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INVITED LECTURES

Known Polish Anatomists and Their Biographical Sketches

Mirosław Topol
Department of Normal and Clinical Anatomy, Interfaculty Chair of Anatomy and Histology, Medical University of Lodz, Poland

The rich history of anatomical sciences has been dominated by western anatomists for various reasons. In Poland the development of anatomical sciences started in the eighteenth century. In fact, the really flourishing development of anatomy took place in five Polish universities in the nineteenth and twentieth centuries. However, there is little information about most known Polish anatomists; therefore, it is the aim of this presentation to understand the careers and contributions of a select few Polish anatomists as we know of them today including one Polish female anatomist.

More About the Scientist

Prof. Mirosław Topol graduated from Medical Academy in Lodz obtaining his PhD in 1983 in Anatomy Department. He is now the full professor and Head of Interfaculty Chair of Anatomy and Histology and Department of Normal and Clinical Anatomy of Medical University of Lodz. He is a board member of the Polish Anatomical Society, member of European Association of Clinical Anatomy, and European Federation for Experimental Morphology. His primary area of research interest was vessel and bronchial patterns of the lungs including lymph drainage and vascular anastomoses, as well as vascularization of some other organs. However, recently also anatomy of the upper and lower limb regions including some nervous structures.

Biological Microscopy: Past, Present and Future

Tatsuo Ushiki
Niigata University Graduate School of Medical Sciences, Niigata, Japan

The invention of the microscope and its development are greatly related to the progress of biology. Development of electron microscopy especially lead to the elucidation of ultrastructure of cellular components, resulting in the rapid progress of the field of cell biology. Currently, various microscopic techniques such as confocal laser scanning microscopy, two-photon laser scanning microscopy, super-resolving microscopy and scanning probe microscopy have been also introduced to investigate live cells in relation to their structure and function. Correlative microscopy and the three-dimensional reconstruction by computers are also in the limelight. I would like to talk about the inportance of microscopy in biology, by introducing our previous and present studies using different microscopic techniques including light microscopy, scanning electron microscopy, and scanning ion conductance microscopy.

More About the Scientist

Professor Tatsuo Ushiki is an Executive Vice President of Niigata University, Japan. He is also Professor of Microscopic Anatomy, Niigata University Graduate School of Medical and Dental Sciences. His main interest is the development of three-dimensional imaging of biological structures by microscopy. He has been studying the ultrastructure of various tissues and organs by using different techniques of microscopy. He has been studying the three-dimensional ultrastructure of various tissues and organs such as lymphoid tissues, peripheral nerves, and connective tissue fibers by using various technique of scanning electron microscopy. He has been also interested in the application of scanning probe microscopy to imaging of biological samples such as chromosomes and culture cells. Recently, he is engaged in studies on the topographic imaging of biological samples under liquid conditions by scanning ion conductance microscopy.

Professor Tatsuo Ushiki was President of the Japanese Society of Microscopy (2017-2019), and Executive Directors of the Japanese Association of Anatomists (2019-present). He is also Secretary of International Committee of Symposia of Morphological Sciences (2016-present), and Board Member of International Scanning Probe Microscopy Conference (2010-).

Normal and Variant Morphological Terminology – Past, Presence and Future

David Kachlik
Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, Czech Republic

The terminology is an unavoidable base for clear and non-misleading medical communication. The fields of morphology (anatomy, histology, embryology) are happy to have an elaborated official nomenclature in Latin since 1895 and 1977, resp. their official version in English, nowadays the world science language, are available for the neuromorphology and embryology only (since 2017). Federative International Programme on Anatomical Terminology (FIPAT) is an organ of International Federation of Associations of Anatomists (IFAA), responsible of revision and re-edition of morphological nomenclatures. The last version of the anatomical nomenclature was issued in 1998 (Terminologia Anatomica), of the histological nomenclature in 2007 (Terminologia Histologica) and of the embryological nomenclature (Terminologia Embryologica 2) in 2017, the combined anatomical and histological terminology of the nervous system and senses (Terminologia Neuroanatomica) appeared in 2017 as well.

Variant anatomy is rather old subbranch of anatomy but its terminology has never been systematised. Terminologia Anatomica contains only 149 entries, the largest number can be found in the Cardiovascular system chapter. It is necessary to make up a system for systematic yet simple, clear and understandable denomination of anatomical variations which seems to be a long-lasting and tremendous task. On the contrary, the developmental defects are already processed at the basal level in the Terminologia Embryologica, but rare anomalies and complex syndrome have not yet been incorporated either.

More About the Scientist

David Kachlík graduated from the Third Faculty of Medicine, Charles University in Prague, Czech Republic in 1998, obtained Ph.D. in Experimental Surgery at the same institution (Blood supply of the large intestine) in 2006 and worked there as teacher and clinical anatomist in research concerning limb musculoskeletal system, vessels, vasa vasorum, peripheral nerves, variational anatomy, history of anatomy, and morphological terminology and nomenclature. Since 2015 he has been Professor of Anatomy, Histology and Embryology, and Head of the Department of Anatomy at the Second Faculty of Medicine, Charles University in Prague, Czech Republic. He is a member of Federative International Programme on anatomical Terminology (part of IFAA).

News of the Pyramidal Tract

Diedrich Graf von Keyserlingk
Professor Emeritus, Free University of Berlin and RWTH Aachen, Germany

Fundamental features in the human brain are the molecular specify of nerve circuits and the topographic organization. Direct chemical contact of markers with the molecules is needed for identification the circuits. The topographic organization of polymers of the molecules may be evaluated by physical methods from distances. If the distance is far enough, the physical method may be applied even from outside of the person, which means patience in the living state. The anatomist and morphologist have the task to bridge the knowledge from the biochemical normal or pathological event to its physical appearance. Some morphologists concentrate on the biochemical part of the task other on the physical part with the bridging from inside to outside. The scientist in question belongs to the latter type. He was taught already as student by experiments in the department of electron microscopy to visualize biological tissues with an electron microscope - a quite new technique for medicine at that time. The kind of experiments and the kind of biological material in the department was free to decide for the interested student, everything was new. At that time (in the middle of the past century) freedom of research and teaching was the undisputed right and practice of universities and their scientists. The contract with the Head of Anatomy I, University Aachen in 1983 explicit claimed self-reliance of research and teaching. Indeed, electron microscopy in Aachen was replacing by CT (Computer Tomography) and MRT (Magnet Resonance Tomography) concentrating on clinical applications determined research activities was done from that time on. As bridging morphological between patience's brain disturbance and consecutive CT and MRT images digital brain atlases were established. Already available stereotactic atlases were improved and applied. The anatomy of the white matter of the human brain became now quite better known as was some years ago, thanks to several new physical methods applying in brain research including the 3D-PLI (3D- Polarized Light Imaging). The interpretation of the patience`s disturbance by physical images cannot be done in simple way because simple linear dependencies do not exist. New concepts like fuzzy logic and artificial intelligence have to be applied. For more details the special publications should be consulted. While 3D-PLI took an impressive worldwide development and improvement, the microwave measurements of brain tissue for its identification did not. Perhaps this idea will also get a future.

More About the Scientist

In 1970, prof. Graf von Keyserlingk performed the first electron microscopic visualization of contractile filaments in the mammalian non-muscular cells by glycerin-extraction and ATP-contraction. In 1998, he also identified the three-dimensional orientation of myelinated nerve fiber and collagen fiber tracts by polarization light. In 1999-2002, prof. Graf von Keyserlingk completed the microwave dielectric measurements in tissues localizing in the human brain. Prof. Graf von Keyserlingk is one of the first researchers who applied in studies stereology (Weibel), image processing, fuzzy logic and artificial intelligence (Kohonen). He is the author of 148 scientific publications, nine textbooks for medical students and specialists and has over 1700 citations since 1977. Prof. Diedrich Graf von Keyserlingk was supported by Grant from Europe AIM, Project N 2003 COVIRA (COmputer Vision in Radiology) that had the final task – to create the Digital Anatomy Atlas.

Comparative Neuroanatomy of Heart

Tomokazu Kawashima
Department of Anatomy, School of Medicine, Toho University, Tokyo, Japan

In cardiac regulating system, the cardiac nervous system has been paid less attention than others such as cardiac conduction system. However, the cardiac nervous system not only controls life-maintaining functions such as heart rate, blood pressure, blood flow and pain delivery but also plays key role to development and progression of heart failure.

Recent research data on molecular physiological mechanisms and clinical alterations continue to be accumulated and thus these evidences clarify that research on the cardiac nervous system will accelerate in the future. On the other hand, the underlying morphological foundation as an anatomical map on complicated cardiac nerve plexuses still remains incompletely understood.

Until now, I have conducted evolutionary anatomical studies of the extrinsic cardiac nervous system in humans, non-human primates and its relatives, and some experimental mammals to gain a broader perspective on the anatomical modifications and evolution of this system within placental mammals. Our comparative anatomical researches contribute to clarify anatomical standing issues and obtain the evolutionary-developmental basis on the cardiac innervation.

The somatic skeleton, muscles and nerves are modified for functional adaptations, but the autonomic nervous system probably is **not** and possibly preserves its evolutionary history in close alignment with phylogeny. Therefore, the evolution of the mammalian autonomic (cardiac) nervous system should be demonstrated by its comparative anatomy without concern for derived features reflecting ecological, kinematic (locomotion), and dietary adaptations as seen in somatic systems.

This session will provide our current understanding on "Comparative Neuroanatomy of Heart".

More About the Scientist

Prof. Tomokazu Kawashima obtained MA in Anatomy and Physiology from Tokyo Medical and Dental University in 2000 and PhD in Anatomy from Tokyo Women's Medical University in 2006. He is now an Associate Professor of Anatomy, Toho University, Japan. During his postdoctoral period at the Smithsonian Institution (Washington, USA) in 2007-2009, he engaged studies on the mammalian comparative anatomy of the cardiac nervous system and is still collaborating with the staff. He has been studying the anatomy of the cardiac regulating systems such as the cardiac innervation and conduction system from comparative, evolutionary, and clinical viewpoints. He has been also interested in the evolutionary and functional morphology of mammalian specialized muscles.

He is a managing editor of the Anatomical Science International published form the Japanese Association of Anatomists (2019-present).

New Views on the Functional Histology of the Human Uterine Tube

Ivan Varga
Faculty of Medicine, Comenius University in Bratislava, Slovak Republic

The first accurate morphological description of the uterine tubes traces back nearly 460 years to the work of an Italian anatomist Gabriele Falloppio from 1561. The uterine tubes are also known as the Fallopian tubes, an eponym chosen in his honor. The histological findings in normal uterine tubes have been described sporadically in the scientific literature. The major reason for the lack of investigation in this regard has been the success of in vitro fertilization techniques which enable bypassing of the uterine tubes, so these organs have become somehow neglected, since they are no longer vital for successful conception. This has resulted in the lack of interest in medical community to study tubal morphology. The last decade of research confirmed the presence of newly discovered population of cells within the interstitium of female genital organs, including uterine tubes. The real significance of these cells – telocytes – however is still at the level of highly hypothetical conjectures. Nevertheless, there is an abundance of preliminary research suggesting that these cells serve as functional interconnectors between other cell populations within the wall of the uterine tubes. This important action of telocytes in tissue homeostasis has been viewed as an implication for their possible involvement in the pathogenesis of a wide range of tubal diseases such as infertility, or tubal pregnancy. However, telocytes are not the only "mysterious and neglected" structures within the histology of the uterine tubes. Since 1904, no one has paid any comprehensive attention to lymphatic drainage of the uterine tubes at the level of lymphatic capillaries. The habilitation thesis authored by a German physician Paul Kroemer was the first to describe the lymphatic lacunae inside the tubal folds (by the method of injection of Indian ink), which he named "Lymphbahnen" ("lymphatic channels"). Despite this first description has been existing for more than 110 years, there is no mention of these lacunae in most of the current literature. This status quo is even more striking when we consider that these lymphatic lacunae may be responsible for the thickening of the fimbriae during the oocyte pick-up and the maintenance of the tubal fluid. Similarly, the histological literature also ignores the issue of nomenclature regarding the epithelial cells of the uterine tubes, even though this tubal epithelium may be the source of high-grade ovarian carcinomas. A detailed identification of intraepithelial immunologically active cells can elucidate the questions regarding the immune suppression within the uterine tubes. In our study, we identified intraepithelial memory / regulatory T-lymphocytes. Intraepithelial regulatory T-lymphocytes can be involved in the process of immune tolerance of non-self cells (sperm) and partially non-self cells (those of developing embryo), preventing the activation of local immune responses. Additionally, we described intercalary/peg cells as actively dividing cells, challenging their notion as effete or degenerating cells mentioned in histological textbooks. Finally, the histological nomenclature should be corrected so that peg/intercalary cells are no longer considered synonymous with secretory cells.

More About the Scientist

Professor Ivan Varga studied and obtained PhD in Anthropology and in Pathological Anatomy and Forensic Medicine at Comenius University in Bratislava. Currently, he is the Vice-Dean for Science and Research at the Faculty of Medicine, and the Deputy Head of the Institute of Histology and Embryology at the same university.

His current research interests include the development and microscopic anatomy of the human lymphoid and female reproductive organs, ultrastructural characterization of the human mesenchymal stem cells and their application in regenerative medicine, reproductive biology, and new trends in histology and embryology teaching. As an author / co-author he published seven textbooks, three monographies and more than 80 scientific papers. He contributed to chapters in Nerves and Nerve Injuries (Elsevier, 2015) and Bergman's Comprehensive Encyclopedia of Human Anatomic Variation (John Wiley & Sons, 2016). His textbook Memorix Histology received the "Jaroslav Jirsa Price" – the best textbook of 2016, according to the decision of the Rector of Charles University in Prague. His articles have been cited more than 600-times in SCOPUS or Web of Science databases.

Professor Ivan Varga is a Member of the Executive Board of the Slovak Anatomical Society, an Honorary Member of the Asociación Argentina de Anatomía Clínica, a Member of the American Association of Anatomists and a Member of the Advisory Board for the Netter Atlas of Human Anatomy 7th Edition. He is also a Member of the Editorial Boards of journals Clinical Anatomy, Revista Argentina Anatomía Cliníca and Morphologicheskie Vedomosti. In 2015 he was the President of the 7th International Symposium of Clinical and Applied Anatomy, which took place in Bratislava.

Contribution of Morphology to Forensic Identification

Rimantas Jankauskas
Department of Anatomy, Histology and Anthropology, Vilnius University, Lithuania

Notwithstanding recent advances of forensic genetics, forensic anthropology still plays a crucial role in identification. It is based on cross-matching of "ante-mortem" (including individual morphological peculiarities of missing person) and "post-mortem" (sex, biological age, stature, pathologies and other morphological individualising traits of usually skeletonised remains) data. Recently, two cases of identification – of A. Ramanauskas-Vanagas, the last commander of Lithuanian anti-Soviet resistance, executed in 1957, and executed leaders of anti-tsarist uprisal of 1863-1864 – that evoked vivid reactions in society, were performed successfully using this approach. In my presentation I will highlight morphological aspects of these identifications.

