2+ transport.

Variability of thiobarbituric acid reacting substances in saliva

ESC-ID 817
Author Behuliak M, Pálffy R, Clec P
Country Slovakia
University Comenius University, Faculty of Medicine
Department Institute of Patophysiology

Aim: Salivary thiobarbituric acid reacting substances (TBARS) have been proved as a potential marker of intraoral oxidative stress and periodontal status. This study was aimed at the analysis of intra- and interindividual variability of TBARS in saliva.

Methods: Twenty two young healthy volunteers (12F & 10M) collected saliva samples daily in the morning during a period of 30 consecutive days. Salivary TBARS were measured spectrophotometrically. Time series analysis was done using standard statistical methods.

Results: Repeated measures ANOVA showed significant between day variations of salivary MDA (p <0.001). The dynamics did not differ between genders, however, the data was not synchronized, and thus, gender differences in endogenous dynamics cannot be ruled out. Intraindividual variability was very high in both genders with coefficients of variation of more than 70%. Interindividual variability was higher in men than in women (63% vs. 20%; p <0.001).

Discussion: The relatively high intraindividual variability indicates that repeated samplings and subsequent measurements are needed for individual diagnostics. Gender differences and interindividual variability will be taken into account in running clinical studies on patients with periodontal and dental diseases. Factors influencing the variability of...
salivary TBARS including infradian biorhythms should be uncovered by further studies.

The antitumoural effect of the combined Doxorubicina - PDT diet in Walker tumour

ESC-ID 91
Author Szocs A
Country Romania
University Medicine and Pharmacy Cluj-Napoca
Department Medicine

Aims: The study shows the evaluation of cytotoxic and antitumoural effect in the combined treatment Doxorubicina - photodynamic therapy (PDT) on an experimental pattern for Walker tumour.

Methods: The study was made on Wistar male rats, with the medium weight of 200 grams, with Walker carcinosarcoma 256 subcutaneous administration. After three weeks from the inoculation the animals were divided into 3 groups: Group I (n = 10) - treated with 5,10,15,20 tetra (4-sulfophenyl) porfirine (TPhSP) 10 mg/kg body and irradiated after three hours with laser I = 685nm, D = 100 J/cm2 for 15 minutes; Group II (n = 10) – treated with combined diet Doxorubicina 3 mg/kg and photodynamic therapy made 15 minutes after the administration; Group III (n = 10) - treated with Doxorubicina (Dox) 3 mg/kg corp; Five animals from each group were sacrificed after 24 hours and 5 animals were mentained for 14 days for appreciating the tumoural regression. Blood and tumoural tissue samples were taken from each group and the parameters of oxidative stress were established: the total malondialdehida (MDA), SH groupings and the hydrogen ability donating (DH).

Results and conclusion: The combined treatment photodynamic therapy - Doxorubicina was more efficient than the simple treatment with doxorubicina or than photodynamic therapy, with the considerable inhibition of the tumour growth (p <0.05). In the case of group II, after 24 hours from the treatment, malondialdehida has a considerable growth (p <0.05). In the case of group II, after 24 hours from the treatment, malondialdehida has a considerable growth (p <0.05). In the case of group II, after 24 hours from the treatment, malondialdehida has a considerable growth (p <0.05). In the case of group II, after 24 hours from the treatment, malondialdehida has a considerable growth (p <0.05).

Alterations in excitation-contraction coupling induced by swimming in rat papillary muscle

ESC-ID 361
Author Scridon A, Serban RC, Perian M, Barbat G, Dobreanu D, Sabau M
Country Romania
University University of Medicine and Pharmacy Targu Mures, Romania
Department Physiology

Introduction: As response to a variety of mechanical, hemo-dynamic, hormonal and pathologic stimuli, the myocardium adapts to increased workloads through the hypertrophy of individual muscle cells. OBJECTIVE This study used swimming, as this exercise training is recognized for its efficiency in inducing myocardial hypertrophy in rats. The purpose of the study was to investigate the role of electrophysiological changes in physiological cardiac hypertrophy.

Ultrasound: an adjunct of tissue plasminogen activator, reduced ischemic brain injury and perfusion

ESC-ID 376
Author Bodaghabadi M, Shabanzadeh AP
Country Iran
University Tehran University of Medical Siences
Department Physiology

Object: Ultrasound has been used in neuroprotection after cerebral ischemia. Application of ultrasound may make strong fibrinolytic medications. In this study the effect of ultrasound alone or in combination with tissue Plasminogen activator (tPA) on brain ischemic injury were examined and we studied whether US is protective in the brain injured ischemic.

Methods: We performed four studies. In the first study, rectal and brain temperature were compared. In the second part, we studied whether US alone or in combination with tPA is neuroprotective in thromboembolic stroke. The infarction volume, edema and neurological deficits were after middle cerebral artery (MCA) occlusion. In the third and fourth
study, we studied if US in combination with tPA can improve perfusion deficits or blood-brain barrier (BBB) permeability in rats after MCA occlusion. 

Results: The results showed that temperature in the brain was approximately 0.50°C lower than the rectal temperature. Results indicate that US alone reduces the infarct volume by 30% compared to the control group (P<0.05). US improved neurological deficits and reduced brain edema significantly, (p<0.05). Result in the studies showed that administration of US significantly decreased perfusion deficits but not BBB permeability.

Conclusion: These studies suggest that US is protective in an embolic model of stroke and that it may be caused by decreased perfusion deficits in brain injured rats.

Expression of tight junction proteins claudin-2 and claudin-16 in mammary epithelium of mice

The wall of the mammary gland alveolus is a monolayer of secretory cells which have tight junctions in their apical section separating the alveolus cavity from the intercellular liquid. It has been shown that major barrier functions of tight junctions are primarily attributed to proteins of the claudin family, which are expressed in a tissue- and organ-specific manner. Transepithelial permeability is allowed by a selective pore function of claudins within the tight junction strands. Claudin-2 has been shown to constitute pores selective for Na+, K+ and other small cations, whereas claudin-16 (paracellin-1) mediates paracellular Na2+ and Mg2+ permeability.

In the process of milk production in the mammary gland, ion influx into the alveolus cavity can take place transcellularly and/or paracellularly through the para-liquid takes place. Ion influx into the alveolus cavity and the intercellular liquid takes place. Ion influx into the alveolus cavity can take place transcellularly and/or paracellularly through the para-cellular pathway. Calcium transport into the milk has been fully attributed to the exocytosis of secretory vesicles, in which the increased cal-cium concentration is generated by the Na2+-AOPase of Golgi membranes. Regarding Na+ and K+ transport transcellular as well as paracellular pathways are discussed.

Aims: The aim of this study was to analyze the expression of the tight junction proteins claudin-2 and claudin-16 in the secretory epithelium of the mammary gland. These two claudins were selected because they may play a role in paracellular ion transport because due to their ability to form cation-selective pores.

Methods: Female mice (n = 6) were taken for the experiment during the lactation period on the 10-15th days post partum. After killing mammary glands were prepared and expression of oc-cludin, claudin-2, and claudin-16 was analyzed by means of immunohistochemistry and confocal laser-scanning microscopy.

Results: In the apical part of secretory cells on the mamma-ry gland tissue, the common marker protein for other tight junction proteins, occludin, was clearly detectable. Regarding the two cation-selective proteins of interest, only claudin-16 but not claudin-2 was detectable. Claudin-16 expression was located in the apical part of the cells at the borders of the alveolar cavity. Claudin-16 was not significantly distributed in any other parts of the alveolus. Claudin-16 colocalized with occludin indicating a localization within the tight junc-tion meshwork.

Conclusion: We found that claudin-16 is located in the tight junction meshwork of mammary gland epithelium. Because the typical lumen-negative voltage of >30 mV would drive cations into the lumen, we conclude that claudin-16 may provides a significant pathway for paracellular Na2+ transport into the alveolus cavity.

Serum erythropoietin level is enhanced during brain hypoxia in critically ill patients with neurosurgical pathologies

New functions of haemopoetic hormone erythropoietin were found in the CNS in last decade. Epo was shown to be endogenous neuroprotector in human brain. Although many articles are focused on its neuroprotective action and first clinical were started, little is known about Epo regulation during brain hypoxia in human. There is little evidence indicating that brain may control renal production of Epo in a humoral way. In particular, increased cerebral hydrostatic pressure leads to increased renal synthesis of Epo in rodents. In order to test this hypothesis in humans, we investigated serum Epo levels in critically ill patients with SAH, TBI and brain tumors.

Methods: Epo concentrations were serially determined in 100 serum samples of 19 critically ill patients with neurosurgical pathologies, including 11 patients with SAH, 5 patients with TBI and 3 patients with brain tumors. Clinical and laboratory data were collected from clinical register in retrospective way. Brain ischemic or hypoxic conditions was detected by MRI, CT, TCD and ICP data.

Results: Samples were divided in 2 groups by presence or absence of brain hypoxic or ischemic conditions. Great inverse correlation between hemoglobin and Epo levels was detected in non-hypoxic samples (r = -0.52, p <0.001). In contrast, no correlation between hemoglobin and Epo levels was found in hypoxic probes (r = -0.022). Epo levels were much higher in hypoxic probes (55 ± 34), than in non-hypoxic ones (21 ± 15), (p <0.001).

Conclusion: Patients with brain hypoxic syndrome exhibit an inadequate Epo response to hemoglobin concentrations, indicating another mechanism of Epo regulation is involved. Brain hypoxia is likely to be the candidate for this role. Increased serum Epo level correlated with development of brain hypoxic syndrome and ischemia. Thus, Epo could serve as a marker of brain hypoxia in humans. Epo is a very attractive candidate for this role due to proposed non-invasive methods. Future investigations are to reveal the mechanisms underlying Epo response to the brain hypoxic syndrome.
Tight Junctions (TJ) regulate paracellular permeability across epithelial cell layers. Claudins, a family of TJ transmembrane proteins, provide an intercellular seal but may also convey specific ion permeability. Claudin-10 has previously been described to exist in two isoforms. While isoform a is restricted to kidney and uterus, isoform b is found in a great variety of tissues. In the present study, claudin-10-transfected cell layers were characterized with respect to expression, localisation, and physiological function.

Methods: Mouse and human claudin-10 isoforms were stably transfected into high-resistance Madin-Darby canine kidney (MDCK) cells, which endogenously do not express juncional claudin-10. Vector-only transfected cells served as controls. Cells were cultivated on filter supports and used for immunostaining and subsequent confocal laser scanning microscopy (CLSM) to investigate claudin-10 localization. Ion selectivity analysis was performed by measuring dilution/biionic potentials in an Ussing chamber set-up.

Results: CLSM revealed junctional distribution of all mouse and human claudin-10 isoforms. Investigation of ion selectivity indicated that claudin-10 isoform b overexpressing cells had reduced transepithelial resistance due to a clearly higher paracellular permeability for cations compared to control cells and to cells transfected with isoform a. Within the monovalent cations, claudin-10 isoform b- transfected cell layers exhibited a preference for Na+ > Li+ > K+ > Rb+ > Cs+ corresponding to the Eisenman sequence X for alkali metals, while vector controls and isoform a followed Eisenman sequence IV: K+ > Rb+ > Cs+ > Na+ > Li+. No differences between corresponding mouse and human isoforms were found.

Conclusion: While the tight junctions of vector controls and claudin-10 isoform a-transfected cell layers exhibit weak field-strength binding sites for monovalent cations, expression of isoform b causes a cation permeability characterized by strong field-strength binding sites. Thus claudin-10 isoform b develops a cation-selective pore across the tight junction.

Detection of oxidative stress by the application of hydrogen peroxide sensor

Introduction: Hydrogen peroxide (H$_2$O$_2$) causes oxidative stress. It plays role in different physiological and pathological conditions. H$_2$O$_2$ owing to its high reactivity, is in very low concentration in biological matrices. There has been no opportunity to detect oxidative stress in vivo so far.

Aim: Our purpose was to measure the interstitial H$_2$O$_2$ level, using a H$_2$O$_2$-specific microelectrode placed into the subcutaneous area.

Methods: Electroenzymatic glucose sensor commercial product of MiniMed Medtronic Company (CGMS, Minimed Medtronic, USA) was used for the experiments. The sensor measures interstitial glucose concentration through the detection of hydrogen peroxide, evolved in an enzymatic reaction. The reaction is catalyzed by the glucose-oxidase enzyme. We inactivated the enzyme using a protein-precipitating agent (sulfosalicylic acid, SSA). The applicability of this „inactivated” glucose sensor was tested in buffered aqueous media, using different concentrations of H$_2$O$_2$. The sensor was calibrated by amperometric measurements (using advanced electrochemical workstation, AUTOLAB 12). Further in vitro experiments were made in human plasma samples. In vivo experiments were carried out in diabetic and non-diabetic Whistar-rats, using inactivated and non-inactivated sensors. We detected the interstitial glucose-, and H$_2$O$_2$-levels.

Results: The inactivation (SSA, 5%, 15 minutes) did not change the H$_2$O$_2$-sensitivity of the sensor, while the glucose-sensitivity decreased to the zero. In in vitro amperometric measurements, carried out in PBS solutions, the current increased proportionately with the H$_2$O$_2$-concentration. The lower limit of H$_2$O$_2$-detection was approximately 5 umol/l. In plasma samples, the current answer to H$_2$O$_2$ adding was much smaller and the life time of H$_2$O$_2$ was shorter than in PBS solution, indicating the very potent antioxidant capacity of the human plasma. We perform in vivo experiments as well. In some of our preliminary experiments, however, when high glucose doses were injected, higher amperometric current transients could be seen with H$_2$O$_2$-electrodes implanted in diabetic rats. Further studies are in progress for gathering detailed information about in vivo applicability of the H$_2$O$_2$ sensor.

Conclusion: Detecting H$_2$O$_2$-level in vivo can give us new information about the interstitial oxidative stress poesessions.

Influence of physical exercise on lipid peroxidation process and oxidative stress

ESC-ID 201
Author Brnic O, Andrijasevic V
Country Serbia
University University of Pristina
Department Department for Pathophysiology

Despite the many health benefits of exercise, there is a plenty of evidence suggesting that endurance exercise is associated with oxidative stress. Physical exercise is characterized by an increase in oxygen consumption by the whole body and particularly by active muscles. This process is accompanied with a rise in the production of reactive oxygen species (ROS), that may be responsible for a series of physiological and biochemical changes that occur during exercise. Measuring free radical production directly is difficult, primarily because of the short life-span of the species. The majority of studies investigating the effects of exercise on oxidative stress have focused on markers of free radicals induced tissue damage. Malondiadehyde (MDA) is the final product of lipid peroxidation, and increased concentration of this compound is believed to be an indirect reflection of increased free radicals concentration. In this paper MDA plasma levels were determined in 30 students in rest and after treadmill running pro-
tocol (Bruce Treadmill Protocol). It has been found that after the treadmill test, the level of MDA in plasma had increased from 3.04 µM/L to 4.39 µM/L (p <0.0001). This results suggest that strenuous exercise increase production of free radicals, causing increased lipid peroxidation process. The question for further investigation would be the benefit antioxidant supplements in preventing exercise induced oxidative stress.

Hippophaea rhamnoides interferes with insulin release via L-type Ca 2+ chanel-mediated pathway in rat islet β-cells

ESC-ID 718
Author Sarlea SV, Joanta AE, Nicoleta D, Bojan M, Socaciu C, Moldovan R, Hobai S
Country Romania
University “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania
Department Department of Physiology

Aims: Diabetes Mellitus (DM) continues to be a world public health problem, having a cascade of complications such as cardiovascular disease, chronic renal failure, retinal damage, nerve damage, and microvascular damage, which may cause erectile dysfunction (impotence) and poor healing of wounds. Recent studies showed that complications are generated by the Oxidative Stress induced by the hyperglycaemia. Polyunsaturated fatty acids, such as oleic acid have an antioxidant effect, being found in high concentration in Hippophaea rhamnoides. Our aim is to explore the in vivo molecular mechanism of action of oleic acid by using a molecular modeling simulation software, Molegro Virtual Docker (MVD) and to observe the antioxidant effect of Hippophaea rhamnoides in different circumstances related to insulin release from rat islet β-cells.

Methods: Experiments were performed on white, male, Wistar rats, organized as follows: group 1: control-standard diet, group 2: rats fed a diet enriched in sea buckthorne: (Hippophaea rhamnoides), group 3: rats fed a diet enriched in sea buckthorne and glucose, group 4: rats treated with L-type Ca 2+ channel blocker (Nifedipine), group 5: fed a diet enriched in sea buckthorne and glucose, group 6: rats treated with L-type Ca 2+ channel blocker (Nifedipine), fed a diet enriched in sea buckthorne and glucose, group 7: rats treated with Nifedipine, fed a diet enriched in glucose. Glycemia level, oxidative stress markers (lipid peroxidation, carbonyl proteins) and antioxidant defence (superoxiddismutase, catalase) were evaluated by estimation of levels of thiobarbituric acid-reactions in biological fluids after 1 and 2 weeks of experiment.

Results: It was noticed a significant increase in glycemia level in group 5 as compared to group 4. These data suggest through an indirect manner that Hippophaea rhamnoides stimulates Ca 2+ influx through L-type Ca 2+ channels in -cells, followed by insulin release. Also, it was observed an increase in oxidative stress markers and a decrease in antioxidant defence of the analysed tissues in animals from group 3 and 5. A possibility is that long-term exposure to fatty acids from Hippophaea rhamnoides, corated to glucose administration could induce long-lasting elevation of Ca 2+, thereby evoking the Ca 2+ toxicity and consequent dysfunction of islet β-cells. By using the MVD software we’ve observed the in vivo binding mechanism of oleic acid and its antioxidant effects on oxidative damaged cells and also the best binding positions for the best antioxidant effect.

Conclusion: Present data suggest that the fatty acids consisted in Hippophaea rhamnoides interferes with insulin release via L-type Ca 2+ channel-mediated pathway in rat islet β-cells. A new approach regarding fitotherapeutical alternatives in diabetes mellitus has to be considered.

Treatment with aminophylline diminished meconium-induced acute lung injury in rabbits

ESC-ID 472
Author Redfors B, Bulikova J, Mokra D
Country Sweden
University Commenius University
Department Department of Physiology

Introduction and aim: Methylxanthines act as non-selective phosphodiesterase inhibitors and antagonists of adenosine receptors and may diminish lung edema formation, inflammation, vasoconstriction and bronchoconstriction induced by meconium aspiration. The aim of our study was to evaluate the effects of aminophylline treatment on the lung function of meconium-instilled rabbits.

Method: Air-ventilated adult rabbits received 4 ml/kg of either saline (Sal group, n = 5) or meconium suspension (25 mg/ml) intratracheally. From this moment, all animals were oxygen-ventilated. When respiratory failure developed, meconium-instilled animals received aminophylline (2 mg/kg) intravenously at 0.5 and 2.5 h after meconium instillation (Mec+Amin group, n = 7) or were left without treatment (Mec group, n = 8). All animals were ventilated for additional 5 h after the first dose of treatment. Lung functions, blood gases and white blood cell count (WBC) were evaluated in regular time intervals. In the right lungs, lung edema was determined by wet/dry weight ratio. Oxidative damage to lipids and proteins of the lung homogenate was evaluated by estimation of levels of thiobarbituric acid-reactive substances, dityrosine and lysine-lipid peroxidation products. The left lungs were saline-lavaged and differential WBC was estimated microscopically in the sediment of the lavage fluid.

Results: Intravenous aminophylline significantly improved oxygenation, reduced intrapulmonary shunting, central venous pressure and lung edema. Aminophylline also significantly decreased the number of neutrophils in the lavage fluid and increased the numbers of total WBC and neutrophils in the blood. Furthermore, aminophylline prevented oxidative damage to proteins and lipids in lung homogenate.

Conclusion: Aminophylline enhanced the pulmonary function and diminished lung edema and inflammation in a rabbit model of meconium aspiration syndrome.

Event related potentials after acute bouts of exercise at different intensities

ESC-ID 242
Author Milankov V
Country Serbia
University Medical faculty, University of Novi Sad
Department General Studies

Abstract: Several articles about physical activity in detail analyze the engagement of the whole body during acute bouts of physical exercise. The idea that physical activity directly influences cognitive functions is still not empirically confirmed. The aim of this experiment was to give inlihgth on the
influence of physical activity at different levels on the amplitude and latency of P300 component of cognitive potentials. After registering cognitive event related potentials in young healthy adults (over Fz and Cz regions), participants underwent a controlled exercise on a bicycle ergometer. Each exercise lasted for 10 minutes with successive increase of intensity to 60%, 75% and 90% of maximal values of pulse and holding this level of intensity for six minutes. Immediately after finishing each bout of exercise, event related potentials were registered again. The amplitude of P300 wave after exercise intensity achieved at 60% (Fz 10.46 ± 5.40 mV; Cz 14.42 ± 3.23 mV) and 75% of maximal values of pulses (Pmax) (Fz 11.31 ± 5.7 mV; Cz 15.74 ± 3.14 mV) were statistically higher (p<0.05) than the amplitude of P300 at rest (Fz 8.88 ± 3.72 mV; Cz 12.33 ± 3.15 mV) and after 90% of maximal pulse (Fz 8.76 ± 3.05 mV; Cz 12.63 ± 3.31 mV). There were no differences (p>0.05) between the amplitudes after 60% and 75% of Pmax, as well as between the values obtained at rest and after 90% of Pmax. There were no statistically significant differences (p>0.05) among the latencies of P300 registered at rest (Fz 321.69 ± 19.89 ms; Cz 321.67 ± 19.87 ms) and after 60% of Pmax (Fz 314.00 ± 26.16 ms; Cz 314.22 ± 25.73 ms), 75% (Fz 315.20 ± 13.93 ms; Cz 313.70 ± 13.68 ms) and 90% of Pmax (Fz 315.11 ± 21.25 ms; Cz 315.89 ± 21.85 ms). Physical activity evidently influences cognitive functions. In the zone of intermediate intensities the amplitude of P300 increases, at submaximal intensities it decreases to the values obtained at rest. The latency of P300 does not change significantly after different intensities of exercise.

Psychiatry I

The long term effects of early exposure to cannabis on the behaviour and neurochemistry of an animal

ESC-ID 110
Author Wiethoff S, Wildgruber D, Kreifelts B, Grodd W, Ethofer T
Country Germany
University Tübingen
Department Department of Psychiatry

Background: Epidemiological evidence has found cannabis to be a risk factor for schizophrenia in susceptible individuals and worsens symptoms in schizophrenia sufferers. The isolation reared rat is an animal model of some of the aspects of schizophrenia, and can be studied for the effects of cannabis in the pathogenesis of schizophrenia, which would not be possible in human studies. Exposure to Δ-9-THC (the active compound of cannabis) in the initial period of isolation aims to model its long-term effects in the developing rat brain. An additive effect of Δ-9-THC on the effects of isolation rearing may confer cannabis as a risk factor in precipitating and worsening the symptoms of schizophrenia. Objectives: To use the behavioural profile, and levels of BDNF protein in the hippocampus, of the isolation-reared rat as parameters for testing the long term effects of early exposure to Δ-9-THC and whether this exacerbates the outcomes of isolation rearing.

Methods: Male Lister Hooded rats were isolated post weaning (23 days) and treated with Δ-9-THC (2mg/kg, I.P.) for the first week of isolation. Behavioural tests (locomotor activity, prepulse inhibition (PPI) of the response to a startle stimulus, novel object discrimination and hyperactivity after PCP treatment) were performed in weeks 6-7 of isolation. Acute locomotor response to PCP (3mg/kg, I.P) was measured before the rats were culled and levels of hippocampal BDNF were measured using Western immunoblotting.

Results: Isolation rearing caused hyperactivity in a novel environment and impaired PPI. D-9-THC, alone, reduced body weight and increased rearing after PCP treatment but did not affect locomotor activity, PPI or novel object discrimination. A D-9-THC x isolation rearing interaction increased locomotor activity in the first 10 minutes after PCP administration. Hippocampal BDNF was significantly reduced in Isolates.

Conclusion: The behavioural profile of our Isolates is consistent with previous studies, supporting the validity of the isolation rearing model for studying schizophrenia in rats. The dosing regimen of D-9-THC in this study did not have a long term effect on rat behaviour, but interestingly did cause a decrease in body weights suggesting a persistent effect of D-9-THC on feeding or metabolism. Although an interaction of D-9-THC and isolation was significant in locomotor activity after PCP treatment, there is not enough evidence to suggest that D-9-THC exacerbates the effects of isolation rearing under these test conditions.

Processing of emotional prosody - influence of acoustic parameters

ESC-ID 7
Author Cui H, Mok J, Graylen S, Baxter A
Country United Kingdom
University University of Nottingham
Department School of Biomedical Sciences

Objective: Prosody is an important factor in human communication. Previous neuroimaging studies have demonstrated the existence of voice-sensitive areas in the associative auditory cortex adjacent to the middle part of the superior temporal sulcus (mid STS) [1]. Recently it has been shown that these voice-sensitive areas respond stronger to angry [2, 3] and happy [3] than to neutral prosody. However it is unclear whether this effect is driven by differences in basic acoustic parameters or reflects higher behavioural relevance of emotional relative to neutral prosody. In this fMRI study we presented prosodic words and employed covariance analyses to evaluate the contribution of several acoustic parameters (duration, intensity and pitch) to hemodynamic responses of mid STS cortices.

Methods: 24 healthy right handed subjects (12 f, 12 m, mean age: 25.1 years) participated in an event-related fMRI-study performed on a 3 T scanner (Siemens TRIO, Erlangen, Germany). 50 semantically neutral adjectives and nouns spoken in neutral, happy, alluring, fearful or angry intonation were presented in a passive-listening paradigm. These stimuli were balanced for word frequency and number of syllables, normalized to same peak intensity and evaluated in two prestudies to ensure that the intended emotional category is recognized and to balance the emotionally spoken stimuli for arousal. Functional imaging data were analyzed with SPM2 to compare hemodynamic responses to emotional versus neutral intonations. Data extracted from right mid STS were submitted to separate covariance analyses for each of the acoustic parameters. Regression residuals of these analyses
were evaluated by paired t-tests to clarify whether stronger responsiveness to emotional stimuli was still significant after correction for basic acoustic parameters.

**Results:** Emotional prosody compared to neutral prosody revealed stronger hemodynamic responses in mid STS, left temporal pole and hypothalamus. In right mid STS, responses were stronger for all four emotional intonations relative to neutral intonations (all paired T(23) >1.9, p <0.05). Responses in these regions showed a significant correlation with duration (r = 0.32), mean intensity (r = 0.19), mean pitch (r = 0.10) and pitch variability (r = 0.09) of the stimuli. Comparison of regression residuals obtained from these analyses revealed higher values for emotional as compared to neutral stimuli (all paired T (23) >2.0, p<0.05).

**Conclusion:** In agreement with previous findings [2, 3] emotional prosody elicited stronger activations in mid STS than neutral prosody. This effect was significant for each of the four emotional categories suggesting a general role of mid STS in processing of emotional prosody. Activation of mid STS correlated with duration, mean intensity, mean pitch and pitch variability of the stimuli. However, none of these parameters could solely explain the stronger responsiveness to emotional comparing to neutral stimuli indicating that mid STS integrates several acoustic cues to a common percept to decode emotional signals in the human voice.

**The BDNF response to exercise**

**Introduction:** Brain Derived Neurotrophic Factor (BDNF) is a member of the neurotrophic factor family that plays a key role in regulating survival, growth and maintenance of neurons (1). In addition BDNF plays a role in learning and memory (2). BDNF crosses the blood-brain barrier and levels of BDNF, both within the brain and in the peripheral, are suppressed in patients with neuropsychological diseases such as depression and dementia (3,4,5,6). Exercise decrease depression rate, prevents or delay the onset of dementia and facilitates cognitive functions (7,8,9). We aimed to investigate whether systemic BDNF levels were regulated by exercise in healthy young males and whether contracting skeletal muscle would express BDNF.

**Materials and Methods:** Twenty healthy, physically active but untrained men (mean ± SD age: 25.6 ± 3.5 years) participated in the study. Blood and skeletal muscle biopsies were obtained at the time points 0; 2; 3; 5; 8; 24; 48 and 72 hr. Immunohistochemistry revealed stronger hemodynamic responses in mid STS, left temporal pole and hypothalamus. In right mid STS, responses were stronger for all four emotional intonations relative to neutral intonations (all paired T(23) >1.9, p <0.05). Responses in these regions showed a significant correlation with duration (r = 0.32), mean intensity (r = 0.19), mean pitch (r = 0.10) and pitch variability (r = 0.09) of the stimuli. Comparison of regression residuals obtained from these analyses revealed higher values for emotional as compared to neutral stimuli (all paired T (23) >2.0, p<0.05).

**Conclusion:** This is the first study to show that exercise leads to BDNF expression by human skeletal muscles. That skeletal muscle expresses BDNF suggests that muscle may contribute to the systemic levels of BDNF. However, the time kinetics indicates that the acute exercise-induced increase in serum BDNF most likely has its origin from other cells, such as platelets. The exercise-mediated increase in BDNF may promote some of the positive effects seen in patients with neuropsychological diseases and enhance learning and memory.

**Modern approaches to therapy of the depressive frustration accompanied by suicide behaviour**

**Introduction:** The problem of depressive frustration is one of actual for the theory and practice of modern medicine. After anxiety, depression is the most common health disorder. **Aim of work:** The purpose of research - optimization of therapeutic approaches to correction depressive frustration accompanied suicide behaviour at persons of young age. **Methods:** The study group constituted 75 patients (from 17 to 35 years ) which are made suicide attempt and have such diagnoses as: the mixed disturbing-depressive frustration, short-term depressive reaction, sharp reaction to stress and the prolonged depressive reaction. As have shown results of research in a clinical picture of depressive frustration of the surveyed patients dominate depression of mood - 79.5 % and affect of melancholy - 44.6 %, asthenic symptoms - 35.7 %, disturbing symptoms - 68.9 %. Results of the biochemical researches lead by us have shown, that there is increase of serotonin concentration in blood and reduction of a level of melatonin in blood at our patients. As occurrence both depressive frustration, and the suicide behaviour speak with the monoamine theory, optimum for their therapy are selective serotonin reuptake inhibitors, so in our work was used stimyloton (the active component of a preparation sertraline is selective serotonin reuptake inhibitor in a brain). The advantages of using stimyloton of our research are: a wide spectrum of efficiency concerning all kinds of depressive frustration; creation of stable concentration of a preparation in blood.

**Results:** After reception of stimyloton within 3 weeks it has been noted decrease in parameters to 13 and less points on a scale of Hamilton, 15 and less points on a scale of Montgomery-Asberg, 7 and less balls on the Hospital scale of alarm and depression that testifies to absence of depressive and disturbing episodes at our patients.

**Conclusion:** Our research has shown high efficiency of this therapy in patients with depressive frustration, accompanied suicide behaviour.
Perceptions about the cause and management of schizophrenia

Background and aims: Culturally different people have variable perceptions regarding the cause of schizophrenia. Patients are generally considered dangerous and are isolated, although studies show that good family and social support, early diagnosis and management are good prognostic factors. We aimed to determine help seeking behavior and perceptions regarding etiology of schizophrenia and correlate it with sociodemographic factors.

Materials and methods: This cross-sectional, questionnaire based study was carried out on a convenience sample of 404 people sitting in the waiting areas of the outpatient department of Aga Khan University Hospital Karachi. Medical staff, students, psychiatric patients, their attendants, and children were excluded. Univariate binary logistic analyses were done to identify associations. Data was analyzed on SPSS v.14.

Results: The mean age was 31.4 yrs and 77% were males. The majority were graduates and employed. Only 30% of the participants attributed 'mental illness' as a main cause of psychotic symptoms while a large number thought of 'God's will' (32.3%), 'superstitious factors' (33.1%), 'loneliness' (24.8%) and 'unemployment' (19.3%) as the main cause. Mental illness as the single most important cause was reported by only 22%. Only 40% reported psychiatric consultation to be the single most important management step that they would choose for a relative with psychotic symptoms. Other choices included spiritual healing (19.5%) and Social changes (10.6) while 14.8% of respondents said that they would to nothing or counsel him themselves. Sex, ethnicity and religious inclination were significantly associated with reporting the correct etiology (p<0.05). Ethnicity, employment status and education were significantly associated with reporting the correct management option (p = 0.001).

Conclusion: Despite majority of the study population being well educated, only a few recognized schizophrenia as a mental illness and many held superstitious beliefs. Educational programs regarding cause and management of schizophrenia should be implemented to increase public awareness.

Stock differences in Sprague-Dawley rats

Sprague-Dawley rats are often used as the genetic background for creating transgenic animals. Since strain and stock differences often result in contradictory outcomes of equally conducted behavioral tests, we investigated the behavior of two different stocks of Sprague-Dawley rats: RjHan:SD (Janvier, France) and Shoe:SPRD (originally derived from DIMED Schönwalde GmbH, Germany, and now bred at our department). Both stocks originated from the “Zentralinstitut für Versuchstierzucht – Hannover”. RjHan:SD rats and Shoe:SPRD rats were tested for their anxiety-related behavior and motor activity in the elevated plus-maze (EPM) and open field test (OF). Furthermore, their habituation abilities were investigated in the hole board test (HB) on two consecutive days. Additionally, the animals were individually tested in feeding boxes where food intake, water intake and activity were measured over one hour. Rats were adapted to this procedure for three days. While exposed to the EPM the Shoe:SPRD rats showed significantly more U-turns, in the OF entries into the center area were significantly reduced. This indicates a probably higher anxiety-related behavior of the Shoe: SPRD rats, whereas differences in motor activity could not be found in these two tests. Both stocks habituated to the HB in a comparable manner indicated by significantly fewer nose pokes on the second observation-day. In the feeding boxes food intake and drinking rate seemed to be increased in the RjHan:SD rats, which is supported by their significantly higher activity rate. The results show that, although all animals were from the same strain, stock differences could be documented for each test. The RjHan: SD rats seem to be less anxious than the Shoe:SPRD rats even though some of the measured parameters are not always significantly different. The breeding differences seem to have an impact not only on the anxiety-related behavior but also affect feeding experiments. The findings indicate that replacement of laboratory animals, although they are from the same strain, can not easily be implemented when they are from different stocks.

Placebo or botulinum for patients with focal dystonias?

Background: Placebo has played an important role in medical treatment for decades, however its effects have only recently become subject of extensive research. This study evaluates the effectiveness of placebo compared to botulinum type A in treatment of focal dystonias and also considers the variables that influence placebo response in an individual.

Methods: A randomized double-blind crossover study was conducted and comprised of 39 patients with torticollis or blepharospasm. Placebo responders were identified and served as cases and non-responders as controls in a case-control study. The examined variables were described with descriptive statistics and differences between cases and controls were calculated using t-test and chi-square test. The probability that a variable will influence the placebo response was calculated using unconditional univariate and multivariate logistic analysis.

Results: The start of initial improvement, duration of the drug effect, percentage of improvement and occurrence of side effects were compared between placebo and botulinum injections; initial improvement occurred after 5.9 days in both injections, however a statistically significant difference was observed between the two injections for the other three variables: duration, improvement, side effects (t = 4.21, p ≤ 0.001; t = 4.68, p ≤ 0.001; t = 2.08, p = 0.04). 56% placebo
responders were identified by both definitions and placebo response was found to be influenced by four different variables – the patients who had not been treated by alternative medicine (OR = 6.43; CI = 1.51-27.45) or had a monthly income lower than 830$ (OR = 3.8; CI = 0.96-15.19) or had only primary school education (OR = 5.2; CI = 0.95-28.5) or were younger than 61 years (OR = 1.43; CI = 0.40-5.12) were more likely to be placebo responders. Odds ratios did not change after controlling for confounders and effect modification.

Conclusion: The study established a causal relationship between placebo response and alternative treatment, level of education, income and age. In future, when considering administering placebo, these variables and the extent of their influence on placebo response could help determine the patients who would most likely benefit from it.

Radiology I

Characterization of atherosclerotic plaques in human coronary arteries with 16-slice-multidetector computer tomography

**Objective**: We investigated, if 16-slice-MDCT allows correct classification of coronary plaques into calcified or non-calcified and further sub-classification of noncalcified plaques into either lipid-rich with a necrotic core or fibrous.

**Materials and Methods**: Coronary arteries of 30 isolated hearts were filled post mortem with a contrast medium and scanned with a 16-slice-MDCT imager (Light Speed 16 pro, GE/MS, Milwaukee, USA). The images were reformatted perpendicular to the axis of the coronary arteries (AW 4.2 software, GEMS) and analysed by establishing attenuation profiles of the coronary cross sections (ImageJ 1.33n software, NIH, Bethesda, MD, USA). Results were compared to the correlating histopathological sections of the arteries.

**Results**: Analysis of 195 CT-cross sections showed a sensitivity and specificity for the correct classification of calcified plaques of 100% and 97.3% and for noncalcified plaques of 80.8% and 95.1% respectively. The attenuation of epicardial fat ranged from -119HU to 23HU (median: -71HU), and from 93HU to 625HU (308HU) for the contrast medium. Calcified plaques showed an attenuation between 333HU and 1944HU (1089HU), noncalcified plaques between 26HU and 124HU (52HU). Further sub-classification of noncalcified plaques showed attenuation values between 26HU and 67HU (median: 44HU) for lipid-rich plaques with a necrotic core and from 37HU to 124HU (median: 67HU) for fibrous plaques.

**Conclusion**: Coronary atherosclerotic plaques can be reliably identified and classified as either calcified or noncalcified by 16-slice-MDCT in post-mortem studies. Further differentiation of noncalcified plaques in either lipid-rich or fibrous is not reliably feasible due to substantial overlap of the attenuation. Key-words: tomography; coronary disease; atherosclerosis; plaque.

A comparison of direct digital radiography with conventional periapical radiography in vertical root fractures

**ESC-ID** 9
**Author** Bonabi M, Niktash A
**Country** Iran
**University** Shahed
**Department** Radiology

**Objective**: The main purpose of this study is to compare the diagnostic potential of direct digital radiography with conventional periapical film for detecting experimental root fractures.

**Materials and methods**: 50 extracted single rooted human teeth were endodontology instrumented and divided into two groups, a control group of 24 teeth without root fracture and a test group of 26 teeth in which root fractures were produced experimentally. Each tooth was casted in a block made of composition of acryl and gypsum and imaged using parallel technique with a CCD-based digital system and E-speed film with substantial soft tissue phantom. Then radiographs were repeated with different horizontal angulations to mesial and distal shifting as 15°. At least 300 radiographs were taken with these two systems. The images were interpreted by 5 observers (3 endodontics, 2 oral and maxillofacial radiologists) without prior knowledge of the distribution of the root fractures. Sensitivity, specificity and reliability of each imaging systems was calculated separately. The degree of intraobserver and interobserver agreements were expressed as Kappa value. The differences between the radiographic systems were then assessed by X2 – test at the 95% significant level.

**Result**: The sensitivity and specificity values of digital system and conventional radiography were assessed as 36.4 ± 6.1, 48.8 ± 5.2, 87.2 ± 7.2, 93.6 ± 5.4 respectively. The difference between two systems was significantly different. (p = 0.027 for sensitivity and p = 0.008 for specificity) There was substantial intraobserver agreement in both digital technique (Kappa = 0.84 ± 0.08) and conventional radiography (Kappa = 0.81 ± 0.04). There was poor interobserver agreement in digital system (Kappa = 0.18 ± 0.15), however it was good for conventional radiography (Kappa = 0.32 ± 0.17) and this difference was statistically significant. (p = 0.002) In this study sensitivity of digital system and conventional radiography in detection of buccolingual root fractures better than mesiodistal root fractures. The difference between site of vertical root fracture was statistically significant (p = 0.041 for conventional system and p = 0.011 for digital system).

**Conclusion**: Conventional radiography shows a better view of vertical root fractures in comparison with digital system. When the decision has been made about the presence of vertical root fracture, it is preferable to take the radiographs with the different horizontal angulations to be sure whether the roots are intact or fractured to avoid wrong treatment planning.
Distribution of white matter lesions in multiple sclerosis

ESC-ID  276
Author  Stankovic A
Country  Serbia
University  Nis
Department  Medicine

Introduction: Multiple sclerosis (MS) is the most common acquired demyelinating disease. Recommended diagnostic criteria for MS incorporate magnetic resonance imaging (MRI) into the well established diagnostic workup that focuses on detailed neurological history and examination and a variety of paraclinical laboratory examinations.

Aim: The aim of this study was to determine distribution of MS white matter lesions on MRI.

Material and Methods: This is a prospective study of 42 patients with MS. There were 32 (76.19%) female and 10 (23.81%) male. The mean age was 38.86 ± 9.13 years. All patients underwent MRI examination with next protocol: pre- and postcontrast T1W (TR 400-700, TE 5-25), PD (TR 2000-3000, TE 20-40), T2W (TR 2000-3000, TE 70-120). Postcontrast T1W scans were performed 5 minutes after intravenous administration of gadolinium-DTPA (0.1ml/kg). Pearson’s x² test and Student’s t test were performed for statistical analysis.

Results: MR findings in all patients were in accordance with the Diagnostic criteria for MS: 2005 revisions to the “McDonald Criteria”. Number of supratentorial lesions (23 ± 23.60) was statistically significantly higher comparing with infratentorial lesions (10 ± 23.60). The most frequent localization of the white matter lesions was juxtacortical and periventricular (38; 90.48% patients). Number of these white matter lesions were juxtacortical (39; 92.86% patients) lesions (t 5.24; p < 0.001). The most frequent localizations of the statistically significantly higher comparing with infratentorial localization of MS white matter lesions was juxtacortical and periventricular (38; 90.48% patients). Number of these lesions was statistically significantly higher (p < 0.001) in comparison with other localization with an exception of pons plaques (p < 0.01). There was no significant difference of the number of lesions with the other localization. MS plaques were found in corpus callosum in 25 (59.52%) patients. Pons was the most frequent infratentorial localization of MS plaques (27; 64.9% patients). MS plaque in n. opticus was found only in one patient. Enhancement of MS plaques after intravenous administration of gadolinium-DTPA was found in 5 (11.90%) patients.

Conclusion: Supratentorial distribution of white matter lesions was predominant in our study. The most frequent localization of MS white matter lesions was juxtacortical and periventricular.

Cerebro-spinal irradiation in pediatric posterior fossa tumor patients causes permanent alteration of neural metabolite concentrations as detected by single voxel MR proton spectroscopy

ESC-ID  532
Author  Rueckriegel S, Blankenburg F, Henze G, Pablo PH, Bruhn H
Country  Germany
University  Charité Universitätsmedizin Berlin
Department  Department of Pediatric Oncology and Hematology

Objectives: Neurotoxicity of posterior fossa tumors, chemotherapy, and cerebro-spinal irradiation in children leads to neurologic and neurocognitive long-term deficits.

Survivors sequelae are more pronounced in high-grade tumor patients who have received chemotherapy and cerebro-spinal irradiation than in patients who have undergone resection only. By comparing these two otherwise similar patient groups we intended to isolate the important neurotoxic effect of cranio-spinal irradiation. Metabolite concentrations in white matter (WM) and in gray matter (GM) as determined by quantitative single voxel proton MR spectroscopy (MRS) served as a measure of neurotoxicity of irradiation and chemotherapy.

Methods: 23 medulloblastoma survivors and 1 ependymoma (WHO III) survivor (age ± SD: 13.0 ±5.2y; interval therapy to examination ± SD: 46 ±31.7m) formed the first group that had received chemotherapy and cranio-spinal irradiation including a boost on the posterior fossa. 19 pilocytic astrocytoma (WHO I) survivors and 1 ependymoma (WHO II) survivor were included in the second group (age ± SD : 11.9 ± 4.7y interval ± SD: 30.4 ± 24m). All patients were subjected to MR imaging and MRS on a 3T-MR system equipped with an 8-channel headcoil (3T Signa Excite, GE Healthcare) after ethically-approved written informed consent had been obtained. MRS was performed in parietal WM (4 ml) and parietal midline GM (8 ml) using the PRESS technique (TR/TE/NEX = 6000ms/30ms/64). Quantification of the resonances of creatine and phosphocreatine (Cre), N-acetylaspartate and N-acetylaspartylglutamate (tNAA), choline-containing compounds (Cho), glutamate (Glu) and myo-inositol (mI) was done with the operator-independent LCModel method.

Results: In the high-grade tumor group concentrations of Cho (p = 0.004, Mann-Whitney-U-test), tNAA (p = 0.029), and Glu (p = 0.045) were significantly decreased in WM, and mI (p = 0.035) was increased in GM. Mean linewidth at FWHM as a criterion of spectrum quality was 6.7 Hz in GM and 4.9 Hz in WM.

Discussion: The decrease of brain metabolite concentrations in irradiated patients supports the notion that cranio-spinal irradiation has a lasting effect on neural tissue. Predominantly in WM decreases of Cho, a marker of membrane turnover in astrocytes and oligodendrocytes, and tNAA, an indicator of neuronal integrity and myelination, were noted.

Conclusion: MRS in vivo provides valuable data on the molecular level for a better understanding of the mechanisms and extent of irradiation-induced neurotoxicity. More extended longitudinal follow-up studies are warranted to correlate the predictive value of metabolite quantification vs. neurocognitive outcome.

Axial and peripheral manifestation in spondyloarthritis: A whole body MRI approach

ESC-ID  90
Author  Burova E, Althoff C, Sieper J, Hamm B, Hermann KG
Country  Germany
University  Charité Universitätsmedizin Berlin
Department  Dept. of Radiology

Background: Spondyloarthritis (SpA) is a group of similar diseases with distinct clinical features and a common genetic predisposition, its best known representative being ankylosing spondylitis. Patients predominantly suffer from axial disease (spine and sacroiliac joints). Peripheral arthritis and
enthesitis are common findings in some subtypes of SpA and may need additional imaging depending on clinical presentation. The aim of the study was to evaluate whole body MRI (WB-MRI) as the sole imaging procedure in SpA patients.

Material and Methods: 39 Patients with known SpA and at least one painful joint region/spinal segment underwent MRI using a whole body scanner (Siemens Avanto 1.5 Tesla). Coronal and sagittal T1-weighted fast spin echo and short tau inversion recovery (STIR) sequences were acquired using parallel imaging techniques and special coil devices. Total scan times were between 60 to 70 minutes. Images of the spine, thorax, pelvis, upper and lower extremities were read by two experienced musculoskeletal radiologists. Global joint pain was recorded on a 0-10 visual analogue scale (VAS).

Results: Patients included in the study were considerably active with a mean VAS pain value of 6.5 ± 1.8. 35 patients (90%) showed axial inflammatory lesions while 28 patients (72%) showed a peripheral involvement (mean of 1.6 inflamed joints) and the plantar fascia (8%). Peripheral enthesitis was found at the Achilles tendon (13%), the sternoclavicular joint (21%), the hip (13%), and the shoulder (13%). Most frequent peripheral findings were arthritis of the ankle (38%), the knee (23%), and enthesitis of spinal ligaments (18%). Most frequent peripheral finding was arthritis of the ankle (38%). The knee (23%), the sternoclavicular joint (21%), the hip (13%), and the shoulder (13%). Peripheral enthesitis was found at the Achilles tendon (28%) and the plantar fascia (8%).

Conclusion: With the use of parallel imaging techniques, high-resolution WB-MRI is possible within acceptable scanning times. Based on our data, peripheral manifestation of SpA seems to be underestimated until now. Thus, WB-MRI has the potential to serve as “one stop shop” in patients with active SpA.

Ultrason sound image of thyroid gland in obese children - is there similarity with Hashimoto’s thyroiditis?

ESC-ID 477
Author Bernad A, Szczerski J, Grodecki L
Country Poland
University Medical University of Gdansk
Department medical

Background: It is suggested that obese children may show similar ultrasound (US) image of thyroid gland to those with Hashimoto's thyroiditis due to the morphological changes caused by obesity.

Aims: The aim of the study was to evaluate US features of thyroid in obese children.

Methods: Two groups underwent US examination: 58 obese children (30 girls), mean age 12.76 ± 3.28yr, height SDS 0.66 ± 1.35, BMI SDS 7.35 ± 3.14 and 62 non-obese children (54 girls) of the same age (12.71 ± 2.94) with Hashimoto’s thyroiditis as a control group. TSH, rT4, rT3 and anti-thyroid antibodies were performed in all children.

Results: US examination of the obese group (OG) revealed: reduction in thyroid echogenicity in 27 children (46.6%), isthmus enlargement in 39 children (67.2%), mildly increased intraparenchymal blood flow in 3 cases (5.2%) and the presence of the thyroid adjacent lymph nodes in 6 (10.3%). Simultaneous occurrence of all above elements was noted in 3 patients (5.2%) of the OG and in 59 children (95.16%) of the Hashimoto's thyroiditis group (HG). In the OG mean thyroid volume was 10.37ml ± 8.2, no differences were found between patients with and without ultrasound changes (8.8ml ± 4.11 vs. 12.17ml ± 11, p >0.1). Thyroid volume in HG was higher (20.35ml ± 14.65) than in the OG (p <0.001). The isthmus width (3.74mm±1.4) was above norm but lower than in HG (6.92mm±2.21, p <0.001). Positive antibodies were found in all HG and in 3 children (5.2%) of OG patients. Two of those 3 patients in OG were suspected to have Hashimoto's thyroiditis after the US examination, with thyroid volumes of 52.5 and 31.8ml and with TSH above norm respectively.

Conclusion: We conclude: isthmus enlargement and reduction of thyroid echogenicity in ultrasound examination are typical changes to obese children and should not be evaluated as signs of Hashimoto's thyroiditis, especially when thyroid is not enlarged.

Space Medicine

Rapid fluid shifts along the body axis during simulated and real microgravity

ESC-ID 340
Author Noack T, Kowoll R, Fraßl W, Koralewski E, Gunga HC
Country Germany
University Charité Universitätsmedizin Berlin
Department Radiology

Rapid fluid shifts was investigated for the two NSCLC cell lines, A549 and H460. Both, A549 and H460, have the same p53 wild-type status, but show a marked difference in cellular radiosensitivity following ionising irradiation and caffeine. In irradiated and non-irradiated G1-synchronized cells 2 mM caffeine led to a temporary delay in the G1-S transition. The percentage of S phase cells was also reduced by caffeine. Furthermore 2 mM caffeine significantly reduced the radiation-induced p53 and p21 protein increase close to the control level. 5 mM caffeine resulted in an increase in apoptosis in irradiated and non-irradiated only in H460. 2 mM caffeine caused a reduction in the mRNA of cyclin D1, gadd45, p21 and hsp-70. However, the gene expression of the proteins Bax, Fas-1 and Tradd was increased by caffeine. No change was found at the mRNA level for the proteins Cdk2, E2F and the caspases. These data indicate that the caffeine effect on cellular radiosensitivity and cell cycle progression is primarily exerted at the protein level than gene level and seems to be independent of p53.

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the tissue thickness (TT) with ultrasound. The aim of the present study was to evaluate the rapid fluid shift ($t \leq 25$ sec) in a simulated (head-down-tilt HDT) and real (parabolic flights PF) microgravity experiment and to compare the use of two ultrasound devices (USD) to measure the TT.

Methods: We investigated the changes in TT on forehead and shinbone with two different USD. The first device Krautkraemer measured in A-Scan and the second device Collagenoson in A-/B-Scan. 1) HDT: HDT (-6°) is used to simulate the fluid shift on earth. During the HDT experiment TT were recorded on 12 healthy volunteers in lying position. We measured manually with booth USD in normal position 0° (Phase I), head-up-tilt 60° (Phase II), head-down-tilt -6° (Phase III), head-up-tilt 60° (Phase IV) and normal position 0° (Phase V). 2) PF: PF is used to create short ($t \leq 25$ sec) periods of changing gravity between 0 Gz and 1.8 Gz. During the PF campaign TT were recorded in 9 healthy volunteers in lying position. We measured manually with booth USD one day before PF (Phase I), hypergravity at the ascending leg of parabola (Phase II), microgravity at the top of parabola (Phase III), hypergravity at the descending leg of parabola (Phase IV) and after the flight (Phase V).

Results: In the HDT group, the results showed non significant increase or decrease of TT in all volunteers during the measurement with A-Scan and A-/B-Scan. In the PF group, we measured an increase in TT of the forehead with a maximum in microgravity (Phase III) in comparison to baseline data and postflight data with booth USD. Furthermore, we found differences in measurements between Krautkraemer and Collagenoson. Krautkraemer and Collagenoson values showed the same kinetics but in their absolute values varied individually during the different phases.

Conclusion: In conclusion, short terms of real microgravity conditions during parabolic flights induce a rapid fluid shift ($t < 25$ sec) along the body axis from the legs to the head in human beings. Furthermore, we concluded from our HDT study and from data from PF, that it is questionable to use the HDT model to describe rapid fluid shifts ($t < 25$ sec) during simulated microgravity along the body axis on earth. This has to be investigated further.

Outlook: Interestingly we were able to observe that the fluid shift induced by weightlessness during PF seems to have a remarkable influence on the heat transmission from the skin to the environment. We have found an increase in superficial skin temperature during the microgravity phase during PF.

Can facilitation increase the HOFFMANN-reflex under short-time micro-gravity conditions during a parabolic flight?

ESC-ID 971
Author Schlabs T, Wagensiel B, Betzler F, Gewies M, Abels W, Schulz J, Kowoll R (†), Gunga HC
Country Germany
University Charité Universitätsmedizin Berlin
Department Center for Space Medicine Berlin

Background: It is important to understand the sensory, motor and perceptual factors that influence man’s ability to adapt to different gravitational forces. Experiments performed on Space Shuttle, Skylab and MIR revealed that changes in a person's neurovestibular system occur during middle- and long-term exposure to weightlessness – changes related to the inner ear, equilibrium and awareness of body or limb orientation. When returning to Earth an astronaut’s or cosmonaut’s microgravity-adapted neurovestibular system must re-adjust to the gravity environment. Several studies on the HOFFMANN-reflex (H-reflex) conducted during parabolic flights have shown an increased reflex response under short-time micro-gravity (micro-g) conditions [1, 2]. The mechanisms on this effect still remain uncertain. In our neurophysiologic study we examined the influence of short-time (about 20s) micro-g conditions (+GZ = 10_-g) on the HOFFMANN-reflex of the triceps-surae muscle while a technique called facilitation was applied. This facilitation is commonly known as the JENDRASSIK maneuver (JM) [4]. The objectives of our study were 1) to ascertain that the micro-G environment alone leads to an increased reflex response of the conditioned triceps-surae muscle, and 2) to find out whether facilitation performed during micro-G results in a further amplification of the reflex response.

Methods: Our study was conducted during the 9th Student Parabolic Flight Campaign of the European Space Agency in Bordeaux from 6th till 15th of September 2006. We tested 4 subjects on two flight days and performed measurements in three different gravitational conditions (+GZ = 10_-g; +GZ = 1g; +GZ = 1.8g). To elicit the H-reflex we stimulated the Ia fibers of the tibialis nerve and measured the reflex response of the soleus muscle by deflecting an electromyogram (EMG). The amplitude of the EMG was a measure of the strength of the H-reflex.

Results: Valid data has been collected from three subjects. We found a significant increase of the reflex-response in micro-g on two subjects, a decrease on one subject. Facilitation leads to a further amplification but the elevating effect in micro-g (+GZ = 10_-g) is weaker than in normo-G (+GZ = 1g). Thus, reflex response in general can not be increased endlessly, and therefore the JM effect declines through micro-g (which itself has an increasing influence on reflex response already). Furthermore there occurred an unexpected, not yet explainable phenomenon in the normo-G phase of the flight: the reflex-response was declined while JM was applied.

Surgery I

Appendicitis - clinical presentation of tumor. Analysis of appendixes' histopathological examination results

ESC-ID 772
Author Rynkiewicz-Szczepanska E, Szczepanski W, Matus L, Wojskowicz P
Country Poland
University Medical University of Bialystok
Department I Department of General Surgery

Aim: For a couple of years all appendixes, that were resected in the I Department of General Surgery of the Medical University of Bialystok, are being sent to histopathological examination. Aim of the study was to analyze the results of these examinations to check the frequency of occurrence of tumors manifesting themselves as appendicitis and the influence of those accidental findings on patient’s further life.
Effect of various power densities of low level laser therapy on normal and impaired skin wound healing in rats

ESC-ID 780
Author Gál P, Mokrý M, Vidinský B, Depta F, Kosejová E, Kostišová L, Harakacová M
Country Slovakia
University Pavol Jozef Safarik University in Kosice
Department Department of Medical Biophysics

Introduction: Low-level laser therapy (LLLT) belongs to modern experimental approaches used in wound healing therapy. However, at present time there is no general agreement about the exact way how LLLT influences wound healing. Moreover, little is known about the direct links between the parameters (wavelength, power density and dose) of laser radiation and the process of wound healing. Nevertheless, it has been well documented that HeNe laser using a dose of 3-4 J/cm² is capable to accelerate wound healing (1,2,3), but measurement of the optimal power density is still unknown. Therefore, the aim of our study was to find the optimal delivering form of such a therapy (wavelength = 635 nm; daily dose = 5J/cm²; tested power densities [mW/cm²] = 1, 5 and 15) to promote wound healing in normal healthy and steroid-treated rats.

Material and Methods: Ten-months-old male Sprague-Dawley rats (n = 72) were included into the experiment and randomly divided into two groups of 36 animals (non-steroid and steroid treated group). In general anesthesia, four round full thickness skin wounds, 4 mm in diameter, were performed on the back of each rat. Three wounds of each rat were daily irradiated using a GaAlAs diode laser (635 nm) to achieve total daily dose of 5J/cm², while the fourth wound was not irradiated and served as control. One of the three treated wounds was irradiated with the power density of 1mW/cm², the second of 5mW/cm², and the third of 15mW/cm². Eight animals from both groups were sacrificed on post operative day (POD) 2, 6 and 14. The tissue specimens were processed routinely for light microscopy (hematoxylin-eosin and van Gieson) and histologically evaluated using both quantitative and semi-quantitative (4) methods.

Results: In non-steroid group, significant acceleration of epithelization (POD 2 – 0mW/cm² vs. 15mW/cm² (p <0.04), POD 6 – 0mW/cm² vs. 15mW/cm² (p <0.02)) as well as higher amounts of collagen fibers in stimulated wounds were observed (POD 2 – 0mW/cm² vs. 5mW/cm² (p <0.01), 0mW/cm² vs. 15mW/cm² (p <0.01), POD 6 – 0mW/cm² vs. 5mW/cm² (p <0.01), 0mW/cm² vs. 15mW/cm² (p <0.01)). Histological evaluation of steroid-treated group showed earlier regress of inflammatory phase in all stimulated wounds in comparison to their non-stimulated control wounds on POD 2 (PMNL/Ma – 0mW/cm² vs. 1mW/cm² (p <0.01), 0mW/cm² vs. 5mW/cm² (p <0.01), 0mW/cm² vs. 15mW/cm² (p <0.01)). Moreover, significant reduction of the granulation tissue area was observed in wounds treated at 5 and 15mW/cm² (0mW/cm² vs. 5mW/cm² (p <0.05), 0mW/cm² vs. 15mW/cm² (p <0.05)).

Conclusion: Routine histopathological examination of resected appendices, even though only about 1% of the results is different than expected inflammation, should be continued. It gives an opportunity to find a tumor in an early stage of its growth and for a radical operation, which lead to much better prognosis.

Aim: Ischemia/reperfusion injury (I/R) is one of the major problems in intestinal transplantation. We have evaluated the efficacy of TNF-alpha inhibitors to mitigate I/R injury in a rat small bowel transplant model.

Methods: Orthotopic small bowel transplantation was performed in an isogenic model in male Lewis rats. Grafts were flushed with UW solution and stored for 6hrs at 4°C before implantation. The TNF-alpha inhibitor groups either received pentoxifylline (P) (25 mg/kg bw i.p., pre reperfusion, PODs 1-5) or etanercept (E) (1 mg/kg bw s.c.; pre reperfusion, PODs 1,3,5,7). Controls (C) received no further treatment. Tissue samples of the proximal and distal small bowel were taken 20min, 12hrs, 7d, and 6mths after reperfusion (each n = 6 per time point and group) for histopathology (H&E stain) and immunohistology (CD4, CD8, CD25, ED1, ZO-1, HSP70). Lung sections were stained for MPO (specific staining of neutrophil granulocytes). Significant differences were evaluated by multfacorial analysis using two-way ANOVA and Bonferroni post test.

Results: 7 day survival was significantly improved following TNF-alpha inhibition (75% vs. 60% in controls). Beyond POD 7 survival was 100% in all groups. TNF-alpha inhibition significantly decreased I/R injury in both treatment groups. Histological signs of I/R-injury as well as immunohistologically assessed graft infiltration of T cells (CD4+, CD8+, CD25+) and ED1+ macrophages (selected data: C vs. E and P/distal 20min: 54 ± 2 vs. 36 ± 2 and 35 ± 1; 12hrs: 55 ± 1 vs. 37 ± 2 and 32 ± 2; 7d: 64 ± 1 vs. 30 ± 1 and 27 ± 3 cells/quarter of field of view, all p<0.001) displayed significant reduction over the whole observation period. Moreover,
Non-invasive evaluation of the gracilis muscle innervation by means of surface electromyography electrode in the context of dynamic graciloplasty procedure

**Introduction:** One of the advanced surgical methods of fecal incontinence treatment is dynamic graciloplasty (DGP). The aim of DGP is formation of a new anal sphincter from gracilis muscle. In DGP gracilis muscle is transposed and a neuromuscular implant is implanted. Transposition is dependant on innervation zone (IZ) location in gracilis muscle. Also, a choice of one of the two available muscles must be made.

**Aim:** The purpose of the study was a non-invasive assessment of the innervation zone location of the gracilis muscle of both thighs by means of surface electromyography (sEMG) on a group of healthy volunteers.

**Materials and methods:** The group included 20 volunteers: 18 male and 2 female (on average 22.6 yrs, SD ± 2.4, on average high of 182.6 cm, SD ± 9.3). The tests were conducted by means of 16 electrodes spaced in 1 cm gaps on a flexible array. Motor unit action potentials from both thighs were detected during maximal contraction of the gracilis muscle, then extracted and identified. On the basis of the acquired signal recordings IZ was established. IZ location on both thighs was compared. Results were statistically analyzed.

**Results:** A statistically significant difference was found (Wilcoxon test, p <0.001) between gracilis IZ location on each volunteer’s thighs. On average, IZ was located 7.032 cm from inguinal fold on one thigh and 8.07 cm on the other thigh. Also a statistically significant correlation was found (p <0.001) between dominance of the volunteer’s leg (right vs. left) and the IZ being located closer to the inguinal fold.

