

Abstracts

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CORRELATION OF MISMATCH REPAIR PROTEINS MLH1, MSH2, MSH6 AND PMS2 WITH ANTIAPOPTOTIC PROTEIN SURVIVIN IN COLON LESIONS

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Introduction: Defective function of mismatch repair proteins (MMRP) contributes to development of variety of cancers. Antiapoptotic protein survivin is involved in pathogenesis of human malignancies and it is widely expressed in majority of malignant tissues. Both MMRP and survivin are considered to be powerful prognostic parameters. The purpose of this study was to determine the association of MMRP with survivin in benign and malignant colon lesions. Methods: The MMRP status and survivin were retrospectively assessed by immunohistochemistry on paraffin-embedded formalin-fixed samples from 41 benign colon polyps and 73 grade 1 and 2 colon carcinomas. Results: In polyps, 34/41 samples were positive for MLH1 staining (82,9%), 38/41 for MSH2 and MSH6 stainings (92,7%), and 35/41 for PMS2 staining (85,4%). Survivin expression was found in 1/41 sample only (2,4%). In panel of carcinomas, immunoreaction was detected in 64/73 samples for MLH1 (87,7%), 72/73 samples for MSH2 (98,6%), 71/73 for MSH6 samples (97,3%), and 67/73 for PMS2 samples (91,8%). Survivin was shown in 24/73 samples (32,9%). In carcinomas, the statistical analysis confirmed a significant correlation between the expression of MMRP and survivin ($p < 0,001$), a significant difference in the intensity of MMRP and survivin immunoreactivity ($p < 0,001$), and a significant difference in percentage of MMRP and survivin labeled cells ($p < 0,001$). Furthermore, the correlation was found in panel of polyps between expression of MMRP and survivin ($p < 0,001$). Conclusions: Our results suggest that MMRP may suppress the survivin expression and

its antiapoptotic activity in grade 1 and 2 colon carcinoma. This work was supported by project VEGA 1/0050/11.

CLINICAL ANATOMY TEACHING ON CADAVERS IN PREGRADUATE AND POSTGRADUATE MEDICAL EDUCATION

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A rapid increase of the importance of mini-invasive techniques in many medical disciplines, ranging from internal medicine (bronchoscopy, gastroscopy) to surgical disciplines (laparoscopy, thoracoscopy) occurs in last ten years. Then the growing need for mastering these techniques, both in terms of theory and practical skills, in particular increases too. In response we built a unique multimedia educational centre for topographic and clinical anatomy, endoscopy and microsurgery at our Anatomical Institute. The Educational Centre offers cadavers fixed by a special method for a repeated use, in order to imitate different medical procedures. We fully equipped a small operating theatre, rooms are interconnected by an audio-video network with a wi-fi Internet. We equipped also a graphic studio for creation of educational materials. Demonstration for medical students includes simulations of arthroscopies, laparoscopies, bronchoscopies and gastroscopies. For young physicians training courses are organized, to get acquainted with instruments for a wide range of endoscopic and microsurgical procedures in a similar topographical situation. Postgradual students can use e-learning materials designed for the selected practical course in advance to their enrollment. Distant educational components together with the practical

cadaver courses are a good approach in clinical anatomy education and surgical skills improvement. The centre provides an advanced approach to the practical training in the field of mini-invasive and endoscopic methods concentrated in one place and, in fact, represents the maximum progress achievable in this respect, before the students enter the real world of medical practice. Supported by RP MSMT and EU Funds OPPC CZ216/3100/24018 (INO/02/01/0017/2010).

NEW REMARKS TO VISUALISATION AND IDENTIFICATION OF IMMUNOCOMPETENT CELLS IN HUMAN CORNEA

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The corneal transplantation is currently common procedure - in the Czech Republic it is about 600 operations per year. There is a lot of conditions which have influence to grafts survival, e.g. number of endothelial cells present in the donor cornea, immunological mechanisms. A principle of white elements series naturally occurring in the cornea is unclear. The aim was to innovate and improve procedure for display cells of white line in the various layers of the cornea. The physiological corneas were dissected and examined under a surgical microscope, and only those without any signs of inflammation or other abnormalities were used in the studies. The corneas were cleared from attached lens, conjunctiva, and excess limbal tissue. The corneal stroma and epithelium were then separated after a 20-minute incubation at 37°C in PBS containing 20 mM EDTA. After separation, the corneal stromas were fixed for 30 minutes at 4°C in 1% paraformaldehyde-PBS followed by extensive washing with PBS. After fixation, the corneal tissue was permeabilized with 0,2-0,5% TWEEN20 aqueous solution for 20 minutes at 37°C. Following corneal tissue was incubated overnight at 4°C with 100 µL antibody antihuman CD45 FITC/CD14-PE DUAL TAG TM diluted in 0,2% TWEEN/PBS. Tissues stained with primary antibody were fixed again with 1% paraformaldehyde-PBS, rinsed with PBS, placed on slides, mounted with Prolong Gold Antifade Reagent (Life Technologies), and coverslipped. All slides were examined by fluorescence microscopy on a 1 × 40 microscope (Nikon, Eclipse). The procedure showed individual immuno-competent cells. Cells were evaluated from the quantity and type point of view. This is a preliminary work and it is taken as a first step in research of cross-linking methodology to reduce rejection episodes in corneal transplantation. Supported by GAUK 282011/2011

RADIATION-INDUCED LONG-TERM ALTERATIONS IN THE FOREBRAIN UNDER EXPERIMENTAL CONDITIONS

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Introduction: Ionizing radiation can induce significant injury to normal brain structures. The present study was dealt with effect of fractionated doses of gamma rays on specific cell types housed the rat brain's neurogenic region. Methods: Adult male Wistar rats received whole-body exposure with fractionated doses of gamma rays (a total dose of 5Gy) and were investigated thirty, and sixty days later. Immunohistochemistry and confocal microscopy were used to determine density of young neurons - neuroblasts derived from anterior subventricular zone (SVZa) and brain resident and activated microglia distributed along and/or adjacent to subventricular zone-olfactory bulb axis (SVZ-OB axis). Cell counting was performed in four anatomical parts along the well defined pathway, known as the rostral migratory stream (RMS) represented by the SVZa, vertical arm, elbow and horizontal arm of the RMS. Results: Gradual decline of neuroblasts was seen in course of sixty days after radiation treatment mostly visible in rostral parts of the migratory pathway. Population of resident and activated microglia showed different dynamic of cell distribution, however data about their cell density are still under evaluation. Conclusion: Preliminary data suggested that radiation response of young neurons arisen from the SVZa may play contributory role in development of more adverse radiation-induced late effects and therefore may have implications for clinical radiotherapy. Supported by a VEGA grant 1/0050/11 and projects "center of excellency for research in personalized therapy (CEVYPET)", code: 26220120053 and "Identification of novel markers in the diagnostic panel of neurological diseases" co-financed from EU sources and European Regional Development Fund.

CLINICAL AND CLINICALLY ORIENTED ANATOMY FOR MEDICAL STUDENTS

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Medicine curriculum in the University of Cordoba was changed in 1973. Until that time Anatomy was developed into four different courses: descriptive anatomy, topographic anatomy, surgical technique and surgical anatomy in the first 4 years of the career. Since 1973 the course of Normal Anatomy (gross anatomy) curriculum included radiological aspects.

Later (1980) endoscopic contents were added. However, those topics were only developed by some professors during theoretical lessons as illustrative examples of clinical application of anatomical knowledge. After 1990 new contents related to diagnostic images were introduced. On 1999, the governmental entity that regulates university careers determined that clinical aspects had to be included in medical basic courses and Anatomy had to specifically involve the identification of anatomical structures in diagnostic images and surface anatomy. Since then, our theoretical lessons include clinical concepts for all subjects, practical activities are developed not only with cadavers but also with diagnostic images and clinical aspects are also evaluated in all instances. Depending on institutional possibilities, students visit some hospitals to observe the practical application of their anatomical knowledge; and depending on their particular interest, they can choose an optional course related to a specific anatomical region or medical specialty. Our students have received with enthusiasm these clinical contents as they feel they motivate their study and provide the basement for that knowledge. Our curriculum does not include a course of Clinical Anatomy. We are developing an optional course of Clinical Anatomy in English, but it only develops some few topics. An introduction of clinical anatomy contents is taught at the beginning of each clinical course, but usually they are not developed by the professors of Anatomy. However, the tradition of our university determines that most of the clinical professors (mainly in surgical courses) have spent some time in Anatomy at any moment of his/her training.

NADPH-d HISTOCHEMICAL STUDY OF THE RAT HIPPOCAMPUS

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Introduction: The hippocampus is a complex region of the brain that has an important clinical significance. Unlike humans, the hippocampus in the rat is located on the dorsal side of the thalamus. The aim of this study was to investigate the presence of nitroergic neurons in the rat hippocampus. **Methods:** Study was carried out on 12 Wistar rats of the both sexes at the age of 3 months under physiological conditions. The coronal sections of each brain sample were examined by nicotinamide dinucleotide phosphate-diaphorase (NADPH-d) histochemical method. **Results:** The distribution of the NADPH-d positive neurons in hippocampus and dentate gyrus of adult rat was investigated. Two thirds of the NADPH-d neurons were located in hippocampal regions CA1, CA2 and CA3, one third was found in the dentate gyrus. The highest number of labelled cells was located in the radiate layer. In the CA1 and CA2 areas of hippocampus the NADPH-d positive interneurons were found mostly in pyramidal and radiate layers, whilst in the CA3 region they were distributed throughout the thickness of whole pyramidal layer. The NADPH-d positive cells in the dentate gyrus were located near the granular layer. Most of the stained neurons were bipolar and oriented

radially in the pyramidal layer of CA1 and CA2. In CA3 layer they were uniform in the size and in multipolar or fusiform shape. **Conclusions:** According to our investigation we can conclude, that the distribution of the NADPH-d positive cell was higher in the ventral part of the rat hippocampus. There were observed no differences between the NADPH-d positive neurons of both hemispheres. Supported by Grant VEGA 1/0154/11.

FORMATION OF TOOTH-BONE INTERFACE IN REPTILES

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Vertebrates exhibit a great variability in type of tooth attachment, which is also depending on the existence or absence of the teeth replacement. Acrodont, pleurodont and thecodont attachments with several subtypes can be distinguished. In reptiles, we can find state of complete ankylosis of tooth into the jaw as well as teeth localized in the alveolar bone accompanied by periodontal apparatus similar to mammals. Acrodonta class (comprises chameleons and agamas) is characteristic by the direct connection of dental tissues to the jaw surface by mineralized connective tissue. This fusion starts in chameleons during the course of their prehatching stages. Developmental processes and molecular bone/tooth communication, which participate to tooth attachment into surrounding bones, have not been fully elucidated yet. We analyzed the origin of chameleons embryonic connective tissues produced between the tooth and the bone. Transversal histological sections of mandibles were used from selected embryonic stages. We applied Hematoxyline-Eosin staining and immunohistochemical analysis on alternative sections. Furthermore, detailed views of tooth/bone attachment were obtained by microCT analysis of chameleon heads. The tooth base in the chameleon formed after mineralization of the tooth crown. The enamel layer did not develop in the area of the tooth base and thus the tooth base was formed only by the dentine. Odontoblasts produced a layer of predentine that connected the dentin to the supporting bone, with both tooth and bone protruding out of the oral cavity and acting as a functional unit. CT scan showed that tooth and bones were separated by bony lamellae whose resorption connected the tooth pulp and the bone marrow at post-hatching stages. In contrast to chameleon, the ankylosis is pathological phenomena in mammals following the tooth implantation or tooth eruption failures. Teeth malfunctions arise with disturbances in the occlusion. Furthermore, the alveolar bone shows a progressive demineralization, which leads to tooth loss. As cellular and molecular mechanisms of ankylosis development are not known, chameleons may provide new and useful information to study the molecular interaction at

the tooth-bone interface in physiological as well as pathological conditions. This project is supported by the University of Veterinary and Pharmaceutical Sciences Brno (Grant 61/2013/FVL).

INFLAMMATORY ACTIVATION OF THE SATELLITE GLIAL CELLS IN THE DORSAL ROOT GANGLIA OF RAT EXPERIMENTAL NEUROPATHIC PAIN MODELS

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The satellite glial cells (SGC) envelope the primary sensory neurons of the dorsal root ganglia (DRG) and react intensely to various types of nerve injury that induce neuropathic pain. Unilateral chronic constriction injury (CCI, n=64) and spare nerve injury (SNI, n=32) of the rat sciatic nerve were used as experimental models of neuropathic pain (NPP). NPP induction was tested by withdrawal threshold of mechanoallodynia and thermal hyperalgesia. Expression of TNF α , IL-6, their receptors and signaling proteins (TNFR1, TNFR2, IL-6R, gp130, STAT3, SOCS3) and mRNAs were investigated bilaterally by immunohistochemistry and in situ hybridization in both lumbar (L4-5) and cervical (C7-8) DRG 1, 3, 7 and 14 days from surgical treatment. The SGC were identified by colocalization with GFAP or GS. Ipsilateral hind paws of all rats operated on CCI/SNI displayed mechanoallodynia and thermal hyperalgesia while contralateral hind paws and forepaws of both sides exhibited no significant hypersensitivity. Expression of cytokines, their receptors and signaling proteins demonstrated inflammatory activation of the neurons and their SGC not only in DRG associated but also non-associated with injured nerve. A distinct expression of cytokines and their receptors was predominantly identified in SGC surrounding large-sized DRG neurons. Moreover, inflammatory activation of SGC was also observed in DRG of sham-operated rats indicating other kinds of trigger mechanisms than after traumatic nerve injury. The results suggest that inflammatory activation of SGC contributes to ectopic activation of the DRG neurons associated with injured nerve, but in other DRG may stimulate conditioning of neurons to nerve injury. This work was supported by the project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund and the EU 7th FP under the "Capacities" specific programme (Contract No. 286154 – SYLICA).