More About the Scientist

R. Jankauskas graduated from the Faculty of Medicine, Vilnius University, and since 1982 is employed at Department of Anatomy, Histology and Anthropology. His scientific interests include various aspects of biological anthropology of past populations in Lithuania, that are implemented during close collaboration with archaeologists, and forensic anthropology.

ORAL PRESENTATIONS

An Interpretation of Some Anatomical Details in Michelangelo's David Statue

Dzintra Kažoka
Department of Morphology, Institute of Anatomy and Anthropology, Rīga Stradiņš University, Latvia

Highlights. The fascination of David's statue is in the precision with which the details of the anatomical structures are reproduced. Michelangelo and his master work demonstrate different parts of human anatomy and figure. There are a number of details in the composition elements of the statue that require consideration.

Background. The symbol of the Renaissance or David has been studied for many years by different experts. It is known from documents that for Michelangelo Buonarroti (1475–1564) this statue was his visual ideal of male beauty with a very muscular physique. In comparison with other sculptor's works, in this statue certain anatomical details and proportions of the body are atypical in sizes and positions. The aim of this study was to take a special look at the David statue of the Italian sculptor and to interpret some anatomical details, according to review of the existing literature.

Material and Methods. Data were collected from several articles and scientific publications in English in the PubMed, Scopus and medical history sources.

Results. The harmony of body parts working together has general role of the image of the David. The bilateral symmetry of the torso is maintained on a gently curving axis. The head and hands are disproportionally larger for his body. The cheeks are smooth, nostrils are slightly flared and the upper lip is just a little bigger than the lower one. David's right leg holds his full weight. The other leg is relaxed (contrapposto position) and moved slightly forward. The hips have shifted with one side being higher than the other. The shoulders and arms give the statue a more dynamic look. His right shoulder drops slightly below his left one. David`s right arm hang loose halfway down his thigh, but his left arm is bent to shoulder height. In the arms are visible bulging veins. The imposing right hand symbolizes the pondered action. In his right hand he holds the stone. The bulging veins of the right hand together with expression of the facial features and are not consistent with the calm stasis of the body. The elbows appear calloused and rough.

Visual analysis of the statue shows that David has a focused look in his eyes. Some authors indicate that there are visible differences between the positions of both eyes. The right eye is in the primary position, but the left eye appears to be looking out to the left. From the statue`s perspective the left eye fixates on the viewer but the right eye appears to be looking at the distance away from the viewer.

Discussion. Michelangelo played with opposites or contrasts and, according to this, an accurate male anatomy moved this work out from other notable sculptures from Italy during the Renaissance. The special attention to the marble statue of David showed that some parts and structures of the human figure were in different dimensions.

Conclusions. All anatomical details served as a window into possible new readings of the David for previous and current time.

Levator Palpebrae Superioris Muscle – Its Typical and Not Typical Course

Robert Haładaj, Mirosław Topol
Department of Normal and Clinical Anatomy, Medical University of Lodz, Poland

Introduction. In the anatomical literature the levator palpebrae superioris muscle, coming from a lesser wing of the sphenoid bone, is classified as the eyeball muscle or the mimic muscle. On its further course it broadens and decreases in thickness, continuing as the levator aponeurosis. Its contraction causes the eyelid to move upwards.

Material and Methods. 56 orbits were dissected. After removal the orbital roof, the shape of the levator palpebrae superioris and its anatomical variations (i.e. the presence accessory muscular bands or atypical formation of the muscle) were assessed.

Results. In 29 cases (51.8%) the levator palpebrae superioris showed typical morphology. In 5 (8.9%) cases the additional fibers were well developed and formed accessory medial slips of the levator. In 6 cases (10.7%), additional muscular slips were attached to the trochlea of the superior oblique muscle In 8 cases (14.3%) the additional muscular slips were attached to the lacrimal gland.; In 7 cases (12.5%), the additional slips were attached both to the lacrimal gland and the trochlea of the superior oblique muscle. In one (1.8%) case the double origin of the levator palpebrae superioris was observed.

Conclusions. Plastic surgeons should be aware of anatomic variations of the levator palpebrae superioris muscle both in planning and in conducting surgeries of the upper eyelid.

Intramuscular Innervation Pattern of Extraocular Rectus Muscles (Superior, Inferior, Medial and Lateral) in Humans

Robert Haładaj, Grzegorz Wysiadecki, Mirosław Topol
Department of Normal and Clinical Anatomy, Interfaculty Chair of Anatomy and Histology, Medical University of Lodz, Poland

Introduction. The goal of the study was to examine innervation pattern of extraocular rectus muscles, including the detailed distribution of the intramuscular sub-branches of the oculomotor and abducens nerves.

Materials and Methods. 10 specimens of each out of the four rectus muscles (superior, inferior, medial and lateral) were harvested (with the orbital segments of the oculomotor and abducens nerves), fixed in 10% formalin solution, and stained with Sihler's whole mount nerve staining technique.

Results. In all rectus muscles, intramuscular distribution of each nerve sub-branches showed a Y-shaped ramification, forming the terminal nerve plexus. The plexus ended in about half of the length of each rectus muscle. Results obtained by Sihler's stain also suggested, that the nerves supplying horizontal rectus muscles (lateral and medial) could be divided into sub-branches that reached functionally distinct superior and inferior compartments of the muscles.

Conclusions. Detailed knowledge of intramuscular nerve distribution within the extraocular rectus muscles may be crucial for understanding their function during complex eyeball movements. Sub-branches running to the horizontal rectus muscles are divided into two groups that supply the superior and inferior compartments of the muscles, respectively.

Distribution and Potential Role of Nitrergic Neurons Within the Sources of Cardiac Innervation

Ieva Navickaitė, Neringa Paužienė, Dainius Haroldas Pauža
Institute of Anatomy, Faculty of Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania

Study Highlights. This study is focused on nitric oxide (NO) as a neurotransmitter in the cardiac innervation. It highlights the possible origin of nitrergic cardiac innervation and potential role of NO in neural control of the heart.

Background. In the nervous system, nitric oxide is produced by neuronal NO synthase (nNOS). Despite an ample distribution of nNOS(+) neuronal somata and nerve fibres (NFs) within the heart, previous morphological investigations showed that a significant part of these NFs arise from the extrinsic sources. Furthermore, the exact role of NO in the cardiac innervation is still under discussion. Therefore, this study aimed to examine the potential source and role of nitrergic cardiac innervation.

Material and Methods. Sections of the medulla oblongata, nodose ganglia, vagal nerve and its cardiac branches, dorsal root ganglia of C8–Th5 spinal nerves and stellate ganglia from twenty rats were examined. Double immunohistochemical staining for nNOS and choline acetyltransferase (ChAT) was performed. Preparations were analysed employing laser-scanning microscope. The data are presented as mean±standard error.

Results. In the nucleus ambiguus, the most abundant population of nitrergic neurons was observed in pars compacta. Most of them were biphenotypic with ChAT (13.1±1.3%), while only solitary nNOS(+) neuronal somata were found. In the semicompact part, the amount of nNOS(+)/ChAT(+) neurons decreased (5.1±2.1%), while they were absent in the loose part. In the dorsal nucleus of the vagus nerve, nNOS(+)/ChAT(+) neurons composed 7.7±1.6%, while only nNOS(+) ones – 2.4±0.8% of the whole nucleus. Small nNOS(+) neuronal somata were amply distributed within the solitary nucleus. Moreover, a dense meshwork of nitrergic NFs was present within the solitary nucleus, dorsal nucleus of the vagus nerve and pars compacta of the nucleus ambiguus. The most abundant population of nNOS(+) neurons was observed in the nodose ganglia (36.5±1.9%). A lot of nitrergic NFs spread along the vagus nerve and entered its cardiac branches. In the examined dorsal root ganglia, a small population of nitrergic neurons was observed. Only solitary nNOS(+) neuronal somata were found in the stellate ganglia.

Discussions. Ample distribution of nitrergic neuronal somata within nodose ganglia and solitary nucleus and NFs positive for nNOS in the vagal nuclei imply that NO may play a role in vagal afferent innervation of the heart and integration of sensory information within the brainstem circuits. However, despite a sparse distribution of nitrergic neuronal somata within the vagal nuclei, we cannot deny the possibility that NO can also be involved in the parasympathetic cardiac innervation.

Conclusions. The most abundant population of nitrergic neurons was found in the nodose ganglia, so nitric oxide is most probably involved in the sensory function of the heart. However, NO can also play a role in the parasympathetic cardiac innervation.

Comparative Anatomy of Sinoatrial Node and its Innervation in Experimental Animals

Hermanas Inokaitis, Kristina Rysevaitė-Kyguolienė, Neringa Paužienė, Dainius Haroldas Pauža

Institute of Anatomy, Lithuanian University of Health Science, Kaunas, Lithuania

Study Highlights. This study has revealed the significant differences in distribution of the sinoatrial pacemaker cells in different animal species.

Background. Mouse, rat, rabbit ant pig are the most commonly used animal models in experimental cardiology. However, many cardiac researchers feel difficulties to choose the most proper animal model as the peculiarities of the cardiac conductive system sinoatrial among these species are little known.

Material and Methods. The hearts from four mice, four rats, three rabbits and for piglets were examined using the whole mount preparations. Distribution of the sinoatrial nodal cells (SAN) and neural components were visualized by a multiple immunohistochemical staining for HCN4 (pacemaker cells) and PGP9.5 (protein gene product 9.5 as general neural marker) or ChAT (cholinergic neural marker).

Results. In all the examined species, HCN4 cells were similarly distributed around the orifice of the right cranial vein (RCV). The main mass of cells surrounded RCV from the frontal part to the lateral, where it extended throughout the terminal sulcus in the whole intercaval region (ICR) almost reaching the caudal vein (CV) in hearts of mouse, rabbit and pig. However, these cells reached CV and "hid" under it in the rat heart. The distribution of HCN4 positive cells in the dorsal part of the RCV was different in the mouse compering to the rabbit and pig. In the mouse, they widely scattered and the main massive of cells disappeared without clear limits. In rabbit and pig, they gathered into a thin bundle. The feature specific only for the rat was a mass of HCN4 cells of cells observed in the medial side of RCV that extended through the whole ICR to the CV. In other species, only some HCN4 positive cells from the main mass extended to the medial side of RCV, but did not reached it. The whole area occupied by HCN4 positive cells occupied had a highly dense meshwork of nerve fibers in all species examined. The mouse and rat ICR had some larger nerves. Only in the rabbit ant pig, the ICR contained epicardial ganglia and solitary neurons. However, the size and number of ganglia in the pig was significantly higher in respect to the rabbit ICR.

Discussion. Current study extends the understanding of the wider distribution of the sinoatrial nodal cells than it was considered earlier. In addition, our findings do support in part the hypothesis that larger animals have the complicated cardiac conductive system and its innervation. Possibly, the much more wider distribution of the HCN4 positive cells could be referred to internodal pathways described previously.

Conclusion. The species-dependent anatomy and neuroanatomy of cardiac conductive system has to be considered by cardiac physiologists in both the choose of the animal models and interpretation of their data.

Synovial Arthritis-Associated Fibroblasts: Exploring the Role of Podoplanin-Positive Cells

Mihails Tarasovs[1,2], Sofija Semenistaja[1], Sandra Skuja[1], and Valerija Groma[1]
[1]Institute of Anatomy and Anthropology, Riga Stradins University, Latvia;
[2]Department of Internal Diseases, Riga Stradins University, Latvia

Study Highlights. The study highlights the significance of synoviocytes assessment in human arthritis.

Background. Fibroblast-like synoviocytes (FLSs) regulate local homeostasis, coordinate inflammatory responses, and mediate articular tissue damage in experimental and human arthritides. Recent studies evidence usefulness of a cell surface glycoprotein podoplanin (PDPN) testing in common disabling joint pathologies like osteoarthritis (OA) and rheumatoid arthritis (RA) in humans. In this study, we examined PDPN expression in inflammatory synovial tissues and correlated these data with synovitis scoring assessments.

Material and Methods. Synovial membrane tissues collected from 40 OA and 7 RA subjects (range 36–87 years, and 54–79 years, respectively) who underwent the joint replacement surgery were processed and used in the study. FLSs were characterized by the expression of CD31, CD34, PDPN, α-smooth muscle actin (α-SMA) assessed immunohistochemically and statistically. Synovial macrophages were identified by CD45+ CD14+/CD68+ phenotype whereas synovial lymphocytes as B lymphocytes when revealing CD45+ CD3¬ CD19+ and T lymphocytes when revealing CD45+ CD3+. Local inflammatory status was confirmed by expression of tumor necrosis factor alpha (TNF- α). Synovial histopathology was scored according to Krenn and Morawietz system. The SPSS version 24.0 software was used for statistical analysis.

Results. In RA samples, PDPN was expressed in both types of synoviocytes being more heavily expressed in the hyperplastic lining layer as well as stromal macrophages and fibroblasts. Similar observations were made in the inflamed OA synovium. Furthermore, PDPN was expressed in inflammatory synovium invading cells of RA and OA subjects. Among the synovial membrane samples examined, TNF-α was expressed in both OA and RA-affected synovium. In the lining layer, an over-expressed TNF-α was demonstrated when covering the sublining lymphoid follicles. A moderate to strong correlation (0.588; p=0.021) was found when PDPN expression in the synovial sublining was compared to TNF-α in the synovial lining. The correlation between PDPN expression in the synovial sublining and invading giant cells was strong and negative (–0.0706; p=0.001). There were slightly more PDPN-positive cell/per visual field in younger arthritic patients vs. those in older (1.85 vs. 1.68) when comparing means.

Discussion. Synovial inflammation, remodeling, and progressive joint destruction are common arthritis-associated processes mediated by synoviocytes and cells of the immune system. Our findings related to arthritic FLSs expressing PDPN are consistent with recent data suggesting these respond to the local pro-inflammatory milieu and contribute to joint inflammation.

Conclusions. Synoviocytes are important players in the development of chronic, inflammatory, and destructive response in joint affected by arthritis. PDPN is a promising biomarker for evaluating synovitis in RA and inflammatory OA.

Cam-Delam: An *in Vivo* Method for Visualizing and Evaluating Delamination Capacity of Cancer Cells

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Highlights and Background. The spreading of cancer from the primary tumor to other places in the body, and the formation of secondary tumors, so called metastases, is the major cause of death for cancer patients. A prerequisite for cancer cells to leave the primary tumour and to invade other tissues is to degrade the basal membrane, including the basal lamina, a biological process called delamination. Today, there is no available method to score cancer cells capacity to initiate delamination. Such a model could be useful both at the clinics to characterise cancer cell biopsies, as well as for pharma companies in their attempt to screen for novel cancer drugs.

Materials and Methods. We present the CAM-Delam method, an in vivo assay to visualize cancer cells ability to degrade the basal lamina within a few days. Briefly, the method includes seeding cancer cells on the chick chorioallantoic membrane (CAM) to evaluate its capacity to delaminate and invade.