**Conclusion:** The gracilis muscle IZ location could be assessed using sEMG in healthy individuals. Non-invasive surface electromyography may be useful in preoperative evaluation of patients who are to be prepared for the dynamic graciloplasty procedure. The sEMG test is a useful way of assessing the gracilis muscle because of its safe and non-invasive character. Additionally, a statistically significant asymmetry was found in the case of innervation zone in the left and the right gracilis at the same person, which means the operator can choose more usable gracilis muscle in the context of dynamic graciloplasty procedure.
The intraoperative evaluation of sentinel lymph node with use of frozen sections examination technique

ESC-ID 295
Author Wierzbka KT, Borys M, Maliszewski D, Wozniacki P
Country Poland
University Medical University of Gdansk
Department Surgical Oncology Dept.

The intraoperative evaluation of sentinel lymph node (SLN) has become subject of scientific discussion. The considered techniques are: imprint cytology and frozen sections examination (FSE). The study presents results of FSE in patients with breast cancer, in whom iSLN was dissected.

Material and methods: 45 female patients (mean age - 59.7; range 28-79) operated in Surgical Oncology Department, Medical University of Gdansk, Poland were enrolled in the prospective, ethics board approved study. All patients gave written, informed consent to participate in the study. The criteria of inclusion were: breast cancer diagnosis confirmed by cytological and/or histopathological examination, malignant lesion in mammography and no axillary lymphadenopathy. In 2 patients tumor was in T1a stage, in 6 - T1b, in 20 - T1c and in 17 patients T2. In 42 patients ductal cancer was diagnosed, in 2 patients lobular cancer and in 1 patient Paget disease of the breast. In all patients surgical treatment was performed – in 7 patients breast conserving treatment, in 38 modified radical mastectomy. All patients were operated by the same surgeon, with student assistance. SLN was identified by the authors alternatingly with: blue-dye technique (methylene blue-dye ) and combined technique (methylene blue-dye + Nannocis with Tc99m). The SLN was identified by tracking stained lymphatic vessel and/or with hand gamma probe. We have frozen half of the SLN in liquid nitrogen, then sliced it perpendicular, parallel to the cutting surface, in levels 200 micrometers apart. Afterwards we stained the frozen sections with hematoxilin - eosin .Thereafter SLNs were evaluated by a single pathologist blinded for the results of final pathological examination(PE). All specimens were bar-coded.

Results: SLN was identified in 43 of 45 patients. 49 lymph nodes (LN) were dissected (range 0-2). Metastases in PE were confirmed in 43 LNs ( in total 779 lymph nodes were excised) In patients in whom SLN was free of metastases in PE no other metastases in LN were diagnosed. PE confirmed metastases in 11 SLN from 8 patients. FSE was concomitant with PE in 9 SLNs. Positive Predictive Value (PPV) of FSE was 100%, Negative Predictive Value (NPV) - 95,1%, sensitivity - 81%, specificity - 100% and accuracy 95.9%. In 49 FSEs no false positive results were counted. The accordance of FSE and PE was 95.9%. The relation between results of FSE and PE of metastatic SLN reached statistical significance (p <0.001) in Fisher exact test.

Conclusion: FSE performed correctly gives high PPV and NPV. Lack of false positive results proves technique safe and useful in intraoperative diagnostics of SLN.
The new method of the ethanol sclerotherapy of the thyroid cystic nodules

The wide relevance of miniminvasive treatment of benign thyroid nodules is established practice in contemporary medicine. The aim of this study is the elaboration of harmless and effective method of ethanol destruction of cystic transformed thyroid nodules.

Materials and methods. We propose a method of the ethanol repeated lavage. The first step is the cyst puncture under the control of ultrasonography, using the 21G needle. The tip of the needle is located in the center of cyst cavity. The total aspiration of the cyst liquid is executed. The capacity of cyst is evaluated and after that 95% ethanol is injected into the cyst. The volume of ethanol equals of 50% of cyst volume. After the one minute exposition ethanol is ejected. Subsequently we inject the same volume of ethanol again without extracting the needle. In this way we make from two to five introductions of ethanol with one minute exposition and subsequent aspiration. When the aspirate becomes colorless and flaskless we stop the procedure and remove as much ethanol from the cyst as possible. Efficiency of the procedure is controlled by sonography, color Doppler mapping, power Doppler mapping. The ethanol repeated lavage method was applied in clinic to 30 patients with cystic transformed benign thyroid nodules. Among them there were 28 women (93.3%) and 2 men (6.6%). The ages of patients were 22-64 years. The volume of cysts was 15.3-36.5 ml (average value was 23.7 ml). The cystic component occupies more than 60% of the whole nodule volume. We include in our research group only patients with solitary node, regular rates of thyroid hormones and TSH, without increasing of antithyroid antibodies. All patients undergo only one course of sclerotherapy. Average amount of procedures was 1.8.

Results: Volume of the nodes after six months after treatment was 1.2-3.4 ml (average volume 2.7). There was total obliteration of cystic cavity in 29 cases (96.6%). All nodules retain the distinct boundary. There were no cases of hypo- or hyperthyroidism, increasing level of antithyroid antibodies after treatment. We didn’t register any complications.

Conclusion: Introduced method of the ethanol repeated lavage is miniminvasive and efficient technique for the treatment of patients with benign cyst thyroid nodules.

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Tumour necrosis factor-alpha stimulates liver regeneration after partial portal vein ligation – Experimental study on porcine model

Introduction: Portal vein ligation (PVL) could multiply the future liver remnant volume (FLRV) in spite of affection of only one of liver lobes by malignant diseases. PVL of portal branch of with malignancy afflicted liver lobe initiates compensatory hypertrophy of contralateral non-occluded lobe. After portal vein ligation increases serum level of Tumour necrosis factor-alpha (TNF-α). The study also observed influence of TNF-α at general status of organism mainly at homeostasis and secondary induction of inflammation.

Methods: Under general anaesthesia the laparotomy and ligation of portal branches for caudate and right lateral and right medial liver lobes were performed. The recombined porcine TNF-α or physiological solution was applied into non-occluded portal vein branches. The following biochemical and immunoanalytical parameters were estimated: Bilirubin, Urea, Creatinine, Alkaline phosphatase, Gama glutamyl transpeptidase, Cholinesterase, Aspartate aminotransferase, Alanine aminotransferase, Albumine, C-reactive protein, Tumour necrosis factor-alpha, Interleukin-6, Hepatocyte growth factor, Insulin-like growth factor, Transforming growth factor-alpha and Transforming growth factor-beta. The compensatory hypertrophy was controlled by periodic ultrasonography. The pathological examination was performed after sacrificing of animals.

Results: The absolute volume of hypertrophic lobes increases after application of TNF-α more rapidly whereas the control group has no changes in hypertrophic liver lobes volumes in first three days practically. The acceleration of growth of hypertrophic liver lobes was maximal at the 7th postoperative day in comparison with control group (p-value <0.05). The secondary effects, which could be hypothesized after application of key pleiotropic cytokine (changes in immune reactions and homeostasis), were not observed during application and also in the whole postoperative period. On the contrary application of TNF-α prevented the increases in serum level of AST (Aspartate aminotransferase), ALT (Alanin aminotransferase), GGT (Gama glutamyl transpeptidase) and Creatinine in postoperative period.

Conclusion: The presented study is newly established experimental model of portal vein embolization on big animal, which is physiologically very similar to human body and the results could be transmittable to human liver surgery. TNF-α confirmed the key role of studied cytokine in priming of regenerating liver parenchyma after portal vein ligation with protective effect on hepatocytes in postoperative period.
Effects of controlled reperfusion on muscle function after 5 hours of ischemia and two or four hours of reperfusion in a rabbit hind-limb model

ESC-ID 585
Author Krepuska M, Foerster K, Boeker T, Dallmeier DE, Heilmann C, Schlensak C, Beyersdorf F
Country Hungary
University Semmelweis / Albert-Ludwigs-Universitaet Freiburg Faculty of Medicine
Department Cardiovascular Surgery

Objectives: Acute limb ischemia is associated with high mortality and amputation rate despite surgical revascularisation due to ischemia-reperfusion(IR)-injury. “Controlled reperfusion” while restoring physiological blood flow is a promising tool to reduce IR-injury and implies control of flow and pressure and application of a hyperosmolar buffer-solution containing metabolic substrates, radical scavenger, and calcium chelator. This study aims at the effects of controlled reperfusion on muscle function.

Methods: Rabbits were anaesthetised; both Mn. tibiales anteriores were dissected and attached to force transducers. Contraction force was measured following stimulation at 4mA and 50Hz. Ischemia was induced by clamping the left A.iliaca comm. and A.femoralis. Collaterals were ligated and ischemia was maintained for 5h. The right limb served as individual control. Subsequently, controlled reperfusion (CR, 9.33ml/min) was performed for 30min by perfusion of the left A.iliaca comm. with reperfusion-solution mixed with blood (ratio 1:6). Afterwards, physiological blood flow was allowed for 1.5h or 3.5h (n = 6, respectively). Controls underwent 5h of ischemia and consecutive 2h or 4h (n = 5, respectively) of uncontrolled blood reperfusion (UCR). Muscle contractility was assessed intermittently. Kidneys were examined at the end of the procedure for activation of transcription factors AP-1 and NF-kB employing gel shift assays.

Results: Muscle force decreased due to ischemia, reaching 12.1±4.2% (CR) and 8.6±4.7% (UCR) of control limb force after 5h. Muscle force recovered to 39.6±14.1% of control limb force after 2h of uncontrolled reperfusion. Initial 30min of controlled reperfusion led to a recovery of 52.4±20.5% (CR vs UCR, p = 0.28) after 2h. Prolonging reperfusion to 4h resulted in muscle force recovery of 47.7±11.8% in the UCR-group, while the CR-group showed an increase to 59.4±7.9% of control limb force after a 30min+3.5h-observation period (CR vs UCR, p = 0.09). Control limbs exhibited 104.4±9.8% (p = n.s.) of basic muscle force throughout procedure. Activation of AP-1 and NF-kB was observed in kidney tissue.

Conclusion: We developed a rabbit model of acute limb ischemia-reperfusion injury verified by evaluation of muscle force. Systemic reperfusion injury is indicated by activation of the inflammatory transcription factors AP-1 and NF-kB in the kidneys. This model is intended to optimize the solution for controlled reperfusion in patients.

Evaluation the effect of recombinant human fibroblast growth factor (bFGF) and erythropoietin (EPO) in prevention of ischemic necrosis of skin flaps in rat model

ESC-ID 640
Author Salavati A, Fayazzadeh E, Tafhi HA, Rabbani S, Dehpour AR
Country Iran
University Tehran University of Medical Sciences
Department Iranian Tissue Bank, SSRC

Aim: Impaired wound healing in ischemic tissues such as skin flap edges resulting from inefficient perfusion is one of the major cause of complications in plastic surgery [1]. Previous studies showed that some growth factors may influence revascularization and healing of ischemic skin wounds [2-7]. In this experimental study, we investigated and compared the effect of recombinant human basic fibroblast growth factor (bFGF) and erythropoietin (EPO) in prevention of skin flap necrosis in rats.

Materials and methods: 30 Sprague-Dawley rats weighing 250-300 g were randomized into 3 groups. After creating a 8*2 cm random pattern skin incision on dorsum of animals, in group 1 (control), 1cc of normal saline solution; in group 2 (EPO) erythropoietin (100 U/kg); and in group 3 (FGF) basic fibroblast growth factor (2.5 µg) were injected subcutaneously in the skin flap area. Four days later, a full-thickness caudally based skin flap was raised with undermining subdermal tissues of the skin. Areas of ischemic (SI) and necrotic (SN) zones were assessed and compared in all groups on the 4th and 10th post-operative days. Macroscopic measurements were conducted by using image analysis; five different flap areas were quantified: total, preserved, necrotic, and ischemic and viability. Viability areas comprised the sum of ischemic and preserved areas. On the 10th post-operative all rats were sacrificed and entire flap and the necrotic areas were cut and sent to pathologist for microscopic assessments. Pathologist and image analyzer were blind about the type of treatment of each sample.

Results: Area of the necrotic zones(SN) as well as ratio of the necrotic zones area to the area of total discolored zones(SN/[SI+SN]) were substantially higher in control group comparing to 2nd and 3rd groups (p <0.001). Further, these values were significantly higher in the 2nd group (EPO) than those in the 3rd group (FGF) (P <0.01). Vascular density in ischemic areas of the control group was less than those in 2nd and 3rd groups.

Conclusion: Local administration of erythropoietin or basic fibroblast growth factor in skin flaps can remarkably decrease tissue necrosis via angiogenesis and wound healing improvement. More over, using basic fibroblast growth factor, necrosis in ischemic zones of skin flaps is decreased meaningfully comparing to erythropoietin.
Perinatal hypoxia and asphyxia as intracranial hemorrhage risk factors in premature newborns

ESC-ID 399
Author Gebrovsky VV, Volkadov OV, Krishnan V
Country Malaysia
University Crimea State Medical University
Department Surgical Diseases

Aim: Determining the optimal methods of diagnostics and treatment of intracranial hemorrhage in premature newborns. Materials: 976 premature newborns with perinatal hypoxia and asphyxia was treated in Crimean Republic Children Hospital between 2001 and 2006. From this total, 124 babies were suffering from intracranial hemorrhage.

Methods: This research studies the risk factors of the premature newborns with perinatal hypoxia and asphyxia which is complicated by intracranial hemorrhage. Focus is on the investigation of various kinds of massive intracranial hemorrhage, traumatic squeezing of the brain by intracranial hemorrhage, stagnation of liquor, prevention and treatment of severe complication such as post ventricular hydrocephaly. Ventricular Peritoneal shuntin was done on 52 patients.

Results and conclusion: The polyetiological development of intracranial hemorrhages in premature newborns is due to the insufficiency of the anastomosis between the short and long branches of the basic cerebral arteries, basilar and periventricular vessels, and the insufficiency of the herminal matrix of the vessels. The perspective of the investigation is to improve the cerebral circulation in the extracranial segments of the cerebral vessels, collateral circulation and cerebral perfusion, and to strengthen the vascular walls. The emergency aid focuses, firstly on eliminating the causes of squeezing, dislocation, and liquor occlusion, and also to normalize the intracranial pressure. For this, VENTRICULO-SUBGALEAL SHUNTING modification was used to proved to be effective. (Copyright Pattern N18211). 12 emergency neurosurgical operations on premature newborns were performed with clinico-neurological and supplementary examinations. (neurosonography, CT scan). The results saw improvement of these children and regression of the occlusive liguro-hypertension symptoms.

Intraoperative colonoscopy in patients with obstructing colon cancer

ESC-ID 655
Author Pedziwiatr M, Major P, Matlok M
Country Poland
University Jagiellonian University, Cracow
Department 2nd Department of Surgery

Background: The development and widespread use of the colonoscopy led to the identification of colonic cancers not only during one of the diagnostic procedures but also during operation. So called intraoperative colonoscopy in patients whom complete preoperative colonoscopy allows to look for additional cancer lesions and polyps in the whole large intestine. Objective: to analyze efficacy of intraoperative colonoscopy in patients with colon cancer in whom complete preoperative colonoscopy was not possible.

Method: 57 (38 men and 19 women) patients treated from January 2002 to February 2007 in 2nd Department of Surgery, Jagiellonian University, Cracow due to obstructing colon cancer were included into retrospective study. In all of these patients the complete colonoscopy was not performed before the operation.

Results: In 12 patients (21%) intraoperative colonoscopy revealed an additional cancerous lesion, not detected before the operation. In 6 cases the new finding induced surgeon to extend resection. In 5 (9%) patients surgeon detected an additional synchronous malignancy and polyps confirmed by further pathologic investigations. In 4 of these patients the operation needed to be extended. Furthermore, in 7 (12%) cases intraoperative procedure revealed polyps in the proximal part of the intestine. In 5 of these cases polyps were removed endoscopically and in 2 the resection was extended. Although the intraoperative colonoscopy increased the operation time it was not statistically significant.

Conclusion: Thanks the intraoperative colonoscopy patients with additional findings avoided further operations and progression of the disease, what is obviously the most important aspect of this procedure.

Surgical treatment of peritoneal metastases from colorectal origin

ESC-ID 917
Author Tonev A, Ivanov K, Ignatov V, Kolev N, Minev I, Ivanov G
Country Bulgaria
University Medical University of Varna
Department General and Operative Surgery

Introduction: Besides the lymphatic and hematogenous routes of metastasing, colorectal cancer frequently gives rise to transcoelomic spread in the peritoneal cavity, which, ultimately, may cause peritoneal carcinomatosis. During the course of disease of a patient with a gastrointestinal cancer, it is thought that there are 2 time points at which intraperitoneal spread or seeding of cancerous tumor emboli may occur.

Aim: We present our experience in the Clinic of General and Operative Surgery upon the subject and analyze the outcome and results from different techniques of surgical treatment.

Results: The peritoneal surface is involved in approximately 10-21% of patients with colorectal cancer at time of initial presentation (synchronous metastases) and in 20-50% of patients who develop recurrence (metachronous metastases). The extent of carcinomatosis was evaluated as a major prognostic and was best assessed by laparoscopic or open explorative surgery. Noninvasive imaging techniques have significant false negative rate in terms of small peritoneal lesions.
Giant unresectable lymphangioma of the trunk managed via intra-tumor injections of Bleomycin

ESC-ID 444
Author Umar K, Asmatullah K, Usman A, Saulat F
Country Pakistan
University Aga Khan University (AKU)
Department Medical College

Introduction: Surgical excision used to be the mainstay of treatment for lymphangiomas until recently when sclerotherapy has produced striking results, with complete disappearance of the lesion on most occasions. This is done by injecting sclerosing agents like bleomycin (BLM) and OK-432 (picibanil) directly into the tumorous mass. We hereby describe a case of a large lymphangioma of the trunk that was successfully treated by intra-tumor bleomycin injections leading to a near-complete resolution of the lesion. Case History A 22 years old girl presented with a mucopurulent, bloody, painful discharge from her wound in the trunk. Chest X-Ray showed a soft tissue abnormality with multiple calcifications in the right upper abdomen and lower thorax. MRI revealed a huge lobulated mass wrapping around the right half of the trunk with crescentric appearance of axial sections. The maximum dimensions of the mass in vertical and side planes were 27 cm and 19 cm, respectively. Most likely diagnosis was lymphangioma. Due to the extensive involvement of tissues, surgical excision was not a favorable option so it was decided to treat it with local injections of bleomycin. This turned out to be a great success as the lesion almost completely regressed.

Conclusion: Bleomycin sclerotherapy is viewed as an important landmark in the treatment of lymphangiomas. It undermines the need for primary surgery and is a very effective non-invasive option on its own with no reported complications. Our case showed a dramatic resolution of the huge lesion following bleomycin injections.

Therapeutic approach of echinococcosis in children, a challenge facing present and future surgeons!??

ESC-ID 719
Author Mihajloska E, Cadikovski V
Country Macedonia
University Medical Faculty-Skopje, Macedonia
Department Department for children’s surgery, Medical Faculty

Aim: Human echinococcosis remains a serious health problem for the Mediterranean countries, among them Macedonia. As there is no effective medical therapy, surgery is still the treatment of choice. Working on finding the best diagnostics and surgical treatment as well as treatment and therapy of echinococcosis with unusual location in children is a challenge facing our community and medical science now and in the future.

Method: A total number of 162 children(107 male and 55 female) aged between 5 and 17 years with abdominal(66%), lung(30%) and other(2.6%) echinococcosis were operated on at the Clinic of Pediatric surgery in a 10 year period(1995-2005). In all patients ultrasound of abdomen and plain-X-ray of the lungs were done before and after surgery. In this way, regression of the disease was also followed up. All children received Albendazole therapy by protocol. We used Echinococcetomy, partial resection, segmentectomy, and other methods case dependant.

Results: We have an affirmation of very effective surgical approach, having ambitious objectives, in the realization of the whole extraction program in a single surgical session. This study shows that 150 patients of 162 had successful operation. Only in 6 children (4.86%) intervention was necessary due to hydatid cyst relapse in the existing pericystic cavities. In 2 cases we punctuated a cyst in the hepary with a size of 4cm, and the content was evacuated with application of 25% NaCl, with ultrasound guidance. Percutaneous drainage of the contents of residual pericystic cavities (liver and lungs-left lobe) was performed helped by ultrasound in 4 cases.

Conclusion: Today, the indication for surgical treatment is laid down for all cases of echinococcosis. Adjuvant therapy is recommended as it minimizes recidivism, but it is a principal therapy only in cysts smaller than 2cm (and requires permanent echosonografic surveillance). One-stage surgery is superior to a classic two-stage approach as it decreases the morbidity, hospital stay and costs. There is no room for improvisation; in fact, it calls for dedication and experience.

SIRS due to destructive pancreatitis: Efficacy of Passive Immunotherapy

ESC-ID 735
Author Plegutsa OM, Sydorchuk RI, Kulachek FG, Knut RP
Country Ukraine
University BSMU
Department Surgery

Due to immune disorders playing a key role in development of Systemic Inflammatory Response Syndrome (SIRS) passive immune therapy is considered to be a method of choice for destructive pancreatitis (DP) patients. Existing remedies (specific hyper immune serum, specific antibodies and immunoglobulins) are expensive and require exact validation of pathogens. The aim of the study was to evaluate the efficacy of using the DP convalescent donors plasma for passive immunotherapy of DP.

Methods: The study was conducted both experimentally and clinically on 50 Wistar line rats and 20 inbred dogs. Totally 46 patients with DP were also involved into the study; 26 formed control group; 20 patients were selected as convalescent donors of plasma. 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 DP forming the waveform curves. Serum antibodies titres to main pathogens were slightly higher due to antibiotics and detoxication therapy. Operation by itself decreased titres from 4.34 ± 0.29 to 3.28 ± 0.31 (E.coli), from 5.48 ± 0.92 to 3.11 ± 0.62 (P.aeruginosa). Antistaphylococcal antibodies titres decreased from 7.44 ± 0.93 before surgery to 4.43 ± 0.54 after. Repeated operations alter antibodies concentrations even more significantly. The highest levels of antibodies were found in patients who underwent successful treatment of DP one to two months prior to investigation. Their
plasma was used in treatment of DP patients. Intravenous administration of two-dose 100-200 ml of hyper immune plasma per day prevented following decrease of antibodies levels and in 98% case increased them (20.57 ± 1.44%). The cost of treatment was 15-35% lower if compare with traditional methods (control group).

Conclusion: There is an exact evidence of hyperimmune plasma using efficacy in patients with destructive pancreatitis; it is more cost effective if compare with traditional methods of immunotherapy.

Long-term outcome of kidney transplantation from anti-HCV positive donors

ESC-ID 480
Author Darocha A, Majkowska M, Kwiatkowski A
Country Poland
University Medical University of Warsaw
Department General and Transplantation Surgery

Due to the shortage of organs available for transplantation procurement of kidneys from marginal donors is inevitable. Not infrequently these donors are infected with hepatitis C virus (HCV). Machine perfusion (MP) of kidneys prior to transplantation (KTx) has been used in our centre since 1994. The aim of this study was to determine the effect of transplanting kidneys from anti-HCV positive donors to anti-HCV positive recipients.

Material and methods: 765 KTx performed between 1994 till 2006 had been included in the study, in which 259 kidneys recipients were anti-HCV positive. Since 1996 kidneys from anti – HCV positive donors had been collected in order to transplant to anti-HCV positive recipients. Of 259 anti-HCV positive recipients 60 received kidney from anti-HCV positive donor (HCV+/HCV+ group). The remaining patients received kidneys from seronegative donors (HCV-/HCV+ group). Control group consisted of 506 seronegative recipients whose received kidneys from seronegative donors (HCV-/HCV- group). All kidneys from anti-HCV positive donors prior to KTx were preserved with MP. We investigated recipient’s liver function [ALT, AST, ALP, bilirubin], graft survival, patient survival and return to HD treatment in a 10-year follow-up.

Results: In 20% of patients in HCV Group and 17% of patients in Control Group, within 2 years after transplantation, a transient rise of AST and ALT (up to 240 i.u.), eventually returning to normal levels, was observed. There were no significant differences in the mean levels of AST, ALT, ALP and bilirubin between the groups. Graft survival didn’t differ between the groups and was: 78% (13/60); 70% (140/199); 77% (388/506) in HCV+/HCV+; HCV-/HCV+; HCV-/HCV- group, respectively. Patients survival was: 95%(57/60); 85%(170/199); 90%(459/506), respectively (p = NS).

Conclusion: Transplantation of kidneys collected from anti-HCV positive donors to anti-HCV positive recipients increases the number of kidneys available for transplantation and does not worsen the prognosis of liver insufficiency development. The long-term results of graft and patients survival are similar in all groups.

Pulmonary metastasis due to renal cell carcinoma in Iceland - How many could benefit from surgery?

ESC-ID 494
Author Oddsson SJ, Hardarson S, Petursdottir V, Jonsson E, Einarsson GV
Country Iceland
University University of Iceland
Department Medicine

Introduction: The incidence of renal cell carcinoma (RCC) is high in Iceland and about 30 new cases are diagnosed every year. Symptoms are often absent which results in over 30% of patients presenting with metastases (most often pulmonary) when diagnosed. The prognosis in such cases is usually dismal. However, recent findings show that pulmonary metastectomy in a selected group of patients can improve life expectancy a lot and up to 49% 5-year disease free survival has been reported. The aim of the study was to analyze the patients diagnosed with RCC and pulmonary metastasis in Iceland to try to evaluate the how many could possibly benefit from surgery according to recent studies.

Material and Methods: This is a retrospective study which includes all patients that were diagnosed alive with RCC in 1971-2000 (n = 701). Information was obtained from clinical records, The National Cancer Registry and pathology reports. Tumors were classified with the TNM staging system.

Results: Patients with lung metastasis were 130 (18.5% of all RCC patients). In 56 the metastases were only in the lungs and 8 were diagnosed incidentally (14%). Precise information about metastases was obtained from 36 patients. 28 (78%) had more than one metastasis and 17 (47%) had bilateral metastases. 8 patients had a sole metastasis and the average age when diagnosed was 63 years (6 males and 2 females). From 1984-2000 a total of 39 patients were diagnosed with lung metastasis within 3 months from the diagnosis of RCC. Only one had a metastatectomy.

Conclusion: Approximately one in five patients has a lung metastasis when diagnosed with RCC. In 44% of cases the metastasis is only in the lungs and 22% are single. Recent findings suggest that some of these could benefit from metastatectomy which has been rare in Iceland. It has to be kept in mind that the study is retrospective (35 years) and the potential of such surgery has only recently been described.
Anatomy II

Morphometric and stereological analyzing of ganglion structures of myenterical nervous plexus in frontal wall of rectum and sigmoid colon

ESC-ID 149
Author Andrijašević V, Brnić O
Country Serbia
University University of Pristina, Kosovska Mitrovica
Department Department for Anatomy

Introduction: Myenteric plexus is very important for functioning of gastrointestinal tract. It represent one regulative level of autonomic nervous system which is placed in the wall of the gastrointestinal tract and it is directly exposed to the pathogenic influences from the out side.

Main goals: The purpose of this study is to analyze the myenteric plexus as a part of autonomic nervous system in the particular parts of the colon (rectum and sigmoid colon).

Material and methods: In the research were used 30 samples of tissue of the frontal wall of sigmoid colon and rectum. First, it was determined their volume by picrometer. After that, the samples were elaborated histologically and colored with HE, Cresyl-violet and AgNO3 method. The samples were placed into a shouldered series of cuts (thickness of the cut 7µm and 50µm cut degr ease). Stereological analysis was performed by the test system M42, which was calibrated on the objective focus 40X. In the analysis it was determined the absolute volume of the ganglion structures of the myenteric plexus and the absolut number of ganglion cells in the ganglion structure. The analysis of the variance and the t-test were used as statistics methods for this research.

The results and conclusion: The structures of the myenteric plexus were showed as wide, irregular ramified structures, hard edged from the smooth muscles surround. Ganglion cells are large, oval or polygonal caracteristic cells. The absolute volume of the ganglion structures and the absolut number of ganglion cells have the tendency of decrease going from rectum to sigmoid colon, but the decrease is not statistically significant. The differences on the level of significant p<0,05 are important only if are compared the absolute volume of ganglion structures and the absolut number of ganglion cells of myenteric plexus of ampullar part of rectum and initial part of sigmoid colon. Basically, ganglion cells of the myenteric plexus are the most numbered in the tissue samples from the ampullar part of rectum, and the least numbered are in the tissue samples of the initial part of sigmoid colon.

An anatomical study of the anastomosis between the recurren laryngeal nerve and the sympathetic trunk

ESC-ID 170
Author Galazka A
Country Poland
University Warsaw Medical University
Department Department of Biostructure

Reccurent laryngeal nerve and sympathetic trunk are important features of the neck anatomy. The presence of the nerval anastomosis between the two structures could be interesting from anatomical and surgical view. Objective: To determine the presence and branching patterns of the anastomosis between the recurren laryngeal nerve and the sympathetic trunk

Methods: Microdissection of 15 formalin preserved foetuses (30 sides).

Results: In one case the nerve was inappropriate for dissec tion. The anastomosis appeared in 23 cases. In 15 cases it was a single branch connection. On 8 sides a multibranch connection appeared. In only 4 cases one of the branches of the anastomosis joined recurrrent nerve in the section superior to the inferior thyroid artery. In 19 cases the connection was situat ed inferior to the inferior thyroid artery.

Conclusion: The astomosis between recurren laryngeal nerve and sympathetic trunk is a frequent feature of the foetal anatomy. The clinical significance of this anatomical feature is yet to be evaluated.

Rat’s ovaries structural asymmetry in normal condition and in an experiment

ESC-ID 513
Author Lyashchenko O
Country Ukraine
University Crimean State Medical University
Department Medical Biology Department

Introduction: For many years the statement about the equality of the pair organs existed and doctors, especially surgeons, considered that a twin organ remaining after a one-sided ovariectomy can fully compensate for the removed one. Aim: The aim of our research was to reveal age organ metric differences in between the ovaries of the rats in normal condition and after one-sided ovariectomy on normal ovary and polycystic ovary having been performed.

Matherials. and methods: The experiment was carried out on 30 female Vistar rats, weight 74-110g (the age of the rats 1 month old). Ten (10) rats served as a control. For solving this problem we created an experiment. We performed a one-sided ovariectomy on all of the rats who were under experiment ( on the rats with intact ovaries and rats with artificial created polycystic ovary syndrome). The terms of observations were determined at a 60, 90 and 180 days interval. We counted the weight of the rats, weight of the removed ovary, volume of the ovary and the length of the fallopian tube.

Results: The maximal asymmetry of the mass, volume of the ovaries and the length of the fallopian tube was revealed in 60 and 90 days interval which is probably connected with the heterogenic growth of the female genital glands in this period of time. In case of a one-sided ovariectomy being performed on intact ovaries the asymmetry phenomenon was revealed in 90 and 180 days of observation. The mass of the ovary and the length of the fallopian tube which is connected with the compensational processes in a single ovary remaining after ovariectomy. In case of a one-sided ovariectomy being performed on polycystic ovaries the asymmetry phenomenon was revealed in all of the terms of observations. The strongly marked tendency of the asymmetry, taking place in the polycystic ovary was revealed in all of the cases observed in the experiment and the right side dominated over the left one. These results are showing the hidden potential of the ovaries involved in the polycystic ovary syndrone.

Conclusions: In the experiment carried out it was proved that the structural asymmetry is taking place in the ovary in
control group as well as in the experimental group. In case of a one-sided ovariectomy being performed on polycystically changed ovaries a very clear asymmetry phenomenon was revealed (the right side predominated over the left one). This information is very important for the surgeons and gynecologists in case of a long-term prognosis of the reproductive health of the women after one-sided ovariectomy and women suffering from polycystic ovary syndrome.

The morphometric study of lungs during human prenatal development

ESC-ID 651
Author Wawrzyniak J, Winiewska J, Bryczkowska A, Siedlecki Z, Ciezciski J
Country Poland
University Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń
Department Department of Normal Anatomy

The aim of the study was to analyse dimensions, development and skeletotopy of fetal lungs during human prenatal life. The study was carried out on 35 human fetuses of both sexes (18 female, 17 male) between 16th and 25th week of intrauterine life. The fetuses were fixed in 10% formalin solution. Fetal age was determined by crown-rump (CR) measurement on the basis of Iffy tables. Using anatomical dissection and statistical analysis eight following dimensions of fetal lungs were measured: (1,2) the longitudinal dimensions of right and left lung, (3,4) the transverse dimensions of right and left lung, (5,6) the sagittal dimensions of right and left lung, (7,8) the longitudinal dimensions of hilus of the right and left lung. For every fetus skelototopic location of following seven lungs' parts to ribs was measured: (1,2) apex of right and left lung, (3,4) base of the right and left lung, (5,6) hilus of the right and left lung, (7) the transverse fissure of the right lung. No gender differences were observed (p >0,05). Every measured dimensions of the right and left lung correlated statistically significant with the fetal age (r = 0,50, p <0,05), the correlation coefficient was the highest for the longitudinal dimension of right lung (r = 0,69, p <0,05). The size of the hilus of the right lung correlated statistically significant with the fetal age (r = 0,44, p <0,05) and the size of the hilus of left lung was statistically irrelevant. For every fetuses the location of right and left lung hilus and horizontal transverse fissure of right lung to ribs were correlated with fetal age (p <0,05) and raised during prenatal development.

The morphometric study of the middle cranial fossa in human fetuses

ESC-ID 653
Author Wiszewska J, Wawrzyniak J, Bryczkowska A, Siedlecki Z, Ciezciski J
Country Poland
University Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń
Department Department of Normal Anatomy

The middle cranial fossa contains temporal lobes of the brain, cavernous sinuses, internal carotid arteries, cranial nerves: III, IV, V, VI and the middle meningeal arteries. It is the site of various pathologies and it is used in neurosurgical intracra-

Different types of variations in the distribution of the middle meningeal artery branches which supply the trigeminal ganglion

ESC-ID 742
Author Ristic N, Stojanovic P, Jelenkovic O
Country Serbia
University University of Nis
Department Anatomy

Introduction: Middle meningeal artery, a side branch of the mandibular segment of the maxillary artery enters in the middle cranial fossa through the foramen spinosum, supplying mandibular part of the trigeminal ganglion. The aim of the study was to determine external morphology and the types of ramification of the middle meningeal arterial blood vessels which supply the human trigeminal ganglion.

Material and methods: The examination was performed on 100 fetal trigeminal ganglia. Fetuses of both sexes, age over 2 lunar months, were obtained from the Gynecology clinic after spontaneous or artificial abortion. The age of the fetuses was determined by measuring the crown-rump distance. After the application of the contrast material "micropaque" in the common carotid artery, a microdissection of the cavum semilunare dura mater, under the control of the surgical microscope and an analysis of the arterial blood vessels were made.

Results: Four morphologic types in the distribution of the middle meningeal artery branches which supply the trigeminal ganglion has been detected type I - only one branch for the mandibular part of the ganglion, its inferior side; type II - one common arterial trunk gives off two branches for the mandibular part of the ganglion; type III - a common trunk gives off three branches for the ganglion; type IV - "V" type, two branches arise from the same part of the arterial trunk, like the Latin "V".

Conclusions: Mandibular part of the trigeminal ganglion is supplied by the four different morphologic types of the middle meningeal artery and less commonly by the branches of
Anatomical peculiarities and histoarchitectonics of the ileocecal junction during the fetal period of human ontogenesis and in newborn infants

ESC-ID 763
Author Pronyaev DV
Country Ukraine
University BSMU
Department Anatomy

A correlation, dynamics of the forming and structural variations of the component elements of the ileocecal junction during the fetal period of human ontogenesis have been studied by means of methods of micro and macroanatomization, morphometry, roentgenography, photodocumenting. The form of an ileal eminence considerably alters during the fetal period from a round papilla at the beginning of the fetal period, an oval one along the axis of the ascending colon in the middle of the fetal period – to an oval one perpendicularly to the axis of the ascending colon papilla and a lip like form of the ileal eminence at the end of the fetal period. The ileocecal valve begins to form in the tenth month of the prenatal period of ontogenesis. In newborn infants the ileal eminence is represented by an oval one and a lip-like form of the ileal eminence in the majority of cases. Newborn infants exhibit the signs of the formation the cecum as an increase of an outpouching of the opposite ileal eminence of the large intestine and the formation of a border between the vermiform appendix and the large intestine. The ileo-cecinal portion of 15 fetuses and 5 human newborns have been studied by means of the histologic method. Certain regularities of morphologic transformation of the ileo-cecal junction have been established during an early stage of human ontogenesis. The processes of forming the definitive shape of the ileo-cecal junction are similar with those that occur in case of invagination. The important conditions determining the dynamics of forming and variants of the structure of the components of the ileocecal junction at an early stage of human ontogenesis, are the structure and localization of the muscular layer of the intestinal wall at a certain stage of development.

Morphological characteristics of ileum in the case of Crohn’s disease

ESC-ID 103
Author Elek M, Gvozdenovic N
Country Serbia
University University of Novi Sad
Department Anatomy

Introduction: Filed with barium contrast, ileum is a shape of multiple-wriggled band and has feathered look. Bright stripes represent circular Kerkring’s folds of the mucosa (plicae circulares), and dark stripes represent thin layer of barium milk between two mucosa folds.

Aim: The aim of this research was to establish validity of enteroclysis in detection of morphological changes of ileum in the case of suspicion of Crohn’s disease and to establish the type of morphological changes of ileum in the case of Crohn's disease.

Materials and Methods: We compared two groups of people who voluntarily accepted to be examined: a control group of people and a group of examined people. The control group was consisted of 11 healthy people, i.e. who did not have any symptoms of gastrointestinal tract. The group of examined people was consisted of 16 patients with Crohn's disease. We did monocontrast and doublecontrast of enteroclysis with both groups. Afterwards, we defined parameters which we compared between these groups.

Results: The values of the width of luminary of ileum were between 1,9 and 2,5 cm in the examined group and between 2,3 and 3,0 mm in control group of people. The most often numbers of folds of mucosaes on 1 cm of wall of ileum were 2 (62,5%) and 1 (18,75%) in examined group and 4 (45,45%) and 5 (27,27%) in control group. The width of wall of ileum was from 1,4 to 2,2 mm in examined group and from 1,0 to 1,4 mm in control group. The width of folds of mucosaes of ileum in the examined group was from 2,0 to 2,7 mm and in the control group was from 1,0 to 1,9 mm.

Conclusion: According to this research project, we concluded that there are, according to statistics, significantly lower values of the width of luminary of ileum and the numbers of folds of mucosaes on 1 cm of wall of ileum in the examined group of people in contrast to control group of people. Also, we concluded that there are, according to statistics, significantly higher values of the width of wall of ileum and the width of folds of mucosaes of ileum in the examined group of people in contrast to control group of people. The determination of these parameters can facilitate to make diagnosis of Crohn's disease.
**Anesthesiology and Reanimation II**

**Intramuscular injection of halothane 5 and 6 vol% detects probands at risk for malignant hyperthermia**

ESC-ID 118  
Author Gardill A, Schuster F, Metterlein T, Roener N, Anetseder M  
Country Germany  
University Julius-Maximilians-Universität Würzburg  
Department Anaesthesiology

**Aim:** In malignant hyperthermia (MH) susceptible individuals, anaesthesia with volatile anaesthetics and depolarizing muscle relaxants may lead to a potentially lethal metabolic syndrome of skeletal muscle metabolism [1]. Since diagnosis of MH susceptibility by the in-vitro contracture test requires an invasive muscle biopsy [2] and genetic mutation is causative for MH found in less than 45% of susceptible individuals [3], alternative and less invasive methods to detect MH susceptibility are sought for. We present a minimally-invasive metabolic test with intramuscular measurement of carbon dioxide as an immediate product of metabolism after local injection of halothane 5 and 6 vol%, assuming to allow a clear diagnostic assignment of MH susceptible (MHS) and non-susceptible (MHN) individuals.

**Method:** With approval of the local ethic committee 8 MHS and 8 MHN probands with a personal or family history of MH and a previously performed diagnostic in-vitro contracture test [2] were investigated. Following local anaesthesia two G18 introducer cannulae were placed ultrasound guided into the lateral vastus muscle. Afterwards two PCO2 probes with attached microtubing catheter for halothane injection were inserted. Following equilibration, single boli of 200 µL halothane 5 and 6 vol% dissolved in soy bean oil were injected simultaneously. PCO2 was measured spectrometrically in 1 min intervals. Probands’ vital signs were monitored throughout the investigation. Serum creatine kinase and myoglobin were quantified before, at the end and 24 hours after the study. Peripheral venous blood gas samples were taken 60 min after intramuscular halothane injection. Data as median and interquartile range (IQR). Mann-Whitney-U-test for differences between MHS and MHN individuals. Wilcoxon test for comparison of creatine kinase and myoglobin before and after the investigation. P < 0.05.

**Results:** Baseline recordings of PCO2 did not differ between the studied groups. Intramuscular maximum PCO2 was significantly increased in MHS compared to MHN probands following halothane 5 vol% (67 [53 – 85] mmHg vs. 44 [40 – 45] mmHg; p = 0.03) and 6 vol% (108 [98 – 121] mmHg vs. 50 [41 – 59] mmHg; p = 0.01). The calculated maximum rate of PCO2 increase was significantly higher in MHS than in MHN participants following halothane 5 vol% (138 [43 – 189] mmHg/h vs. 12 [-(-5) – 27] mmHg/h; p = 0.02) and 6 vol% (246 [189 – 279] mmHg/h vs. 34 [13 – 71] mmHg/h; p = 0.01). The hemodynamic and metabolic parameters did not differ between groups. Interestingly, 24 hours after the investigation creatine kinase increased significantly only in the MHS group.

**Conclusion:** Intramuscular injection of halothane 5 and 6 vol% leads to a significant PCO2 increase in MHS compared to MHN patients reflecting a local hypermetabolic reaction. Therefore the presented test system allows a diagnostic assignment of MHS and MHN individuals. The observed creatine kinase increase reflects a local, but clinical irrelevant muscular damage. For individual differentiation halothane 6 vol% seems to be more suitable and should therefore be implemented in the further development of a minimally-invasive metabolic test.

**Biochemistry II**

**Choline protects isolated rat hepatocytes from ciprofibrate and tumor promoters-induced oxidative stress**

ESC-ID 878  
Author Kamenov A  
Country Serbia  
University University of Nis  
Department Department of Biochemistry

Fibrates are now available and widely used drugs in hyperlipidemic disorders, and among them ciprofibrate represents a novel, one of the most active class. As a hepatic peroxisome proliferators constitute a well known class of chemical carcinogens. Xanthine oxidase serves as a rate-limiting enzyme in nucleic acid, as well as other cell purine compounds degradation by terminal oxidation with the critical importance as a generator of free oxygen radicals. The aim of the study was to elucidate the effect of ciprofibrate on rat liver xanthine oxidase (XO) activity and to compare its effect with the effect of protein kinase C activator-phorbol 12-myristate 13-acetate (PMA) and choline. The activity of xanthine oxidase as well as the lipid peroxidation level were assessed using a primary rat hepatocyte culture system. Fresh rat hepatocytes were isolated by collagenase technique Exposure of hepatocytes to ciprofibrate led to substantial increase in xanthine oxidase activity (p <0.001) and the lipid peroxidation level. Obtained results confirm that the ciprofibrate is capable of inducing the enzymes of the oxidation pathway. The activity of xanthine oxidase was slightly increased (p <0.05) after the incubation with PMA, also the lipid peroxidation level (p <0.05), indicating that PMA may induce reactive oxygen species via purine catabolic pathway. Since the role of reactive oxygen species in tumor promotion is well postulated, our results indicate that xanthine oxidase activation can be one of the mechanisms of liver tumor promotion. The exposure of isolated hepatocytes to ciprofibrate and PMA simultaneously expressed higher xanthine oxidase, similar to that observed after ciprofibrate exposure. When hepatocytes were exposed to choline the activity of xanthine oxidase did not increase significantly, but the lipid peroxidation level had decreased compared with the control level. It was also important to adress the potential of choline to counteract the increase of xanthine oxidase and lipid peroxidation level during simultaneous exposure of hepatocytes to ciprofibrate and choline, indicating about possible protective effects of choline against carcinogenesis during fibrate therapy.
High serum homocysteine levels accompany poor cardiac function in coronary artery disease patients

ESC-ID 186
Author Afraz Z, Nabatchian F, Ghanadian N, Shabani A, Pasalar P
Country Iran
University Medical sciences/Tehran University
Department Biochemistry/Faculty of Medicine

Background and aim: higher level of plasma homocysteine concentration is related to increased risk of atherosclerotic cardiovascular diseases. Hyperhomocysteinemia is related to decreased level of nitric oxide production whilst artery diameter is related to ejection fraction (EF) and the angiographic extent of atherosclerotic cardiovascular disease in patients with coronary artery disease. The aim of this study was to investigate the effect of age and plasma homocysteine level on cardiac function and the extent of coronary vessel involvement in angiographically proved patients with Coronary artery disease (CAD).

Material and Methods: a cross sectional study on 166 angiographically proved CAD patient who were on optimal medication was performed. Eligible patients were sequentially recruited into two groups: early-onset CAD which presented disease under the age of 55 years and late-onset CAD which presented disease over the age of 65 years. Cardiac function and the involvement of cardiac vessels were evaluated by measuring ejection fraction through angiography. homocysteine was measured using ELISA and lipid profile was also determined.

Results: Homocysteine levels were inversely correlated with cardiac EF (p<0.01) and was significantly higher in male (p<0.001). Higher levels of Triglycerides (TG) were associated with extensive coronary involvement (p=0.003), but we did not find any significant correlation between cholesterol and vessel involvement. However, our preliminary work showed that homocysteine levels were inversely correlated with LDL cholesterol (p<0.05). Higher vessel involvement was observed in late onset CAD (p<0.001).

Discussion: homocysteine causes direct toxic damage to endothelial cells in both in vitro and in vivo models. Impaired production of endothelium-derived Nitric oxide elevation of triglycerides and oxidation of LDL, are associated with higher level of vessel involvement. Lower level of homocysteine in premenopausal women may have protective effect against CAD. We concluded that higher level of plasma homocysteine is related to poor cardiac function in coronary artery disease patients.

Analysis of degradation of support fixed peptides by the proteasome

ESC-ID 555
Author Hovestaedt M, Tapia V, Kuckelkorn U, Volkmer R, Holzhuetter H
Country Germany
University FH-Aachen
Department Abt. Jülich

Aims: Proteasomes are cellular, multicatalytic protease complexes that degrade intracellular proteins into smaller peptides. Proteasomes are involved in many regulatory and metabolic processes. Thus, it is particularly important to research the specificity and kinetics of proteasome-mediated degradation. The Degradation of immobilized substrates is largely unknown.

We try to create a model of the digest of integral proteins. Pp89-derived peptide from cytomegalovirus (Kloe411) in the solid-phase and the 20S proteasome are used. The cleavage probabilities in solution-phase of the Kloe411 peptide serve as reference. The work aims on following questions: Are immobilized peptides correctly presented to the proteasome? Is it possible to compare the activity of the proteasome in solid- and solution-phase assays? Furthermore, is it possible to compare cleavage sites of soluble and support fixed peptides?

Material Methods: Peptide Synthesis Peptides were synthesized using standard SPPS on a Syro 2000 machine, purified through HPLC and identified through MS. Synthesis included CB4-1 epitopes for functional control of peptide immobilization and a pp89-derived peptide (Kloe411) from murine cytomegalovirus, which has been well described as a solution-phase substrate of the proteasome. Peptide Immobilisation Three different solid-phase supports were coupled with specific linkers to promote peptide presentation. Tids or aminohexan were coupled to the amino-functionalized glass slides, β-alanine linker was coupled to cellulose membranes and Rink-linker, to polystyrol beads. The Kloe411 peptide was immobilized to s-p supports Isolation of the Proteasome The 20S Proteasome was isolated from mouse erythrocytes. Incubation of the proteasome The proteasom is dissolved in buffer and are incubate for different times. Spotting of the control-peptides For the qualitative control of the immobilisation success, CB4-1 peptides lay on the proximally platforms: β-Ala-membrane and glass-slides. Analytic part Chemielumininsenz By the Immunodetection were the coupling linker qualitative proven. Thereto uses antibody which binds specific on the epitope from the CB4-1 peptides. Electrospray-Ionisation MS (ESI) The probes were be analysed with ESI. The founded masses were ranged in a cut-map.

Results: The peptides were stable immobilized on three support types. Following sequences were be found: Support Sequenz Glass slides Cellulose Beads 16-25 N-Term x x 8-15 other fragments x 8-25 other fragments x x 5-22 other fragments x 5-25 other fragments x 5-15 Precursor x

Conclusion: Initial peptide load was estimated through photometric control of Fmoc cleavage. Although, peptide load, especially for glass slides was low, up to 30% of the reference fragments were found. The diverse support surfaces are different in structure and chemistry. This could be one reason for the different peptide loadings observed. Finally the surface of the glass slides seems to be the best conditions for proteasome digest. The reason for the good presentation is either the loading of the substrate or the surface service type.

Upregulation of antigen-processing machinery components and increased expression of chemokines (MDC and TARC) at mRNA level involved in dendritic cell migration to lymph nodes in acute lymphoblastic leukemia cells after CD40 stimulation

ESC-ID 564
Author Blecharczyk B, Rembizska M
Country Poland
University Medical Academy of Biystok
Department Medical

Heading: Immunotherapy is one of the newest therapeutic methods in cancer. It is possible now to convert neoplastic
cells from acute lymphoblastic leukemia (ALL) in children into dendritic cells presenting cancer antigens to host T-cells, possibly leading to the elimination of the leukemic clone and cure the patient. In the future immunotherapy the knowledge about dendritic cells (DC) function is one of the important issues. For example the production of chemokines involved in T-cell migration and antigen presentation by leukemia-derived DC should be elucidated. The aim of our study was to assess the gene expression for chemokines (such as MDC, TARC and CCR7), costimulatory and adhesion molecules and antigen-processing machinery (APM) components in acute lymphoblastic leukemia (ALL) cells before and after CD40L and IL-4 stimulation at messenger RNA (mRNA) level.

**Material and Methods:** Mononuclear cells from bone marrow or peripheral blood were collected from children with B-cell precursor acute lymphoblastic leukemia (BCP-ALL) at the time of diagnosis. After thawing ALL cells were washed, resuspended in medium and cultured at concentration 1x10^5/ml and stimulated or not with CD40L and IL-4. The cells were incubated at 37°C in 5% CO2 for 96 hours. Elements of the antigen-processing machinery (MB1, LMP2, LMP7, LMP10, TAP1, TAP2, calnexin, calreticulin, tapasin, ERP57, zeta, delta) and gene expression of chemokines (MDC, TARC and CCR7) were determined by real-time PCR technique. The expression of important costimulatory and adhesion molecules considered as DC markers (CD40, CD54, CD80, CD83, CD86) and phenotypic characterisation of neoplastic cells before and after culture were determined at the mRNA (PCR) and protein (flow cytometry) levels.

**Results:** We confirmed the possibility of turning ALL cells into dendritic cells after CD40L stimulation. We found increased expression of mRNA for MDC, TARC and CCR7 after culture. Also the significant rise in expression of nearly all APM components after CD40L stimulation was observed.

**Conclusion:** All-derived dendritic cells express chemokines involved in T-cell migration and show up-regulation of antigen processing machinery. Our pre-clinical findings could be useful in designing of immunotherapeutic trials for the treatment of children with ALL.

**Method and Results:** Human epidermal keratinocytes were obtained from voluntary donors and cultured in serum-free growth medium. Human monocytes were isolated fromuffy coats using CD14 magnetic microbeads and, then, added to the keratinocyte culture. Under treatment with a cytokine cocktail consisting of GM-CSF, TGIF-beta, and IL-4 the monocytes in this coculture changed morphology and surface markers to cells resembling dendritic cells. These "dendritic cell-related cells" reacted to known contact allergens by dose-dependent up-regulation of the dendritic cell maturation marker CD86 as measured by flow cytometry. All of the tested contact allergens elicited a positive CD86 reaction: TNBS (2,4,6-trinitrobenzenesulfonic acid; a strong contact allergen often used as a model allergen), PPD (para-phenyldiamine; used in black hair dye), HCA (hexyl cinnamic aldehyde; used in axilla deodorant fragrances), 4-aminoacetanilide and 2-amino-p-cresol (cleavage products of a yellow textile dye). Cells from different donors showed similar amounts of CD86 up-regulation with only little donor-to-donor variance. In contrast, SDS (sodium dodecyl sulphate) which is known to provoke an irritant contact dermatitis (as opposed to the allergic contact dermatitis) did not elicit a CD86 response.

**Conclusion:** This novel in-vitro assay was able to detect five known contact allergens. Keratinocytes and monocytes can be cryopreserved prior to use which makes the assay convenient and easy to handle. This is necessary for the high-throughput screening of chemicals that is needed in the context of REACH. This is the only known in-vitro assay with the capability to sense HCA as a contact allergen. If combined with an in-vitro skin penetration assay, this assay could be a complete substitute for the LLNA in mice.

**Epidemiology/Social Medicine/Public Health II**

**Assessment of reasons of the drug abuse among teenagers and students**

**ESC-ID** 283  
**Author** Temov K, Micevski D, Izmaylov A, Georgieva Z, Komssa-Penkova R  
**Country** Bulgaria  
**University** Medical University – Pleven  
**Department** Alliance “Health in Our Hands”

**Introduction:** Young people are the most at risk for the use of psychoactive substances. The facts about Bulgaria show that nowadays we have the rejuvenation of drug abuse. Aims: •To view (discover) the main motives for the use of drugs by teenagers and students in Pleven. •To compare our observations with the results of other studies those were carried out before 7-10 years.  
**Materials and Methods:** •The communication as promoters of prevention of drug abuse with teenagers and students. •We used the results of our questionnaire (2004) that was filled by teenagers from 6 schools and students of MU-Pleven. •The information from Internet and other sources about drugs, some aspects of drug abuse, and opinions of youngsters about this was collected with our aim.  
**Results:** Results showed that the most common reasons for the use of marijuana for example among medical students are curiosity and the pursuit of delight: 32 % -for curiosity 29 %
Conclusions: Drug abuse is one of the biggest problems of our society. The knowledge regarding drugs, their effects, risks and the reasons leading to the increase in its abuse are important factors in the prevention of drug abuse.

Can chronic mental stress lead to chronic distress syndrome?

ESC-ID 490
Author Geisler BP, Gähler A
Country United States of America
University Harvard University
Department MGH-Institute for Technology Assessment

Background: Both acute and chronic psychological stress can cause bodily changes. Chronic distress syndrome (CDS), which is anecdotally speculated to be caused by excessive workload and to be primarily occurring in high level executive managers, is a form of chronic mental stress. Symptoms of CDS are heart pounding or arrhythmia and fainting. CDS might be attributable to increased catecholamines in the myocardium.

Methods: The National Comorbidity Survey-Replication (NCS-R), a nationally representative survey to study the prevalence of mental illnesses, was assessed for the exposure "work of hours for pay/profit in an average week" and being a manager, and for the outcome "attack when all of a sudden - you became very uncomfortable, - you either became short of breath, dizzy, nauseous, or your heart pounded, - or you thought that you might lose control, die, or go crazy". We used univariate and multivariate regression analyses to study a possible association.

Results: In n=109 subjects CDS was present; the comparison group was comprised by n = 2,015 individuals. There was no association between the working hours and CDS, neither when looking at the variable continuously, nor after stratifying in three groups (up to 45 hours, 45-54 hours, and >54 hours). The combination of working ≥45 hours/week and being a manager, however, had a crude odds ratio of 2.77 (95% confidence interval: 0.67; 11.43). The association persisted after adjusting for age, gender, and socio-economic status.

Conclusions: Our results show that the combination of the amount and the type of work undertaken by an individual can be a risk factor for CDS, whereas the amount or type of work alone does not suggest a risk. As CDS is not well-studied and might have been overlooked in the past, it deserves more scientific attention.

Living in the shadow. Life and perceived social restriction in a former leprosy colony in North-East Brazil

ESC-ID 598
Author Lesshaft H, Rieckmann N, Barbosa JC, Heukelbach J, Liesenfeld O
Country Germany
University Charité Universitätsmedizin Berlin, Federal de Ceará (Fortaleza, Brazil)
Department Microbiology/Social Medicine/Public Health

Leprosy is one of the most ancient infectious diseases in the history of mankind, and for thousands of years, leprosy has been loaded with myths and fears. Therefore it can lead not only to physical impairment and deformation, but also to severe psychological trauma through stigmatisation and exclusion from society. Leprosy is still endemic in several countries in Asia, Latin America and Africa. In Brazil, the isolation of leprosy patients was compulsory by law since the 1920s, and many affected individuals were confined to “leprosy colonies” until the 1980s. Today, leprosy is curable with multi-drug therapy and the isolation of patients is obsolete. Nevertheless, we still find 33 former “leprosy colonies” in Brazil, voluntarily inhabited by around 3,000 ex-leprosy patients. This study aims to present a picture of the inhabitants of the former leprosy colony “Centro de Convivência Antônio Diogo” (CCAD) in the municipality of Redenção (Ceará State) in North-East Brazil, with focus on their social restrictions. The CCAD is inhabited by 90 ex-leprosy patients and 86 not leprosy affected family members. Socio-demographic data of all 176 inhabitants were collected from the archive of the institution. In addition, a questionnaire was used to collect demographic, clinical, socio-economic and social data only of the ex-leprosy patients. Subjectively perceived social restriction was measured using the so-called Participation Scale. The leprosy-affected inhabitants of the CCAD were significantly older than the not affected inhabitants (Mean age = 61.5 years and 20.0 years, respectively)

Factors associated with maternal report of varicella zoster virus infection in pre-school aged children in the UK

ESC-ID 796
Author Bartington SE, Peckham C, Dezateux C
Country United Kingdom
University University College London
Department Institute of Child Health

Background: Many countries are currently reviewing their policies for childhood vaccination against Varicella Zoster...
Virus (VZV).(1) Information regarding the age of acquisition of VZV in young children and the factors associated with infection is important for determining changes in VZV epidemiology and modelling the impact of vaccination. Maternal report of VZV history has been shown to be very reliable.(2) We examined factors associated with VZV infection in early childhood, using information from the Millennium Cohort Study (MCS).

Methods: We analysed maternal report of VZV for 14382 singleton children aged 32 to 55 (median 37) months between September 2000 and January 2002 in the UK. Social, ethnic and demographic information was obtained, and mothers were asked to report whether their child had ever had chickenpox at home interview. Multivariable Poisson regression with survey commands was performed to report crude and adjusted Rate Ratios (RR).

Results: Varicella history was positive for 6181 (45.5%), negative for 8063 (53.5%) and unknown for 138 (1.0%) children. The reported prevalence was highest in Wales (46.0%) and lowest in Northern Ireland (39.4%). In multivariable analyses after adjusting for local area type and infant age, a positive history was more common in girls [RR 1.04; 95% CI: 1.00,1.08], children with white [1.21; 1.10, 1.33] non lone parent [1.11; 1.04,1.19] or high socio-economic status [1.33; 1.19,1.50] mothers; families with more than one child [1.31; 1.20,1.45]; or children attending daycare nurseries [1.09;1.03,1.15].

Conclusions: Almost half of three year olds in the UK have acquired VZV infection providing further evidence for an increasing prevalence of VZV in UK pre-school age children. Ethnic, social and demographic variations in VZV prevalence reflect the child’s wider social contact, and opportunities for acquisition of infection.

Supplement to overlooking of the epidemiological characteristics of the people suffering from myocardial infarction in the region of Nish in 2006

ESC-ID 798
Author Todorovic B, Velickovic D
Country Serbia
University Faculty of Medicine-University of Nish
Department Department of Epidemiology

Introduction: The myocardial infarction represents the one of the states of ischaemic illness of heart which arises in consequence of abrupt and complete intermission of circulation in one of the coronary artery, which causes the irreversing ischaemia and necrosis myocard. A aim of this study was to determinate epidemiological characteristics of myocardial infarction and incidence rates in the region of Nish in 2006.

Material and methods: The data on patients were obtained from the municipal population register of myocardial infarction in the region of Nish and Toplica. The research included all the registered myocardial infarction affected patients in the territory of the area of Nish during the 2006. The descriptive epidemiological study was applied. Unstandardized incidence rates were calculated, while the population data were taken from the Census of 2002. Rates were calculated per 100 thousand inhabitants.

Results: In the area of Nish in 2006 the total registered number was 981 cases (out of which 620 were male and 361 female patients). The average annual unstandardized incidence rates was 256.96 (330.17 in male and 186.1 in female population). Men suffered from myocardial infarction by 1.7 times more than women. With both genders the illness is not registered under the age of 24. The lowest rate were register in age of group 20-24, while the highest rate were register in age of group 80-84, which indicates that with getting older the rate of incidents increases and that it reaches its maximum at the age between 80 and 84. In this age of group specific incidence rate was 1663.82 in male and 1082.92 in female population. In relation to residence the highest rate were register in City of Nish (295.96) and Svriljig (266.14), thereafter following Niska Banja, Gadzin Han, Merosina, Razanj and Aleksinac, while the lowest rate were register in Doljevac (168.71). Among the patients, most were retired 63.6%, there were were unemployed 18.9% and workers 6.8%. The greatest number cases were in July (119).

Conclusion: Cardiovascular disease is the most common cause of illnesses and dyings of the inhabitants and that is way it is necessary to undertake adequate preventive measures to decrease the number of ill and dead people.*

Study on awareness and attitude level of intern medical students towards palliative care and barriers to providing it, Tabriz University of medical sciences, Iran

ESC-ID 802
Author Maghbooli L, Maghbooli M, Aghamohammadi D, Myandoab AT, Azar DK
Country Iran
University Tabriz University of Medical Sciences
Department Students Research Center

Introduction: Palliative care is a service designed to provide relief of symptoms that interfere with quality of life when treatments won’t change the time course of the illness. It is care given to improve the quality of life of patients who have a serious or life-threatening disease. This is an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial and spiritual. Some goals of Palliative Care are: 1) To prevent or treat as early as possible the symptoms of the disease, side effects caused by treatment of the disease. 2) To prevent or treat psychological, social, and spiritual problems related to the disease or its treatment. 3) To help people with life-threatening disease to live more comfortably. Palliative care is Comfort care, supportive care and symptom management. Physicians who care for dying patients often find this work fulfilling, a source of pride, and even a privilege. Making excellent care for dying patients regularly available will require improvements in systems for service delivery and in professional education.