COMPARATIVE ANATOMY OF HUMAN, PORCINE AND DEER EYEBALL

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Introduction: In higher organisms the eye collects light from the environment, regulates its intensity, focuses,

forms an image, converts this image into a set of electrical impulses, and transmits these impulses to the visual cortex through the optic pathway. From the morphological point of view the porcine eyes are structurally similar to the human eyes. The deer visual system is designed to function in extremely low-light conditions. The aim of this work was to study, observe and compare the morphology of the human, porcine and deer eyes. Methods: Two human eyes were obtained from 70 years male cadaver, 10 porcine eyes were gained from a local butcher shop and 4 deer eyes from a local hunting association. The eyes were fixed in 10% formalin, processed by the paraffin embedding technique, and stained by hematoxylin-eosin. The photomicrographs were taken by light microscope equipped with digital camera. Results: The Porcine corneal thickness is almost twice that of the human cornea, and lacks Bowman's layer. The anterior radius of the human lens is 10 mm, and the posterior radius is 6 mm, with a central lens thickness of 4 mm. These values are smaller than those of the porcine eye. The porcine retina shows great similarity to the human retina. Deer ciliary muscles surrounding the lens are small. Also, their lens is quite thick. Deer's ciliary muscles are too weak to change the shape of such a thick lens. The tapetum lucidum is a shiny, blue-green colored membrane attached to the deer retina which enhances their vision in low light and is responsible for the eyeshine. This structure doesn't exist in human or porcine eye. Human pupil is round, whereas deer have a horizontal slit pupil. The deer's pupil extends nearly the entire width of the eye. The deer retina has a horizontal visual streak in which cones are highly concentrated along a wide band. Humans are trichromats. Deer is best described as dichromats. Conclusion: This work describes the anatomy of the deer, porcine and human eyeball collected from our histological study and findings described in the literature.

MYELOID CELLS POLARIZATION IN TUMOR'S MICROENVIRONMENT

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Introduction: The process of tumorigenesis and growth is an interplay between tumor cells and several types of host cells, without which, tumors will most likely to be unable to grow. Macrophages are constantly described as crucial players in the process. Their function however, is a matter of contradiction as they are described as both protumoral and antitumoral. Methods: In this work we summarise the current state of knowledge concerning the role of macrophages and other myeloid cells in the tumoral process. We will attempt to explain their paradoxical behavior with respect to tumor type, based on their polarization and subtypes. Results: Myeloid cells involved in the tumor microenvironment are of great adaptivity. Their

polarization is the key factor for determining their future function. We present a hypothesis of the possible polarization of these cells in different tumor based on their phenotype and behavior. Conclusion: Our presented schemes offer an interpretation of tumor behaviour with respect to the population of myeloid cells in the microenvironment. Myeloid cells and macrophages are involved in several signalling cascades in the tumor microenvironment. Understanding their function helps provide an insight into the tumoral process with perspective future redefinition of tumor staging and possible targets for intervention and therapy.

THE EFFECT OF EPIGALLOCATECHIN GALLATE ON RESPIRATION OF ISOLATED RAT HEPATOCYTES AND ITS ANTIOXIDATIVE ACTION ON TERT-BUTYL HYDROPEROXIDE-INDUCED OXIDATIVE DAMAGE OF HEPATOCYTES

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Introduction: Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea. It is an important antioxidant which has a positive impact on organism. Recent data in the literature have shown that EGCG may exert also undesirable effects. Oxidative stress is one of the most important mechanisms by which toxic agents influence cells, mitochondria and respiratory chain enzymes. . The goal of our work was to study the effect of EGCG on respiration of isolated rat hepatocytes and observe if antioxidative effect of EGCG would improve damage of hepatocytes induced by tert-butyl hydroperoxide (t-BHP - model substance inducing oxidative damage). Methods: All experiments were performed on adult Wistar male rats with body weight 230-320g. Hepatocytes were isolated by two-step collagenase perfusion of the liver. Oxygen consumption by hepatocytes was measured at 30°C using High Resolution Oxygraph2K (Oroboros, Austria). Hepatocytes were incubated with EGCG in concentration range 5 - 400 µM for 5 and 10 minutes, respectively. In the second part of our study we measured respiration of hepatocytes damaged by exposure to 0.25 mM t-BHP for 5 min. and compared the results with respiration of hepatocytes coincubated with 50 µM EGCG. Conclusion: We proved that EGCG increases hepatocyte respiration after addition of glutamate-malate (Complex I) and decreases respiratory control index. These results indicate uncoupling of oxidative phosphorylation caused by EGCG. Increased respiration after addition of cytochrome c to hepatocytes incubated with EGCG

indicates damage of outer mitochondrial membrane. These changes correlate quite well with the concentration of EGCG in the medium. Oxidation of succinate (Complex II) was in the presence of EGCG also significantly decreased. EGCG exerts only mild effect on respiratory changes induced by oxidative effect of t-BHP. This work was supported by research program: PRVOUK P37/02.

IMPORTANCE OF HYPOXIA-INDUCIBLE CARBONIC ANHYDRASE IX IN BREAST CANCER

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Introduction: Breast cancer is heterogeneous disease with variable clinical presentation, histomorphological parameters and biological behavior. The traditional morphological and biological parameters are commonly judging in assessment of the prognosis and therapeutic response. However, it was proved that these conventional features lack the power to reveal the true tumor heterogeneity, individual malignant growth variability and appropriate therapeutic management. Among new potential tumor markers, the tumor-associated and hypoxia-inducible carbonic anhydrase IX was found to be representing an attractive diagnostic biomarker as well as a novel prognostic factor. CA IX attracts a significant interest owing to its strong association with neoplasms and its absence from corresponding normal tissues. Material and Methods: The aim of this study was to assess the expression of CA IX in 38 benign and 60 malignant breast lesions. Immunohistochemical staining was performed using monoclonal antibody M75 (Institute of Virology, Slovak Academy of Sciences, Bratislava). The intensity of staining, percentage of labeled cells and subcellular localization of CA IX were assessed. Chi-square test was used to demonstrate the correlation in CA IX expression of benign and malignant breast tissues. Results: We found three expression patterns of CA IX. CA IX was absent or weak cytoplasmic immunopositivity was dominated in fibroadenoma cases. Moderate to strong membrane immunoreaction was predominantly found in carcinoma cases. We noticed combined membrane and cytoplasmic CA IX expression in only 3 carcinoma cases. The statistical analysis confirmed significant correlations between the assessed parameters in fibroadenoma and carcinoma. Conclusion: The results of our study support the possible importance of CA IX for breast carcinogenesis and suggest its potential use as a valuable diagnostic as well as a new independent prognostic parameter in breast carcinoma. *Supported by Grant VEGA 1/0050/11.*

COMPARISON OF SIZE AND NUMBER OF THE SPLEEN LYMPHATIC FOLLICLES UNDER VARIOUS PATHOLOGIC CONDITIONS

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Introduction: The human spleen is an important lymphoid organ participating in immune reactions against all types of circulating antigens. One of the current problems in evaluating spleen morphology features under conditions of disease is being concerned with evaluation of white and red pulp volume. A typical example of a disease, accompanied by white pulp volume and follicle structure changes is the autoimmune thrombocytopenic purpura (AITP). **Material and methods:** We analysed 42 bioptic samples from human spleens. 24 samples were taken from cases of AITP, 18 samples originated from reference group of persons with traumatic spleen rupture or with either traumatic spleen rupture or some nonautoimmune disorders. Sections of paraffin-embedded samples were stained with hematoxylin and eosin. Digitalized images were analyzed by "AnalySIS^D" © "software (Olympus Soft Imaging Solutions GmbH, Münster, Germany). **Results:** The mean diameter of white pulp areas in AITP group was 498.44 µm, whereas in reference group it was 435.25 µm. The mean count of white pulp areas was 11.33 in one image. There were 5.21 mean count of white pulp areas in one image of reference group. The difference was statistically highly significant ($P > 0.01$). **Conclusion:** The autoimmune thrombocytopenic purpura was followed by an increase of size and number of lymphatic follicles of human spleen in comparison with spleens from reference group.

NEW ELECTIVE SUBJECT IN PREGRADUATE STUDY OF GENERAL MEDICINE

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This paper presents a new subject called Selected Topics in Topographical Anatomy. The subject was authorized by Scientific Council of our Faculty since the winter term 2013/14. Author of this proceeding was encouraged by similar poster seen at last year's Congress of Morphology. Meaning of this elective subject is to complete and improve knowledge of topographical anatomy and to join it with manual skill especially. So the subject is intended to be a continuation of current work of our voluntary dissection club. Many specimens and several presentations in Students scientific conference and congress of Czech

Anatomical Society arose from this activity during last 10 years. Proposition of this subject reflects steady students' interest: this academic year we faced twofold excess of demand over our technical capabilities. The applicants are especially the students of the second year and students of „repeating year“. However, transformation from a voluntary activity to the official subject registered in the Study Information System should bring benefits for both teachers and students - both attendance discipline and evaluation of the participants by credit. The extent of the subject is planned as ten three-hour-long meetings. The schedule is suggested to respect parallel run of Czech and English education. Both student groups must be led in different languages but we will support mutual contact. It may result in higher anatomical experience and good extracurricular relations as well. Study materials are made by author of this presentation in form of E-learning courses.

EVALUATION OF THE SHAPE OF THE ROOF OF THE ETHMOID CELLS AND SPHENOID SINUS

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Objective: Variations and incidence of the different types of the shape of the roof of ethmoid cells and sphenoid sinus at the level of optic nerve were evaluated by computed tomography. **Material and Methods:** FiftyThree CT scans of the adults (older than 18 years) were retrospectively reviewed. Images were assessed in axial plane with 1 mm section thickness. **Results:** Six types concerning shapes of the roof of the ethmoid cells and sphenoid sinus at the level of optic nerve in axial plane were differentiated. The most common type was type in which anterior diameter of ethmoid cells and posterior diameter of sphenoid sinus was narrowed and middle part was enlarged (shaped like a barrel). This type was detected in 39.6% of patients. The other types: type 2 – sphenoid (posterior) diameter is larger than ethmoid (anterior), type 3 – ethmoid dimensions were wider than sphenoid diameter, type 4 – approximately identical dimensions in anterior and posterior portion, type 5 – irregular shape and type 6 – ethmoid labyrinth and sphenoid sinus had an hour-glass shape. The incidences evaluated in type 2 – 6 were 28.3%, 7.5%, 3.8%, 11.3% and 9.4% of cases, respectively. **Conclusion:** Types 3 and 6 are unsafe for surgery due to the risk of iatrogenic damage of the orbit, optic nerve and internal carotid artery. Evaluation of CT-scans can help and simplify surgeons in their orientation and recognition of the relations of the neurovascular structures, paranasal sinuses and orbit during the surgical procedures. Configuration of the shape of the ethmoid cells and sphenoid sinus is a topic which is not well documented in the literature although it provides a comprehensive view about the pneumatization of the posterior paranasal sinuses and their intimate relations to the adjacent neurovascular structures.

IDENTIFICATION OF FUNCTIONAL AND NON-FUNCTIONAL P53 IN HUMAN NEPHROBLASTOMA BY P53 AND MDM2 IMMUNOHISTOCHEMISTRY

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Introduction: Mutations or deletion of suppressor gene p53 are the most common genetic abnormalities occurring during cancerogenesis in the majority of cases of human neoplasm. p53 gene, localized on the short arm of chromosome 17 (17p13), encodes nucleic phospho-protein, and affects several cell functions (induction of many genes, the regulation of the cellular cycle and apoptosis control). Under the condition of mutation of p53 gene, cancer cells remain intact and survive. The index of p53 expression is not an independent prognostic factor in nephroblastoma in children, but it may be helpful in the identification of high risk and low risk patients. **Objective of study:** We investigated expression of MDM2, as potential marker of p53 function. Recent studies have shown that expression of MDM2 might correlate with p53 activity. **Patients:** This study involved 25 children (17 girls and 8 boys), aged 7 months to 10 years, treated for unilateral Wilms' tumor. 25 tissue samples of Wilms' tumor and 5 samples of normal human kidneys were obtained from the Department of Pathological Anatomy, Jessenius Faculty of Medicine in Martin. All the tumors had classical triphasic histology, showing epithelial, mesenchymal and blastemal components in varying proportions. Anaplasia was not detected in any nephroblastoma tumor samples. **Antibodies and method:** mouse anti-p53, clone BP53-12 (Alexis Biochemicals, Enzo Biochemicals, Farmingdale, NY, USA) and mouse anti MDM2, clone SMP14 (Santa Cruz Biotechnology, inc.). For detection p53 and MDM2 we have used indirect immunohistochemical method. **Results:** Our study revealed weak or undetectable reaction of p53 in the majority of Wilms' tumors. Positive immunoreaction was observed only in two cases of nephroblastoma samples with clinical stage I. None tissue samples have shown MDM2 expression. **Conclusion:** The recent studies suggest that the presence of p53 in Wilms' tumor is non-functional (mutant p53), because it is not able to activate MDM2 gene. Nevertheless, immunohistochemical detection p53 at the time of diagnosis might assist in choosing specific chemotherapeutics to improve prognosis and therapy. This work was supported in part by a grant VEGA 1/0224/12 partly by grant VEGA 1/0928/11 and partly by grant VEGA 1/0925/11.