Results. Our results, testing different cancer cell lines, show that the delamination capacity of cancer cells can be divided into four categories; 1) intact laminin, 2) altered, but not damaged laminin, 3) damaged laminin without cell invasion, 4) damaged laminin with cell invasion/ micro-metastasis. We validated the CAM-Delam method in functional assays with cancer cell lines already known for their metastatic capacity. The results show that PC3, (Prostate cancer) SW620, (Colon cancer), and A549 (Lung cancer), cells induce delamination at different time points. Moreover, U251 (Glioblastoma), a non-metastatic cancer cell line, did not show any delamination capacity. However, by exposing the U251 cells to Cobalt (II) chloride, a chemical inducer of hypoxia, a delamination capacity was ectopically induced.

Discussion. The simplicity of this method together with consistent and reliable results highlight the strength and usefulness of the CAM-Delam assay to determine delamination, i.e metastatic, capacity of specific cancer cells. Our results emphasize the usefulness of the CAM-Delam assay not only to score delamination capacity as a measurement of metastatic aggressiveness of specific cancer cells, but also to unravel molecular mechanisms regulating delamination.

Conclusions. A possible future application of the method is to analyse human cancer biopsies to score the aggressiveness of individual cancer types, which would complement the current TNM staging of cancer. In addition, the CAM-Delam method might be useful to enable identification of patient specific cancer therapies.

Orphopathogenic Aspects of Cleft Lip Palate Affected Tissue

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Study Highlights. Tissue repair after the surgical intervention on cleft lip palate (CLP) patients depends on the coordinated action of multiple tissue growth and remodelation factors. This study aimed to evaluate a relative number and appearance of the tissue factors: matrix metalloproteinase-2 (MMP2), tissue inhibitor of metalloproteinase-2 (TIMP-2), bone morphogenetic protein 2/4 (BMP 2/4), transforming growth factor beta; (TGF-β), Wnt3a gene (Wnt3a), Runt-related transcription factor 2 (Runx2), basic fibroblast growth factor (bFGF) and osteoprotegerin (OPG) in hard tissue of CLP patients during the first time surgical intervention.

Background. CLP is one of the most common facial deformations that affects quality of life. The number and distribution of the tissue factors that may have a role in it have not been widely investigated.

Materials and Methods. The research involved 43 patients with CLP with 24 bone tissue and 36 cartilage tissue samples. The control material of bone was obtained from seven patients from facial cleft unrelated surgical operations. Immunohistochemistry was used to assess the levels of tissue factors and the semi-quantitative census method was used for quantification of immunological structures. Data were analysedusing the Mann-Whitney U-test and Spearman's rank correlation coefficient.

Results. No statistically significant difference (p> 0.05) in numbers of OPG, Runx2, Wnt3a, TGF-β, TIMP-2 positive cells between the CLP group and the control group was obtained. Statistically significant (p< 0.05) increased amount of MMP2 and bFGF positive cells was detected in the CLP group, both - in cartilage and bone - compared to the control group. Statistically significant (p=0.012) increased number of BMP 2/4 positive cells was found in the cartilage of the CLP patients in comparison to the control group.

Discussion. MMPs are responsible for tissue remodelation, cell proliferation, and tissue repair. Summarizing the research data, it indicates that loss of MMP results with dentoalveolar tissue defect. Our study shows thattissue are more requisite for repair and indicate the predominance of tissue degradation. bFGF is an angiogenic factor and have an important role in wound healing. Research report that new bone formation was observed after bFGF applications to the bone defects. Our results suggest that increased proliferation and hypertrophy processes takes place in case of CLP. It is concluded that BMP applications accelerate fracture healing. Our results of increased number of BMP 2/4 positive cells in cartilage suggest increased tissue proliferation and healing capacities in cartilage that exceeds over bone healing.

Conclusions. Increased appearance of MMP2, bFGF in hard tissue of the CLP patients indicates the predominance of tissue degradation. Increased number of BMP2/4 positive chondrocytes suggests the elevation of cartilage growth and thus better regeneration of cartilage in comparison to bone in CLP patients.

Gender-Related Sodium Valproate Effect on Thymocytes NKCC1 RNA Expression and on Rat Thymus

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Study Highlights. Sodium valproate (VPA) effect on rat thymus, its structure and thymocytes Na-K-Cl cotransporter (NKCC1) RNA expression depends on gender and gonad hormones.

Background. VPA is a histone deacetylases inhibitor which inhibits cell proliferation and increases cell apoptosis. The NKCC1 is a recognized tumorigenesis marker responsible for sodium, potassium, and chloride (Cl-) influx into thymocytes. The intracellular Cl- concentration is one of the critical messengers in cell proliferation and differentiation processes. The study aim was to investigate the effect of VPA on thymus weight, its structure and NKCC1 RNA expression in thymocytes.

Material and Methods. Wistar rats, age 4 to 5 weeks, were investigated in VPA-treated gonad-intact and castrated males and females and in their control groups (n=6 each). The VPA 300 mg/kg/day treatment duration was 4 weeks in drinking water. The thymocyte NKCC1 (the Slc12a2 gene) RNA expression was determined by the real-time PCR method; thymus cortical and medullary areas structural changes were assessed in slides stained with H and E; Hassall's corpuscles numbers per 1 mm2 (HCs) were determined immunohistochemically. Results. Castrated male VPA-treated rats showed a significant thymus weight loss after VPA treatment compared with their control ($p<0.05$). The VPA treatment caused a 6-fold increase of HCs in males ($p < 0.05$) and more a 4-fold one in gonad-intact females ($p<0.05$). The HCs of the castrated male control was higher than of the gonad-intact male control ($p<0.001$). No significant VPA effect was found among castrated controls and VPA-treated castrated rats of both genders. The NKCC1 RNA expression was found to be significantly higher in the gonad-intact male control compared with female ones ($p=0.04$). Compared with the respective controls, the Slc12a2 gene RNA expression level was found to be decreased in the VPA-treated onad-intact males ($p=0.015$), and no significant VPA effect was found in gonad-intact females ($p>0.05$).

Discussion. VPA inhibits the proliferative capacity of thymocytes and induces the differentiation of thymic epithelial cells to HCs. VPA induces an increase of HCs in animals of both genders. Thymocytes show a gender-related difference in the NKCC1 RNA expression; the VPA treatment decreased NKCC1 expression in the thymus of gonad-intact males.

Conclusions. The NKCC1 RNA expression downregulation by VPA could be important for the further VPA pharmacological studies in oncology.

The Morphopathogenesis of Intraabdominal Adhesions in Infants

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Study Highlights. The appearance of transforming growth factor β (TGFβ), hepatocyte growth factor (HGF), basic fibroblast growth factor (FGF-2), fibroblast growth factor receptor-1 (FGFR1), vascular endothelial growth factor (VEGF), protein gene product 9.5 (PGP 9.5), chromogranin A (CgA), interleukin (IL) -1, 4, 6, 7, 8, 10, tumor necrosis factor alpha (TNF-α), human beta defensin-2 (HBD-2), matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of matelloproteinase-2 (TIMP-2) were determined in adhesions.

Background. The morphopathogenesis of intraabdominal adhesions is a complex process, characterised by the accumulation of extracellular matrix, tissue hypoxia and inflammation (Christodoulidis et al., 2013; Coccolini et al., 2013).

Materials and Methods. The study material was obtained from 49 patients who underwent abdominal surgery due to bowel obstruction. All factors were detected using immunohistochemistry and were assessed according to semiquantitative counting method.

Results. Significantly more TGFβ positive structures were found in adhesions. HGF positive cells were observed occasionally. Lower amount of FGF-2 and higher amount of FGFR1 positive structures were found in adhesions.

More VEGF positive macrophages were observed more in adhesions. PGP 9.5 findings in the study group were lower. In the adhesions group, mostly few CgA positive structures were observed.

The amount of IL-1α, IL-4 and IL-8 was significantly lower in adhesions. Adhesions demonstrated a moderate number of IL-6, IL-7, IL-10, TNFα and HBD-2 positive structures.

A positive reaction for MMP-2 and TIMP-2 was seen, but the number of TIMP-2 positive cells was significantly higher in the controls.

Discussion. An increased TGFβ finding, a decreased HGF finding and a disbalance in FGF-2/FGFR1 and MMP-2/TIMP-2 was observed, overall facilitating fibrosis, tissue remodelation disorders and adhesion development. Neoangiogenesis and an increase in VEGF positive macrophages are characteristic. Possibly, hypoxic injury caused a decrease in PGP 9.5 positive structures. The decrease in IL-1, IL-4, and IL-8 are the most significant changes in inflammation regulating cytokines. The inflammation in adhesions is also linked to the increase of the antimicrobial peptide HBD-2, promoting the maintenance of inflammation.

Conclusions. The increase in TGFβ-containing structures indicates the growth/regeneration potential of loose connective tissue. The lack of HGF and the imbalance between MMP-2/TIMP-2 proves of increased fibrosis and might promote formation of adhesions. The less distinct FGF-2 and more prominent FGFR1 prove of a compensatory stimulation of receptors. The decrease in PGP 9.5 positive structures and the increase in VEGF positive macrophages, indicates a hypoxic injury and tissue ischemia. The less IL-1 and more IL-10 finding points out a local tissue protection reaction. The decrease in IL-4 and IL-8 positive structures could confirm the prolongation of the inflammatory process.

Strontium Enriched Hydroxyapatite-70 and Tricalcium Phosphate-30 Ceramics Improve Local Bone Regeneration in Ovariectomized Osteoporotic Rabbits Femur

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Study Highlights. In our study 11 different bone sample groups from osteoporotic and healthy rabbits femoral neck area were analyzed. We found that Sr-HA70/TCP30 biomaterials induce most noticeable local changes of bone regeneration.

Background. Sr can incorporate into ion exchange during bone remodeling via similarity to calcium ions, which allows Sr to affect some osteoblast and osteoclast mediated processes and improve bone regeneration.

Material and Methods. Osteoporosis was induced in 36 female rabbits (ovariectomy and glucocorticosteroid course). Bone defect in femoral neck area was filled with hydroxyapatite 30% (HA) and tricalcium phosphate 70% (TCP) (n=7), 5% Sr-enriched HA30/TCP70 (n=7), HA70/TCP30 (n=8) and Sr-HA70/TCP30 (n=7) granules. Sham surgery was performed in 6 rabbits. Bone samples from both legs were obtained 12 weeks after surgery. Ten healthy rabbits composed a control group. Bone samples were analyzed by histomorphometry. Immunohistochemistry and semi-quantitative analysis were done to evaluate expression of osteoprotegerin (OPG), nuclear factor kappa beta 105 (NFkB-105), osteocalcin (OC), bone morphogenetic protein 2/4 (BMP-2/4), collagen I (Col-1α), matrix metalloproteinase 2 (MMP-2), tissue inhibitor of matrix metalloproteinase 2 (TIMP-2), interleukin 1 (IL-1) and interleukin 10 (IL-10).

Results. Our study showed that Sr-HA70/TCP30 induced higher expression of all above-mentioned factors compared to intact leg and even higher expression of OC, MMP-2 and NFkB-105 compared to Sr-HA30/TCP70. HA70/TCP30 induced higher level of NFkB-105 and IL-1 compared to HA30/TCP70. Sham group showed higher expression of only Col-1α compared to operated leg. Sr-HA70/TCP30 showed higher amount of OC, NFkB-105, BMP 2/4, TIMP-2, IL-1 and Col-1α immunopositive osteocytes compared to healthy bone. Expression of OPG positive osteocytes were higher compared to pure ceramics and sham bone samples, while similar expression of OPG was found compared to Sr enriched granules.

Discussion. None of the previously published studies on Sr-enriched biomaterials provide more than two experimental groups and there is a lack of osteoporotic conditions in most of the studies. Therefore, we focused on simulating an analogous level of osteoporosis similar to postmenopausal bone disease. We found an increase of OPG after implantation of Sr-enriched HA/TCP granules compared to pure HA/TCP. The level of OPG was even higher than in healthy bone. It is important to emphasize the OPG role in the bone remodeling, because it decreases bone resorption activity, which is the dominant action in osteoporosis.

Conclusion. Sr-HA70/TCP30 increased expression of OPG, OC, NFkB-105, BMP-2/4, MMP-2, TIMP-2, Col-1α, IL-1 and IL-10 compared to intact leg bone and improved mineralization, extracellular matrix turnover and cellular activity in comparison to Sr-HA30/TCP70. However, less considerable changes of analyzed factors were found between Sr enriched, pure ceramics and sham in osteoporotic bone after 12 weeks of implantation.

Chronic Inflammation, Remodeling and Antimicrobial Defense Aspects of Copd Morphopathogenesis

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Study Highlights. Local findings of more pronounced inflammation, remodeling and antimicrobial defense associated markers of IL-1α, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-α, MMP-2, TIMP-2, TGF-β1, hBD-2, and hBD-3, whereas mostly low numbers of Hsp-70 and hBD-4 immunoreactive cells within the airway wall provide valuable information and support the relevance of these biomarkers in the morphopathogenesis of COPD.
Background. Intercellular signaling networks with high complexity cause a spectrum of mechanisms achieving COPD that still question many uncertainties.

Materials and Methods. Immunoreactive cells in bronchial tissue obtained from 40 COPD patients and 49 healthy control subjects were detected by biotin-streptavidin immunohistochemistry method for the following markers of IL-1α, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-α, MMP-2, TIMP-2, TGF-β1, Hsp-70, hBD-2, hBD-3, and hBD-4.

Results. In a healthy lung, we found moderately increased numbers of immunoreactive cells for all examined markers. Overall the highest numbers (from mostly moderate (++) to abundance (++++)) of IL-1α, IL-4, IL-7, IL-8, IL-10, IL-12, MMP-2, TIMP-2, and TGF-β1 immunoreactive cells were marked increasingly in the blood vessel wall, connective tissue and bronchial epithelium of COPD affected lung, respectively. However, bronchial wall glands and smooth muscle were the compartments with the lowest numbers of overall all examined markers. We found statistically significant ($p<0.05$) higher numbers of immunoreactive cells positive for all examined interleukins, TNF-α, MMP-2, TIMP-2, TGF-β1, hBD-2, and hBD-3 in the COPD affected lung compared to control group, but not for Hsp-70 and hBD-4.

Discussion. Moderately increased numbers of immunoreactive cells for all examined markers in control group indicate basal levels of various mediators released at relative health status. Various cells and their communication with other tissue structures design and shape signaling pathways to form local immunity in lung even at health status. Numerous studies explain COPD association with increased local expression of various mediators, like cytokines, remodeling, and regulatory factors, antimicrobial defense factors. Statistically significant ($p<0.05$) higher numbers of immunoreactive cells positive for all examined markers, but not for Hsp-70 and hBD-4 in COPD affected airways when compared to control group indicate activated numerous signaling pathways in COPD and promote the local significance of these markers in COPD morphopathogenesis.

Conclusions. COPD affected lung tissue exhibits mostly inflammatory response patterns of increased IL-1α, IL-4, IL-8, IL-12, and TNF-α, moreover, especially in the airway epithelium. Increased MMP-2 and TGF-β1, but decreased Hsp-70 proposes pronounced tissue damage and remodeling in COPD. High numbers of hBD-2 and hBD-3 immunoreactive cells may highlight antimicrobial activity in COPD within stable regulation of local immunity.