Aim: The goals of the study were to explore medical students’ attitudes and opinions about end-of-life care and barriers to providing palliative care.

Method and Material: This was a cross-sectional study and the target population was 154 completing medical students of Tabriz University of Medical Sciences. Data were collected

October 7, 2007 EUROPEAN JOURNAL OF MEDICAL RESEARCH 123
using a questionnaire developed specifically for this study (consists of: demographic characteristics, awareness and attitude about end-of-life care). Then, the information was processed by SPSS statistical software version 12.0.

**Results:** This study consisted of 87 women, 67 men intern students of Tabriz University of medical sciences. 43 of students (27.9%) could not define palliative medicine. Although 97 of students (63%) reported having sufficient awareness about end-of-life care, the mean score of knowledge (about diagnose and treat various types of pain, World Health Organization approach to pain, common symptoms in the terminal phase and ...) for all students was 11 out of 20. Only 11 (7.1%) of students answered more than 70% of the questionnaire correctly, although 128 of them (83.1%) answered at least 50% correctly. 117 of students (76%) believed that care for dying patients should focus on pain and psychological disorders and 66.8% said that should use nonpharmacologic means for controlling pain. We detected low score by awareness questions about pain management for 96 students (76.8%) who agree with the statement (125 of all students 81.1%), “I feel comfortable taking a pain history and writing pain medications”. 56% of them prefer don’t use opioids for end of life patients. We asked 3 open-ended questions regarding: • Common symptoms which occur at the end of life and describe at least two therapies for each. • Common adjuvant therapies and describe situations where they may be effective. 3. Physicians, nurses, managers, ... in hospitals and clinics of Iran. Who take part in public health project “Your health is in Your hands” implemented in rural areas of Pomorskie Province in Poland. Patients were divided into 5 groups depending on their age and gender according to actual screening criteria for breast, colorectal, cervical cancers and guidelines for breast self-examination. Clinical data were obtained from own questionnaire conducted among patients who took part in the project. Foregoing data were subjected to statistic analysis. 19 patients (12%) of all students 24.5% women in group 20 – 39 years old and the mean period between examinations was 27 months. What is more about 70% questionnaires women perform breast self-examination (average frequency: 1.89 month) although breast self-examination correlates with non-performing mammography (p = 0.02). The main screening test to prevent cervical cancer – cytology was executed by 71.7% obliged women. Only 20.9% of the examined population had colonoscopy as colorectal cancer screening. Analysis revealed that there is a significant correlation between performing screening tests (USG, mammography and colonoscopy) and economic activity (adequately p = 0.002; p = 0.02; p = 0.02). Further statistical analysis demonstrated that there is a correlation between breast cancer screening and level of education (p = 0.03) or familiar history of cancer (p = 0.014).

**Conclusion:** The knowledge about screening is very low in rural population of Poland therefore this group should be included with intensified program preventing cancer. Foregoing data revealed that screening performed is insufficient to protect this population properly. Although some patients were aware how to detect early stages of developing neoplasm and understood the relevance of screening tests, this knowledge didn’t result in regular participation in screening program.
The prevalence of hypertension and diabetes mellitus and their associated risk factors in Sudan (Darfur)

ESC-ID 47
Author Alamin MS, Homeida M
Country Sudan
University University of Medical Sciences and Technology
Department Medicine

Introduction: Hypertension and diabetes are an important public health challenge in both economically developing and developed countries. Significant numbers in individuals with both diseases are un aware of their condition, and among those who were diagnosed, treatment is frequently inadequate. Measures are required at a population level to prevent the development of hypertension and diabetes and to improve awareness, treatment and control of diabetes and hypertension in the community. The aim of this study is to determine the prevalence of diabetes and hypertension, and to identify the associated risk factors in five different cities in Sudan.

Materials and Methods: This is an observational, community based study which was achieved by measuring blood pressure and blood glucose in people aged 18 and above who are residing in Khartoum (The capital), Nyala (Darfur region), Port-sudan(Main port in sudan), Shendi (north) and Malkal (south). Before that a written questionnaire were filled. The written questionnaire questions about relevant risk factors (age, gender, family history, socioeconomic status, level of education, occupation, alcohol consumption, smoking and obesity). In addition to the questionnaire there was a check list considered of weight and height value to calculate the BMI of each individual, the level of blood glucose and the reading of blood pressure.

Results: The questionnaire and the check list results: We statically analyzed and the results showed that the total prevalence of diabetes being 16.1% and of hypertension being 30.3% in the five cities. The risk factors which affect hypertension in this study were age, smoking, Obesity, socioeconomic status and stressful job. While the risk factors for developing diabetes mellitus were socioeconomic status and stress.

Conclusion: The prevalence of hypertension and diabetes is increasing in Sudan.

Heart Surgery

The early outcomes of coronary artery bypass grafting in patients with advanced left ventricular dysfunction: Comparison with whom had normal left ventricular ejection fraction

ESC-ID 260
Author Karamustafaoglu B, Ozucer B, Kuertugo M
Country Turkey
University Istanbul University
Department Istanbul Faculty of Medicine

Backgrounds: Coronary artery bypass grafting is indicated in patients with coronary artery disease and impaired ventricular function, however revascularization of ischemic myocardium in patients with severely impaired global left ventricular function remains a surgical challenge. The aim of this study is to compare the demographic, pre-intra and post operative variables in CAGB patients with Ejection Fraction equal or less than 20% with ones who has EF greater than 20%.

Methods: Between January 2002 and March 2006, 11259 patients underwent coronary artery bypass grafting in Tehran Heart Center. 135(1.2%) patients with markedly reduced left ventricular function (EF equal or less than 20) were compared with 11124 (98.8%) patients who had EF greater than 20%. The ejection fraction were determined by echocardiography and angiography. Demographic, Pre-intra and post operative data of two groups were analyzed by univariate analysis.

Results: In group-A (EF¡Ü20) mean age of patients was 57.16¿9.21 and 87.4% were men. In group-B (EF>20) mean age of patients was 58.70¿9.82 and 73.9% were men. group-A had the highest percentage of patients who were men (P = 0.001). Patients in group-A compared with group-B had more previous MI, CHF, Cardiogenic Shock, arrhythmia, mitral and tricuspid insufficiency. These patients also had more NYHA class IV and more previous preoperation creatinine level as well as emergent operation status, CPB and Aortic cross clamp time, IABP use, total ICU stay, arrest and mortality (P <0.05). It is notable that number of grafts, incidence of LIMA use as conduit, postoperative MI, AF and stroke were similar in two groups. Hospital mortality in groups A and B was 3.7% and 1.3% respectively (P = 0.037).

Conclusion: Coronary Artery Bypass Grafting is still one of the best choice procedure for Ischemic cardiomyopathy and our results revealed 3.7% of mortality rate for this group of patients that is adequate rate for CAGB.

Role of ankle brachial pressure index measurement in arterial injury management

ESC-ID 260
Author Karamustafaoglu B, Ozucer B, Kuertugo M
Country Turkey
University Istanbul University
Department Istanbul Faculty of Medicine

The arterial injuries with hard signs are directly operated due to the short ischemic period of success. These hard signs are external arterial bleeding, rapid expanding hematoma, palpable thrill, audible bruit, obvious arterial occlusion (painlessness, pallor, paresthesia, pain, paralysis, poikilothermia). Since 6-8 hours of ischemic period are very important for the diagnosis and treatment of vascular injuries, simple and fast methods of examination are more appropriate in the management of suspicious arterial injuries, which carry soft signs with a high risk. These soft signs are history of arterial bleeding at the scene, proximity of trauma to major artery, diminished unilateral distal pulse, small nonpulsatile hematoma, neurologic deficit, abnormal flow-velocity waveform on Doppler ultrasound. Patients with hard signs in the physical examination should be directly operated. For patients with soft signs the first level of investigation must be Ankle Brachial Pressure Index (ABPI). Only with ABPI we can decide whether the patient needs further investigations or can be followed up conservatively. In the literature, if the ABPI is 1 or higher, the risk of arterial injury is less than 5%. When the ABPI is lower than 0.9, the probability of arterial injury is.
increases to 30-90%, so when the index is below 0.9 further imaging should be performed. These patients are investigated by Duplex USG and/or angiography for the definitive diagnosis. As Duplex USG is operator-dependent, angiography is the gold standard investigation. Between January 1997 and January 2001, 1772 patients with peripheral arterial injury or the suspicion of peripheral arterial injury admitted to the Department of General Surgery, Trauma and Emergency Medicine of Istanbul Faculty of Medicine. They were analyzed retrospectively. With physical examination, hard signs of arterial injury had been seen in 283 patients (15.97%), who had been directly operated. The rest, 1489 patients (84.03%) without any hard sign in the physical examination and any operation sign in doppler pulsation were investigated with ABPI. 1343 patients (75.79%) with ABPI higher than or equal to 1 were accepted as healthy and followed up conservatively. The rate of missed arterial injury in the followed up group was 0.8% (11 patients - mortality and morbidity 0%). 146 patients (8.24%) had ABPI lower than 1, they were detected by Duplex USG and/or angiography. 39 of them (26.71%) had arterial injury. These data show that in suspicious arterial injuries the sensitivity of ABPI lower than 1 is 78%, specificity 92.56% and diagnostic accuracy 92.07%. In conclusion, physical examination finds hard signs in 16% of the patients, ABPI excludes arterial injury in 76% of the patients. The rest (8% of the patients) is the group where angiography and/or Duplex USG can be selectively applied for the diagnosis of arterial injury. According to us this is the most reliable, cost-effective algorithm of arterial injury management.

**On pump versus off pump coronary artery bypass surgery. Preliminary results from a nation-wide study in Iceland**

**Objective:** In recent years, off pump coronary artery bypass (OPCAB) surgery has gained increasing popularity as the treatment of choice for multivessel coronary artery disease. Numerous studies, most of them with small numbers of low risk patients, have indicated lower rates of complications following OPCAB when compared to conventional CABG. However, OPCAB is technically more challenging and recently the advantages of OPCAB over CABG have been debated. In Iceland, OPCAB surgery was started already in 1999 and for several years around 30% of bypass procedures are performed off pump. The aim of this retrospective study was to compare these two techniques with main focus on perioperative and short-term postoperative complications.

**Material and methods:** This is a retrospective study that includes all patients operated in Iceland with coronary artery bypass surgery from June 2002 to June 2004. Cases with concomitant bypass surgery (i.e. together with valve procedures) were omitted. Patients were divided into two groups, CABB-group (n=150) and OPCAB-group (n=53), the latter group including three patients that were converted to CABB.

**Results:** Patient demographics were very similar for both groups, including age, gender and the extent of the coronary artery disease (table 1). The OPCAB-procedures took longer and bleeding was increased when compared to the CABG-group (table 1). A trend for increased rate of complications was observed for the CABG-group, including perioperative myocardial infarction, atrial fibrillation and operative mortality, however, the difference was not statistically significant. Rate of perioperative stroke was comparable in both groups, or 2% and there was a trend for increased hospital stay (1 day difference) for the CABG-group.

**Conclusion:** This preliminary data indicates that there is a trend for lower rate of complications following OPCAB when compared to CABG. Our data also shows that OPCAB can be performed with low risk and mortality, even in patients with extensive coronary artery disease and low ejection fraction. However, OPCAB takes longer and bleeding is increased.

**Off-pump surgery: Demystifying the Myths – Comparison of patient outcomes in off-pump versus conventional procedure**

**ESC-ID** 220
**Author** Khan SF, Siddiqui FJ, Sharif H
**Country** Pakistan
**University** The Aga Khan University
**Department** Department of Surgery

**Background and Aims:** Coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB) has been tremendously effective as a treatment for Ischemic Heart Disease. Nevertheless, CPB is associated with a recognized risk of morbidity. The possibility of delivering similar or superior outcomes through CABG, while obviating the need for CPB is appealing. Our primary aim was to determine whether CABG without CPB (off-pump) decreases postoperative morbidity and mortality as well as myocardial damage in comparison with conventional CABG. A secondary aim was to identify any cost-saving associated with the off-pump technique in comparison to the conventional procedure.

**Methods:** The study reviewed patients undergoing either conventional or off-pump CABG at AKUH from 1995 onwards till 2004. All patients undergoing isolated coronary artery bypass grafting without any concomitant surgical procedure were included. Both elective and emergent procedures were considered eligible without any restrictions as to age, sex, American Society of Anesthesiologists (ASA) status, prior cardiac surgery or ejection fraction. There were 31 patients during the last 10 years who underwent off-pump CABG at our hospital along with 337 who underwent conventional CABG. A retrospective review of patient records was undertaken to record outcomes for morbidity and mortality. Several patient characteristics and risk factors for adverse outcome were noted for the two groups as well. Categorical variables were compared using the chi-square test or the Fisher’s exact test as appropriate. Continuous variables were compared using the independent samples Student’s t test. The tests were two-sided and &p<0.05; of less than 0.05 was considered significant.

**Results:** Some pre-operative patient characteristics varied between the off-pump and conventional CABG groups while others were similar. In terms of outcomes, from the off-pump patients, 74.2% had prolonged stays as compared to 37.1% in the conventional CABG group with a p-value ≤0.001. In the conventional CABG group 88.7% of the patients had post-
operative renal impairment (defined as a Serum Creatinine value above 1.3 mg/dl) as compared to 61.3% in the off-pump CABG group with a p-value of < 0.001. Of the other outcomes reviewed, there was no significant difference in morbidity, mortality or expenditure between the two groups.

**Conclusions:** Off-pump CABG is as safe a procedure as conventional CABG with no significant difference in outcomes or expenditure between the two. Hence, it can be incorporated into mainstream cardiac surgical practice. It may well be a superior choice in well-selected patient groups.

### Quality of life of patients after mitral valve surgery combined with surgical ablation of atrial fibrillation

**ESC-ID** 240

**Author** Dobrowski F, Roman R, Machowski M

**Country** Poland

**University** Medical University of Warsaw

**Department** Faculty of Medicine

**Background:** Patients referred to mitral valve surgery (MVS) in 30 to 50% suffer from atrial fibrillation (AF) related heart valve disease. Since AF is recognized as risk factor for death, stroke and heart failure after surgical treatment of mitral valve disease concomitant surgical ablation of AF may result in better long-term outcomes. However there is little data showing quality of life (QoL) in these patients, specially in term of sinus rhythm maintenance. The aim of presented study is to analyze QoL in long-term follow-up after MVS and concomitant surgical ablation of AF.

**Material:** Study involved 30 consecutive patients (18 females and 12 males) at mean age of 57.9 (± 8.3) years who underwent uncomplicated MVS with concomitant surgical endocardial ablation ablation using radiofrequency probe or cryothermy. In all patients the same ablation lesion pattern was applied.

**Methods:** After mean time 2.25 (± 2.1) years blinded investigators performed quality of life measurement with use of short form 36 (SF-36) questionnaire. SF-36 contains of 36 questions in main QoL-domains: current general health, general health state in comparison to preoperative state, physical functioning, limitations in physical functioning, importance of physical limitations due to health problems, social functioning, body pain, emotional problems, role of physical limitations due to health problems, social functioning, body pain, emotional problems, role of physical limitations due to health problems, emotional problems, role of physical limitations due to health problems, social functioning, body pain, emotional problems, role of physical limitations due to health problems, emotional problems, role of physical limitations due to health problems, social functioning, body pain, emotional problems, role of physical limitations due to health problems, role of physical limitations due to health problems. For each answer the points were collected and more points were given for better status. Maximal points number possible to obtain was 149. After total data collection study was unblinded in terms of sinus rhythm or AF occurrence.

**Results:** Mean number of collected points was 110.2 (±12.1). Mean general health status was 2.6 (± 0.7) points (max. 5 possible). Mean health status improvement after surgery was 4.4 (± 1) points (max. 5 possible). There were 23 (76.6%) patients in sinus rhythm (SR) and 7 (23.3%) in AF. There were no significant differences between SR and AF groups in terms of age and time after surgery (p>0.05). Significant difference was observed only in role of physical limitations. Patients who sustained SR had better status in that domain than patients in AF: 5.3 (± 1.2) vs 4.0 (± 1.6) points, respectively (p=0.03). There was trend towards higher number of total QoL points collected in patients with SR that in AF: 112.2 (±12.4) vs 103.4 (±17.9), respectively (p=0.15), however did not reach statistical significance.

**Conclusion:** Study showed good QoL in patients after MVS with concomitant surgical ablation for AF. Significant impact of SR on QoL have been observed only in one domain. General trend towards better QoL in SR group gives rationale for more statistically powered trials.

### Surgery for Coarctation of the Aorta: A Nation-wide Study from Iceland

**ESC-ID** 385

**Author** Gunnarsson SI, Torfason B, Sigfusson G, Helgason H, Gudbjartsson T

**Country** Iceland

**University** University of Iceland

**Department** Medicine

**Objective and aims:** Coarctation of the aorta is a common congenital defect of the heart. It is most commonly located on the thoracic aorta, distal to the left subclavian artery [1]. The coarctation obstructs blood flow from the left ventricle, causing hypertension in the upper body and reduced blood flow to the lower extremities. Heart failure with shock can be observed in extreme cases. Most commonly coarctation is treated with surgical resection or balloon angioplasty, however, in less severe cases medication and observation can suffice [1]. No studies have been made in Iceland on the efficacy of surgical treatment for this disease. The aim of this study was to evaluate the results of surgical treatment for aortic coarctation in Iceland.

**Materials and methods:** These are the first results from a study that includes all Icelandic children with coarctation of the aorta diagnosed in the period between 1990 and 2006. This study focuses exclusively on children (under the age of 18) who were operated on for this condition. In this retrospective study data was obtained from medical reports as well as diagnosis and operations lists from Landspitali University Hospital. Symptoms and signs of diagnosed patients and indications for surgery as well as complications and the ultimate efficacy of surgery were examined.

**Results:** A total of 67 children were diagnosed in the 17 years the study spanned, 38 of whom were operated on in Iceland (22 boys and 16 girls, average age 37 months, range 3 days to 17.8 yrs). Twentysix patients (68%) manifested symptoms, those of heart failure and claudication being the most common ones. Eleven patients were diagnosed incidentally and one in utero. On examination, 33 patients (87%) had a systolic murmur and 32 patients (84%) were diagnosed with weak or absent femoral pulses. In 80% of cases the coarctation was distal to the left subclavian artery. Eight patients required acute surgery, in most cases for severe heart failure and shock. The average gradients before and after surgery were 50 and 12 mmHg, respectively. The most common surgical procedures were end-to-end anastomosis (n = 31) and subclavian-flap aortoplasty (n = 5). The average operation time was 134 min. (range 80-260) and the aortic closure time 24 min. (range 11-70). In one case an extracorporeal circulation (ECC) connected to the femoral artery, was used. The most common post-operative complications were paroxysmal hypertension and heart failure. No cases of postoperative paraplegia or other paralysis were detected. The median length of stay was 9 days (range 4-127). Seven patients (18%) required balloon dilation after surgery and one patient needed to be reoperated for complications following a balloon angioplasty. No other patients required surgical intervention.
for recoarctation. All patients survived the operation and all except one are currently alive (April 2007). This patient died three months after the initial operation due to other congenital disorders.

Conclusions: The incidence of aortic coarctation in Iceland is similar to that of neighbouring countries [2]. More than half of diagnosed patients underwent surgery. Results of surgical treatment are usually good with a relatively low rate of both intra- and post-operative complications. Operative mortality was 0% and long-term prognosis is excellent. Still a significant number of patients have hypertension postoperatively that is in most cases not related to recoarctation.

SLPI, a fundamental molecule in ischemia/reperfusion and cardiac transplantation

Aim: Protease activation as well as inflammatory responses contribute to organ damage in response to ischemia and reperfusion (IR). In this study we investigated the role of protease inhibitor SLPI in IR-injury and cardiac transplantation.

Methods: Hearts from SLPI-/-mice were heterotopically transplanted immediately or challenged with 10h of cold ischemia (CI) and then transplanted. C57BL/6 isografts (WT) undergoing the same procedure served as controls. In selected groups 200mg of recombinant SLPI (rSLPI) were added to the preservation solution or given i.v. After evaluation of graft function, hearts were removed at various time points. Morphology was investigated by histology. SLPI gene expression was analysed using quantitative RT-PCR. SLPI protein expression was studied by immunohistochemistry (IHC). SLPI, TNF-α, TGF-γ, Cathepsin-G and Elastase activity were analysed employing ELISA and Western Blot.

Results: At 15 min, recovery of graft function was normal in WT and SLPI-/mice transplanted without CI (4.0±0.0). In contrast, SLPI-/ hearts transplanted after 10h of CI showed no or marginal recovery of organ function (0.6±1/4 vs. 0.85). At 24h cardiac function in SLPI-/ (2.5±1/4 vs. 0.89) was less compared to WT (3.6±1/4 vs. 0.55). Single administration of rSLPI i.v. had no effect; however, when rSLPI was added to the preservation solution organ function comparable to WT mice was observed (3.6±1/4 vs. 0.55). A mild mononuclear cell infiltrate and small focal necrosis were found in all groups at 24h. At 10 days, post ischemic inflammation as well as myocyte necrosis were significantly higher in the SLPI-/ group (2.5±1/0.5 vs. 1.8±1/3.4 and 1.6±1/4.5 vs. 0.2±1/3.4). Myocyte vacuolization as a sign of sublethal ischemic injury was present at high level in SLPI-/ mice undergoing CI only. SLPI gene expression was detected in WT mice at 12 and 24h after reperfusion. Gene transcription at 12h was significantly higher after prolonged CI (7.99 vs. 1.57 orders of magnitude) and was associated with significantly decreased TGF-γ activity. SLPI protein was first observed at 24h, high levels of SLPI protein were found at 10 days. SLPI positive cells were mainly identified as macrophages (IHC). High intragraft levels of SLPI activity were found early as well as 10 days after application of rSLPI. TGF-γ was effectively abolished by application of rSLPI to the preservation solution, levels of TGF-γ activity were significantly lower in both WT and SLPI-/ animals. TNF-α was found in all animals without any significant difference. Investigation of Elastase and Cathepsin G activity revealed high protease levels early after reperfusion in all groups. At 10 days Elastase and Cathepsin G activity were still high in both WT and SLPI-/ groups, however application of rSLPI resulted in abrogation of protease activity in WT groups whereas protease activity in SLPI-/ animals remained unaffected.

Conclusion: We conclude that SLPI has a substantial effect on protease activity and the inflammatory response in ischemia and reperfusion in the context of cardiac transplantation. This effect is related to the inhibitory property on TGF-γ as well as proteases such as Cathepsin G and Elastase. In addition, SLPI seems to be crucial for recovery of organ function early after heart transplantation. We therefore propose that SLPI represents a promising target for modulating the destructive processes of posts ischemic inflammation while preserving its restorative nature.

Internal Medicine Cardiology II

Adaptogenic manifestations of extractum fluidum Rhodiolae when using in patients with chronic heart failure of ischemic genesis

Aim: To study the effect of application of Extractum Rhodiolae Roseae Fluidum (ERRF) on the indices of diurnal monitoring of arterial pressure (DMAP) in patients with chronic heart failure (CHF) of degree IIa of ischemic genesis. Thirty seven patients aged 49.2±5.7 years were examined, 14 women and 23 men. DMAP was performed during 48 hours. During the first 24 hours the patients continued to receive the remedy of basic therapy – angiotensin-converting enzyme inhibitor (ACEi) in an individually selected dose. Next day the patients additionally took ERRF in a single dose of 10 drops at 10.00 and 13.00. An analysis of the results of DMAP after ERRF intake indicated a reliable increase of the readings of diurnal systolic arterial pressure (SAP) by 14.82% (p<0.05) and diastolic arterial pressure (DAP) by 20.72% (p<0.01) in the group of patients under study (15-40,54% of the persons) with a low initial reading of AP (the average daily reading made up 110.46±3.31 mm Hg). In the second group (9-24,32% of the patients) (SAP was within the bounds from 121 to 139 mm Hg) the indices of SAP and DAP had only a tendency to be on the rise. In the third group (13-35,14% of the patients), patients with border-line readings of AP from 140 to 159 mm Hg, an elevation of the SAP diurnal reading was observed during the second 24-hour period of monitoring by 5.22% with a trustworthy difference (p<0.01), untrust worthy changes were marked for DAP. Thus, ERRF manifests adaptogenic properties against a background of basic cure in

The object of our research was an estimation of the effect of Extractum Rhodiolae Roseae Fluidum (ERRF) on the indices of diurnal monitoring of arterial pressure (DMAP) in patients with chronic heart failure (CHF) of degree IIa of ischemic genesis. Thirty seven patients aged 49.2±5.7 years were examined, 14 women and 23 men. DMAP was performed during 48 hours. During the first 24 hours the patients continued to receive the remedy of basic therapy – angiotensin-converting enzyme inhibitor (ACEi) in an individually selected dose. Next day the patients additionally took ERRF in a single dose of 10 drops at 10.00 and 13.00. An analysis of the results of DMAP after ERRF intake indicated a reliable increase of the readings of diurnal systolic arterial pressure (SAP) by 14.82% (p<0.05) and diastolic arterial pressure (DAP) by 20.72% (p<0.01) in the group of patients under study (15-40,54% of the persons) with a low initial reading of AP (the average daily reading made up 110.46±3.31 mm Hg). In the second group (9-24,32% of the patients) (SAP was within the bounds from 121 to 139 mm Hg) the indices of SAP and DAP had only a tendency to be on the rise. In the third group (13-35,14% of the patients), patients with border-line readings of AP from 140 to 159 mm Hg, an elevation of the SAP diurnal reading was observed during the second 24-hour period of monitoring by 5.22% with a trustworthy difference (p<0.01), untrust worthy changes were marked for DAP. Thus, ERRF manifests adaptogenic properties against a background of basic cure in
Does coronary artery disease increases the prevalence of abdominal aortic aneurysm? (Preliminary results)

ESC-ID 882
Author Armata M, Walczuk A, Olszewski R, Skrobowski A
Country Poland
University Medical University of Warsaw
Department 1st Faculty of Medicine

Background: In course of Coronary Artery Disease (CAD) inflammatory mechanisms play a main role in atherosclerotic plaque destabilization. Recent data indicated that inflammatory mechanisms play the similar role in the widening of a abdominal aorta also.

Aim: The purposes of this study were determined whether an association exists between CAD and abdominal aortic aneurysm (AAA).

Methods: 43 patients (21F; 22M; aged 38-74) with acute Coronary Syndrome admitted to Cardiological Department were included to the study. Ultrasound examination of the abdominal aorta was performed during hospitalization period using Biosound Esaote MyLab30CV machine. The abdominal aorta were measured in 3 times in longitudinal plane and in 3 times in transverse plane. AAA was defined as a diameter 30mm or more.

Results: The abdominal aorta were visualized in 41 (95.34%) patients. In 7 (17.07%) patients (3F, 4M) AAA were detected. Our results show that it is more than in general population (people between 45 and 54 years old: men-1.3%; women- 0%; people between 75 and 84 years old: men- 12.5%; women- 5.2%)

Conclusion: The results suggest that rapid screening of the abdominal aorta should become a part of standard echocardiographic examination in patients with ACS during hospitalization period.

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Echocardiografic evaluation of the effect of Abciximab in the patients with acute myocardial infarction

ESC-ID 879
Author Zeynel S, Pejkov H
Country Macedonia
University Ss. Cyril and Methodius University – Skopje
Department Institute for Heart Diseases

Introduction: Abciximab is a Fab fragment of the chimeric human – murine antibody 7E3. In the process of abciximab production, the chimeric 7E3 antibody is processed with papain, a proteolytic enzyme for obtaining the Fab fragment. The pathways of platelet activation terminate on GP IIb/IIIa and the result is platelet aggregation. Abciximab prevents the platelet aggregation and thrombus formation trough the mechanism of binding with GP IIb/IIIa receptors. Objective: Evaluation of the left ventricular function after stent implantation in the patients treated with PCI + Stent + Abciximab in comparison with patients with PCI + Stent.

Patients and Methods: For elaboration of this study were used the data from the Institute for cardiovascular diseases, Clinical Center – Skopje. For the aims of this study were evaluated and compared the results of 96 patients within the period from December 2002 – December 2003. The echocar-
diography analysis was made at the time of the control reconography after nine months.

**Results:** The analysis shows that the patients with PCI + Stent + Abciximab have a better EF compared with the group treated without Abciximab. 6.3% have EF > 50%, 66.7% have EF from 41-50% and the other EF < 40%. In the group with PCI + Stent patients with EF > 50% were not found, 43% have EF from 41-50%, and in 50% EF was bellow 40%.

**Conclusion:** The results suggest the administration of abciximab contribute for the significant improving of the left ventricular function in the patients in which abciximab was administered compared with patients without treatment with abciximab.

### Arterial hypertension in patients with nonalcoholic fatty liver disease

**ESC-ID:** 845  
**Author:** Badau CI, Casoinic FE  
**Country:** Romania  
**University:** University of Medicine and Pharmacy Cluj Napoca  
**Department:** Cardiology

**Aim:** Hypertension represents a public health problem due to its high prevalence and significant impact on cardiovascular morbidity and mortality, being an independent cardiovascular risk factor with a strong, positive and continuous correlation with all forms of atherosclerotic vascular diseases. Nonalcoholic fatty liver disease (NAFLD), the hepatic expression of the metabolic syndrome is associated with cardiovascular risk factors such as type 2 diabetes mellitus and abdominal obesity. The purpose of our study was to assess the prevalence of hypertension in patients with ultrasonographically testified nonalcoholic disease compared with controls and the general population (data from SEPHAR study(1)).

**Material and methods:** The studied group consisted of 104 patients ultrasonographically diagnosed with NAFLD, in which alcohol consumption and other causes of chronic hepatopathy have been excluded. A control group of 100 subjects without ultrasonographical proof of hepatic steatosis has been used. Subjects with NAFLD were allocated to two subgroups: simple steatosis and steatohepatitis, based on the levels of transaminases. In both NAFLD and controls blood pressure was measured. The diagnosis of hypertension was established according to ESH/ESC guidelines (2).

**Results:** The prevalence of hypertension has been significantly higher in subjects with NAFLD compared with controls (67% versus 22%, p<0,01) and compared with the prevalence of hypertension reported in general population by SEPHAR (67% versus 40%). The prevalence of newly diagnosed hypertension was 35% (25/70 patients). Patients with steatohepatitis had a higher prevalence of hypertension than those with simple steatosis (69% versus 66%).

**Conclusions:** The prevalence of arterial hypertension in NAFLD is significantly higher compared with controls and the general population. There is a significant prevalence of undiagnosed AHT in NAFLD subjects.

### Gender differences in the angiographic parameters obtained during PCI performed in patients with acute myocardial infarction

**ESC-ID:** 843  
**Author:** Dziechcinska D, Mlezko S, Zaremba M, Wrobel M, Olszowski D, Gasior M, Polonski L  
**Country:** Poland  
**University:** Silesian Medical University in Katowice, Faculty of General Medicine in Zabrze  
**Department:** 3rd Department of Cardiology, SCHD

**Introduction:** Percutaneous coronary intervention (PCI) is a preferred intervention therapy in acute myocardial infarction both in men and women. Its' effectiveness can be assessed on the basis of several criteria including angiographic parameters such as good final flow (TIMI equal 3) and residual stenosis in the infarction related artery less than 20%.

**Aim:** The aim of the study was to compare parameters obtained during PCI in men and women especially concerning the infarct related artery and also evaluation of the angiographic effectiveness of PCI.

**Material and methods:** We analysed 3946 patients admitted to Silesian Center for Heart Diseases in Zabrze, Poland, in years 1998 - 2005 because of acute myocardial infarction (MI). Among patients included in the study there were 1078 women and 2868 men who have undergone PCI after admission.

**Results:** The right coronary artery (RCA) being the infarct related artery was more frequent in women (55,15% vs 53,57% p <0,0001) whereas the left circumflex artery (CX) in men (25,63% vs 22,92%, p = 0,00483). No statistically important gender differences were noticed in case of left anterior descending artery (LAD) and left main coronary artery (LM). These two groups didn't also differ in the occurrence of multivessel coronary disease. The initial TIMI flow equal to 0 was present in 65,53% women and 61,56% men (p <0,0001). The final TIMI 3 score was obtained more often in men (91,10% vs 89,88%, p <0,0001). Men dominated slightly over women in the number of those who had residual stenosis (6,36% vs 6,08%, p = 0,00748).

**Conclusions:** In women RCA is more often the infarct related artery than in men, whereas men dominate if CX is concerned. Male patients gained better results in case of angiographic parameters used to evaluate the effectiveness of PCI as more men had good final flow (TIMI 3) despite relatively more frequent residual stenosis.

### The influence of chronic total occlusion’s angiographic parameters on success of recanalisation procedure

**ESC-ID:** 842  
**Author:** Klapkowski A, Podlesko A, Krys A, Jaguszewski M, Masiewicz E, Duda M  
**Country:** Poland  
**University:** Medical University of Gdansk  
**Department:** First Department of Cardiology

**Introduction:** Chronic total occlusion is a lesion which totally occlude coronary arteries. International studies suggests that approximately one-half of patients with significant coronary disease have at least one chronic total occlusion (CTO).
Recanalisation of CTO is one of the most difficult procedures in interventional cardiology. With comparison to non-occlusive lesions, CTO recanalisation is associated with lower procedural success. It is caused by inability to cross the lesion. Technical advances in the design of angioplasty equipment (especially specialized wires) have improved recanalization rates. Moreover the recanalization success depend on operator experience which is higher every year. Failed procedures can lead to acute adverse events but successful CTO recanalization is associated with patients improvement in long-term survival rates. The influence of angiography parameters on recanalization success in not well documented yet. 

**Aim of study:** The goal of the study is to investigate the influence of angiographic parameters on recanalization success of chronic total occlusion’s lesions in coronary arteries. 

**Materials and Methods:** Studied group consists of 401 patients (72,5% men, 27,5% women) who underwent the CTO recanalization procedure in I Department of Heart Disease in Gdansk between 2003 and 2005. Their angiography videos were described with the usage of special made form. 

**Results and Conclusion:** In studied group there was 64% successful recanalization (in patients who underwent recanalization in 2003,2004 – 60%, in 2005 – 70%). The most frequent cause of failure was problems with crossing lesion by wire – 90,4%. We investigated that in women population recanalization was significantly more successful than in men (74,3% vs 60,6%, p<0.01). The analysis of angiographic parameters of primary lesion shew statistically significant association of vessel size (arteries < 2,5 mm in diameter – 73,4% ineffective recanalisations of the CTO; p<0,001) and side branch take-off from occlusion side (branches are present in 66,5% of ineffective CTO recanalisations; p = 0.001; OR = 2,3), with procedure outcome. Other parameters, which improve possibility of failure was: TIMI 0 before procedure (p<0.0002), massive calcifications(p<0.006).

**Multivessel disease in diabetic and non-diabetic patients with myocardial infarction. 2-year clinical outcome**

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According to recent research, patients with diabetes mellitus and myocardial infarction have significantly higher mortality than patients without diabetes. Moreover, those patients differ in relation to clinical parameters and risk factors. 

**Aim:** The aim of our study was to: 1) compare clinical characteristic and prognostic risk factors in patients with myocardial infarction and multivessel disease 2)determine the impact of diabetes on 2-year clinical outcome 

**Materials and methods:** We analyzed medical records of patients hospitalized in Silesian Center for Heart Diseases between 1998 and 2005. Hospitalization due to myocardial infarction was the inclusion criterion. In our study we enrolled 1312 patients with multivessel disease treated with percutaneous coronary intervention (PCI). In this group, 998 patients had diagnosis of diabetes on admission, and 314 did not have such diagnosis. We analyzed 2-year mortality. 

**Results:** The compared groups did not differ in relation to prior myocardial infarction, cardiogenic shock, anterior infarction, fibrinolysis before PCI, infarction related artery, left ventricular ejection fraction, stenting, reocclusion, maximum CFP levels and mean hospitalization duration. In diabetes group there were more women, mean patient age and mean chest pain duration were higher, patients in this group were more often hypertensive and less often smokers. Reinfarction and 3-vessel involvement were also more frequent in the diabetes group. We observed significant difference in mortality between non-diabetic and diabetic both in in-hospital and 2-year observation – 5.51% vs. 11.15%; p = 0.0006 oraz 13.33% vs. 27.39%; p <0.0001, respectively. Multivariate analysis confirmed the impact of diabetes on prognosis; the analysis revealed, that diabetes was an independent risk factor of 2-year mortality in patients with multivessel disease. 

**Conclusion:** Although the differences in initial clinical characteristic and mortality predictors are non-significant, prognosis of patients with diabetes is clearly worse.

**Does gender influence one-year prognosis in patients ≥ 75 years old with acute myocardial infarction treated by percutaneous coronary intervention?**

**Introduction:** Age is an independent factor which influence prognosis in patients with myocardial infarction. It is interesting if gender may influence in-hospital and one-year mortality in 75 years old or older patients with acute myocardial infarction (AMI) treated by percutaneous coronary intervention (PCI). The aim of our study was to compare the results of treatment according to gender in elderly patients with AMI treated by PCI. 

**Material and method:** We examined 184 consecutive patients ≥ 75 years old with acute myocardial infarction admitted to III Chair and Department of Cardiology Medical University of Silesia, Silesian Center for Heart Diseases in Zabrze, Poland, between 1998 and 2005. Patients were analysed in two groups according to the gender. For the purpose of this study selected parameters were compared during inhospital and long-term observation. 

**Results:** We identified 94 men and 90 women in analysed group. 75 years old or older women in comparison to men had higher level of glicaemia at admission (11 mmol/l vs 9.11 mmol/l; p = 0.0104), more often suffered from diabetes (40% vs 15%; p = 0.0001) and hypertension (77% vs 56%; p = 0.0036). However, men were more often smokers (41% vs 13%; p = 0.0001) and trend towards more frequent reoclusion (7% vs 2%; p=0.0971) was observed. We have also observed lower in-hospital (8% vs 12%) and one-year (17% vs 23%) mortality in women according to men, however no statistical significance for these parameters was observed. In multivariate analysis independent factors influencing one-year mortality in this group of patients were (relative risk
The early outcomes of coronary artery bypass grafting in diabetic women

ESC-ID 809
Author Zon AM, Drohomirecka A, Cichon R
Country Poland
University Heart Centre MEDINET
Department Cardiac Surgery

Women are reported to be at increased risk of poor outcome after coronary artery bypass grafting (CABG). Diabetes which is a frequent co-morbidity diagnosed in women with coronary heart disease may affect additionally adversely the results of CABG. The aim of this study was to evaluate the early outcomes of CABG in female diabetic patients. The study group consisted of 124 consecutive female patients undergoing isolated CABG: 49 (39.5%) diabetic women (DW) and 75 non-diabetic women (nDW) Mean age, Euroscore, rate of urgent operation, mean left ventricular ejection fraction, prevalence of hypertension, chronic obstructive pulmonary disease, unstable angina, peripheral vascular disease, history of myocardial infarction, stroke or TIA were comparable in both groups. Body mass index was greater in diabetic patients (DW: 28.69 ± 3.68 vs nDW: 26.75 ± 3.76 kg/m²; p <0.006). The prevalence of left main stenosis (DW: 20% vs nDW: 21%, NS) and three vessel coronary artery disease (DW: 63% vs nDW: 53%, NS) as well as the previous percutaneous coronary intervention (DW: 16% vs nDW: 23%, NS) did not differ between both groups. The rate of off -pump coronary artery bypass grafting was comparable in both groups (DW: 8% vs nDW: 5%;NS) The average time of extracorporeal circulation and aorta cross clamp did not differ between both groups (NS). The number of grafted coronary vessels (DW: 2.41 ± 0.67 vs nDW: 2.59 ± 0.65, NS) and frequency of use of arterial grafts (DW: 86% vs nDW: 89%. NS) were similar in both groups. The average time of mechanical ventilation (DW: 8.54 ± 4.28 vs nDW: 7.32 ± 3.25h, NS) and intensive care unite stay (DW: 28.16 ± 25.39 vs nDW: 22.94 ± 15.1 h, NS) were comparable. The number of patients who needed an inotropic support (DW: 18 % vs nDW: 11%;NS) or intra-aorta balloon pumping application (DW: 4% vs nDW: 3%; NS) did not differ between groups. The peak serum concentration of the myocardial- specific isoenzyme of creatine kinase (DW: 36.44 ± 75.01 vs nDW: 34.04 ± 62.88 ng/ml, NS) as well as the rate of the peri-and postoperative myocardial infarction (DW: 9% vs nDW: 4%;NS) were relatively high but statistically not different in both groups. Postoperative stroke and/or TIA occurred with similar frequency in both groups (DW: 4% vs nDW: 1.3%). The inhospital mortality was low and did not differ in women without diabetes and women with diabetes (DW: 0% vs nDW:1,3%; NS). The early outcomes of CABG in diabetic women were comparable to those observed in women without diabetes. Relatively high rate of myocardial ischemia was noticed in all women but it did not result in the increase of intra-hospital mortality.

Determinants of vital capacity among patients with heart failure.

ESC-ID 786
Country Poland
University Medical University of Silesia
Department III Department of Cardiology, SCHD Zabrze

Aim: The aim of this study was to define relations between vital capacity and global and local body mass composition in patients with heart failure and between land selected markers of progression of hear failure.

Method: We analyzed data from 193 patients with heart failure: 164 males, 29 females, age: 52.6 ± 9.9, LVEF: 25.9 ± 8.3, NYHA class: 2.6 ± 0.7, ischemic etiology of heart failure; in 64.8%. All patients were treated in an optimal way. They were examined by spirometry and DEXA scanning. Correlations between vital capacity and clinical markers of heart failure severity and between DEXA parameters were analyzed. We calculated Pearson’s linear correlation product and using the multifactorial regression modeling we examined independent predictors of vital capacity.

Results: Negative correlation was found between vital capacity and: duration of heart failure symptoms (-0.23, p = 0.002), NYHA class (-0.27, p = 0.0002) and total fat mass (-0.034, p = 0.0000). Positive correlation was noticed between VC and: peak VO2 (0.44, p = 0.0000), total muscle mass (0.25, p = 0.001), central muscle mass (chest + abdomen) (0.021, p = 0.007), peripheral muscle mass (upper and lower limbs) (0.29, p = 0.0001). Neither BMI nor NTproBNP correlated with vital capacity. In the multifactorial regression independent predictors of vital capacity were: age (p = 0.03), duration heart failure symptoms (p = 0.01), ischemic etiology of heart failure (p = 0.02), total fat mass (p = 0.0008) and total muscle mass (p = 0.0000). NYHA class was not an independent predictor of vital capacity.

Conclusion: Vital capacity in heart failure patients might by partly dependent on body mass composition and body mass distribution. Exercise intolerance which is a hallmark of heart failure (HF) depends mortally on circulatory, pulmonary and muscular functions. In HF all of these organs undergo pathologic remodeling and wasting impaired vital capacity (VC) may cause exercise intolerance but determinants and clinical correlates are poorly defined.
The prevalence of circulating 20S proteasomes and the role of anti-proteasomal antibodies in congestive heart failure

ESC-ID 699
Author Bartel K, Egerer K, Kuckelkorn U, Klingel K, Staudt A, Gericke C
Country Germany
University Humboldt University
Department Clinic for Cardiology

Aim: The proteasome, a major cytosolic antigen-processing complex, is crucial for the generation of a majority of MHC class I restricted peptides and is involved in various cellular regulatory pathways. Anti-proteasomal antibodies (APAs) and circulating proteasomes (cProteasomes) have been detected in several autoimmune diseases and hematologic malignancies. Here, we investigated the relevance of cProteasomes and APAs in congestive heart failure with particular regard to patients suffering from dilated cardiomyopathy (DCM). The pathogenesis of DCM remains a matter of discussion. However, several studies suggest a contribution of auto-antibodies to ongoing cardiomyopathy. Patients undergoing immunoadsorption (IA) therapy show a benefit in their clinical outcome. Therefore, we analysed APAs in sera and column eluents (CEs) in DCM patients during IA.

Methods: Sera of healthy blood donors as well as of patients with DCM, borderline and chronic myocarditis were screened for APAs and cProteasomes by sandwich ELISA. Furthermore, we performed Western blot analysis to confirm our ELISA data. Two-dimensional (2D) electrophoresis with purified 20S proteasomes enabled the detection of primary target subunits of APAs. A subgroup of DCM patients was treated with IA. CEs containing eliminated IgG were investigated by one- and two-dimensional western blot analysis.

Results: The levels of cProteasomes were significantly increased in patients with DCM, borderline myocarditis and chronic myocarditis in comparison to healthy blood donors as shown by ELISA. These data were confirmed exemplarily by Western blot analysis. Cardiac inflammation characterized by CD3 T cells and CD68 macrophages in endomyocardial biopsies was not associated with increased cProteasome levels. The subgroup of DCM patients treated with IA showed elevated individual levels of APAs in sera as well as in CEs, 2D western blot analysis revealed proteasomal subunits beta5 and beta7 as primary targets of APAs in CEs. APAs were considerably decreased in relation to total IgG content during each IA session. Interestingly, we did not detect any relevant changes in APA content during consecutive IA sessions.

Conclusion: Individual levels of cProteasomes are markedly elevated in patients with congestive heart failure. These proteasomes and any of their subunits may present targets for APAs. Indeed, some DCM patients show enhanced serum levels of APAs. The decrease of APAs in sera and CEs after each IA session indicates their effective elimination and suggests a correlation with the clinical benefit of patients following IA therapy. However, constant levels at the beginning of consecutive sessions indicate a rapid reconstitution of APAs. This observation is to some extent in contradiction to the prolonged clinical benefit of DCM patients treated with IA. Our data suggest that efficient elimination of APAs does not unequivocally contribute to the improved clinical outcome of DCM patients undergoing IA.
Use of self-measurement devices in clinical practice: Is device calibration and accuracy retained during and after robust use? (Microlife Pro M & 3AC1)

ESC-ID 550
Author Ahmed N, Sheppard E, de Greeff A, Shennan A
Country United Kingdom
University Guy's, King's and St Thomas' School of Medicine
Department Medicine

After a century of clinical use, the mercury sphygmomanometric technique of blood pressure (BP) measurement is finally hearing its death knell. Several factors have given the need for change some impetus, namely the toxicity of mercury to the environment. However with the disappearance of mercury sphygmomanometer, there have been concerns on finding an adequate replacement. One of the favoured candidates for replacing mercury sphygmomanometers are automated devices which incorporate oscillometric technology to derive blood pressures. The lifespan of automated blood pressure machines is thought to be reliant upon a capacitive sensor that has been stated to vary between 10,000 to 30,000 measurements (Reinders et al, 2005). Devices designed for robust use are thought to be equipped with a sensor that has a longer lifespan and have additional monitoring functions (i.e. temperature etc.), all of which drive up the cost of the device. This study examined use of low-cost self-measurement devices in clinical practice and whether device calibration and accuracy is retained during and after robust use. Two oscillometric devices (Microlife Pro M & 3AC1) which validated according to the International Protocol in an adult population (O'Brien et al, 2002). Devices were then distributed for use in clinics and wards at Guy’s, King’s & St Thomas’ Hospitals. Thereafter a Microlife 3AC1 device which had undergone 10,000 inflations was validated in a pregnant population according to British Hypertension Society (BHS) guidelines (O’Brien et al, 1993). Calibration checks were carried out on devices that had approached 1,000 inflations. The Microlife Pro M passed the International Protocol in adults and can be recommended for clinical use according to the International Protocol and also met Association for the Advancement of Medical Instrumentation (AAMI) criteria (mean difference <5mmHg and standard deviation <5mmHg) (Arlington, 1993). The Microlife 3AC1 failed phase 2.2 of the International Protocol in an adult population in this study, but has been previously shown to pass the protocol (Topouchian et al, 2005). Having undergone a intensive period of clinical use the Microlife 3AC1 was re-validated and achieved the highest possible grade for accuracy in pregnancy (A/A) according to the BHS criteria. It also met the necessary AAMI requirements. All the devices that underwent calibration checks, were still accurate to ±3 mmHg, meeting the European Standard specification.

One year follow up of high risk patients with acute coronary syndrome and peripheral disease after percutaneous cardiovascular interventions

ESC-ID 469
Author Wróblewska ź, Piñowicz M, Stolarczyk M, Zabek A, Brzeziński M
Country Poland
University Jagiellonian University
Department Collegium Medicum

**Background:** The coexistence of peripheral artery disease (PAD) increases death and stroke rates in patients with coronary artery disease (CAD). Due to many comorbidities these patients are often treated conservatively without revascularization. Complex percutaneous cardiovascular interventions for CAD and PAD may improve prognosis and long term outcome for this group of patients.

**Methods:** We included 67 patients with confirmed CAD. All patients underwent percutaneous coronary intervention (PCI) for non ST elevation acute coronary syndrome (Braunwald IIB, IIC) before/after/simultaneously with peripheral angioplasty. Major adverse cardiac and cerebrovascular events (MACCE) during one year follow-up were defined and assessed: death (cardiac and non-cardiac), urgent/non-urgent revascularization (surgical or repeat PCI, repeat PTA), myocardial infarction (MI), stroke, amputation.

**Results:** The mean age of patients was 60.9±8.3 years and men constituted 82%. Four patients (6%) had one-stage PCI and PTA procedure. One hundred lesions in lower limb arteries were treated and 53 (53%) with stent implantation. In one year follow-up, there were 3 total deaths (4.5%) and only 1 cardiovascular death, 1 MI, 1 urgent coronary revascularisation, 2 strokes and 8 repeated PTAs and no amputations. Altogether 22% patients suffered from MACCE. In multivariate regression analysis, only age (p = 0.001) and previous MI (p = 0.04) were independent predictors of one year MACCE.

**Conclusions:** Patients with concomitant CAD and PAD could safely undergo percutaneous cardiovascular interventions. Multilevel intervention could provide promising long term follow up.

Hospital networking and population derived invasive facility centre distribution increased mechanical reperfusion among patients with acute coronary syndromes in the Krakow Network Region in Poland

ESC-ID 466
Author Piñowicz M, Wróblewska ź, Stêpieñ I, Zasada W, Boroñ A
Country Poland
University Jagiellonian University
Department Collegium Medicum

**Background:** Until 2005 one high volume percutaneous coronary intervention (PCI) centre provided 24/7 duty for the population of 3.2 million inhabitants in the Krakow Hospital Network Region in Poland. In August and November 2005 two additional round-the-clock duty PCI centres were launched in remote municipal hospitals (Local1 and Local2). The aim of the study was to assess the influence of new hos-
Hospital networks on treatment strategies of Acute Coronary Syndromes (ACS) in the Region.

Methods: 29 non-PCI centres participated in the Registry of Acute Coronary Syndromes in February-March 2005 (Registry 1) and in December 2005-January 2006 (Registry 2). While Registry 2 was conducted, three PCI centres provided round-the-clock PCI for the Krakow Region.

Results: The total of 1404 patients with ACS were enrolled, of which 695 in Registry 1 and 709 in Registry 2. In comparison to Registry 1, a non-significant trend towards more frequent mechanical reperfusion of ST-Elevation Myocardial Infarction (STEMI) patients with chest pain onset <12 hours was observed in Registry 2 (54% vs 60%; p = NS). A steep and significant rise was observed particularly among STEMI patients treated in non-PCI centres outside of the Krakow City (51% vs 78%; p = 0.001). In the newly established Local1 and Local2 PCI networks the reperfusion rates for STEMI patients with chest pain <12 hours were 78% and 88% respectively immediately after these 24/7 PCI centres were launched. The transfer rate for invasive treatment of Non ST-Segment Elevation Acute Coronary Syndromes (NSTE ACS) has increased from 13.8% in February-March 2005 to 19% in December-January 2005/2006 (p = 0.031). The in-hospital mortality for patients remaining for conservative treatment in community hospitals has decreased among NSTE ACS patients (6.3% vs 3.9%; p = 0.045) and remained unchanged in STEMI (21.3% vs 19%; p = NS). The fibrinolysis rate for STEMI <12 hours decreased from 15% to 7% (p = 0.15) in the entire Region.

Conclusions: Optimal PCI centre distribution, based on a population structure, improves local adherence to guideline-recommended invasive approach in high risk ACS patients. The Krakow Hospital Network Region model has proved that one high volume 24/7 PCI centre with a network of cooperating non-PCI centres for a population of 0.5 million is sufficient to provide invasive treatment according to ESC guidelines for eligible patients. The Local Networks achieved almost optimal recommended reperfusion rates. However, more educational effort needs to be done to increase percentage of patients with reperfusion therapy.

Relationship of generalized periodontitis and atherosclerosis in patients with concomitant ischemic illnesses

ESC-ID 463
Author Vivcharenko VI, Stasyuk NO, Neiko NV
Country Ukraine
University Ivano-Frankivsk State Medical University
Department Therapeutic Stomatology

Nowadays the generalized periodontitis is the most researchable disease in modern stomatology. Now one can name more than 300 types of microorganisms that can be found in a periodontal pocket. They cause a local inflammation and formation of bacterial plate and chronicisation of process during 5 - 20 days. Atherosclerosis is one of the most frequent reasons of the blood circulation system diseases, that shows up as a syndrome of ischemia of different organs and thromboses. Modern researches of molecular biology exposed the mechanisms of humoral and cellular reactions which form the inflammation stages, however the major factor initiating atherosclerosis, is still the article for discussion. Numerous researches that confirm or deny the fact of microorganisms influence on the processes of atherogenesis. During a few last years the amount of scientific researches grew in relation to the study of periodontitis and atherosclerosis connection. A mechanism which is the basis of this dependence is incomprehensible to the end, however local and system inflammation have a considerable role (the microorganisms of oral cavity is the start factor of inflammation). In an experiment on a biological model it was shown, that bacteriemia of the noted microorganism causes formation of atherosclerotic plates in an aorta and coronal vessels of guinea-pigs without changes of lipid exchange and increases the size of plates in case of hypercholesterolemia. Other mechanism of connection of periodontitis and atherosclerosis that deserves attention, is an individual genetically conditioned immune reply, which shows up as formation of special hyperinflammatory monocite phenotype (MO+) that produce many (up to ten) inflammatory factors (PGE2, IL-1, TNF and others) and play a leading role in formation of atheroma and bywall blood clots. So, the connection between periodontitis and atherosclerosis can depicted by the followings statements: 1. Periodontitis raises the risk of ischemic illness twice. 2. The microorganisms of oral cavity promote the formation of local and system inflammatory process which causes the formation of new and the increase of sizes of existing atherosclerotic plates.

Assessment of the Fe status range in the patients that angiography is indicated for them in Tehran Emam Khomeini Hospital in 2005-2006

ESC-ID 351
Author Parhizgar SE, Nematipour E, Nabati M, Hajalipour S, Nozari Y
Country Iran
University Tehran University of Medical Sciences
Department Cardiovascular Researches Center

Background: Free iron catalyzes the production of free radicals in myocardial ischemic injures. There is a correlation between dietary Iron intake and Increase of rate of myocardial infarction. In one study Say et al showed that increase in body Iron storage accompanied by severity of functional abnormality and coronary perfusion. Mais there is not correlation between extensions of CAD in hypercholesterolemic patient. objective: The studies showed that Ferritin is a strong factor in progression of carotid diseases. They found a relationship between blood donors and decrease in rate of myocardial infarction and cardiovascular disease. IHD is a common disease in Iran and causes large amounts of mortality and morbidity. There is no investigation about relationship between Fe status and coronary artery stenosis. Then we decided to assess the correlation between Fe status and coronary artery stenosis in a case-control study. method: In this study we enter 120 patients as cases and 120 patients as controls. The coronary artery angiography as has indicated for them. We collected the data (demographic and clinical with question sheet). 5 cc bloods are taken from patients for biochemical tests as FBS/TG/Cholesterol and Ferritin. Coronary Angiography was performed by specialist andgensini score that shows the severity and extension of coronary artery stenosis is determined.

Results: There is a correlation between serum Ferritin and severity of coronary artery stenosis in males in cases. (P: 0.002) Also there is correlation between gensini score and systolic blood pressure in males and females.(P: 0.042)
Percutaneous occlusion of atrial septal defects: Is a pediatric patient a bigger challenge?

**ESC-ID**: 793

**Author**: Taraszkiewicz M, Wozniak S, Meyer-Szary J, Kwiatkowski P, Sabiniwicz R

**Country**: Poland

**University**: Medical University of Gdańsk

**Department**: Pediatric Cardiology

**Background**: From congenital heart diseases atrial septal defects are among the most common. Patent foramen ovale (PFO) represents 17-38% of the total and ASD II 6-10%. ASD II is twice as prevalent among woman. Both defects can be treated with percutaneous occlusion of the defect. This study summarizes the safety, efficacy, and clinical application of this method in our department.

**Patients**: The total number of performed procedures of interatrial communications (ASD + PFO) occlusion in years 1998-2005 was 184, 92 children (below 18 yr.) and 92 adults. There was an increase in the number of procedures 1998-2001: 11, 2002: 10, 2003: 27, 2004: 37, 2005: 58. The age range of the patients was 4-76 years (M = 27,9 yr.). Man to woman ratio in the pediatric group was 1:1,5 and among the adults 1:2,5.

**Results**: Type of a defect is the most prominent difference among the groups. 86 children had ASD II, 3 multiple ASD II and 3 PFO while 68 adults had ASD II and 24 PFO. The size of ASD II in the children was 5,4-28mm (M = 13,42mm) and in the adults 6,5-30mm (M = 18,81). The radiation time was for children 2,2-34,6min (M = 8,27min) and for the adults 0,5-25,3min (M = 5,5min). The complications were less frequent in children (12% of children). Transient supraventricular tachycardia (SVT) occurred 2 times during the procedure. There was a need to remove the set and reimplant a new one in 9 children. Among the adults the complications were more varied (28% of adults): 1 left atrium perforation and a resulting heart tamponade, 2 transient SVT, 3 transient coronary ischemic incidents (air embolism). 4 times the set was reimplanted and one patient underwent a cardiectomy. Late complication occurring approximately 3 weeks after was 12 paroxysmal atrial fibrillation.

**Conclusions**: ASD and PFO are among the most commonly treated congenital heart defects. Percutaneous occlusion has a set place in the treatment of this conditions giving very good results with a low risk. Therefore there is a constant growth of the number of this procedures. Despite the shorter average procedure time and less complications, children are subjectively considered as more challenging patients.

To investigate the correlation between coronary atherosclerotic heart disease (CAD) and periodontal tissues and inflammatory markers

**ESC-ID**: 338

**Author**: Morteza RB

**Country**: Iran

**University**: Tabriz

**Department**: Dentistry

Forty-five patients with CAD (CAD group) and 70 patients without CAD (control group) were compared with their pathological changes of periodontal tissues and inflammatory
markers [high sensitive C reactive protein (hsCRP), interleukin-1beta (IL-1beta), and tumor necrosis factor-alpha (TNF-alpha)].

**Results:** Univariate analysis showed that the prevalence of PD was 74.88% in CAD group and 25.12% in control group ($P < 0.01$). Gingival index and plaque index were statistically different between two both groups ($P < 0.01$). Logistic regression analysis showed that in addition to pulse pressure and low density lipoprotein cholesterol, periodontal disease index was a higher risk factor of CAD.

**Conclusion:** PD can cause CAD. The improvement of public oral health plays an important role in the prevention and treatment of CAD.

### Photothermal nanodestruction of atherosclerotic plaque with using of adult stem cells

**ESC-ID**

44

**Author**

Kharlamov AN, Gabiniskiy YL, Smolenskaya OG, Novoselova OS

**Country**

Russia

**University**

Ural State Medical Academy

**Department**

Internal Medicine

**Introduction:** Metal nanoshells are a novel type of composite spherical nanoparticle (NP) consisting of a dielectric core covered by a thin metallic shell which is typically gold. When nanoparticles are irradiated, they absorb energy, which is quickly transferred through nonradiative relaxation into heat and accompanied effects, and eventually leads to irreparable damage. We describe a new method for management of coronary artery (CA) disease and destruction of atherosclerotic plaque (AP).

**Material and Methods:** Cores of silica NPs were fabricated as described by Stober et al. (1968). Gold shells were grown using the method of Duff et al. (1993). We used the nanoshells with a 60 nm core radius and a 10 nm thick shell which absorb light with an absorption peak at ~815 nm. Adult stem cells (ASCs) from adipose (fat) tissue were used for delivery of NPs to AP. ASCs received at lipospiration were cultured in the medium with NPs (2 x 109/mL). Our 17 patients with stable angina were divided into two treatment groups: nanoshells + near-infrared (NIR) laser (8) and intracellular (ASCs) nanoshells + NIR laser (9). Firstly, CA were investigated with using of intravascular ultrasound (IVUS) mapping. Secondy, NPs (109/mL) and ASCs were injected to the most favourable place of plaque’s core above media with using of NOGA (Biosense Webster) and modified AXIOM Artis dFC/dBC Magnetic Navigation (Siemens). Thirdly, in 3-5 days AP were irradiated under a laser emitting light (820 nm, 5 W/cm$^2$) for 6-7 minutes with using of catheter and FilterWire EX Embolic Protection System (Boston Scientific) with the consequent IVUS-control. Clopidogrel, bivalirudin and epifibatide were used for antithrombotic therapy (just 1 month). Patients had taken the medicines for blood pressure and cholesterol level control.

**Results:** Magnetic resonance temperature imaging demonstrated that plaques reached temperatures 38.1 ± 6.5 C within 4-6 minutes. 97.4%/ 64.1% (p<0.001) of plaque’s value were demolished in ASCs group and second group accordingly with restoration of vessel blood flow/ local hemodynamics/ artery lumen in 100% of cases. IVUS had showed total degradation and loss of plaque’s typical structure in ASCs group. Destruction of plaque’s fibrous cap with outlet of atherothrombotic mass was observed in 100% of patients.

Thrombosis and thromboembolism were prevented with special therapy (see above). We had no complications such as a dissection, a perforation, an acute thrombosis, a myocardial infarction, a hemodynamic shock or a death. In 1 and 12 months after procedure IVUS had showed normal reendothelialization and restoration of vessel structure. We had no long-term cases of restenosis, thrombosis, progression of atherosclerosis or toxic phenomena.

**Conclusion:** Combining advances in biophotonics and nanotechnology offers the opportunity to significantly impact future strategies towards the therapy of CA disease. ASCs are perspective deliverer of NPs to APs.