PREFRONTAL CORTEX OF 7-DAYS OLD RAT IN PHYSIOLOGICAL DEVELOPMENT AND AFTER EXPOSURE TO VITAMIN A

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Introduction: The prefrontal cortex is with respect to their "control" functions probably the best connected

place of brain. Prefrontal association area in front of motor cortex in front of the hemispheres and has extensive connections with the structures of the limbic system, with significant effect on the formation the behavior of the organism. It's the planning part of the cortex, develops after birth and the neural networks are organized through of emotional impulses and learning. Our work is focused on the physiological development nitrergic neurons in the prefrontal cortex of the rat brain and the condition after administration of retinoic acid, which is the biologically active form of vitamin A. Vitamin A is essential for embryonic development but also for an adult. In addition to vitamin A deficiency even excess can be harmful. Teratogenic effects were observed in pregnant mammals, which were given high doses of vitamin A. In this case, the described multi-organ damage, including the effect on the central nervous system. **Methods:** Studies were carried out on six male Wistar rats, who have been prenatally administration RA on E14-16. Brains of 7-days old rats were dissected out and postfixed in the fixative. The brain was cut on cryostat and sections were subjected analysis after staining by NADPH-diaphorase. Present of nitrergic neurons were visualized as intensely stained structure with processes. **Results:** In microscopy image of control rats were intensely darkly blue stained bipolar neurons. Their dendrites have an average length 24.5 of microns. The experimental rats after prenatal administration of retinoic acid have also intensely coloration bipolar neurons and an average length of dendrites was 23.9 microns. We found the presence of bipolar cells and multipolar neurons were not present. **Conclusions:** The results are confirmed by the neurotransmitter nitric oxide utilization in these types of neurons but we conclude that retinoic acid administered prenatally at E14-16 probably does not affect the morphology of nitrergic neurons in the prefrontal cortex of the 7 days old rats. Supported by Grant VEGA 1/0154/11

COMPARISON OF SUBCUTANEOUS ADIPOSE TISSUE MORPHOLOGY USING THE METHODS OF CLASSICAL EXCISION AND FINE NEEDLE BIOPSY

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Objective: Characterization of selected morphological features of subcutaneous and visceral adipose tissue in pathologically obese patients is part of our long-term research on the role of tissue macrophages and, morphometric analysis of adipocytes. The aim of the present study was to determine whether subcutaneous adipose tissue is morphologically altered by thin biopsy needle insertion in comparison with the classical excision method. This information was needed for

ensuring valid and comparable research data. Methods: Adipose tissue biopsy was obtained from patient subcutaneous tissue before sleeve resection of the stomach, by fine needle biopsy (needle Medax or Cook Quick-Core® Biopsy Needle) and using the classic tissue excision method. The material was fixed in neutral formalin and processed within 24 hours in paraffin blocks. 5µm sections were stained by hematoxylin-eosin. Results: The biggest difference between the materials obtained via the two methods logically related to the amount of tissue available for evaluation. Classical excision provides an area of around 100 mm² of tissue. In needle biopsy the sample was 20 times smaller (around 5 mm²). The quality of the sections was, however, comparable. A smaller sample area somewhat limits our ability to analyse the basic structure of all parts of the tissue (thickness and arrangement of the connective septa/morphology of the larger blood vessels) but it provides enough adipose tissue for evaluating any changes in size of adipocytes, response of macrophages and/or presence of preadipocytes. We performed morphometric evaluation of the average size of adipocytes and found values: 82 µm (excision), 87 µm (Cook) and 83 µm (Medax) confirming that the method of collection does not affect the size of fat cells. Conclusion: Data from specimens obtained by the classical excision method and by fine needle biopsy are equivalent and comparable. The study was supported by Grant of the Czech Ministry of Education donated by University of Ostrava with the number SGS27/LF/2013.

A FEW HISTORIC REMARKS REGARDING THE QUESTION OF PAPILOMATOSIS

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Papillomatosis and warts are mentioned in ancient epics, such as the Kalevala. They are also mentioned in basic religious scripts: the Bible, Babylon's Talmud, the Koran and several Buddhist scripts. Genital warts were already known during classical antiquity, as many Greek and Roman authors refer to them. Early history of the disease is reviewed by Bafverstedt and Oriol. The authors describe that certain synonyms, such as the words "FIG" or "CONDYLOMA" are still used in present times. Record of the disease in the middle ages did not significantly differ than in ancient times, but the term wart was used to describe characteristic known genital lesions. Breakout of syphilis in end of the 15th century drew more attention to genital disease. Most of which at that time were referred to as "venereal poison". Causal distinctions between the diseases known today as syphilis, gonorrhoea or genital warts were not defined. In the 18th century, Hunter presents an exact description of genital warts as a manifestation of syphilis. He does not distinguish them from *condylomata lata*. The first note of genital warts as a separate disease entity was by Bell in the end of the 18th century. His observations were further supported in the 19th century by Jourdan and Ricord. As the idea

that genital warts were a sign of syphilis was slowly abandoned, another false opinion emerged; namely that they are related to gonorrhoea. They were referred to as gonorrhoeal warts throughout most of the 19th century. Meanwhile, Martin describes that many of the patients suffering from genital warts do not show signs nor history of gonorrhoea. After the isolation of gonococci in 1879, it is determined that about half of the patients do not have gonococci. The assumption that genital warts are induced by cutaneous irritation from detritus, smegma and genital discharge prevail, as the theory of nonspecific irritation becomes widely accepted in the professional community for many years. The theory was still strongly supported by Cronquist in the early 20th century. The idea that genital warts could be related to those on the skin elsewhere in the body was first noted in 1893 by Gemy, driven by their histological resemblance. Even though, certain histological differences between the two are present, histological similarity between warts in the genital area and elsewhere in the skin does not demonstrate an identical aetiological agent. Several pieces of evidence support the close relation between warts in the genital region and elsewhere on the skin. Clinical evidence described by authors in the late 19th and early 20th centuries is the fact that patients with genital warts present also with warts on different areas of the skin. The very identification of the human papilloma virus was by Richard Shope in 1930. From there on, history of this entity is generally well known and widely accessible in several publications.

LIQUOR-NERVE BARRIER PERMEABILITY IN THE SPINAL NERVE ROOTS AND DORSAL ROOT GANGLIA INDICATED BY FLUORESCENT-CONJUGATED DEXTRAN

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Diffusion of the molecules from liquor to the spinal nerve roots (SNR) and dorsal root ganglia (DRG) is one of the possible ways how is the neuroinflammation spread to the primary sensory neurons. The aim of our study was to demonstrate the diffusion capacity of fluorescent-conjugated dextran (FluoroRuby, FR; FluoroEmerald, FE) from liquor in subarachnoid space to DRG and SNR of cervical and lumbar segments. Ten Wistar rats (male, 250-300g) were used in our experiments after anesthesia with a mixture of ketamine and xylazine. In the first group of animals (n=5), FR or FE was applied intrathecally to the cisterna magna and to the cisterna lumbalis in the second group of animals (n=5). After 18-24 hrs, the rats were deeply anaesthetized, perfused transcardially by Zamboni's fixative and DRG together with their SNR were removed. Distribution of FR and FE was observed on cryostat sections and simultaneously the immunohistochemical reactions for resident (ED2) and activated (ED1) macrophages, satellite glial cells (GFAP, GS), proliferating cells (Ki-67), antigen presenting cells (MHC-II) and chemokine MCP-1 were performed. Strong red or green fluorescence present in SNR and DRG demonstrated diffusion of fluorescent-conjugated dextran from liquor. Fluorescence of FR or FE was localized in the satellite glial cells and neurons

of DRG at the level of their application. The intensity of dextran fluorescence was higher in cervical DRG after lumbar application of FR/FE than in lumbar DRG after cervical application. In addition, diffusion of FR/FE from intrathecal space into DRG caused strong immune reaction presented by ED1+ macrophages and increased immunofluorescence for MHC-II a MCP-1. The dextran particles were localized in interstitial spaces of SNR, but in comparison with DRG the fluorescence intensity was significantly lower. Our results showed an easy diffusion of molecules from liquor to DRG and SNR. This indicates that neuroinflammatory mediators can spread by the road of intrathecal space from the point of origin to remote DRG and SNR. Supported by MUNI/A/0846/2012.

HISTOMORPHOLOGICAL STUDY ON PRENATAL DEVELOPMENT OF THYMUS

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Introduction: The thymus is a primary lymphoid organ derived from pharyngeal region. Development of this organ is a series of epithelial/mesenchymal inductive interactions between neural crest-derived arch mesenchyme and pouch endoderm. The Thymus is located in adulthood behind the sternum and in front of the heart. The function of the Thymus is to produce and "educate" T-lymphocytes (Tcells). These cells are critical to the adaptive immune system. Methods: Our findings are based on study of 18 human embryos from 5th to 8th week of development and 26 human fetuses from 15th to 25th week of development. Paraffin sections were stained with hematoxylin, eosin, Masson's trichrome staining and combination Periodic Accid Schiff stain (PAS)-reaction. Histological examination was performed by light microscope LEICA DM2500. Results: We described the changes in the internal structure as well as the changes in location of this organ in the body during development. We photographically documented morphological changes in thymus and migration from area of pharyngeal pouches to its final position – anterior to the heart and posterior to the sternum, visualised by the light microscopy. In this work we describe the development of the first thymic primordia, a gradual increase in proliferation and cell growth, and initiation of thymic cortex and medulla differentiation. Furthermore, we observed rapid growth of the thymus in the fetal period, growth and expansion of cortex at the expense of medulla associated with constriction of interpseudo-lobular septation. These findings were confirmed by morphometric measurements of lobules and connective tissue septa. We observed the presence of the Hassall's bodies in the thymic medulla, their size, shape and frequency of occurrence. Conclusions: This morphological study offers an overview of normal development of thymus. *This study was supported by the VEGA Grant of Slovak Ministry of Education No. 1/0902/11 (Thymus in human ontogenesis).*

DIFFERENCES BETWEEN ARTERIAL AND VENOUS CORONA MORTIS

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Introduction: Arterial corona mortis does not correspond to the classical description stating that it is a communication between the *ramus pubicus arteriae obturatoriae* et *ramus pubicus arterie epigastricae inferioris*. It is a vessel crossing the *ramus superior ossis pubis* and entering the *canalis obturatorius*. It should be considered either as the *arteria obturatoria aberrans* (if replacing the usual *arteria obturatoria*) or as the *arteria obturatoria accessoria aberrans* (if merging with the usual *arteria obturatoria*). The venous corona can be present in three forms – aberrant, aberrant accessory or as a communication with *vv. pubicae*. Material and method: Study of 450 specimens of dissection and section cadavers was performed. Results: The incidence of arterial corona mortis was 25% in total. The *arteria obturatoria aberrans* was present in 20% of cases, the *arteria obturatoria accessoria aberrans* in 5% of cases. The incidence of venous corona mortis was 91 % in total. The *vena obturatoria accessoria aberrans* was present in 83% of cases. The *vena obturatoria aberrans* and *vena obturatoria accessoria aberrans* was a direct tributary of the *vena iliaca externa* in 10% of cases. The corona mortis traversed the *ramus superior ossis pubis* at about 55 mm in average, measured from the midpoint of the *symphysis pubica* (spanning from 20 to 90 mm). Conclusion: The clinical relevance of corona mortis consists in the danger of bleeding in certain nosological and therapeutical units, such as fractures of pubic bone and their surgical treatment, hernia repair, prostatectomy, angiographic embolization or harvesting of an arterial graft. This work was supported by the Grant: PRVOUK 38.

HUMAN GLOTTIS - RECONSTRUCTION BASED ON MICRODISSECTION AND MRI+CT EXAMINATION

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The modified high resolved microMRI and CT examinations of the intact and slightly prefixed microdissected human glottal apparatus helps us to measure a spatial changes of the three strip-like layered vocal fold, where lamina propria can be described as the compressible mass of collagen bundles and intercellular matrix. It is known that even

very small lateral, ventral or rotating shifts of the arythenoid cartilage during phonatory movements result in changes of the spatial arrangement of the collagenous pattern in that fold. That pattern is recognized as one of important physical values characterizing tone and timbre of voice. At our study, the 3D digital model of the human larynx was created giving us the detailed view on the epithelial cover, lamina propria and muscular strips of the vocal folds (the binary data yielding voxels with side lengths of 0.110 x 0.120 x 0.300 mm). Thus, the vibration of that folds can be mapped with high resolution. Using the DICOM data collection (mostly based on microMRI examinations of the dissected human larynx) was adapted to create an artificial 3D digital model, where the changes in elasticity of the vocal structures can be simulated. Supported by GACR P101/12/1306 (Biomechanic modelling of human voice – way to 3D artificial vocal folds)

ABBOT SEKA SKULL – POSTMORTAL OR MALADY CHANGES

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It is not too much known about etiology and mechanisms resulting in a brushite occurrence on the cadaverous bones, examined hundred years after death. In our case the body of Ferdinand Seka, abbot of the premonstrate monastery, was morphologically examined, to determine processes resulting in long time lasted postmortal, eventually premortal body changes. The well-kept Seka's skeleton was found inside metal coffin placed in well closed and brick lined crypt where humidity and temperature were stable for tens years. His bones were only partially covered by remnants of clothing and soft tissue. No adipocire or continuing putrid processes were observed; on the other side the top of skull, facial skeleton, both tibiae, iliac crests and ventral surface of vertebrae in the abdominal area, all of them soft tissue-free, were covered by the thick layer of the brushite crystals. No signs of the congenital malformations were found, skull trajectories as well as the sutures and bone crests and lines were recognized continuous; TMJ gap was clearly seen. All cervical, lower thoracic and first three lumbar vertebral bodies were flattened, mutually connected together by osseous bridges. The left supernumerary floating rib was firmly fused with last thoracic vertebra. The high resolved CT examination showed a numerous shallowed depressions on the squamous bones of the skull cap and deformed vertebral bodies. The margins of these defects were etched by brushite crystals, while disorganized diploe undermining them deeply to the bone mass. These findings are in high contrast to numerous, relatively sharply bordered and round pathologic defects following tumorous metastases (e.g. myelomas). It can be assumed that defects can be caused more due to growth of brushite necrogenic crystals than ones developed from salts crystallizing out of body. Supported by GACR P101/12/1306 (in part)

SYSTEMIC OR REGIONAL ANATOMY FOR DENTAL STUDENTS?

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Introduction: With the reduction of time devoted to teaching anatomy, there is a need to manage learning of head and neck structures for dental students in a detailed way. Anatomy of the rest of body should be taught as well, in reduced manner. There is a question if students can handle better learning of systemic or regional anatomy. Methods: Two groups of students underwent learning of head and neck structures, one by the only systemic and other by combined systemic and regional approach. A new study material was prepared for dental students from regions of the trunk and limbs. Anatomy of limbs and pelvis was reduced the most, as well as the walls of the trunk. Anatomy of organs and systems of the trunk. There were created small books of anatomy structures which serve as a guide for students in the dissecting room. Results: The time originally allocated to anatomy teaching for dental students was cut in 1/3 of previous teaching. Students of both groups began to study anatomy by learning structures of the skull and systemic anatomy of the head and neck in a detailed way – joints, muscles, vessels and nerves. Following, the anatomy of the central nervous system, trunk and limbs was taught in a reduced manner. This way of learning was completed for one group of students, while the other group of students learned regional anatomy of head and neck areas additionally instead of originally taught structures of the limbs and trunk. These students showed better understanding of anatomy, important for their clinical practice. Conclusions: Teaching of systemic anatomy of head and neck structures in dental students in the beginning was beneficial when accomplished by following regional anatomy teaching at the end of course. This study was supported by the Grant KEGA 006UPJS-4/2011.