Effects of Fluorine and Boron on the Two Generations of Rats Teeth Enamel, Embryos Lower Jaw and Long Bones Formation

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Aim of the Study. Examine the effects of different fluorine and boron concentrations in drinking water on two generations of rats teeth enamel and their embryos lower jaw and long bones formation.

Materials and Methods. 25 female rats were divided into 5 groups depending on fluorine and boron concentration in drinking water: F3 – 3 mg/L, F12 – 12 mg/L, B3 – 3 mg/L, B12 – 12 mg/L and the control group (K) – received tap water. Two generations of rats were analyzed. 494 embryos were fixed in ethanol for analysis of ossification centers. Rats lower jaw front incisors were dissected and prepared for histological examination. The thickness of the enamel was measured every 120 μm (1689 measurements). The Kruskal–Wallis (for bones data analysis) and ANOVA and the Tukey's HSD tests (for enamel data analysis) were applied.

Results. In both generations, rats teeth enamel differed: B3 group (I – 21±2.4 μm, II – 48±1.8 μm) was thinner and B12 group (I – 61±11.7 μm, II – 734±158.2 μm) – thicker than control group (I – 33±2.3 μm, II – 481±46.8 μm). In B12, F3 and F12 groups of second generation significant thickening were observed compared to the first generation but only F3 and F12 groups were significant thicker than control group. In second generation of F3 (18.3±0.5 mm) and F12 (18.3±0.5 mm) groups lower jaw was significantly longer than in control group (17.7±0.5 mm). Increase of front limb bones in second generation of F12 group embryos were observed too. In B3 and B12 groups the bones ossified unevenly.

Conclusions. 1. Rats teeth enamel were thinned by 3 mg/L of boron in drinking water, while 12 mg/L of boron or 3 mg/L and 12 mg/L of fluorine significantly thickened enamel compared to control groups. 2. The increase of fluorine in drinking water causes not only thickening of the enamel but also an increase of the length of the embryos jaw and limb bones.

Worldwide Variation in Prevalence of Low Birth Weight, Prematurity and Stillbirth Rate in Relation to Geographic Region, Climatic, Socioeconomic Factors and Food Consumption

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Study Highlights. Low birth weight (LBW) is one of the most important biological predictors of infant's mortality, greater risk for poor health (cardiovascular diseases, metabolic syndrome, visceral obesity, type 2 diabetes, intellectual and neurological disabilities) in later life (Knop et al., 2018; Blencowe et al., 2019; Fall and Kumaran, 2019).

Background. There is a lack of knowledge on changes in prevalence of LBW, prematurity and stillbirths in global Lithuanian population of newborns, hence, the aim of present study was to analyse general changes of these indices in global Lithuanian population of neonates during the last decades, to compare local and worldwide situation in relation to geographic, climatic, socioeconomic factors and food consumption.

Material and Methods. Data on LBW newborns (n=30,664), preterm live-born newborns of 22-36 gestational weeks (n=33943) and stillbirth neonates (n=3,877) were analysed for the 1995-2015 period from Lithuanian Medical Data of Births. Lithuanian indices were compared with analogous data from the other 57 countries. Principal component analysis was used to reveal interrelationship between the country's prevalence in LBW, prematurity, stillbirths, also geographic, climatic, socioeconomic factors and food consumption.

Results. During the 1995-2015 period, the prevalence of LBW newborns in Lithuania fluctuated between 4.2% and 5.0%, the prevalence of prematurity – from 4.8% to 6.0%, the prevalence of stillbirths – from 0.46% to 0.72%. The prevalence of LBW worldwide (in 2015) fluctuated from 2.4% in Sweden to 27.8% in Bangladesh, the prematurity – from 5.2% in Kazakhstan to 19.1% in Bangladesh, the stillbirths – from 1.8% in Finland and Netherlands to 43.1% in Pakistan. The principal component analysis showed that first factor included the prevalence of stillbirths, also the main socioeconomic indicators of the country or region (Gross Domestic Product per capita, Inequality-adjusted Human Development Index), food (milk, meat, sugar, fruits) consumption and population size. However, LBW and prematurity were not connected into the first, but appeared under the influence of the second factor together with few geographic and climatic factors and population density.

Discussion. Most of the studies agree on growing importance of socioeconomic, demographic, environmental and behavioural factors related to LBW, prematurity and stillbirths (Kana et al., 2017; González-Jiménez and Rocha-Buelvas, 2018; Bansal et al., 2019). However, present study showed that stillbirths and LBW together with prematurity worldwide are influenced by slightly different main factors.

Conclusions. 1. During the last few decades, the prevalence in LBW, prematurity and stillbirths in Lithuania, were one of the lowest in the world. 2. Worldwide, stillbirths were more related to country's socioeconomic indicators, however, LBW and prematurity – more to region's geographic and climatic factors.

Making Stillbirths Visible: Changes in Indicators of Lithuanian Population During the 1995–2016 Period

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Study Highlights. Stillbirth is a global health problem. Adverse socio-economic status, lack of an education, low incomes are associated with an increased risk of stillbirth even in high-income European countries (Flenady et all; 2016).

Background. Comprehensive analysis on various aspects and possible factors of stillbirths in global population of newborns in Lithuania has never been done. The aim of the study was to determine socio-demographic and health related indicators of women whose pregnancies have finished by stillbirths during the 1995–2016 period, as well as physical indices of stillbirth neonates and the most common causes of their death.

Material and Methods. Data of 3877 stillbirths were selected from Medical Data of Births. Socio-demographic and health related indicators of mothers, physical indices of stillbirth neonates were analysed. Data were processed by MS Excel 2016, SPSS 21.0.

Results. The prevalence of stillbirths decreased from 0.72% in 1995 to 0.46% in 2016, $p<0.001$. Since the 2002, a decreasing tendency of foetal deaths at birth has been observed (2002 – 25.1%, 2016 – 15.8%, $p<0.05$). The mean age of mothers who delivered stillbirth newborns was 28.65 (±6.42) years. In comparison to the general population, significantly more women were over 35 years old (21.3% vs. 13.5%, $p<0.001$), had primary or secondary education (57.9% vs. 47.6%, $p<0.001$), were unmarried (23.8% vs. 17.9%, $p<0.001$); significantly more women smoked before pregnancy (20.8% and 8.2%, $p<0.01$) and during pregnancy (13.9% and 4.9%, $p<0.01$), also consumed alcohol (1.6% and 0.13%, $p<0.01$). The majority of stillbirths (65.6%) were preterm, of which approximately one third (34.9%) were less than 28 weeks of gestation. The mean birth weight of term and preterm stillbirth neonates was significantly lower than that of live born babies (3048±102.8 g vs. 3457±23.0 g, $p<0.001$; 1383±90.1 g vs. 2286±18.7 g, $p<0.001$). Slightly more than one quarter (26.2%) of stillbirth neonates weighed below 1000 g. In comparison to general population, these pregnancies were significantly more frequently complicated by genital infections, preeclampsia, bleeding during pregnancy, and the imminent miscarriage. Antenatal and intranatal hypoxia, caused by pathology of placenta or umbilical cord, were the most common reasons of foetal deaths.

Discussion will be presented.

Conclusions. During the 1995–2016 period, the prevalence of stillbirths in Lithuania has diminished, and since 2002, the prevalence of foetuses who died during the birth decreased as well. Advanced age of the mother at pregnancy, lower socio-economic status were risk factors for the stillbirth. The incidence of complications during the pregnancy which ended by stillbirth was higher than in the case of general population. The majority of stillbirth newborns were premature. The average birth weight of term and preterm stillbirth neonates was significantly lower than that of the general population.

Prevalence of Prematurity in Lithuania During the 1995–2015 Period in Relation to Maternal Age, Antenatal and Perinatal Pathologies

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Study Highlights. In 2014, approximately 10.6% of all live births globally were preterm. Prematurity was the leading cause of death worldwide for children under 5 years of age in 2016. The rate of preterm births has increased worldwide, predominantly, because of artificially conceived multiple pregnancies, however, the variety of causes of preterm births are not fully understood.

Background. Detail analysis of possible factors related to prematurity in global population of newborns in Lithuania was not preformed. The aim of this study was to analyze the prevalence of preterm births in Lithuania during the 1995 to 2015 period in relation to sub-category of prematurity, maternal age, antenatal and perinatal pathologies.

Material and Methods. A retrospective cohort analysis of Lithuanian Medical Data of Births from 1995 to 2015 included 33 943 live-born newborns of 22–36 weeks of gestational age (GA). The dynamics of singleton, twin and multiple pregnancies were analyzed with respect to sub-category of prematurity based on GA according WHO, the changes of maternal age, also antenatal and perinatal maternal pathologies.

Results. During the last two decades, the prematurity rate in Lithuania varied from 4.8% in 1997 to 6% in 2013. The birth rate of moderate to late preterm newborns for singleton, twin and multiple pregnancies was higher than birth rate of extremely ($p<0.01$) and very preterm ($p<0.05$) newborns during the study period. Mothers of singleton preterm newborns were older in 2015 (29.56±5.66 y) than in 1995 (26.22±6.24 y) ($p<0.05$). Similar results were obtained for mothers of preterm twins ($p<0.05$) and multiples ($p>0.05$). Very weak negative correlation between mother's age and newborn's GA was revealed ($r=-0.04$, $p<0.05$). Decreasing trend in the clinical occurrence of urinary infections ($p<0.05$), premature rupture of membranes ($p=0.002$), threatened miscarriage ($p<0.01$), anemia ($p<0.05$), genital organs' infection ($p<0.01$) was obtained from 1995 to 2015. The upward trend of previously diagnosed infertility, stimulated ovulation and artificial fertilization was revealed.

Discussion. The established mothers' ageing trend in Lithuania was mostly observed in extremely preterm and multiple pregnancies. Though the prevalence of maternal pathologies was decreasing, challenges of artificially conceived pregnancies greater attention to preterm births.

Conclusions. 1. Prevalence of preterm births in Lithuania during last two decades was similar to high-income countries. 2. Moderate to late preterm newborns represented the major proportion of newborns between singleton, twin and multiple preterm pregnancies. 3. The age of mothers of premature newborns was rising both with the study years, and the number of fetuses. 4. The GA of preterm newborns decreased with the rising age of mothers. 5. The occurrence of antenatal and perinatal maternal pathologies had a diminishing trend, while artificially assisted pregnancies increased.

Premenstrual Syndrome of 15-25 Year Old Lithuanian Females in Relation to Lifestyle Peculiarities

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Highlights. Various lifestyle peculiarities (smoking, alcohol consumption, exercising, eating and sleeping habits, caffeine use) in relation to PMS were analyzed (Rad et al., 2018; Acikgoz et al, 2017; Tsai et al, 2017). However, results vary across the countries.

Background. PMS in relation to lifestyle peculiarities was not studied in young Lithuanian females. The aim of this study was to analyze the severity of PMS in relation to lifestyle peculiarities (smoking, alcohol consumption, physical and sexual activity) of adolescent and young adult Lithuanian females.

Material and Methods. Present study was conducted on 15-25 years old females, not using oral contraceptives at the moment of filling the questionnaire (N=5448). An anonymous online questionnaire was designed to evaluate 10 most common symptoms of PMS using a 5-point Likert scale (1–never, 5–always occurring). Several lifestyle peculiarities were investigated: smoking, alcohol consumption, physical and sexual activity. IBM SPSS Statistics 24 and Microsoft Excel were used for statistical analysis.

Results. Severity of PMS differed statistically significant among women of diverse frequency of smoking (p=0.001), alcohol consumption (p=0.0001) and sexual activity (p=0.004). No significant relationship between physical activity (p=0.293) and PMS symptoms was found. The most severe PMS symptoms among 15-20 years old females were experienced by those, who smoked less than 10 cig. per week (M=3.70; SD=0.72), consumed alcohol few times a month (M=3.68; SD=0.697) and were sexually active 2-3 times per month (M=3.72; SD=0.675), while the mildest symptoms were felt by women who were non-smoking (M=3.57; SD=0.744), not consuming alcohol beverages (M=3.51; SD=0.762), and sexually active less than once per month (M=3.57; SD=0.75). Among 21-25 years old females, the most severe PMS were felt by those who smoked <10 cig. per day (M=3.70; SD=0.687), consumed alcohol once per week (M=3.73; SD=0.71), and were sexually active 2-3 times per month (M=3.68; SD=0.721), while the mildest symptoms were felt by women who smoked more than 10 cig. per day (M=3.57; SD=0.757), were not consuming alcohol (M= 3.47; SD=0.8), and were sexually active 2-3 times per week (M=3.56; SD=0.748).

Discussion. Heavy drinking and smoking is related to dysmenorrhea (Windham et al. 1999; Lyngso et al. 2014). Recently, it was proved that heavy alcohol intake might increase PMS risk as well (Fernandez et al. 2018), and our study confirms that. Positive effect of physical activity on PMS is known (Samadi et al. 2013; Zhang et al. 2014), however, our study did not confirm this relation, but sexual activity was related to PMS severity.

Conclusion. 1. Among adolescent females, the mildest PMS was felt by those who did not consume alcohol beverage, did not smoke, and were less sexually active (once per month). 2. Among young females, the mildest PMS was felt by those who did not consume alcohol, and were sexually active 2-3 times per week.

Premenstrual Syndrome of Older Adolescent and Young Adult Lithuanian Females in Relation to Body Size and Shape

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Highlights. There are many studies on various PMS aspects; however, studies on intensity of PMS in relation to body size (adiposity, body mass index) are scarce and controversial (Bertone-Johnson et al., 2010; Hong Ju et al., 2015; Mizgier et al., 2019). Besides, association between PMS and body shape were not established at all.

Background. Several small-scale studies have been conducted on PMS symptoms in relation to body size of 10-18 years old Lithuanian girls (Tutkuviene et al., 2011; 2015). However, there is a lack of larger-scale studies on PMS, body size and shape in adolescent and young adult Lithuanian females. The aim of this study was to analyse the association between intensity of PMS, body size and shape in older adolescent and young adult Lithuanian females.

Material and Methods. An anonymous online questionnaire was designed to evaluate 10 most common symptoms of PMS according to a 5-point Likert scale (1 – never, 5 – always occurring). Study was conducted including women from 15-25 years old, not using oral contraceptives at the moment of filling the questionnaire (n=5448). To determine body shape more accurately, pictures representing five female body types (apple, inverted triangle, hourglass, rectangle, pear) were provided. The height and weight data were self-reported. BMI was divided into six intervals: <18.5; 18.6-19.9; 20-24.9; 25-29.9; 30-39.9; >40. IBM SPSS Statistics 24, Microsoft Excel and R-Commander were used for statistical analysis.