### Internal Medicine

**Endocrinology II**

**Positive effects of nicotine in diabetic angiopathies**

**ESC-ID**

48

**Author**

Padia D

**Country**

Ukraine

**University**

Crimea State Medical University

**Department**

Department of Internal Medicine

**Aim:** To demonstrate the decrease of Neuropathies, Retinopathies and Vasculopathies in Diabetic patients, through the neo-vascularization effect of Nicotine (Nicotine patches)

**Material and Methods:** 30 patients (males) have been included in research with Diabetes Mellitus (Type I and Type II) hospitalized in the 6th City hospital of Simferopol from the year 2003 to 2006. In 30 patients we hypothesized that activation of nAChRs with nicotine would accelerate wound healing in diabetic foot wound full-thickness (0.8 cm) dorsum part of the foot and that is why we stick nicotine patches in the close proximity of the wound. At the same time In diabetic animals an nicotine agonist (epibatidine, 10-10 mol/L) or antagonist (hexamethonium, 10-4 mol/L) of nAChRs as well as the positive control basic fibroblast growth factor (bFGF, 25 µg/kg) were also tested.

**Results:** Nicotine significantly accelerated wound healing as assessed by closure rate and histological score. The effects of nicotine were equal to bFGF and were mimicked by epibatidine and blocked by hexamethonium. Histomorphometry revealed increased neovascularization in patients treated with nicotine. Furthermore, capillary-like sprouting from vascular explants was significantly enhanced by nicotine.

**Conclusion:** Nicotine agonist-induced stimulation of nAChRs decreases retinopathies, neuropathies and other micro-vasculopathies in diabetic patients by promoting angiogenesis.
Glucose variability is correlated with glucose status in a group of patients with type 2 diabetes evolution

| ESC-ID | 374 |
| Author | Morosanu A, Roman G, Hancu N, Gribovschi M, Nita C |
| Country | Romania |
| University | University of Medicine and Pharmacy |
| Department | Diabetes, Nutrition and Metabolic Diseases |

**Introduction:** Actual and long-term evolution of blood glucose levels remains the most important determinant of diabetes and diabetes chronic complications. Glucose fluctuations, generally or postprandially, are related to oxidative stress in a higher extent than sustained hyperglycemia. Glucose excursions can be expressed by glucose variability and by mean amplitude of glucose excursions (MAGE), parameters that are determined by continuous glucose monitoring. MAGE is known to be related to urinary 8-iso PGF2α & 8&9HETE; excretion in persons with type 2 diabetes. Aim To evaluate the relation between glucose variability, MAGE and glycated haemoglobin (A1c), parameters of glucose distribution on glycemic domains, anthropometric parameters, sex, diabetes treatment, currently and prospectively in persons with type 2 diabetes.

**Method and study group:** 30 persons with type 2 diabetes, 16 women, 22 men, median age 64 (39-69) years, mean diabetes duration 14 (0-17) years, 14 persons insulin-treated and 16 subjects on oral therapy performed continuous glucose monitoring for 3 days. 10 of the insulin treated subjects performed a second continuous monitoring after 3 months. The subjects completed a diary during continuous monitoring which included capillary glycemia, treatment doses, meal hours, carbohydrates intake/every meal, physical exercise, stress, emotions. We determined interstitial glucose levels in the abdominal subcutaneous tissue by continuous glucose monitoring system – CGMS standard (Medtronic MiniMed) - 17 persons and by Guardian RT (Medtronic MiniMed) - 13 persons. We assessed glycated haemoglobin A1c, anthropometric parameters (weight, body mass index – BMI, waist circumference). Based on continuous monitoring data we calculated glucose distribution on glycemic domains (number, mean, area under the curve – AUC for further ranges; < 70 mg/dl - hypoglycemic, > 180 mg/dl - hyperglycemic, 70-180 mg/dl – intermediate, 90-130 mg/dl – optimal), glucose variability (standard deviation) and MAGE. For the second monitoring we evaluated the same parameters.

**Results:** Weight (p<0.05). BMI (p<0.05) and waist circumference (p<0.05) increased at second visit. A1c, glucose variability and MAGE decreased after 3 months (p<0.05). 2. Glucose variability and MAGE were directly correlated with A1c initially and after 3 months (p<0.05). 3. Glucose variability and MAGE were inversely correlated with weight initially and after 3 months (p<0.05). 4. Glucose parameters for glucose > 180 mg/dl were directly correlated with A1c, while glucose parameters for glucose between 70-180 mg/dl and 90-130 mg/dl were inversely correlated with A1c, initially and at 3 months (p<0.05). 5. Glucose variability and MAGE were directly correlated with glucose parameters for glucose > 180 mg/dl, and inversely correlated glucose parameters for glucose between 70-180 mg/dl and 90-130 mg/dl, both initially and at 3 months (p<0.05). 6. Glucose variability and MAGE were significantly higher in insulin treated persons and women at the first visit. A1c was higher in women (p<0.05) and in insulin treated persons (p>0.05).

**Conclusion:** Glucose fluctuations are directly related to long-term glucose status (A1c), hyperglycemic domain and inversely related to parameters of normoglycemia. A1c, glucose variability and MAGE decreased after three months, due to specific treatment adjustments based on continuous glucose monitoring.

**Obesity, quantity and risky distribution of body fat among population of Novi Sad**

| ESC-ID | 344 |
| Author | Bojana A, Crnobrnja V, Bibic Z, Stokie E |
| Country | Serbia |
| University | University of Novi Sad, Medical Faculty |
| Department | Endocrinology, Diabetes and Metabolic Disease |

**Introduction:** Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Obesity has been shown to predispose to various diseases, particularly cardiovascular diseases, diabetes mellitus type 2, dyslipidemias and osteoarthritis. That is the reason why is so necessarily to detect precisely obesity, quantity and body fat distribution.

**Aim:** The aim of this research was ratifying weight status (overweight and obesity), quantity of body fat and its distribution, with an emphasis on pathological values, related to risk of developing complications.

**Materials and Methods:** We measured body height, weight and based on those parameters calculated body mass index – BMI. Waist circumference was determined at the same time and quantity of body fat mass -FAT% (using bioelectrical impedance method). Group of 387 persons was examined (of both gender), age 18 to 86 years. Obesity was defined by BMI and FAT%, and central obesity by waist circumference.

**Results:** Obesity was registered in 19.20% persons, overweight in 58.65%. Overweight was more frequent among men, and obesity among women. The highest percentage of obese persons, both sexes, was verified among elder than 60 years. Excessive body fat was established in 60,98% persons (55.76% male, 64.86% female). Increased visceral fat, presented with waist circumference, was detected in 53,49% persons (57,66% women, 47,88% men). It is important to emphasize the results in the group with normal BMI- even 29,22% of them had excessive body fat (31,68% female, 24,50% male). Increased intraabdominal fat mass was registered in 31,13% of persons with normal BMI.

**Conclusion:** More than half of examined population was overweight or obese! That shows how important is complete and precise diagnostic of overweight and obesity. BMI alone revealed high presence of overweight and obesity. Establishing quantity of body fat, and its distribution in the body (visceral, androgenic obesity) is necessary because two thirds of persons have excessive body fat, and half have increased content of intraabdominal fat mass, which has a much stronger correlation, particularly with cardiovascular disease, than BMI alone. Also, analyzing BMI alone, could hide very serious health problem of obesity among people with normal BMI values. It should be pointed out that even one third of them had excessive body fat and central obesity. Key words: obesity, body fat, waist circumference.
Evaluation of BDCA-1+, BDCA-2+ dendritic cells and lymphocytes’ subpopulations in patients with polyglandular autoimmune syndromes

ESC-ID 255
Author Przybylski P, Glazer M, Plew D, Radej S, Wiktor K
Country Poland
University Medical University of Lublin
Department Department of Clinical Immunology

Introduction: Polyglandular autoimmune syndromes (PAS) are rare immune endocrinopathies characterized by the coexistence of at least two endocrine glands failure, based on immune-mediated destruction of endocrine tissues. Pathogenesis of autoaggression in endocrine autoimmunity is currently unrecognized. The aim of study: Examination of BDCA-1+, BDCA-2+ dendritic cells and lymphocytes’ subpopulations in patients with PAS.

Material and methods: Peripheral blood mononuclear cells (PMBCs) were isolated from heparinized blood from 10 patients with PAS (2-type 2, 2-type 1, 6-type 3), 5 patients with isolated Addison Disease, 5 patients with isolated Graves Disease and 15 healthy volunteers by density gradient centrifugation. Isolated PMBCs after rinsing were incubated with following antibodies BDCA-1, BDCA-2, CD19, CD123, CD4, CD62L, CD25, CD8 and CD28. After incubation expression of antigens were analyzed using an flow cytomtery. Statistical analysis was also performed.

Results: In all cases with PAS total number of lymphocytes were appreciated. In comparison to control group significant abnormalities in level of CD19 positive, CD4 positive and CD8/CD28 among CD8 positive subpopulations were obtained. Among the others examined groups other results were not statistically important.

Discussion and Conclusions: Although mechanisms of autoimmunity in PAS are not clearly defined, lymphocytes seem to play crucial role in this pathology. Results of our study indicate abnormal levels of T and B lymphocytes in examined patients. Subpopulations responsible for regulation of immune response and effector cells tend to be disturbed. Understanding of pathological immune response in PAS require future investigations and more patients inclusion.

Depression associated with atenolol intake

ESC-ID 87
Author Kharazmi K, Rahimi B, Rikhtegar R, Maleki A
Country Iran
University Tabriz University of Medical Sciences
Department Students Research Department

Object and background: Antihypertensive drugs are one of the most common used medications in elderly patients. Amongst these drug beta-blocker are the most prescribed group. In the other hand depression one of the important and common psychiatric disorder in more prevalent in highly aged groups because of special social condition and physical limitations. As beta blocker drugs are able to partially diffuse to brain tissue and according to their ability to change adrenergic system activity and also relation between hemodynamic of brain and depression it is possible that these drugs could provoke depressive symptom. Our aim was to investigate any association between depression and atenolol tablet intake.

Method and materials: the study was done as a historical cohort. Exposure group consisted of 173 men aged between 50 to 65 years referred to hospitals pharmacy who have been taking atenolol 50mg per day for 6 to 8 months. Patients who have taken other hypertensive drugs before excluded. Control group was selected in the same size of exposure group and consisted of men who referred to pharmacy for purchasing any non-medical material. Selection of control group was adjusted with first group based on age and education level. Both groups were asked to fill Beck depression inventory (BDI). The cut off point of our study was 12 based on the finding of Frause-Smith study.

Results: the estimated risk ration for depression in exposure group compared to control group was 1.21 but this difference was not significant. However comparison of BDI score in two group by students t-Test revealed that those who have taken atenolol significantly had higher score (p = 0.04)

Conclusion: our data show that atenolol intake in mentioned dosage could not be assumed as a risk factor of depression. However regarding to higher score in atenolol taking patients it can be concluded that perhaps atenolol can cause some depressive symptom but not lead to depression. Maybe more studied would be necessary to elucidate this medications effect in higher doses and in higher-risk patient for depression.

L-Thyroxine suppressive therapy impact on autonomic nervous system and heart rhythm in middle-age patients

ESC-ID 273
Author Kovalchuk V
Country Ukraine
University O. O. Bogomolets National Medical University
Department Endocrinology

Cardiac failure and/or atrial fibrillation are well-know causes of increased cardiovascular mortality in patients of elder age with subclinical hypothyroidism. Nevertheless the mechanism of exogenous thyrotoxicosis impact on heart is not clear in patients of middle age, who take suppressive therapy with L-thyroxine over a long period of time. The aim was to study the impact of exogenous hyperthyroidism on autonomic nervous system and heart rhythm in middle-age patients. Assessment of heart rate variability (HRV) and heart rhythm disturbances was made by ECG Holter monitoring in 24 patients aged 40-59 years. All patients after thyroidectomy received a suppressive therapy with L-thyroxine to achieve a level of serum TSH <0,1mU/L. Healthy persons (n = 15) of the same age were taken as a control. Patients were divided into 2 groups. 1st group (n = 8) characterized with significant increase of time and frequency domain measurements comparing with patients of the 2nd group and close to healthy of the same age. On the contrary in patients of 2nd group (n = 16) significant lowering of time and frequency domain measurements was observed comparing with healthy and 1st group. Accordingly, the analysis of ECG Holter monitoring showed that in 2nd group next to lowered values of HRV, a significant increase of average heart rate per day, minimal and maximal heart rate and average heart rate at night was found. Also in 2nd group episodes of sinus tachycardia parox-
ysms were established two time more often and its duration was significantly increased comparing with patients of the 1st group. Also, a tendency for disparity in duration of suppressive therapy in 1st and 2nd groups was established. The exogenous subclinical hyperthyroidism which lasts more than 1 year due to L-thyroxine suppressive therapy intake, causes autonomic nervous system disturbances that appear in lowering of HRV measurements in middle-age. This intensifies myocardial irritability and increases the risk of fatal arrhythmic complications.

Factors associated with occurrence of partial remission in patients with type 1 diabetes mellitus

ESC-ID 183
Author Uruski P, Uruska A, Niedzwiecki P, Kasprzak M, Spornia A
Country Poland
University Poznan University of Medical Sciences
Department Department of Internal Medicine and Diabetology

Introduction: Clinical partial remission of type 1 diabetes, which can occur soon after introducing insulin therapy, plays important role in further clinical course of the disease and development of its chronic complications.

Aim: Aim of the study was to compare lifestyle and metabolic control parameters in patients with clinical remission with non-remitters.

Material and Methods: 136 patients (59 females, 77 males, age 24 ± 5.1 years) with newly diagnosed type 1 diabetes admitted to the Department of Internal Medicine and Diabetology in years 1995-2006 were qualified to the study. Partial remission was defined as HbA1c <7% and total exogenous daily insulin requirement <0.5 U/kg. The occurrence of remission was assessed during regular visits at out-patient clinic. Style of life was assessed on the basis of questionnaires. Mann-Whitney test, chi2 test and logistic regression were used in statistical analysis.

Results: 98 subjects achieved partial remission. When compared with patients who didn’t experience remission remitters showed lower: total daily exogenous insulin dose, glycaemia, serum triglyceride levels (TAG) and body weight loss before diagnosis of diabetes. Significantly higher HDL-cholesterol levels were observed in partial remission group. Using logistic regression significant association between insulin requirement (OR = 33.56, 95%CI: 1.27-886.85, p <0.03), TAG (OR = 1.005, 95% CI: 1.0-1.01, p = 0.045) and occurrence of remission was found. According to the analysis of questionnaires group with remission characterized better education, less stressful live, more seldom drinking coffee and smoking, more money to treatment and negative family history.

Conclusions: Metabolic disturbances prior to diagnosis, as well as lifestyle determine occurrence of remission phase in patients with type 1 diabetes.

Internal Medicine
Gastroenterology

The flavonoid quercetin seals the epithelial barrier in colonic caco-2 cells

ESC-ID 533
Author Schlichter S, Amasheh MM, Tavalali S, Fromm M, Schulzke JD
Country Germany
University Potsdam
Department Gastroenterology, Infectiology and Rheumatic

High dietary intake of fruits and vegetables is associated with a reduced disease risk. A group of secondary plant compounds, the flavonoids, are supposed to be important in this respect (Heyek et al., 1997; Lamson et al., 2000). Although there are numerous data suggesting that flavonoids influence molecular cell structures there is only limited information about their effects on intestinal barrier function. Paracellular properties of epithelial cells are defined for the most part by the tight junctional complex with the corresponding tight junction (TJ) proteins occludin and the claudin gene family (Anderson 2001; Tsukita et al., 2001). Therefore, the aim of our study was to elucidate the effects of quercetin, a common flavonoid, on the barrier function of the colonic epithelial cell line Caco-2. Caco-2 cells grown on permeable supports formed a differentiated, polarized monolayer. Quercetin was applied in concentrations of 50 to 200 µM to the mucosal and serosal culture medium. Barrier function was measured as transepithelial resistance (Rt). Expression and distribution of TJ proteins were performed by Western blotting, Taqman RT-PCR and immunofluorescence staining analyzed with confocal laser scanning microscopy. Furthermore, promoter activity of a TJ protein was determined by reporter gene assays. Paracellular transport was characterized by mucosal to serosal 3H-mannitol tracer fluxes and by 22Na and 36Cl tracer flux analyses in both transepithelial directions. Addition of quercetin to the Caco-2 monolayer increased Rt in a concentration-dependent manner. The action of 200 µM quercetin was already observable after 4 hours and reached maximal levels after 48 hours (163 ± 9 % vs. untreated cells 104 ± 4 % of initial resistance). The Rt rise was associated with an elevation of the claudin-4 expression, a barrier-sealing TJ protein, whereas TJ proteins occludin, claudin-1, claudin-3, and claudin-7 remained unchanged. The mRNA expression and the promoter activity of claudin-4 were also increased by the flavonoid. Immunofluorescent staining primarily revealed a strong increase of claudin-4 localized within the TJ as well as in subjunctional regions. Functional analyses revealed a reduced paracellular permeability of Na+ and Cl- by quercetin whereas the flux of the paracellular marker 3H-mannitol was not altered. We demonstrate that the flavonoid quercetin increases the expression of claudin-4 within the tight junction which caused a decrease of the paracellular permeability for Na+ and Cl-. This leads to a sealing of the intestinal barrier. Thus, this novel direct effect of quercetin may be utilized for the treatment or prevention of diarrhea-causing intestinal barrier defects in inflammatory bowel diseases.
The clinical and biological particularities of patients with chronic hepatitis C and associated extrahepatic manifestations

ESC-ID: 912
Author: Lilea GC, Popescu A, Ciocalteu AM, Bordu SI, Bicicusa V, Petrescu F
Country: Romania
University: Universitatea de Medicina si Farmacie Craiova
Department: Semiology

**Aim:** We wanted to assess the incidence of extrahepatic manifestations in patients with chronic hepatitis C, and also the clinical and biological particularities of these patients.

**Materials and methods:** We have used a group of 338 patients diagnosed with chronic hepatitis C, all admitted and treated in the Clinica Medica II of the Spitalul Judetean de Urgenta Craiova. We have assessed the extrahepatic manifestations through anamnesis, general examination, dermatological examination, chest X-ray, functional ventilation tests, EKG, abdominal ultrasonography, neurological examination, ophthalmological examination, blood tests, biochemical tests and immunological tests.

**Results:** Of the 338 patients, only 90 (26.6%) have shown extrahepatic manifestations. Of the 90 patients, 53 were women (66.25%) and 27 were men (33.75%), of a mean age of 46.24 years and a mean time since infection 22.4 years. The incidence of these manifestations in the entire group was: 14.7% (50 patients) with cutaneous manifestations, 20.4% (69p) with articular manifestations, 3.5% (12p) with respiratory manifestations, 4.43% (15p) with cardiovascular manifestations, 7.9% (27p) with xerostomia, 10.65% (36p) with renal manifestations, 15.9% (54p) with asthenia, 1.7% (6p) with tyroid manifestations, 0.8% (3p) with ocular manifestations, 16.5% (56p) with diabetes mellitus, 8.8% (30p) with hematologic manifestations and 25% (85p) with immunological modifications. From the biochemical changes, the only correlations of a high risk of extrahepatic manifestations were with elevated blood levels of total bilirubin (>1.4mg/100ml), alkaline phosphatase (>245ui/l) and a-globulines (>2.4g/100ml).

**Conclusions:** The patients with chronic hepatitis C tend to develop a series of extrahepatic manifestations, of which the most common are the articular, neurological and metabolic ones. The onset of these manifestations was correlated with: feminine gender, old age, long time since infection, and elevated blood levels of total bilirubin, alkaline phosphatase and a-globulines. These patients have also shown immunological modifications, the most frequent being: cryoglobulinemia, the presence of rheumatoid factors, antinuclear antibodies and elevated levels of IgG.

The clinical and biological characteristics of chronic C hepatitis with vasculitis syndromes

ESC-ID: 874
Author: Bordu SI, Popescu A, Ciocalteu AM, Lilea GC, Bicicusa V, Petrescu F
Country: Romania
University: Universitatea de Medicina si Farmacie Craiova
Department: Semiology

**Aim:** We have described the clinical, biological and immunological features of patients with chronic C hepatitis and vasculitis syndromes.

**Materials and methods:** We have conducted a retrospective study on 83 patients with chronic C hepatitis.

**Results:** Of the 83 chronic C hepatitis patients, only 20 (14 F, 6 M, mean age 43 ± 9 years) had clinical manifestations of cryoglobulinemic vasculitis: purpura 80%, urticaria 5%, livedo reticularis 5%, cutaneous ulcers 40%, artralgias 50%, myalgias 20%, symmetrical distal sensory polyneuropathies 50%, proteinuria 25%, renal insufficiency 20%, diffuse abdominal pain 25%, elevated ALAT 55%, rheumatoid factor 75%, low C3 90%, cryoglobulinemia 100% and hyperglobulinenia 20%. 2 patients (1M, 2F, mean age 52 ± 12 years) had clinical manifestations of polyarthritis nodosa: fever 100%, weight loss 100%, arthritis 66%, multifocal sensitivomotor motoneuropathies 66%, malignant hypertehsion 66%, abdominal pain 66%, elevated CRP 100%, and increased ESR 100%.

**Conclusions:** HCV infection may be associated with cryoglobulinemia vasculitis and polyarthritis nodosa. Because of the different therapeutic strategies, cryoglobulinenia vasculitis should be distinguished from polyarthritis nodosa in HCV-infected patients.

Salmonella mediated antioxidative and anti-inflammatory gene therapy in dextran sodium sulfate treated female Wistar rats

ESC-ID: 834
Author: Pălfy R, Behuliak M, Celec P
Country: Slovakia
University: Comenius University, Faculty of Medicine
Department: Institute of Patophysiology

**Aim:** To analyze the effects of Salmonella-mediated gene therapy using Cu-Zn superoxid dismutase (SOD1) and a mutant of monocyte chemoattractant protein-1 (7ND-MCP1) on chemically induced colitis.

**Methods:** Dextran sodium sulfate (DSS)-induced colitis in female Wistar rats was treated with SOD1 and 7ND-MCP1 encoded by plasmids carried by Salmonella typhimurium SL7207 applied daily during one week. Fecal consistency, clinical status and body weight were monitored during the whole experiment. Malondialdehyde (MDA) and advanced oxidative stress in plasma and even in inflammatory markers in colon were measured as markers of oxidative stress. Interleukin 1, interleukin 6 and tumor necrosis factor alpha were quantified in colon and plasma samples using ELISA.

**Results:** Fecal consistency and MDA showed a slight improvement in SOD1 and 7ND-MCP1 + SOD1 groups, but not in the 7ND-MCP1 group. Surprisingly, no differences were found in body weight increase, markers of oxidative stress in plasma and even in inflammatory markers in colon homogenates. Plasma concentrations of IL1, IL6 and TNFalpha were lower in the treatment groups than in the DSS group. However, DSS induced inflammation could only be confirmed by plasma IL1 and TNFalpha but not IL6.

**Discussion:** Although some parameters showed a tendency to be improved by the bacteria-mediated antioxidative and anti-inflammatory gene therapy, the inflammation in the experimental DSS group was rather minor in comparison to other studies. Whether this is caused by the choice of gender and strain of the experimental animals will be studied.
Different patterns of arginine level and arginase activity in blood serum of patients with acute pancreatitis and pancreatic cancer.

ESC-ID 701
Author Kraj L, Kerdie M, Kules J, Grabon W, Baranczyk-Kuzma A
Country Poland
University Medical University of Warsaw
Department Chair and Department of Biochemistry

Introduction: Arginase (EC 3.5.3.1.) is an enzyme that is found mainly in the liver, where it catalyzes the final reaction in the synthesis of urea. The enzyme is also present in other human tissues and in blood serum. Its substrate, arginine, is involved in a variety of physiological and pathological processes, including biosynthesis of nitric oxide and polyamines - compounds that play an important role in cell proliferation and differentiation. Cancer and inflammatory states are the most prevailing pancreatic illnesses. Earlier studies from our laboratory have shown that serum arginase activity is increased in blood serum of patients with acute pancreatitis. However, there are no available studies comparing arginine level and arginase activity in blood serum of patients with acute pancreatitis and pancreatic cancer.

Aim: The aim of this study was comparison of arginine level and arginase activity in blood serum of patients with acute pancreatitis and pancreatic cancer.

Materials and methods: Arginine activity and arginine level were studied in blood serum of patients with acute pancreatitis (n = 30) before and after treatment and those with pancreatic cancer before surgery (n = 15). The control group included healthy blood donors. Arginase activity was determined according to Chinard (J Biol Chem, 1952, 199: 91-95). Arginine level was assayed from the amount of ornithine formed by standardized arginase preparation (Clin. Chim. Acta 1980, 106: 333-337).

Results: The increase in arginase activity was observed in both studied groups of patients, but it was more pronounced in pancreatic cancer compared to those with acute pancreatitis. The enzyme activity was lower after an effective treatment of pancreatic inflammation. Arginine level was decreased in patients with acute pancreatitis but increased in those with pancreatic cancer.

Conclusions: In patients with acute pancreatitis an increased serum arginase activity coincided with lowered arginine concentration. Depletion of serum arginine may reflect both an impaired systemic synthesis of this amino acid and enhanced degradation due to increased arginase activity in the blood. Determination of arginase activity and arginine level in blood serum may be used as an adjunct test for evaluation of therapeutic outcomes in patients with acute pancreatitis. Conversely, in patients with pancreatic cancer an increased serum arginase activity was associated with increased arginine concentration. It suggests there is a mechanism by which tumor cells affect arginine metabolism to maintain adequate systemic pool of this amino acid in spite of increased arginase activity. Further studies are needed to evaluate the mechanism of tumor effect on systemic arginine metabolism.

Use of alternative therapies in irritable bowel syndrome

ESC-ID 603
Author Neda CI, Moraru IG
Country Romania
University "Iuliu Hatieganu" Cluj Napoca
Department General Medicine

Background and aim: Irritable bowel syndrome (IBS) is an invalidating condition without a perfect therapy. Therefore many patients use also alternative therapies. There are no data on the use of alternative therapies in IBS. We looked for this until now hidden aspect of IBS management

Material and methods: 162 consecutive patients with IBS referred to a tertiary gastroenterological center were questioned about the use of alternative therapy using a specially developed questionnaire. All of them accepted to answer to the questionnaire after careful instruction.

Results: 99 out of 162 (61%) patients admitted to have used one or more alternative therapies, usually together with the observance of medical dietary or pharmacological prescriptions. These were: homeopathy 12%, herbal therapy 76%, acupuncture 3%, bioenergetical resonance 11%, others 14%. Users were mainly females of lower educational levels, unlike in USA where alternative therapies users usually belong to higher educated categories. Some patients avoided answering and needed supplementary information about the purpose of the questionnaire.

Conclusion: These data show the importance and the magnitude of the use of alternative therapies by patients with IBS.

Buffer properties of unstimulated saliva as noninvasive method of differential diagnostics of gastroesophageal reflux disease

ESC-ID 496
Author Afanas'ev MV, Khomutova EV
Country Ukraine
University M.Gorky Donetsk State Medical University and L. M. Litvinenko Institute of Physical-Organic and Coal Chemistry
Department Therapy 2

Gastroesophageal reflux disease (GERD) is the most prevalent acid dependent diseases at world. Frequent recurrences of GERD require the second invasive cost expensive analysis, e.g. upper endoscopy, and ambulatory 24-h esophageal pH monitoring. The objective of this research is working up of novel cheap method of GERD diagnostics with and without reflux oesophagitis.

Materials and methods: The studied population consisted of 45 patients with and without reflux oesophagitis (aged 18–55 years). Diagnosis of GERD was verified of upper endoscopy, ambulatory 24-h esophageal pH monitoring. Investigations of buffer properties of unstimulated saliva carry out with using to acid base titration of patient's unstimulated saliva with and without reflux. Ratio between integral buffer capacity at interval _Í 7- 9 and 7 - 4: BC7- 10 / BC7-4 was used as diagnostic variable.

Results: Among examined patients with GERD 21 ones were without reflux oesophagitis and 24 patients with reflux oesophagitis. Males dominate among patients with GERD combined with reflux oesophagitis whereas the amounts of
males and females were equal inside the sample of patients with GERD without reflux esophagitis. At patient with and without reflux esophagitis reliable differences of ratios BC7-10 / BC7-4 of unstimulated saliva were obtained (\( \pm \) 0.001). Median, second, and third quartiles for the both groups were equal 1.00 (0.92 – 1.26) and 1.44 (1.35 – 1.65), respectively.

**Conclusion:** It was determined that saliva of the patients with much severe GERD has increased buffer value under pH much 7. This fact suggests that saliva of such patients has excessive amount of alkaline products. Hence there is a good reason to believe that increasing of buffer capacity of saliva is rather the disturbing factor than protective one. It was shown that ratios BC7-10 / BC7-4 of unstimulated saliva can be uses as cheap, noninvasive method of differential diagnostics of GERD with and without reflux esophagitis.

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**A case-control study on risk factors of osteoporosis in patients with Crohn's Disease**

**ESC-ID:** 689  
**Author:** Vahedi H, Montahen S, Kazzazi AS, Hosseini S, Abtahi A, Malekzadeh R  
**Country:** Iran  
**University:** Tehran University of Medical Sciences  
**Department:** Digestive Disease Research Center

**Back ground and aim:** Osteoporosis and osteopenia have been reported frequently in patients with inflammatory bowel disease, especially Crohn's Disease (CD) [1]. It has been suggested that the pathogenesis of osteoporosis in patients with CD is multifactorial [2]. Corticosteroid use, disease duration, low body weight or body mass index, calcium and vitamin D deficiency, small bowel resection, smoking, previous fracture, gender and increasing age have been implicated [3,4].

**Aim:** To determine the prevalence of osteoporosis in patients with CD and possible risk factors.

**Methods:** In this case-control study, one hundred fifteen patients with CD (30 cases and 85 controls) from 20 to 80, recorded at Gastroenterology Clinics of Shariati Hospital, Tehran, were studied. Data collection was done through convenience sampling method. Diagnosis was based on Lennard Jones criteria[5]. Hormone replacement therapy (HRT) and steroid consumption for other reasons were the exclusion criteria. Dual-energy x-ray absorptiometry (DXA) measurements of bone mineral density (BMD) were obtained. A Tscore < -2.5 was chosen as denoting osteoporosis. cases (T Score < -2.5) and controls (T Score > -2.5) were asked about age, sex, body mass index (BMI), corticosteroid cumulative dose (CCD), previous fracture, history of bowel resection, site and duration of disease, smoking and consumption of Ca-vitamin D as osteoporosis risk factors, through a face-to-face interview [6]. SPSS 13, independent sample T-test, chi-square, Logistic regression model and multivariable modeling technique were used for analysis.

**Results:** Mean age (SD) at onset of disease was 31.9(14.9) and 29.7(10.8) in cases and controls, respectively. Male / Female ratio was 1/1.3 in cases and 1/1 in controls. Osteopenia (-2.5\( \leq \) T-Score;\( \leq -1\)) and normal BMD (T-Score > -1) were found in 49% and 51% of controls, respectively. BMI less than 25 was found in 86.7% of cases and 70.6% of controls. Lower BMI associated with higher osteoporosis rate (p<0.05). Multivariable modeling technique revealed lower osteoporosis risk in (<10 gram) and (10-35 gram) of CCD rather than (>35 gram). (p = 0.04). CCD of 10-35 gram, provided the lowest osteoporosis risk in its J-shaped curve (p<0.05). Previous fracture was reported in 16.7% of cases and 5.9% of controls (p<0.05). Duration of disease was more than five years in 63.3% of cases and 42.7% of the controls (p<0.05). Age, sex, bowel resection, site of disease, smoking and consumption of calcium-Vit D did not show any relationship with osteoporosis (P>0.05).

**Conclusion:** Among several evaluated osteoporosis risk factors, CCD, duration of disease and BMI had considerable role in occurrence of osteoporosis in CD patients. It is suggested that, the lowest osteoporosis risk in 10-35 gr CCD, may be due to its effects on reducing the inflammation of CD.

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**Comparison of the effect of water and antisecretory drugs upon gastric pH**

**ESC-ID:** 689  
**Author:** Giannoulis E, Yiasemidou M, Karamanolis G, Triantafyllou K, Ladas SD  
**Country:** Greece  
**University:** University of Athens  
**Department:** School of Medicine

**Heading:** Heartburn, the cardinal symptom of gastroesophageal reflux disease (GERD), is a very common problem. Treatment to relieve heartburn is based on acid-suppression drugs (antacids, H2-antagonists, PPIs). Onset of action of anti-secretory agents is of pivotal importance in GERD, particularly in approaches with increasing trend, such as “ondemand” therapy (patients control their own drug utilization intermenttently, as dictated by their symptoms).

**Aim:** To compare the onset and duration of gastric acid suppression by some of acid-inhibiting drugs and of water administration.

**Method:** A randomized, cross-over study was performed in 10 fasting, H.pylori (-) healthy subjects (4 men; mean age: 26 yrs). A single oral dose of each of the following drugs was received with a wash-out period of 72h between each study: water (200 ml), aluminium-magnesium hydroxide (400 mg), ranitidine (150 mg), omeprazole (20 mg), esomeprazole (40 mg) and rabeprazole (20 mg). Gastric pH was recorded using an antimony catheter for 6h after drug intake. Results are showed as median, range.

**Results:** Water administration increased gastric pH (pH4>4) in 8/10 subjects after 1.1 (1,2) min. The time needed to pH4=4 was for antacid 1.8 (0.5) min, for ranitidine 45.3 (10,68) min, for omeprazole 187 (63,315) min, for esomeprazole 99 (30,167) min, and for rabeprazole 161 (55,230) min. Gastric pH remained >4 until the end of recording in 3/10 subjects with ranitidine, in 9/10 with rabeprazole, and in all subjects with omeprazole and esomeprazole administered. The time that pH remained >4 were for water 4 (2,11) min, for antacid 15 (1,42) min, and for ranitidine 132 (120,275) min.

**Conclusion:** Water and antacid immediately increased gastric pH, while PPIs showed a delayed but prolonged effect. Temporarily early relief of GERD symptoms may be justified by water administered with the drug.
Polyradiomodification in combined treatment of rectal cancer

ESC-ID 51
Author Gordeyev SS
Country Russia
University I.M. Sechenov Moscow Medical Academy
Department Department of Oncology

Using preoperative radiation improves local recurrence rates in rectal cancer patients. Nevertheless, some patients develop local relapse because of the lack of radiation effect on the radioresistant hypoxic tumor cells. Increasing radiation dose induces severe toxicity and complications. Therefore, development of combined treatment schemes using radiosensitizing agents is considered very promising by many investigators to further improve local control and disease-free survival without increase in complication rate. The aim of this study is to assess safety and efficacy of concurrent radiotherapy, local hyperthermia, metronidazole and xeloda in the preoperative treatment of patients with rectal cancer.

Methods: 66 patients with T3-4N0-1M0 rectal cancer who underwent combined treatment in the department of proctology of N.N. Blokhin cancer research center between January 2004 and February 2007 were randomly assigned to different types of preoperative polyradiomodification treatment. All patients had short-term, large-fraction preoperative radiotherapy 5 x 5 Gy in 5 days in combination with local application of metronidazole on the 2nd and 4th day of the radiation. Local hyperthermia was used as a second radiosensitizing agent in 14 patients on the 3rd, 4th and 5th day of the radiation. Xeloda was used in 24 patients dosed 1500 mg/m² every day of the radiation. Both radiomodifying agents were used in the same doses for 28 patients. Following criteria were used to estimate the treatment efficacy: metronidazole toxicity, the degree of radiation patomorphosis, the rate of recurrences and local relapses, disease-free survival rate.

Results: Only a single application of metronidazole could be used for 11 patients because of the acute toxicity. 5 patients developed toxicity after the second application. Third degree of radiation pathomorphosis was found in 16 patients. No recurrences, local relapses or distant metastases were found during the time of the follow-up. The study continues.

Conclusions: Preliminary results show high efficacy and low toxicity of the investigated polyradiomodification treatment regiments. Nevertheless, additional studies and results of the retrospective randomized trials are needed to compare different polyradiomodification regiments and better define their efficacy.

Expression and activity of beta-arrestins in GI epithelia in health and cancer

ESC-ID 428
Author Fischer JC, Richter JF, Fromm M, Schulze JD, Schumann M
Country Germany
University Charité Universitätsmedizin Berlin
Department Gastroenterology

Background: b-arrestins (b-art) are intracellular proteins that bind and thereby inactivate phosphorylated G protein coupled receptors (GPCR). In this process they also trigger GPCR endocytosis and become scaffolds for alternative intracellular signalling events that include non-receptor tyrosine kinases. Recently, they have been implicated in tumor growth.

Aims: (1) To show evidence that arrestins are functional in gi epithelial cells. (2) To evaluate the expression level and activity of arrestins in the mucosa of colorectal cancer vs normal mucosa.

Methods: HT-29/B6 cells were cultured with standard culture media. Human intestinal mucosa biopsies were taken either during colonoscopy or after surgical resection of colorectal carcinomas of the sigmoid colon. b-art expression was measured with western blotting. b-art1 phosphorylation status was also analyzed by western blotting using a phosphorylation-specific b-art1 antibody.

Results: After preincubation of differentiated HT-29/B6 cells with GPCR antagonists for muscarinic, neurotransin and LPA receptors the corresponding agonists were added. Dephosphorylation of b-art1 at serine-412 was monitored as a marker of b-art1 activation. b-art1 phosphorylation was significantly reduced by 36 ± 5%, 28 ± 6% and 22 ± 2% for carbachol, neurotransin and LPA, respectively. Analysis of the b-art1 and b-art2 expression in the ileum, the ascending, transversum, descending colon and the rectal mucosa (endoscopic biopsies) revealed significantly higher expression levels in the aboral gi tract for b-art1 but not b-art2. Mucosal samples of surgical resections of colon cancers (exclusively sigmoid cancers) and the adjacent healthy tissue revealed a significantly reduced level of b-art1 but not b-art2 (b-art1 level in carcinoma samples: 62 ± 10% of adjacent healthy mucosa). Interestingly, phosphorylation of b-art1 at serine-412 was significantly reduced in the neoplastic (b-art1-Ser412P level in carcinoma samples: 81 ± 7% of adjacent healthy mucosa), implying an increased activity of b-art1 in colorectal malignancies.

Discussion: This study shows evidence that arrestins are expressed and functional in gi epithelial cells and are differentially expressed and active in the mucosa of colorectal cancers versus the adjacent healthy mucosa.

Calcium intake and obesity

ESC-ID 199
Author Kartashova KM
Country Ukraine
University Kharkov State Medical University
Department General Practice-Family Medicine

Objective: The association between daily calcium intake, body composition and plasma lipoprotein-lipid concentrations was studied. Design: Adults aged 20–65 y (35 men, 35 women) were studied. Subjects who consumed vitamin or mineral supplements were excluded. Subjects were divided into 3 groups on the basis of their daily calcium intake: groups A (< 600 mg), B (600–1000 mg), and C (> 1000 mg).

Results: Daily calcium intake was negatively correlated with plasma LDL cholesterol, total cholesterol, and total:HDL cholesterol in women and men after adjustment for variations in body fat mass and waist circumference (P < 0.05). In women, a significantly greater ratio of total to HDL cholesterol (P < 0.05) was observed in group A than in group C after correction for body fat mass and waist circumference. In women, body weight, body mass index, waist circumference were significantly greater (P < 0.05) in group A than in groups B and C, even after adjustments for confounding variables. Comparable trends were observed in men, but not after adjustment for the same covariates.
Comparison of 2-week quadruple therapy with omeprazole, colloidal bismuth subcitrate, amoxicillin and clarithromycin (OBAC) versus 1-week omeprazole, metronidazole, amoxicillin, clarithromycin (OMAC) for the eradication of Helicobacter pylori in Iranian patients with dyspepsia: Randomized clinical trials (RCT)

ESC-ID: 223  
Author: Zamani F, Alizadeh S, Shakeri R  
Country: Iran  
University: Iran University of Medical Science  
Department: Gastroenterology

**Heading and aim:** Quadruple therapies are considered the standard regimen for Helicobacter (H). Pylori eradication, but the optimal duration of these regimens is still controversial. The aim of this study was to compare the efficacy of 1-week quadruple therapy with OMAC versus 2-weeks with OBAC in H. pylori-positive patients.

**Methods and Material:** A total of 60 H. pylori-positive patients (50% female) suffering from dyspepsia were enrolled in the study and randomly allocated into the following two groups: group A (n:30) received a 1-week quadruple therapy (20 mg Omeprazole b.i.d., 500 mg Metronidazole b.i.d., 1000 mg Amoxicillin b.i.d., 500 mg Clarithromycin b.i.d.); group B (n:30) received a 2-week quadruple therapy (20 mg Omeprazole b.i.d., 240 mg Colloidal Bismuth subcitrate b.i.d., 1000 mg Amoxicillin b.i.d., 500 mg Clarithromycin b.i.d.). Two months after treatment Endoscopy was repeated and Rapid urease test and Histology assessment for H. pylori eradication were performed.

**Results:** At intention to treat and per protocol analysis, 2-week therapy with OBAC achieved a significantly higher eradication rate than 1-week therapy with OMAC (86.66% versus 70%) and (89.65% versus 70%) respectively. Compliance and tolerability were good for all regimens. (OBAC: 96%, OMAC: 100%).

**Conclusion:** Two-week therapy with OBAC clinically lead to a significant increase of H. pylori eradication rate compared to 1-week therapy with OMAC.

Biochemical effects of Skvalen in the rats gastric mucosa during the ulceration

ESC-ID: 236  
Author: Rudenko I, Kovalyova V, Dvorshchenko K, Ostapchenko I  
Country: Ukraine  
University: National Taras Shevchenko University of Kyiv  
Department: Biological

**Aim:** Despite the progress in the conservative treatment of the stomach ulcer many patients have some complications of the disease, which are growing progressively worse. So the new methods of alimentary functions correction, treatment, diagnosis and prevention of the life-threatening states are needed. The goal of this study was to explore the influence of skvalen on the biochemical processes in the gastric mucosa during the experimental ulceration in rats.

**Material and methods:** White Wistar rats of both sex 130-150 grams weight were used during the experiment. The research were carried out using the aspirin (acetate), stress and ethanol methods of experimental ulceration. Skvalen was obtained by the extraction from the amaranth oil. Skvalen preparation diluted 1:10 in seed-oil was administered per os to rats in doze of 20 microliters 3 times a day. The diene conjugate content was evaluated in heptane-isopropanolic extract using spectrophotometric method. Schiffs bases were evaluated using fluorimetric method. Separation and quantitative measurements of adenine nucleotides on Silufol was carried by [Zarubina 1986]. The protein content of gastric mucosa was analyzed by electrophoresis on a 10% polyacrilamid gel [Laemmli 1970]. Lipid extraction from gastric mucosal cells was made by densitometry [“Camag” Switzerland]. The proteins concentration was made by [Lowry et all].

**Results:** It was determined, that the administration of skvalen pulls off the edema and the hemorrhage, decreases the number of erosions and ulcers in gastric mucosa in all used models of ulceration. Activation of lipid peroxidation (LPO), violated cell energy state and changed content of lipids and proteins were also found during ulcer development. Skvalen shows clearly defined antistress, immuno-modulating and bacteriostatic properties. The introduction of skvalen for 5 days normalised lipid peroxidation products content (diene conjugates, TBK-active substances and Schiffs bases) as well as antioxidant enzymes activity (superoxidismutase and catalase). The cell energy state was defined by permanent ATP, ADP, AMP concentration and total nucleotide concentration. The nucleotide balance becomes restored in general gastric mucosal cells fraction by the 5-th day after introduction of skvalen. In the protein content testing it was determined that by the 3-d day of ulcer development total protein composition in gastric mucosa changed against control. After skvalen introduction statistically significant restoration of proteins with molecular weight of 89, 95 and 99 kDa was determined. The complex exploration of gastric mucosal cells lipid composition found out decreased cholesterol level and triacylglycerol level. The introduction of skvalen normalizes cholesterol content and shows a tendency to normalization of triacylglycerol level by the 5-th day.

**Conclusion:** The obtained results show that skvalen has strongly pronounced antioxidative, cytoprotective, immunomodulating effect and can be used as a basis for pharmacological preventive and complex therapy drug creation. It is the only drug that can heal stomach ulcer by the 5-th day and has no known side effects or contraindications.

Non-invasive method of detecting liver fibrosis by Fibrotest/Actitest in chronic C hepatitis patient

ESC-ID: 315  
Author: Chakravarty AA, Shah A, Viral MP, Maheshwari S, Verma A  
Country: Romania  
University: Victor Babes University of Medicine and Pharmacy  
Department: General Medicine

**Aim:** To establish the efficacy of Fibrotest compared to liver biopsy in chronic hepatitis C patients.
Method: 74 consecutive patients with chronic C hepatitis admitted in the department of gastroenterology were included in this study. They were all treatment naïve and underwent a liver biopsy, as well as routine biochemical tests. One pathologist – unaware of the Fibrotest/Actitest results – graded and staged liver biopsies according to Metavir system. The Fibrotest/Actitest biochemical markers-alpha2 macroglobulin, apolipoprotein A1, haptoglobin, GGT, bilirubin, and age, sex were determined and used to compute the Fibrotest/Actitest results. For statistical analysis Medcalc software version 9202 has been used.

Results: The majority of patients were females (48 females, 26 males), mean age 51.6 ± 9.39 (SD). The area under ROC for significant fibrosis (F2-4) versus no or minimal fibrosis (F0-1) was 0.717 (95% CI 0.6-0.815). For a cutoff value of 0.51, with the following sensitivity, specificity, positive and negative predictive value, respectively: 72%, 74%, 83% and 60.6%. The adjusted AUROC according to the prevalence of each stage of fibrosis was 0.843.

Conclusion: Our results indicate that Fibrotest/Actitest can replace successfully the liver biopsy with good confidence. This confirms other studies and indicates that we must accept this new method for evaluating the liver fibrosis at least in patients with chronic C hepatitis.

Internal Medicine
Haematology and Oncology II

Intracellular compartmentization and DNA binding of anticancer drugs depend on their extracellular concentration

ESC-ID: 275
Author: Ignatova E
Country: Russia
University: Moscow State University
Department: Faculty of Basic Medicine

Purpose: It is known that pharmacological inhibitors of functional activity of ABC-transporters, the major determinants of multidrug resistance (MDR), regulate intracellular distribution of MDR related drugs in particular cellular compartments. We proposed that extracellular MDR-drug concentration might influence its intracellular compartmentalization. The hypothesis is that at high extracellular doses of MDR-drugs the pumps would be incapable of effluxing all the toxins out of the cells, and the amount of effluxed drug would be negligibly low compared with its intracellular uptake. The ultimate goal of escalating the extracellular drug concentration should be the predominant accumulation of Dox in the nucleus than in the cytoplasm.

Materials and methods: To test this hypothesis the subline of K562 human leukemia cells - K562i/S9, that expresses P-glycoprotein (Pgp) after the MDR1/Pgp gene transfer (no drug selection) were incubated with increasing concentrations of MDR-drug doxorubicin (Dox) from 7.7 x 10^-7 to 7.7 x 10^-5 M for 5 min and cell associated fluorescence was measured on a flow cytometer.

Results: Two subpopulations representing the cells with high and low fluorescence (‘bright’ and ‘dull’ cells) were shown with all studied Dox concentrations. In the range of extracellular Dox concentrations between 7.7 x 10^-7 to 7.7 x 10^-6 M mean fluorescence of cell suspension increased proportionally. In contrast, when the amount of Dox in the incubation medium exceeded 7.7 x 10^-6 M, an inverse correlation was observed, namely, mean fluorescence of cell suspension decreased with elevation of extracellular Dox concentration, and the number of ‘dull’ cells increased at the same time. We attribute the drop of Dox fluorescence to its redistribution.
from the cytoplasm to the nucleus because it is the intercalation of Dox into the DNA that is known to dramatically (down to 40-fold) quench the fluorescence of this drug. 

**Conclusion:** Extracellular Dox concentration is a factor that regulates the drug distribution between the cytoplasm and the nucleus in MDR cells. One mechanism of this phenomenon could be the bypass of ABC-transporters with high doses of Dox, leading to the increased cytoplasmic drug concentration and therefore nuclear accumulation. This provides the basis for the explanation of more efficacy of high dose chemotherapy compared with gradual increase of drug concentration within the conventional therapeutic range.

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**Septic shock – predictors of mortality in a group of hematological patients**

ESC-ID 875  
Author Szewczyk J, Kryczka K, Ziarkiewicz M, Waszczuk-Gajda A, Jedrzejczak WW  
Country Poland  
University Medical University of Warsaw  
Department of Hematology, Oncology and Intern

**Introduction:** Septic shock is defined as sepsis-induced hypotension that persists despite fluid resuscitation and is associated with tissue hypoperfusion. It is a severe complication in hematological malignancies aggravated by the concomitant immunsuppressive therapy and one of the direct causes of death in this group of patients.

**Aim of the study:** To analyze the onset and the course of septic shock in a group of patients with severe blood disorders, and to determine which factors were associated with higher mortality and which were prognostic for better outcome.

**Material and methods:** We have analyzed retrospectively medical records of 83 patients (39 women, 44 men; median age 55 years) with underlying hematological malignancies (Acute leukemias and myelodysplastic syndromes-55%, lymphomas-45%). The clinical status of each patient was evaluated by means of Acute Physiology and Chronic Health evaluation Score (APACHE II) as well as Organ Failure Score (OFS). The statistical relationships in the collected data were evaluated using the Fisher’s exact test.

**Results:** Septic shock developed in the evaluated patients in the 15th day of hospitalization as a median. Overall, 31 patients (37%) survived shock incident. Age and chronic organ failure as well as negative blood culture were poor prognostic factors (p <0.05) whereas primary hematological illness did not influence the survival rate. Higher APACHE II score was associated with higher mortality (p <0.05) (average APACHE II – 23 points). The presence of major acute organ failures (OFS above 35 points) was associated with increased patient mortality (p <0.05). Higher death rate was associated with liver dysfunction (p <0.05) (bilirubin >1.2 mg% (p <0.05)) and respiratory insufficiency (p <0.05), whereas other system dysfunctions were insignificant. Presence of acidosis was significantly associated with higher mortality (p <0.05). The median pH of non-survivors was 7.29, in comparison to 7.39 in the group of survivors. There was no relation between the presence of neutropenia and survival (p = 0.16). Afebrile patients were more prone to die in comparison to those who reacted with fever (p <0.05).

**Conclusions:** APACHE II score is a good prognostic scale for septic shock outcome in hematological patients. The severity of multiple organ failure was associated with higher mortality. Acidosis, the need for mechanical ventilation and liver dysfunction were the most important predictors of mortality and therefore challenge an intensive treatment of those clinical states. As fever appeared to be an advantageous response to sepsis routine temperature control practices should be taken under consideration.

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**P 14 ARF regulate checkpoint control and induce cell death**

ESC-ID 784  
Author Milojkovic A, Hemmati PG, Güner D, Gillissen B, Wendt J, von Haefen C, Daniel PT  
Country Germany  
University Free University  
Department Biology

Tumor suppressor gene p14ARF is encoded by the human INK4A gene locus and has been shown to play a unique role in 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 re-transfected with procaspase-3. P14ARF induces strong G1 arrest in both cell lines. Cell cycle checkpoint abrogation by the kinase inhibitor caffeine was accompanied by loss of cell viability measured as a number of PI positive cells in both cell lines. Apoptosis was executed via the mitochondrial pathway as evidenced by breakdown of the mitochondrial membrane potential, cytochrome c release and caspase activation but only in caspase-3 proficient cells. MCF-7 cells, which have lost caspase-3 undergo a non-apoptotic cell death so-called autophagic death. To visualize autophagy we used GFP tagged light-chain 3 protein, which is concentrate in the autophagosomes during the autophagic process. Microscopic studies revealed punctuated fluorescence pattern by GFP-LC3, which was not the case in caspase-3 proficient cells. Loss of the CDK inhibitor p21CIP/WAF-1 facilitates p14ARF-induced apoptosis in caspase-3 dependent manner. These data indicate that cell cycle arrest programs interfere with p14ARF-induced apoptosis strictly depending on caspase-3 and that loss of the executioner caspase-3 facilitates induction of autophagic cell death.

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**Bax/Bak independent induction of apoptosis by BH3-mimetic small molecule compounds**

ESC-ID 977  
Author Gebhardt N, Gillissen B, Preissner R, Daniel PT  
Country Germany  
University Charité Universitätsmedizin Berlin  
Department of Hematology, Oncology and Tumor

Gossypol, HA14-1 and BH3I-2’ are small organic compounds which are considered as antagonists to antiapoptotic Bcl-2 and Bcl-xL. Due to this activity they were therefore suggested as suitable agents for the induction of apoptosis in Bcl-2 or Bcl-xL overexpressing cancer cells. Here, we show that Gossypol, HA14-1 and BH3I-2’ induce apoptosis inde-
ependent from protein expression of Bcl-2 or Bcl-xL in human cancer cell lines Jurkat (T-lineage ALL), BJAB (Burkitt-like lymphoma) and HCT-116 (colon carcinoma). This indicated an off-target effect of these Bcl-2 and Bcl-xL antagonists. To functionally prove such an off-target effect we investigated whether induction of apoptosis by these compounds is dependent on multidomain proapoptotic Bcl-2 homologues Bak and Bak. Notably, Gossypol, HA14-1 and BH3L-2 all induced apoptosis independently of Bak and Bak in HCT-116 Bak/Bak single and double deficient cells. This indicates that induction of apoptosis by these compounds is independent of the Bcl-2 family and therefore indicates off-target activities.

The effect of different T-cell depletion regimens on hematopoietic recovery and survival in allogeneic hematopoietic stem cell transplantation

ESC-ID 774
Author Judickas S, Ambrazevicius M, Rainys D, Griskevicius L
Country Lithuania
University Vilnius University
Department Medicine Faculty

Aim: Allogeneic stem cell transplantation provides a possibility for cure of malignancy. Despite improvements in clinical outcomes, graft-versus-host disease (GVHD) remains a significant and potentially lethal complication. T-cell depletion of the donor graft offers the potential prevention of GVHD without morbidity associated with immunosuppressive drugs such as methotrexate and cyclosporine. Most conditioning protocols include T-cell modulation with antithymocyte globulin (ATG) or Campath-1H (alemtuzumab) for GVHD prevention. The best type of T-cell depletion as well as the dose of T-cell depleting agent remains unknown. The aim of our study was to evaluate the influence of different T-cell depletion regimens in allogeneic hematopoietic stem cell transplantation on hematopoietic recovery and survival.

Methods: A total of 63 cases of allogeneic stem cell transplantation, performed in Vilnius University Hospital during 2000-2006, were enrolled in retrospective analysis. The first group of 26 had total doses of Campath-1H ≤ 50 mg, the sub-sequent 16 had Campath-1H > 50 mg, and the final group of 21 had ATG in their conditioning. The groups were similar as regards age, sex, risk group, CMV status, HLA match and ABO match. Reduced intensity conditioning was applied more often in Campath-1H > 50 mg group than in the other two groups (P<0.05).

Results: The recovery of neutrophil count was longer in Campath-1H > 50 mg group than in Campath-1H ≤ 50 mg group (median 23 days and 14.5 days respectively; P <0.05). The recovery of platelet count was fastest in Campath-1H ≤ 50 mg group (median 10 days; P<0.05). The need of erythrocyte and platelet transfusions was lowest in Campath-1H ≤ 50 mg group (median 1.5 doses and 2 doses respectively; P<0.05). The number of days with fever and intravenous antibiotics was higher in ATG group than in Campath-1H > 50 mg group (median 2 and 20 days versus 0 and 11 days respectively, P<0.05). 11 (42.3%) patients in Campath-1H > 50 mg group, 4 (25%) patients in Campath-1H ≤ 50 mg group and 11 (52.4%) patients in ATG group developed acute GVHD. 6 of 9 deaths in Campath-1H ≤ 50 mg group, 2 of 2 deaths in Campath-1H > 50 mg group and 2 of 3 deaths in ATG group were due to transplant related toxicity. The overall survival was better in ATG group, but the difference has not reached statistical significance.

Conclusion: The early results showed a trend in survival for the ATG-treated patients. The platelet engraftment was fastest in Campath-1H ≤ 50 mg group. Campath-1H ≤ 50 mg group required less erythrocyte and platelet transfusions.

Evaluation of patient knowledge of diabetics among Iranian type 2 diabetic patients

ESC-ID 921
Author Morteza A, Izadi SM, Nakhjavani M
Country Iran
University Tehran University of Medical Science
Department Endocrinology and Metabolism Research Center

Introduction: Diabetes is a major health problem in developing countries like Iran. Nearly 8 million of Iranians have diabetics. This rate is much higher in some provinces like yazd. Patient knowledge of disease like what is diabetics, how to use medication, diet and monitoring of blood sugar are important factors in prevention of diabetic complications. The aim of this study was to estimate the patient knowledge of disease (diabetes) among Iranian diabetic patients.

Method: One hundred and fifty five type 2 diabetics visited at Tehran imam hospital diabetic’s clinic during November 2006 to March 2007 randomly selected and participated in this study. Every patient filled a questionnaire. It had general questions about age, sex, duration of diabetes. It evaluated the patient knowledge of disease by the following fields: 1) What is diabetics( general knowledge) 2) how to use medication 3) diabetics diet 4) how often they monitor their blood sugar and if they have the instrument at home .Each right answer received 1 point and each wrong answer received 0 point. Total education was the average of all these parts. We used neuropathy disability score NDS to assess the neuropathy of each leg. Other data like height, weight was collected from the patient files.

Results: There was 121 patients responded to all the questions and took all the exams .34 patients excluded because of the lack of enough information .There was 78(64.5%) female and 43 (35.5%) male with the mean age of 53.7 ± 11.4 , diabetes duration of 8.4 ± 7.1 years and BMI 26.8 ± 4.6 . 86(71.04%) did not had the general knowledge about diabetics. 25 (20, 66%) did not know how to use their drug. 78 (64.46) did not know anything about diet. 10 (8.21%) did not answer any parts of the questionnaire. 107 (88.42%) answered just one or 2 parts of the questionnaire. There was just 4 (3.03%) who answered all the parts completely. None of them had regular monitoring of their blood sugar. None of them knew what should he/she do if they eat more in a party. There was no relation between total education and neuropathy disability score for each leg. There was a significant relation between total neuropathy score and duration of diabetes (correlation coefficient = 0.273, p = 0.003 for right leg and correlation coefficient = 0.337, p <0.0001for left leg). 14% had high levels of diabetic neuropathy (NDS >3).

Conclusion: As a conclusion we should pay more attention on patient education in Iranian diabetic clinics. If they do not have the appropriate knowledge about the disease, they do
not use the medication properly. Most of those who were more educated were those who had much more duration of disease, so they were more experienced! Most of the patients get good points on how to use medication, but they did had general education about the disease and diet. This shows that the physician should pay more attention on these parts as well. The neuropathy disability score (NDS) is so high among them. It has a significant relation with the duration of diabetics. This shows that they were not control. The bias of the study was that we assessed all these data in a medical university clinic. Most of the patients were so old. They were illiterate and hard to change their diet. These data are not the representative of all diabetics in Iran.

Ways of death in Burkitt's lymphoma: Apoptosis or mitotic catastrophe

Burkitt's lymphoma is a fast-growing non-Hodgkin's lymphoma that originates from B cells. It is a relatively rare disease in Western countries, but is common in Central Africa, where it is associated with infection by the Epstein–Barr virus. Although a rare condition in adults, it is relatively common in children, making up about 30% of all childhood non-Hodgkin’s lymphomas. It has been shown that following genotoxic stress Burkitt's lymphoma (BL)-derived cells trigger cell cycle checkpoints at the G2/M transition leading to G2/M arrest and default apoptosis. In some cases they are also known to override the G2/M checkpoint leading to mitotic catastrophe, which is recognized by unscheduled mitotic progression, resulting in polyploidy and multi- and/or micronucleation. As the mechanism behind mitotic catastrophe seems to be due to aberrant regulation of mitotic checkpoint activation, we decided to do a comparative study of various Burkitt's lymphoma cell lines in presence of the mitotic poisons paclitaxel (Taxol) or nocodazole, which induce mitotic checkpoint activation. In total we screened 19 cell lines on these cells to get further insight into the mechanism.

Analysis of knowledge and needs of information on head and neck neoplasms among oncological patient

Background: Knowledge about oncology plays an important role in prophylaxis, early diagnosis and future treatment. Head and neck tumours comprise more than 5% of human neoplasms. In most cases early symptoms of those neoplasms are easy to recognize. For that reason patients should be open-eyed. However, patients usually visit doctors with neoplasms in advanced stages.

Aims: Evaluation of knowledge and needs of information on head and neck neoplasms among oncological patients.

Methods: To analyze this problem a questionnaire was prepared. First group comprised 50 oncological patients treated in the Department of Maxillofacial Surgery of the Jagiellonian University in Cracow. Average age of patients was 60 (18-90). Second group comprised 50 patients treated in the Swoszowice Health Resort who did not suffer from head and neck tumours. Average age of these patients was 59.6 (25-80). Part of the questionnaire assessed knowledge about head and neck oncology, including awareness of carcinogenic factors, symptoms, types of treatment and prognosis. In this part of the questionnaire patients could get maximum 20 points. Patients also expressed their opinion about needs of new information on head and neck oncology. In addition the questionnaire completed by patients from the first group assessed their awareness of oncological illness.

Results: Patients' oncological knowledge: Patients from the I group got 9.74 points (49% of correct answers) on average. Patients from the II group got 9.15 points (46%). Patients' education: patients with higher education got 11.9 points (59.5%), patients with secondary education got 9.5 points (47.5%), patients with primary education got 7.3 points (36.5%). Patients age: patients under 50 years old got 11 points (55%), patients above 50 years old got 9 points (45%). Patients' place of living: patients from a small city got 10.3 points (51.5%), from a medium city got 9.8 points (49%), from a big city got 9.7 points (48.5%), from a village got 8.5 points (42.5%). Patients' opinion: 83 patients (83%) would like to get more information about head and neck oncology, 17 patients (17%) would not. Most patients (71%) would like to know everything (diagnosis, types of treatment, prognosis) about their illness. In most patients' opinion (80%) physician should inform patients' family about cancer. Oncological patients' awareness of their illness: Only 11 patients (22%) from the first group gave correct diagnosis and 26 patients (52%) could localized properly their tumour.