INFLAMMATORY REACTIONS IN THE DORSAL ROOT GANGLIA OF RATS WITH PACLITAXEL-INDUCED NEUROPATHIC PAIN

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¹*Department of Anatomy, Medical Faculty, Masaryk University.* ²*Central European Institute of Technology (CEITEC) Masaryk University. Brno, Czech Republic.* Paclitaxel, chemotherapeutic agents used for the treatment of solid tumors, results in many patients in peripheral neuropathy often accompanied with neuropathic pain. Mechanisms responsible for the development of neuropathic pain are still not well understood. The aim of present study was investigate the cellular and molecular changes in dorsal root ganglion (DRG) of rat model of paclitaxel-induced neuropathic pain. Six rats were treated with cumulative dosage of Paclitaxel 2mg/kg and survived for 3 and 7 days from last administration. Three naive rats served as control. The cryostat sections through cervical and lumbar DRG was incubated under the same conditions with antibodies against: GS (satellite gliall cells, SGC), GFAP (activated SGC), ED-1 (activated macrophages), Ki67 (proliferating cells), TNF α and IL-6 and

their receptors (pro-inflammatory cytokines), SOCS3 (suppressor of cytokine signalling), STAT3 (transcription activator), TLR4 (membrane receptor implicated in induction of neuropathic pain) and COX2 (enzyme implicated in inflammation). Only small weak of GFAP positive satellite cells were observed in DRG of naive rats, but their number was markedly increased in animals surviving 3 and especially 7 days. Double immunofluorescence staining with Ki67 and GS showed only scattered proliferating cells including a small number of SGC. The activation of SGC was associated with expression of TNF α and IL-6 and their receptors in neurons of the small and medium size as in hypertrophic SGC around large neurons. In comparison to naive rats, the ED-1 and SOCS3 immunostaining was decreased whereas the STAT3 was increased in DRG of Paclitaxel treated rats. No significant increase TLR4 and COX2 was also observed in comparison with naive DRG. Our results provide evidence on inflammatory reactions in DRG of Paclitaxel-treated rats that differ from neuropathic pain models based on nerve injury. Supported by the project CEITEC (CZ.1.05/1.1.00/02.0068)

MECHANISMS ASSOCIATED WITH INDUCED NEURONAL DEATH AND ISCHEMIC TOLERANCE

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Transient forebrain or global ischemia induces neuronal death in vulnerable CA1 pyramidal neurons with many features. Because clinical trials of pharmacological neuroprotective strategies in stroke have been disappointing, attention has turned to the brain's own endogenous strategies for neuroprotection. A brief period of ischemia, *i.e.*, ischemic preconditioning can afford robust protection of CA1 neurons against ischemic challenge. The molecular mechanisms underlying ischemic tolerance are not fully understood. Therefore, we investigated the effect of ischemic preconditioning on neural cell apoptosis in rats. Intracellular MAPK signalling pathway is presumed in the mechanisms of ischemic damage/protection. ERK protein is part of this cascade leading to survival of neurons after injury. However, high levels of p38 protein are associated with cell death. We have studied neurodegeneration as well as post-translation changes in MAPK pathways after global IR injury in rat hippocampus. Global forebrain ischemia was induced by 4-vessels occlusion. Rats were preconditioned by 5 min of sub-lethal ischemia and 2 days later, 15 min of ischemia with reperfusion period of 1h, 3h, 24h and 72h was induced. The result showed that ischemic preconditioning may attenuate the neural cell death

and DNA fragment in the hippocampal CA1 region. We demonstrated occurrence of survival of selectively vulnerable neurons after induced tolerance. Further western blot study suggested that ischemic preconditioning down-regulates the p-p38 protein, a well known marker of apoptosis and up-regulates p-ERK protein, which is associated with survival of neural cells. These findings suggest that ischemic preconditioning have a neuroprotective role on global brain ischemia in rats through the same effect on inhibition of apoptosis. Supported by VEGA 213/12, 1/0050/11 and by project "identification of novel marker in diagnostic panel of neurological diseases" co-financed from EU sources and European Regional Development Fund.

BLOOD SUPPLY OF THE SUPRAMESOCOLIC PART

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Introduction: The supramesocolic region is the superior compartment of the peritonealcavity, it lies between diaphragm and transverse colon. The aim of this project is to study the main topographical, structural, functional and pathological parts of blood vessels of the supramesocolic region. The high importance of this study is the understanding of the blood circulation of important organs of the human body and the correlation of the variations of these blood vessels with severe pathological situations. Methods: We used two male and two female cadavers from the anatomy department. Results: The root of all the arteries that supply the organs of the supramesocolic space is the abdominal aorta. The first unpaired visceral branch of the abdominal aorta is the celiac trunk which gives off three branches: the left gastric artery, the splenic and the common hepatic. The hepatic portal vein and the inferior vena cava are the biggest veins of the supramesocolic area into which all the smaller veins of the area drain. The portal vein after metabolic procedure in the liver, drains into the inferior vena cava via the hepatic veins. The portal vein is formed from the union of the superior mesenteric and splenic veins at the level between L1 and L2. Small branches which drain directly to portal vein are right and left gastric veins. In the right and the left gastric veins, also drain some esophageal veins. Other veins which drain directly into portal veins are: cystic veins, prepyloric and paraumbilical vein (which communicate with subcutaneous veins of the abdominal wall). In specific regions of the body, the drainage area of hepatic portal vein communicates with the inferior and superior venaecavae. The regions of these portocaval anastomoses related to the supramesocolic region are: the esophagus and the abdominal wall. Conclusion: The study of the anatomy and the variations of blood vessels of supramesocolic area is useful for planning and conduct of surgical and radiological procedures of the upper abdomen.

INFLAMMATORY CELLS IN HUMAN ATRIAL SAMPLES FROM PATIENTS WITH ATRIAL FIBRILLATION

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Introduction: The processes underlying the initiation and development of atrial fibrillation (AF) are still not sufficiently explored. In this study we focused on morphological and functional changes in endomysium of atrial myocardium with a special attention to inflammatory infiltration. **Methods:** We analyzed atrial biopsies obtained from patients undergoing bypass or mitral valve surgery. The patients had a regular sinus rhythm or were suffering from atrial fibrillation. The atrial samples were fixed with 4% paraformaldehyde and embedded into paraffin. For visualization of different types of leukocytes the following markers were detected immunohistochemically: CD45 as a pan-leukocyte marker, CD3 for T-lymphocytes, CD68 for monocyte/macrophages and mast cell tryptase for mast cells. **Results and Conclusion:** In all atrial samples from both groups examined in the pilot study we detected CD45+ cells in atrial myocardium as well as in endocardium and epicardium. Apparently, CD45+ cells formed a heterogeneous group of cells. We also detected CD68+ cells, CD3+ cells and mast cells. A dual immunostaining showed CD45+/CD3+ and CD45+/CD68+ double positive cells. There were also CD45+ and CD68+ cells with elongated shape sending out long cellular processes, which may correspond to activated macrophages and/or dendritic cells. We performed a semiquantitative analysis of these cells and found that they are more frequent in auricular samples from patients with atrial fibrillation. The difference reached statistical significance in the right atrial samples. Our results document that CD45+ cells are a heterogeneous cell population in atrial myocardium from patients undergoing open heart surgery and that these cells can be detected regardless of the heart rhythm. The finding of higher frequency of CD45+ leukocytes with elongated processes in the AF samples suggests the activation of inflammatory cells in this arrhythmia. This work was supported by the Research Program of Charles University – PRVOUK P25/LF1/2.

PONTICULUS POSTICUS

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The ponticulus posticus is a common anatomical variation of the atlas, the first cervical vertebra. It is the product of the complete or incomplete ossification of the posterior atlanto-occipital membrane over the vertebral artery groove resulting in the formation of a foramen (arcuate foramen) containing the vertebral artery and the posterior branch of the C-1 spinal nerve.

The vertebral artery exits from the transverse foramen, passes over the posterior arch of the atlas and forming a groove called the sulcus of the vertebral artery. This sulcus is situated on the posterolateral margin of the posterior arch of the atlas. The posterior occipital ligament extends from the posterior arch of the atlas, is connected to the posterior margin of the foramen magnum. This ligament is a broad but thin membranous sheet blending with the dura. Its lateral parts are known as the atlanto-occipital ligaments. These ligaments are incomplete at their inferior margins, affording with the sulcus of the vertebral artery an opening for the passage of the vertebral artery and suboccipital nerve. At times an anomalous ossification center occurs in the ligament and bridges the sulcus. This bony arch - the ponticulus posticus - encloses the foramen and through this the suboccipital nerve and vertebral artery pass as they course over the upper surface of the first vertebra. The clinical significance of the ponticulus posticus is far from clear. It has been associated with vertebro-basilar insufficiency symptoms, various types of headaches, hearing loss, Barré-Lieou syndrome, photophobia and migraine. However, little epidemiologic evidence for this exists. Literature Koutsouraki E, Avdelidi E., et al. Kimmerle's anomaly as a possible causative factor of chronic tension-type headaches and neurosensory hearing loss: case report and literature review. *Int J Neurosci*. 2010 Mar;120(3):236-9 Wight S., Osborne N., Breen A.C. Incidence of ponticulus posterior of the atlas in migraine and cervicogenic headache. *J Manipulative Physiol Ther*. 1999;22(1):15-20.

CLINICAL ANATOMY OF THE ROOTS OF CRANIAL PARASYMPATHETIC GANGLIA

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Introduction: As the quality, depth, and effectiveness of medical education improves, new methods become available for the teaching of anatomy; the use of more sophisticated models, computer generated images, and interactive computer programs. Though there are arguments to the contrary, the strong consensus among anatomical academics appears to be that dissection courses remain as central tools for teaching macroscopic anatomy. Dissection lays a firm foundation for the development of medical language and provides a platform for developing 3-D anatomical knowledge. Clinical anatomical knowledge is fundamentally important to the study and practice of medicine. However, the level of appropriate anatomical knowledge necessary for general and special medical and surgical practice is still under discussion. Cadaveric studies can contribute to the refinement of the indications for cranial surgery and better delineated the relevant anatomy. **Methods:** The extended transcranial, lateral transmandibular, and trans-

sphenoidal approaches provided wide access to the middle cranial fossa, orbit, infratemporal fossa, and also the nearby situated pterygopalatine fossa. Soft and hard tissues were removed using standard methods. High resolution photographic techniques are required to demonstrate the topography of the neurovascular, muscular and other structures located in the examined areas. The topography of the examined roots of parasympathetic ganglia was photographically documented. Results: The detailed photodocumentation of this study provides a view of structures until now only partially documented. Three parasympathetic ganglia are located in hardly accessible areas of the head – inside the orbit, infratemporal fossa, and in the pterygopalatine fossa. No detailed photographs have been found in current anatomical textbooks and atlases in relation to the morphology of fibers (roots) connected to the ciliary, otic, and pterygopalatine ganglia. Conclusions: This study focused on the detailed display of sensory, sympathetic, and parasympathetic roots of ganglia to provide relevant photodocumentation and an improvement in human anatomy teaching. This study also confirms that cadaver dissection provides an excellent opportunity for the integration of anatomy and clinical medicine into the early clinical training of undergraduate dental and medical students. Supported by Grant KEGA 3/7291/09 and 006UPJS-4/2011.

TUMOR NECROSIS FACTOR ALPHA AND ITS RECEPTORS UPREGULATION IN DORSAL ROOT GANGLIA OF RHEUMATOID ARTHRITIS MODEL

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Introduction: Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory disease that affects approximately 1% of the population. Tumor necrosis factor α (TNF α), that are also important in pathogenesis of RA, binds to two receptors, the type 1 TNF receptor (p55, TNFR1) and the type 2 TNF receptor (p75, TNFR2), that are expressed on many types of the cells. The aim of our present study was to compare a distribution of TNF α and its receptors, TNFR1 and TNFR2, in the lumbar dorsal root ganglia (DRGs) and changes of their amount in time. Methods: We used a rat model of unilateral antigen-induced arthritis (AIA). For induction of AIA, rats (n=14) were immunized by an intra-articular injection of antigen [500 μ g methylated bovine serum albumin (m-BSA)] in saline emulsified with 500 μ l of complete Freund's adjuvant (FCA) into the knee joint. The knee joints of control rats were injected by 500 μ l of saline and the naïve rats were without AIA induction and injection of saline. All animals were left to survive for 1, 2, 4, 14 and 21 days. TNF α , TNFR1 and TNFR2 proteins were investigated in L3 and L5 DRGs by immunostaining and Western blot analysis. Results: Elevated levels of

TNF α , TNFR1 and TNFR2 were found bilaterally in L3 and L5 DRGs in comparison with naïve and control rats. One day after induction of AIA, we observed robust bilaterally increase of TNF α and its receptors in both investigated ganglia. In contrast, from 2 to 14 days after immunization we found almost similar levels of proteins in the all observed ganglia when compared to those of control and naïve rats. Twenty one days after AIA, we observed bilateral increase of TNF α , TNFR1 and TNFR2. Conclusion: The present results have shown that AIA is associated with a bilateral elevation of TNF α and its receptors in DRGs on 1. and 21.days are linked to relapse RA. Acknowledgment: Supported by the project CEITEC (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

CONGENITAL ANOMALIES OF THE UPPER LIMB

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After the disaster in Černobyl's nuclear power plant number of developmental anomalies and cancer disease in Belarus exploded. The ionizing radiation causes simple and double DNA ruptures and nascent radicals lead to chromosome aberrations and mutations (Gazjuk et al. 1997). Because of the higher sensitivity of male sex fetus during the fetal development, increased number of injured fetus spontaneous miscarriages was expected in this group. We examined a young man with the multiple congenital anomalies of the right upper limb. Gravidity of his mother has begun in April of 1986 - two months after the explosion in Černobyl's nuclear power plant. Mother and pediatricist expected the influence of radiation on the fetal development. They considered the congenital anomalies of the right upper limb as the sequel of the radiation. We established the developmental anomalies of the bones and muscles of the right upper limb. Initial physical examination showed: agenesis of the right pectoralis major and minor muscles, hypoplasia of the supraspinatus and infraspinatus muscle, hypoplasia of the bones and muscles of the forearm and hypoplasia and brachysyndactyly of the fingers. These anomalies were compatible with the diagnosis of Poland syndrome. We report the case of a young man with the multiple congenital anomalies of the right upper limb located on the right side fingers. These anomalies were compatible with the Poland syndrome diagnosis. Classical Poland Syndrome is characterized by unilateral, partial or complete absence of the sternocostal head of the major pectoral muscle and brachysyndactyly of the fingers on the same side. The incidence is 1:30 000, with a higher frequency among males (Supulveda 2009). In 75% of the unilateral cases it is located in the right hemithorax (Ferraro, Perrotta et Rossano 2005).