Results. Severity of PMS differed statistically significant in women of diverse body type (p=0.001) and different BMI (p=0.015.) No correlation between PMS and height was found (r=-0.0009; p=0.96). The strongest PMS in women of 15-20 years old was detected for those having hourglass body type (M=3.66; SD=0.72) and BMI bigger than 40.0 (M=4.31; SD=0.51), while the mildest PMS in this age group was felt by women with rectangle body shape (M=3.49; SD=0.73) and BMI less than 18.5 (M=3.45; SD=0.75). In 21-25 year old females, the most intensive PMS was felt by men having apple-shaped body (M=3.66; SD=0.76) and BMI of 25-29.9 (M=3.67; SD=0.75), however, the weakest PMS in this age group was felt by women with rectangle body shape (M=3.53; SD=0.74) and BMI less than 18.5 (M=3.45; SD=0.75).

Discussion. This study confirmed few other studies that women with higher BMI tended to feel stronger PMS (Deuster et al., 1999; Bertone-Johnson et al., 2010); however, very recent study from Poland (Mizgier et al., 2019) has reported opposite results. Moreover, our results showed that women having apple-shaped body felt stronger PMS, and that might be related to metabolic disorders (Ofori et al., 2019).

Conclusion. 1. The mildest PMS in both age groups was felt by women with rectangle body shape and BMI<18.5. 2. The most severe symptoms of PMS were felt by adolescent girls having BMI>40.0, and apple-like body shape, while women aged 21-25 years felt strongest PMS having apple-shaped body and BMI of 25-29.9.

Premenstrual Syndrome in Young Lithuanian Females Passing From Adolescence to Adulthood in Relation to Education, Professional Status and Social Media Usage

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Highlights. Premenstrual syndrome (PMS) is related to fluctuating general health status of the majority of menstruating females. However, PMS studies are scares, there is no common consensus on PMS prevalence and possible factors. Recently, biopsychosocial etiology of PMS started to prevail (Zendehdel and Elyasi, 2018).

Background. PMS in relation to education and social media usage was not studied in larger-scale population of young Lithuanian females. The aim of present study was to analyze the prevalence of the most common PMS symptoms in large population of young Lithuanian females with respect to above mentioned factors.

Material and Methods. An anonymous online questionnaire (age, education, professional status, social media usage were recorded) was designed to evaluate 10 most common symptoms of PMS according to a 5-point Likert scale (1 – never, 5 – always occurring). Women from 15 to 25 years old, not using oral contraceptives at the moment of filling the questionnaire were included in this study (N=5448). IBM SPSS Statistics 24 and Microsoft Excel were used for data analysis.

Results. Severity of PMS differed statistically significant among women of diverse education (p=0.01), professional status (p=0.0001) and intensity of social media usage (p=0.0003). Most severe PMS among women of 15-20 years old was experienced by ones with higher education (M=3.74; SD=0.814), studying and working simultaneously (M=3.71; SD=0.671) and using social media every hour (M=3.67; SD=0.719). The mildest symptoms in this age group were felt by females with basic education (M=3.60; SD=0.728), not occupied at the moment (M=3.40; SD=0.872) and using social media once a day (M=3.31; SD=0.791). The most severe PMS among females of 21-25 years old was experienced by ones with higher education (M=3.67; SD=0.734), studying and working simultaneously (M=3.68; SD=0.725) and using social media every hour (M=3.65; SD=0.736). The weakest symptoms in this age group were felt by women with basic education (M=3.37; SD=0.833), not occupied at the moment (M=3.48; SD=0.809) and using social media few times a day (M=3.52; SD=0.762).

Discussion. Stressful everyday routine may have a negative impact on PMS severity (Deuster et al., 1999). A positive effect of social-media-based support in females with PMS was noticed recently (Nam and Cha, 2019). Our study showed that young adolescent females felt much stronger PMS than young adult females, besides, extreme use of social networks and stressful everyday routine was related to the severity of PMS symptoms in all young females.

Conclusions. 1. Overall, women of 15-20 years old felt more severe PMS symptoms in comparison to 21-25 years old females. 2. The mildest PMS' symptoms were experienced by non-occupied, having basic education and moderately using social media females. 3. The most severe PMS was felt by women with higher education, which were studying and working simultaneously and using social media extensively.

Evaluation of Body Fat in Preschool Children With Bronchial Asthma

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Study Highlights. To evaluate the differences in waist circumference, hip circumference and upper arm measurements in preschool children with bronchial asthma (BA).

Background. World Health Organization (WHO) recommends using body mass index (BMI) to evaluate nutrition in patients of all ages. However, there are more factors, like skinfold (SF) thickness and SF derived parameters, that help determine the body composition and nutrition.

Material and Methods. The study group consists of 42 children (23 boys and 19 girls), age 5 to 7 years, with BA, but no other chronic conditions. The control group includes 85 children (44 boys and 41 girls), age 5 to 7 years, without any chronic conditions. The measurements taken were body height, body weight, hip circumference, subscapular SF, abdominal SF, triceps SF and biceps SF were measured. Using these measurements in formulas, the following parameters were determined: BMI, percent body fat (%BF), pediatric body adiposity index (BAIp), central to total subcutaneous fat ratio, total upper arm area (TUA), arm muscle area (AMA), and arm fat area (AFA).

Results. The mean age of the girls was 6.03±0.82 years; in boys 6.13±0.69 years. All the mean measurements were determined in both genders of the study and the control group. The Mann-Whitney U test was performed to compare means.

There was no statistically significant difference in BMI and%BF between study and control groups of both genders ($p>0.05$). However, the study groups of both genders showed a statistically significantly larger BAIp ($p<0.05$).

In girls, the subscapular SF and abdominal SF were statistically significantly larger in the study group ($p<0.05$ and $p=0.05$, respectively).

In boys, only the subscapular SF was statistically significantly larger in the study group ($p<0.05$).

Central to total subcutaneous fat ratio in girls was higher in the study group ($p<0.05$); in boys the difference was not statistically significant ($p>0.1$).

In girls, TUA and AMA were larger in the study group ($p<0.05$)

In boys, TUA, AMA and AFA were not statistically significantly different between the study and control groups.

Discussion. In determining body fat, %BF has no advantage at identifying children who are at increased risk of cardiovascular disease based on different blood biochemical values (Freedman et al, 2013). BAIp, however, is valid for population studies, and cannot be applied individually (El Aarabaoui et al, 2013). The present study several higher body fat values in preschool girls with BA, compared to healthy girls, which is consistent with previous studies (Wadden et al, 2018; Umlawska, 2015).

Conclusions. In preschool children with BA, the BAIp is larger than in healthy preschool children. There are no differences between preschool children with BA and healthy children in BMI and %BF. In preschool girls with BA, the central subcutaneous fat measurements are higher than in healthy girls.

Prevalence of Posture Symmetry and Type According to Sedentary Behavior Among Pre-School Aged Children in Riga

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Study Highlight. The asymmetrical posture was found in 76.1% of children included in the research. Disturbances of posture type were found in 58.7% of children. Symmetrical posture and standard posture is more common in children watching TV less the 1 hour a day or not using a computer.

Background. The tendency to sedentary behavior has become common among pre-school aged children. Sedentary time promote psychosocial and physical health, including the changes of posture. The objective of our research was to analyze the posture in pre-school aged children according the sedentary behaviour - watching TV, using a computer.

Material and Methods. In research 959 children were included of age 4 to 7 years. The questionnaire was filled up by parents afterwards the measurements were performed. In the questionnaire there was asked about the time of watching TV and using computer during the daytime. The body height, posture type and symmetry were analyzed from the measurement data.

Results. In the research 53.3% (n=511) girls and 46.7% (n=448) boys were included. Symmetric posture was identified for 23.9% (n=229) children but asymmetric posture for 76.1% (n=729) children. Standard posture was found in 41.3% (n=396) children, disturbances in posture was found in 58.7% (n=562) children. Standard posture was found in 36.5% (n=172) children using a computer compared to the children not using a computer – 46.2% (n=224). Symmetrical posture was found in 21.4% (n=101) for children using computer opposite to children not using computer - 26.4% (n=128).

Standard posture was found in 40.4% (n=180) for children watching TV over 1 hour a day opposite to children watching TV less than 1 hour a day - 42.3% (n=216). Symmetrical posture was found in 22.9% (n=102) for children watching TV over 1 hour a day compared to children watching TV less than 1 hour a day - 24.9% (n=127).

In the group of children watching TV over 1 hour a day and using a computer the symmetrical posture was found in 15.8% (n=29) (p=0.003). In the group of children watching TV less 1 hour a day and not using a computer the symmetrical posture was found in 24.7% (n=55) (p=0.890). In the same group of children watching TV over 1 hour a day and using a computer standard posture was found in 37.0% (n=68) (p=0.207). In the group of children watching TV less the 1 hour a day and not using a computer the standard posture was found in 50% (n=112) (p=0.002).

Discussion. In Latvia this is the first study of several somatometric and morphofunctional measurements in the group of the pre-school age children. The results of our data showed that the sedentary behavior in children aged 4 to 7 years scause disturbances in symmetry and type of posture.

Conclusions. Children watching television for more than 1 hour a day are more likely to experience disabilities of posture. Computer use has a significant effect on the development of disturbances of posture type among pre-school aged children.

POSTER ABSTRACTS

Laterality of Human Body: Is it Really Related to Intelligence?

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Study Highlights. There are many speculations as concerns the relationship between laterality of human body and intelligence, besides, crossed-laterality for a long time was related to poor intelligence and worse academic abilities. Although very last studies are denying these statements, still there are discussions with respect to relationship between laterality of human body, intelligence and health. Besides, leg dominance in relation to intelligence still is purely understood.

Background. The aim of present research was to establish the prevalence of handedness and leg dominance (footedness) in 18-23 years old males and females in relation to inherited non-verbal intellect evaluated by the test of Raven's Progressive Matrices (RPM), and with respect to gender, birth status (full-term or premature birth), hand and foot injuries, also regular sporting.

Material and Methods. In total, 104 males and females of 18-23 years of age were investigated. All participants filled in an anonymous questionnaire (age, gender, height, weight, sporting habits, injuries of hands and legs were recorded). Handedness was determined using R. C. Oldfield (1971), and leg dominance – Waterloo (Elias et al., 1998) questionnaires. In order to evaluate inherited intellect, all participants underwent test of Raven's Progressive Matrices (RPM). Microsoft Excel and IBM SPSS 23.0 programmes were used for data analysis. We compared means of RPM in different groups and frequency of questioned features in different groups as well.

Results. There were 50.96% (n=53) participants in the same-laterality groups: right-handers right-footers (43.27%; n=45), left-handers left-footers (4.8%; n=5), both-handers both-footers (2.88%; n=3). There were only 1.92% (n=2) of participants in the crossed-laterality groups: right-handers left-footers (n=1) and left-handers right-footers (n=1), while the rest of participants (47.11%; n=49) did belong to the mixed groups: right-handers both-footers (41.34%; n=43), left-handers both-footers (4.81%; n=5), both-handers right-footers (0.96%; n=1). Right-handers had significantly higher RPM test results (M reached 53.37 percentile) than left-handers (M – 34.27 percentile); p=0.026. Both-handers had significantly higher RPM test results (M reached 65.50 percentile) than left-handers; p=0.011. RPM test did not differ between same-laterality, crossed-laterality and mixed groups (p>0.05). Frequency of hand and foot injuries, regular sporting, premature birth status did not differ in the same-laterality and mixed (crossed) laterality groups, also between right-handers, left-handers and both-handers as well (p>0.05).

Discussion. There is no consensus as for the relation of human intelligence and laterality of the human body. Present study is unique, because non-verbal, inherited intellect (determined by RPM test) was investigated in relation to handedness and footedness. Further and more extensive studies are needed in order to objectively determine the relationship between intelligence and crossed-laterality of human body, also with respect to general health status.

Conclusions. 1. The majority of investigated young people was right-handed, 10% - left-handed and only few percent – both-handed; almost a half of youngsters were right-footed and only few percent – left-footed. 2. RPM test was significantly higher in right-handers than in left-handers, also it was higher in both-handers than in left-handers. 3. Same-laterality and mixed-laterality groups had no significant differences in RPM results. 4. Laterality was not related to frequency of hand or foot injuries, regular sporting and premature birth status, however, further studies are needed to confirm or deny these pilot results.

Differential Diagnosis for an Adrenocortical Non-Adenoma: A Case Report

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Study Highlights. Tumours of the adrenal gland are mostly represented by adenomas, pheochromocytomas and adrenocortical carcinomas. In contrast, here we report an unusual case of a primary vascular tumour within adrenal gland.

Background. Adrenal gland is an endocrine gland composed of the cortex and medulla, each having different morphological structure and function. Benign tumours represent 70–85% of adrenal neoplasms. Most of these benign tumours within adrenal gland are adenomas, either hormonally active, or non-active. About 20% of all benign adrenal tumours originate from neural crest cells (Majbar et al., 2014). Primary vascular tumours in adrenal gland are rather uncommon (Zheng et al., 2018). Vascular neoplasms infrequently become symptomatic or hormonally active (Arkadopoulos et al., 2009; Ng et al., 2008).

Materials and Methods. Here we present a rare case of a primary vascular adrenal tumour in order to increase awareness of the broad differential diagnosis of adrenal mass lesions.

Results. A 53-year-old female was brought to a hospital because of abdominal pain in the left middle quadrant. Ultrasonography disclosed an unspecified mass between spleen and pancreas. Emergency computed tomography (CT) was suspicious for adrenal tumour showing non-homogenous appearance by contrast enhancement. Adrenalectomy was done, and tissue material was submitted for pathology evaluation with the clinical suggestion of hormonally inactive left adrenal tumour, possibly pheochromocytoma. Grossly, a smooth mass was evident, measuring 8.3×6.8×6.5 cm. It was dark red, showing yellow spots on the cut surface. Histologically, it contained dilated vascular spaces that were lined with flat endothelial cells and filled with erythrocytes. Fibrosis of vascular walls, as well as widespread central necrosis was found. There was preserved atrophic adrenal tissue in periphery. Immunohistochemistry was performed and revealed absence of pancytokeratin CK AE1/3AE, positive expression of CD31 and CD34, as well as Ki-67 of 5–7%.

Discussion. Cavernous haemangioma is a typical tumour in the skin and liver, being rarely found within adrenal gland. However, it is clinically significant because of the possible complications, including rupture and haemorrhage, as previously described by Forbes in 2005. Due to the mass effect, radiologic findings, the significant size and benign growth pattern of the neoplasm, in the presented case it could be removed to exclude malignancy and/or pheochromocytoma that also can have similar presentation (Machairas et al., 2018).

Conclusion. Primary adrenal neoplasms have a broad differential diagnosis. Histological diagnosis is of utmost importance to prevent unnecessary overtreatment.

Dissection - the Tool Improving the Quality of Anatomy Studies

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Highlights. The dissection of human bodies and organs systems is one of the tools to study the structure of human. Students are able to observe the location of the organs in situ, evaluate the positional relationship of organs, and compare their individual, gender, and aged features. Students, who were acquainted with systemic anatomy during dissection were developed the holistic understanding about human body and made sense of permanent anatomy knowledge.

Background. To review the significance of the human body dissection in anatomical curricula in the Institute of Anatomy.