Conclusions: 1. Higher level of knowledge about head and neck oncology was observed in patients with higher education, under 50 years old and living in cities. 2. Most patients want to get more information about head and neck oncology especially from doctors. 3. In patients' opinion a doctor should give them full information on oncological disease:
Factors affecting the length of hospital stay of children with Acute Lymphoblastic Leukemia

Introduction: Acute Lymphoblastic Leukemia (ALL) is the most common Hematologic malignancy in children. These patients are admitted in hospital most frequently for chemotherapy, neutropenia and infection. Few studies have assessed the predictive factors for the length of stay (LOS) for children with ALL. In this study we determined the factors associated with the LOS of children with ALL, admitted for neutropenia or infection and aimed to perform a predictive model.

Methods: In this cross-sectional study, the records of episodes of admission of children with ALL admitted to Ali-Asghar children hospital for Neutropenia or infection from July 2005 to Jan 2007 were reviewed retrospectively. Episodes of admission for chemotherapy or those resulting in death were excluded. During the study, demographic and health-related data of 80 episodes of admission from 52 patients were recorded. We performed the analysis by SPSS v.13 using One-Way ANOVA, Correlation and Multiple linear regressions for data analysis.

Results: The mean LOS was 8.82 (SD = 6.55) day. Mean age of patients in admission was 98.23 (SD = 41.50) month. Reasons for admission were neutropenia (58.8%), Pneumonia (16.3%), Sepsis (13.8%), Chicken pox (8.8%) and meningitis (2.5%). LOS was longer for neutropenia (9.34, SD = 7.86) and shorter for chicken pox (6.25, SD = 1.73). Hospital ward, diagnosis and antibiotic response were statistically related to LOS (p values: .002, .002, .009 respectively). In a multiple linear regression, no statistically or clinically significant model was performed. The distance between Tehran and the patients’ living towns was not statistically related to LOS (Power: 0.76). Discussion Although some studies showed that LOS in children with ALL admitted for infection or neutropenia could be predicted by some factors in admission, our results showed few factors related to it. This made constructing a predictive model impossible. A greater sample size may be needed to perform such a model. Because of the well support of NGOs, socioeconomic factors such as income, insurance and their living town no longer affect the LOS.
The survey of the effective factors on low quality of life in children with leukemia under intrathecal injection

ESC-ID 157
Author Hedayat Z, Fatemeh G, Fatemeh A, Shahla A
Country Iran
University Iran University
Department Medical

Introduction: Leukemia is the most common kind of cancer in children who referral to the hospitals and medical centers in Iran. One of the most common treatments in these children is Intrathecal injection which can be very painful and effect in their quality of life therefore their QoL is lower than normal children. Our aim of this qualitative study is finding the cause of this low QoL.

Method and Material: We observed the hospital, IT room, IT process and children behavior through the whole time of IT process. And also we randomly chose 15 children from that group, and interview with them and their parent and another key participation such as Oncologists, supervisor, nurses and health care staff and residents in charge of performing IT process.

Results: Analysis of codes witch extracted from researchers' observation and interview wear done. And thematic analysis method showed four major themes: 1. Pain, including both the pain caused by the treatment procedure and the general pain as the result of the disease Side effects and decrease in general health. 2. Fear of the IT (Intrathecal) process, recurrence of the disease, death, etc. 3. Parent behavior with their children about their disease and treatments. 4. Problems related the staff's communication and outdoor environment.

Conclusion: This qualitative study demonstrates that the pain stage in the children under IT treatment is significantly lower than the general postulation and also there are other factors involved in turning the IT process to an unbearable and frightening procedure for children. Fear of the hospital environment, recalling previous pain experiences during communication with other children, parent who don't speak with their child honestly and don't describe the situation for them all have serious impacts on IT experience more than the pain itself. Therefore it is suggested to speak with children about there problem and provide a calm, happy environment for the children in the hospital, making them busy with games before the IT process to make them relax and distracted from the pain, instead of making them waiting at the door of the IT room, panicking.

Induction of mitochondrial apoptosis by p14ARF critically depends on PUMA in p53-proficient cells

ESC-ID 729
Author Müer A, Hemmati PG, Gillissen B, Overkamp T, Wendt J, Dörken B, Daniel PT
Country Germany
University Charité Universitätsmedizin Berlin, Campus Berlin-Buch
Department Department of Hematology, Oncology and Tumor

The p14ARF tumor suppressor protein plays a central role in mediating apoptosis, cell cycle arrest, and senescence in response to various cellular stress signals. We previously reported that p14ARF is capable of triggering p53-independent cell cycle arrest and apoptosis programmes, respectively. Whereas the p53-independent induction of mitochondrial apoptosis by p14ARF is fully independent from Bax, the activation of mitochondria in the p53-proficient cells is mediated either by Bax or Bak. However, the upstream signaling requirements for the activation of Bax by p14ARF remain unclear. We show here that the induction of mitochondrial apoptosis upon adenovirus-mediated expression of p14ARF critically depends on the presence of PUMA in p53-proficient cells. In contrast to PUMA-proficient HCT116 wild-type cells, isogeneic HCT116 cells homozygously deleted for the puma gene are almost completely resistant towards p14ARF-induced apoptosis as evidenced by a lack of nuclear DNA fragmentation and activation of caspases upon expression of p14ARF. Notably, apoptosis induction by p14ARF in p53-proficient cells is mediated exclusively by pro-apoptotic Bax, but not Bak. A N-terminal conformational change of the Bax protein is followed by activation of mitochondria, i.e. a breakdown of the mitochondrial membrane potential, and induction of caspase-9 (LEHD) -like and caspase-3/7 (DEVDD) -like activities. Whereas the concomitant loss of p21 strongly enhanced p14ARF-induced apoptosis in PUMA-proficient cells, the deletion of p21 in PUMA-deficient cells did not sensitize for apoptosis induced by p14ARF. Finally, we show that reconstitution of PUMA fully restores sensitivity towards p14ARF-induced apoptosis. Taken together, these data demonstrate that p14ARF-induced mitochondrial apoptosis critically depends on the BH3-only protein PUMA and Bax in p53-proficient cells and is independently modulated by the cell cycle regulator p21.

Induction of apoptosis by the Betulinic acid analogue Ursolic acid via the mitochondrial intrinsic pathway

ESC-ID 756
Author Herrberger C, Füllbeck M, Richter A, Preissner R, Daniel PT
Country Germany
University Charité Universitätsmedizin Berlin
Department Department of Hematology, Oncology and Tumor

Some triterpenoids have long been known to have anti-inflammatory activities and are often utilized in folk medicine in Asian countries. Since the 70’s Betulinic acid (BA) has been described as a promising anti-cancer agent, but its
mechanism of action has not been solved so far. Ursolic acid (UA), a BA analogue, is a pentacyclic triterpene carboxylic acid and can be found in various plants. UA is described as an anti-inflammatory and anti-proliferative substance that has been shown to induce apoptosis in a great variety of cancer cells e.g. myeloid leukaemia, epithelial, lymphoma or colon carcinoma cells. In our study T-cell leukemia (Jurkat) cells were used to investigate the UA induced apoptosis. Caspase-8 and FADD double knockout cells underwent apoptosis after treatment with UA. To confirm these data we blocked caspase-8 in Jurkat wild type cells but as expected, cells still died on apoptosis after treatment. This indicates that UA is not acting via the intrinsic apoptotic pathway. However, we could observe the collapse of the mitochondrial membrane potential, accompanied by cytochrome c, Smac, and AIF release. In succession we showed that the caspase cascade is activated, culminating in the activation of caspase-3, the major executioner of programmed cell death. This indicates that UA induces apoptosis in Jurkat cells by triggering the intrinsic apoptotic pathway. Although the intrinsic pathway is activated we could not observe a dependency on Bcl-2, using Bcl-2 deficient cells. To further examine the role of caspase-3, a caspase activity assay was performed. We could clearly show a strong activation of caspase-3 after UA treatment. Furthermore, the key role of caspase-3 in UA induced apoptosis was confirmed using a caspase-3 deficient cell line. In these cells the apoptotic rate was significantly decreased. The role of the upstream initiator caspase-9 is still not clear and needs to be further investigated. Taken together, all data imply that UA acts via the mitochondrial intrinsic apoptotic pathway in a Bcl-2 independent manner.

The prevalence of ABO blood grouping in patients with esophagogastric cancers in some hospitals at Tehran, Iran, 2005

**ESC-ID** 509

**Author** Omid A, Mehrdad M

**Country** Iran

**University** Tehran Medical University

**Department** Medical University

**Objectives:** Malignant diseases of the stomach and esophagus are common sufferings throughout the world Including Iran especially the north of Iran. These Malignancies are severely invasive and dreadful. Several factors are involved in the causation of these cancers. These include ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and socio-economic status of the patient. In this study prevalence of ABO blood grouping diet, smoking, alcohol, nitrates, hot tea, and soc

**Methods:** The total numbers of patients were 64 that were selected by method of total sampling. The diagnoses were confirmed through histopathological reports. 40 patients were male and 24 were female. Relative prevalence ratio for male to female was 2:1. Of the total numbers 50 had gastric cancer and 14 suffered from esophageal cancer.

**Results:** 61.9 % of the patients having Gastric cancer had blood group A of ABO blood grouping system, That shows meaningful statistical difference as compared with remaining blood groups (P value = 0.02). Among patients with cancer of esophagus the prevalence of blood groups A&amp;O have relatively high but no meaningful statistical difference was observed. The commonest presenting clinical symptom of the patients suffering from gastric cancer was Abdominal pain.

**Discussion:** We suggest that clinical symptoms and signs of these two cancers should be explained to the whole population specially blood group A.

**Internal Medicine Immunology II**

The state of cellular immunity and caspase activity in patients with chronic pulmonary obstruction diseases and intestinal dysbiosis

**ESC-ID** 751

**Author** Koval H, Olenovych OA

**Country** Ukraine

**University** BSMU

**Department** Clinical Immunology and Endocrinology

**Introduction:** In the majority of patients with chronic pulmonary obstruction diseases (CPOD) intestinal dysbiosis and cellular immunity disorders develop.

**Material and Methods:** During the examination of 98 patients with CPOD and intestinal dysbiosis, they were divided into 5 groups depending on dysbiosis degree (normobiocenosis, dysbiocenosis of I, II, III, IV degrees); control group was formed of healthy people. The level of T-lymphocytes with CD3+, CD4+ and CD8+ phenotype was detected in advance, and it was established, that the decrease of cell amount was proportional to intestinal dysbiosis degree, mostly significant in dysbiosis of III and IV.

**Results:** The lymphocyte apoptosis, which effectors are caspases (particularly, caspase-8 and caspase-3), were investigated in patients with CPOD and intestinal dysbiosis to define the mechanisms of cellular immunity lowering. The caspase-1, is known to send a stimulating signal for the production of cytokines, which improve the functional activity of phagocytic blood cells. The results of investigation of caspase activity are as follows. An activity of caspase-8 of lymphocytes lystate of the patients of first 3 groups didn’t differ from control indices, while in patients with CPOD and dysbiosis of III and IV degrees it increased by 41.5% and 95.7% accordingly. The caspase-3 activity didn’t significantly differ from control index both in patients with CPOD and normobiocenosis and dysbiosis of I and II degrees. In case of dysbiosis of III degree the activity of this enzyme increased by 55,2% as compared with control, and it was of the highest level in dysbiosis of IV degree, being 2,3 times higher, than in patients of control group. The insignificant increase of caspase-1 in patients with normobiocenosis was found, while in patients with CPOD and disorders of species composition of intestinal microflora this index was significantly higher than control indices: in dysbiosis of I degree – by 58,0%. II degree – by 76,5%, III degree – by 2,2 times, IV degree – by 3,3 times.

**Conclusion:** Thus, the activity of caspase-8, caspase-3 and caspase-1 in lymphocytes lystate becomes high enough in dysbiosis of III and IV degrees, though it doesn’t show proportional dependence on the degree of intestinal microflora disorders. We consider the increase of caspase activity in lymphocytes lystate in patients with CPOD and intestinal dysbiosis to be a reason for cellular immunity lowering.
Early diagnosis of liver fibrosis

ESC-ID  140
Author  Boldizhar R
Country  Belarus
University  Gomel State Medical University
Department  Infection Diseases

**Introduction:** Dissemination of chronic virus hepatitis (CVH) is about 5% in population. It connects with passing this infection by different ways. Stage, duration, prognosis, threat of cirrhosis transformation CVH definite stage of fibrosis. There is one main difference between acute and chronic liver inflammation – fibrosis. That’s why we can not agree with R.G. Knodel et al. (1981). He talks about 0 fibrosis stage (no fibrosis). But when the CHV start we can not see in liver collagen fibers, we only can see changing portal spaces: if usually they are triangular, in this case they become oval or round. Besides portal spaces processes of collagen tissue synthesis also touch sinusoid capillaries. Ito lipofibroblasts, which are in perisinusoid space, start synthesis of connective tissue elements. This elements close distance between vessel cells. Probably, any injury of liver start Ito cells to produce collagen. As inductors of this process we can talk about liver cells lysis products, especially damaged membranes peroxides. It is very important using those methods, which allows definite negligible amount of connective tissue. The aim of our investigation is definition helpful of immunogistochemical method for detection procollagen fibers in liver tissue for early diagnosis of fibrosis.

**Material and methods:** We used examples of liver tissue patients with CHV situated in Gomel Region Infectious Clinical Hospital from 2001 till 2004 which was underwent painting of hematoxilin and eosin. The same examples was underwent adaptation of monoclonal antibodies to procollagen, as visual system we used dab-chromagen of SLAB (DAKO) company. The hallmark of AIDS is the declination in the number of the patient's CD4+ T cells. Apoptosis is considered to be one of the major mechanism responsible for the depletion of CD4+ cells. The goal of our research is to check the correlation of immunosuppression in HIV-positive children with the level of Fas-mediated apoptosis. Out of 28 children who were selected for this research, were from age group of 11 months to 9 years, amongst them 13 (46,4%) were girls and 15 (53,5%) were boys. Children were at different stages of HIV-infection. Out of them 4 children (14.2%) were at the I stage of the disease, 14 (50%) - at II and 10 (35,7%) - at III stage of the disease, children were assigned these stages according to CDC of HIV/AIDS classification (2002 year). According to the level of immunosupression children were divided in the following groups: in 4 children (14.2%) immunosupression was absent, in 11 children (39.2%) there was moderate level of immunosupression, in other 13 children (46.4%) there was severe immunosupression. To evaluate the intensiveness of the apoptosis we examined the level of Fas-receptor (CD95) by observing the reaction of monoclonal antibodies under immunofluorescence (“Status”, Russia). The results obtained from the immunological examination of HIV-positive children showed that regressing decrease of CD4 was attended with the increasing level of CD95. The highest level of CD95 was defined in children with severe immunosupression. It is statistically reliable comparing with the level of CD95 in healthy children (p<0,05) and with CD95 level in children with moderate degree of suppression (p<0,01). The data of our research testify about the increased FAS-mediated apoptosis in children with HIV-induced immunodepression. Consequently the determination of the level of Fas-receptor in the blood of HIV children may serve as a beneficial guideline in monitoring the development and progression of HIV infection in children.

Prevalence and antimicrobial resistance of Shigella serotypes isolated in Tehran pediatrics center between 2001-2006 and changes in shigella resistance pattern over time

ESC-ID  872
Author  Mashoori N, Mahboobi N
Country  Iran
University  Tehran University of Medical Sciences
Department  Faculty of Medicine

**Introduction:** Shigellosis is one of the most common causes of dysentery in children which causes lots of morbidity and mortality. The epidemiology and antibiotic susceptibility of Shigella subgroups changes over time. Appropriate antibiotic treatment of shigellosis depends on identifying resistance patterns. So, updated knowledge of shigella susceptibility is necessary for appropriate empirical antibiotic treatment. In this survey, we studied the prevalence of shigella subgroups and their antimicrobial resistance in Tehran pediatrics center between 2001-2006 and we compared its resistance pattern in 2001-2002 to 2005-2006.

**Materials and Methods:** In a total of 682 isolates of Shigella from stool cultures in Tehran pediatrics center, shigella subgroups and their antimicrobial susceptibility to CAZ, TOB,
CT, CFM, CRO, Chloramphenicol, Kanamycin, Naldixic acid, Cephalothin, Bacterim, Ampicillin, Gentamicin and Amikacin was determined antimicrobial susceptibility test. (Shigella subgroups was determined in 397 isolates). Then Antimicrobial susceptibility and prevalence of shigella subgroups were compared in years 2001-2002 to 2005-2006. Results: Among 397 isolates, the most common subgroup of Shigella was Sh. flexneri (48%) and Sh. sonnei (45%) and other results were Sh. dysenteriae (2.9) and Sh. boydii (1.2%). Shigella isolates were totally most sensitive to CRO (95.4%), CT (93.6%) and CB (93.4%). Sh. flexneri was most sensitive to CRO (93.6%), CB (92.9%) and Naldixic acid (88.5%), and Sh. sonnei was most sensitive to CT (97.6%), CRO (97%) and Chloramphenicol (93.2%). Shigella isolates were totally most resistant to Bacterim (86.4%) and Ampicillin (83%). Sh. flexneri was most resistant to Ampicillin (94.7%) and Bacterim (89.4%), and Sh. sonnei was most resistant to Bacterim (90%) and Ampicillin (70.5%). In 2001 to 2002, the most common subgroup was Sh. flexneri which was most sensitive to CAZ and TOB and most resistant to Ampicillin and Bacterim. In 2005 to 2006, the most common subgroup was Sh. sonnei which was most sensitive to Chloramphenicol and most resistant to Bacterim. Conclusion: In this study the most common subgroup of Shigella was Sh. flexneri which was more sensitive to CRO, CT and CB. These results should be considered in empiric therapy of Shigellosis. This study also shows, despite changes in shigella subgroups resistance over time they are still sensitive to Naldixic acid, which is one of the most antibiotics used in treatment of shigellosis. Comparison of shigella subgroups and its antimicrobial susceptibility in different years shows that shigella resistance pattern changes over time. So, for appropriate antibiotic therapy its susceptibility pattern should be determined.

Diagnostic and prognostic value of non-specific laboratory tests in imported malaria

ESC-ID 304
Author Nikolic J, Radeka T
Country Serbia
University Belgrade
Department Infectious Diseases

Introduction: Imported malaria is a challenging diagnostic and therapeutic problem, with a compulsory need for prompt and efficient diagnosis.

Aim: The objective of this study was to assess role of non-specific laboratory tests in diagnosis of malaria according to their sensitivity, specificity and positive predictive values. Their association with disease severity and therefore prognostic value was determined through comparison with parasitemia, occurrence of complications, recovery duration and organ failure.

Material and methods: A retrospective case-control study included 64 patients, diagnosed and treated for malaria in Institute for Infectious and tropical diseases Clinical Centre Serbia, from January 1999 to December 2006. and healthy control (n = 11).

Results: Thrombocytopenia, leukopenia, hyperglycemia, hypcholesterolemia and elevation of transaminases, lactat dehydrogenase (LDH) and creatinine were associated with malaria. Although thrombocytopenia had highest sensitivity (84%), hyperglycemia, leukopenia and hyperbilirubinemia were the most specific (100%) and with the strongest positive predictive values (100%). Malaria complications were associated with anemia, leukopenia, thrombocytopenia, hypoprotenemia, hyponatriemia and elevation of LDH and creatinine. Hypcholesterolemia, anemia and fibrinogen elevation had a significant effect on recovery duration. Hepatospleno-megal in malaria was associated with thrombocytopenia and LDH elevation, as jaundice was associated with anemia.

Conclusion: Non-specific laboratory test are significant diagnostic and prognostic tools, particularly combined with the microscopic detection of parasites, useful for avoiding misdiagnosis and evaluation of disease severity.

Neurology II

SICH: Do clinical and CT features differ in the very elderly patients?

ESC-ID 584
Author Florczak-Rzepka M, Gniatkowska I, Gocea K, Kucharska A
Country Poland
University The Medical University of Warsaw
Department Neurology

Background and purpose: The number of people aged 80 years and over is increasing in most populations. Spontaneous intracerebral haemorrhage (SICH) carries high morbidity and mortality rate. Thus, our aim was to identify and quantify whether there are any significant differences in the most important clinical and CT factors related to the outcome between patients in two age groups (<80 and ≥80 years old).

Material and methods: 149 consecutive patients (40 patients aged ≥80) with the diagnosis of SICH hospitalized at the Department of Neurology, the Medical University of Warsaw in years 2000-2005 were included in the study. We carried out an univariate analysis of retrospectively collected data on clinical and CT features on admission, 30-day mortality and clinical outcome according to a modified Rankin Scale (mRS) at discharge.

Results: The coronary artery disease was significantly more frequent in patients ≥80 years (49% vs. 17%, p = 0.003). Compared with their older counterparts the younger patients had a trend towards fewer cases with intraventricular haemorrhage (18% vs. 34%, p = 0.14) and more favourable GFR (81 ml/min vs. 72ml/min, p = 0.10). The difference in blood pressure, Glasgow coma scale (GCS), disturbances of consciousness, limb paresis and its severity, respiratory insufficiency, haematoma site and diameter wasn’t statistically significant. There was no difference between both groups as far as the influence of the analysed factors on the 30-day mortality is concerned.

Conclusions: Our data indicate that clinical and CT factors as well as their impact on the outcome do not vary in patients under and above 80.
Therapy compliance after the acute ischemic stroke in young adults

ESC-ID: 618
Author: Popovic N, Budimkic M, Jovanovic D
Country: Serbia
University: University of Belgrade
Department: Institute of Neurology

Introduction: Adherence to antithrombotic and risk factor treatment is essential for the efficiency of secondary prevention of acute ischemic stroke. Poor adherence and compliance could reduce the effects of any stroke prevention strategies.

AIM: The aim of our research was to see the rate of compliance to recommended discharge therapy among young adults with ischemic stroke, the existence of significant difference between the adherence to risk factor treatment before and after the ischemic stroke, and the determinants that could influence the compliance.

Material and methods: We analysed 60 patients with a diagnosis of acute ischemic stroke who were treated in the Department of Emergency Neurology in the Institute of Neurology in the period from 2002 to 2005 and who were not over 55 years old in the moment of the stroke. Compliance with therapy was evaluated retrospectively at least one year after the stroke through telephone interview using a specially designed questionnaire.

Results: At least one year after the ischemic stroke 46 patients (78.3%) still use antithrombotic therapy. Among 13 patients who stopped taking therapy 12 (92.3%) initiated the discontinuation by themselves. When risk factor therapy is concerned evaluated compliance with antihypertensive therapy was 70.7% with antilipidemic 25% and 91.7% with therapy of diabetes. Statistically significant difference was found comparing the adherence to antihypertensive therapy before and after the stroke. No statistically significant determinants (age, smoking, stroke severity) for better adherence were identified.

Conclusion: Compliance with antithrombotic therapy among young adults more than one year after the ischemic stroke is rather good. For the risk factor therapy it is concluded that there is a much better adherence to it after the stroke compared to the period before the disease, but is still not good enough. These facts stress the need of better education of our patients about the importance of taking their medicines regularly and persistently and about the necessity of risk factor control, in order to improve compliance to secondary prevention therapy of acute ischemic stroke.

The effect of antiepileptic drug therapy on malondialdehyde level in plasma and Erythrocytes in patient with epilepsy

ESC-ID: 619
Author: Pesic I, Topalovic A, Basic J
Country: Serbia
University: Nis
Department: Department of Neurology, Dep. of Biochemistry

Aim: It has been demonstrated that oxidative stress can contribute to neuronal loss and may be involved in degeneration of neurons in epilepsy, schizophrenia, Parkinson’s and Alzheimer’s disease. Since the intensity of the lipid peroxidation in erythrocytes reflects the state of the cell membranes in different tissues, including the brain, the estimation of oxidative stress in erythrocytes as well as in plasma of epilepsy’s compared to healthy subjects was studied. The concentration of malondialdehyde (MDA) as a parameter of oxidative stress was measured in erythrocytes of patients with mono and poliytherapy of epileptic seizures before and after exposing of erythrocytes to hydrogen-peroxide (H₂O₂).

Method: The examined group of epilepsy’s consists of 30 patients, age 42 ± 13, treated at Clinic of Neurology in Clinical centre Niš. Patients were divided into two groups: I-11 patients on poliytherapy; II – 19 on monotherapy (Valproate 6, Carbamazepine 6, Lamotrigine 7). The control group was composed of 11 voluntary blood donors of the same sex and of approximately similar age. Lipid peroxidation was estimated spectrophotometrically by measured MDA in plasma (µmol/l) and in erythrocytes (nmol/g of hemoglobin) depended on thiobarbituric (TBA)-reactive-product. The level of lipid peroxidation products was examined in fresh untreated erythrocytes and after exposing 3 mM H₂O₂ (dissolved in isotonic saline 0.05M phosphate buffer containing 2 mM/l sodium azid to inhibit catalase activity).

Results: A significantly lower MDA value in plasma (2.68 ± 2.47 µmol/l) has been determined on patients taking polytherapy (2.68 ± 2.47 µmol/l) compared to MDA values of patients taking monotherapy (8.02 ± 6.80 µmol/l; p < 0.05) and healthy subjects (6.53 ± 3.65 µmol/l; p < 0.05). The concentration of MDA in erythrocytes for epilepsy’s in both groups, patients on monotherapy (16.31 ± 7.34 nmol/g of hemoglobin), and on polytherapy (22.04 ± 8.18 nmol/g of hemoglobin), is significantly higher compared to the control group (12.27 ± 5.57 nmol/g of hemoglobin; p < 0.05). After the H₂O₂ oxidative stress induction in patients medicated with antiepileptic mono (30.03 ± 10.40 nmol/g of hemoglobin) and poliytherapy (41.67 ± 19.63 nmol/g of hemoglobin), the concentration of MDA in relation to the referent value for all groups of examinees increases significantly (p < 0.001).

Conclusion: No difference between erythrocytes of unhealthy and healthy examinees has been found in their response to the additional factors of oxidative stress. Exposure of erythrocytes from patients on monotherapy to H₂O₂ produced higher level of MDA (66.97%) than those from poliytherapy (47.11%; p < 0.05). Taken together these data provide a rational basis for assessing possible benefits of polytherapy treatment of epilepsy’s.

Neuronal changes in the spinal cord in multiple sclerosis

ESC-ID: 589
Author: Muller-Wielsch K, Vogt J, Omari KM, Nitsch R, Raine CS, Zipp F
Country: Germany
University: Charité Universitätsmedizin Berlin
Department: Cecile-Vogt Clinic for Molecular Neurology

Aim: Multiple Sclerosis (MS), leading cause of disability among young adults in Western countries, is a putative autoimmune disease of the central nervous system, traditionally regarded as a disease that affects white matter only. Yet in recent years, grey matter damage, known to occur in MS but never emphasized, has become a topical subject (1,2,3). The latter findings focused mainly on neuronal damage in the brain, while our study explored neuronal damage of the spinal...
controls, as well as of 15 EAE cases and 3 healthy controls. In Part I we examined 10 im transverse sections of fresh frozen spinal cord of 14 MS cases, 3 cases with other neurological diseases and 3 healthy controls were fixed and stained with cresyl violet. Spinal cord levels were defined microscopically by assessing anatomical landmarks. We evaluated sections from corresponding heights with high-precision design-based stereology under light microscope at a maximum magnification of 20x. Different neuron populations were defined according to morphological criteria and size (6). Part II: We examined 10 im transverse sections of fresh frozen spinal cord of 14 MS cases, 3 cases with other neurological diseases and 3 healthy controls, as well as of 15 EAE cases and 3 healthy controls. EAE was induced with myelin oligodendrocyte glycoprotein in mice at the age of 8-12 weeks. Animals were clinically graded for neurological status and sacrificed 15d, 20d, 40d, 63d after sensitization for EAE. 3 healthy animals were sacrificed at 8-14 weeks to serve as controls. Sections were fixed, stained with SMI-31 for phosphorylated NF and SMI-32 for non-phosphorylated NF, and developed with diaminobenzidine. We evaluated the results under light microscope at a maximum magnification of 20x (human) and 40x (murine).

Results and Conclusion: In part I we found a significant decrease of neuronal cell counts in all MS cases. Alpha motor neurons were reduced by 54 ± 18% (p<0.01), overall neurons even by 74 ± 11% (p<0.001). In part II MS cases showed a significant increase in SMI-31 reaction of anterior horn neurons compared to healthy controls, particularly in lumbar spinal cord. In the murine study, only slight differences between EAE cases and controls were apparent in older animals. The different degree of reaction in murine tissue in part II might be due to a reduced function of the antibodies on mouse tissue (although a Mouse-on-Mouse-Kit was used). Alternatively, it may support the hypothesis that irreversible neuronal damage in MS occurs only years after disease onset (7). Older results from our group showed a significant decrease of neuronal cell counts in EAE as well (8). Taken together, our findings show strong evidence for neuronal cell damage in the spinal cord both in MS and EAE.

The color feature does not improve reaction times to the visually presented cues

| ESC-ID | 588 |
| Author | Jahantabakshi A, Malekpour M |
| Country | Iran |
| University | Tehran University of Medical Sciences |
| Department | Neurology |

To find out the level of processing of pre-attentive objects we preferred to use cues, i.e., a transient visual stimuli that may attract attention automatically or voluntarily (peripherally or centrally presented cues). In one of our recent experiments (Authors, Unpublished experiment), we observed that color similarity of cue and target does not improve subject’s performance in a peripherally cued detection task. In this paper we describe another experiment which had two kinds of symbolic cues presented in the fixation point (at the center) and showed the subjects where to deploy their attention (right or left) according to their “shapes. Subjects were asked to detect the target (i.e. a simple detection task) or to discriminate between two alternate targets, different in some features except color (i.e. a common discrimination task) We compared the reaction time (RT) in both types in same feature condition (target’s color was similar to cue’s) and different feature condition (target’s color was different from cue’s). Results showed significant difference between them. So we concluded that with increased exposure, color-as an unattended feature of the central cue- can also get processed and improve subjects performance.

Nonviral gene transfer into murine mesenchymal stem cells

| ESC-ID | 581 |
| Author | Scheibe F, Priller J |
| Country | Germany |
| University | Charité Universitätsmedizin Berlin |
| Department | Experimental Neurology/ Molecular Psychiatry |

Aim: Mesenchymal stem cells (MSCs) from bone marrow have been used for therapeutic purposes in a variety of mouse models of neurological disorders. To date, no protocol has been established for the transfection of murine MSCs. We aimed to determine whether MSCs can be genetically modified efficiently by ex vivo gene delivery using transient transfection. Method: Murine bone marrow-derived MSCs were isolated from adult C57BL/6 mice and propagated in vitro for 7 passages. MSCs were characterized by determining their differentiation potential into adipocytes, osteocytes and chondrocytes and by FACS analysis of surface marker expression. Transient transfection of MSCs with pEGFP-N2 plasmid vector (Clontech) was performed using nucleofection, lipofection and magnetofection. Transfection efficiencies were measured by FACS analysis of enhanced green fluorescent protein (EGFP) expression at 48 hours after transfection. Stable transfections were obtained using selection with G418 disulfate.

Results: Murine MSCs were found to express the stem cell marker Sca1 and also CD29 (Integrin β1). They did not express hematopoietic cell surface epitopes like CD11b, CD34, CD45, CD45R/B220, Ter119/Erythroid cells, Ly6e/b/Ly6c and CD3ε. Moreover, we found no evidence for the expression of endothelial markers like CD31 (PECAM-1), Flk1 (VEGF-R2) or VCAM1 (CD106). Murine MSCs differentiated into adipogenic, osteogenic and chondrogenic lineages in vitro. Nucleofection was found to be the most efficient way of gene transfer into murine MSC with a transfection rate of 60.23% ± 2.06%, followed by magnetofection with 29.51 ± 1.71% and lipofection with 22.84% ± 1.95%. We were able to generate stably transfected MSCs without loss of their differentiation potential. Stably transfected single cell-colony forming units (sc-CFU) were propagated and retained their ability to differentiate into adipocytes and osteocytes.

Conclusion: We describe an efficient nonviral procedure for genetic engineering of murine MSCs using nucleofection.
Polynuropathy in patients on continuous ambulatory peritoneal dialysis (CAPD)

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<tr>
<td>Author</td>
<td>Radeka T, Rudic I, Nikolic J, Matanovic D</td>
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<td>Country</td>
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**Heading:** Polynuropathy is a common complication in dialysis patients, it’s detected in more than 65%. The most sensitive diagnostic methods are nerve conduction velocity (NCV) studies for early detection of polynuropathy.

**Aim:** The aim of this study was to find the frequency of polynuropathy in patients on CAPD and correlations between electrophysiological parameters and age, duration of CAPD treatment, biochemical parameters and dialysis adequacy. Method: We studied 52 patients on CAPD (average age 58.29 years, average dialysis duration 29.37 months) to assess levels of biochemical (urea, creatinine, glycemia) and electrophysiological parameters (motor conduction velocity (MCV), terminal latency (TL), F wave latency of n.peroneus and n.tibialis, sensor conduction velocity (SCV) of n.suralis).

**Results:** Over 80% patients had pathologam parameters. The most sensitive were latency of F wave and SCV of sural nerve. Also our study showed significant corelation between electrophysiological and biochemical parameters, dialysis adequacy, age and duration on CAPD.

**Conclusion:** Our study showed that NCV studies are sensitive, noninvasive diagnostic method for early detecting of polynuropathy changes. The most sensitive electrophysiological parameters of polynuropathy are F wave latency and SCV of n.suralis.

Determination of the prevalence and associated factors of restless legs syndrome (RLS) in Iranian non-traumatic patients who referred to neurology and orthopedic clinics

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<tr>
<td>Author</td>
<td>Zamani B, Mehrabani M, Danesh YJ, Bakhti S, Ferehsheh SM</td>
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<td>Country</td>
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<td>Iran University of Medical Sciences</td>
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**Introduction:** Restless legs syndrome (RLS) is a poorly understood sensory-motor neurological disorder whose prevalence in Caucasian populations ranges from 10-15%. The patient reports unpleasant sensations in the lower limbs with dysesthesia resulting in an urge to move the legs. The symptoms occur during periods of inactivity, increasing in the evening and at night. Moving the legs provides relief. These problems could affect patients’ quality of life and may need to be treated. Although common, RLS often escapes diagnosis and many patients are referred to different clinics. Objective: Therefore, the aim of this study is to evaluate the prevalence and associated factors of restless legs syndrome (RLS) in Iranian patients who referred to neurology and orthopedic clinics.

**Patients and Methods:** A total of 1103 non-traumatic patients consecutively admitted to the neurology and orthopedic clinics of Rasoul-e-Akram, Shafa Yahyaee and Milad hospitals were studied. The diagnosis of RLS was based on the criteria adopted by the International Restless Legs Syndrome Study Group Rating Scale (IRLS). Also another variables such as demographics, chief complaint, family history of RLS and systemic diseases were recorded in the check list. The data were analyzed using SPSS v.13 software. Chi2 and Independent T-test were used in analysis.

**Results:** Out of 1103, RLS was detected in 116(10.5%) patients. The prevalence of RLS among patients who referred to neurology and orthopedic outpatient clinics was 9.7% and 11%, respectively, whereas, only 3(2.7%) patients were admitted with chief complaint of RLS symptoms. The prevalence of RLS was significantly higher in women than men (15% vs. 4.3%, P<0.001) and the mean age of RLS patients was significantly higher than non-RLS patients [47.9(SD = 15.34) years vs. 40.71(SD = 17.8) years, P<0.001]. Additionally, Thyroid diseases (11.2% vs. 4.7%, P = 0.033), family history of RLS (28.4% vs. 0.6%, P = 0.001) and upper limb involvement (15.5% vs. 0.1%, P <0.001) were more reported in RLS patients.

**Conclusion:** The prevalence of RLS in the general population is poorly known and it differs in different countries. The results of our study show the considerable prevalence of RLS in Iranian patients. However, most of them are referred to orthopedic clinics than neurology. In addition, thyroid diseases and family history of RLS are associated with RLS. It seems that increasing both patients and physicians knowledge about RLS may lead to better diagnosis, treatment and improvement of RLS symptoms.

The assessment of the efficacy of Diazepam loading dose therapy of Delirium tremens

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<tr>
<td>Author</td>
<td>Bylicka E, Cichecki JK, Grotkowska A, Romanowicz M, Wojciek A</td>
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<td>Country</td>
<td>Poland</td>
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**Introduction:** Delirium tremens is a potentially fatal form of alcohol withdrawal syndrome. Alternative to the existing methods of treatment is Diazepam loading dose therapy [Diazepam given orally 20 mg every 1-2 h until the improvement of the clinical condition is achieved].

**Aim:** The objective of this study was to assess the efficacy of diazemepam loading method. We also wanted to estimate the incidence and frequency of particular delirium tremens’ symptoms. For a better individualization of therapy we evaluated the significance of different data obtained from case history and clinical status in single and total loading dose of drug used.

**Material and Methods:** Our retrospective cohort study was performed in Nowowiejski Hospital in Warsaw. Out of over 200 cases of patients suffering from Alcohol Withdrawal Syndrome with Delirium (according to ICD-10), a group of 44 men at the age ranging from 28 to 62 years [mean 46.12] who met inclusion criteria: clinical state assessed and measured by means of the Clinical Institute Withdrawal Assessment for Alcohol scale [CIWA-A], full biochemical measurements and anamnesis was chosen for further investigation. Patients’ clinical state was evaluated with CIWA-A: nausea and vomiting, tremor, sweating, occurrence of halluc-
cinations, tactile disturbances, auditory disturbances, visual disturbances, clouding of sensorium, quality of contact, thought disturbances, seizures, headache, flushing of face. **Results:** Total dose of diazepam varied between 20 and 280 mg [mean: 71.36 mg] and the duration of delirium ranged from 1 to 32 h [mean 7.49h] in experimental group. Whereas in group of patients treated with traditional method based on previous studies it ranged between 2 and 124h (mean: 33.8).

Following the CIWA- A scale evaluation, we found that 94.5% of patients presented tremor and sweating, 83.6% flushing of face, 81.8% thought disturbances and only 3.6% had seizures during therapy. The occurrence of hallucinations and sensory disturbances we noticed at about 70%. There was no positive correlation between the amount of diazepam used and the time of alcohol abuse, withdrawal or last drinking bout.

**Conclusions:** The results confirm advantage of diazepam loading dose therapy over diazepam in divided doses treatment. This method reduces symptoms in short time and prevent complications. Therapy guided by CIWA- A scale seems to be the best individualization of treatment.

**The relationship between patent foramen ovale (PFO) and migraine headache**

**ESC-ID** 272  
**Author** Motamed MR, Fereshtehnejad SM, Najimi N, Mehrpour M, Am N  
**Country** Iran  
**University** Iran University of Medical Sciences  
**Department** Medicine  

**Introduction:** Migraine is defined as an often familial symptom complex of periodic attacks of vascular headache, usually temporal and unilateral in onset. Approximately 12% of population or 27 million people in the United States alone have migraine headaches. Many studies have been done to evaluate different risk factors for migraine headaches. Recently, the probable association between migraine with aura and right to left shunts has been found. These shunts are usually across a large patent foramen ovale (PFO).

**Objectives:** The aim of this study was to evaluate the prevalence of PFO among migraineurs by means of Transcranial Doppler Ultrasonography (TCD).

**Methods:** A total of 56 migraine patients consecutively admitted to the neurology outpatient clinic of Firouzgar hospital were studied. The diagnosis of migraine was based on the criteria adopted by the International Headache Society (IHS). All the migraineurs underwent TCD in order to detect PFO in their atrial septum. Subsequently, Transesophageal Echocardiography (TEE) was done in migraineurs with TCD evidence of PFO. The data were analyzed using SPSS v.13 software. Chi2; Independent T-test and Mann-Whitney U-test were used in analysis.

**Results:** The mean age of the patients was 30.75(SD = 1.17) years. The migraineurs were 14(25%) males and 42(75%) females. Additionally, 26(46.4%) had migraine with aura and 30(53.6%) had migraine without aura. The result of TCD shows that the prevalence of PFO was 32.1% (18 out of 56) in migraineurs; which is significantly higher than normal population (20%, P = 0.023). By the way, the prevalence of PFO was higher in migraineurs with aura (38.5%) than migraineurs without aura (23.7%). PFO was confirmed in 14 out of 18 (77.8%) migraineurs with aura by means of TEE.

**Conclusion:** PFO is more common in patients with migraine headache especially in migraineurs with aura. The association between migraine with aura and PFO suggests that the two entities are comorbid and may share a similar inheritance pattern. Many migraineurs may also benefit a significant improvement in frequency of headache attacks following PFO closure.

**Knowledge and attitude towards epilepsy among secondary school students in Gdansk**

**ESC-ID** 724  
**Author** Dagmara S, Wójtowicz J, Kass M  
**Country** Poland  
**University** Medical University of Gdansk  
**Department** Department of Adults’ Neurology  

**Introduction:** Epilepsy is one of the most prevalent neurological disease. Even today, people, that suffer from epilepsy, may experience social difficulties. The attitude towards epilepsy is influenced by the level of knowledge of this disorder.

**Aim:** The aim of the study was to establish the knowledge and attitude towards epilepsy of secondary school students in Gdansk.

**Material and methods:** The study population included 150 secondary school students in Gdansk (51 males and 99 females). The study instrument was a questionnaire containing 24 items about demographic data and general knowledge of epilepsy.

**Results:** More than 35% know the person suffering from epilepsy. The most common symptoms of epilepsy proffered by students include convulsions (95.3%), loss of consciousness and sudden stiffening (64%), muscle jerks of arms or legs (24%). The knowledge of seizure first aid is unsatisfactory. 70% of students would remove sharp object from around the person in order to prevent injury, 60% of respondents would loosen clothing around the person’s neck. However, 70% of students thought that inserting any objects in the person’s mouth is necessary; half of the respondents would try to hold the person down or restrain him or her is necessary. Twelve percent would be afraid to work with a person with epilepsy. 80% of the students considered epilepsy a hindrance to driving.

**Conclusions:** The knowledge of epilepsy among secondary school students is still unsatisfactory. Educational programs that would inform about this disorder and about seizures are needed.

**Plasticity related gene-3: a new brain specific protein involved in brain development?**

**ESC-ID** 317  
**Author** Savaskan NE, Moolenaar W, Nitsch R, Bräuer AU, Velmans T  
**Country** Germany  
**University** Freie Universität Berlin  
**Department** Institut für Zell- und Neurobiologie, Charité

In the central nervous system, regeneration of damaged axons is inhibited. Long-distance axon elongation is mainly restricted to the axon-formation phase during development. Recently a novel set of molecules were identified in the mammalian
Prevalence of various types of headaches among patients with cerebral stroke-preliminary evaluation

**ESC-ID** 631  
**Author** Mochort M, Sawicka M, Kozera G, Nyka W  
**Country** Poland  
**University** Medical University in Gdansk  
**Department** Medicine

**Introduction:** Headaches are one of the most common neurological symptoms. The International Headache Society Classification includes the primary headaches (migraine, tension-type, cluster headaches) and the secondary headaches (which are the symptom of other diseases).

**Aim of the study:** We aimed to retrospectively analyze prevalence of various headache types among patients with cerebral stroke.

**Materials and Methods:** The analysis of questionnaires filled in by patients with cerebral infarct hospitalized on AMG Neurological Ward in 2006/2007 was made. Studied group consisted of 36 patients - Tricity and region residents - 17 women in age 21 - 81 years (median 71) and 19 men in age 47-79 years (median 62). The results of analysis were confronted with data form control group composed of northern Poland residents.

**Results:** Data of 36 patients were analyzed (34 with ischemic and 2 with haemorrhagic stroke). 61% (22) of patients form studied group suffered from headaches before admission to the hospital because of stroke. The most common type of headache among patients with cerebral infarct was the secondary headache (72.7%). The primary headaches occurred in 6 cases (27.3%), 1 patient suffered from migraine, 3 from cluster headaches and 2 from tension-type headaches. Patients evaluated the intensity of their pain on average on 6 points in 0 to 10 points scale.

**Conclusion:** Results of our study showed that prevalence of headaches among patients with stroke and control group is similar, however patients from study group suffered more often form secondary headaches, which is the consequence of their past history of other diseases like hypertension, glaucoma, sinusitis etc.
Maximizing neuronal differentiation in striatal foetal neuronal stem cells

ESC-ID 737  
Author Ghanbar M, Fricker-Gates R  
Country United Kingdom  
University Keele University  
Department Neuroscience

**Aim:** To increase the proportion of neuronal cells derived from embryonic stem cells in once induced into differentiation in order to maximise the yield of neurones for use in transplantation. Introduction: Cell replacement therapy may provide a realistic future alternative for many neurodegenerative diseases. In particular, Huntington’s disease has been the focus of some researchers in their bid to recreate the striatal neural circuitry in this autosomal dominant condition. Although the art of transplantation is yet to be fully mastered, it seems by far the most appealing solution for such patients, and many animal models have been developed to test the efficacy of neural transplants. One choice of cells for neuronal replacement may be foetal neural stem cells. However, a major limiting factor in the progression of this field lies with being able to retain an adequate percentage of neurones derived from stem, once the stem cells proliferate in vitro. Current work shows that with time in culture the majority of neural stem cells end up as glia rather than neurones (1). One explanation for this may be that the in vitro environmental development for cell proliferation may not contain crucial signals present in the embryonic brain that drive neuronal differentiation.

**Method:** In this experiment, we try to test out different growth factors that have been shown to be pivotal in the development of the brain, in a bid to recreate an environment parallel to that in vivo. Factors used were retinoic acid, platelet derived growth factor, brain derived neurotrophic factor, neurotrophin-4, fibroblast growth factor-8 beta, and sonic hedgehog, all individually and in combinations.

Embryonic day 14 rat lateral ganglionic eminences were used to provide the source of striatal neural stem cells. These were plated with different combinations of growth factors in a standard differentiation medium. Cells isolated were also cultured in flasks for proliferation, with cells being replated from the cultures for differentiation on days 7 and 21. Immunocytochemistry was undertaken with markers specific for neurones (α-III tubulin) and gamma amino butyric acid (the neurotransmitter present in striatal neurones).

**Results and conclusion:** Preliminary results suggest that different neurotrophic factors may influence both differentiation of neural stem cells to GABA neurones and their survival/proliferation. More detailed results will be presented.

**Ophthalmology II**

**Autologous mononuclear cells are effective in improvement sclera regeneration**

ESC-ID 776  
Author Avetisov SE, Pylaev AS, Pavliuk AS, Trufanov SV, Baranov P  
Country Russia  
University Russian State Medical University  
Department Medico-Biological

**Introduction:** Progressive myopia is one of the most actual problems in ophthalmology. In spite of progress in prevention and therapy for this disease, it leads to irreversible changes of fundus of eye and considerable decrease of visual acuity among young people. The cause of the disease is morphological and structural insufficiency of scleral connective tissue. There is an ongoing search for alternative methods of treatment. We offered a cell therapy method for improvement of mechanical properties of sclera by amending it’s regeneration. Between fibroblast, mesenchymal stem cells and mononuclear fraction of blood, we have chosen the last one. Mononuclear fraction of blood consists of different cells. Monocytes and collagen-positive cells are able to differentiate into fibroblasts. Other cells can produce a wide range of cytokines and growth factors after activation, thus ‘simulating’ the processes of regeneration as if it was tissue damage. We assessed the effectiveness of mononuclear cells application for enlargement of sclera thickness.

**Material and methods:** Four chinchilla rabbits (eight eyes) underwent an operation of sclerectomy in upper quadrant in 5 mm away from the limbus. A superficial scleral flap (4 mm², 50% of sclera thickness) was excised and the conjunctiva was sewed above the defect. In one week after the operation the stitches were removed and suspension of autologous mononuclear cells activated by Toll-like receptors ligand (0.2 ml, about 80 000 – 100 000 cells) was injected under the conjunctiva in the operated area. The controls were: intact eyes, operated eyes without injection of cells, not-operated eyes with injection of cells (the amount of cells injected is equal in all groups). In six months the animals were sacrificed, the eyes were enucleated, fixed in formaldehyde and histological mounts were prepared. The quantitative criteria for evaluation of the mounts were sclera thickness and nuclei-tissue ratio.

**Results:** In six months the scleral tissue in three groups (intact eyes, sclerectomy, sclerectomy + injection of cells) looked like dense regular connective tissue with well-ordered fibers. In the group which underwent the cells injections in not-operated eyes – a not-ordered connective tissue banks were formed on the border between conjunctiva and sclera. The rows based on the assessed criteria (sclera thickness, nuclei-tissue ratio) in increasing order: intact sclera (210 μm, 0.05), sclera after sclerectomy (270 μm, 0.11), intact sclera after injection of activated cells (340 μm, 0.13), sclera after sclerectomy and injection of activated cells (440 μm, 0.14). The differences were statistically significant (Student t-test 0.05). Thus we can conclude that the specific density of sclerocytes in research group was significantly higher then in control groups (2.5 times increase of the nucleus-tissue ratio). The sclera thickness was 1.7 times larger then in control groups.

**Conclusion:** The subconjunctival injection of activated autologous mononuclear cells and scleral injury lead to...
increase of sclera thickness in later period. The mechanical properties of sclera seem to improve. The highest result in sclera thickness is reached in the case of combining injury and cell injection. These facts show the effectiveness of the cell therapy method in treatment of progressive myopia.

**Supine cyclotorsion and asphericity**

ESC-ID 672  
Author Lehnert R, Mueller M, Liekfeld A  
Country Germany  
University Charite Universitätsmedizin Berlin  
Department Ophthalmology

**Background:** Customized ablation to correct refractive errors is becoming more and more important today. Preoperative examinations are performed in a seated position and laser-surgery in a supine position. It is well known that cyclotorsions occur but until now there haven’t been any investigations on possible changes in astigmatism, root mean square (RMS) and asphericity.

**Methods:** 18 eyes of 14 individuals were measured in both seated and supine positions with a mobile videokeratoscope Keratron Scout (Optikon).

**Results:** A change from seated to supine position caused cyclotorsion in left eyes from -9° to 7° (p = 0.56) and -8° to 8° (p = 0.53) in right eyes. The average amount change of the cyclotorsion was 3.6°. The overall power of astigmatism increased to 0.3 D and decreased to 0.2 D compared to astigmatism in a seated position (p = 0.06). The Root Mean Square (RMS) of corneal wavefront increased to 0.20 μm and decreased to 0.24 μm in comparison to the same in a seated position (p = 0.36). The asphericity average changed from -0.19 in a seated position to -0.16 in a supine position (p = 0.04).

**Conclusion:** Cyclotorsion is statistically not significant when changing from a seated to a supine position as there occurs both excyclo- and incyclotorsion. Since individual cyclotorsion is clinically relevant, this has been taken into account by the latest laser systems. Changes in astigmatism and statistical significant changes in asphericity may be the reason for suboptimal customized refractive surgery. It must be considered whether the preoperative measurement for laser ablation in supine position should not be carried out in supine position as well.

**Contributions to the histological study of pterygium**

ESC-ID 440  
Author Ciocalteu AM, Popescu A, Lilea GC, Craitoiu S  
Country Romania  
University Universitatea de Medicina si Farmacie Craiova  
Department Histology

**Introduction:** Pterygium is an epithelial hyperplasia associated with a fibrovascular growth, originating in the cornea-conjunctive junction. This disease raises a number of debate issues: relatively high frequency, relapses following surgical excision, great variations in clinical aspects (ranging from locally invasive to mild dysplasia and, although it is a relatively benign process, even in-situ carcinoma), and grave impairment of the eye’s functionality in the more severe cases (by extending over a great surface of the cornea, by inducing irregular astigmatism, by creating lesions in the corneal stroma and by covering the visual axis).

**Aim:** The aim of this study is to contribute to the clarification of some aspects related to the etiopathogenesis of the disease which, in spite of many advanced theories, is insufficiently explained.

**Materials and methods:** We have used pieces of primary and relapsed pterygium, and also normal conjunctive membrane, from patients with surgical excision of pterygium followed by a conjunctive membrane autograft. The materials were processed histologically by paraffin inclusion, then followed by both routine and immunohistochemical staining. We have also used tests for assessing the lachrymal function.

**Results:** Histological examination has shown a proliferative epithelium of several layers, decreasing in girth towards the advancing head of the pterygium. At the site where it crosses the normal cornea. Underneath the advancing head there is an accumulation of fibrous and/or elastic connective tissue. At this site we have found inflammatory cells and blood vessels which could supply both stimulating signals and nutrients for proliferation and tissual invasion. Another characteristic trait of the pterygium is the loss of basal membrane, a natural barrier separating the epithelium form the stroma underneath it.

**Conclusions:** We have described several histological traits of the pterygium which help elucidate some aspects of its etiopathogenesis. These may also contribute to improving the surgical therapy employed and to developing non-surgical strategies, in order to reduce relapse, inflammation severity, tissual invasion and angiogenesis.

What is the target intraocular pressure in pseudoexfoliative glaucoma?

ESC-ID 358  
Author Glowacka DA, Sawicka A  
Country Poland  
University Medical University of Bialystok  
Department Ophthalmology

**Introduction:** Pseudoexfoliation syndrome frequently leads to secondary open-angle glaucoma. Rise in intraocular pressure is attributed to the blockage of trabeculum by pseudoexfoliative material and/or iris pigment and trabeculum endothelium dysfunction. Beside pharmacotherapy and surgery, laser trabeculoplasty is a method of treatment.

**Aim:** The aim of the study was to assess the target intraocular pressure in patients with pseudoexfoliative glaucoma after treatment with laser trabeculoplasty.

**Material and methods:** The retrospective study covered 9 patients (4 females and 5 males) with diagnosed one-eye open-angle pseudoexfoliative glaucoma. They were all patients of Ophthalmology Out-Patients Clinic at the Medical University Hospital in Bialystok, Poland. The mean age equaled 71.6 ± 4.9 years. In this selected group the fellow eye served as a control. The disease remained in the one-eye form during the observation period of 35.4 ± 12.7 months, including 28.7 ± 17.6 months after laser trabeculoplasty. The Nd-YAG laser procedure covered 180 or 360 grades of trabeculum circumference (parameters: 40 and 80 burns, respective ly; 100msek / 800mW / 50 microm). The intraocular pressure was measured with Schiötz tonometer before and every 3 months after laser trabeculoplasty. Visus was assessed according to Snellen chart with the best ocular correction.
Statistical analysis was conducted with the Mann Whitney test.

Results: Despite pharmacological treatment, the mean intraocular pressure values before laser trabecuoplasty equaled 35.2 ± 12.3 mmHg (norm: 16 ± 6 mmHg). The mean intraocular pressure (mmHg ± SD) was: 20.0 ± 8.7, p < 0.005 after 3 months; 15.7 ± 1.7, p < 0.0005 after 6 months; 21.0 ± 5.1, p < 0.005 after 9 months; 16.6 ± 6.9, p < 0.005 after 12 months; 19.6 ± 9.1, p < 0.005 after 15 months; 18.1 ± 4.5, p < 0.0005 after 18 months; 18.0 ± 5.4, p < 0.005 after 21 months and 17.5 ± 2.8, p < 0.0005 after 24 months. Comparing the involved and fellow eyes the mean intraocular pressure values before the laser trabecuoplasty statistically significantly differed (p < 0.00005). However, the difference was not statistically significant after the therapy (p = 0.071). The visus difference (number ± SD) in the involved eyes before (0.76 ± 0.3) and 24 months after (0.67 ± 0.3) the applied therapy was not statistically significant (p = 0.397).

Conclusions: Laser trabecuoplasty is an effective method of reducing and stabilizing the intraocular pressure and secures from visus deterioration. However, the further results are dependent of topical medical treatment for glaucoma.

Central corneal thickness and medically uncontrolled primary open-angle glaucoma

ESC-ID: 65
Author: Ghasami Esfe AR, Abdollahi A, Attarchi H, Mehran S, Bozorg V
Country: Iran
University: Tehran University of Medical Sciences
Department: Ophthalmology/Radiology

Aims: Current evidences have determined clearly the role of central corneal thickness (CCT) in progression and development of Primary Open-Angle Glaucoma, but its effect on the level of glaucoma severity remains uncertain. This study was designed to expand the available knowledge about the relationship between central corneal thickness and glaucoma severity with comparing two medically controlled and medically uncontrolled glaucoma groups.

Patients and Methods: Patients with past diagnosis of open-angle glaucoma, who were seen at glaucoma clinic of Farabi Hospital, participated in this retrospective case – control study. Patients in Case group were those with primary open-angle glaucoma that intra ocular pressure (IOP) reduction of their affected eyes with maximum dose of medications has not been sufficient and because of visual field loss with or without increased in cup-to-disc ratio, they had been got elected for trabeculectomy. Control group was containing patients with primary open-angle glaucoma whom their disease has been under control with medical treatment and they were not considered for glaucoma filtering surgery.

Results: Central corneal thickness and medically uncontrolled primary open-angle glaucoma. It seems, like some recent evidences central corneal thickness could not predict the level of glaucoma severity and this is contrary to well recognized ability of central corneal thickness to predict development of primary open-angle glaucoma.

Orthopedics and Traumatology II

Osteometric indices in patients with cervical discopathy and spondylosis

ESC-ID: 307
Author: Kot M, Lukomski T, Anczykowski G
Country: Poland
University: University of Medical Science in Poznañ
Department: Neurosurgery and Neurotraumatology

Introduction and the aim of the study: Degenerative changes in cervical spine lead to a compression of nerves and vascular structures. Patients with progression of neurological symptoms are qualified to surgical treatment. The aim of this study was to establish dependence between osteometric indices and neurological condition of patients with cervical discopathy and spondylosis.

Material and methods: Clinical analysis concerned 47 patients operated in Clinic of Neurosurgery and Neurotraumatology in Poznañ in 2003 to 2005. Material included 30 males (64%) and 17 females (36%), aged from 32 to 77. One level discopathy was present in 15 patients and multilevel - in 32 patients. Authors used JOA (Japanese Orthopaedic Association) scale to assess neurological condition of patients. On the basis of plain radiogram and MRI scanning of cervical spine the following osteometric indices were established: lordosis index, pincer’s index (sagittal diameter of spinal canal in the place of discopathy), Pavlov’s index (proportion between the diameter of vertebral body and diameter of vertebral cana), intervertebral space height index. Evaluation of pathological changes of T1, T2, PD-dependent time of MRI scanning was performed too.

Results: Statistical analysis revealed significant association between neurological condition (JOA scale) and the extension of operation and mode of stabilisation. Moreover distinguished indices could be useful in planning the extension of operation and mode of stabilisation.
Clinical use of Dynastab -N (wrist) external fixator in the treatment of articular and periarticular fractures of the radius distal part

ESC-ID 519
Author Deszczynski J, Stolarczyk A, Nagraba L, Mitek T
Country Poland
University Medical University of Warsaw
Department Orthopedics

Introduction: Fracture of the distal radius is the most common in the upper limb. Common statements that these are simple fractures with good prognosis for treatment are not so clear nowadays, as we observed a large number of unsatisfactory results in the treatment of those fractures.

Materials and methods: The purpose of this work is the analysis and clinical, radiological evaluation of early results of functional treatment of distal radius fractures with the aid of Dynastab N (wrist). In period from August 2004 to May 2005 11 patients were qualified for treatment with Dynastab N according to Cooney’s classification. There were 11 patients, four men, seven women, mean age 46 years. Follow up period was at least 1 year.

Results: The use of Dynastab N allowed for early movement of the wrist. The results were qualified according to the clinical criteria of Garland - Wherley in Sarmiento modification.

Resorbable scaffolds as a potential support for bone tissue formation in vitro

ESC-ID 788
Author Wójewódzka J, Wojewódzka M, Mikulska A
Country Poland
University Medical University of Warsaw
Department Biophysics and Human Physiology

The classical scheme of tissue engineering is engraftment of 3D biomaterial overgrown by patients own cells. Resorption of the scaffold is expected to occur after implantation. This is, however, difficult to be controlled. So our perspective aim is to obtain a construct of cells and extracellular matrix on a biomaterial, which would be already resorbed in vitro i.e. at the preimplantation stage. This futuristic Tissue Engineered Product (TEP) would imitate an autologous piece of bone. The aim of this study is optimising a scaffold for such a purpose as the first step toward TEP. MG63 cells were cultured on 8 three dimensional materials from the group of commonly used polyesters (PLA and PGA). Two types of original polymer were used as well as polymers enriched with calcium carbonate particles added in order to neutralise the acids concentration, especially deep within the scaffold pores. The expected benefit from CaCO3 was not achieved probably because it was hidden under the material surface, thus unable to alkalise the medium and at the same time, worsening the surface topography (SEM) favoured by cells. It is suggested that the better results could be achieved if the technology of obtaining CaCO3 enriched scaffolds would result in its accessibility on the surface. The promising result is the availability of a set of materials which undergo the controlled resorption in vitro within the period which should be proper for bone tissue formation in culture.

Demineralized bone matrix as an effective tool in tissue engineering

ESC-ID 951
Author Wojewódzka M, Wójtowicz J
Country Poland
University Medical University of Warsaw
Department Department of Biophysics and Human Physiology

Introduction: Demineralized bone matrix (DBM) bone inducing abilities were confirmed both in animal experiments and clinical applications in vivo. Thus, it is expected that the addition of DBM to human derived cells (HBDC) may be the promising differentiation agent used for preparation of bone tissue engineered product (TEP) in vitro. The positive influence of autogenic DBM on HBDC was proved. The aim of this study is to verify osteoinduction potential of autogenic radiation sterilized DBM fabricated in tissue bank.

Materials and Methods: In the experiment DBM in three different multiform shaped particles was used: powder ($\varnothing = 200\mu m$), granules ($\varnothing = 1mm$) and flakes ($\varnothing = 4mm$). Samples were sterilized by irradiation (25kGy). Investigated materials were placed in 24-well culture dish. HBDC were cultured in direct contact with DBM samples in standard medium (DMEM) supplemented with 10% FCS, L-ascorbic acid and antibiotic, under standard conditions (37°C, 5% CO2, humidified atmosphere). Control wells contained no DBM. Cell viability, proliferation, differentiation were assessed. Cell morphology in direct contact with tested native osseous tissue was characterized in microscope on 4th, 7th and 8th day of cell culture. After the longest culture period alkaline phosphatase activity (ALP Sigma Kit), total protein measurement (BCA PIERCE assay) and osteocalcin - specific marker of terminally differentiated osteoblasts (ELISA) concentration were performed.