IMMUNOLocalISATION OF INFLAMMATORY MEDIATORS IN ARDS

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Acute respiratory distress syndrome with its high incidence rate is common condition in patients in intensive care unit. With its high mortality rate (32-45%) it is still challenge for critical care medicine. Behind clinical appearance of ARDS patient pathophysiological and histopathological changes in lungs are in progress. Diffuse alveolar damage is associated with ARDS, pulmonary microvascular injury and inflammatory cell populations within lung tissue. In second stage (exudative) stage of ARDS various cell populations are localised within lung tissue compromised also many cells of inflammatory origin. The purpose of this study was to evaluate the histopathological alterations and to determine the role inflammatory cells in exudative phase of ARDS via detection of cell positivity for inflammatory markers. The necrotic lung sample of patient suffering from car accident with developed ARDS were harvested immediately fixated in 4% paraformaldehyde and embedded in Paraplast wax, sectioned (4-5µm) and stained with H&E, and by immunohistochemical means using anti-MPO, anti-VEGF and anti-CD163 marker. Myeloperoxidase (MPO) immunoreaction was present in cytoplasm of neutrophil granulocytes. Their localisation was predominant within lumen of alveoli and in small numbers also in interstitial tissue. Sporadically alveolar macrophages were also positive for MPO as brown cytoplasmic stain. MPO expression was very intense in PMNL filling every alveolar space, which indicates high stage of inflammatory response. Expression of vascular endothelial growth factor (VEGF) was localized mostly in secretory granules of PMNL within lumen of lung alveoli. Also focally macrophages were positive for VEGF, but immunoreaction was absent in large alveolar macrophages. Endothelial positivity for VEGF was absent. CD163 expression was cytoplasmic positivity in cells of monocyte-macrophages system. Their predominant localization was in lumen of alveoli and only small amount of these cells were in interalveolar septa. Our findings indicate that various inflammatory cells within ARDS are different in expression of inflammatory mediators.

ACUTE JEJUNAL DAMAGE AFTER SMALL INTESTINE ISCHEMIA/REPERFUSION INJURY

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Ischemic-reperfusion (IR) injury of small intestine is a serious complication of several surgical procedures with devastating injury to small intestine and distant organs. Small intestine as primarily injured organ is open to ischemic as well as reperfusion injury. In

reperfusion period very rapid changes occur in jejunal tissue which includes inflammatory and proliferatory changes as well. The aim of our study was to determinate expression of MPO and PCNA in jejunal tissue. Male Wistar rats (n=18) were divided into 2 groups: ischemia-reperfusion group (IR, n=12) and control group without ischemic insult (C, n=6). In both experimental groups ischemia of cranial mesenteric artery was performed in duration of 60 minutes and after reperfusion period was followed (IR1, IR24). Histopathological damage of small intestine was determined by evaluation of histopatological injury (Park-Chiu score), immunohistochemical (anti-PCNA, anti-MPO). MPO expression was an cytoplasmic expression of cell localized within lamina propria mucosae. Expression was significantly increased ($p<0.001$) after one hour of reperfusion compare to control group. After 24 hours after reperfusion number of MPO positive cells was decreased compare to IR1, but incidence was still higher than in control group. Proliferating cell nuclear antigen (PCNA) expression is nuclear expression of proliferating/repairative cells. Expression of PCNA was identified by strong nuclear positivity in jejunal crypts stem cells. PCNA-positivity in epithelial layer in IR1 group was increased, almost twice compare to control group. After 24 hours of reperfusion, difference between IR24 and control group decreased. Expression of PCNA in lamina propria mucosae was decreased in IR1 group comparing to control group. After 24 hours of reperfusion PCNA expression was comparable to control group. Results of our work point out on rapid changes in inflammatory cell population and acute proliferative changes in small intestine after IR injury. Supported by APVV-0252-07, CEMIO-ITMS-26220 120058.

IMMUNOLocalISATION OF SUPPORTING AND CONTRACTILE CELLS AND FIBERS IN THE DOG PROSTATE

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Although, the mechanisms of prostate cancer development and progression have been extensively studied this process is not fully understood. The aim of the study was to localize the main components of stroma and secretory epithelium of normal dog prostate gland. For this purpose the immunohistochemical staining and the monoclonal antibodies directed against cytokeratin 18 (CK 18), smooth muscle actin (α -SMA), desmin, and elastin were used. The expression of selected intermediate filaments and elastin was found in lining epithelium, smooth muscle cells and elastic fibers. α -SMA and desmin have been observed in smooth muscle cells (SMC) localized in the outer zone of the capsule. Elastic fibers in this capsule form dense elastic membranes. Smooth muscle cells positive for α -SMA and desmin and elastic fibers in the form of dense meshwork were seen in thick stromal septa. Individual smooth muscle cells strongly stained with α -SMA and desmin were present between the secretory alveoli.

Elastic fibers in this space were seen to support the secretory alveolar epithelium. Around ducts which enter the urethra an accumulation of elastic fibers was observed. Pericytes and smooth muscle cells of blood vessels positive for α -SMA and desmin were present in connective tissue stroma in connection with blood capillary network. The adventitia of larger blood vessels present in large septa was abundant in elastic fibers. Strong positive reaction for CK 18 was present in the secretory cells and in cells of ducts. Supported by National Reference Laboratory for pesticides UVLP.

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHANGES IN THE PERITUBULAR TESTICULAR TISSUE OF RAT TESTIS

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In humans and other mammals, cadmium cause various damages to different organs depending on the dose, route, ways, and duration of exposure. Cd adversely affects a number of organs and tissues of the body, including lung, pancreas, testis, placenta, and bone, with kidney and liver being the two primary target organs. The aim of this work was to study histological and immunohistochemical changes in the peritubular testicular tissue of rat testis after per oral administration of cadmium chloride. After 5 days cadmium exposure an advanced change in the boundary testicular tissue were observed. Dissarrangement of collagen fibres and peritubular cells, dilatation of peritubular space and oedema, as well as damage of blood vessels with thrombosis were observed. Changes in the boundary tissue were accompanied with the desquamation of the germinal epithelium. Immunohistochemically, positive reaction for α -smooth muscle actin and desmin was present in tunica media of larger testicular blood vessels. No positive reaction for vimentin was seen in endothelial cells of blood capillaries, but this was seen in large blood vessels. The myofibroblasts forming single incomplete layer in the lamina propria of seminiferous tubules were positive for desmin and α -smooth muscle actin. Vimentin reactivity in the myofibroblasts and in the supporting Sertoli cells as well as Leydig cells in damaged testicular tissue was not observed. An increase in fibroblasts and free inflammatory cells positive for vimentin was observed in the peritubular space concentrated mainly on the periphery of the testis. Supported by National Reference Laboratory for pesticides UVLP.

ANATOMICAL VARIABILITIES OF FORMATION AND COURSE OF MUSCULOCUTANEOUS NERVE

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Introduction: This work was aimed in elucidating of the anatomical variations of the musculocutaneous nerve formation and course and their possible impact on the

clinical picture of its innervation disorders. Musculocutaneous nerve is one of the terminal branches of the brachial plexus, providing the motor innervation to the anterior brachial muscles and the sensitive innervation to the lateral aspect of the forearm. Method: Bilateral musculocutaneous nerve course and formation was studied on the fifty adult cadavers- the dissection was performed on the bodies with the slightly abducted limb after the revision of the supraclavicular part of each brachial plexus. Results and conclusions: In 6 cases we have found the anastomotic branches to the median nerve. In 3 cases we observed its division even before entering the coracobrachialis muscle, in 1 case it ran parallel with the biceps brachii muscle and issued branches towards it. In observed 3 cases additional direct branches from lateral fascicle entered the biceps brachii muscle and the median nerve. In 8 cases it was missing, in 4 cases it emerged from the median nerve more distally on the shoulder. We hope that this description of the found anatomical variabilities of the musculocutaneous nerve formation and course will be useful for performing the surgical procedures in this anatomical region and to prevent surprise from the operational findings.

BRADYKININ PRECONDITIONING GUIDES THE NUMBER OF DEGENERATED NEURONS AND THE LEVEL OF ANTIOXIDANT ENZYMES AFTER SPINAL CORD ISCHEMIA

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Bradykinin (Br), a biologically active nonapeptide that mediates inflammatory response and increases vasodilation, has been used for possible acquisition of ischemic tolerance in spinal cord neurons. New Zealand white rabbits were preconditioned with Br 48 h prior to 20 min of abdominal aorta ligation followed by 24 and 48 h of reperfusion. The activity of SOD and catalase was measured and degenerated Fluoro Jade B stained neurons were statistically analysed. Tarlov scoring system was used to assess neurological outcomes of rabbit hind limbs. Analysis of Fluoro Jade B positive neurons revealed significant decrease of degenerated neurons after Br preconditioning prior to 20 min of ischemia and 24 h of reperfusion. SOD activity was significantly increased in the ischemic group *versus* control, but in both Br preconditioned groups the activity was significantly lower if compared to ischemic group. CuZn-SOD activity was significantly decreased in Br preconditioned group after 24h of reperfusion *versus* ischemic group. Significant increase of Mn-SOD activity was registered in the ischemic group and decreased activity was detected in both Br preconditioned groups *versus* ischemic group. Catalase activity showed significant increase between Br preconditioned group after 24 h of reperfusion and 48 h of reperfusion. Important significant decrease of catalase activity was found in the Br preconditioned group after 24 h of reperfusion *versus* ischemic group. Tarlov scoring system was significantly better in Br preconditioned groups where rabbits were not paraplegic. The findings of our experiments suggest

that Br administered intraperitoneally plays an important role in acquisition of ischemic tolerance in the spinal cord neurons. The results can be used for new strategies during therapeutic window in therapy of ischemic injury to CNS.

INTERACTION OF SOFT AND BONY STRUCTURES ON THE BASE OF THE SKULL

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The atlanto-occipital fusion and accompanying changes at the external base of the skull and posterior cranial fossa were the inspiration for the observation of the structures of posterior cranial fossa on the skulls without evident pathological process. Atlanto-occipital fusion with the rotation of the atlas caused the unilateral narrowing of jugular foramen with the contralateral compensatory enlargement of the opening as well as numerous subsequent changes on the internal base of the skull, particularly deepened and enlarged impressions of dural venous sinuses. The comparison with the skulls without evident pathological changes showed the occurrence of unequal size of jugular foramina and the corresponding differences of the impressions of dural venous sinuses. The right-left asymmetry of the structures in the posterior cranial fossa has been known but it has not been explained yet. Observed significant changes on the internal base of the skull at atlanto-occipital fusion indicated the interaction of vascular and skeletal structures, but mainly they raise the question of the mechanism how the bone structures changes are a consequence of the influence of the soft vascular structures.

ACTIVITY OF ANTIOXIDANT ENZYMES IN RAT TESTICLE AFTER DEPRENYL ADMINISTRATION

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Objective: Numbers of infertile couples in western countries increase and assisted reproduction techniques must be applied to solve this kind of problems. Male factor contributes to about 50% of such cases. Monoamine oxidases (MAO) inhibitors, such as deprenyl, have been proven to have therapeutic value in several neurological diseases. But there are many evidences that MAO inhibitors administered in low doses are capable to trigger production of antioxidant enzymes and in such way are able to protect many types of cells from apoptosis. The aim of this study was to evaluate administration of two deprenyl doses on activity of antioxidant enzymes such as superoxide dismutases (SOD) and catalase (CAT) in rat testicle. Material and Methods: All procedures performed with animals adhered to the permission granted by the Committee for Ethical Control of Animal

Experiments at Šafárik University and the permission of the State Veterinary and Food Administration of the Slovak Republic. 15 male Wistar rats (390g, aged 8-9 weeks) were randomly divided into 3 groups and were injected intraperitoneally daily for 30 days with either saline (control animals) or with low-dose of deprenyl (LDD, 0,0025mg/kg per day, Sigma M003) or with high-dose of deprenyl (HDD, 0,25mg/kg per day) dissolved in saline. After the last drug administration males were killed by lethal dose of thiopental and the activities of antioxidant enzymes were measured by spectrophotometric methods. Results: We have found that total SOD activity in LDD (3.13±0.32) and HDD (3.08±0.30) groups was elevated compared to controls (1.65± 0.12). The similar ratio between experimental (LDD 2.52±0.32; HDD 2.72±0.32) and control (1.33 ±0.12) groups was noted in copper/zinc superoxide dismutase (CuZn-SOD). On the other hand, activity of manganese superoxide dismutase (MnSOD) was increased in animals from LDD group only (0.62±0.08) compared to HDD (0.36±0.12) or even control (0.32±0.03). Concerning catalase activity, in both experimental groups elevated activity of enzyme (LDD 34.91±6.91; HDD 37.88±13.17) was recorded compared to control (15.62±6.01). Results are given in U/mg of protein. Conclusion: We have found that administration of deprenyl resulted in increasing of both SOD and CAT enzyme activities in rat testicle. Interestingly, high dose of deprenyl (HDD) did not cause elevation of MnSOD activity compared to 100 lower dose of the same drug. Our results show that endogenous antioxidants SOD and CAT, which play considerable protective role in mammals, increase in testicle after administration of deprenyl, a potent MAO inhibitor. This work was partially supported (30%) by the Agency of the Slovak Ministry of Education for the Structural Funds of the EU, under project ITMS: 26220120058, partially by VEGA grant 1/0928/11, partially by VEGA grant 2/0066/12, partially by VEGA grant 1/0925/11 and partially by VEGA grant 1/0224/12.