Materials and Methods. We reviewed the dissection curricula in 2014-2019.

Results. In 2014-2019 were dissected 22 human bodies in the Institute of Anatomy. The first year students of Medicine and Odontology faculties dissected bodies and organs according the study requirements. In "Locomotion", "Homeostasis and Excretion", "Respiration and Circulation", "Reproduction and Inheritance", "Basics of Neurosciences", "Immune response", and "Intensive Care and Emergency Medicine" modules the second, third and fifth year students were dissected the human bodies and systems, which were related to the module's tasks. In the dissection course students were used the guidelines for dissection and for practical works students had self dissection tasks.

Students of Medicine, Odontology, and Nursing faculties were well cared for dissection on their elective studies in the Institute of Anatomy. Every year surgery and orthodontics residents were improving their dissection skills in the Institute of Anatomy. Beside course work students had possibility to dissect in extracurriculum time, the number of interested students increases strongly. Students were prepared the anatomical specimens, which were used for anatomy teaching and stored in the anatomical museum.

Discussion. Our described curricula coincided with the opinion of I. Memon (2017), who did overview of different undergraduate and postgraduate medical curricula, elective courses and voluntary activities across the world. He was concluded - as dissection is a time-consuming activity it is still essential part of anatomy studies, and is important for the training of future competent professionals.

Conclusions. The dissection of human bodies in the curriculum of anatomy studies need to be presented in acceptably way and it is the integral tool for learning anatomy in the Institute of Anatomy.

Sodium Dichloroacetate Effect on Glioblastoma U87 MG Tumor Growth and Invasion into Chicken Embryo Chorioallantoic Membrane

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Study Highlights. Sodium dichloroacetate (DCA) treatment has a significant impact on glioblastoma U87 MG cell line tumor (GB) growth and invasion into the chicken embryo chorioallantoic membrane (CAM).
Background. Glioblastoma is the most common and lethal brain cancer, is highly resistant to standard therapies. DCA is a pyruvate dehydrogenase kinases inhibitor; it stabilizes the pyruvate dehydrogenase complex escaping pyruvate dehydrogenase deficiency, inhibits NKCC1, enhances the mitochondrial function. The study aim was to evaluate the DCA effect on GB growth and invasion into CAM.

Materials and Methods. Fertilized Cobb-500 chicken eggs were incubated at 37°C and 60% relative air humidity. A window was opened in the shell of the egg on the 3rd day of embryo development (EDD3) and covered with a sterile transparent tape. The GB was grafted onto CAM on EDD7. The study groups were as follows: control (non-DCA-treated GB, n=17), 5 mM DCA-treated (n=14), and 10 mM DCA-treated (n=14). GB growth, neoangiogenesis, and its invasion into CAM were assessed through EDD9–12 by biomicroscopy in vivo. At EDD12 the specimens were harvested, fixed in a 10% formalin solution and embedded into paraffin; 3 μm sections were stained with H–E for morphological study. The p53, EZH2, PCNA expression in GB were determined by immunohistochemistry.

Results. Neoangiogenesis was induced in non-DCA-treated GB and was inhibited by the DCA treatment. The DCA treatment significantly reduced tumor invasion frequency into CAM: it was a 12-fold lower in 5 mM DCA-treated ($p<0.0001$) and 1,7-fold in 10 mM DCA-treated ($p=0.044$) compared with control. A significant p53 protein expression decrease was found in both DCA-treated groups ($p=0.008$ and $p=0.017$ respectively). The EZH2-positive cells in control were equally distributed in the whole GB area, while in 5 mM DCA-treated GB EZH2-positive cells were located more in the GB periphery. The treatment with 5mM DCA caused a significant decrease in EZH2 expression ($p=0.004$). The DCA treatment caused a significant tumor cell proliferation decrease: the lower PCNA expression was determined in both DCA-treated groups ($p<0.0001$ and $p=0.002$ respectively) with no significant difference between DCA-treated groups.

Discussion. The DCA treatment suppresses the neoangiogenesis and tumor cell proliferation in GB and interferes with p53 and EZH2 molecular pathways in GB progression. DCA has a pharmacological effect in GB therapy.

Conclusions. DCA treatment significantly inhibited GB growth, its invasion into CAM, neoangiogenesis, and tumor cell proliferation.

The Reflection of Human Health Problems in Latvian and Lithuanian Folk-Songs

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Study Highlights. There are more than 1.2 million texts of Latvian folk-songs (Dainas) and 30,000 melodies and 11456 Lithuanian folk-songs (Fs) and 7005 melodies in "Lietuvių liaudies dainynas". First collections of Lithuanian Fs – 7000 texts, and 2000 melodies were recorded by A. Juška (1819- 1880). The collection of Dainas called "The Cabinet of Folksongs" is inscribed in the UNESCO Memory of the World Program. K. Barons (1835-1923) was a person who travelled and pick up most of the Fs in Latvia. Folk-songs are dedicated to all bright and sad events of human life like the birth, death, love, family, war, job, and also about health, sufferings, and their treatment. Researches of Fs about medical topics in Europe was done by J. Kupcis (1932), A. Maceina (1955), K. Arons (1999), D. Penkala-Gawecka (1995), F. Stefansson (2005), R. Saukand (2005), M. Zavjalova (2016) etc. More Fs there are about health healing but there are few publications about the manifestation of different diseases in Dainas (Fs) of Lithuania and Latvia.

Background. The aim of the study was to find out the variety of health problems discussed in Latvian and Lithuanian Fs.

Material and Methods. We have analyzed an available folk literature and Internet resource in Latvian, Lithuanian, and English languages. We found more than 1000 Fs in Latvian and Lithuanian Fs about different health problems but very few are translated in English and German (Auziņa-Szentivayi, 2018; Reza (1825), Katzenelenbogen (1935).

Results. Folksong tellers of Latvia and Lithuania were from all districts of both countries. In Fs we have found reflection of paediatric problems, young people and elderly diseases in various dialects and even different languages of one country. We have analyzed Fs about traumatology during the wars and even family conflicts. There are songs with advises not to climb up in trees, to use swings carefully, fire and beat injuries in family. From internal diseases mainly are described head ache and heart pain. From gynaecology and obstetrics there are mentioned sterility, delivery problems and bleeding during it. From infectious diseases we have analyzed Fs about sexual transmissive disease, cough and plaque. Without human diseases in folksongs there is reflection of bad habits of people many centuries ago: like smoking and alcoholism, and mental sufferings.

Discussion. Folk-song is still alive in all Baltic countries. Especially people feel them during Folk- song and dance festivals of Baltic countries. Texts of Fs are still used in modern music. During last 2 decades there are organized many ethnomedical symposia in Germany and in the Scandinavian countries.

Conclusions. Our study has proved that health problems in the past were so similar to today's diseases. Fs of Latvia and Lithuania recall the same sufferings of inhabitants. Folk-songs like messages were sent to our and next generations to inform people about different disease and how to avoid and treat them.

Cytokines, Antimicrobial Factors and Proliferation Markers in Cholesteatoma in Ontogenetic Aspect

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Complex characterization of cholesteatoma tissue proliferation, pro- and anti-inflammatory, local protective factors, in the ontogenetic aspect was studied in this work with an aim to find out those factors what are responsible for this benign tumour aggressiveness.

Background. Cholesteatoma is a benign lesion found in the middle ear. It is divided into three layers: cystic layer, matrix and perimatrix. Matrix contains the same layers as normal skin, perimatrix - mainly inflammatory cells which induces osteolytic process in surrounding bone. The ontogenetic changes in cholesteatoma is not yet understood. Also the complex research with correlation of different tissue factors is not done yet in cholesteatoma.

Material and Methods. Four cholesteatoma specimens were obtained from children (age 9-17), five cholesteatoma specimens - adults (age 23-75). Seven deep external meatal skin controls were obtained from 9 cadavers in collection of Institute of Anatomy and Anthropology. Besides routine histological slides also immunohistochemistry was provides to detect proliferation marker Ki-67, immunoregulatory cytokines IL–1 and IL–10, and defensins - HβD-2 and 4. Results were evaluated semiquantitatively and by help of Spearmen's coefficient and the Mann-Whitney test.

Results. Controls showed moderate number of Ki-67 positive cells, while in the cholesteatoma only up to few positive epitheliocytes were detected. HβD-4 immunoreactive cells didn't differ between the control and tumour tissue, while HβD-2 positive cells showed few positive epithelial cells in control with moderate number of cells in perimatrix. There were only occasional IL-1 positive cells in control, while the perimatrix of tumour demonstrated few to moderate number of such cells. Finally, IL-10 in controls showed moderate number of cells, but cholesteatoma matrix and perimatrix – moderate to numerous number of cells.

Discussion. Proliferation marker decrease in tumour tissue probably might suggest the specific stage of disease with decrease of cellular activity. Ununiform appearance of different defensins in cholesteatoma more likely connects to the specificity of tumour development. Pro- and anti-inflammatory balance seems to be very stable in the tumour tissue what is proved by correct their relation.

Conclusions. Cholesteatoma tissue demonstrated low appearance of Ki-67 proves the indistinct proliferation activity in the already developed tumour. From defensins, HβD-2, but not HβD-4, seems the most important factor for antimicrobial defence. The lower IL-1 and higher IL-10 positive structure relation indicates the correct balance between pro- and anti-inflammatory cytokines in cholesteatoma. Changes in tissue factors in cholesteatoma do not correlate to the age.

A Pilot Study of Tumors Formed Using A549 Lung Cancer Cell Line and Treated With Navp Behavior on Chicken Embryo Chorioallantoic Membrane

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Study Highlights. A549 cell line formed tumors on chicken embryo chorioallantoic membrane (CAM). Tumor growth and invasion depends on a different NaVP dose.

Background. Lung cancer is the leading cause of cancer-related mortality in the world. A549 lung cancer cell line is widely used in cancer research and tumor growth. Sodium valproate (NaVP), a histone deacetylase inhibitor, has been shown to display potent antitumor effects by inhibiting cell growth and proliferation. CAM is a useful tool to evaluate tumor growth, invasion and drug research.

Material and Methods. Fertilized Cobb-500 chicken eggs were used for the study. On day 3 of embryo development, a small window was drilled in the eggshell. 1×10^6 A549 cells supplemented with rat tail collagen and special medical sponge were transferred on CAM at day 7 of embryo development. Tumor growth were evaluated macroscopically days 9-12 of embryo development. At day 12 chorioallantoic membranes were collected, fixed in buffered formalin and paraffin blocks were made. CAM's were evaluated using H&E histological staining, statistical analysis was made. Four different groups were investigated: non-treated (control) group (n=11), 4 mM NaVP group (n=11), 6 mM NaVP (n=10), 8 mM NaVP (n=10).

Results. Tumors with adhesion to the CAM epithelium were formed on 90.1% cases in control group, 72.7% cases in 4 mM NaVp group, 70% 6 mM NaVP group and 70% 8 mM NaVP group. Invasion into CAM was observed in 27.3% cases in control group, but 6mM and 8 mM NaVP treatment significantly diminished it and it was detected only in 10% of cases. In vivo biomicroscopy was carried out on days 9–12 of embryo development. Neoangiogenesis in tumors was induced: the tumor was surrounded by newly formed blood vessels and a clearly expressed "spoked wheel" pattern was present.

Discussion. NaVP showed potential antitumor effect in our study. NaVP inhibits A549 cell growth and proliferation. Number of A549 cell tumors with adhesion to the CAM epithelium decreased when treated with NaVP.

Conclusion. Our pilot study showed a different A549 tumor dynamic on the chicken embryo chorioallantoic membrane, when treated with different NaVP concentrations. NaVP suppresses tumor growth and invasion when concentration is higher than 4mM.

Sacroccocygeal Teratoma With Features of Fetus in Fetu: A Report of Rare Case

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Study Highlights a rare case of sacrococcygeal mature teratoma with features of fetus in fetu (FIF).

Background. FIF is a rare congenital abnormality with an incidence of 1: 500,000 births that is characterized by the incorporation of one or more partially developed fetuses into of the body of a normally developed fetus. Fever than 200 cases worldwide have been reported. FIF is most commonly found intraabdominally. When it is located in sacrococcygeal region, FIF can mimic teratoma. It is distinguished from mature teratoma by the presence of axial skeleton with organized limbs and organs. FIF and teratoma may also coexists.

Materials and Methods. Clinical and histopathological data of autopsy case of radiologically diagnosed sacrococcygeal teratoma were reviewed in order to demonstrate rare pathology

Results. 33 year old patient had termination of pregnancy in 22nd week of gestation due to sacrococcygeal teratoma and polihydramnion found in last prenatal ultrasound scan. The patient delivered stillborn foetus which was sent to post mortem study. Department of Pathology received male baby weighing 903 g. Crown heel length was 28 cm. The external examination of baby revealed well-encapsulated sacrococcygeal external mass. The mass measured 9x8x6 cm and had a smooth, skin covered surface. By opening of surface, a fetiform structure in the middle of tumour was observed. The structure consist of well-developed lower limb with toes and upper limb with rudimentary, deformed wrist as well as part of vertebral column and intestinal tract. Around the fetiform structure multicystic tumour tissue were found. The lesion was completely external, no presacral component of sacrococcygeal teratoma was detected. No feeding blood vessels were identified. Histological investigation of the mass revealed a well differentiated teratoma with multiple tissue types including mesenchymal tissue, mature cartilage, partially calcified bone tissue, skin with the skin derivates, intestinal structures, lymphatic tissue and pancreas. A final diagnosis of sacrococcygeal teratoma with features of FIF was made.

Discussion. Clinically FIF can be differentiated from a teratoma by the presence of vertebrae and limbs, however there have been controversies regarding its differentiation from well differentiated teratoma. Spencer has suggested that an FIF must have one or more of the following conditions: 1) be enclosed within distinct sac; 2) be partially or completely covered by normal skin; 3) have grossly recognizable anatomic parts, 4) be attached to the host by a pedicle containing a few relatively large blood vessels. In our case we favour diagnosis of teratoma with features of FIF because of multicystic appearance in main tumour mass, sacrococcygeal localisation and no feeding blood vessels found.

Conclusions. Differential diagnosis of congenital sacrococcygeal mass lesions should include both teratoma and FIF, although these two entities can overlap.

Frequency and Morphological Spectrum of Multifocal Thyroid Neoplasms

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Background. Although thyroid cancer is comparatively rare, it is the most common endocrine malignancy, constituting 3% of cancer burden in women and 1% in men (Dal Maso et al., 2017). Furthermore, the incidence of thyroid cancer is steadily growing (Sui et al., 2017; Price et al., 2017). The treatment options for multifocal thyroid cancer have aroused heated discussions (Mazeh et al., 2011; Riss et al., 2012).

Aim. The aim of this study is to explore the frequency and morphological spectrum of multifocal thyroid neoplasms, diagnosed between 2012 and 2016 in a single local university hospital.

Methods. In a retrospective study, 945 patients with morphologically confirmed thyroid neoplasms were included Tumours were diagnosed according to the most recent classification (2017) and criteria issued by World Health Organisation (Lloyd et al., 2017). Statistical analysis included descriptive methods (mean±standard deviation), calculation of 95% confidence interval (CI) according to Altman et al., 2000 and Mann-Whitney test (IBM SPSS Statistics 23; Armonk, USA). $p<0.05$ was considered statistically significant.