Results: Microscopic observation of cell morphology showed the proper attachment and spreading of cells with particular affinity to DBM particles in all cases. The presence of DBM particles were found to significantly stimulate alkaline phosphate activity (ALP). Protein staining (PIERCE) showed that amounts of protein were about the same in all the investigated populations. Osteogenic markers were higher in cultures with DBM supplementation comparing to the control.

Discussion and Conclusions: The results obtained show the effectiveness of autogenic, radiation sterilized DBM gained, and CaCO3 used in the experiment was found to be well tolerated by cells. The process of material resorption was advanced after the longer culture. Materials resorption probably resulted in a local overrunning the lethal acids concentration, especially deep within the scaffold pores. The expected benefit from CaCO3 was not achieved probably because it was hidden under the material surface, thus unable to alkalise the medium and at the same time, worsening the surface topography (SEM) favoured by cells.

Demineralized bone matrix particles induced osteogenesis in vitro.

Materials and Methods: In the experiment DBM in three different multiform shaped particles was used: powder ($\varnothing = 200\mu m$), granules ($\varnothing = 1mm$) and flakes ($\varnothing = 4mm$). Samples were sterilized by irradiation (25kGy). Investigated materials were placed in 24-well culture dish. HBDC were cultured in direct contact with DBM samples in standard medium (DMEM) supplemented with 10% FCS, L-ascorbic acid and antibiotic, under standard conditions (37°C, 5% CO2, humidified atmosphere). Control wells contained no DBM. Cell viability, proliferation, differentiation were assessed. Cell morphology in direct contact with tested native osseous tissue was characterized in microscope on 4th, 7th and 8th day of cell culture. After the longest culture period alkaline phosphatase activity (ALP Sigma Kit), total protein measurement (BCA PIERCE assay) and osteocalcin - specific marker of terminally differentiated osteoblasts (ELISA) concentration were performed.

Results: Microscopic observation of cell morphology showed the proper attachment and spreading of cells with particular affinity to DBM particles in all cases. The presence of DBM particles were found to significantly stimulate alkaline phosphate activity (ALP). Protein staining (PIERCE) showed that amounts of protein were about the same in all the investigated populations. Osteogenic markers were higher in cultures with DBM supplementation comparing to the control.

Discussion and Conclusions: The results obtained show the effectiveness of autogenic, radiation sterilized DBM gained.
from tissue bank as the differentiation factor in a culture of cells to be used in TEP. This may be of a high practical value, especially due to the growing interest in the potential use of stem cells which are to be differentiated in vitro prior to therapeutic implantation.

**Quantitative assessment of growth factors in reaming aspirate, iliac crest and platelet preparation**

**Aim:** Autogenous bone transplants, allografts, bone substitutes or stimulation with growth factors or the growth factors containing platelet-rich plasma (PRP) play an important role in the treatment of bone defects and non-unions. These materials vary on their biological and material-specific properties as well as on their mode of harvesting or their fabrication. Autografts have been an established treatment option since many years. Iliac crest is widely used in clinical practice. Although results are excellent, their use is associated with a high incidence of graft site morbidity[1]. Reaming debris, which can be harvested using a new Reaming-Irrigating-Aspirating system could be a valuable alternative. It is easy to harvest and delivers a high amount of graft, which contains cells and growth factors. Another clinically available source for autologous growth factors is PRP. It is obtained from venous blood using a simple, standardized technique. The aim of this study was to compare the quantity of various growth factors within iliac crest, bony reaming debris, reaming irrigation fluid, and PRP.

**Methods:** Iliac crest, reaming debris and irrigation samples were obtained from patients that underwent surgery to harvest graft material for defect filling. The iliac crest samples were pulverized using a cooled mill (Retsch, Haan, Germany). The reaming debris was homogenized with an ultra turrax (IKA-Labortechnik, Staufen, Germany). The homogenized bony material was then diluted for 2 h in PBS plus Proteinase inhibitor at 4°C. A definite amount of the PBS puff er was added, according to their measured total protein concentrations, to a homogenous total protein concentration in all samples. After extraction the samples were centrifuged at 12,000 rpm and the supernatant was stored at -80°C. Volunteers donated blood, which was then further processed into Platelet Rich Plasma using a commercially available enrichment-system (GPS, Biomet Merck Biomaterials, Darmstadt, Germany). For further analysis Platelet Poor Plasma (PPP) was obtained from the same samples. All samples were stored in PBS plus Proteinase Inhibitor at -80°C before final analyzing was performed. The content of growth factors in the individual samples was determined by the ELISA method, the total protein concentration using the Coomassie Protein Assay (Pierce, Perbio Science GmbH, Germany). The growth factor concentration was correlated to the weight of the bony materials or the volume of the liquid products.

**Results:** Elevated levels of FGFα, PDGF, IGF-I, TGF-β1 and BMP-2 were measured in the reaming debris as compared to iliac crest curettings. However, VEGF and FGfb were significantly lower in the reaming debris than from iliac crest samples. In comparing PRP and PPP all detectable growth factors, except IGF-I, were enhanced in the PRP. In the reaming irrigation FGFα (not measurable in PRP) and FGFB were higher, but VEGF, PDGF, IGF-I, TGF-β1 and BMP-2 were lower compared to PRP. BMP-4 was not measurable in any sample.

**Conclusion:** Using ELISA technique, we quantified comparable growth factors values in reaming debris and iliac crest. PRP contained a higher concentration of growth factors compared to PPP except for IGF-1. In the reaming irrigation growth factors were also measurable. The use of bony reaming debris alone or in combination with reaming irrigation fluid may be an alternative method of obtaining significant amounts of autogenous bone rich on growth factors.

**In vivo tracking of locally transplanted mesenchymal stem cells following skeletal muscle trauma**

**Aim:** Functional deficiencies after severe skeletal muscle trauma remain to be a serious problem in orthopaedic and trauma surgery. Transplantation of autologous bone marrow derived stem cells improves muscle contraction force after a blunt crush trauma of the soleus muscle in the rat (Matziolis et al. 2006). Until now the only possibility of gathering information about the fate of the transplanted cells has been the histological analysis of the target tissue. The behaviour of the transplanted cells in a traumatized muscle between the date of transplantation and histological analysis is unknown. Stroh et al. could observe systemically administered mononuclear cells in the ischemic brain using citrate-coated very small superparamagnetic iron oxide nanoparticles (VSOP) and high-field magnetic resonance imaging (MRI) (Stroh et al. 2006).

**Methods:** The presented study was designed to track locally administered bone marrow derived stem cells (BMDSC) in vivo after a severe crush trauma of rat skeletal muscle using 7 Tesla MRI.

**Results:** Labelling of the BMDSC at iron concentrations between 0.375 mM to 6 mM yielded vitality values of the cells of more than 95%. Proliferation assays showed that incorporation of VSOP did not lead to cytotoxic effects. The relaxometry of 1x106 cells loaded with increasing concentrations showed a decrease of the transverse relaxation time up to 62% of the control. The iron content increased up to 6.33µg/1x106 cells. Labelled cells generating a hypointense
signal within the traumatized muscle could be detected clearly in all used scan modes. Even over a period of 6 weeks detection of the cells was possible. The volume of the reconstructed cell pools showed a decrease of 60-70 percent after 42 days. Since the centre of gravity was identified nearly at the same place in the muscle over 42 days, we conclude that the transplanted MSC did not migrate significantly within the traumatized muscle. The histological results showed fusion events between the transplanted cells and muscle fibers. The location of the cells observed in the MRI scans corresponded with the histological results of the Prussian Blue staining.

**Conclusion:** Our results demonstrate that very small iron oxide nanoparticles can be an efficient tool for in vivo detection of mesenchymal stem cells after local transplantation into severely traumatized skeletal muscle.

**Predictors of blood transfusions in emergency and elective spinal surgery**

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<th>ESC-ID</th>
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<tr>
<td>Author</td>
<td>Fitzpatrick P, Butler JS, Queally J, McCormack D, Synnott K, Pyonton AR</td>
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<td>Country</td>
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<td>University</td>
<td>University College Dublin</td>
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<td>Department</td>
<td>UCD School of Medicine and Medical Science</td>
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**Background:** The blood loss and transfusion requirements of patients undergoing emergency and elective spinal surgery have always been considered to be significant. However, there is a paucity of data on the determinants of blood transfusion requirements. Patients undergoing spinal surgery at the National Spinal Injuries Unit (NSIU) at the Mater Misericordiae University Hospital between 1 Jan 1996 and 31 Dec 2005 (n = 1596). The Hospital Inpatient Enquiry (HIPE) System and NSIU databases were used to identify our study cohort. Data was collected from the MMUH transfusion laboratory database and supplemented with data from the Hospital Inpatient System (HIS) and the medical records of patients. Risk factors evaluated included age, sex, preoperative haemoglobin, reason for surgery, type of surgical procedure, level of surgery and surgical approach. We evaluated risk factors based on units of blood transfused. **Results:** There were 1596 spinal surgical procedures performed at the NSIU over a ten-year period. Of these cases, 22% (350/1596) required a blood transfusion. Preoperative haemoglobin, tumour surgery and number of posterior levels surgically fused were the greatest determinants of transfusion requirement.

**Conclusion:** Several risk factors in the spinal surgery patient were identified corresponding to increased transfusion requirements. A greater awareness of the risk factors associated with transfusion is required in order to optimize patient management.

**External fixators with parallel and with convergent pins – Comparing of stability balances**

**ESC-ID:** 203
**Author:** Mitkovic M, Rancic M
**Country:** Serbia
**University:** University of Nis, Medical Faculty
**Department:** Medicine

**Aim:** The aim of this study was comparing of stabilities of external fixator with parallel pins, with the position in the same plane, and external fixator with convergently oriented pins, with position in different planes.

**Material and methods:** We used external fixator Orthofix which provides at list 4 pins to be inserted in the same plane, and external fixator Mitkovic which provides at list 4 pins to be inserted in different planes. These fixators were applied on each tube like long bone model, made of wood, cut on the middle with gap of 8mm. On these bone models fragments, there were applied 10kg of loads, in 2 directions perpendicular on long axis of bone models, with position in 2 planes (plane A and plane B) perpendicular to each other with gap throw long axis of bone model: in the use of Orthofix fixator one of these directions was in the plane of all pins, and in the use of Mitkovic fixator each of these directions was in the plane of one of pair of pins. At the same time there were measured fragments movements produced by the used loads.

**Results:** Fragments movements were: 1. Orthofix fixator: a) plane A - 0,3mm, b) plane B - 1,1mm; 2. Mitkovic fixator: a) plane A - 0,9mm, b) plane B - 1,6mm.

**Conclusion:** According to comparation of 1a:1b and 2a:2b relations it can be seen that balanced stability in two perpendicular planes of external fixator with convergent pins is significantly higher in comparison to external fixator with parallel pins. As natural long bones have balanced stability in different perpendicular directions in that regard, it can be concluded that external fixators with convergent pins are more suitable for long bone fracture fixation.

**Stimulation of fracture healing by local application of a Bisphosphonate**

**ESC-ID:** 162
**Author:** Back D, Greiner S, Alidoust M, Schmidmaier G, Wildemann B
**Country:** Germany
**University:** Charité Universitätsmedizin Berlin
**Department:** Medicine – Traumatology

**Objective:** Zoledronic acid (ZOL) a nitrogen containing bisphosphonate [3] has been shown in vitro and in vivo to inhibit osteoclastic activity and to regulate cell proliferation, differentiation and gene expression in osteoblasts [1]. Its antiresorptive effect is clinically used in the treatment of bone consuming pathologies like tumor associated bone metastases or tumor induced hypercalcaemia. Because of their impact on bone cells there might be a possible benefit in treatment of fractures by local application of this potent substance. Moreover, local application could be able to increase the therapeutic fence, local efficiency and reduce systemic side effects [2]. Aim of the study was to analyze the effect of local application of ZOL from a biodegradable Poly(D,L)-Lactide
Do running shoes go the distance?

ESC-ID  451
Author  Clinghan R, Abboud R
Country  United Kingdom
University  Dundee
Department  Orthopaedics

Aim: Running shoe manufacturers suggest that you should change a pair of running shoes every 300 miles. This study aims to investigate whether the cushioning capabilities of more expensive running shoes are more durable than cheaper alternatives and do running shoes provide enough protection from impact force after 300 miles of wear. Method Three pairs of neutral UK size 10 running shoes from three different manufacturers were purchased at three different price ranges: low (£40-45); medium (£60-65) and; high (£70-75). The right shoe of each pair was tested in a universal testing machine that simulated 300 miles of wear. The left shoe was not tested and acted as a reference. After testing, plantar pressure was recorded in both shoes from 12 subjects (mean age 26.3, SD = 10.2) with the Pedar® in-shoe pressure measurement system, over approximately 15 footfalls.

Results: Midsole stiffness increased with wear. Mean peak pressure was relatively lower in most of the tested shoes. No significant difference in mean peak pressure was observed after 300 miles with respect to brand and cost. In the majority of shoes tested, mean instant of peak pressure occurred significantly earlier in medium and high cost shoes than low cost shoes.

Conclusion: Decreasing mean peak pressure with increasing midsole stiffness may be accounted for by a change in foot and leg kinematics. A cheap running shoe is as good (if not better) than a more expensive running shoe. No brand of shoe provided superior durability of cushioning.

Monitoring and appraisal of bone union inflexibility of fractures treated by Dynastab DK external fixator

ESC-ID  524
Author  Deszczysiński J, Mitek M, Stolarczyk A, Nagraba L, Mitek T, Lesnial M
Country  Poland
University  Medical University of Warsaw
Department  Orthopedics

This work focuses on problems of heuristic techniques based on artificial intelligence. Mainly about artificial non-linear and multilayer neurons, which were used to estimate the bone union fractures treatment process using orthopaedic stabilizers Dynastab DK. The author utilizes computer software based on multilayer neuronal network systems, which allows to predict the curve of the bone union at early stages of therapy. The training of the neural net has been made on fifty six cases of bone fracture which has been cured by the Dynastab stabilizers DK. Using such trained net, seventeen fractures of long bones shafts were being examined on strength and prediction of the bone union as well. Analyzing results, it should be underlined that mechanical properties of the bone union in the slot of fracture are changing in nonlinear way in function of time. Especially, major changes were observed during the forth month of the fracture treatment. There is strong correlation between measure number two and measure number six. Measure number two is more strict and in the matter of fact it refers to flexion, as well as the measure number six, to compression of the bone in the fracture slot. Consequently, deflection loads are especially hazardous for healing bone. The very strong correlation between real curves and predicted curves shows the correctness of the neuronal model.

Management of haemophilic knee flexion contractures: Trapezoid supracondylar femoral extension osteotomy

ESC-ID  903
Author  Heidari P, Mortazavi SMJ, Motamedi M
Country  Iran
University  Tehran University of Medical Sciences
Department  Iranian Tissue Bank, Orthopedics Ward, SSRC

Background: Recurrent intraarticular bleeding in haemophilic patients could lead to a fixed flexion contracture of the knee [1]. Conservative corrective measures are to be tried first; these include serial casting[2], reversed dynamic sling [3, 4], and extension desubluxation hinges [5]. In severe contractures (>30°) corrective surgical procedures have to be done. Hamstring release and posterior capsulotomy [6, 7], and supracondylar femoral extension osteotomy [8], have been advocated.

Aim: To report indications, technique, and results of trapezoid supracondylar femoral extension osteotomies in the management of severe haemophilic flexion contractures of the knee joint.

Methods and Materials: From 2001 to 2005, 9 male haemophilic patients (11 knees) with severe (mean degree of contracture of 51.4°) unresponsive fixed knee flexion contractures underwent trapezoid supracondylar femoral extension osteotomies. The patients had either severe haemophilia (PDLLA) coating of osteosynthetic implants on biomechanical fracture stability.

Material and Methods: Standardized midshaft fractures of the right tibia of 5-month-old female Sprague-Dawley rats (n = 49) were stabilized with uncoated, PDLLA-coated and ZOL/PDLLA-coated titanium Kirschner wires. For detection of systemic side effects, blood analyses, body weight and temperature were taken throughout the experimental period. X-ray examinations were performed regularly. Tibiae were dissected 42 and 84 days after fracture for mechanical torsional testing.

Results: No systemic side effects could be detected. Radiological evaluation showed significantly increased bridging in the ZOL-treated group at 28 and 42 days, without any difference between the groups at 84 days. Biomechanical testing of the tibiae showed significantly higher maximum load and torsional stiffness in the ZOL-treated group compared with the controls at 42 days. Maximum load of the control groups reached the value of the ZOL coated group at 84 days. Torsional stiffness of the ZOL-treated group remained significantly higher than the uncoated group, whereas there was no significance between the ZOL- and PDLLA-coated groups anymore.

Conclusion: Results demonstrate that local application of ZOL from a PDLLA coating is an effective pharmacological path to accelerate the achievement of biomechanical stability in fractures.
type A or B. All were unable to walk without an assistant device, climb the stairs, and use means of public transportation. Two patients with bilateral knee involvement were wheelchair-bound. The angle of deformity was measured using an anteroposterior and lateral x-ray films in maximum knee extension. An isosceles trapezoid osteotomy with the lesser parallel side on the posterior cortex was to be done. The prolongation of non-parallel sides should have crossed at the deformity angle. A factor activity of 100% was achieved before the operation and was maintained to >80% for two next days. The osteotomy was done and reduction was obtained using an AO condylar blade plate. Physiotherapy was begun on 3rd postoperative day 3 times a week. Full weight bearing was not allowed for 3 months. Clinical results were assessed according to recommendations of Orthopaedic Advisory Committee (OAC) of World Federation of Haemophilia [9]. Radiographic result classification was based on Pettersson score [10].

Results: There were no serious complications after surgery. All of the patients had a subjective feeling of improvement in routine daily activities. All were able to extend their knees to full extension. Increased range of motion of the operated knees was observed in all patients except one with an ankle-lowered knee before operation. The mean range of motion increased from 68.6° to 98.2°. The OAC physical examination score decreased in all knees. There were 9 good and 2 fair OAC score results. Pain was decreased in 4 patients, increased in 2, and unchanged in the remaining. All 4 patients with frequent joint bleeding before the procedure (bleeding score 3), reported a marked reduction in bleeding episode (two bleeding score 0 and two bleeding score 1).

Conclusion: The trapezoid supracondylar femoral extension osteotomy should be considered as an alternative in the management of severe, fixed flexion contracture of the knee joint that is unresponsive to conservative measures in patients with haemophilia.

Otorhinolaryngology

Low level laser therapy positively influences healing of tracheal anastomoses in rats

ESC-ID 247
Author Grendel T, Sokolsky J, Polakova M, Hudakova L, Sabo J, Longauer F
Country Slovakia
University Pavol Jozef Safarik University in Kosice
Department Department of Medical Biophysics

Tracheal stenosis presents a difficult management problem in medical treatment (1). Late diagnosis could often lead to the death of patients because of stifling (2). Most nonmalignant upper tracheal stenoses are caused by prolonged endotracheal intubation or tracheotomy (3). Low-level laser therapy (LLLT) belongs to modern experimental approaches used in wound healing therapy (5). Nevertheless, there is no evidence about the effect of LLLT on tracheal wound healing. The aim of our study was to evaluate the effect of LLLT on the healing of tracheal anastomoses in Sprague-Dawley rats. Male Sprague-Dawley rats (n = 60) of similar age (8-10 months) were randomly divided into two groups: control and laser treated. Animals were anesthetized with combination of ketamine, xylazine and tramadol. During the surgery, additional inhalation anesthesia (oxygen, nitrous oxide and halothane) was used. Animals were placed in the supine position and intubated with endotracheal tube. Consecutively, the wall of trachea extending from second to fifth tracheal ring was vertically incised and immediately closed with absorbable sutures (4). In laser treated group (n = 30), the laser radiation was applied daily during the first seven postoperative days (POD) (830 nm, 450 mW, 60 J/cm²). Six animals from both groups were sacrificed on POD 3, 5, 7, 14 and 28, respectively. Trachea was excised, stained with hematoxylin and eosin and histopathologically evaluated. Reepithelization, polymorphonuclear leukocytes (PMNL), tissue macrophages (TM) and neangiogenesis were semi-quantitatively evaluated in coded slides. The histological changes were marked by the scale of four points (0, 1, 2 and 3). Statistical analysis was performed using the Mann-Whitney U test. Area of granulation tissue and the PMNL/TM ratio was calculated using the software and the unpaired Student's t-test was used for statistical analysis. Histological evaluation revealed acceleration of reepithelization in laser treated group (3, 5 POD, p<0.05). At 7, 14, 28 POD the process of reepithelization was completed in both groups. The PMNL/TM ratio was lower in stimulated wounds (3 POD, p<0.05). Thus, the acute inflammation was more reduced. The area of granulation tissue was significantly smaller in laser treated group (7, 28 POD, p<0.05). In addition to these results, no significant differences in other evaluated parameters were observed. In conclusion, LLLT positively influenced tracheal anastomoses healing by acceleration of epithelization, inhibition of acute inflammation and reduction of granulation tissue. Although granulation tissue is needed for integrity and stability of injured tissue, it may be sometimes excessive causing tracheal stenosis (4). Since we showed the reduction of granulation tissue, we hope that LLLT opens new approach to the management of tracheal stenoses. However, further research is needed to confirm the exact mechanism of these effects.

Influence of hypertrophy of pharyngeal tonsil on dysfunction of auditory tubes in children

ESC-ID 237
Author Jakhongir K, Sayfiddin A
Country Uzbekistan
University Tashkent Medical Academy
Department Treatment

Heading: Contradictory information about the role of hypertrophy of pharyngeal tonsil in formation and state of dysfunction of auditory tubes are given in literature. Most authors are saying that hypertrophy of pharyngeal tonsil plays key role in formation of dysfunction of auditory tubes. Aim: The purpose of the given research was the estimation of influence of a hypertrophy pharyngeal tonsil on current dysfunction of auditory tubes at children with studying parameters before and after adenotomy.

Methods: We had been investigated 19 children in the age of 5 till 8 years. Among them girls - 8, boys - 11. In development have been included children at whom other factors resulting in dysfunction of auditory tubes have been whenever possible excluded, i.e. a hypertrophy of pharyngeal tonsil, was unique reasonable factor of the given disease. At all patients before operation estimated complaints, carried out endoscopic research of an ear, a cavity of a nose and a nasopharynx, research of hearing acuity (acumetry) and tympanometry. In
given endoscopic researches of a nasopharynx at 6 patients the hypertrophy of pharyngeal tonsil covered more than 10 – 13, and in 3 less than 13 heights of shovel. According to the international classification of hearing disorder at 6 patients it is revealed I degree and 13 – II degree of reduction of hearing. It was registered tympanogram type C on classification of J. Jerger in all patients. Estimation of influence of operation on dysfunction of auditory tubes began in 1 month, i.e. after calm down of the reactive phenomena in a nasopharynx. Repeated investigations of children carried out in 3 and 6 months after surgical intervention. In the postoperative period treatment of dysfunction of auditory tubes it was not carried out.

Results: By the end of time of observation in 17 children have not presented complaints. Endoscopic picture corresponded to norms and it was registered tympanogram type A, i.e. at these patients full clinical recovery is achieved. At 2 children clinical improvement that was expressed by complaints to moderate reduction in hearing is established, i.ä. in them was I degree of deafness with positive dynamics after operation, presence of insignificant indrawn of ear drums though at them registered tympanogram type A. For the period of observation efficiency of adenotomy at an estimation of results only on full clinical recovery has made 89.5 %.

Conclusions: The hypertrophy of pharyngeal tonsil is the causal factor of development of dysfunction of auditory tubes at children. Adenotomy is positively influences a functional condition of ear organs as a whole and an auditory tube in particular at children with dysfunction of an auditory tubes.

Otoscopic and audiological evaluation of children after operational treatment of secretory otitis media
ESC-ID 84
Author Kiciel M, Kornatowska K, Kulinska M, Szafarowski T, Steczen M
Country Poland
University Medical Academy in Warsaw
Department Medical

Introduction: Secretory otitis media (SOM) is one of the causes of conductive hearing loss in children. Its presents with fluid in middle ear without features of acute inflammation. Typical for conductive hearing loss in SOM is air-bone gap in pure-tone audiometry and type B or C impedance audiometry. Operational treatment are tympanocentesis or ear drainage. If SOM is accompanied with adenoid and palatine tonsils hypertrophy, an adenooidectomy or adenontonsillotomy is conducted.

Aims: The aim of this work was otoscopic and audiological evaluation of children after operational treatment of SOM. Additionally the occurrence of factors which could potentially protect or lead to the disease was estimated.

Materials and methods: Students Science Society in the Pediatric Otolaryngology Department was permitted by the Bioethical Comission of Medical Academy to conduct an independent scientific project. The analysis was based initially on 41 patients (19 girls, 21 boys) aged 5 to 17 years (mean 8.8) with SOM and were subjected to tympanocentesis with adenotomy / adenotonsillotomy between 2003-2006. Patients had otoscopic measurement with ear microscope to assess the state of tympanic membrane and audiological measurement (pure-tone and impedance audiometry). Children were divid-
Modern therapeutical attitude in cervical oesophageal neoplasm (Procedures of pharyngo-oesophageal surgery)

ESC-ID 254
Author Topala VF, Stanescu GA, Mocanu H, Manu AD
Country Romania
University Titu Maiorescu-Bucharest
Department Faculty of Medicine - ENT Department

Introduction: Resection of pharynx and total oesophagectomy - Long term follow-up; V over 9 years period. - Various technical procedures of reconstruction: •Musculo-cutaneous flaps. •Free transfer of internal organs with vascular micro sutures •Transposition of the left colons with vascular micro sutures

Aim: The problem of surgical technique o Surgical techniques described lead to an increase in the patients lifespan.

Material and methods: X 43 patients operated between 1996-2005 X Hypofaringeal and oesophageal epidermoid carcinoma X Total circular faringo-laringectomy, total or partial oesophagectomy X 36 patients with preop. cohab-totherapy

Oncological results: Rate of survival: •1 year survival rate 74% (39 cases) •2 years survival rate 58% (22 cases) •3 years survival rate 33% (16 cases) - Causes of death (19 cases studied): fe 14 patients (74%) pulmonary metastasis fe 5 patients regional relapse

Plastic results: o Musculo-cutaneous flaps: +24 cases studied: X 8 myo-dorsal flaps X 16 myo-pectoral flaps •Postop. evolution: X 6 benign salivation fistulas X 2 major sutures disunions that lead to a surgical re-intervention •In 18 cases followed between 1-5 years the functional result was good o Free transfer of internal organs with vascular micro sutures

Postop. evolution: •2 benign fistulas: - 1 sigmoidian - 1 jejunal • 1 patient died through vascular rupture o Transposition of the left colons with vascular micro sutures •2 cases: postop. benign salivation fistula.

Conclusions: X We are interested in the surgical technical problems, the onchological problems and the quality of the patients life X The frequency of metastasis shows that the real problem remains controlling the evolution of the cancerous disease X We consider surgery as main indication in the management of ENT neoplasm.

Pathology II

Pathohistological and proliferative changes in rat liver treated with monosodium glutamate

ESC-ID 136
Author Mladenovic P
Country Serbia
University University of Nis
Department Medical Faculty

Background: The neonatal administration of monosodium glutamate (MSG) causes lesions of a number of structures of the central nervous system to rats (nucleus arcuatus, eminencia mediana) as well as a number of complex metabolic and endocrine disorders in adult period. The effect of MSG on liver is performed indirectly, through the increased level of corticosterone in plasma and directly through specific receptors on hepatocytes (mGlu5 receptors).

Aim: The aim of our research was to find the type, volume and the mechanisms of generating pathological changes on rat livers treated with MSG.

Material and methods: Both male and female white rats are used in the experiment. The rats are devided into two groups of ten animals: experimental and control group. The experimental group is treated with MSG in dose of 4 mg/kg/TT on the 2th, 4 th, 6 th, 8 th, and 10 th day of neonatal life. The control group is treated with physiological solution in dose of 4 mg/kg/TT (equal dose) on the 2th, 4 th, 6 th, 8 th and 10 th day of neonatal life. The rats are sacrificed 3 months later and liver cats are coloured in the following methods: 1. Hematoxylin eosin (HE) histological staining 2. Sudan black B histological staining 3. Immunohistochemical staining (Ki 67-LSAB-2) 4. Immunohistochemical staining (Bcl-2-LSAB-2)

Results: The following pathohistological changes are noticed on the preparations received from the experimental group: 1. Focal fatty changes, which leads to initial phase of fatty liver degeneration (steatosis) 2. Diffuse hypermia with the dilatation of sinusoides and the central vein (venula centralis) 3. Regenerative proliferation of hepatocytes in perportal zone of liver lobules. 4. Decaying of hepatocytes due to the type of apoptosis, without obvious zonal distribution. We have noticed decaying of hepatocytes due to the type of apoptosis according to the specific shape and color of hepatocytes in apoptosis (Hematoxylin eosin staining), and according to the significantly lower expression of antiapoptotical protein Bcl-2 on the liver cats of experimental group then on the liver cats of control group (Bcl-2-LSAB-2).

Conclusion: According to the results of research we can firmly claim that in rat livers, which are treated with MSG, develop some focal fatty changes, diffuse hypermia with the dilatation of sinusoides and central veins, regenerative proliferation in perportal zone of liver lobules and the decay of hepatocytes due to the type of apoptosis.

Investigation of HER-1 expression and HER-1 and uPAR gene status in triple negative versus other breast carcinomas

ESC-ID 174
Author Pintens S, Vanden Bempt I, Neven P
Country Belgium
University Katholieke Universiteit Leuven (K.U.Leuven)
Department Medicine, Biomedical Science

Background and Aims: Recently, it has been hypothesized that HER-1 overexpression plays a role in triple ER-/PR-/HER-2- negative breast carcinomas. As HER-1 might be a target for therapy, we investigated the HER-1 status in triple negative compared to grade-matched non-triple negative breast carcinomas. In addition we aimed to investigate the urokinase Plasminogen Activator Receptor (uPAR) gene status in tissue sections of breast cancer and to study its relation to the HER-2 gene status.

Materials and methods: In total, 57 high-grade breast carcinomas were studied: 25 triple negative cases and 32 grade-matched controls with variable ER/PR/HER-2 phenotypes. For all cases, formalin-fixed paraffin-embedded material was
available. HER-1 expression was examined by immunohistochemistry (IHC) using the standardized PharmDx kit (Dakocytomation). Cases with membrane staining in more than 10% of invasive tumour cells were regarded as showing HER-1 overexpression. Copy numbers of HER-1, HER-2 and uPAR were separately determined by Fluorescence in situ Hybridisation (FISH) using dual-probe kits (PathVysion, Vysis). A mean ratio of the gene to the chromosome copy number greater than 2 was considered amplified for that gene.

Results: Overall, HER-1 overexpression was found in 20 out of 57 (35.1%) high-grade breast carcinomas, all of them being ER negative. Out of the 25 triple negative cases, 13 showed HER-1 overexpression (52.0%). HER-1 gene amplification was found in 5 cases (8.8%) and was always associated with HER-1 overexpression. Of note, HER-1 gene amplification was a generalized event, as it was found in more than 90% of the tumours cells in all 5 cases. By contrast, HER-1 expression was more variable throughout the tumour. Still, 3 out of 5 HER-1 amplified cases also showed generalized HER-1 overexpression. Out of 57 cases, 26 were amplified for HER-2. Only 1 of these cases showed concurrent amplification of the uPAR gene (1/26, 3.8%). Thereby, the mean uPAR gene copy number was 10 compared to 20 for HER-2. No uPAR gene amplification was found in non-HER-2 amplified breast carcinomas. While uPAR gene amplification was rare, we frequently observed aneuploidy of the chromosomome 19 (35.1%).

Interpretation and Conclusion: HER-1 overexpression was restricted to ER-negative breast carcinomas, half of which were triple negative. HER-1 overexpression was accompanied by HER-1 gene amplification in only a minority of the cases. Therefore, HER-1 gene amplification is unlikely to be a driving mechanism for HER-1 overexpression. Nevertheless, it might delineate a particular subgroup of ER-negative breast carcinomas that might benefit from therapy with HER-1 inhibitors. FISH analysis on tissue sections indicates that uPAR gene amplification is extremely rare in breast cancer. Whereas related to HER-2 gene amplification, it is unlikely to play an important role in the pathogenesis of breast cancer.

Possible role of S100 protein in iron resistance of liver cells
ESC-ID 560
Author Svirina D, Rozentale B, Isajevs S, Knipshe G, Markovs J
Country Latvia
University University of Latvia
Department Faculty of Medicine

Introduction: The liver is one of the organs most affected by iron toxicity. There are many different proteins, which control iron homeostasis in the liver - hepcidin, ferroportin, transferrin, ceruloplasmin, hephaestin, etc. It is known that some proteins can maintain iron homeostasis in non-specific manner. We hypothesize, that the calcium-binding protein-S100 can also participate in the regulation of iron metabolism. The aim of this study was to investigate the distribution of immunoreactive S100 protein and iron deposition in the target liver cells (principally, iron-storing hepatocytes and iron-recycling macrophages) in chronic hepatitis C patients with and without liver hemosiderosis.

Methods: Immunohistochemical method was used to identify S100 positive cells. Hemosiderin accumulation was confirmed using Perl’s Prussian blue staining.

Results: The macrophages and hepatocytes of patients with hepatitis C without hemosiderosis exhibited weak, variable expression of S100 protein. On the other hand, in patients with hemosiderosis S100 protein positive macrophages and hepatocytes showed strong and diffuse staining in the cytoplasm. Besides, S100 protein was located at the same places as siderosomes. There was a significant correlation between
the hepatocellular iron grade and expression of S100 protein. **Conclusion:** The present results demonstrate that hepatic siderosis was accompanied by induction of S100 protein, which may be due to prolonged cell activation. There would be a high probability that S100 protein may be involved in iron resistance of liver cells by mediating intracellular diffusion and deposition of metal ions.

**Pediatrics II**

Etiological aspects of diarrhoeal acute syndrome among patients examined in pediatric clinic in Prishtina

ESC-ID 459
Author Sermaxhaj B, Bajrami E
Country Kosova
University University of Prishtina
Department Medicine

The diarrhoeal acute syndrome is caused by different, bacterial, viral and parasitical carriers. Using the nutritious terrains (places) for bacteries isolation, retolox test ( for the rota virus) as well as microscopical looking, regarding to the parasitological examination of the faeces (feces stool) are attained the following results: Faeces examination was positive at 67,3% of the examined persons (children), while 32,7% was negative. Bacteriological examination (checking up) at 92,8 % was positive, virological (rotavirus) at 6,8% whereas parasitological examination at 0.2% of the cases. In the bacteriological examination mostly was isolated salmonellae (40,5%) and E. coli (3,4) of the case. From the sallmonellae gender (types), more often was isolated salmonella wien (36,7%), Salmonellae Gluetester (21,8%), but rarely salmonellae Stanley and Salmonellae Gatuni. As for E. coli types, more often is isolated serotype 0119: B14 (at a cause 1,5%). From the shgella’s types, more often is isolated shigel-laev Flexneri. As for not convenient frequency, etiology and prognosis, the diarrhoeal acute syndrome represents in Kosovo a social and a medical problem. Key words: the diarrhoeal acute syndrome, etiology.

Analysis of risk factors for arteriosclerosis and heart disease in children with hematologic neoplasms

ESC-ID 959
Author Swiecicka A, Mojsak B, Sawicka- Zukowska M, Panasiuk A
Country Poland
University Medical University in Białystok
Department Pediatric Oncology

**Heading:** Recent studies have suggested a relationship between cancer and serum lipids. It is possible that one of the possible pathways of oncogenesis is connected with lipid abnormalities. On the other hand, above norm lipid values increase risk for developing cardiovascular disease. The aim of this study was to analyse values of blood lipid profiles (total cholesterol = TC, triglycerides = TG, high-density lipoprotein cholesterol = HDL), fibrinogen and bone mass index (BMI), which are risk factors for arteriosclerosis, in subjects with childhood cancer.

**Methods:** The study group consisted of 49 subjects (30 boys), who were diagnosed with acute lymphoblastic leukemia (ALL- 40) or lymphomas (9). All subjects were treated at the Department of Pediatric Oncology in Białystok. Data was assessed 3 times: at the moment of diagnosis (mean age of the group- 8,84 years), during treatment (mean age- 9,53 years) and after cessation of the therapy (mean age- 11,9 years). The control group included 41 healthy children (21 boys) of mean age 11,8 years. Statistical analysis was conducted using Statistica Package 6.0.

**Results:** Mean values at the moment of diagnosis: TC = 143,3 mg/dl, TG = 123 mg/dl, HDL 33,9 mg/dl, fibrinogen = 357,2 mg/dl, BMI 17,05 kg/m². Mean values assessed during therapy: TC = 168,4 mg/dl, TG = 151,6 mg/dl, HDL 53,02 mg/dl, fibrinogen = 378 mg/dl, BMI 17,2 kg/m². Mean values after completed treatment: TC = 166,9 mg/dl, TG = 108,6 mg/dl, HDL 55,1 mg/dl, BMI 19,42 kg/m². Mean values in the control group: TC = 154,36 mg/dl, TG = 86,6 mg/dl, HDL 58,56 mg/dl, fibrinogen = 351,65 mg/dl.

**Conclusion:** Statistically significant alterations in blood lipid profile were observed at the moment of diagnosis (below norm HDL concentration and above norm TG), which suggests that neoplastic process itself provokes these changes. Some authors point to correlation of HDL concentration with proliferation of cancer cells. Higher concentration of TG and TC could be z result of glucocorticoids and asparaginase administration. Increase in HDL is connected with cessation of blasts proliferation. After the termination of the treatment TG concentration stabilized on the with-in norms level, however it was higher than in the control group. BMI values were higher at this point of the study. Since both TG and BMI are recognized risk factors, further follow-up of children after completed antineoplastic treatment should be conducted, in order to minimalize heart diseases later in their lives.

A-gene polymorphism and fractures in childhood cancer survivors

ESC-ID 943
Author Panasiuk A, Sawicka- Zukowska M, Rybak A
Country Poland
University Medical University in Białystok
Department Pediatric Oncology

**Heading:** Osteoporosis is the most prevalent bone disorder, which consequences in increased bone fragility and susceptibility to nontraumatic fractures. In its’ multifactorial pathogenesis 50-80% is contributed to genetic status, other being diet, decreased physical activity, hormonal disorders and pharmacotherapy. Childhood cancer and its’ treatment (frequent and long hospitalizations, steroido- and chemotherapy, inadequate calcium and vitamin D absorption) are additional environmental factors disrupting physiological bone turnover and could cause osteoporosis. Estrogen (E) maintains balanced rates of formation and resorption in the healthy bone by affecting lifespan and activity of osteoclasts and osteoblasts. It increases osteoclasts’ apoptosis and osteoblasts’ formation, which results in shorter lifespan of the former and longer in the latter. E deficiency prolongs the resorption phase and shortens bone formation. Genetic polymorphism of
Congenital disorders of glycosylation – Case report

ESC-ID 887
Author Murzyń J, Piotrowicz-Wójcik K, Ożyniec K, Kruczek P
Country Poland
University Jagiellonian School of Medicine
Department Pediatrics

Introduction: Congenital Disorders of Glycosylation (CDG) are a very rare group of inherited metabolic disorders caused by deficient glycosylation of glycoconjugates, such as glycoproteins and glycolipids. Depending on the impaired glycosylation track, the disease can be divided into O-linked or N-linked defect. The first reported cases date from nineteen eighteens. Eleven types of CDG have been discovered up to now and the majority of them belongs to N-linked defects. Although the CDG symptoms are not strictly specified and can vary from one case to another, the disease may be suspected in patients with one or more of the following: hypotonia, failure to thrive, dysmorphic, mental and psychomotor retardation, optic atrophy, stroke-like episodes, protein losing enteropathy, hypoglycemia, endocrinal disorders, unusual fat deposits generalized, dysmyelination, cardiomyopathy with pericardial exudates, impaired liver function and recurrent infections.

Aim: We aimed to investigate a case of CDG in order to emphasise the rising importance of accurate and careful diagnostics of this disease. Method: We report a 11-month-old patient treated and diagnosed in University Children's Hospital of Cracow.

Results: Initially the infant was admitted with following symptoms: bilateral cataract, exanthema, impaired weight gain, muscular hypotonia, hepatomegaly, icterus. Next admissions to hospital were related to recurrent infections,
anorexia and progressing weight loss. During diagnostics classic galactosemia, infections (including TORCH infections) and structural organ defects were excluded. Because of the entire clinical picture a disorder of glycosylation was suspected. Levels of antithrombin, factor XI and protein C were measured. Decreased levels of those factors suggest diagnosis of CDG. The diagnostics included also electrophoresis of glycoproteins: transferrin and alpha-1 antitrypsin. The electrophoresis was abnormal and different from electrophoresis of the infant’s parents. In order to verify the diagnosis a skin fibroblast culture was established. The culture was used for molecular tests.

Conclusion: It is not possible to classify the described case into any of the 11 known CDG types. Due to a small number of scientific records concerning those disorders and progressing researches, it is possible that the described case is a new type of congenital disorders of glycolisation.

**Evaluation of incidence and types of isolated ventricular septal defect in fetuses**

**ESC-ID**: 848

**Author**: Kapcanska A, Kuran J, Kraska A, Papierz J, Sliwka E

**Country**: Poland

**University**: Medical University of Warsaw

**Department**: 2. Department of Obstetrics and Gynecology

**Background**: Ventricular septal defect (VSD) is the most common congenital heart disease (CHD) in children. It is less frequently diagnosed in fetuses because the diagnosis of small VSDs can be difficult in prenatal life due to equal pressure in both ventricles. VSD can be an isolated lesion or may be a part of more complex CHD.

**Aim**: To evaluate the prevalence of isolated VSD in fetuses and types of VSD in relation to chromosomal aberrations and other fetal abnormalities and assess perinatal follow-up of the fetuses with VSD.

**Material and methods**: Retrospective analysis of computer database of Perinatology and Perinatal Cardiology Unit of the 2nd Department of Obstetrics and Gynecology, Warsaw Medical University between 2004-2006. There were 75 (25%) fetuses with isolated VSD out of 295 fetuses with CHD who were examined in the Unit at that time. Fetuses with VSDs were analyzed group according to the following: type of VSD, karyotype, other congenital malformations and the follow-up.

**Results**: Mean maternal age was 30.8 ± 6.2 yrs, median: 30. Mean gestational age at the time of diagnosis was 27 ± 6.7 weeks (from 12 to 39 weeks), median: 26. There were following types of VSD: muscular 27pts (36%), perimembranous 26pts (35%), inlet 18pts (24%), multiVSD 3pts (4%), subaortal 1pts (1%). Karyotype was identified in 34pts (45%). In 16 (47%) cases was normal [VSD perimembranous 5, VSD muscular 5, inlet 5, VSD subaortal 1]. In 18 (53%) was abnormal, in which 47+18,XX/XY in 11 pts; 47+21,XX/XY in 6pts and 46,XX,del5p,15,1 in 1 pt. Out of 11 fetuses with trisomy 18, 10 (91%) had inlet VSD. 5 (83%) out of 6 fetuses with trisomy 21 had perimembranous VSD. The fetus with deletion had multi VSD. In fetuses with chromosomal abnormalities 16(89%) had coexisting congenital malformations, while in the fetuses with normal karyotype the rate was 62.5% (10 out of 16). Amongst 27 fetuses with muscular VSD (none of them had diagnosed trisomy) 19pts (70%) were born alive, 1 died after birth, 1 pregnancies was terminated, 1 last and in 6 cases the follow-up is unknown. In the group of fetuses with perimembranous VSD the numbers are: 19 (73%), 0, 2, 0, 1, 4 respectively. In the group of 17 patients with inlet VSD, 5 (29%) died either in utero or after birth, 11 were born alive and 1 pregnancy was terminated.

**Conclusions**: 1) Fetal inlet and big perimembranous VSD is a strong marker of chromosomal abnormalities, so such diagnosis should be an indication for fetal karyotyping. 2) Fetal muscular isolated VSD with otherwise normal USG result is a benign lesion and other investigations are not necessary. 3) Prognosis of fetal isolated VSD is different to postnatal VSD due to comitant chromosomal aberrations and other organ malformations. 4) Relatively high rate of fetuses with isolated VSD and normal karyotype have concomitant extracardiac malformations.

**Urinary loss of erythropoietin after intravenous vs. subcutaneous Epoietin-beta application in preterm infants**

**ESC-ID**: 825

**Author**: Langer J, Martus P, Obladen M, Dame C

**Country**: Germany

**University**: Charité Universitätsmedizin Berlin

**Department**: Klinik für Neonatologie

Recombinant Erythropoietin (rEpo) is used for the treatment of the anemia of prematurity. Not only erythroid progenitor cells but also endothelial cells (Anagnostou 1994), neurons and astrocytes ((Li 1996) express the Erythropoietin (Epo)-receptor. For that reason the long term effect of rEpo treatment during anemia of prematurity is currently under reevaluation concerning the development of the retinopathy of prematurity (Ohlsson 2006) and the high-dose application of rEpo for neuroprotection is considered. The potential urinary loss of Epo after intravenous (i.v.) vs. subcutaneous (s.c.) Epoietin-beta application is analyzed in our study. Every 14 days the urinary loss was determined before, 4 h and 8 h after the treatment of 250 U Epoietin-beta/kg (i.v./s.c.) using ELISA-technique (detection limit 2.5 mU/ml; reported as median in mU/ml (range)) in 21 preterm infants. Before application, Epo was detected in 9/67 urine samples (4.3 mU/ml; (2.9-59)). Four h and 8 h after Epoietin-beta application, respectively we found Epo in 32/54 and 31/52 urine samples. The Epo concentration determined 4 h and 8 h after i.v. application was significantly higher than after s.c. application (4 h: i.v. 120 (3.5-377) vs. s.c. 21 (2.8-200) mU/ml; 8 h: i.v. 102 (7.6-305) vs. s.c. 38 (6.6-81) mU/ml; each with a p-value < 0.05. In longitudinal analyses obviously higher urinary Epo-concentrations were found 4 h after i.v. application in preterm infants with a gestational age < 31 weeks compared with later developmental stages (25+0 till 27+6: 120 (31-199); 28+0 till 30+6: 173 (3.5-377); 31+0 till 33+6: 53 (15-92)). The higher renal loss of Epo after i.v. application in preterm infants with a gestational age < 31 weeks could limit the efficiency preventing anemia of prematurity.
Bed-side chest ultrasound examination in diagnosis of pediatric pulmonary thromboembolism

ESC-ID 628
Author Kosiak M, Korbus A, Kosiak W, Potaz P
Country Poland
University Medical University of Gdansk
Department Scientific Students' Ultrasonography Group

Objective: Pulmonary thromboembolism (PTE) occurs in groups of children suffering from selected clinical condition. Prevalence of PTE in children is associated with: central venous line, congenital heart disease, infection, malignancy, surgery, immobility, hypovolemia or SLE. The clinical presentation of PTE is often very subtle masked by the underlying clinical condition. Diagnostic strategies for pulmonary thromboembolism in children are extrapolated from studies based on adult patients. Sonographic image of PTE has been already evaluated based on adult patients. There have been limited studies to determine the diagnostic value of sono-graphic features in pediatric PTE.

Aim of the study: To present usefulness of chest ultrasound examination in diagnosis of pediatric pulmonary thromboembolism (PTE).

Material and methods: In the first stage of our study chest ultrasound examination was performed in three teenage patients with deteriorating clinical condition: 13 years old boy suffering from primary antiphospholipid syndrome, 14 years old boy with Wegener granuloma disease and 15 years old girl with urosepsis. In the second stage: Chest ultrasound examination was performed in three male newborns with congenital heart disease and risk of pulmonary thromboembolism. Two boys were treated for HLHS and one boy for CoA and PDA. In all six cases ultrasound examination was performed as bed side ultrasound examination using GE Logic 500 equipment with linear probe 8,2-11,0 MHz.

Results: In all children the chest sonograms showed bilateral peripheral subpleural hypoechoic lesions: triangular or oval in shape, accompanied by a small effusion. In teenagers the lesions were: average 1,0 x 2,2 cm (0.5-4.5) large. In newborns lesions were: average 0.5 x 1,8 cm large. In all children chest ultrasound examination was the first imaging test that suggested of PTE. Based on chest ultrasound examination further diagnosis was directed to confirm PTE. In teenagers it was possible to perform CT and confirm the diagnosis. In newborns, because of critical condition of children, other imaging tests were not performed. Treatment was started based ultrasonographic results.

Conclusions: Although sonographic features of PTE in chest ultrasound examination were evaluated based on adults patients, presence of those features should be considered in diagnosis of pulmonary thromboembolism in children. In our opinion bed side chest ultrasound examination is especially useful in children with high risk of PTE and critical physical condition. Extended study based on large group of patient is necessary to evaluate diagnostic value of chest ultrasound examination as one of the first method in children with risk factors of PTE.

The use of Quadrant Impedance Measurement (QIM) to evaluate the alveolar recruitment under High Frequency Oscillatory Ventilation (HFOV)

ESC-ID 624
Author Pitterle M, Burkhardt W, Blassnig N, Lüb V, Simbruner G, Rüdiger M
Country Austria
University Medical University Innsbruck
Department Pediatrics, Neonatology

Introduction: High Frequency Oscillatory Ventilation recruits atelectatic lung areas and improves the oxygenation by adjusting the Mean Airway Pressure (MAP). In clinical routine this is tested by punctual blood gas analysis. QIM allows the measurement of changes in impedance in four thoracic quadrants depending on tidal volume (Tidal Impedance, TI) and on the endexpiratoric thoracic air volume (Residual Impedance, RI). Thus RI could be suitable for continuous bedside monitoring of recruitment. Hypothesis: The variation of the Mean Airway Pressure during High Frequency Oscillatory Ventilation results in a variation of the Residual Impedance.

Methods: Newborn piglets were surfactant depleted with bronchoalveolar-lavage and treated with HFOV. For different Mean Airway Pressures (9 – 12 – 15 – 18 – 21 – 24 – 27 – 24 – 21 – 18 – 15 – 12 – 9 – 6 cmH2O) the Residual Impedance (RI) in the four quadrants was measured by QIM, then summed up resulting in Total Residual Impedance (TRI). Blood gas analysis and vital parameters were measured.

Results: 5 piglets with a weight of 1,4 kg ± 0,2 were examined. Starting from a baseline (6 cmH2O) the percentage of variation of TRI was calculated as well as the difference between each time point (in %). Every constant ∆MAP (3 cmH2O) resulted in a change of TRI, which ranged between 0.54 and 6.09%. With a maximum MAP of 27 cmH2O the TRI finally reached 26.46% ± 4.03 above the baseline. The highest increase in PaO2 was produced by changes of MAP from 12 to 15 (ΔPaO2 101.58 mmHg ± 76.12) and 15 to 18 cmH2O (ΔPaO2 95.16 mmHg ± 38.30). A MAP of 27 cm H2O showed a PaO2 of 432.26 mmHg ± 182.89. The reduction of MAP resulted in a decrease of TRI, which ranged between 0.62 and 7.07% per 3 cmH2O. Interestingly at the end of the experiment TRI values remained above the baseline (+4.7% ± 1.32).

Discussion: The variation of MAP caused a variation of TRI measured by Quadrant Impedance Measurement. Hence this method is suitable for continuous bedside monitoring of alveolar recruitment. However, until now Total Residual Impedance could not be correlated with a defined volume. A comparison with sophisticated measurement of the Functional Residual Capacity (FRC) is pending.
Bone maturation in patients with multiple pituitary hormone deficiency and isolated growth hormone deficiency treated with recombinant human growth hormone

ESC-ID 474
Author Mnichowska A, Wierzbicka I
Country Poland
University Jagiellonian University Collegium Medicum
Department Faculty of Medicine

Introduction: Somatotropic pituitary insufficiency is characterized by isolated growth hormone deficiency (IGHD). In multiple pituitary hormone deficiency (MPHD), the patient presents with additional deficiency of thyreotropic hormone, gonadotropins and prolactin. In both forms, starting at an early age, the patients manifest decreased growth rate speed and delayed bone age: MPHD is also characterized by thyroid and suprarenal gland failure and no puberty. Therapeutic management of both IGHD and MPHD consist of recombinant human growth hormone (rhGH) therapy; in MPHD, other hormonal deficiencies are additionally supplemented. rhGH is generally believed not to increase bone maturity in patients with IGHD and MPHD. Objectives: To assess bone maturity in patients with IGHD and MPHD treated with rhGH. Materials: A retrospective analysis of 37 patients (14 with IGHD, 23 with MPHD) at the age corresponding to the period of puberty, i.e. 12 girls between 10-14 years, 19 boys aged between 10-16 years of age, treated with rhGH for at least 2 years before puberty.

Methods: Body height measurement made every 3 months expressed in standard deviation and as the height age (HA). Puberty estimation based on the Tanner’s scale. Bone age (BA) estimation based on the left wrist’s X-ray pictures with the use of the Greulich-Pyle method. During rhGH therapy, estimation of growth and bone maturity with the use of height charts and the KIGS 4.0 software. At the time of expected puberty, estimation of favorable final height factors: the ratio of BA/chronological age(ChA)<1, BA/HA<1, and unfavorable final height factors: the ratio of BA/ChA>1, BA/HA >1.

Statistical methods: the t-Student test, Chi-Square analysis and Statistica 6.0 software.

Results: Favorable final height factors were observed in 70% of all the patients; the majority of them were diagnosed as MPHD. Both unfavorable factors (BA/ChA>1 and BA/HA >1) were observed 5 times more frequently among patients with IGHD (57%) than with MPHD (13%); these children formed a group with an unfavorable prognosis. In this group, we observed spontaneous puberty three times more frequently (91%) than spontaneous or induced puberty in the group with favorable prognosis (27%). Moreover, spontaneous puberty in this group was observed significantly earlier than in the group with favorable prognosis. Ninety-five per cent of the patients without spontaneous puberty were in the group with favorable prognosis.

Conclusions: rhGH therapy administered to patients with GH deficiency does not affect bone age acceleration. Bone age acceleration of patients treated with rhGH is caused by spontaneous puberty and sex hormone (FSH, LH) activity, alone or in synergy with rhGH.

Legumain DNA vaccination for active anti-neuroblastaoma immunotherapy

ESC-ID 446
Author Woehler A, Huebener N, Gaedicke G, Lode HN
Country Germany
University Charité Universitätsmedizin Berlin
Department Pediatrics

Background: Neuroblastoma is the most common extra cranial solid tumor in infancy accounting for about 8% of childhood malignancies. Despite multi-modality treatment such as surgery, chemotherapy, radiation and autologous bone marrow or stem cell transplantation, the 5-year survival rate especially of neuroblastoma stage 4 patients remains poor. Therefore, the search for alternative therapies is a challenging task. A promising approach is active immunotherapy with DNA vaccines. Legumain, a member of the asparaginyl endopeptidase family, functions as a stress protein being upregulated under hypoxic conditions present during tumor growth. In fact, it is highly expressed in most human solid tumors and is known to promote cell migration, tissue invasion and metastasis.

Aim: We hypothesize that novel legumain DNA vaccines can break self-tolerance of the immune system and induce an active anti-neuroblastoma immune response in vivo. Method: First, the expression of legumain in murine NXS2 neuroblastoma as well as four human neuroblastoma cell lines was evaluated by RT-PCR, Real-time PCR and Western Blot. Second, we generated two novel DNA vaccines: one encoding for the whole cDNA sequence of murine legumain (mLGMN) while the other vaccine consisted of three mLGMN derived epitopes with high predicted binding affinity to MHC class I representing a so called minigene vaccine (mLGMN-Mini). Both constructs were cloned into ubiquitin expression vector pCMV-F3Ub. The vector design provides for a stable ubiquitination of the minigene peptide and therefore facilitates proteasomal degradation and, subsequently, antigen presentation. Third, the vaccines were tested in our syngeneic neuroblastoma mouse model for its efficacy to suppress subcutaneous tumor growth and tumor weight. For this purpose, A/J mice (H2-KkDd) were vaccinated 3 times every 7 days by oral gavage of 1.0e008 Salmonella typhimurium SL7207 (Aro-) carrying the vaccine constructs or empty vector control. Furthermore, the role of specifically activated cytotoxic T cells was evaluated.

Results: We could show for the first time that Legumain is expressed in human neuroblastoma cells and therefore represents a suitable T cell antigen for active immunotherapy of neuroblastoma. Importantly, after tumor cell challenge mice vaccinated with the LGMN encoding vectors presented with decreased tumor growth and lower primary tumor weights compared with mice vaccinated with the empty vector. Furthermore, in a Cr51 cytotoxicity assay we could show that the immune response induced by DNA vaccination is mediated by cytotoxic T lymphocytes, CTLs.

Conclusion: Active immunotherapy with legumain encoding DNA vaccines is effective against neuroblastoma in vivo and might therefore be a promising adjuvant strategy against one of the most challenging tumors in childhood.
The evaluation of prevalence and due to age and sex distribution of enuresis in school

**ESC-ID:** 411  
**Author:** Barak M, Chiniforoush M, Amanzadeh Z, Sadri SF  
**Country:** Iran  
**University:** Islamic Azad University  
**Department:** Pediatrics

**Introduction:** Enuresis is one of the crucial problems that both parents and kids have had from many old years. This issue can be found even in old texts that belongs to 1550 years ago. In spite of huge developments in medical science, scientists still don’t know a lot about it. Therefore, we have decided to study enuresis, its prevalence and due to age and sex distribution of it.

**Methods and Materials:** This study is a cross-sectional one which has been done during a period of one year. Elementary schools have been selected in gelen, which were both girls and boys included. 700 questionnaires were given to the students to be filled, which 640 out of 700 were filled. Then we assessed all the resulted information by SPSS.

**Results:** From all valid filled questionnaires, 110 students suffered from enuresis which is 17.18 percent of overall volume of these 110 students, 48 of them were females (43.1%) and 62 of them were males (56.9%). Assessing age distribution showed that 22 of them (20%) were between 6 to 8 years, 64 of them (58.18%) were between ages of 8 to 10 and 30 of them (27.24 present) were limited in the ages of 10-12 years old and 12 of them (10.9%) were in ages limited to the ages of 12-14.

**Conclusion:** With the respect to the results the prevalence of enuresis in the Ardebil is 17.18 percent. Comparing to the other countries such as Malaysia 8 present, Turkey 12/4 present, *Sweden 7.1 present, china 4.2% and Pakistan 9.7% is a lot of more. If we compare the sex ratio of two genders, females were 43.1% and males were 56.9 present and due to the total volume, 7,49% were female students, 9068% were male students, which is pretty close to the real statistics. Analyzing of age group distribution shows that age group between 8 to 10 is the most and the age group between 12-14 is the least in case of quantity. Based on this study, enuresis is a prevalent disease in Ardabil. And efforts to know the reasons, will help to solve the problem as well. It seems to be essential in other studies to assess different etiologies of enuresis.

Assessment of Relation between hyperbilirubinemia in neonatal period and childhood deafness

**ESC-ID:** 371  
**Author:** Mahboubi H, Javadian P, Nazari R, Mojtabah M, Mojtabah A  
**Country:** Iran  
**University:** Tehran University of Medical Sciences  
**Department:** Pediatrics

**Objective:** Hyperbilirubinemia is the commonest morbidity in the neonatal period and remain an important cause of childhood deafness. Auditory brainstem responses (ABR) were evoked, before and after exchange transfusion (ET) in an effort to determine whether hyperbilirubinemia is associated with acute effects on brainstem function on neonates.

**Methods:** ABR were measured in 12 full term newborns with severe hyperbilirubinemia [Total bilirubin concentration (TBC) ≥20 mg/dl and direct bilirubin ≥2 mg/dl] before ET and 6 days after the ET. The mean of birth weight on admission were 3066 ± 477 gr and gestational age was 38.5 weeks respectively. The follow up of ABR was performed at 90 days of life. None of the babies have had evidence apparent bilirubin encephalopathy. Data were collected by SPSS and analyzed by repeated measure and t test.

**Result:** In two infants, wave-absence before exchange transfusion was followed by appearance of the wave after ET. Other significant alteration observed in the group after ET were reduction of interpeak latencies (brainstem transmission time 5.16 ± 0.32 after ET compared with 5.39 ± 0.39 before ET, P < 0.05). Interpeak latency for wave V after ET showed marked improvement (P < 0.05). These improvement are considered to have been brought about by removal of bilirubin from the body and from the brainstem during ET.

**Conclusion:** Acute brainstem toxicity appears to occur in a percentage of infants with hyper bilirubinemia at serum levels commonly seen in clinical practice. These changes appear to be rapidly reversible with ET. Therefore we recommend ABR for screening and early detection of bilirubin otoxicity.

Retinoic acid therapy in teenagers hematologic diseases

**ESC-ID:** 239  
**Author:** Miron OT, Malanacea RI, Cirdei C, Moldovanu I  
**Country:** Romania  
**University:** Grigore T.Popov University  
**Department:** Pediatrics

**Background:** Acute promyelocytic leukemia (AML-M3) is a subtype of acute myeloid leukemia characterized by a balanced reciprocal translocation between chromosomes 15 and 17. All-trans-retinoic acid (ATRA) binds retinoic acid receptor alpha resulting in differentiation of leukemic promyelocytes into mature cells and produce complete remission resulting in an excellent survival compared to conventional chemotherapy alone. Material and Method: two AML-M3 teenagers, a 14 years boy and an 11 years girl were studied; they were admitted in St Mary Hospital, Iasi, Department of Hematology and Oncology between 2000-2003. In this interval from a total of new 56 cases of acute leukemia, 12 had AML type. Our patients came in hospital with anemic, infection and hemorrhagic conditions. ATRA treatment was given together with specific chemotherapy and we performed a clinical and biological survey.

**Results:** ATRA was followed by a good response of leukemia, and both patients reached a complete remission. Nowadays they are both alive, off therapy, disease free for 5 years.