ELECTRONIC COURSES IN TUITION OF HISTOLOGY AND EMBRYOLOGY

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Aim of the project was to introduce electronic courses as a new kind of tuition in histology and embryology. The courses were prepared on a free source e-learning software platform, Moodle (Modular object-oriented dynamic learning environment) that allowed to introduce texts, images, files, books, and a wide range of interactive tools, such as video links, URL, audio recordings, quizzes, forums and offered the opportunity to put assignments online for lecturer's review. Moodle also permitted to modify and check level, date and length of the courses. As an alternative to conventional lectures, we prepared seven electronic courses that covered the topics of cytology, basic and special histology and embryology. Students adapted to the online courses easily and their evaluation was positive for several reasons, namely popularity of electronic tools and their interactivity. Moreover,

students could literally access their course material any time with a possibility for multiple revisions and practice. Our first experience confirmed that electronic courses in histology and embryology can be used as an equivalent to conventional lessons. Stimulating results outweighed laboriousness and time-consuming preparation of the courses. Therefore we plan to extend this mode of tuition to practical classes as well. Supported by project IT Medik.

MODULATION OF EVI-1 EXPRESSION IN CHICKEN EMBRYOS

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Introduction: *EVI-1* (ecotropical viral integration site 1) was first described as a common locus of retroviral integration in myeloid tumors found in AKXD mice (Mucenski et al., 1988). Cloning of chicken cDNA corresponding to this locus revealed that the *EVI-1* gene encodes a transcription factor belonging to the zinc-binding family located on chromosome 9 of the chicken genome. *EVI-1* expression, displayed by *in situ* hybridization, was localized in the brain and intermediate mesoderm at stage 13 of chicken embryos. In the head of later stages, *EVI-1* expression was situated in all pharyngeal arches with the most prominent expression in the second and the third pharyngeal arch. Furthermore, *EVI-1* was expressed adjacent to the apical ectodermal ridge. As this structure is a source of FGFs, we tested whether endogenous FGF signaling was necessary to maintain *EVI-1* expression. Material and methods: We used chicken embryos between stages 20 and 24. FGFR inhibitors SU5402 (1mg/ml) or PD161570 (1mM) were applied into the limb bud. *EVI-1* expression was analyzed by whole mount *in situ* hybridization and results were confirmed by qPCR analysis. For gain-of-function approach, we applied FGF2 (1mg/ml) soaked beads into the wing buds at stage 20. Results: Downregulation of *EVI-1* expression was observed 24 h after SU5402 treatment. As SU5402 is known to increase apoptosis in the surrounding cells, we also used another FGFR inhibitor PD161570. We observed a downregulation of *EVI-1* expression similar to SU5402 treatment. Furthermore, qPCR analyzes revealed downregulation of *EVI-1* expression at 6 h, 16 h as well as 24 h after injection, however $\Delta\Delta C_t$ values were statistically significant only at 16 h time point. When FGF2 beads were applied into wing at stage 20, *EVI-1* expression was upregulated both 16 h and 24 h after implantation but values were statistically significant only for 16 h time point. Surprisingly, the expression was downregulated after 6h, opposite to the later time points. Conclusion: Based on our results, *EVI-1* may be a novel gene mediating the effects of FGF signaling during limb development. As there is late response to FGF ligand as well as to FGFR inhibitor treatment, we expect that the effect is not direct but mediated through more complex signaling

within the mesenchyme. This project is supported by the GA CR (304/09/0725) and IPAG (RVO:67985904). Reference: Mucenski, M.L., Taylor, B.A., Ihle, J.N., Hartley, J.W., Morse, H.C., 3rd, Jenkins, N.A., Copeland, N.G., 1988. Identification of a common ecotropic viral integration site, *Evi-1*, in the DNA of AKXD murine myeloid tumors. *Molecular and cellular biology* 8, 301-308.

EIGHTH CRANIAL NERVE - CHANGES OF ITS NAME DURING THE TIME

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Introduction: the aim of the study was to create a review of the historical development of the Latin terminology of the eighth cranial nerve. Methods: the study was done in a form of a literary study of the originals of the most important anatomical textbooks from Vesalius (1543) up to the present time. Further, the terminology of the nerve was followed in the MEDLINE database, with respect to the years, in which the articles were published. Tolerancy was +1 year from the valid or recommended terminology. Results: It was found out, that during the centuries the amount of the described cranial nerves changed from the original seven (Galenos, Vesalius) through eight (Jessenius 1601) and ten (Willis 1664) up to the final twelve (Sömmering 1798). While the correct amount of the cranial nerves was stabilised before the end of the 18th century, the terminology of the eighth nerve have been changing in different forms up to the half of the 20th century. Conclusions: the final form of its name obtained the eighth nerve in the P.N.A. (1960) terminology. Supported by project of Charles University in Prague PRVOUK P38.

VASA VASORUM OF THE PORCINE CORONARY ARTERIES

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Introduction: According to the recent data, the neoangiogenesis of the vascular system supplying the walls of the human coronary arteries – vasa vasorum (VV) – seems to play very important role in the etiopathogenesis of several pathological processes (atherosclerosis, thrombosis, medionecrosis etc.). On the other hand, a detailed morphological description of the human as well as of the porcine coronary VV is still

not available. The aim of the study was an anatomical study of the architectonical arrangement of the coronary VV and of their inner structure in the normal porcine heart. Contemporarily, the results obtained should verify the validity of either classical opinion of T. Sömmering (1800), that all blood vessels approach to the vascular wall from the adventitia or of the recently valid theory of Gössl et al. (2003), affirming that some parts of the arterial system of VV originates directly from the lumina of coronary arteries and ramify in the arterial wall from its inner side. Methods: fresh hearts of ten normal pigs (120-140 kg) were used for the study. The coronary arteries were either injected (India ink, Dentacryl, Mercocox Resin) or processed for light microscopy. Results: analysis of the injected specimens as well as of the histological sections univocally demonstrated that the blood supply of the walls of the coronary arteries ensure in the whole extent only the adventitial VV, approaching the arteries from the close neighbourhood. Arterial branches of the VV system, which originate directly from the arterial lumina (so-called vasa vasorum interna after Gössl) and participate on the supply of the inner layers of the arterial wall, were not detected in any case. All parts of the VV system, e.g. arteries, capillaries and veins, are distributed only in the adventitia and in the outermost thin layers of the media of coronary arteries. The density of their branching is substantially lower than that of the previously described VV of the human great saphenous vein. Finally, a richness of valves closing the lumina of all veins of VV was described. Conclusions: it can be concluded that on the supply of the walls of porcine coronary arteries participate only the adventitial VV, while the arteries entering the arterial walls from their lumina directly were not described. The results obtained can be used in further studies, oriented on the architectonics and structure of the VV of the coronary arteries of human heart, too. Supported by project of Charles University in Prague PRVOUK P38.

CELLULAR HETEROGENEITY OF MALIGNANT BRAIN TUMOR

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Introduction: Most primary brain tumors originate from glial cells and are named gliomas. They may be classified as benign or malignant tumors. Glioblastoma multiforme, the most aggressive form all gliomas is characterized by extensive heterogeneity at the cellular and molecular level. Heterogeneity of gliomas is still poorly understood and is likely the key to understand failure of glioblastoma treatment. In addition, the cellular heterogeneity among and within gliomas contribute to diagnostic difficulties of these neoplasms. Methods: Three glioma cell lines (GL-15, 8-MG-BA, 42-MG-BA) established from glioblastoma multiforme were used for this study. The immunostaining was performed with antibodies against intermediate filament (IF) proteins. Results: These results show the co-expression of glial and non-glial IF proteins in three glioma cell. Glial fibrillary acidic protein (GFAP) is specific for astroglial cells, vimentin

for cells of mesenchymal origin and cytokeratins (CK) are characterized for epithelial cells. Immunofluorescence analysis revealed the presence of three IF proteins in all glioblastoma cell lines. High percentage of GFAP-positively stained cells and low percentage of CK-positive cells were found in GL-15 cell line. On the other hand, low number of GFAP-positively stained and higher number of CK-positively stained cells was observed in 8-MG-BA cell line. However, all cells showed strong intensity for vimentin staining in examined three glioblastoma cell lines. Conclusion: Our results demonstrate a marked heterogeneity of IF staining in all glioblastoma cell lines. Double labeling confirms the co-expression of epithelial and glial marker proteins in cultured glioma cells. These findings may have a diagnostic value in the differential diagnosis of primary and metastatic brain tumors. Supported by project: Centre of Excellence ITMS 26240120023

FUNCTIONAL MORPHOLOGY OF THE UTERINE TUBE – HISTOLOGICAL EXAMINATION OF 50 UTERINE TUBES OF WOMEN WITH DIFFERENT AGE AND WITH VARIOUS DIAGNOSES

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Introduction: The Fallopian tube has until recently been a neglected structure, bypassed by in vitro fertilization and seen only as a tube that transports the oocyte or early embryo to the uterus. More recently, its role is even more undervalued after the introduction of techniques of assisted reproduction, in which the Fallopian tubes become like unnecessary. The Fallopian tube performs several important functions. It captures the oocyte after ovulation, maintains and controls the migration of spermatozoa to the site of fertilization. It provides the special microenvironment for fertilization; nourishes the early embryo while it is being carried to the uterus and amplifies signals from embryo to the mother. Material and Methods: In our article we conducted a systematic review of relevant articles found in PubMed, Scopus and ISI Web of Knowledge, focused on the new insights into the functional morphology of Fallopian tube. This theoretical knowledge we completed with our findings which are based on the histological study of 50 uterine tubes of women with different age and various diagnoses. The specimens were fixed in formalin for 24 h, embedded in paraffin, and 5µm thick sections were used for routine histology and histochemistry (PAS reaction, acid phosphatase). Preliminary results: By light microscopy, mucosa consists of lining epithelium and lamina propria and has longitudinal folds. In the infundibulum and ampulla these folds are high and branched. In the isthmus of the uterine tube the mucosal folds are low. The uterine tube is seen to have a single layer of columnar epithelium which often appears pseudostratified because of crowding of cells of different height. The two major cell types are the

ciliated cells and non-ciliated secretory cells. Ciliated cells are most numerous in the infundibulum and ampulla and are found predominantly on the apex of the mucosal folds. They have very pale cytoplasm and large, pale, and oval nuclei located near the apexes of the cells. Non-ciliated (peg) cells are secretory cells that produce the fluid that provides nutrient-rich medium for the oocyte and preimplantation embryo as they pass through the tubal lumen. Also their secrete aid in capacitation of spermatozoa. These secretory cells have granular cytoplasm and darkly stained nuclei which usually lie at the bases of the cells. In the center of each mucosal fold we found central lymphatic vessel, similar to the lymphatic vessel located in the intestinal villi. The function of these lymphatic vessels during normal and pathological stages is unknown

MINIMUM INTERCAUDATE DISTANCE – A RELIABLE MARKER OF ENLARGED BRAIN LATERAL VENTRICLES IN WOMEN

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Aim: To evaluate the association between the size of lateral ventricles of the brain in women differing in diagnoses. **Methods:** We measured the linear minimum intercaudate distance in the group of women who were divided according to diagnoses into three subgroups (F1 – F3). Subgroup F1, controls, was made up of 132 women. Subgroup F2 comprised of 32 women after having suffered a head injury. Subgroup F3 comprised of 20 women, suffering from neurological and psychiatric diagnoses. Examinations were carried out under standardised conditions: all patients had transverse CT (Computed Tomography) scanning of the entire brain, contiguous CT scans, final slice thickness 4 mm. Measured values were detected by Osiris software. Recorded characteristics were statistically described and tested for differences. **Results:** The linear minimum intercaudate distance was found to be significantly longer in subgroup F3 than in controls (F1) with $P = 0.0002$. We found non-significant difference between controls and subgroup F2 ($P = 0.386$). **Conclusions:** The linear minimum intercaudate distance is a reliable marker in distinguishing group F1 from F3, and can be used as a reliable marker when evaluating the enlargement of brain lateral ventricles in women as well.

MONITORING OF P53 AND MDM2 IN HUMAN LUNG CANCER CELLS

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Introduction: The protein p53 induces cell growth arrest (apoptosis) in response to endo- or exogenous stimuli. P53 levels in normal cells is highly determined by MDM2 protein (murine double minute-2) - mediated degradation of p53. Mutation of TP53 (gene encodes a protein p53) is common in human malignancies and alters the conformation of p53. The result is a more stable protein which accumulates in nuclei of tumor cells with losing of function. Mutant p53 is stabilized, and by immunohistochemistry (IHC) it is possible to detect this form very clearly. As a potential marker of p53 function is expression of MDM2 protein. MDM2 overexpression represents at least one mechanism by which p53 function can be abrogated during tumorigenesis. **Patients and Methods:** Lung carcinoma samples were obtained from patients who underwent radical resection (lobectomy or pneumonectomy and lymphadectomy). The pathological diagnosis was based on WHO criteria. In our study we investigated expression of p53 and MDM2 protein that might improve IHC as a marker for p53 status. Immunohistochemistry (IHC) is a major method for observation of both proteins. Proteins were IHC detected in 136 samples of primary lung carcinoma. For IHC staining the following primary antibodies were used: anti-p53 protein (DO7) (BioGenex Laboratories Inc.) and anti-MDM2 protein (SMP14: sc-965) (Santa Cruz Biotechnology Inc.). Immunostaining results of p53 positive samples were compared to IHC expression of MDM2 positive and MDM2 negative samples. **Results:** Strong brown nuclear staining was visible in p53 and MDM2 positive cells. Only 9 samples (7%) were simultaneously p53 and MDM2 positive. In 46 (34%) cases elevation of p53 was combined with MDM2 negative expression. Other tumour samples were either negative in both proteins (71/52%), or p53 negative and MDM2 positive in 10 (7%) tumour samples. **Conclusions:** Absence of p53 staining in most studies indicates absence of p53 mutation, and on the contrary, the positive expression of p53 is a sign of p53 mutations with losing of function. In our study in 34% of cases extensively high level of p53 without increased level of MDM2 was identified. These are tumours with inactivating mutations that stabilize p53. On the other hand, tumours with high level of stabilized wild-type p53 protein and simultaneously with increased MDM2 staining (7%) represent group with functional p53. **Acknowledgments:** The work was supported by Grand VEGA 1/0224/12, 1/0925/11, 1/0928/11.