Results. In the study, 1367 cases of thyroid neoplasms were identified, affecting 945 patients: 820 (86.8%; 95% CI=84.7–89.3) females and 125 (13.2%; 95% CI=7.1–18.9) males. Among them, 278 (29.4%; 95% CI=23.7–34.3) patients had 702 (51.4%; 95% CI=47.3–54.7) multifocal thyroid neoplasms.

The most common multifocal thyroid tumours were as follows: papillary microcarcinoma – 36.3% (95% CI=30.1–41.9%), follicular adenoma – 35.0% (95% CI=29.0–41.0%), papillary carcinoma – 18.1% (95% CI=11.3–24.7), follicular carcinoma – 5.8% (95% CI=1.3–13.3%), Hurtle cell adenoma – 2.3% (95% CI=4.9–8.9%); Hurtle cell carcinoma and medullary carcinoma –1.1% each (95% CI=5.9 – 7.9%).

Discussion. Multifocality of thyroid neoplasms is a significant factor influencing the choice of treatment (Mitchell et al., 2016). It is widely discussed in the context of papillary carcinoma (Wang et al., 2017) but less studied in regard to medullary (Essig et al., 2016), Hurtle cell (Lopez Penabad et al., 2003) or follicular tumours. Here we present a large study showing the frequency of multifocal thyroid tumours in the local population and the biological potential of the involved neoplasms. Considering the known geographic heterogeneity (Dal Maso et al., 2017), such data are valuable to personalise the diagnostic and treatment options for individual local patients.

Conclusions. 1. Multifocal thyroid neoplasms are common – they occur in 29.4% of patients with morphologically diagnosed thyroid tumours.

2. The most frequent multifocal thyroid tumours are papillary microcarcinoma (36.3%), follicular adenoma (35.0%) and papillary carcinoma (18.0%).

3. Since the most common thyroid tumours are papillary microcarcinoma and follicular adenoma, each thyroid nodule should be evaluated carefully. Biological potential of papillary microcarcinomas should be evaluated.

Variations of Basal Cell Carcinomas According to Gender, Age, Location and Histopathological Subtype on Head and Neck

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Background. Basal cell carcinoma (BCC) is the most common malignant cutaneous neoplasm with an annual incidence increasing worldwide. According to the literature and our observations, the age of patients affected by BCC has been shifted from 50 to 40 and even 30 years. The most common histopathological subtypes of BCC recognized are nodular, mixed, infiltrating, adenoid, micronodular, superficial, and basosquamous with the first two reported to be predominant (Koyuncuer, 2014). Progression of BCC is slow, and it rarely metastasizes due to its downregulated vascularization, however it often appears locally invasive and shows destructive growth. The recurrence rates of BCC reported by the literature are greatly varying – from 10 up to 67% (Lara, 2017), whereas the distinguished rates of residual tumors vary between 7% and 45% (Ríos-Buceta, 2007). It has been pointed out that despite sufficient progress achieved in diagnostics of BCC and progress made in its treatment options, recurrent, aggressive, and metastatic variants of the tumor still pose a significant challenge for the healthcare system.

Material and Methods. Seventy nine patients presented with BCC of head and neck treated prospectively in Department of Maxillofacial Surgery. In total 46 (58.2%) female and 33 (41.7%) male patients were enrolled. The age range was 32-95 years. The clinical data of patients were obtained with respect to duration and type of the lesion at the time of presentation, clinical features, anatomic location, and course of the tumor. The skin colors were assessed as I-III types according to the Fitzpatrick Classification Scale. Only fully excised primary and recurrent BCC with 10 mm deep indention into healthy tissue were used in this investigation. Sixty one of 79 (77.2%) were primary tumors whereas 18 (22.8%) – recurrent BCC. The histopathology of the tumor was assessed by two independent observers following the World Health Organization classification system for BCC. The disease relapse was monitored over a 2-year follow-up period. The diagnosis of BCC before surgical excision was confirmed dermoscopically when the classical features, including lack of pigment network and the presence of at least one of the following criteria were detected: ulceration, maple-leaf like structure, blue-gray globules, blue-ovoid nests, arborizing vessels and spoke-wheel structures.

Results. Among 79 patients, 15 (19%) presented with the nodular histopathological subtype of the tumor, 18 (23%) – superficial, 10 (12%) – infiltrative, 7 (9%) – micronodular subtype, and 29 (37%) – mixed subtype. The most frequent combinations of the mixed BCCs included nodular-infiltrative, superficial-nodular, and nodular-micronodular subtype. No statistical differences in sex distribution were found among histological subtypes (p=0.102). Analysis of the anatomical location lesioned confirmed that the nose and cheek were predominant regions affected by both primary and recurrent tumors constituting 36.7 and 29.1%, respectively. Furthermore, according to our study, the nose area was very susceptible to tumor recurrence – nine of 18 cases (50%). Furhermore, the prevalence of the mixed subtype in nasal recurrent BCC was very high – seven of 9 cases (77.8%).

Conclusion. In the present study, we assessed histopathologically the occurrence and distribution of 79 primary and recurrent BCCs developed in the head and neck region. We found that the gender distribution and the age at the time of diagnosis were similar comparing this study to former ones. According to our study, more aggressive mixed-type bccs were localized on the cheeks and nose and relapsed after surgical removal.

Furthermore, histopathological characteristics of BCC of the head and neck have changed over time, and new studies deepening our knowledge about the biology of BCC.

Anatomical Variations of the Levels of Origin of the Lateral Femoral Cutaneous Nerve

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Goal. The aim of the research was to trace anatomical variations of the levels of origin of the lateral femoral cutaneous nerve.

Materials and Methods. Fifty cadavers (100 sides) fixed in 10% formalin solution were dissected. The dissection comprised exposure and excision of the lumbar plexus, together with the roots of the lateral femoral cutaneous nerve (LFCN), followed by retrograde intraneural fascicular dissection using microsurgical instruments. Results. Several types of LFCN origin from the lumbar plexus were observed. Typically, the LFCN appeared as a single trunk arising from dorsal divisions of the ventral rami of the lumbar plexus. The most prevalent origin of the nerve was from the L2 and L3 roots (62 cases; 62%). The LFCN took an origin from the L1-L2 level in 16 cases (16%) and from the L2 nerve in remaining 12 cases (12%).

Conclusions. Considerable variability in the origin and the course of the LFCN was observed, which should be taken into account during clinical assessment of nerve lesions.

Correlation Between Bioelectrical Activity and Skeletal Muscle Tone in Relation to the Level of Pain Perceived in Clinical Trials

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Introduction. As is well known, the bioelectrical activity of the muscle is in close correlation to its resting tension and remains in close correlation with the severity of pain. The aim of the study was to examine the level of dependence between these parameters in patients with lumbar spine pain.

Material and Methods. The medical experiment was conducted among 180 patients of both sexes (67 women, 113 men) suffering from lower back pain. The surface electromyography (sEMG) and the Szirmai myotonometer as well as the VAS pain scale were used to evaluate the parameters studied.

Results. The more the resting muscle tone increases, the bioelectric activity increases. This in turn causes an increase in pain.

Conclusion. There is a direct proportional correlation between the resting muscle tone and its bioelectric activity and the level of pain experienced in patients with lumbar spine pain.

The Human's Auxiliary Organs of Vision in Historical Perspective

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Goal. The goal of the study was to investigate the development of knowledge about the anatomy of the auxiliary organs of vision in humans.

Materials and Methods. In order to carry out the research, queries were conducted in the resources of the Welcome Library and the national library of medicine. Also, other numerous historical sources and studies were analyzed.

Results. Knowledge about the organ of vision and its auxiliary apparatus has been developing since ancient times. Already Avicenna described dural sheath of the optic nerve. In the anatomical figures of De Humani Corporis Fabrica of Vesalius extraocular muscles are illustrated incorrectly, on the basis of the dissection of animals (the retractor bulbi muscle may be recognized in some figures). Eustachio was the first to correctly illustrate extraocular muscles. Oculomotor nerve was probably known to Herophilus (C335-280 BC), it can be also found in some plates of the treatise of Estienne. Credit of discovery of the trochlear nerve is given to Achillini (1520). Abducens nerve was first described as fourth pair nerves from brain by Fallopio (1561). Modern anatomical techniques (for example Sihler's stain) and imaging techniques allow to deepen the knowledge about the anatomy of structures located in the orbital area.

Conclusions. Knowledge about the anatomy of the eye and its auxiliary organs has been developing since ancient times and has always had great clinical significance.

Variability of the Cephalic Index in Modern Youth from Belarus of 16-17 Years Old

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Study Highlights. Regional features of the head shape in modern youth of 16-17 years old were revealed in our study.

Background. In Belarus, the process of brachycephalization of the shape of the skull in the local population was established during the second millennium AD, i.e. the head became more rounded, the cerebral part of the skull became shorter and wider. Now there is a tendency to debrachycephalization.

Material and Methods. In the 2000s anthropometric measurements of 246 youth of 16-17 years old were conducted in Miory, Vitebsk region (northern Belarus) and in Minsk (center of the country). For comparison, we used data on young men of 17 years from the southern part of Belarus (Pinsk, Brest region). Head length and width were measured, the cephalic index was calculated. The scheme of P. Broca was used for classify of the head shape. Pearson's chi-square test was used to evaluate differences between the percentages.

Results. Among Minsk youth, dolichocephalic and subdolichocephalic morphotypes are significantly more common than among their peers from the northern part of Belarus. So, dolichocephalic morphotypes are 5 times and subdochiocephalic morphotypes – more than 2 times more common among girls in Minsk (in both cases $p<0.05$). In young men, differences reach a significant level only in the case of subdolichocephalic morphotype (an almost 3-fold excess in Minsk residents; $p<0.01$). At the same time, brachycephalic forms have an advantage among young men of the Vitebsk region: brachycephals are found in 39.3% of cases against 17.8% of their peers from Minsk ($p<0.001$). In Minsk girls, both subbrachycephalic and brachycephalic morphotypes are recorded much less frequently than in girls of the north of Belarus ($p<0.05$ for subbrachycephalic morphotype). While brachycephalic morphotypes prevail among young men in the north and south of Belarus (51.9 and 39.3%, respectively), then in the central part of the republic the subbrachycephalic type is the leading one (32.7%). The differences reach the significance level only according to the brachycephalic morphotype (Minsk-Pinsk, $p<0.001$).

Discussion. Such differences can be caused by both epochal trends (debrachicalphalization) and environmental factors (migrations to a big city, panmixia, etc.). Minsk is the largest city in the republic, where people from all over the country migrate. In addition, the ecology of a large city can also influence changes in human morphology.

Conclusions. Young people aged 16-17 from the northern and southern regions of Belarus have the most common brachycephalic head shape. In the central region the subbrachycephalic morphotype is the leading one.

The Study of Hexose Transporters and Mucin in Birds Small Intestine

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Study Highlights. The aim of the study was to immunolocalize the hexose transporters GLUT-2 and GLUT-5 and to detect mucin subtypes and the density of goblet cells in the broiler's chicken duodenal and ileal epithelium.

Background. Although small intestine plays an important role in food digestion and absorption there is few information about localization of hexose transporters as well as of quantitative and qualitative differences of mucopolysaccharide mucin produced by the goblet cells of ingested food in birds small intestine.

Material and Methods. Material from duodenum and ileum was collected from 12 chicken (Gallus gallus domesticus) divided into two age groups, six birds in both groups: chicken immediately after hatching and 30 days old chicken. Specimen were fixed with 10% formalin, embedded into paraffin, slices 7 μm thick were cut followed by immunohistochemical staining with polyclonal primary antibodies Rabbit anti-GLUT-2 and Rabbit anti-GLUT-5 carried out according to the manufacturers quidelines (IHC kit, Abcam, UK). For identification of the epithelial neutral mucopolysaccharides periodic acid-Schiff method was used; for identification of the epithelial acid mucopolysaccharides Alcian Blue at pH 1.0 and Alcian Blue at pH 2.5- periodic acid-Schiff method were used.

Results. The duodenal and ileal enterocytes of both age groups stained strongly by GLUT-2 and GLUT-5. The intestinal goblet cells remained mostly unstained, some moderate staining was noted in goblet cells in 30 days old chickens' ileal epithelium. In 30th postnatal day number of both types of mucin containing cells was higher in ileum than in duodenum. From the 1st day after hatching to age of 30 days of life the density of goblet cells per area unit tended to increase in both segments of the broiler's small intestine.

Discussion. The integral membrane proteins GLUT-2 and GLUT-5 facilitate the transport of hexoses across epithelial cell layers that separate distinct compartments in organism. In the present study GLUT-2 and -5 expressed in duodenal and ileal enterocytes of equal strenght in the both studied age groups. As mucus layer coating the gastrointestinal tract serves as front line of innate host defense it is important to understand the differences of mucin produced by intestinal goblet cells. In our study increase of neutral and acid mucin containing cells per field of microscope-view in both parts of the small intestine was detected from first day after hatching towards 30 day of life.

Conclusions. The hexose transporters GLUT-2 and GLUT-5 were immunolocalized in the epithelium of broiler's small intestine. The quantitative and qualitative differences of mucus secretion noted in our study are probably connected to specific roles of the small intestine different segments during nutrients absorption.

Comparison of the Efficacy of Ultra-High and Low Temperature Processed Milk on the Viability of Periodontal Ligament Cells

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Backgraund. One of the main factors in a long term tooth prognosis after avulsion is selecting the right media for transportation. The ideal media should be able to maintain periodontal cells viable until replantation is possible and be easily obtainable in the event of trauma. One of the recognised mediums for avulsed teeth is milk. There are many types of differently processed milk and they all have distinct qualities.

Material and Methods. The aim of this in vitro study was to compare the efficacy of ultra-high and low temperature processed milk on the viability of periodontal ligament cells.

Forty mature extracted human teeth with healthy periodontal tissue were randomly and equally divided into three groups (n=10) according to the storage medium: ultra-high temperature processed milk (UHTPM), low temperature processed milk (LTPM), saline solution (control) and tap water (negative control). After extraction teeth were left to dry for 15 minutes simulating trauma, then placed in a storage media. After 30 minutes the scrapings of the periodontal ligament were collected in Falcon tubes containing 0.5 mf of collagenase in 2.5 mL of phosphate buffer saline and were incubated for 30 minutes and centrifuged for 5 minutes at 800 rpm. Cells' viability was analysed by Trypan blue exclusion. Statistical analysis was performed using SPSS 19.0 software by one-way ANOVA and Tuckey's Post Hoc tests.

Results. LTPM had a significantly higher number of viable cells compared to UHTPM, saline solution and tap water. There was no statistically significant difference between saline solution and UHTPM (p=0.947).

Many of the storage media may be considered ideal because of their cell reconstitution ability. However, the major disadvantage of these media is their unavailability. It is very important that the storage medium be easily available to allow replantation of avulsed teeth within time for better prognosis. Therefore, the present study was conducted to evaluate and compare the efficacy of LTPM and UHTPM as alternate storage media for avulsed teeth.