**Discussion:** Their course was complicated with severe side effects of ATRA: fever, pulmonary infiltrates, weight gain, pleural effusion, Sweet’s syndrome (acute febrile neutrophilic dermatosis) and they needed extra inhospital days in ICU. Surgery and Pediatric Hematology

**Conclusion:** Some of the new drugs discovered in hematology research field can dramatically improve overall survival of previously fatal diseases. However side effects are to be considered to improve the quality of life of patients.
Obesity is one of the most common diseases of endocrine system, marked by the excessive accumulation of fat tissue. Different disorders in functioning of many organs and body systems, cardio-vascular for the first place, appear without timely correction of metabolic changes. The aim of our investigation is to reveal functional features of cardio-vascular system in children with obesity on the base of clinic-laboratory and instrumental data. We have made retrospective analysis of 109 case histories of children with exogenic constitutional obesity staying in endocrinology department of City Children's Clinic in Samara from 2000 to 2006, 68 of them are boys and 41 girls at the age from 3 till 17. Control group contains 50 children of the same age. On admission overweight among all the complaints was on the first place in 100% of all cases. Nearly half of the children complained on periodical elevation of arterial blood pressure, headaches and pains in heart area. Most of the children were not examined before or were examined in out-patient departments. Doctor’s recommendations were not followed. Most children had average weight and height at birth, but a quarter of newborns were more then 4000gr and 55sm. Having analyzed the control group one can see that children with obesity are more predisposed genetically to obesity, diabetes mellitus, hypertension, ischemic heart disease then healthy ones. On examination in hospital more frequent elevation of diastolic blood pressure (27.5%) then systolic (18.4%) was revealed. The increase of glucose level more then 5.5mmole/l is noted in 12.3% cases. Changes of glucose tolerant test were revealed in 46.8%. Some changes in biochemical blood analysis concerning lipid exchange are observed: more then half of the children have an increase level of cholesterol and B-lipoproteins. The findings of the cardiogram were significant: sinus tachycardia, sinus bradycardia and respiratory sinus arrhythmia appear more often. In some children peacemaker migration is observed. Conductive disorders are marked by right bundle-branch block in 14.7% and conductive disturbance of Pourkinners fibers in 8.3%. Conductive disturbance of myocardium fibers of right and left atrium is revealed in few cases. Hypertrophy of different parts of the heart was revealed in 13.8% of cases. Having analyzed history cases of children with exogenic constitutional obesity we can get the following conclusions: the presence of elevated diastolic arterial blood pressure together with left atrium hypertrophy is the sign of development of symptomatic arterial hypertension. Changes in the curve of glucose tolerant test with combination of high blood pressure, hypercholesterolemia and hyperlipidemia supposed the presence of metabolic disorders and future development of complete metabolic syndrome which contribute to early appearance of atherosclerosis and cardio-vascular complications.

**Pharmacology and Toxicology II**

The effect of enalapril on skin flap viability is independent of angiotensin II AT1 receptors

**Pharmacokinetics of Ceftriaxone of patients in poor condition**

Introduction: The main aim of the following study done is to determine pharmacokinetics of Ceftriaxone of patients in poor condition, suffering on labor pneumonia and accurate kidneys’ insufficiency. Knowledge and application of pharmacokinetic principles leads to accelerated drug development, cost effective drug use and a reduced frequency of adverse effects and drug interactions.

**Material and Methods:** The investigation was fulfilled in September 2006- March 2007 on the base of chemistry department of Gomel Medical University, Belarus. The experimental data were obtained by taking samples of urea and blood plasma through fixed time terms with later analysis of a drug. The content of Ceftriaxone in test samples was determined by UV-spectroscopic method. 3.

**Results:** On the basis of the results obtained we calculated the principal pharmacokinetic parameters of Ceftriaxone: the Volume of Distribution (Vd), the Clearance (Cl) of a drug , the elimination constant (kel) and the elimination half life (t1/2). Taking a studying system as 1-compartment, we developed a pharmacokinetic model, which is important for precise determination of elimination rate. Thus a rather great difference in kel and t1/2 was revealed between patients suffering from pneumonia and pneumonia plus accurate kidneys’ insufficiency. The given parameters were approximately twice greater for the second category of patients. Thus the average
t1/2 is near 16 hours when for patients with kidneys’ disease it is approximately 30 hours. 4.

The Conclusion: Pharmacokinetics, the study of drug disposition in the body, is an integral part of drug development and rational use. Pharmacokinetic data give an opportunity to make treating of patients more effective and correct the optimal doses of medicines. Thus the doses of Ceftriaxone may be substantially decreased for patients suffering from acute kidneys’ insufficiency without reduction of effectiveness of its action.

Effects of Valproate and Diazepam on induced hyperthermia in rats

ESC-ID 714
Author Papazova D, Popov T, Ferdinandov D, Nikolov Y, Yakimova K
Country Bulgaria
University Medical University – Sofia
Department Pharmacology and Toxicology

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain. GABAergic terminals and receptors are present in the preoptic area of the anterior hypothalamus (PO/AH) – the central temperature controller. Animal studies have shown that GABA-mimetic drugs produce hypothermic effect. Probably the hypothermia induced by GABAergic drugs is mediated by activation of GABAA and/or GABAB receptors. We have investigated the effects of diazepam, a positive modulator of GABA receptors, and valproate, a GABA-aminotransferase inhibitor, on the thermoregulation in rats. The experiments were designed into two main parts: 1) in vivo experiments on conscious rats; 2) in vitro experiments on activity and temperature sensitivity of rat PO/AH neurons. Central (i.c.v.) or systemic (i.p.) administration of diazepam and valproate produced dose-dependent hypothermia. Both GABAergic acting drugs increased temperature sensitivity in warm-sensitive PO/AH neurons. Pretreatment with GABAergic acting drugs, decreased hyperthermic response of morphine-induced hyperthermia in rats, as well as antagonized the first phase of lipopolysaccharide-induced hyperthermia. Our results suggest the involvement of GABAergic mechanisms in thermoregulation, as well as the complicated interactions between GABA and other neuromediators and neuromodulators on the level of thermoregulation.

Ethanol induced vascular smooth muscle contraction independent of nitric oxide synthase activity in concentration dependent manner

ESC-ID 664
Author Berden J, Blaz G, Ziberna L
Country Slovenia
University University of Ljubljana
Department Faculty of Medicine

Aim: The aim of our study was to evaluate possible enhancement of KCl, endothelin (ET-1) and norepinephrine thoracic rat aorta rings reactivity induced by acute ethanol application. Methods: Endothelial integrity of aortic rings (basal tension 2.0 g) was assessed by the degree of relaxation caused by acetylcholine (0.1mM) in the presence of contractile tone induced by norepinephrine (0.1 µM). The rings precontraction by 60 mM KCl, 10 mM ET-1 and 0.1 µM norepinephrine was followed by application of different ethanol concentrations (V/V%): 0.3%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%.

Results: Either case showed ethanol concentration-dependent contraction. Maximal contraction (6.4 ± 0.8%) in KCl precontracted rings was reached at 2.5% ethanol concentration. The maximal contraction in ET-1 precontracted rings (21.4 ± 4.8%) was observed by application of 3.0% ethanol concentration. In norepinephrine precontracted rings maximal contraction (12.5 ± 2.9%) was reached at 2.5% ethanol concentration. In higher ethanol concentrations (2.5-3.5%) the amount of contraction was decreasing from 11.0% to 5.0%. In addition, significant contraction of aortic rings at basal tension was observed only by application of ethanol concentration over 2.0%. Pre-incubation of aortic rings with 0.36 mM N(G)-nitro-L-arginine methylster (NOS inhibitor) and 9.8 µM indomethacin (COX inhibitor) was followed by identical pre-contraction method resulting in higher plateau values. In this case the ethanol induced contraction was also observed although minor responses were reached.

Conclusions: The results showed that the ethanol application induces vascular smooth muscle contraction in concentration dependent manner. The mechanism for this action is independent of nitric oxide synthase activity.

Examination of long-term effects of different routes of drug-administration on physical development and emotional behaviour in rats

ESC-ID 946
Author Schmidt N, Rex A
Country Germany
University FU Berlin
Department Institute of Pharmacology and Toxicology

Background: Attention Deficit Hyperactivity Disorder (ADHD) is the predominant psychiatric disorder during childhood/adolescence with an incidence of 2-7% in children. The psychostimulant methylphenidate and the selective norepinephrine reuptake inhibitor atomoxetine are approved drugs for the pharmacological therapy of ADHD and prescriptions are rising constantly. To our knowledge there are no studies evaluating the long-term behavioural effects of a treatment with atomoxetine or methylphenidate in healthy adolescents. We will assess several facets of emotional behaviour in adult rats following chronic administration of atomoxetine or methylphenidate during peripolescence. Rationale: To differentiate pharmacological effects and behavioural changes induced by procedure and route of drug administration including the accompanying handling of the animals, our aim was to compare different application methods and to determine the method with least effects compared to an untreated control group.

Methods: Four groups (n = 6) of female Sprague-Dawley rats (Schoe:SD) in the age of 28 to 42 days were treated once daily (14 days) with a 10% sucrose solution either using intraperitoneal injections or oral administration using a micropipette or oral administration by gavages or the animals were not handled at all. After a week administration-pause the anxiety-related behaviour of the animals was tested in the Elevated Plus Maze (50 days postnatal) and in the Free Exploratory Paradigm (78 and 79 days postnatal) while habit-
Lithium (Li) is a drug which is used in manic-depressive disorders and its blood concentration should be measured 12 hours after an oral dose and continuously during treatment (Therapeutic Drug Monitoring). Therapeutic and toxic concentration of Li are close together. There are two methods for quantitative determination of Li in serum and urine: Atomic absorption spectrophotometry and atomic emission spectrophotometry (flame photometry). Because sodium (Na) and potassium (K) produce interference in determination of Li in these two methods, we decided to propose a new, simple, cheap and rapid colorimetric (spectrophotometric) method for quantitative determination of Li in serum and urine. For reduction of sample matrix effect, we used a standard addition method. To 5×1 ml of serum, urine and aqueous samples containing 0.5, 0.7, 0.9, 1 and 1.2 mM Li (therapeutic range of serum Li concentration) were added 1 ml of 1.5% violuric acid in distilled water separately. Samples were placed in boiling water bath for 1 hour and cooled. For finding wavelength of maximum absorbance (λMax) of Li-violuric acid complex, the processed aqueous sample containing 1.2 mM Li was scanned from 370 nm to 800 nm by a spectrophotometer. This λMax was selected for quantitative determination of Li in all samples. For study of correctness of this method, to 5A—1 ml of serum, urine and aqueous samples containing 0.8 mM Li were added 1, 2, 3, 4 and 5 ml of a standard solution containing 5 mM Li incrementally and separately and then treated as above. For quantitative measurement of Li concentration of these samples the formula $C_x = (b\lambda - C_s)/(m \times V_x)$ was used where $b$ is calibration curve intercept, $m$ is slope of calibration curve, $C_s$ is Li concentration of standard solution, $V_x$ is volume of sample and $C_x$ is Li concentration of sample. According to our results such a simple method let us measure Li concentration quantitatively in serum and urine more accurately.

### Drug-induced side effects in allergology – prospective study

**ESC-ID** 520  
**Author** Ołyniec K, Piotrowicz-Wójcik K, Skowron M  
**Country** Poland  
**University** Jagiellonian University Cracow  
**Department** Department of Allergology

**Introduction:** From among drug causing hypersensitivity the most common are the nonsteroid antiinflammatory drugs (NSAIDs), however many other drugs may cause similar reactions. These drugs include of example antibiotics, anaesthetics and ACE – inhibitors.

**Aim of the study:** The analysis of character of the allergic reactions caused by drugs, treatment used, occurrence of other allergic diseases and contributing factors. In the study all groups of drugs were included except of the nonsteroid antiinflammatory drugs (NSAIDs) because of their different mechanism of hypersensitivity reaction and the great prevalence which needs further study.

**Methods:** The study was carried out with the use of standardised drug hypersensitivity questionnaire by Demoly extended with informations about the frequency of the previous drug usage. The form includes questions about associat-ed cutaneous, gastrointestinal, respiratory, cardiovascular, psychic and other symptoms like: fever, myalgia, arthralgia or involvement of other organs. Results received from the questionnaire were verified by the use of wide range of additional diagnostic procedures; provocative tests and laboratory analysis.

**Results:** Anaesthetics and ß-lactam antibiotics caused most of the hypersensitivity reactions. After intravenous injection of anaesthetics occurrence not only of many cutaneous symptoms but also dangerous anaphylaxis was observed. The most frequent hypersensitivity reaction after usage of antibiotics was macular exanthema and angioedema. The greatest intensification of cutaneous symptoms was observed a few hours after drug intake. More than 50% of examined patients had other allergic diseases, however there was only a small group of patients with allergic reactions in the family. In the treatment of drug-induced allergic reactions steroids were used as a most popular anti-allergic factors.

**Conclusions:** Systematic reporting and description of drug-induced hypersensitivity reactions allows to obtain significant case study which helps to develop knowledge about frequency and process of drug-induced hypersensitivity reactions.
### Hypersensitivity to nonsteroid antiinflammatory drugs - use of a standardised drug hypersensitivity questionnaire

**ESC-ID**: 516  
**Author**: Piotrowicz-Wójcik K, Ożyniec K, Skowron M  
**Country**: Poland  
**University**: Jagiellonian University Cracow  
**Department**: Clinical and Environmental Allergology

Nonsteroid antiinflammatory drugs, usually abbreviated to NSAIDs, are one of the most commonly used medications. Most people are familiar with over-the-counter, nonprescription NSAIDs. Those medicaments have analgesic, antipyretic and anti-inflammatory effects— they reduce pain, fever and inflammation. NSAIDs are indicated in symptomatic treatment of pain, for instance in the treatment of back pain, dental pain, period pain and migraine. Due to their anti-inflammatory effects, they are widely prescribed for treating rheumatological diseases such as rheumatoid arthritis or osteoarthritis. Despite the fact, that NSAIDs are considered to be very safe, they cause quite often hypersensitivity reactions.

**Aim of the study**: Evaluation of frequency of hypersensitivity reactions to NSAIDs among patients, who are directed to the Department of Allergology of the Jagiellonian University in Cracow with a suspicion of hypersensitivity to drugs. The problem of cross reactions with other NSAIDs, type of most common occurring symptoms and eventual presence of specified predisposing factors were also evaluated.

**Methods**: The study was carried out with the use of standardised drug hypersensitivity questionnaire by European Network on Drug Allergy. Results of the enquiry were verified by provocative tests and diagnostic laboratory analysis.

**Results**: 1. Acetylsalicylic acid (Aspirin) caused the most of the hypersensitivity reactions. 2. The most frequent symptoms included skin reactions (maculopapular exanthema or urtiacrius exanthema) and Quincke's oedema, localized mostly around the face. Skin reactions were frequently accompanied by pruritus. 3. By over half of patients with hypersensitivity to NSAIDs occurred also other allergic diseases such as bronchial asthma, allergic rhinitis, food allergies or atopic dermatitis. 4. Viral infection and fever were the most common predisposing factors. 5. Positive results of provocative tests were observed in about 1/3 of the patients.

**Conclusions**: The standardised drug hypersensitivity questionnaire by European Network on Drug Allergy is priceless in diagnosis of drug reactions. Result of the enquiry should be verified by laboratory analysis and provocative tests, because not every suspicion of drug allergy was confirmed by laboratory tests. That suggests a great influence of concomitant factors on the occurrence of hypersensitivity to NSAIDs.

### Letrozole in the prevention of mammary carcinogenesis in female rats

**ESC-ID**: 567  
**Author**: Svecova I, Rovna T, Kapustova I, Sadlonova V, Kubatka P  
**Country**: Slovakia  
**University**: Comenius University, Jessenius Faculty of Medicine  
**Department**: Department of Pharmacology

**Aim**: The efficacy and toxicity of aromatase inhibitors in the treatment of premenopausal breast cancer patients are discussed among oncologists (Freedman et al., 2006, Goss and Strasser, 2001, Santen et al., 1999). In our experiment, a new method suitable for examining the chemopreventive effects of aromatase inhibitor - letrozole in premenopausal mammary carcinogenesis, was evaluated.

**Method**: Female rats of Sprague-Dawley strain from 33 to 37 days old were used. The rats were adapted to standard vivarium conditions. The animals were taken water ad libitum and standard food for rats. Mammary carcinogenesis was induced in rats by repeated application of N-methyl-N-nitrosourea on postnatal days 44 and 51. This model mimicked the situation in high risk premenopausal women for breast cancer. The animals were divided into 3 groups; each group consisted of 20 animals. Letrozole as a chemopreventive agent was administered in the food. In the first, control group, the food did not contain letrozole. In the second group - LETRO 1 letrozole was administered in the concentration of 1 mg/1 kg of food and in the third group - LETRO 10 in concentration of 10 mg/1 kg of food. Chemoprevention was initiated a week prior to carcinogen administration and lasted till the termination of the experiment, i.e. 17 weeks. The rats were weighed and palpated once weekly during the experiment. The body weight of animals and the presence, number, location and size of each palpable tumour were registered. The food intake of animals was measured. In the last week of the experiment the animals were killed by quick decapitation. Subsequently the blood was taken to examine plasma lipid profile - total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triacylglycerols. During autopsy, mammary tumours, uterus, vagina and other pathologically changed organs were excised. The organs (uterus, vagina) were weighed. The samples of excised organs and tumours were sent for histological analysis.

**Results**: Letrozole in the group LETRO 1 decreased the incidence of the mammary tumours (P<0.00002) in comparison with control group (75 % vs. 5.2 %). As well as the frequency of mammary tumours in the group LETRO 1 was decreased (P<0.00002) compared with control animals (1.60 vs. 0.05). In the group LETRO 10, totally suppression of mammary carcinogenesis was observed. In the groups with letrozole, uterine and vaginal atrophy and decrease in the weight of both organs (P<0.0001) were found at the end of the experiment. In letrozole - treated animals in comparison with control animals, increased plasmatic triacylglycerol concentrations (P<0.0001) were observed. Other parameters of lipid metabolism were not significantly changed. An increase of body weight gain and food intake was found in both letrozole groups in comparison with control group (P<0.0001).

**Conclusion**: This study is the first report on tumour suppressive effects of letrozole in the model of premenopausal mammary carcinogenesis in female rats. The administration of letrozole in premenopausal breast cancer women will be lim-
Patients’ abuse of antibiotics

Introduction: Intensive use and abuse of antibiotics (ABs) resulted in selection of bacteria which are resistant to most, and some of them to all ABs. Results of Palmer and Bauchner’s study show that about 93% of parents think that all infections of ear need AB treament, also throat infections (83%), cold (32%), temperature (58%) and cough (58%) need AB. Study is taken in 8 East European countries show that the most frequent source of ABs abuse are pharmacies (68%) and "leftover" antibiotics (26%).

Aim: The main aims of the study are: 1. the most frequent symptoms as reasons for patients to use ABs without a physician’s recommendation; 2. the most frequent sources of abuse ABs; 3. most frequently abused ABs; 4. how much patients know about unwanted effects as results of abuse of ABs.

Method: We have tried to find the answers to the questions using a questionnaire. This study included five offices that belong to the Center of family medicine in Banja Luka. The questionnaire includes 5 questions. The study also includes information about age, gender and education. Data were analysed by method of likelihood ratio test, chi-squared test and another statistical tests that present a valid ratiocination.

Results: The preliminary sample consisted of 111 respondents: 77 females and 34 males. Every third patients use of ABs without consulting a doctor (33%). 1. The most frequent symptoms as reasons for patients use of ABs without consulting a doctor are: temperature (21%), headache (20%), cough (14%), sore throat (9%) and hoarseness (6%), otitis media, diarrhea and pain during urinate (5%). 2. The most frequent sources of ABs are pharmacies (64%), 18 percent of patients use “shared”antibiotics, and 18% of patients use “leftover” antibiotics. 3. Most frequently abused ABs are: amoxicillin, amoxiclav, cotrimoxazole and doxycycline. 4. Even most patients think that ABs have unwanted effects and shouldn’t be taken without consulting a doctor (83%), every third patients use of ABs without consulting a doctor (31%). They also agree (78%) that uncontrolled abuse of ABs will result in bacterial resistance to ABs, still every fifth patient thinks that ABs are drugs for virus infections (21%). Also most patients (88%) agree that abuse of ABs can damage their and another people’s health, but 23% of patients says that they get an AB from they doctor anyway.

Conclusion: Most patients have a certain level of knowledge about ABs, their unwanted effects and bacterial resistance, and still they abuse them and even ask their doctor for AB, although they have symptoms caused by virus.

Physiology II

Role of glutamate induced apoptosis at thymus level in rats

Introduction: Glutamic acid represents one of the main excitatory amino acids in the central nervous system, where it exerts its effects by binding to ionotropic and metabotropic receptors, thus modulating the intracellular processes. Recent studies have showed the existence of glutamic receptors on lymphocytes and thymocytes. But, immunomodulatory effect of monosodium glutamate (MSG) on the cells of the immune system still remains unknown.

Aim: The current study was design to evaluate the effect of MSG on the thymocyte proliferation and apoptosis.

Material and methods: Experimental and control groups of animals included 10 Wister rats each. Experimental group animals were treated with MSG (4 mg MSG/g BW) for 6 consecutive days and sacrificed 45 days after MSG treatment. Thymocyte proliferation was evaluated by measuring the expression of proliferating cell nuclear antigen by flow cytometry. Apoptosis was detected using the Annexin V-FITC/PI apoptosis detection kit and cells were analyzed using flow cytometry. Expression of Bcl-2 and Bax proteins were determined with flow cytometry using respective monoclonal antibodies. For histopathological analysis, paraffin sections of the thymus were hematoxylin-eosin (HE) stained.

Results: The study results demonstrate that MSG significantly decreased thymocyte proliferatio (p<0.001) induced by Con A and increased apoptosis rate (p<0.001) of the cells during examination period. MSG treatment induced down regulation of Bcl-2 protein while Bax protein levels were not significantly changed. Histopathological analysis of the thymus in experimental animals shows depletion of thymocytes in the cortex, while in the medulla there is hemorrhagia with destruction of normal tissue.

Conclusion: Flow cytometry results along with histopathological analysis indicate that MSG significantly modulates thymocyte proliferation by modulating the apoptosis rate of the cells. The Bcl-2 and Bax expression, after MSG treatment, suggest that down regulation of Bcl-2 protein and resulting change of Bcl-2/Bax protein ratio may be an important event in thymocyte apoptosis, triggered by MSG.

Gastric adaptation to aspirin enhances the mucosal resistance to injury by strong irritants

Introduction: In this study we want to investigate if gastric adaptation to injury during repeated doses of acetyl salicylic acid (ASA) affects the tolerance of the mucosa to other strong irritants?
**Material and methods:** 40 female Rats were divided into 2 groups in group A gastric adaptation was induced by repeated daily doses of acidified ASA (80 mg/kg in 1 ml of 0.2 N HCl) given intragastrically in group B which was control rats with an intact stomach were given daily intragastric vehicle only (1 ml of 0.2 N HCl). After 6 days of adaptation, rats were challenged again with acidified ASA or, for comparison, with strong irritants such as 100% ethanol, 200 mM acidified taurocholate, or 25% NaCl for 2 hour or with water immersion and restraint for 4 hours.

**Results:** The first dose of ASA produced numerous gastric lesions and deep histological necrosis accompanied by a fall in the gastric blood flow, negligible expression of epidermal growth factor (EGF) and transforming growth factor alpha (TGF alpha) or their receptors, and no evidence of mucosal proliferation. As adaptation to ASA developed, however, the areas of gastric lesions were reduced by more than 80% and there was a noticeable decrease in deep necrosis, a partial restoration of gastric blood flow, an approximately four-fold increase in EGF expression (but not in TGF alpha) and its receptors, and an appreciable increase in mucosal cell proliferation compared with vehicle treated rats. Increases in the mucosal expression of EGF receptors and the luminal content of EGF were also found in ASA adapted animals. In ASA adapted rats subsequently challenged with 100% ethanol, 200 mM TC, 25% NaCl, or stress, the area of the gastric lesions and deep histological necrosis were appreciably reduced compared with values in vehicle treated rats. This increased mucosal tolerance to strong irritants was also accompanied by the return of the gastric blood flow towards control levels and further significant increases in the mucosal expression of EGF receptors and mucosal cell proliferation.

**Conclusion:** Gastric adaptation to ASA enhances the mucosal resistance to injury by strong irritants probably as a result of the restoration of the gastric blood flow and increased cell proliferation that may result from increased mucosal expression of EGF and its receptors. Keywords: acetyl salicylic acid, mucosal resistance, EGF, TGF alpha.

**Colloidal Stability of Cholesterol Dispersions**

**ESC-ID** 849  
**Author** Gureev S, Savchenko O, Savenko J, Barsukova O, Sivakov D  
**Country** Belarus  
**University** Gomel State Medical University  
**Department** Medical

**Introduction:** The research work was done to study the effects of multiple factors on colloidal stability of cholesterol dispersions. We examined the effects of calcium, heparin and herudin on critical coagulation concentration (c.c.c) of cholesterol suspension by lead (II) acetate solutions. The obtained data make possible to characterize anticoagulant activity of drugs not only qualitatively but quantitatively as well.

**Materials and Methods:** Cholesterol water-alcohol colloidal solutions were prepared from atherosclerosis spots by ultra sonic degradation method. Cholesterol and calcium content in test solutions were determined by visible molecular spectrometry. C.c.c of cholesterol coagulation by lead (II) acetate was calculated according to the formula: c.c.c = C/Vmin +1.5, where C is (CH3COO)2Pb concentration, mol/L, Vmin is a minimal volume of a salt, which initiates cholesterol visible coagulation, mL.

**Results:** The obtained data revealed that: • Cholesterol content in atherosclerotic spot is in the range 13-82 % by mass and calcium concentration in them varies from 0.1 to 2.5 %; • C.c.c of cholesterol coagulation by lead(II) acetate is directly proportional to calcium content iv test dispersions; the relationship between them is the following: c.c.c = 2.36 ?Ca/ +0.47, where ?Ca? - calcium concentration, per cent by mass; • The analogues linear correlation was determined between colloidal stability of cholesterol dispersions and heparin concentration in test solutions: c.c.c = 8.25?Heparin? +1.5, where ?Heparin? - a volume of 2 % by mass heparin solution, mL • The herudin effect on cholesterol colloidal stability is quite different. In the area of low herudin concentrations the increase in its content gives increase in stability, while in the area of its high concentration the effect is reverse. This is a phenomenon of irregular series well studied by colloidal science. The stabilizing effect of herudin is approximately identical to heparin.

**Conclusions:** • The effect of different factors on cholesterol colloidal stability was on study. • The obtained data make possible to describe the anticoagulant activity of some drugs and calcium not only qualitatively but quantitatively as well.

**Catecholaminemia at rats after immobile stress**

**ESC-ID** 794  
**Author** Saipii R, Nestorovic V  
**Country** Serbia  
**University** University of Pristina  
**Department** Medical Faculty

**Introduction:** In 1935 Selyea published the work in “Nature” magazine under the name of “Syndrome caused by a different causing damages” and called this syndrome “biological stress”. In later works, it was noticed that certain reactions of the organism were caused by numerous physiologic and psychological factors so they called that syndrome “stress”. Selyea found out that numerous factors such as physical factors (coldness, warmth, muscles activity), different kinds of medicaments, different pathologic conditions (disease, injury) could cause stress.

**Aim:** Aim of the work was to measure the level of catecholamine in serum after immobile stress.

**Material and methods:** This experiment was performed in vivo at sexually matured males of laboratory albino rats type Lewis which were 3-6 months old and had weight of 300 g (+50 g). The experiment was performed in two groups consisted of 10 animals. The first group of animals was exposed to violent immobilization while the second one was control group. The blood was taken out of carotids and serum was separated for laboratory analyses. The result: We had very high level of catecholaminemia at animals exposed to immobile stress in comparison to control group which wasn’t exposed to stress because of the fact that immobilization is an acute neurogenic stress which leads to a sudden release of noradrenalin on the nerves endings as well as the great amounts of adrenalin from suprarenal gland.

**Conclusion:** Neurogenic stress – enforced immobilization leads to an activation of the noradrenergic ways and also intensifies the release of noradrenalin on the nerves endings.
Effects of phenylephrine, D600, and sodium nitrite upon membrane potential and isometric tension in rat aortic smooth muscle

ESC-ID 614
Author Isaila O, Ionela LS, Dragomir NS
Country Romania
University University of Medicine and Pharmacy "Gr.T.Popa"
Department Physiology

Membrane potential (MP) is an essential factor in smooth muscle contractility, mainly by its effect upon activity of L-type calcium channels (CaL). There are relatively few electrophysiological studies upon rat aortic smooth muscle. We used a wire myograph, glass microelectrodes (Ag/AgCl, KCl 3M, 40-60 MΩ), a microelectrode amplifier and an AD converter. We studied the dynamic relation between MP and active force in deendothelialised aorta rings from male adult Wistar rats, within the contraction induced by 0.01 mM methoxy-verapamil (D600) or by 0.1 mM NaNO . The resting MP was 49.5 ± 1.6 mV (n = 72). The MP threshold of contraction and the MP value the moment the maximum force was reached were in the case on K+ -41.2 ± 4.1 mV and -14.2 ± 3.7 mV, while for PE they were -48.8 ± 3.5 mV and -27.2 ± 3.8 mV respectively (n = 12 in both cases). For resting MP and depolarization induced by K+ or PE our results are similar to those obtained by others. MP-force dynamics show that depolarization and CaL involvement in the contractile effect of PE is minor. Since the polarizing effect of NaNO is similar in K+ and PE-precontracted rings, it is unlikely to be based on activation of K+ channels. Not being correlated with force, polarization by NaNO seems to be just an additional mechanism within the respective relaxation.

Amplitude changes in motor unit action potential of biceps brachii muscle during fatigue

ESC-ID 479
Author Ragaji A
Country Serbia
University University of Medicine and Pharmacy Novi Sad
Department Physiology

Introduction: Action potential of all muscle fibres of one motor unit is called motor unit action potential (MUAP). Aim: Registration of amplitude changes in MUAP of biceps brachii muscle of dominant arm during development of fatigue in non sportsmen and recreational weight-lifters. Method: Study was performed on 25 persons of both sex divided into two groups. First group consisted of 10 male recreatives – weight lifters. In the second, control group (7 male and 8 female subjects) were students of the Faculty of medicine in Novi Sad. The research was conducted in two phases. The first phase comprised of gathering anamnestic and anthropometric data as well as determination of single maximal voluntary contraction by determination 1 RM (one repetition maximum) of the dominant arm. The other phase of the research comprised the registration of amplitude changes in motor unit action potential, using surface monopolar EMG electrodes, during the static (isometric) contraction, using weight which mass was 30% of 1 RM, until breakdown. The registration was performed twice with time interval of 15 minutes. Results: In both groups, the second measurement lasted significantly shorter than the first. In the analysis of the EMG registration results, linear dependence of the amplitude of MUAP in time was noticed. In both groups the amplitude of MUAP rises during the registration. Significantly higher starting amplitudes of MUAP in the second measurement were registrated in both groups. In comparing the results of recreatives and male non-sportsmen we noticed significantly higher value of starting amplitude of MUAP of recreatives in the first and the second measurement than the values of amplitudes of MUAP of male non-sportists. In analysis of changing rate of amplitudes of MUAP, we found significantly higher values in the second measurement of male non-sportists. Conclusion: There are no significant gender differences in the observed parameters. Amplitude changes in MUAP are in correlation with development of fatigue.

Ethnic and gender dominant side differences in Gipsy and Serbian pupils as established by means of performance tests

ESC-ID 578
Author Uljarevic M, Petrovic S, Bundovska S, Nasic M
Country Serbia
University Nis
Department Physiology

Introduction: While numerous studies have aimed to cast light on the causes of hemispheric asymmetry, there are relatively few studies that deal with ethnic differences in lateralisation. This study is concerned with gender interaction, dominant sides and ethnic affiliation. Aim: 1. to study the dominant side in Gipsy and Serbian children of male and female gender; 2. to establish existence, if any, of ethnic and sex differences in dominant side use. Material and Methods: The sample consisted of 255 pupils (110 of female and 145 of male gender; 166 of Gipsy and 89 of Serbian population), from the first through the seventh grade of elementary school. The pupils were studied with regard to eight lateralized tasks that imply the primary use of one body part in two-hand activities. Results: Hi square test established a statistical gender significance for the following activities in Gipsy population: crossing of forearms on the chest (p = 0.048), using arms for â€œ_back scratchingâ€ (p = 0.01) and applauding (p = 0.04), while in Serbian population it was established for winking (p = 0.04). Multivariant tests showed the difference effect among the subjects with regard to their gender for the following activities: crossing of forearms on the chest (F = 4.407, p = .03), back scratching (F = 8.764, p = .00), winking (F = 4.674, p = .03), as well as with regard to their ethnic affiliation for applauding (F = 5.224, p = .02).

Conclusion: Our results point at the gender differences, as well as at the ethnic differences with regard to the use of the dominant side of the body. Owing to the fact that our study included the elementary school pupils, it would be significant to do a follow-up with the older Gipsy population in order to establish whether the differences found in Gipsy pupils remain or change with years.
In experiments in animals there has been established that cadmium chloride introduction together with gonad-toxic effect results in a variety of impairments in enzyme system, metabolism of some microelements, phosphorus-calcium exchange. This in its turn leads to a sharp decrease in the level of androgens in blood of animals and as a consequence to dehydration of additional sexual glands. The research aim was to study the changes in the mass of additional sexual glands after introduction of cadmium chloride and after allo- and xenotransplantation. The solution of CdCl2 (0.1%) was introduced intraperitoneally to 3-4 months male rats in respect of 3 mg/kg of an animal. In a month after introduction of CdCl2 intratesticular allotransplantation of newborn piglets testis interstice cells was done to the animals. The cells were procured as follows: after extirpation, the testes were removed from protein cover and finely fragmented. After collagenization and primary sedimentation of tubular structures the obtained cell supernatant was separated in sucrose gradient. Cell fraction with the density of 1.047-1.100 g/cm3 was collected and thoroughly washed out with PBS (pH = 7.2-7.4). Afterwards 0.5ml suspension with the amount of cells about 80·10^7/cells was intratesticularly introduced to the rats treated with CdCl2, that corresponded to an average amount of cells obtained from one donor. The part of animals were mortified prior to transplantation, in 4 days after transplantation other one and in a month the rest of animals. In addition, there was investigated the group of animals with CdCl2 but not subjected to transplantation. The mass of inner organs were examined and expressed in respect of 100g of animal¡’s weight. In a month after CdCl2 introduction the animals had normal appearance, mobility and appetite. After dissection of abdominal area there was found an extended connective tissue, that pointed to passed inflammation process, caused with CdCl2. Secondary sexual glands, i.e. seminal vesicles were strongly degraded with the mass of 57,10 mg/100g. In normal animals their weight was 325,29 mg/100g. In a month after transplantation we observed a rise in body mass of seminal vesicles up to 258,85 mg/100g, that statistically and significantly does not differ from normal animals but considerably differed from rats with no transplantation, 531,10mg/100g. Testes in all groups with the injection of CdCl2 kept the features of deep degradation, the mass of 163,32mg/100g if compared with the control 531,10mg/100g. Xenotransplantation also contributed to a rise in the mass of additional sexual glands in the same manner. It is known that the mass of sexual glands correlates strongly with the level of androgens in blood of animals [1]. Thus the introduction of allo- and xenocells of interstice contributed to the fastest recovery of additional sexual glands.

NO and endothelium-derived constricting factor in the spontaneous recovery of L-NAME-induced hypertension

ESC-ID 419
Author Ludovit P, Zicha J, Kunes J, Hojna S, Kojsova S, Pechanova O, Fedor
Country Slovakia
University School of Medicine, Comenius University
Department Institute of Physiology

Aim: Although NG-nitro-L-arginine-methyl ester (L-NAME)-induced hypertension is an attractive model of experimental hypertension, the mechanisms contributing to the maintenance of L-NAME hypertension are not completely understood. Our previous experiments have shown the release of cyclooxygenase-sensitive endothelium derived-constricting factor (EDCF) in femoral artery contributing to constriction following transient relaxation after acetylcholine-stimulation in L-NAME rats. We aimed to investigate, whether the recovery of NO and EDCF-mediated vascular responses in the spontaneous regression of L-NAME hypertension (after cessation of L-NAME administration).

Methods: Four groups of Wistar rats (n = 8 each) were investigated: 5-week control, L-NAME (40 mg/kg/day for 5 weeks), spontaneous recovery (L-NAME for 5 weeks followed by 3-week recovery) and 8-week control. Blood pressure (BP) was measured invasively. NO-synthase (NOS) activity in the aorta was determined by measuring the conversion of radioactive L-arginine to L-citrulline. Using Mulvany wire myograph, the normalized inner diameter of the femoral artery, acetylcholine (ACh)-induced relaxations of norepinephrine (NE)-precontracted femoral and small mesenteric arteries and the influence of L-NAME-preincubation on ACh-induced relaxations were evaluated. In femoral arteries also EDCF-mediated responses induced by ACh were determined.

Results: L-NAME caused systolic and diastolic BP elevation accompanied by reduced inner femoral artery diameter, augmented EDCF-signaling and impaired NO-signaling indicated by decreased NOS activity in the aorta, impaired ACh-induced relaxations (dose-response curve shifted to higher ACh-doses) and abolished sensitivity of ACh-induced relaxations to L-NAME-preincubation on ACh-induced relaxations were evaluated. In femoral arteries also EDCF-mediated responses induced by ACh were determined.

Conclusion: L-NAME administration leads to disruption of NO-signaling in vessels with large and small diameter. This impaired NO-signaling is fully reversible after the cessation of L-NAME, but persistent arterial structural alterations and enhanced formation of EDCF may maintain elevated blood pressure even after the restoration of NOS activity.

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The implication of gastrin as a trophic agent for colonic mucosa (CM) in arising and development of neoplasms in colon is undoubted. But its role in maintenance of the colonic water and electrolyte transport function is not almost studied. The aim of the study was to examine the effect of omeprazole-induced (OM) hypergastrinemia (HG) of different terms on morphofunctional indices (MFI). The implication of gastrin as a trophic agent for colonic mucosa (CM) in arising and development of neoplasms in colon is undoubted. But its role in maintenance of the colonic water and electrolyte transport function is not almost studied. The aim of the study was to examine the effect of omeprazole-induced (OM) HG of different terms on morphofunctional indices (MFI).

Methods: Adult male Wistar rats were used under urethane anesthesia according to a method of isolated colonic loop perfusing technique in vivo [6] for determining of net water and electrolyte transport function. Administration of OM for 1 week increased GL by 207.05% and decreased Jnet NaCl compared with the BG. After 2 weeks of OM administration there weren’t shown the significant changes of studied MFI. Prolongation of OM influence up to 3 weeks entailed significant increasing of GL by 131.10%, JnetCl−, CD and MT and decreasing of JnetNa+. HG for 4 weeks (GL remained higher then in BG by 189.27%) decreased Jnet water, Na+ and NPA and significant increased JnetCl−, CD, MT.

Results: Administration of OM for 1 week increased GL by 207.05% and decreased Jnet NaCl compared with the BG. After 2 weeks of OM administration there weren’t shown the significant changes of studied MFI. Prolongation of OM influence up to 3 weeks entailed significant increasing of GL by 131.10%, JnetCl−, CD and MT and decreasing of JnetNa+. HG for 4 weeks (GL remained higher then in BG by 189.27%) decreased Jnet water, Na+ and NPA and significant increased JnetCl−, CD, MT.

Conclusions: The short-term OM-induced HG alters the electroneutral absorption of NaCl. The prolongation of HG up to 3 weeks displays the first manifestation of the trophic effect of gastrin along with altered electronegative JnetNa+ absorption. OM-induced HG for 4 weeks causes appearance of hyperplastic features and decreases differentiated colonocytes ratio which provokes the decreasing of Jnet water and NaCl absorption.

Influence of physical activity on some anthropometric, dynamometric and ventilatory parameters

Introduction: Determination of body composition is of great importance for clinical purposes and for follow-up of training process in athletes. Aim: The aim of this study was to determine some anthropometric, dynamometric and respiratory parameters in the groups of sportsmen and non sportsmen and to establish correlation between these parameters and level of physical activity.

Materials and methods: The research was performed at the Department of Physiology, University of Novi Sad. Followed parameters were measured: body height (BH), body mass index (BMI), body fat percent (BF%) by bioelectrical impedance (BFbia) and skinfold thickness (BFsft), dynaminometric measurements of muscle strength and ventilatory parameters.

Results: Athletes have had significantly higher values of BMI, BFbia and BFsft compared with non sportsmen (p<0.05). There is a good correlation between BMI and BFbia, BMI and BFsft and BFbia and BFsft in the group of nonsportsmen. In the group of sportsmen there was no correlation found between BMI and BFsft. There is a significant difference (p<0.05) in dynaminometric, but no significant difference in ventilatory parameters between groups.

Conclusion: Athletes have had significantly lower values of BMI and BF%. Athletes have had higher values of dynamometric parameters because of longlasting training process. Ventilatory parameters were similar in both groups because sportsmen were younger. BMI is a measure of heaviness rather than fatness and it is not a good predictor of fat content in sportsmen because of muscle and fat tissue ratio.

The role of glutamate receptors in realization insulin and carbacholin gastric acid secretion in rat

Glutamate receptors are involved in many diseases. NMDA antagonism has been considered as a strategy in the development of drugs for the treatment of anxiety, depression, epilepsy, stroke, cognitive deficits and drug dependence. But chronic administration of these drugs can be reasons of development different disorders in the gastrointestinal tract and also many NMDA antagonists display side effects [2, 4, 5, 6, 7]. Aim: to investigate the role of block of ion channel of glutamate receptors NMDA-type in regulation of gastric acid secretion (GAS) stimulated by carbacholin and insulin.

Methods: The investigations were carried out on white male

The effect of short and long-term hypergastrinemia on the colonic water and electrolyte transport function

The implication of gastrin as a trophic agent for colonic mucosa (CM) in arising and development of neoplasms in colon is undoubted for today [2, 4]. But its role in maintenance of the colonic water and electrolyte transport function is not almost studied [3]. The aim of the study was to examine the effect of omeprazole-induced (OM) hypergastrinemia (HG) of different terms on morphofunctional indices (MFI). The implication of gastrin as a trophic agent for colonic mucosa (CM) in arising and development of neoplasms in colon is undoubted. But its role in maintenance of the colonic water and electrolyte transport function is not almost studied. The aim of the study was to examine the effect of omeprazole-induced (OM) HG of different terms on morphofunctional indices (MFI).

Methods: The research was performed at the Department of Physiology, in Laboratory for Functional diagnostics, Faculty of Medicine Novi Sad. Study was conducted on 50 male subjects, 25 sportsmen and 25 non sportsmen. Followed parameters were measured: body weight (BW), body height (BH), body mass index (BMI), body fat percent (BF%) by bioelectrical impedance (BFbia) and skinfold thickness (BFsft), dynaminometric measurements of muscle strength and ventilatory parameters.

Results: Athletes have had significantly higher values of BMI, BFbia and BFsft compared with non sportsmen (p<0.05). There is a good correlation between BMI and BFbia, BMI and BFsft and BFbia and BFsft in the group of nonsportsmen. In the group of sportsmen there was no correlation found between BMI and BFsft. There is a significant difference (p<0.05) in dynaminometric, but no significant difference in ventilatory parameters between groups.

Conclusion: Athletes have had significantly lower values of BMI and BF%. Athletes have had higher values of dynamometric parameters because of longlasting training process. Ventilatory parameters were similar in both groups because sportsmen were younger. BMI is a measure of heaviness rather than fatness and it is not a good predictor of fat content in sportsmen because of muscle and fat tissue ratio.
rats in acute experiments by method of perfusion of isolated stomach by Ghosh and Shild. The GAS was stimulated by intraperitoneal (i.p.) injection of carbacholin (0.01 mg/kg), and insulin (1.2 U/kg). MK-801 was injected in dose 0.1 mg/kg, i.p. One experimental group of animals was vagotomized and treated with MK-801.

**Results:** It was established that i.p. injection of MK-801 diminished carbacholin GAS by 37% (P<0.01). Depressive effect of MK-801 on carbacholin GAS is abolished by vagotomy. So, in realization GAS are involved central glutamate receptors that exist on the neurons of nucleus tractus solitary (NTS) [8] and activation of this NMDA-receptor’s ion channel by endogenous glutamate increase carbacholin GAS. Treatment with MK-801 increased insulin GAS by 63% (p<0.05). So, stimulation NMDA-receptors by endogenous glutamate inhibits insulin GAS. Insulin GAS is a result of irritation the first of glucosensing zone of hypothalamus by hypoglycemia and than, the nucleus of vagus [1]. It is obvious that in this case we can see two effects: increasing insulin GAS throw irritation NTS by endogenous glutamate and decreasing insulin GAS via stimulation NMDA-receptors in the in glucosensing zone of hypothalamus. The last is stronger, so as a result we can see diminishing insulin GAS. It is evident; there are two types of glutamate receptors [3].

**Conclusion:** We suggested that excitation of ion channel of NMDA-receptors by endogenous glutamate are important for realization GAS stimulated by carbacholin and insulin. In this process are involved NMDA-receptors that realize opposite effect and are located in the NTS and glucosensing zone of hypothalamus.

**Gender differences in cardiovascular responses to one session of slow ‘pranayamic’ breathing**

**ESC-ID** 43  
**Author** Nayar M, Pearson J, Farrish H, Cheema D, Besser J, Short J  
**Country** United Kingdom  
**University** University Nottingham  
**Department** Biomedical Science

**Background:** There is interest in the use of slow breathing as a form of non-pharmacological treatment for cardiovascular disease, but studies to date have largely been restricted to measurements of blood pressure and heart rate, and, to our knowledge, none have considered gender differences, despite the evidence for gender differences in cardiovascular regulation and in the incidence of cardiovascular disease.

**Method:** Cardiovascular responses of 35 volunteers (21 females), age range 20-24 years, were measured before, during and after a 20 cycle session of slow pranayamic breathing (1 cycle = 3s inspiration, 12s breath-holding, 6s exhalation). Use of a Finometer allowed continuous, non invasive, measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR). Cardiac variables were factored to account for body surface area.

**Results:** Baseline values of CO and SV, factored for body surface area, were significantly higher in males (3.7 ± 0.9 l/min/m², 49.1 ± 1.6 ml/m²) than in females (2.9 ± 0.2 l/min/m², 39.4 ± 1.5 ml/m²) while TPR was higher in females (0.8 ± 0.04 u/m²) than in males (0.5 ± 0.03 u/m²). During the breath-holding phase of the first cycle of pranayamic breathing, SBP, DBP and MAP decreased (p<0.05, Friedman’s test) in both genders (-12 ± 3 and -8 ± 2% fall in MAP in males and females, respectively), accompanied by a significant fall in CO (-12 ± 4 and -9 ± 2% in males and females, respectively) which was due to a fall in HR. During this period, there was a significant increase in SV in females but not males. Over the entire 20 cycle session, there was a significant reduction in MAP only in males, while only females showed significant bradycardia. At end of the intervention, the fall in SBP in males was 6 ± 3mmHg and the fall in DBP was 3 ± 1mmHg.

**Discussion:** Males and females showed similar patterns of cardiovascular responses during the first cycle of pranayamic breathing, with the fall in MAP being due to a fall in CO, rather than TPR. It has been suggested that females have less tonic autonomic regulation of blood pressure and a reduced baroreflex buffering capacity, but this is not consistent with the present findings, where a significant fall in BP during the 20 cycles of pranayamic breathing occurred only in the males. Indeed, since bradycardia during the intervention was only significant in females, this could indicate a gender-difference in the capacity for vagal activation. This study only reports on acute modifications with one session of pranayama in normal healthy volunteers. Whether or not the benefits can be maintained, or enhanced, chronically, and whether or not the responses are enhanced in patients with cardiovascular disease, remain to be determined.

**Pharmacological myocardium protection via inhibition of mitochondrial permeability transition pore opening**

**ESC-ID** 569  
**Author** Goshovska Y, Dobrovolskyj F, Shymanskaja T, Sagach V  
**Country** Ukraine  
**University** Bogomoletz Physiology Institute  
**Department** Blood Circulation

It is well-known that hypoxic states induced by ischemia and myocardial infarction cause changes in cell function. Membrane potential decreasing, dissipation of proton gradient, lower ATP production, increasing of free radicals take place during ischemia. All these lead to permeability transition, mitochondrial pore opening, apoptosis or/and necrosis. In result dysfunction of myocardium and heart failure occurs. It was recently demonstrated that reperfusion causes the efflux of mitochondrial factor which can be a marker of myocardial reperfusion injury. The most powerful cardioprotective strategy known is ischemic preconditioning – short periods of ischemia that paradoxically protect myocardium from subsequent prolonged ischemia. Furthermore, prevention of ischemic cardiac abnormalities is also possible with pharmacological preconditioning using chemical agent such as antioxidants (for example tloolox, melatonin etc.). That is why monitoring of novel chemicals that appear to be scavengers of free radicals is urgent question. The aim of the research was to compare two cardioprotective strategies in order to investigate their implication in inhibiting mitochondrial transition pore opening.

**Methods:** The influence of pharmacological pre-treatment with novel chemical (C15H15N3O2S – synthesized on basis of quinazololone) has been studied on isolated guinea pig’s hearts. Cardiodynamic parameters (left ventricular pressure,
coronary flow, dP/dtmin, dP/dtmax), oxygen cost of cardiac work were measured. Mitochondria transition pore-dependent swelling and mitochondrial factor efflux have been measured spectrophotometrically.

Results: it was shown that ischemia cause significant heart reperfusion disturbances and mitochondrial swelling with mitochondrial factor releasing. Such effect of ischemia was eliminated with previous pharmacological application with quinazolinone-base substance and ischemic preconditioning. Left ventricular pressure in the end of reperfusion arranged 67.0 ± 6.6% and 78 ± 5.4% respectively (48% ± 11.6% in control). Oxygen cost of microcardial work were 132% ± 7.8% in pharmacological treated and 116% ± 5.7% in preconditioned hearts (183% ± 5.0% in control). Isolated mitochondria from pretreated hearts swelled significantly less from those in control.

Conclusions: Obtained data suggest that C15H15N3O2S as well as ischemic preconditioning prevent mitochondrial swelling by inhibition mitochondrial transition pore opening. Previous pre-treatment with C15H15N3O2S improved myocardial functions and cardiac recovering after prolonged ischemia. Inessential quantity of mitochondrial factor and absence of coronary contractility are strong evidence for cardioprotective effect of chemical tested. Thus, mitochondrial permeability transition pore is beneficial target for cardioprotection.

Prescription of psychotropic medications by family physicians at a university hospital in Karachi, Pakistan

ESC-ID 398
Country Pakistan
University Aga Khan University
Department Medical College

Background: Studies have shown a high prevalence of psychiatric illnesses among patients in primary health care settings. Family Physicians have a fundamental role in managing these disorders via prescribing psychotropic medications. Information regarding prescribing patterns is limited and studies from across the world reveal variable patterns. Objective: The purpose of this study is to determine the prescription pattern unique to our community, to assess its relationship to various socio-demographic factors and to determine, if patients who have been prescribed these medicines were educated about their disease and the drugs. Design and Setting: Cross-sectional study was conducted at the Community Health Centre (CHC), Aga Khan University Hospital Karachi, Pakistan.

Methods: Information about prescriptions was acquired from the patients visiting the CHC. Three hundred and fifty-four adult patients were interviewed after their consultation. The outcomes of interest were analyzed using SPSS v.14.

Results: Of the total patients interviewed, 20.6% were prescribed psychotropic medications. Prescription of psychotropic medications was significantly associated with increasing age (p = 0.017), female gender (p = 0.001), lower education (0.001) and being married (p = 0.009). Among patients receiving psychotropic medicines, 50% did not know their diagnosis 86% were not explained the adverse effects of the drugs and 33% were not aware of the duration of treatment.

Psychiatry II

Comparison between the effects of oxytocin and alcohol drinking on affective “mind-reading” in men and women

ESC-ID 613
Author Ceanga M, Ioan S, Nastase A, Braga RI, Zagrean AM, Moldovan M
Country Romania
University University of Medicine and Pharmacy "Carol Davila"
Department Center for Excellence in Neuroscience

Background: The ability to make inferences about other persons’ beliefs, intents and desires by interpreting subtle behavioural cues (“mind-reading”) is an indispensable skill for human social interaction. Impairments in “mind-reading” were described in disorders of social dysfunction such as autism, Asperger’s syndrome and schizophrenia. Recently, the neuropeptide oxytocin was reported to improve affective “mind-reading” in men and was suggested to have potential clinical implications for individuals with severe social impairment (Domes G. et al, Biol Psychiatry. 2007).

Aim: Alcohol drinking is historically known as an enhancer of sociability. The aim of this study was therefore to compare the effect of alcohol drinking and oxytocin on “mind-reading”.

Methods: Experiments were carried out in a group of medical students of both genders, occasional drinkers of beer. Only nulliparous women in the second half of their menstrual cycle were included. We compared the effects of a single “standard” dose of intranasally administered oxytocin (24 IU) and 200ml of beer (5% alcohol) on Reading the Mind in the Eyes Test (RMET) Briefly, the participants were presented with a series of 36 photographs of the eye-region of the face of different actors and actresses and were asked to choose one out of four mental state descriptors that best describes what the person in the photograph is thinking or feeling.

Results: No adverse events were reported within 1 week after oxytocin administration in either men or women. Oxytocin improved performance on RMET for both men and women with ~5%. A glass of beer had a remarkably similar effect with oxytocin in increasing “mind-reading” in men and women alike. Both the effects of 200 ml glass of beer and oxytocin appeared more pronounced for the subset of photographs that were more challenging to interpret in terms of the affective mental state. Nevertheless, a 400 ml glass of beer did not further improve the results in men; and decreased women’s “mind-reading” performance.

Conclusions: Our data confirms that a single dose of intranasally administered oxytocin is sufficient to cause a slight but significant increase in affective “mind-reading” in men. Furthermore, we bring novel evidence that this effect of oxytocin is also present in women. Nevertheless, the improvement in RMET performance was not larger after oxytocin than after drinking a single glass of beer. It is therefore possible that the positive effects of both oxytocin and alcohol could simply be explained by a reduction in subjective anxiety during behavioural testing.
Conclusions: The results indicate that female gender, marriage and lower education status were the variables that most influenced psychotropic prescription. Also the practice of explaining the diagnosis, disease process, adverse effects and discussing alternative treatment options to patients seems to be grossly inadequate.

Euthanasia in Belgium and The Netherlands: Legally constructed and medically interpreted

ESC-ID 153
Author Peeters E, van IJperen WJ, Cras P
Country Belgium
University Universiteit Antwerpen
Department Neurology

Aim: Belgium and The Netherlands are the only countries with a euthanasia act. In Belgium there is no clarity concerning demented patients and euthanasia. In The Netherlands euthanasia on demented patients is legally possible. In Belgium there is a debate to include dementia as a legal reason for euthanasia via an advance directive. Two bills have been put forward, but momentarily the political debate is postponed. What are the differences between the Belgium and the Dutch law? What are the possibilities for demented patients in both countries? What are the weaknesses of the proposed Belgian bills? Method: A comparative literature study of the current euthanasia laws in Belgium and The Netherlands, especially the patient-related criteria. We examined the Belgian bills on extension of the euthanasia law towards dementia. Next, we interviewed the Belgian senators who played an important role in the development of the current euthanasia law and bills.

Results: In Belgium dementia is not an exclusion criteria, thus legally it is not impossible to perform euthanasia on a demented person. Nevertheless, the doctor still has to be certain that the request is also considered, repeated and voluntary. Although euthanasia on demented persons is legally possible in The Netherlands, the KNMG (Royal Dutch Society to improve Healthcare) claims that Alzheimer cannot be a reason for euthanasia unless there is also unbearable and hopelessly psychological suffering. So far four cases have been reported.

Conclusion: The Belgian bills show three problems. First of all both bills do not include the suffering of the patient as a criterion. The basis of euthanasia is unbearable physical or mental suffering. Leaving out unbearable suffering as a criterion makes the law too liberal. Secondly, the period of validity as stated in the current law is a method of control that the request is considered every five years. Removing this validity takes away the control. Finally, there is the so called ‘someone else’s problem’: during the progression of dementia, there’s a personality change. Therefore, the drawn up advance directive would not necessarily be valid in the new situation. The latter is the main reason why euthanasia should not be performed on a demented person via an advance directive.

The psychological bias in reporting pain at cold stimuli

ESC-ID 599
Author Ioan S, Nastase A, Braga RI, Zagrean L, Moldovan M
Country Romania
University "Carol Davila" University of Medicine and Pharmacy, Bucharest
Department Physiology, Center for Excellence in Neurosciences

Background: For more than 70 years the only clinical method available to quantify pain perception has been the cold pressor test (CPT). Briefly, the subjects are asked to immerse the non-dominant hand in a cold water bath at 3°C and two behavioral parameters are quantified: (1) pain threshold, as the time to initial pain report and (2) pain tolerance, as the time at which the subject will withdraw from noxious cold stimulation. Although not all CPT studies reported concordant changes in pain threshold and pain tolerance, it remains unclear whether the differences between these parameters reflect different aspects of pain perception.

Aim: The aim of this study was to compare the susceptibility to psychological distractors of pain threshold and pain tolerance during CPT. Method: We tested the effect of color, as a pure psychological distractor (S. Ioan et al., Ev & Ham Behav 2007), on the parameters of CPT (A. Nastase et al., Neuroreport 2007) in a group of students. The subjects were presented with two identical water baths colored in red and blue, half-filled with thermostated water (3-4 °C). The 2 CPTs were carried out in random order in the red or the blue container, allowing one day apart between the determinations. The palm temperature was measured prior and immediately after the procedures using an infrared digital thermometer.

Results: Pain tolerance was not influenced by color and was in concordance with the decrease in the palm's temperature. Pain threshold for red was increased for women and decreased for men (p<0.05). In an additional sequence of experiments women tend to associate red colored water bath with a warmer temperature. This effect was not apparent in men.

Conclusion: Color influences pain perception in CPT by modifying pain threshold. Our data suggest that pain threshold is more susceptible to psychological distractors (red color) than pain tolerance. Pain tolerance represents a more reliable parameter, and this difference between pain threshold and pain tolerance should be taken into account when interpreting the results of CPT.

Effect of chronic pain on social life

ESC-ID 309
Author Gholipour F, Habibi R, Madani SMM, Jourgholami M
Country Iran
University Iran University of Medicine
Department Psychiatry

Introduction: Most people when suffer from pain, have a special reflex. Chronic pain causes limitation of activity, work potential, weak increase and depression. Material and Methods: This study is a descriptive analytic survey, which
has studied 105 chronic pain patients records randomly selected from patients record visited in Multidisciplinary pain clinic from 2001 to 2003.Data has been analysed by descriptive test(SPSS 11.5).

Results: Mean of cases age was 41.15 14.21 years old,which %52 were women and %48 men. %62 have diploma and high school education.%71 patients had manual job and %23 non-manual. Chronic pain during 6 months prior to the visit caused,work disturbance in %34, absence of work in %40 and %42 of cases changed their job to an easier work.Family income had decreased in %23 of patients. Social activity mean rank decreased from 7.12 in healthy time to 4.65 in time suffering from pain and leisure time activity decreased from 6.90 to 3.72, which wilkakson test showed a statistically significant relationship between pain and decrease of social and recreational activity(\( r_{ij} = 0.5 \)).

Conclusion: Concerning the fact that %26 of patients had lost their hope to come back to their routine work and life , this study confirms Wein theory about negative effect of chronic pain on psychological aspects as career and life expectancy.

**Surgery II**

**Influence of liver function to post transplant Tacrolimus blood level after orthotopic liver transplantation**

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<td>Author</td>
<td>Lock J, Stockmann M</td>
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<td>Country</td>
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<td>University</td>
<td>Charité Universitätsmedizin Berlin</td>
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<td>Department</td>
<td>Department of Transplantation Surgery</td>
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**Background:** Orthotopic liver transplantation (OLTX) is a well-studied rescue for end-stage liver disease. One of the most important aims after transplantation is quickly providing a stable blood concentration of immunosuppressants to avoid early graft rejections. In our Clinic we use Tacrolimus as the standard immunosuppressant (starting with 0,1mg/kg/d, following blood level controlled) in combination with methylprednisolone. It is necessary to provide Tacrolimus levels of 10 to 15 ng/ml within five days to avoid graft rejections that occur often at this point. Somehow we often registered a big variety of Tacrolimus levels on the first post operative days (Range 1 - 25ng/ml).

**Methods:** 103 Patient undergoing OLTX were enrolled in our prospective clinical study. We investigated the liver function with the new intravenous LiMAx test (maximal enzymatic liver function capacity) a 13C-Methacetin breath test. 2mg/kg 13C-Methacetin were injected into a intravenous catheter as a bolus. The excretion of 13CO2 was continuously observed for one hour after injection. The blood level of Tacrolimus was analyzed by our transplantation laboratory in common technique.

**Results:** We can show in our study that there is a correlation between the first postoperative LiMAx test result and the blood level of Tacrolimus. If Tacrolimus is administered body weight controlled on the first post operative day, we can nearly predict the blood-level of Tacrolimus on the following day. A high LiMAx test result (>200µg/kg/h, n = 28) as an indicator for a high metabolic capacity of the liver leads to a decreased blood level of Tacrolimus (median: 5.5ng/ml) on the following day. In contrary a low or critical LiMAx test result (<50µg/kg/h, n = 7) can result into a toxic blood level of Tacrolimus (median: 19.7ng/ml) which can impair the renal function and create neurological side effects that could affect the effective post operative mobilization of the patient.

**Discussion:** As described, the quick approach to a sufficient Tacrolimus blood level is essential to avoid early graft rejections and toxic side effects. Most likely this is very important for patients with good post transplant liver function to preserve that status as a basis for the fast recovery of the patient and an early discharge from the ICU. Further more patients with critical liver function might be protected against negative side effects of toxic Tacrolimus level due to a smaller dosage post transplant.

**Conclusion:** As we show in our study it might be possible to predict the accurate dosage of Tacrolimus to provide a sufficient blood level after OLTX. Further investigations could proof the consequence that Tacrolimus therapy should not be started body weight controlled, which has no connection to the enzymatic elimination of the immunosuppressant, though it could be predicted by testing maximal enzymatic liver function capacity. Annotation: Statistical analysis is still in progress. Results with more details will be available soon. Therefore it is possible to provide an actualized abstract within 4 weeks.

**Therapy of locally advanced rectal cancer-importance of surgery and preoperative radiotherapy**

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<tr>
<td>Author</td>
<td>Oljaca S, Nikolic S</td>
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<td>Country</td>
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<td>University</td>
<td>University of Novi Sad</td>
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**Background:** Survival analysis of preoperatively radiated patients with locally advanced rectal cancer is very significant. It estimates effect of diagnostic and preoperative therapy on the stage of cancer, selection of operative procedure and overall outcome of treatment this group of patients.