MACROSCOPICAL RELATIONS OF INTERNAL CAPSULE AND ITS CONNECTIONS

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Introduction: Although the gross anatomy of the brain has been studied by anatomists and clinicians, the intrinsic microanatomical study of the white matter is still neglected. Part of the white matter structures of the brain are association fibers and internal capsule. To the association fibers belong short fibers, superior

longitudinal fasciculus, association fibers of gyrus cinguli, perpendicular fasciculus, uncinata fasciculus, inferior longitudinal fasciculus. The internal capsule lies between the lenticular and caudate nuclei. The internal capsule ends within the cerebrum, just above the midbrain, but the axons that pass through it continue down through brain stem and spinal cord. In the horizontal section the internal capsule exhibits the shape of a hook, made up of the anterior limb, the posterior limb and the genu. Anterior limb includes frontopontine and thalamocortical fibers, the genu contain corticobulbar fibers and the posterior limb contains the corticospinal fibers, corticobulbar fibers and sensory fibers. Methods: We dissected 5 formalinated human brains by using both medial and lateral approach of dissection, and a transversal section. Results: We were able to find and describe the structure and topography of internal capsule by the transversal section of the brain. Medial side dissection revealed the association fibers of gyrus cinguli, fasciculus longitudinalis superior, corona radiata and internal capsule. In lateral side dissection we described insula, lentiform complex, part of the internal capsule, fasciculus longitudinalis superior and short U-shaped association fibers. After superficial dissection of pons and crus cerebri we found continuation of pyramidal tract from posterior limb of internal capsule to crus cerebri and finally downwards where we exposed rubrospinal tract and nigrospinal tract. Dissection from lateral aspect of hemisphere exposed the interconnections of neighboring sulci with short U-shaped association fibers. Conclusion: The dissection of the white matter of the brain and thus the tracts it is very important from educational point of view but also from clinical point of view. It is very crucial for medical students to realize and understand the 3-dimensional topography of white matter in the brain. From clinical point of view it is very important for physicians to evaluate certain imaging methods and to be able to take advantage of revolutionary methods, to be able to diagnose lesions and compressions of white matter.

ANATOMY AND CLINICAL IMPORTANCE OF THE ROTATOR CUFF

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Introduction: Upper limb is crucial for human's everyday life. Arm's movement depends upon shoulder's ability to function normally. Shoulder is the most unstable joint and with the biggest range of movements. The aim of this project is to understand the anatomy and the function of the rotator cuff as well as the study of clinical importance of anatomical variations in the area. Methods: For the project we used three male cadavers. For the model we used bones from the Department as well as silicon glue and powder paint. For the calculation of the anatomic variation of the acromion, we used 50 bones. Results: As a part of this project an anatomical model of the muscles is made so anatomy of the specific muscles

becomes clearer especially to the relation to their function. Rotator Cuff ruptures are one of the major diseases in the Western world. Most of the ruptures do not possess any symptoms. In healthy population between 60 and 69 years of age, the incidence of rotator cuff ruptures are 20% and the corresponding percentage for the population between 80 and 89 years of age is 51%. Rotator cuff ruptures can be distinguished according a. to the width of ruptured tendons, b. according to the depth of the rupture and c according to the duration as acute or chronic. There are anatomical variations that either combined or not cause the rotator cuff ruptures. They are mainly distinguished between intrinsic and extrinsic. The intrinsic factors take place in the tendons unlike extrinsic that take place outside of the tendons. The extrinsic factors include the acromion's type, the acromial slope, the lateral acromion angle, the type of the coracoacromial ligament, presence of osteophytes in the subacromial space, history of acute trauma and presence individual congenital or acquired anatomical variations leading to tendon's impingement to the coracoid process or the supraglenoid tubercle. Conclusion: All of above contribute to the development of the Impingement Syndrome. The causes of the rotator cuff tearing are not yet fully understand. Most probably a combination of extrinsic and intrinsic factors is collaborating to the tear.

IN VITRO STUDY OF LOW-GRADE ASTROCYTOMAS

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Introduction: Gliomas are a heterogeneous group of primary brain and spinal cord neoplasms. Grading of gliomas is based on their microscopic appearances of certain characteristics: atypia, mitoses, vascularity, invasive potential and necrosis. The grade indicates the level of malignancy. Astrocytomas are gliomas that arise from supporting brain cells called astrocytes. Astrocytomas are of two main types: low-grade I-II and high grade III-IV. In this study we examined morphological and cytoskeletal features of low-grade gliomas. Methods: The tissue cultures were derived from five tumor biopsies diagnosed as fibrillary astrocytomas. Immunofluorescence staining with antibodies against glial fibrillary acidic protein (GFAP) and vimentin was performed on primary and secondary cultures up to passage number 10. Results: The first attached cells were observed within 2-3 days after seeding of explants. They were long and thin processes-bearing cells positively stained for GFAP and vimentin. These cells formed confluent layer over 2-3 weeks in culture. At confluence in primary cultures they constituted more than 95%. The remaining cells were flat or spindle-shaped cells with or without poorly developed processes. These cells negatively stained for GFAP and positively for vimentin are usually named as "glia-like" cells. Subculturing of astrocytomas revealed the overgrowth by "glia-like" cells. By subsequent passaging the neoplastic astrocytes with specific morphology and immunophenotype completely

disappeared. The cultures within passage number 5 to 10 were completely comprised from flat GFAP-/vimentin + cells. Conclusion: Many permanent glioma cells line were established from high-grade glioblastoma or anaplastic astrocytoma. However, spontaneous immortalisation of low-grade gliomas is usually unsuccessful. This study demonstrates the overgrowth of neoplastic astrocytes cultures by "glia-like" cells. Supported by project: Centre of Excellence ITMS 26240120023

FIBROSIS AND MICROVASCULAR DENSITY IN HUMAN ATRIAL WALL DURING ATRIAL FIBRILLATION

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Introduction: Atrial fibrillation (AF) is one of the most common arrhythmias in the clinical practice and it is associated with an increase in mortality risk that is strongly related with old age. Its pathogenesis is still not sufficiently explored. One of the generally recognized factors contributing to the initiation and maintenance of atrial fibrillation is structural remodeling of the myocardium. **Aim:** In this project we focused on morphological and functional changes in endomysium of atrial myocardium. We focused on changes in the collagen volume fraction and microvascular density. **Methods:** We studied the morphological changes of atrial biopsies performed at 46 patients (19 patients with AF, and 27 with sinus rhythm (SR)) undergoing bypass or mitral valve surgery. The atrial samples were fixed with 4% paraformaldehyde and embedded into paraffin. Sections from atrium were histologically examined using routine hematoxylin-eosin staining. For quantification of collagen volume fraction (CVF), the sections were stained with the picrosirius staining. CVF was quantified as an area fraction of myocardial tissue section containing collagen fibers labeled with picrosirius staining. **Results:** We found variable amount of endomysial collagen in myocardial samples from both groups of patients. The CVF in the left auricle was 23.8±5.1% in patients with SR and 28.4±6.7% in patients with AF. The CVF in the left atrium was 24.2±3.1% in patients with SR and 29.3±8.8% in patients with AF. The MVD did not differ in samples from patients with SR and AF. **Conclusion:** Our document the variable level of fibrosis in atrial myocardium from patients undergoing open heart surgery. In the left atrial samples from patients with AF we observed an increased CVF. This work was supported by a grant PRVOUK – P25/LF1/2

CORRELATION OF MYHC ISOFORM mRNA AND PROTEIN LEVELS. EFFECT OF ALTERED THYROID STATUS

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We studied the expression of myosin heavy chain (MyHC) isoforms at mRNA and protein levels as well

as fiber type composition in the fast extensor digitorum longus (EDL) and slow soleus (SOL) twitch muscles of adult euthyroid (EU), hyperthyroid (TH) and hypothyroid (HY) inbred Lewis strain rats. Comparison of the results from Real Time RT-PCR, SDS-PAGE and fiber type analysis showed corresponding proportions of MyHC transcripts (*MyHC-1*, *-2a*, *-2x/d*, *-2b*), protein isoforms (*MyHC-1*, *-2a*, *-2x/d*, *-2b*) and fiber types (type 1, 2A, 2X/D, 2B) in both muscles in all thyroid states. Furthermore, we found that mRNA expression of slow *MyHC-1* in the SOL was up to three orders higher than fast *MyHC* transcripts. This finding can explain the predominance of *MyHC-1* isoform and fiber type 1 and the absence of pure 2X/D and 2B fibers in the SOL muscle. At protein level the slow *MyHC-1* isoform formed more than 95% of the total *MyHC* protein and the SOL contained 99% of slow type 1 fibers. The TH status significantly increased expression of *mRNA-2a* at the expense of *mRNA-1*, while the transcript levels of *-2x/d* and *-2b* remained markedly low. The amount of *MyHC-2a* protein and the number of fast 2A fibers was also significantly increased compared to the EU status, while *-2x/d* and *-2b* isoform content were not changed and no 2X/D or 2B fibers were detected. The HY status abolished in the SOL muscle expression of all three fast *MyHC* isoform transcripts and the presence of fast *MyHC* isoforms and fast fibers. In the EDL muscle, we found differences among *MyHC* transcripts, *MyHC* isoform and fiber type proportions not exceeding one order of magnitude in all thyroid states. Based on our data presenting quantitative evidence of corresponding proportions between mRNA level, protein content and fiber type composition, we suggest that the Real Time RT-PCR technique can be used as a routine method for analysis of muscle composition changes and could be advantageous for the analysis of scant biological samples such as muscle biopsies in humans. The study was supported by MYORES LSH-CT-2004-511978, GA304/08/0256, GAAV IAA 601110908, MSM0021620858 and MSMT 7AMB 12SK158 grants and by Research Project RVO: 67985823 (AV0Z 50110509).

ANATOMICAL AUTOPSY IN THE CONTEXT OF THE SLOVAK LAW

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The autopsy for anatomical studies has a long tradition and represents an important part of teaching process at faculties of medicine. The changes in the Slovak legal system after the year 1989 include many laws which defines terms like: public life, health system, human rights and etc. Health care legislative includes following laws: 576/2004 Z.z., 577/2004 Z.z., 578/2004 Z.z., 579/2004 Z.z., 580/2004 Z.z., 131/1010 Z.z. and others. Contemporary legislation includes the terms: anatomopathological autopsy and forensic autopsy. This legislation contains definitions of the conditions, material equipments and personal requirements for the practice of these two forms of autopsy. It is a necessity to define the term autopsy for anatomical studies. In this context the Czech legal definition of the forms of

autopsy and conditions could show the way for legislative changes and could give suggestions for what is currently daily practiced. **Literature** Slovak law n. 576/2004 Z.z. Slovak law n. 578/2004 Z.z. Slovak law n. 577/2004 Z.z. Slovak law n. 579/2004 Z.z. Slovak law n. 580/2004 Z.z. Slovak law n. 131/2010 Z.z. Slovak law n. 211/2000 Z.z. Czech law n. 372/2001 Sb. Slovak directive Directive UDZS n. 8/2007

HISTORY OF SELECTED SKELETAL MUSCLES LATIN TERMINOLOGY

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Introduction: The aim of the study was to present a brief review of the historical development of the Latin anatomical terminology of the human skeletal muscles, from Vesalius up to the present time. **Methods:** Study and analysis of originals of the most known anatomical textbooks from the 16th-19th centuries. **Results:** Into the results of the study were included selected muscles of particular body regions, divided into two groups. The first group is represented by examples of muscles, possessing the same names from Vesalius (1543) up now (temporalis, masseter, rectus abdominis). In the second group are mentioned selected muscles, named by individual authors during the centuries in form of many different synonyms, mostly derived from the muscle function, frequently also very poetic. **Conclusions:** The described heterogeneity of the skeletal muscles terminology was reflected only by the first version of the official anatomical terminology (B.N.A., 1895). Supported by project of Charles University in Prague PRVOUK P38.

FORENSIC OSTEOLOGY AND POSSIBILITIES OF ITS USE IN THE MEDICOLEGAL PRACTICE

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Administrative structure of our institute includes the only Laboratory of forensic anthropology and osteology in Slovak Republic. Establishment of such a specialistic laboratory was influenced especially by significant changes in the violent criminal activity against human lives in SR. Well organised criminality has gained enormous size of before unknown intensity, after rapid political and economical changes in the public life of Slovak society. Also repeated demanding appeals from concerned state and even church institutions required forensic expertises of bone

remains of public figures of a big cultural and historical importance. Praxis in forensic anthropology and osteology of our institute was beneficial even in cases of expertises of primeval animal bone remains. All the appeals were fully accepted by the experts working at our institute and the expertises were successfully performed. By presentation of the following illustrative cases, we would like to offer academic public a partial profile of our scientific activities on the field of forensic anthropology and osteology. **Casuistic expertise No. 1:** Exhumation of the bone remains of a newborn child being murdered and illegally buried by his own mother. **Casuistic expertise No.2:** Extraction, purification and osteometric analysis of the bone remains of Žofia Bosniaková, after thethermic damage by being intentionally fired in April, 2011. **Casuistic expertise No. 3:** Diagnostic procedures of the pathological changes of the primeval cave bear (*Ursus spelaeus spec.*) bone remains done in cooperation with the National Museum in Martin.