**Materials and Methods:** Sixty-three patients with locally advanced rectal cancer (37 men,26 women) received preoperative chemoradiation therapy.54 patients are radically operated,8 palliativly and one patient is not operated.They were operated in the period from june 2000. until october 2004. Using the program packet Statistika™ we arrange are grouped references and presented them with Kaplan-Mayer curve.

**Results:** In our group of 63 patients mean follow up was up 48 months.35 patients are alive and 28 of them are dead.Overall survival for three years is 60%,while for five years is 47,5%.In the group of radically operated patients (54 patients) three year survival is 67,5% and five year survival is 54,2%. Survival by stadiums of radically operated patients shows:stadium 0-three year survival is 100%,five year survival is 0%;stadium I- three year survival is 77,5%,five year survival is 12%;stadium II-three year survival is 85%.five year survival is 35%;stadium III-three year survival is 68%,five year survival is 0%;stadium IV-three year and also five year survival is 0%. In the group of non-radically operated patients three and five year survival is 0%. Odds ratio(Cross-product ratio) shows that chance for survival of
radically operated patients is 13.6 times more then non-radically operated patients.

**Conclusion:** Radical surgery, especially sphincter preserving operations (because of better life quality) in combination with preoperative radiotherapy is the best treatment option for patients with locally advanced rectal cancer. Comparing survival by stadiums we concluded that complete histological regression of disease (stadium 0) has no influence on survival of patients. KEY WORDS. Rectal cancer, surgery, preoperative radiotherapy.

The sentinel lymph node mapping in patients with initial diagnosis of ductal carcinoma in situ of the breast

**ESC-ID** 322  
**Author** Polom K, Polom W, Lianeri M, Murawa D  
**Country** Poland  
**University** University of Medical Sciences Poznan  
**Department** Ist Surgical Oncology Dept. WCO Poznan

**Aim:** Sentinel node biopsy (SNB) is an accepted alternative to lymphadenectomy in the case of invasive breast carcinoma, although the sentinel node's role in ductal carcinoma in situ (DCIS) has been not well defined. The purpose of this study was to determine the diagnostic value of sentinel nodes in female patients with primary DCIS, using core needle stereostatic biopsy.

**Method:** Between the years 2000 and 2006, 183 diagnosed with DCIS underwent SNB to determine lymph node involvement. Those patients with metastases to the sentinel node underwent axillary lymphadenectomy.

**Results:** In the group of 183 patients that underwent SNB, 10 patients (5.5%) showed metastases to the sentinel lymph node. Histopathological studies of the primary lesions of these 10 patients revealed invasive ductal carcinoma in 6 cases (3.5%) and 1 case (0.5%) of invasive lobular carcinoma. Only 3 of the patients (1.5%) were given a final diagnosis of DCIS with metastases to sentinel lymph nodes, of which 2 cases were DCIS and 1 case was DCIS with microinvasion and atypical ductal hyperplasia (ADH). Axillary lymphadenectomy performed on patients with abnormal SNB showed involvement of other axillary lymph nodes in 4 patients.

**Conclusions:** SNB as a diagnostic tool in DCIS remains controversial as the number of cases of axillary lymph node metastases is minuscule. The biggest clinical challenge in this situation is a group of patients with primary diagnosis of DCIS in which invasive components are seen by mammotomic biopsy.

Lung function among patients with pectus excavatum after the minimal invasive repair of pectus excavatum (Nuss procedure)

**ESC-ID** 621  
**Author** Stepak H, Szydlik S, Zwarun D, Jankowska J, Krzyzanowski M, Ka D  
**Country** Poland  
**University** Poznan University of Medical Sciences  
**Department** Thoracic Surgery

**Introduction:** The Nuss procedure was for the first time performed at our clinic in 2002. The operation was mainly carried out for cosmetic effect. However, controversy exists regarding the effects of repair on pulmonary function. We also hypothesized that the placement of a steel bar in the first stage of the Nuss procedure would adversely affect lung function. The aim of the study, a prospective analysis, was to evaluate lung function among patients who underwent the Nuss procedure (Minimally Invasive Repair of Pectus Excavatum - MIRPE). The analysis included spirometric evaluation of the lung function before the Nuss repair, during perioperative period and after removing steel bars from behind the sternum.

**Materials and Methods:** The study group involved patients operated on the pectus excavatum in the Department of Thoracic Surgery in Poznan in years 2002-2004. The study group consisted of 44 patients (5 females and 39 males). Aged between 10 to 32 years old, the mean age was 16. The following spirometric parameters were analysed: vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1s (FEV1), FEV1/VC ratio, peak expiratory flow (PEF), forced expiratory flow for 25% (FEF25), 50% (FEF50) and 75% FVC (FEF75). The results obtained were compared with the value of the preoperative chest deformation, age, height and weight.

**Results:** The values of the FVC, FEV1, MEF25, and FEV1/VC ratio in the study group were significantly higher in postoperative period in comparison with the preoperative period. There was statistically significant correlation between the improvement in spirometric parameters after the Nuss procedure and the impairment of spirometric values in preoperative period. There were no statistical differences between the value of initial chest deformation and spirometric parameters improvement. There were also no statistically significant correlations between age, height and weight of the patient in the study group and spirometric values improvement.

**Conclusion:** There is a statistical improvement in lung function in patients who underwent the Nuss procedure (MIRPE). The improvement in spirometric parameters after the operation does not correlate with age, height, weight and value of the chest deformation in preoperative period, however the improvement in spirometric parameters correlates with the impairment of spirometric values in preoperative period. There is also statistically significant improvement in pulmonary function between perioperative period and after removing steel bars from behind the sternum.

The role of endogenous opioids in impairment of ischemic skin flap survival in cholestatic rats

**ESC-ID** 649  
**Author** Assa S, Nezami BG, Emami H, Talab SS, Dehpour AR  
**Country** Iran  
**University** Medical University of Tehran  
**Department** Medicine Faculty

**Background:** There is an increasing body of data suggesting that endogenous opioids play a key role in inducing oxidative stress, as in cholestatic liver. Our objective was to show the role of cholestasis and the consecutive liver failure in decreasing the survival of skin flaps, and investigating the effect of endogenous opioid system as the possible modulator of this phenomenon.

**Methods:** 42 rats were randomly assigned into 6 groups (7 rats each). Random pattern skin flap was performed in all
groups with elevation of a caudally based dorsal flap (2X8cm) at midline and survival rate was assessed at the seventh day postoperatively. Group 1 served as normal without bile duct ligation and only received flaps. Bile duct ligation was performed in Groups 2 and 3 and skin flaps were elevated after 7 and 14 days, respectively. Groups 4 and 5 served as appropriate sham operated animals for these groups and animals in group 6 received daily intraperitoneal injection of nonspecific opioid receptor antagonist, naltrexon (20 mg/kg) while undergoing the same procedure as group 3.

**Results:** The survival rate was 53.26 ± 6.32% (mean ± SD) for normal and 46.43 ± 2.84, 32.78 ± 4.71 for 7 and 14 days choledochocystic groups, respectively. There was no significant difference in flap survival between normal and sham operated groups. A significant decrease in skin flap survival was observed in 14 days choledochocystic animals of group 3 in comparison with appropriate sham control group. This effect was reversed in naltrexon treated rats (66.53 ± 6.72).

**Conclusions:** bile duct ligation impairs the survival of random pattern skin flaps in rats which is reversed by chronic administration of naltrexon. These results suggest the involvement of opioid system in reduced skin tolerance to ischemia in choledochocystic liver diseases.

**Liver transplantation for hepatocellular carcinoma**

HCC represents about 80% of all the primary tumors of the liver that mostly occurs with cirrhosis. Liver transplantation is an accepted treatment in patients who fulfilled the so called Milano criteria. This is one of the few cases, where cancer is not a contraindication for organ transplantation. The aim of the paper was to analyze the application of the Milano criteria in process of qualification to liver transplantation in patients with HCC secondary to cirrhosis and evaluation of pre-operative cancer treatment prior transplantation. Method: Between 2001 and 2005, 26 patients aged 45 - 57 (average: 59.9) with a diagnosis of HCC underwent liver transplantation at the Department of General, Transplant and Liver Surgery at the Medical University of Warsaw.

**Results:** The etiology of cirrhosis among patients with HCC was as follows: cirrhosis secondary to HBV in 8 cases and to HCV in 12 cases. There were 3 cases of coexisting HBV or HCV and alcohol. In one case an alcoholic cirrhosis was diagnosed and 3 patients had unknown etiology of cirrhosis. In 27.9% of patients tumor recurrence occurred on average 13.3 months (1-25 months) after transplantation. 8 out of 26 patients did not meet the Milano criteria (30.8%). Mortality in that group equaled 62.5%. However in the group of patients that met the criteria mortality was 16.6%. Only 5 patients had a neoadjuvant treatment before OLTx. There was a liver resection in 3 patients, one case of cryotherapy 1 and one of chemotherapy.

**Conclusion:** Our data support liver transplantation as the treatment of choice for patients with HCC fulfilled the Milano criteria. In the group of patients that did not meet the above criteria, a nonsurgical management might be considered a treatment instead of liver transplantation. Neoadjuvant management before OLTx may be a treatment of choice in a selected group of patients.

**Angiography and interventional radiology in diagnostic and treatment of hemorrhagic and traumatic shock**

**ESC-ID** 271
**Author** Boyko VV, Avdosyev YV, Myroshnychenko DA, Mylovydova GE
**Country** Ukraine
**University** Institute of General and Urgent Surgery
**Department** Roentgen Surgery, Hospital Surgery

**Heading:** The severe abdominal hemorrhages (SAH) complicated by traumatic and hemorrhagic shock (THS) is a one of serious problems in modern surgery. Though some success in treatment of this group of patients death rate remained very high – more 30%. Properly tactic of treatment patients with SAH decided a life of patient and result of operative treatment. At last time in complex of surgical treatment (ST) of this group of patients very often using endovascular methods of haemostasis which owing to small invasiveness and high effective turned to a widespread in treatment of different pathology, include SAH.

**Aim:** possibility of using endovascular catheter haemostasis (ECH) in patients with SAH complicated by THS.

**Material and methods:** We have an experience of endovascular treatment of 32 patients with SAH by different etiology complicated THS whose were in IGUS AMSU from 2001 till 2006 years. There are 20 of men and 14 of women from 24 till 72 years old. They were undergoing by different ST including ECH. The causes of SAH were: blunt abdominal injury with disruption of great vessels – 5, multiple fractures bones of pelvis with disruption of branches of internal iliac artery (BIIA) – 2, acute gastointestinal hemorrhage (GIH) from esophageal and gastric varices (EGV) – 16, cancer of stomach – 4, duodenal ulcer – 2, rupture of abdominal aortic aneurism (RAAA) – 2, rupture of common iliac artery – 2. In 20 patients ECH (embolization, intraarterial haemostatic therapy, transumbilical catheterization of portal vein) was performed as alone method of ST. In 14 patients ECH (embolization, temporally balloon occlusion) was performed as first period of ST with aim of stabilization of patient’s status and preparing for abdominal operation (AO).

**Results:** The serious complications after ECH we didn’t occur. After ECH 2 patients with EGV died from rebleeding GIIH and 2 patients died with RAAA in first day after AO. Using ECH and temporal balloon occlusion of branches of abdominal aorta by far expanding possibility ST of SAH of parenchymal organs and large vessels of abdominal cavity and pelvis in that moments when performing embolization of deep location hemorrhaging vessels.

**Conclusions:** ECH is an effective method of ST patients with SAH in THS and indication in profound abdominal bleeding in patients with unstable haemodynamics and high risk of development of DIC-syndrome and multiple organ failure by no effective substitutive therapy and can used: a) in patients with combined severe blunt trauma, injury of retroperitoneal space and small pelvis; b) in patients with GIH and arrosion of visceral branches of abdominal aorta; c) in patients with RAAA and disruption of BIIA.
Factors influencing the survival of Tenckhoff peritoneal dialysis catheters

ESC-ID 840
Author Skonieczny B, Pytka M, Liberek T, Stefaniak T
Country Poland
University Medical University of Gdansk
Department Dep. of Gen., Endocrine and Transplant Surgery

Introduction: The most significant complications that may occur during peritoneal dialysis associated with peritoneal access are those of technical nature.

Aims: To evaluate the factors influencing the survival of Tenckhoff dialysis catheters. To observe the frequency of peritonitis, fluid leak and tunnel infections occurring during peritoneal dialysis.

Material and methods: We have analyzed the prospective survival of 312 consecutive Tenckhoff catheters implanted in 264 patients treated with peritoneal dialysis form Jan 1993 to Dec 2005 in the Department of Nephrology, Transplantology and Internal Medicine, Medical University of Gdansk.

Results: Most of the Tenckhoff catheters (293) were implanted by minilaparotomy procedure. To avoid catheters’ contamination povidone-iodine swab dressing was used every day. There were 7329 patients-months at risk and the peritonitis rate was 1/20.5 patients-months. 78 catheters (25.1%) were removed out of 45 patients because of complications. 66 (84.6%) out of those 78 catheters were removed due to peritonitis, fluid leak appeared in 6 patients (7.7%) and 6 (7.7%) because of exit site/tunnel infections. Malfunction was not a reason to remove any of the catheters. The median catheter survival was 66.3 months, 88.8% of catheters survived more than 1 year and 66.1% more than 3 years. In 220 patients (83%) there was no need to remove the catheter during their peritoneal dialysis therapy. In the multiple hazard Cox regression analysis a lower catheter survival was observed in patients with higher BMI and those transferred from hemodialysis. Patient age, gender, diabetic status, Charlson comorbidity index score and albumin level did not influence significantly the catheter survival.

Conclusions: Minilaprotomy implantation of Tenckhoff catheters is a safe procedure which should be recommended as a gold standard for those patients in which peritoneal dialysis therapy is necessary. This procedure allowed the decrease of the frequency of peritonitis and other complications. Unresponsive to antimicrobial therapy peritonitis was the main problem resulting in the catheter loss.

First experiment of enteroureteroplasty.

ESC-ID 282
Author Kuzminykh SV, Krasilynikov DE
Country Russia
University Saint-Petersburg Pediatric Medical Academy
Department Urology

Introduction: The relevance of the experiment is determined by severity of urodynamic abnormalities in pediatric patients (who suffer from neuromuscular ureteric dysphasia) and absence of single practical solution of this problem in children surgery and urology. Frequently the treatment of these patients consists of surgical “organ”-ectomy, which is performed when there is a loss of renal and ureteral function. Whereas no experiments were reported regarding possibilities of enteroureteroplastics – fixation of ureter with peristalsis seromuscular sheath. Experiment purpose is to study the opportunity of performing enteroureteroplastics and its following influence on upper urinary tract urodynamics.

Materials and Methods: The experiment was carried out with 3 cats (average weight 2.5-3.0 kg). Under GA(general anesthesia) intake of ileum part (length 2-3 cm) on vascular pedicle was made to perform following anastomosis end to end. After the ileum part was dissected at antimesenterical margin and mucosa was separated under microscopic control, it was brought through mesenteric window to left retroperitoneal space. In retroperitoneal fat the middle part of ureter was separated. Transplant was designed in width – 4 times of ureter diameter (1, 2 – 1, 6 cm). Ureter was covered by transplant like sheath and antimesenterical margin was sutured. Antibacterial therapy and IV(intravascular) urography (on 10-11th day) were leded. On 14th day under GA kidney and ureter were taken for histology. Condition of anastomosis and peristalsis of middle part of ureter were estimated.

Results: Postoperative period in all cases was without complications. IV urography showed no obstructive changes. Intra-operative examination showed no inflammation in the area of anastomosis, peristalsis was normal; ureter was in normal position inside of the seromuscular sheath. US(ultra sound) of kidney revealed no parenchyma changes, neither changes of collecting system. During sheath anastomosis histology the union of ileum transplant and ureter surface was revealed to be without mucosal formation and without cavities with exudation and transudation.

Conclusion: The mentioned surgery brought no negative influence on upper urinary tract urodynamics. Formation of peristalsis seromuscular sheath was achieved in all cases. The results of the experiment have shown the perspective of following research of sheath enteroureteroplastics in experiment.

Surgical emergencies in elderly

ESC-ID 244
Author Rosu I, Bartus I, Coros M
Country Romania
University University of Medicine and Pharmacy
Department General Medicine

Background: Considering the increase of life expectancy, a higher number of elderly patients turn to the surgery services with different affections, both in elective and emergency conditions. We can even talk about a real geriatric surgery adapted to these patients, who besides the stigma of the age, are affected from a series of other affections that increase the risk of morbidity and mortality.

Objectives: The aim of the study was to analyze the characteristics of elderly patients’ surgical emergency pathology and to establish several prognostic factors of their evolution.

Materials and methods: We did a retrospective study on 22.803 patients admitted in the first and the third Clinics of Surgery of the County Emergency Hospital from Targu-Mures between 1999 and 2006, the results being statistically processed.

Results: The elderly patients (> 69 years) represented 15.10% (n = 3441) of all cases and 20.70% of all emergency

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admissions. Almost half of elderly (n = 1645) were admitted in emergency conditions, compared to only a third (32.53%) of the younger patients. Almost 77% of elderly admitted in emergency conditions were suffering from other illnesses, mostly cardiovascular and pulmonary ones. In this group, the malignant affections represented 16.72% of all cases, meanwhile in the younger one it represented only 6.98% - significant difference (p<0.01). Abdominal emergencies represented 83.59% of all cases, mostly due to intestinal occlusion and digestive hemorrhages of different etiologies. The operability ratio of the elderly was of 68% close to the younger ones (72%). The global mortality was 3.95% in the elderly patients admitted in emergency conditions, comparing with only 1.61% in those admitted in elective conditions. (p<0.01). The postoperative mortality rate was 2.14% in emergency, comparing to 1.34% in elective conditions.

Conclusions: Surgical emergencies affect elderly people in a much higher percentage than the younger group. (p<0.01) Neoplastic affections give a higher percentage of emergency admissions in patients over 70 years old, comparing to those younger. The emergency condition, the elder age and neoplasms are the most important factors of negative prognostics, and much more important when they cumulate, increasing the mortality rate up to fivefold compared to the elderly patients admitted in elective condition. Key words: elderly, emergency.

Vascularizeted matrixes in reconstruction of hollow and parenchimatosis organs

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<td>Author</td>
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Purpose: Most oncological patients undergo removal or subtotal resection of hollow and parenchimatosis organs. Postoperative pathological states are connected in considereable degree with lack of functional epithelium. That is why the aim of this work is to investigate the possibility to create vascularizeted matrixes for reimplantation authoepithelium, after laboratory growing (increasing) the number of cell or transplantation of donor’s stem epithelial cell.

Methodology: The experiment was conducted with 30 dogs and 60 rats. All experiments were performed under general narcosis and in postoperative period all animals were given analgetics, antibiotics and stimulators of regeneration. 3 dogs and 6 rats were the donors of embrional tissues in adult organism were investigated and the possibility of using cells cultures received by laboratory increasing the numbers of cells were shown.

Conclusion: The possibility of using demucoused intestine transplants as a vascularizeted matrixes base by transplantation functional epithelium was experimentaly shown. It was for the first time when the vascularizeted matrixes with arterial and portal blood supply was created. Some methods of epithelium of hollow and parenchimatosis organs transplantation were developed and studied. Operations can be conduct-ed both in one and in several stages including endoscopy. The methods can be used in reconstruction of any hollow and some parenchimatosis organs. Clinical use of the presented work will allow the oncological patients who underwent operations to improve their live conciderably and give them a possibility to return to normal life of full value, thanks to restoration of the extracted organs. It will also give a chance for women who underwent their uterus deprivation on different reasons to become mothers.

Enhanced early immune response following the transplantation of organs from elderly donors

ESC-ID | 133 |
| Country | Germany |
| University | Charité Universitätsmedizin Berlin |
| Department | Dept. of Surgery, Virchow-Klinikum |

Aim: Previously, we described an advanced chronic allograft nephropathy following the engraftment of elderly donor organs. Here, we studied the early immune response in detail in a model of strong histoincompatibility. Method: Kidneys from young or old (3 or 18 months) DA donor rats were grafted into 3 months old LEW recipients. Alloreactivity was evaluated by ELISPOT (intracellular IFN-gamma), cytokine production was measured by ELISA. Frequency and activation of intragraft and peripheral B cells and dendritic cells (DCs) of donor or recipient origin were analyzed by days (d) 3 and 10.

Results: Older grafts demonstrated enhanced signs of acute rejection (d10: tubulitis, interstitial haemorrhage, fibrinoid changes; p<0.05 vs young grafts). The number of allreactive cells had increased (IFN-gamma+ cells/5E5 splenocytes: 375 ± 124 vs 242 ± 59), while levels of IFN-gamma, IL-6 and IL-4 were elevated significantly (d10, IFN-gamma: 20 ± 3 vs 12 ± 2 ng/ml; IL-6: 0.8 ± 0.1 vs 0.4 ± 0.1 ng/ml; IL-4: 6 ± 1 vs 6 ± 1 pg/ml, p <0.05). Although the total amount of OX62+DCs was higher in younger grafts (d10: 14.2 ± 10.4% vs 4.1 ± 2.2%, p = 0.052), the number of activated, donor-derived cells within the dendritic cell population (RT1Aab+CD86+ and RT1Aab+MHC class II+) was significantly elevated in recipients of older grafts (p <0.05). Overall, elevated amounts of CD45RA+ B cells were detected in older kidneys (d3: 15 ± 3 vs 10 ± 4%, p <0.05). At the same time, frequencies of donor-derived B cells (RT1Aab+) had increased in all compartments (graft: 5.0 ± 3.4 vs 2.3 ± 0.8%, ns; spleen: 1.7 ± 0.4 vs 0.8 ± 0.2%, p <0.05; lymph nodes: 1.1 ± 0.3 vs 0.4 ± 0.02%, p <0.05). The number of intragraft and peripheral activated, donor-specific cells within the B cell population (RT1Aab+CD86+ and RT1Aab+MHC class II+) had increased in recipients of older grafts (d3 and d10: p <0.05). In parallel, amounts of B cells staining for ICAM-1 were sig-
significantly elevated (CD45RA+CD54+, spleen: 30 ± 2 vs 14 ± 2%, p <0.0001: graft: 14 ± 2 vs 5 ± 1%, p <0.01).

Conclusion: Our results demonstrate an enhanced early immune response following the transplantation of elderly grafts associated with increased amounts of activated donor-derived B cells and DCs. Beside the production of alloantibodies, activated B cells of donor origin may play a role as antigen presenting cells in this context.

Results of morbid obesity surgical treatment – 1 year follow up

ESC-ID 119
Author Szalas J, Ziemianski P
Country Poland
University Medical University of Warsaw
Department Medicine

Vertical Banded Gastroplasty (VBG) and Roux-en-Y Gastric Bypass (RYGB) are two methods for morbid obesity surgical treatment in our Department. The aim of the study was to compare the outcome of these procedures defined as an excess body weight loss and number of early post-operative complications.

Material and methods: We achieved outcome of 60 patients (48 women and 12 men, mean age 42.1, BMI 48.0, Body Fat % - 51.1) treated for morbid obesity with bariatric surgery in our Department. Prospective study was undertaken in order to compare VBG with RYGB in obese patients with a BMI >40 kg/m². Based on specific criteria including eating behavior 30 patients (24 women and 6 men, mean age 41.4, BMI 49.1, Body Fat % - 53.1) were selected for RYGB and 30 (24 women and 6 men, mean age 43.2, BMI 44.8, Body Fat % - 50.6) - for VBG. All patients have undergone complete follow-up evaluation at 1, 2, 3, 6, 9 and 12 months postoperatively.

Results: The excess weight loss >50% after 12 months was significantly greater in the RYGB group (73.3% versus 46.6% in VBG, p<0.02). The percentage of initial body weight loss after 12 months was higher in the RYGB group as well (30% for VBG versus 39% for RYGB p<0.05). Number of early post-operative complications in the VBG group (delayed wound healing in 6 and urinary tract infection in 2 patients) was lower than in RYGB group (delayed wound healing in 6, urinary tract infection in 2, pneumonia in 1 and pulmonary embolism in 1 patient) (VBG vs RYGB: p<0.05). Number of late post-operative complications in the VBG group was also lower than in GB group (5 post-operative hernias in VBG and 6 in RYGB group) (VBG vs RYGB; NS).

Conclusions: Roux-en-Y Gastric Bypass provides better weight loss effect than Vertical Banded Gastroplasty, but it is related to greater number of post-operative complications. Roux-en-Y Gastric Bypass is a first choice method of morbid obesity surgical treatment in our Department.

Pretransplant MELD score and post liver transplantation survival in Scotland

ESC-ID 52
Author Liong KK
Country United Kingdom
University The University of Edinburgh
Department The Scottish Transplant Unit

Aim: Since February 2002, the model for end-stage liver disease (MELD) score has been used for allocation in liver transplantation waiting lists in the United States replacing the Child-Turcotte-Pugh score. MELD incorporates three standardized laboratory parameters to generate an objective score [6(mild disease) to 40(most severe)] that is rapidly available. Studies have demonstrated that the MELD score is associated with post-transplant patient survival. The aim of our study was to evaluate how accurate the MELD score is to predict post-transplant survival in adult patients with chronic liver disease in the Scottish population. We also evaluated how accurate the MELD score was to predict survival in adult patients with chronic liver disease who were on the waiting list for liver transplant.

Material and Methods: The study included 232 patients who underwent elective liver transplantation between November 1992 and February 2006 in a single center, the Scottish Liver Transplant Unit. We also studied 36 patients who were on the waiting list for liver transplant between October 2000 and March 2006 in the same centre. We excluded patients with hepatocellular carcinoma, who received an urgent liver transplantation, who underwent retransplantation or multi-organ transplantation and those younger than 17 years old. Pretransplantation MELD score was calculated based on the United Network for Organ Sharing formula: (0.957 x In(creatinine[mg/dL]) +0.378 x (bilirubin[mg/dL]) + 1.12 x In(INR) + 0.643) x 10. All the biochemical parameters used were obtained from the laboratory of the Royal Infirmary of Edinburgh to avoid dynamic variables. Patients were stratified according to MELD score <10, 11-18, 19-24, 25-35, >36 and followed to the most recent visit or death. We collected data on patients age, gender, ethnicity/race, aetiology of liver disease, history of renal disease/dialysis, co-morbidity, presence of varices, ascites and encephalopathy, serum bilirubin, serum albumin, prothrombin, INR, and serum creatinine for demographics purposes. Survival analysis was performed using the Kaplan-Meier method. Patients surviving was analysed at 1, 3, 6, 12, 24 and 60 months for adult patients who underwent liver transplantation and 1, 3, 6 and 12 months for adult patients who died while on waiting list. P values less than 0.05 were considered to be statistically significant.

Results: The mean age at transplant was 54.4 (SD 11.2) years and the main indications for transplantation were choledochal disease and alcoholic liver disease (62.5%). Other indications were multiple aetiologies(12.7%), hepatitis C(5.2%), metabolic(2.2%) and hepatitis B(1.3%) and other aetiologies(16.2%). Patients with higher MELD scores had higher tendency to have acquired ascites, varices and encephalopathy. There was no significant correlation between the pre-transplant MELD score and survival at 3, 6, 9, 12, 24 and 60 months. Although there was a trend towards a difference in 60-months patient survival, it did not reach statistical significance (p = 0.877). All patients on transplant waiting list and not transplanted died at 12 months. The main cause of death was multi-system failure (41.7%). The association between
patient survival and UNOS MELD categories was not significant at 1, 3 and 6 months on patients on transplant waiting list. However, there was a statistically significant difference (p = 0.039) in 2-month patient survival.

Conclusion: MELD score was not the sole determining factor to assess organ allocation. There was insufficient evidence to show that there was an association between MELD score and post-transplant survival rate in our population. We observed that the MELD score may be useful to predict patients survival in the first two months on waiting list. Larger studies are needed to confirm our findings.

The use of albumin sealant in the inguinal hernioplasty

Background: Inguinal hernia repair is one of the most commonly performed operations in general surgery (S.S. Awad, S.P. Fagan, 2004). The tension-free techniques proved their effectiveness by sufficient reduction of the recurrence level in these patients. But publications of the last years witness that number of complications after these operations still remains high. Most common of them are: development of seroma, hematoma, suppuration of postoperative wound, atrophic and cicatrize changes in muscles, caused by their ischemia, which can lead to hernioplasty insufficiency. Few publications predict biologic sealants as an alternative method of mesh fixation, instead of sutures, and prophylactics of complications in postoperative period (S. Canonico, 2003). Albumin sealant is widely used in the cardio-vascular surgery, but possibility of its employment in the inguinal hernioplasty requires validation. The aim of the study was to reveal the possibility of use of albumin sealant instead of sutures for the mesh fixation in the inguinal hernioplasty.

Materials and methods: 37 male patients undergoing inguinal hernia repair formed two groups. In all cases we implanted prolene hernia mesh using I.L. Lichtenstein technique. 12 patients of the 1st group underwent hernioplasty with use of albumin sealant for the mesh fixation and in 25 patients of the 2nd group we performed the standard technique with prolene sutures. For the evaluation of this method in the postoperative period we used such criteria as recurrence rate, length of the inguinal pain, length of the hospital stay and development of postoperative complications.

Results: There was no reliable difference between studied groups in length of hospital stay, in both groups it was 7.12±0.42. The length of inguinal pain in the 1st group was 4.02±1.51 days and in the 2nd 5.28±0.99 days (P<0.005). In one case a patient of the 2nd group felt the inguinal pain over the three months. In the postoperative period in patients of the 1st group we registered no complications. Two patients of the 2nd group developed seromas and one hematoma which were cured conservatively. The one year follow-up showed that in both groups of patients no recurrences of hernia were registered.

Conclusion: The use of albumin sealant in the surgical treatment of inguinal hernias helps to prevent the development of complications and sufficiently shorten the length of the inguinal pain in the postoperative period.

The accuracy of endorectal ultrasonography in staging rectal cancer after preoperative chemoradiation

Background: Preoperative staging of rectal cancer is considered essential to tailor treatment for individual patients. The aim of the present study was to evaluate the accuracy of endorectal ultrasonography in preoperative staging of rectal cancer.

Materials and Methods: Forty-two patients with locally advanced rectal cancer (25 men, 17 women) had preoperative chemoradiation and then underwent endorectal ultrasonography with a 7.5-MHz probe. The results of examinations were compared with the histology of resected specimens.

Results: Histopathology showed 5 stage T0, 2 stage T1, 10 stage T2, 23 stage T3, and one stage T4 tumor. The overall accuracy of endorectal ultrasonography for T stage was 73.8%, with 19% patients overstaged and 7.1% understaged. The overall accuracy for N stage was 66.7%. Sensitivity has been 64.2%, specificity 67.8%, overstage 21.4% and understage 11.9%.

Conclusion: Endorectal ultrasonography showed high accuracy in rectal cancer staging. Due to its advantages, endorectal ultrasonography should be diagnostic modality of first choice in predicting the stage of rectal cancer. KEY WORDS: Endorectal ultrasonography, rectal cancer, preoperative chemoradiation therapy.

Difference of TNF alpha gene expression in Haemangiomia and Focal Nodular Hiperplasia (FNH)

Background: Hemangioma and FNH are the most common benign liver tumors. Specific changes of gene expression in liver and tumors may play an important role in the growth of liver tumor. The purpose of the study was to identify the level of expression of one of growth factors-TNFα in benign tumors and surrounding liver tissue.

Methods: Liver tissue was obtained from 22 patients during hepatic resection. Gene expression was evaluated by qRT-PCR (TaqMan, Applied Biosystems, USA) in benign tumors and compared to the expression level of corresponding surrounding healthy liver.

Results: The expression of TNFα gene within FNH was 2-fold increased compared to adjacent normal liver tissue. But the expression of TNFα gene in hemangioma were 0.8-fold decreased compared to nearby non-neoplastic tissue.
Conclusion: Our data suggests that expressions of cytokines involved in the tumor growth such as TNFα is altered on transcriptional level in these two benign liver tumors compared to adjacent nontumorous liver tissue. It might lead to changes on the protein level and play an important role in regulating tumor growth.

Virology

Phenotypic and genetic characterization of Varicella-Zoster virus mutants resistant to acyclovir, brivudin and foscarnet

ESC-ID 815
Author Bleymehl K, Schöfer H, Doerr HW, Cinatl jr. J, Schmidt-Chanasit J
Country Germany
University Johann Wolfgang Goethe University Frankfurt am Main
Department Institute for Medical Virology

Introduction: Varicella-Zoster virus (VZV) is the causative agent of chicken pox (varicella) in children. After primary infection a lifelong latent infection is established in the dorsal root and trigeminal ganglia. VZV can reactivate and cause shingles (zoster) in elderly or immunocompromised persons. Resistant VZV strains have emerged following prolonged treatment of VZV infection in immunocompromised patients. Therefore, determination of the mechanism of resistance may help to diminish the appearance of drug-resistant VZV.

Methods and Results: Three wild-type VZV strains isolated from vesicle smears of herpes zoster patients as well as the Oka vaccine strain were propagated in human retinal pigment epithelial cells (hRPE) and human foreskin fibroblasts (HFF). Resistant mutants were obtained by exposure of purified cell-free VZV at increasing concentrations of acyclovir, brivudin and foscarnet. The 50% inhibitory concentration (IC50) was determined for every resistant mutant. Single base changes resulting in amino acid substitutions were observed in the nucleotide sequence of the DNA polymerase gene, thymidine kinase gene and glycoprotein genes and phenotypic analysis reveals a slow-growth phenotype of resistant mutants.

Conclusion: Base changes resulting in amino acid substitutions in drug-resistant VZV mutants not only occur within the thymidine kinase gene or the DNA polymerase gene. The mutations were found also to occur within the glycoprotein genes and be responsible for a different phenotype.

Production and the quality of exopolysaccharides by submerged culture of an anthomopathogenic fungus

ESC-ID 4
Author Chun-Ping X, Jong-Won Y
Country The Netherlands
University University of Groningen
Department Department of Biomedical Engineering

Introduction: Although many studies have examined the effect of culture conditions on the production of microbial polysaccharides (EPS), little is known about the influence on the product quality, particularly molecular properties. The objective of this study was to investigate the effect of shear stress on the production of EPS from an anthomopathogenic fungus, Paecilomyces tenuipes C240 in a stirred-tank reactor (STR) and in an airlift reactor (AR).

Methods: A series of batch cultures were carried out in either a 5-L stirred-tank reactor with six-blade Rushton turbine impeller or in a 5-L airlift reactor. The mycelial dry weight was measured after the mycelial pellet was repeated washed then dried. To measure EPS concentration, samples were centrifuged and the filtrate was mixed with Ethanol. The weight of the EPS was estimated after the precipitated EPS was diazlyzed then lyophilized. Morphology was examined under a light microscope through a CCD camera. The ethanol precipitates of the EPS components were partitioned by Sepharose-CL-6B chromatography. Standard dextran series were used for determination of molecular weight of EPS. Carbohydrate composition was analyzed by gas chromatography and the amino acids in EPS were assayed by an amino acid analyzer with a high performance ion exchange column.

Results: The optimal agitation rate for the production of EPS in the STR was 150 rpm with the mycelial morphology of hairy pellets, where the final concentration and the specific production rate of EPS were 2.33 g/l and 0.312 g/h, respectively. However, the maximum concentration of biomass (21.06 g/l) in the STR was obtained at a high agitation speed of 300 rpm. The specific production rate of EPS (0.456 g/g/h) in the AR was significantly higher than that achieved in the STR, in which the typical morphological form of mycelium was a loose clump. The three EPS groups in the STR (designated as STR-I, -II, and -III) were determined to be 1820, 25, 1.8, 1160, and 6.7 kDa, respectively. An agitation rate of 150 rpm in the STR was selected as the optimal culture condition for maximum EPS production (2.33 g/l), which was similar to the level achieved in the AR (2.30 g/l). The carbohydrate composition in each EPS was quite different from each other: the major component was glucose (in STR-I, -II, and -III) and mannose (in STR-II), and arabinose (in AR-II). In contrast, no significant difference in amino acid composition was observed. In conclusion, a combination of medium composition and environmental conditions should be carefully considered to control quality of EPS during the submerged mycelial culture processes of entomopathogenic fungi.

In vitro reassortment between Dobrava virus lineages

ESC-ID 333
Author Kirsanovs S, Klempa B, Krüger DH
Country Germany
University Charité Universitätsmedizin Berlin
Department Institute of virology
nucleocapsid protein, the medium (M) segment encodes the envelope proteins, and the large (L) segment the viral polymerase. Dobrava virus (DOBV) is the most virulent European hantavirus. DOBV strains are hosted by at least two different rodent species, Apodemus flavicollis and Apodemus agrarius. According to their natural hosts, they form two distinct genetic lineages, DOBV-Af and DOBV-Aa, respectively. Both lineages circulate sympatrically in Central Europe. Phylogenetic analyses indicated that genetic reassortment could have occurred during DOBV evolution. Reassortment processes might be the cause of dramatic changes in virulence of viruses with segmented genome as it is known from influenza virus. In this study, the representatives of the DOBV-Af and DOBV-Aa lineages were used for dual infection of Vero E6 cells. Single virus clones were isolated and then screened for reassortment by strain-specific multiplex PCR. Out of 202 tested clones, 63 (31%) were found to be reassortants. In addition, 65 (32%) "diploid" virus particles containing both versions of the particular segment were observed which, however, lost their "diploid" character after repeated passaging. Only two reassortment scenarios could be detected where the M segment was exchanged. Therefore two reassortant clones, each representing one scenario, were selected for further characterization. Sequence analysis of complete S and M, and partial L segment sequences confirmed the reassorted genotype of the virus clones. In vitro reassortment processes between DOBV-Af and DOBV-Aa seem to be a frequent phenomenon. The obtained reassortants will be now used to study pathogenicity determinants of different DOBV lineages and functional consequences of the reassortment process.

MxA-independent inhibition of hantavirus replication induced by type I and type II interferon in vitro

The genus Hantavirus belongs to the family of the Bunyaviridae, characterized by a tripartite single-stranded RNA genome of negative polarity. The three genomic segments code for a nucleocapsid protein, two envelope glycoproteins, and a RNA-dependent RNA polymerase. Hantaviruses are rodent born pathogens. They are transmitted to humans by inhalation of aerosols of excreta from infected rodents, in which the virus persists without any pathogenic symptoms. In humans infection can lead to hantavirus cardiopulmonary syndrome (HCPS) or hemorrhagic fever with renal syndrome (HFRS), depending on the species of hantavirus involved. HCPS caused by Sin Nombre or Andes virus have case fatality ratios of approximately 40 %. HFRS which is mediated by Hantaa virus (HTNV), Seoul virus, or variants of Dobrava virus is associated with fatality rates between 6 % to 15 %. The reasons for the varying degrees of virulence and outcome of a hantavirus infection in humans are still elusive. Interferons (IFN) are key players of the immune system which prevents uncontrolled spread of a viral infection. IFN induce an antiviral state against Hantaa virus (HTNV) but the mechanisms responsible for inhibition are unclear. Type I and type II IFNs induce the expression of a huge pattern of different interferon stimulated genes (ISGs), which results in establishment of an antiviral state protecting cells against infection by several viruses, including hantaviruses. The IFN-inducible MxA-protein is discussed to be important for control of infection with hantaviruses. To characterize the role of endogenous MxA, the inhibition of HTNV induced by type I and type II IFNs was compared in African green monkey kidney (Vero) and human lung epithelial (A549) cells. In this study, IFNa and IFNg reduced production of infectious virions, viral RNA, and nucleocapsid protein with the same efficacy, although expression of MxA-protein was detectable only in IFNa-treated A549 cells. Furthermore, siRNA-mediated knock down of MxA expression did not impair IFNa-induced inhibition. Thus, inhibition of HTNV induced by type I and type II IFNs did not depend on expression of endogenous MxA. Taken together, these data suggest that MxA endogenously expressed in response to type I or type II IFNs does not play a pivotal role for the antiviral state against HTNV.

Knowledge, awareness and practices regarding Dengue fever amongst the adult population presenting at tertiary care hospitals in Karachi

Aim: To assess the knowledge, attitudes and practices regarding Dengue fever in people visiting two major tertiary care hospitals in Karachi, Pakistan.

Methods: A cross-sectional study was conducted amongst people visiting two tertiary care hospitals in Karachi; Aga Khan University Hospital, which is a private sector hospital and caters to the needs of a higher socio-economic class of the city, and Civil hospital Karachi, which is the public sector hospital providing free of cost services to the citizens. A pre-tested and structured questionnaire was administered via face to face unprompted interview to 447 visitors. Knowledge was scored using three criteria the investigators considered most important.

Results: Most people (89.9%) had heard of dengue fever. 172 (38.5%) respondents had "sufficient" knowledge. 115 (66.9%) people in AKUH and 57 (33.1%) people in Civil Hospital fulfilled the criteria for "sufficient" knowledge regarding the mode of transmission, preventive practices and symptoms of dengue. Mosquito spray (54.9%) was the most common preventive practice, and Television was the major source of information considered by the respondents. Univariate analyses revealed significant correlations between sufficient knowledge and education (p<0.001), income (p<0.001) and the hospital presented to (p<0.001). When assessed using multimomial logistics and removal of confounding, Income (p = 0.05) and hospital (p = 0.004) were independent predictors of knowledge in the population.

Conclusion: Although a large number of people had heard about Dengue, our results reveal a low prevalence of sufficient knowledge in the general population. Targeted efforts towards raising awareness on essential aspects of the disease and its prevention are required.
Gynaecology II


ESC-ID 176
Author Momtahen S, Kazzazi AS, Gholipour F
Country Iran
University Iran University of Medical Sciences
Department Pathology

Background: Various histological types of gynecological tumors may develop from different etiological aspects. The observed differences in cancer incidence are mainly due to different individual and social risk factors [1,2,3,4].

Aim: Step toward establishing a nation wide registry is being taken in this study.

Methods: To give a relative frequency of female genital tract malignant tumors in Iran we designed a cross-sectional study included 450 women with definite diagnosis of gynecological cancer based on pathologic reports in five pathologic centers, Medical sciences University of Iran, Tehran, 1995-2005. We evaluated different characteristics of patients with definite histology of uterine, ovarian and cervical cancers. Age, marital status, parity, menopause, smoking and OCP consumption, stage of tumors and histological grade, were assessed. WHO classification of gynecological tumors[5] and International Federation of Gynecology and Obstetrics (FIGO)[6] were used as basis of study. Data collection was done through census sampling method. Vulvar and vaginal malignancies were excluded from this study. Spss 14 and correlation test was used for data analysis.

Results: From all 450 cases, the highest percentage is from the ovary, 55.5%, followed by uterus, 24.9% and cervix 19.6%. The average age of diagnosis was (50 ± 0.7). The most distribution range of age was (48-52), (58-62) and (48-52) for ovarian, uterine and cervical malignancies respectively. 58.8%, 33.9% and 47.7% of ovarian, uterine and cervical malignant patients were not menopausal. 26.4%, 9.8% and 5% of ovarian, uterine and cervical malignant patients were nullipar. 4% of ovarian, 22.3% of uterine and 7.9% of cervical malignant patients had the history of using contraceptive pills during their lives. 6.8%, 10.8% and 48% of ovarian, uterine and cervical malignant patients had the history of smoking. Surface epithelial-stromal tumor was the most frequent type of ovarian tumors (78.4%) with the most frequent subtype (Serous cyst adenocarcinoma, 43.2%). Endometrial adenocarcinoma (70.5%) and squamous cell carcinoma (83%) were most frequent types of uterus and cervical cancers respectively. The most histological grade in ovarian cancers was moderately differentiated (40%), well differentiated for cervical cancers (46%). In tumors of uterus and cervix the most frequent stage of diagnosis was stage IA (25% and 26.1%). The most metastatic diagnosed cases were seen between ovarian tumors (39.7%).

Conclusions: comparison with data published as review articles in NEJM till 2006, the most frequent gynecologic cancer in our study (ovarian cancer), is different; also the median age of our patients is lower than them [2,3,4].

Radiology II

The role of MRI in evaluation of lipomatous soft tissue tumors

ESC-ID 356
Author Isakov I
Country Serbia
University University of Novi Sad
Department Radiology

Introduction: Lipomatous tumors are common mesenchimal lesions that can be both benign or malignant. MRI has become a vital part of the routine work up of a patient with a suspected on lipomatous tumors. The superioriry of this technique stems from its exquisite sensitivity in displaying minor differences in tissue composition. MRI is diagnostically valuable in distinguishing fat-containing tumor from other tumors, because of its ability for detecting fatty components.

Aim: Aim of our study was to compare MRI characteristics between lipomas and liposarcomas by using a different pulse sequences and investigate the role of p.c. study in evaluating of this tumors.

Material and methods: retrospective study included 35 patients (19 men and 16 women) with mean age of 46.28 years. Routine MRI included both T1-weighted and T2-weighted sequences in the axial plane and at least one longitudinal plane and one sequence form from fat suppression and p. c. T1 sequence at least one plane.

Results: Lipomas present as homogenous, well-circumsribted and incapsulated masses of a fatty nature, without enhancement after i. v. contrast administration. Liposarcoma may appear as a well-margined mass, with attenuation values equal to those of simple fat, mimicking a benign lipomatous tumor. However, this type usually has some thickened linear or nodular soft tissue septa, wich enhance considerably more than in lipoma is probably due to the presence of malignant cellularity and inflammatory cell infiltration.

Conclusion: Considering reported results, we conclude that presentation of lipomatous tumors by many different methods is still unexplored. MRI is sensitive method for detection lipomatous tumors, but not always able to distinct lipomas and liposarcomas.

Obstetrics II

Complications at multipli repeated cesarean section

ESC-ID 743
Author Stojevic B, Markovic S
Country Serbia
University Pristina University
Department General

Introduction: Early intraoperation complications at multiply repeated Cesarean section are: hysterectomy Caesarea, lesio vesicae urinariae and laesio intestini.

Aim: Aim was to examine do with increased number of multiply repeated Cesarean sections comes to the increased number of early intraoperaotin complications (hysterectomy
Caesarea, lesio vesicae urinariae and laesio intestine).

**Material and methods:** Study was conducted retrospectively. Data were used from the history of disease from the Gynecology and Obstetrics Department, Kosovska Mitrovica. In a period of ten years on the Pristina University, Clinic of Gynecology and Obstetrics in Pristina there were 2474 repeated cesarean sections. With two cesarean sections were 2041, with three cesarean sections were 343, with the four cesarean sections were 74 and with the five cesarean sections were 16 women. Intraoperative risk in repeated cesarean sections was defined in the early intraoperative complications such as (hysterectomy Caesarea, lesio vesicae urinariae and laesio intestini). We separate the early intraoperations in the women with the second and third cesarean section and early intraoperative complications in the women with the fourth and fifth cesarean section.

**Results:** Results of the study show that there were no statistically important differences.

**Conclusion:** With increased number of multiply repeated cesarean sections doesn’t come to the statistically significant growth of intraoperative complications like hysterectomy Caesarea, lesio vesicae urinariae and laesio intestini.

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**Genetics II**

**The effects of bactofection mediated gene therapy using hypoxia inducible factor 1 alpha gene on markers of oxidative stress and angiogenesis in a model of intestinal ischemia in rats**

**ESC-ID** 928

**Author** Gardlik R, Palffy R, Behuliak M, Hodosy J, Celec P

**Country** Slovakia

**University** Comenius University, Faculty of Medicine

**Department** Institute of Pathophysiology

**Aims:** The aim of this study was to prove whether hypoxia inducible factor 1 alpha (HIF) gene therapy decreases oxidative stress in a model of intestinal ischemia in rats.

**Methods:** Male Wistar rats with a surgically induced ischemia of colon (caecum) or sham operated rats were treated by daily per os application of LB medium or E. coli with plasmids encoding listeriolysin and invasin (needed for the bactofection) and HIF. After one week, rats were sacrificed, plasma and tissue samples were taken for analysis. Markers of oxidative stress – malondialdehyde, advanced oxidation protein products (AOPP) were measured. The expression of SOD1, SOD2 and VEGF was analyzed using real time PCR.

**Results:** No significant differences were found in the analysis of oxidative stress markers. A tendency to alleviate the tissue damage measured by AOPP was seen in HIF treated animals. Interestingly, all treatments reduced VEGF expression in comparison to ischemic group without treatment (p<0.02). No significant differences were found in SOD1 and SOD2 expression, although the results for SOD2 indicated that HIF gene therapy might increase the antioxidant status. The differences were, however, marginally not significant.

**Conclusion:** Our results show some potential for bactofection mediated HIF gene therapy, but further studies are needed to improve the bacterial vector and, thus, the efficacy of gene transfer.
Author Index

Afraz, Zahra ................................................. 120
Ahmed, Naeem ........................................... 134
Akruwala, Chinmay ...................................... 66
Aktürk, Halis Kaan ..................................... 73
Alamin, Mohammed Siddig .......................... 125
Aleksovska, Olgica ...................................... 75
Alizadeh, Shima .......................................... 145
Aly, Zarmeneh ........................................... 18, 146
Amali, Omid ............................................... 152
Ambrushkevich, Anna ................................. 6, 7
Anand Kumar, R ........................................ 61
Andrijasevic, Vuk ........................................ 99, 116
Andriychuk, Denys ..................................... 89
Antoniak, Silvio ........................................... 37, 38
Arlt, Franziska ............................................ 23, 26
Armata, Michal .......................................... 129
Arndt, Robert ............................................ 39
Arunesh, Pandey ......................................... 61
Askari Jirhandeh, Sareh ............................... 150
Assa, Solmaz ............................................. 190
Åström, Maj-Brit ......................................... 102
Atanasov, Georgi ......................................... 108
Back, David ............................................... 165
Badau, Catalina Ileana ................................. 130
Bakhit, Sahar ............................................. 157
Baranov, Petr ............................................. 160
Bartel, Katrin ............................................... 133
Bartington, SE ........................................... 122
Bartington, Suzanne .................................... 122
Bartus, Imola Beata .................................... 13, 192
Bedretdinova, Dina ..................................... 58
Behuliak, Michal ........................................ 96, 141, 199
Berden, Jernej ............................................ 92, 178
Bernad, Anna ............................................. 106
Blecharczyk, Bernadetta ............................... 120
Bhurgi, Hadi .............................................. 187
Bilyavskaya, Svetlana ................................. 45
Bjurman, Christian ..................................... 133
Bleymehl, Karoline ................................... 196
Boboea, Andrei-Cristian .............................. 50
Bodaghabadi, Mohammad ............................ 97
Boelling, Olga ............................................ 21, 27
Bojana, Andrejic ......................................... 138
Boldizhar, Roman ...................................... 153
Bolten, Ulrike ............................................ 37, 38
Bonabi, Maede .......................................... 104
Bonsra Fynn, Richard .................................. 86
Bordu, Silviu Iulian .................................... 3, 141
Borys, Michal ............................................. 47, 49, 110
Brandt, Katrin ............................................ 79
Brnic, Olja ................................................. 99, 116
Bruha, Jan .................................................. 111
Bücker-Gärtner, Carola ............................... 42
Bulikova, Janka .......................................... 100
Bunevicius, Adomas ................................... 79
Burova, Elena Konstantinova ....................... 105
Bylicka, Emilia .......................................... 157
Castaneda Wysocka, Patricia ....................... 191
Ceanga, Mihai ............................................ 187
Chakravarty, Aditi Anupam ........................ 145
Channa, Romasa ....................................... 19, 187
Chumuri, Cindrilla ..................................... 148
Chun-Ping, Xu .......................................... 196
Ciocâlteu, Adriana Mihaela ......................... 3, 161
Clingham, Richard ..................................... 166
Coman, Andreea-Codruta ........................... 64
Cosmina, Margineanu ................................ 1
Costoiu, Lucia-Elena .................................. 93
Cotoi, Corina Gabriela ................................. 170
Cui, Helen ................................................ 101
 Cvetkovic, M ............................................. 2
Cvijanovic, Dane ....................................... 33
Czerniecka, Katarzyna ................................. 110
Dagmar, Sominka ....................................... 158
Danicic, Magdalena ................................... 131
Darocha, Arkadiusz ................................... 115
 Demir, Okan ............................................. 43
Demydenko, Ganna ................................... 44
Deng, Bo .................................................... 46
Dev, Padia .................................................. 36
Devaraj, Prathab ........................................ 35
Dirar, Khalid, M ......................................... 75
Dobrowski, Filip ......................................... 127
Doehn, Jan-Moritz ..................................... 63
 Domby, Marst ............................................ 87
Domuz, Sanela .......................................... 181
Donat, Cornelius ....................................... 70
Dörrler, Felix ............................................. 83
Dunbar, Andrew ......................................... 51
Duplik, Nadezhda ..................................... 55
Durmartkina, Alevtina ................................. 172
Durmus, Tahir ............................................ 31
Dziamidava, Tatsiana ................................ 78
Dziecinska, Daria ....................................... 130
Dziubenko, Natalia .................................... 185
Efremov, Boro .......................................... 65
Egorov, Vladimir ....................................... 9
Elek, Miroslav ........................................... 118
Enigk, Fabian ........................................... 6
Esmaeili, Sara .......................................... 179
Evers, Andrea ............................................ 18
Faber, Patryk ............................................. 74
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knut, Ruslan</td>
<td>195</td>
</tr>
<tr>
<td>Kobelt, Dennis</td>
<td>21, 27</td>
</tr>
<tr>
<td>Kocharyan, Armine</td>
<td>168</td>
</tr>
<tr>
<td>Koka, Kaja</td>
<td>65</td>
</tr>
<tr>
<td>Kosiak, Mateusz</td>
<td>174</td>
</tr>
<tr>
<td>Kostyra, Kacper</td>
<td>40</td>
</tr>
<tr>
<td>Kot, Marcin</td>
<td>162</td>
</tr>
<tr>
<td>Koval, Halyna</td>
<td>152</td>
</tr>
<tr>
<td>Kovalchuk, Volodymyr</td>
<td>139</td>
</tr>
<tr>
<td>Krabben, Géanne</td>
<td>67</td>
</tr>
<tr>
<td>Kraj, Leszek</td>
<td>141</td>
</tr>
<tr>
<td>Krasa, Agata</td>
<td>173</td>
</tr>
<tr>
<td>Krepska, Miklós</td>
<td>112</td>
</tr>
<tr>
<td>Krishnan, Vicknesh</td>
<td>113</td>
</tr>
<tr>
<td>Kruszyna, Lukasz</td>
<td>9, 147</td>
</tr>
<tr>
<td>Ksenia, Teterina</td>
<td>81</td>
</tr>
<tr>
<td>Kuzminykh, Svetlana</td>
<td>192</td>
</tr>
<tr>
<td>Labeledzka, Magdalena</td>
<td>76</td>
</tr>
<tr>
<td>Lada, Artem</td>
<td>24</td>
</tr>
<tr>
<td>Langer, Juliane</td>
<td>173</td>
</tr>
<tr>
<td>Latthe, Kutty Kusuma</td>
<td>35</td>
</tr>
<tr>
<td>Lehnert, Ron</td>
<td>161</td>
</tr>
<tr>
<td>Lesshaft, Hannah</td>
<td>122</td>
</tr>
<tr>
<td>Lilea, Georgiana Carmen</td>
<td>3, 141, 161</td>
</tr>
<tr>
<td>Liong, Ker Keong</td>
<td>194</td>
</tr>
<tr>
<td>Liotta, Agustin</td>
<td>71</td>
</tr>
<tr>
<td>Lock, Johan</td>
<td>189</td>
</tr>
<tr>
<td>Ludovit, Paulis</td>
<td>184</td>
</tr>
<tr>
<td>Lung-Illes, Valentin</td>
<td>150</td>
</tr>
<tr>
<td>Luther, Natalie</td>
<td>11</td>
</tr>
<tr>
<td>Lutsenko, Dmytro</td>
<td>96</td>
</tr>
<tr>
<td>Lyashchenko, Olga</td>
<td>116</td>
</tr>
<tr>
<td>Maciorkowska, Dominika</td>
<td>44</td>
</tr>
<tr>
<td>Maghbooli, Leili</td>
<td>16, 123</td>
</tr>
<tr>
<td>Mahbobi, Nastaran</td>
<td>153</td>
</tr>
<tr>
<td>Mahboubi, Hossein</td>
<td>176</td>
</tr>
<tr>
<td>Mahdian, Abolfazl</td>
<td>125</td>
</tr>
<tr>
<td>Majooni, Mohammad Bagher</td>
<td>94</td>
</tr>
<tr>
<td>Maleki Fard, Elham</td>
<td>41</td>
</tr>
<tr>
<td>Marciniak, Patrycja</td>
<td>50</td>
</tr>
<tr>
<td>Marcinkute, Agne</td>
<td>81</td>
</tr>
<tr>
<td>Markovic, Milica</td>
<td>76</td>
</tr>
<tr>
<td>Markovic, Sladjana</td>
<td>198</td>
</tr>
<tr>
<td>Markovska, Zorica</td>
<td>45</td>
</tr>
<tr>
<td>Martin Pereira, Maria Jose</td>
<td>8</td>
</tr>
<tr>
<td>Marzieh, Roshani</td>
<td>40</td>
</tr>
<tr>
<td>Mielcarek, Monika</td>
<td>25</td>
</tr>
<tr>
<td>Mihajloska, Elida</td>
<td>114</td>
</tr>
<tr>
<td>Mihajlovic, Dusan</td>
<td>86</td>
</tr>
<tr>
<td>Mikolaščík, Eszterka</td>
<td>99</td>
</tr>
<tr>
<td>Milankov, Vukadin</td>
<td>100</td>
</tr>
<tr>
<td>Milojkovic, Ana</td>
<td>147</td>
</tr>
<tr>
<td>Miron, Oana Tatiana</td>
<td>176</td>
</tr>
<tr>
<td>Mitek, Tomasz</td>
<td>163, 166</td>
</tr>
<tr>
<td>Mitkovic, Milan</td>
<td>165</td>
</tr>
<tr>
<td>Mladenovic, Pavle</td>
<td>169</td>
</tr>
<tr>
<td>Mnichowska, Agata</td>
<td>175</td>
</tr>
<tr>
<td>Mochnert, Malgorzata</td>
<td>159</td>
</tr>
<tr>
<td>Mojtahed, Mohammad</td>
<td>95</td>
</tr>
<tr>
<td>Montahen, Shabnam</td>
<td>143, 198</td>
</tr>
<tr>
<td>Morošanu, Andreea</td>
<td>138</td>
</tr>
<tr>
<td>Morteza, Afsaneh</td>
<td>148</td>
</tr>
<tr>
<td>Morteza, Rostam Beigi</td>
<td>136</td>
</tr>
<tr>
<td>Müer, Annika</td>
<td>151</td>
</tr>
<tr>
<td>Muller-Wielsh, Kathrin</td>
<td>155</td>
</tr>
<tr>
<td>Muntaz, Imtiaz</td>
<td>54</td>
</tr>
<tr>
<td>Murzyn, Joanna</td>
<td>172</td>
</tr>
<tr>
<td>Mykytenko, Dmytro</td>
<td>52</td>
</tr>
<tr>
<td>Myroshnychenko, Dmytro</td>
<td>191</td>
</tr>
<tr>
<td>Najimi, Neda</td>
<td>158</td>
</tr>
<tr>
<td>Naram, Aliyea</td>
<td>82</td>
</tr>
<tr>
<td>Nayar, Meenakshi</td>
<td>186</td>
</tr>
<tr>
<td>Neda, Catalin Ionut</td>
<td>141</td>
</tr>
<tr>
<td>Nedeljko, Aleksandra</td>
<td>52</td>
</tr>
<tr>
<td>Nedoszytko, Michal</td>
<td>17</td>
</tr>
<tr>
<td>Neuman, Sara</td>
<td>75</td>
</tr>
<tr>
<td>Niedziela, Jacek</td>
<td>132</td>
</tr>
<tr>
<td>Niedzwiecki, Pawel</td>
<td>140</td>
</tr>
<tr>
<td>Nikolic, Jelena</td>
<td>154, 157</td>
</tr>
<tr>
<td>Nikolic, Slavica</td>
<td>195</td>
</tr>
<tr>
<td>Nikolov, Petar</td>
<td>36</td>
</tr>
<tr>
<td>Nizhnikov, Anton</td>
<td>23</td>
</tr>
<tr>
<td>Noack, Thilo</td>
<td>106</td>
</tr>
<tr>
<td>Nogal, Malgorzata</td>
<td>109</td>
</tr>
<tr>
<td>Norouzi, Sayna</td>
<td>91</td>
</tr>
<tr>
<td>Nowacki, Maciej</td>
<td>82</td>
</tr>
<tr>
<td>Obradowic, Biljana</td>
<td>84</td>
</tr>
<tr>
<td>Oddsson, Saemundur J</td>
<td>115</td>
</tr>
<tr>
<td>Oelschlegel, Robin</td>
<td>197</td>
</tr>
<tr>
<td>Okhovat Ghahfarokhi, Shahir</td>
<td>89</td>
</tr>
<tr>
<td>Ojaca, Sanja</td>
<td>189</td>
</tr>
<tr>
<td>Olyniec, Karolina</td>
<td>179</td>
</tr>
<tr>
<td>Omer, Mohammed Siddig</td>
<td>14</td>
</tr>
<tr>
<td>Omipidan, Suleiman</td>
<td>20</td>
</tr>
<tr>
<td>Omuz, Sanela</td>
<td>31</td>
</tr>
<tr>
<td>Orae, Yazdani, Saeed</td>
<td>71</td>
</tr>
<tr>
<td>Ortner, Daniel</td>
<td>88</td>
</tr>
<tr>
<td>Osadcha, Olena</td>
<td>47</td>
</tr>
<tr>
<td>Padia, Dev</td>
<td>137</td>
</tr>
<tr>
<td>Pakhomov, Oleandr</td>
<td>184</td>
</tr>
<tr>
<td>Pálffy, Roland</td>
<td>96</td>
</tr>
<tr>
<td>Panasiku, Anna</td>
<td>171</td>
</tr>
<tr>
<td>Panfilova, Olga</td>
<td>43</td>
</tr>
<tr>
<td>Papazova, Diana</td>
<td>178</td>
</tr>
<tr>
<td>Parthigar, Seyed Ehsan</td>
<td>135</td>
</tr>
<tr>
<td>Pashevin, Denys</td>
<td>85</td>
</tr>
<tr>
<td>Pesic, Ivana</td>
<td>155</td>
</tr>
<tr>
<td>Pedziwiatr, Michal</td>
<td>113</td>
</tr>
</tbody>
</table>