MORPHOLOGY OF NADPH- DIAPHORASE POSITIVE NEURONS IN RAT AMYGDALA AFTER PRENATAL EXPOSURE TO VITAMIN A

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Introduction. Nitric oxide is specific gaseous neurotransmitter, which does not interact with membrane receptors, but diffuse rapidly to target cells in widely extended area (0,3 – 0,4 mm). The formation of NO is carried out by nitric oxide synthase (NOS). Reduced nicotinamide adenosine dinucleotide phosphate diaphorase (NADPH-d) histochemistry selectively labels neurons, which contain NOS and thus produce NO. Nitrergic neurons were detected in many areas of central nervous system. The aim of our work was mapping NADPH-d positive neurons in the basolateral nucleus of amygdala under physiological conditions and after prenatal exposure to retinoic acid (metabolite of vitamin A). **Methods.** Wistar strain rats were used in the experiment. Pregnant females were exposed to retinoic acid on the 14. – 16. day of gravidity. The dose was 1mg RA/ kg weight of body. Adult progeny of these mothers were anesthetized and transcardially perfused. The brains were removed and consecutively processed by the histochemical methods of NADPH-d. **Results.** We didn't record changes of nitrergic neurons in the amygdala of control animals and animals exposed to retinoic acid. In both cases, we found strongly stained NADPH – d positive neurons, which were mainly located in perihelical portion of basolateral nucleus, close to the external capsule. The body cells were oval or elongated. Most of the cells were multipolar, bipolar neurons occurred sporadically. The number of NADPH-d positive neurons in basolateral nucleus, the size of their bodies and dendrites were the same in both groups of animals. **Conclusion.** Results of our work indicate, that retinoic acid applied on the 14. – 16. day of gravidity has no effect on morphology and number of NADPH-d positive neurons in the basolateral nucleus of amygdala. Supported by grant VEGA 1/0154/11.

EMBRYOLOGICAL VIEW ON ASSOCIATED CONGENITAL ANOMALIES OF PATIENTS WITH MORBUS HIRSCHSPRUNG FROM CHILDREN'S MEDICAL HOSPITAL IN BRATISLAVA

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Introduction: Hirschsprung disease (HSCR) is the most common congenital gut motility disorder and is characterized by a lack of ganglion cells in a variable length of distal gut. Usually this involves just a short segment, the rectum and sigmoid colon, but occasionally there is much greater involvement of the colon and, even more rarely, involvement extending to the proximal ileum. The functional characteristic of the disease is intestinal obstruction, caused by the localized inability of the gut to transmit a peristaltic wave. The aim of our study was analyze the relationship between HSCR and other congenital anomalies or malfunctions. Patients and Methods: We examined 130 patients with Hirschsprung disease from Slovakia for last 10 years. All patients underwent surgical procedure and the diagnosis of HSCR was verified histologically and histochemically. During patients examination we focused not only for morphologically abnormalities, but also functional anomalies. Results: Hirschsprung disease, as a neurocristopathy, has a strong association with numerous congenital anomalies, syndromes and also functional abnormalities. The incidence of associated congenital anomalies in our patients with HSCR was 26.1%. But if we add functional defects (hypothyroidism, malfunction in cellular immunity, neurological deficit) to the morphological congenital abnormalities, the rate of the patients with HSCR with additional defects achieves 50.1%. The most frequent disorder (13.6% of patients) was primary deficiency in cellular immunity. More than 12.3% of patients with HSCR had genitourinary abnormalities, in 10.0% of patients variable degree of psychomotor retardation was observed, and skeletal, muscle and limb anomalies involve 7.7% of patients. In 7.6% cases of patients we found congenital hypothyroidism or agenesis of thyroid gland. More than 6.1% of patients presented with an associated anomaly in gastrointestinal tract. Up to 5.5% patients had a congenital anomaly of heart, 3.8% had ophtalmic and 3.1% had craniofacial anomalies. Down syndrome was a main diagnosis in 3.8% patients. The relationship between HSCR and other anomalies are probably caused by abnormal migration, proliferation, or differentiation, of neural crest cells during embryogenesis. Supported by Grant of Comenius University No. UK/468/2013.

MORPHOLOGICAL CHANGES OF THE HUMAN THYMUS FROM THE 6TH UP TO 25TH WEEK OF PRENATAL DEVELOPMENT

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Introduction: Thymus belongs to one of the most mysterious organ of human body, but plays a pivotal role in the development of the adaptive immune system, an important factor that separates higher vertebrates from the rest of the animal phyla. The most of recently published works about thymus development describe thymic development in mice. The aim of our study is to capture and describe the development of thymus in human embryos and fetuses from 5th up to 25th week of development. Material and Methods: Our findings are based on the study of 12 human embryos from 5th to 8th week of development and of 16 human fetuses from 10th to 25th week of development. The embryos and fetuses were taken from the archive of collections of our institute. We used serially sectioned slides, mostly in sagittal sections. Histological examination was performed by Nikon Eclipse 80i microscope and images were captured with Nikon DS-Fi1 digital camera. Results: Two fundamental stages of the thymus intrauterine development can be distinguished: *thymus epithelialis* and *thymus lymphaticus*. The first primordia of the thymus and parathyroid glands within the endoderm of third pharyngeal pouches can be seen in 8 to 9 mm stages of embryo. We found also an epithelial proliferation in the second pharyngeal pouches, but this "*thymus secundus*" stops its further differentiation. The thymus primordia at the 7th and 8th week of development contain almost exclusively epithelial cells. These cells are arranged at the periphery as a row of prismatic cells. The mesenchyme accumulates around the epithelial thymic primordium, and during 9th to 12th week of development, septa from mesenchyme fold between the epithelial cells and create the "openings" in the capsular surrounding. According to our observations, in the 13th week of development the differentiation of cortex and medulla becomes obvious and is completed from the 17th up to 18th week of development onward. The first developing Hassall's corpuscle we found in the 13th week of development. The striking increase in the number of the Hassall's bodies was observed between the 16th and the 18th week of development, as well as between the 22nd and the 25th week of development. This study was supported by the VEGA grant agency No. 1/0902/11 (Thymus in human ontogenesis).

A CERVICAL THYMIC CYST - AN UNUSUAL CASE OF CONGENITAL ANOMALY OF THE THYMUS (HISTOLOGICAL AND IMMUNOHISTOCHEMICAL EXAMINATION OF ECTOPIC THYMIC TISSUE OF 7-YEAR-OLD BOY)

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Introduction: Ectopic cervical thymus and thymic cysts are rare causes of pediatric neck masses and can be found anywhere along embryologic tract of thymic descent from the angle of the mandible to the superior mediastinum. Most literature about thymic cysts has been published in recent years. Less than 100 cases have been reported, usually as a single case reports. **Case report:** A 7-year-old boy was referred to the Department of Pediatric Surgery in Bratislava with an enlarging, painless mass in the left neck. He had no difficulty in swallowing or breathing. Ultrasound and CT of the neck showed a normal thyroid gland and a cystic swelling attached to the carotid sheath. During surgery, a soft bi-lobed cystic mass measuring 85x25x15 mm was found in the anterior triangle of the neck beneath the sternocleidomastoid muscle. Multiple sections studied from the lesions showed multiple cysts lined by a simple squamous or low cuboidal epithelium (positivity for cytokeratins). In some areas, the cyst lining had been lost and an associated multinucleated foreign-body giant-cell (positivity for CD68) reaction to the cyst contents was present in the surrounding tissue. Collections of cholesterol crystals were also present in the cysts. Around wall of cysts we found well-developed thymic tissue with interspersed Hassall corpuscles. Based on these findings, the diagnosis of multilobular ectopic thymic cyst was established. **Discussion:** An ectopic thymus does not usually cause severe clinical symptoms. Various types of cervical thymus may present as a neck mass, usually laterally, from the angle of the mandible to the manubrium. Since it is rare to diagnose this entity before its surgical removal, the differential diagnosis includes the more common pharyngeal cleft cyst, thyroglossal duct cyst, cystic hygroma, cystic dermoid and lesions of the salivary gland as well as thyroid and parathyroid glands and cervical lymph nodes. Two theories, firstly the persistence of thymopharyngeal duct (*ductus medullaris thymi*) and secondly the degeneration of Hassall corpuscles, are considered plausible in regard to pathogenesis of thymic cyst is proposed. This study was supported by the VEGA grant agency No. 1/0902/11 (Thymus in human ontogenesis).

DIFFERENTIATION BETWEEN WILD-TYPE AND MUTANT P53 BY EXPRESSION OF MDM2 IN HUMAN BREAST CARCINOMA

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Introduction: Immunohistochemistry is a major method for investigating p53, based on observation that mutant p53 protein is frequently stabilized. However there are a number of problems that lead to false-negative and false-positive results. Wild-type p53 is stabilized by physiological stimuli such as hypoxia, specific oncogenic stresses or DNA damage resulting from the presence of free radicals from tumour-associated macrophages or following therapy, leading to positive staining in the absence of mutation. As potential marker of p53 function, we investigated expression of MDM2, important downstream target gene induced by wild-type p53. Recent studies has shown that expression of MDM2 might correlate with p53 activity. This method may show an improvement in the accuracy of identification of p53 status when p53 staining is combined with MDM2 expression. **The study objective:** The aim of this work was to determine the expression of MDM2 protein in p53 positive breast carcinomas. **Methods:** Detection was provided in 30 tissue samples of p53 positive breast carcinomas by using of indirect enzymatic immunohistochemistry. **Results:** Only 10% (3 tissue samples) of p53positive breast carcinomas show the positivity for protein MDM2. **Conclusions:** These finding suggest that immunohistochemically detected protein p53 in most of breast carcinomas is non-functional (mutant p53). Mutant p53 is not able to activate MDM2 gene. Immunohistochemical detection of p53 could serve as prognostic factor of poor outcome since the mutant p53 is detected in 90% cases. Supported in part by a grant VEGA 1/0224/12 partly by grant VEGA 1/0928/11 and partly by grant VEGA 1/0925/11.

AN ANGIOGRAPHIC STUDY ON ARTERIES WITH A HIGH RISK OF INJURY IN PELVIC FRACTURES

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Background: Vascular anatomy of the pelvis and its variation is important especially for pelvic surgeons. Bleeding in pelvis fractures is life threatening situation in people after car accidents, falling and other high energetic trauma to the pelvis. Angiography is able to easily uncover relation of arteries to bones in lesser pelvis and is able to give more information than evaluation on cadaveric dissections made by many authors. Intimate contact with bone of any artery is a risk factor for bleeding in fractures of the pelvis, especially in places of fracture lines course. As intimate contact we took distance lesser than 1 cm. **Material and Methods:** Records of 31 patients were examined. In total 60 hemi-pelvises on CT angiography (Siemens AS+ CT scanner) using 1 mm sections were evaluated. For description of the contact localization the pelvic bone and sacrum was divided

into 40 segments. The distance from bone, diameter and length of intimate contact with bone were measured. This technique detected arteries larger than 1 mm in diameter. Results: The arteries that were in intimate contact with bony structures were branches of internal (IIA) and external iliac artery (EIA). In total 9 branches with intimate contact were registered - stem of IIA, posterior stem of IIA, iliolumbal, lateral sacral, superior gluteal, anterior stem of IIA, obturator, inferior gluteal and internal pudendal arteries. Conclusion: Arteries near the bony pelvic ring which are in high risk to be injured were described. For prediction of higher risk of bleeding the comparison of course of the pelvic fracture line on the actual X-ray with our findings might be used. Supported by SVV Charles University and EU Funds OPPC CZ216/3100/24018 (INO/02/01/0017/2010)

IMMUNOHISTOCHEMICAL QUANTITATIVE EVALUATION OF THE CAPILLARY BED WITHIN THE PSORIATIC LESIONS IN VARIOUS TOPOGRAPHIC REGIONS

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Objective: The augmentation of the microcirculatory bed in psoriatic lesions is not causal but it is significant event in the pathomechanism of psoriasis. The objective of the study was the quantification of the capillary bed and concomitant VEGF expression within the healthy skin and skin of psoriatic lesions in various topographic regions. Material and Methods: Twenty-five skin samples were excised from the patients with untreated plaque-type psoriasis and compared with the control healthy skin. Immunohistochemical staining was performed with anti-human CD34 for the detection of capillary endothelium and with anti-human VEGF for the observation of growth factor expression. Morphometric analysis of the digital images was done by LabView-based programme - Vision Assistant. Results: Evaluation of the capillary area confirmed the expansion of the microcirculatory bed only in the papillary dermis of psoriatic lesions. In the reticular dermis, the capillary bed remained unchanged. The present study revealed a new additional evidence that all psoriatic lesions had comparable level of vascularization regardless of their anatomical localisation although healthy skin showed significant

regional variability. VEGF expression displayed no regional variability but significant differences between healthy skin and psoriatic lesions as for the intensity of immunoreactivity and the percentage of labeled cells both in the epidermis and dermis. Conclusion: Recent advances in the field of pathological angiogenesis in psoriasis can indicate novel potential possibilities in antiangiogenic therapy. Supported by VEGA 1/0060/09

THE DIFFERENT HR-HPV GENOTYPES DISTRIBUTION IN THE SLOVAK POPULATION SINCE 2004 TO 2012

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Introduction. Human papillomaviruses with high oncogenic risk (HR-HPV) are associated with the development of invasive cervical cancer. The 13 HPV types are regarded as oncogenic. A causal relationship between HR-HPV and cervical cancer has been well understood at the molecular level. German virologist prof. Harald zur Hausen - author of this theory - acquired in October 2008 Nobel Prize in physiology and medicine. Patients and methods: We examined since 2004 - 2012 total 7111 patients with atypia (ASCUS) and dysplasia of the cervix (CIN1-3). Exocervical swabs were used for DNA extraction and HR-HPV viral DNA was detected by RT-PCR (AB 7500 FAST) using commercial genotyping kit (HCR AmpliSens HPV genotype-FRT PCR kit, Moscow-Russia). Results: Of the 7111 analyzed samples, 339 was discarded (degraded or discrepant clinical results), representing 5% of the total number of samples. Genotyping was performed in 6772 patient samples. In correlation with published data, HR-HPV 16 and 18 were the most frequent in Slovakia (up to 58.4% of HR-HPV positive samples - the sum of single, double and triple infections including HR-HPV-16 or HPV 18). HR-HPV 33 and 58 are next the most frequent (> 7%), and HR-HPV 31 and HR-HPV 56 (> 4%). Other HR-HPV types are at lower frequencies (<4%). Conclusions: Using HR-HPV detection, we identified the group of patients with HR-HPV infection and higher grade of dysplasia. This group of patients were in higher risk of malignant cell transformation. Patients with persistent HR-HPV infection have been cured by operation (conisation). Early detection of malignant cell transformation can be helpful in eradication of this type of cancer in Slovakia. Supported by Centre of Excellence project ITMS 26240120023