Conclusion. LTPM is able to maintain PDL cell viability of avulsed teeth better than UHTPM, saline solution or tap water.

Prognostic Impact and Correlations of Ki-67 Labeling Index and Cd44 Expression in Gliomas

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Study Highlights. In this study, we evaluated expression of CD44 and Ki-67 labelling index (LI) in glioblastomas (GBMs) and diffuse astrocytomas (DAs) as well as assessed their prognostic significance and mutual correlation.

Background. Ki-67 is strongly related to cellular proliferation. Scientists mostly agree on prognostic role of Ki-67 LI in DAs. Regarding GBMs, results are less conclusive (Yang et al., 2013). CD44 is involved in cell-matrix adhesion, cell migration and cellular signalling pathways (Dzvonek et al., 2015). In addition, CD44 has been identified as a marker of neural stem cells (Liu et al., 2004). Clinical impact of CD44 in gliomas has been debated (Amido et al., 2010). As there is no consensus on those markers, additional research is important.

Materials and Methods. In the current study, 146 and 19 patients, diagnosed with GBMs and DAs, resp., were enrolled. The expression of Ki-67 and CD44 was detected by immunohistochemistry. The presence of nuclear, cytoplasmic and membranous staining was assessed quantitatively as the relative number of positive neoplastic cells (%). Survival was evaluated by Kaplan-Meier test. For survival analysis, Ki-67 LI was classified into high vs low using cut-off of 25% for GBMs and 5.5% for DAs. For CD44, cut-off value of 50% was applied (Popova et al., 2014). Correlations were assessed by Spearman's rank test. Two-tailed $p<0.05$ was considered significant.

Results. The mean Ki-67 LI in GBMs and DAs was 44.4% (95% confidence interval: 41.1–47.6) and 6.4% (4.7–8.0), correspondingly. The mean CD44 expression in GBMs and DAs was 74.1% (69.6–78.7) and 13.5% (7.7–19.2), respectively. Ki-67 LI showed statistically significant survival difference only in DAs ($p=0.037$) but not in GBMs ($p=0.252$). There were no survival differences within GBM and DA groups, regarding the expression of CD44. Levels of CD44 and Ki-67 LI lacked correlation in gliomas.

Discussion. In the current study, Ki-67 was found to be a reliable indicator of worse prognosis in DAs, but not in GBMs. Although proliferation fraction is not included in latest World Health Organisation classification, assessment of Ki-67 may be useful to identify increased proliferative activity in DAs with otherwise typical low-grade appearance, because such cases of DAs might need more careful follow-up and have a worse prognosis. In this study, no prognostic role of CD44 was found in GBMs and DAs. However, according to Verhaak et al., high expression of CD44 indicates mesenchymal subtype of GBM, which lacks response to radiotherapy. Thus, it may have predictive role (Verhaak et al., 2010). In this study, expression of CD44 did not correlate with Ki-67 LI.

Conclusions:

1) Ki-67 LI shows no further prognostic significance in patients with GBMs, but it has prognostic role in DAs.

2) CD44 expression differs by glioma grade but lacks prognostic value within gliomas of the same grade.

3) There are no correlations between CD44 and Ki-67 LI in gliomas.

Ki-67 Labeling Index and Cd44 Expression in Gliomas: Does the Gender Matter?

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Study Highlights. In this study we evaluated gender-related differences of CD44 expression and Ki-67 labelling index (LI) in glioblastomas (GBMs) and diffuse astrocytomas (DAs).

Background. Gender differences in the incidence and outcome of human disease, including gliomas, are broadly recognized but in most cases not adequately understood. Regarding glial tumours, some scientists suggest that outcome and even molecular features of gliomas may differ between males and females (Trifiletti et al., 2016; Yang et al., 2017). There are no data regarding gender-specific assessment of Ki-67 LI and CD44 in glial tumours. As consensus is lacking on those aspects, an additional research is important.

Materials and Methods. In the current study, 146 and 19 patients, diagnosed with GBMs and DAs, respectively, were included. The expression of Ki-67 and CD44 was detected by immunohistochemistry in surgically removed tumour tissues. The presence of nuclear, cytoplasmic and membranous staining was assessed quantitatively as the relative number of positive neoplastic cells (%). Survival was evaluated by Kaplan-Meier analysis. To identify significant associations, Mann-Whitney U test was applied. Two-tailed $p<0.05$ was considered significant.

Results. GBM was diagnosed in 75/146 (51.4%; 95% confidence interval: 43.3–59.5) females and 71/146 males (48.6%; 40.5–56.7). DA was confirmed in 14/26 (53.8%; 34.6–72.7) females and 12/26 (46.2%; 27.0–65.4) males. In GBMs, there was a trend towards lower Ki-67 LI in males (Mann-Whitney U test, $p=0.056$). However, in DAs, Ki-67 LI was statistically significantly higher in males than in females ($p=0.010$). Regarding GBMs, significantly higher expression of CD44 was found in females ($p=0.026$), but there were no differences in DAs. No statistically significant survival differences were observed analysing median overall survival by gender.

Discussion. In the current study, important gender-related molecular differences were found in gliomas. Thus, Ki-67 LI were significantly higher in GBMs in males. Although such association between gender and Ki-67 LI is poorly evaluated in glioma research and the explanation is not clear, a growing number of studies have supported the role of sex hormones in pathogenesis of glial tumours (Sareddy et al., 2016). Regarding CD44 expression, significantly higher values were observed in GBMs in females. Since CD44 has been identified as a marker of neural stem cells (Liu et al., 2004), it may indicate that glioma stem cell population may be altered by gender-specific factors. In this study, no significant survival differences were found between genders.

Conclusions:

1) Both Ki-67 LI and CD44 showed an association with patient's gender, indicating existence of gender-specific molecular differences in glial tumours.

2) In contrast, there were no gender-related survival differences in glioma patients.

Differences in Some Parameters of the Greater Sciatic Notch of Human Hip Bone in Sex Determination

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Highlights. There are differences in components of the hip bone among populations and these variations are related to genetic, environmental factors and ethnicity. The measurements of the Greater Sciatic Notch (GSN) are commonly used to determine the sex of the individuals.

Background. The methods for human sex determination depends on the different bone morphologies presented by men and women. Objectives of this study were to take several measurements of GSN and to evaluate the role of these parameters in sex determination.

Materials and Methods. This study comprised 41 adult human dry hip bones (22 left sided, 19 right sided, 26 male hip bones and 15 female hip bones). The bones without damages and pathological conditions were obtained at random from the skeletal collection of the Laboratory of Anatomy of the Department of Morphology, Rīga Stradiņš University. The GSN was identified in all the hip bones and all measurements were repeated twice with the help of Verniear caliper. On each hip bone several points were marked as follows: the piriformis tubercle was taken as the posterior point (B) of the width (AB). The tip os the ischial spine was taken as the anterior point (A) of the width. Between the base line (AB) and the deepest point (C) of the GSN was determined maximum depth (OC). OB was observed as the posterior segment of width of the GSN. All measurements were made in millimeters and afterwards two indices were calculated: Index I (depth (OC)x100/width (AB)) and Index II (posterior segment (OB)x100/width (AB)). Data collected were tabulated, statistically analysed (IBM SPSS Statistics, version 22.0 was used to analyse the data using the independent sample t-test, Pearson's correlation test) and results were compared with the accessible literature.

Results. The measurements done on the right and left sided hip bone indicated that there was bilateral asymmetry of hip bone. There were noted significant differences in maximum width (AB) and maximum depth (OC) of the GSN between male and female hip bones. Significant differences were found in the Indices I and II of the GSN.

Discussion. Several authors had performed different data and various measurements on human GSN between sex and ethnic groups. The findings of this study confirm that GSN provides relatively high accuracy rates in estimating sex and these results related to the role of this feature obtained in previous studies.

Conclusions. Results suggest that assessment of the measurements of the GSN is very variable and it can be used in sex determination. In future several topics can be studied to broaden knowledge on GSN in sex estimation not only for Anatomists, but also for Anthropologists.

Th-POK Antigen Characteristics in Oral Leukoplakia

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Study Highlights. For oral leukoplakia (OL) transformation rate into oral cancer is from 1%-12% (Warnakulasuriya et al., 2011). Oral cancer cases in Baltic countries accordingly Globocan 2018 are 12.7 patients in Latvia; 6.9 in Lithuania and 6.4 in Estonia per 100,000 of population. Therefore it is important to examine OL as potentially premalignant disorder of oral mucosa (Wanninayake et al., 2019). One of the most controversial immunohistochemical markers is Th POK which has been found to be upregulated in some cancers, lymphomas and gliomas (Fei et al., 2018; Fuquan et al., 2019). It has been described in different tissues as protooncogenic and opposite as an oncosuppressor factor (Guo et al., 2014).

Background. Expression of Th-POK in oral mucosa is not surveyed enough (Sartini et al., 2015). The purpose of our study was to analyze the expression of Th-POK in OL of different its types.

Material and Methods. We have analyzed OL from 38 patients. 7 samples with normal non-keratinized oral mucosa were in the control group. Specimens were stained by hematoxylin eosin and Th-POK antigen detection was done. Intensity of immunohistochemical reaction was classified semiquantitatively as absent (0), weak (1), intermediate (2) and strong (3) but its expression was evaluated accordingly three levels: 1 (1-33%), 2 (34-66%), 3 (67-100% positive epithelial cells). Statistical analysis was done by GraphPad Prism 7.0 version software.

Results. In our research OL was mainly diagnosed in buccal mucosa (54.5%) and tongue (18.1%). The average age of patients was 52.9 years. From 38 OL 34 were conventional but 4- proliferative verrucous type. In control cases Th-POK antigen was expressed in suprabasal layers of oral mucosa, its intensity was from weak to intermediate and of 1st level expression with 27±5.6 labeled cells. In OL Th-POK was not detected in stratum basale but it was expressed in str. spinosum, granulosum and lucidum of oral mucosa. Its intensity varied from strong till weak but expression was mainly of 2nd level. Immunoreactivity with Th POK antigen was present in parakeratotic epithelial cells but lacking in stratum corneum. This marker clearly demonstrate nucleoli and chromatin changes. In OL number of labeled cells varied from 20% till 87% with higher amount of labeled cell in OL of proliferative verrucous type in areas of dysplasia. The average amount of Th-POK in examined OL were 43.7%±9.4%.

Discussion. Our study has proved that Th POK antigen is expressed in suprabasal layers of oral mucosa but is lacking in its cells of basal layer. Th POK is present in normal oral mucosa, its hyperplasia and dysplasia and oral cancer (Kleina et al., 2019).This marker is involved in the cell lineage fate decision (Lunardi et al., 2013).

Conclusions. Th-POK in OL is present not in progenitor cells but in differentiated cells of oral mucosa. It is a marker of proliferation and differentiation processes of oral epithelium and is the information carrier about possible transformation into oral cancer.

Age-Related Morphological Parameters of Wistar Rats' Testes

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Study Highlights. 1. The mean weight and volume of both testes increased with age.

2. The gonadosomatic index of testes was maximal in 2 months old rats.

3. The weight and volume of left testes increased with age. The weight and volume of right testes increased till 1-year-old and decreased in 2-year-old rats.

Material and Methods. This investigation was carried out according to the permission of the State Food and Veterinary Service (No. B1-135, 16.03.2017). Wistar male rats were divided into three age groups: 2 months (n=4), 1-year (n=5) and 2-year-old (n=6). Pairs of testes were obtained after autopsy. Rats' bodies and testes were weighed using KERN 440-21N balance. Volume was determined by water displacement of each testis. The gonadosomatic index was calculated according to the formula: GSI=(testicular weight/body weight)×100. The Statistica Version 5 (StatSoft inc.) program was used for statistical analysis. Data were expressed as mean±standard deviation, and $p<0.05$ was taken as significant.

Results. Body weight of 2 months old rats (317.5±14.82 g) differed significantly from 1-year-old (390.6±24.36 g) and 2-year-old rats (388.33±30.24 g; $p<0.05$).

The mean weight of testes in 2 months old rats was significantly smaller (1.18±0.6 g) than in 1-year-old (1.35±0.17 g) and 2-year-old rats (1.36±0.13 g, $p<0.05$). Weight of left testes was 1.23±0.4 g in 2 months old rats, 1.35±0.15 g in 1-year-old rats and 1.39±0.15 g in 2-year-old rats ($p>0.05$). Weight of right testes was 1.14±0.03 g in 2 months old rats, 1.35±0.21 g in 1-year-old rats and 1.33±0.12 g in 2-year-old rats ($p>0.05$). Left testes weighed more than right in the 2 months old ($p<0.05$) and 2-year-old rats ($p>0.05$). In 1-year-old rats, weight of left and right testes was identical.

The GSI of testes was maximal in 2 months old rats (0.75±0.04%). In 1-year-old rats, GSI decreased till 0.69±0.05% and then it increased marginally in 2-year-old group (0.7±0.06%, $p>0.05$)

The mean volume of testes increased with rats age (from 0.99±0.11 ml to 1.13±0.2 ml, $p>0.05$). Volume of left testes was 1.05±0.06 ml in 2 months old rats, 1.12±0.18 ml in 1-year-old rats and 1.2±0.2 ml in 2-year-old rats ($p>0.05$). Volume of right testes was 0.93±0.13 ml in 2 months old rats, 1.12±0.25 ml in 1-year-old rats and 1.07±0.2 ml in 2-year-old rats ($p>0.05$). Volume of left testes was major than right in 2 months and 2-year-old rats ($p>0.05$). In 1-year-old rats, volume of left and right testes was identical.

Conclusions:

1. The body weight of 2 months old rats was smaller than 1-year-old and 2-year-old rats.

2. The mean weight of testes increased with age. The weight of left testes increased with age. The weight of right testes increased till 1-year old, then decreased. The gonadosomatic index of the testes was maximal in 2 months old rats.

3. The mean volume of the testes increased with age. The volume of left testes increased with age. The volume of right testes increased till 1-year old, then decreased.

Ganglioglioma – A Rare Brain Tumour Associated With Long-Lasting Drug Resistant Epilepsy

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Study Highlights. Gangliogliomas are an increasingly recognized cause of epilepsy in children.

Background. Gangliogliomas are rare central nervous system tumours, which are strongly associated with epilepsy (Cully et al., 2018). We report two cases of gangliogliomas, clinically presenting with drug-resistant seizures.

Material and Methods. The medical documentation was retrieved retrospectively from the archives of Children's Clinical University Hospital, Riga. The microscopic and immunohistochemical features of the tissues were verified.

Results. The first patient was an 1-year-old boy, who presented to the Hospital with epileptic seizures. Despite anti-seizure therapy since that, at the age of 3, seizure frequency increased and magnetic resonance imaging (MRI) revealed right hippocampal mass, radiologically described as a dysembrioplastic neuroepithelial tumour (DNET). The patient underwent surgical tumour resection via pterional craniotomy. Histological examination revealed biphasic tumour of moderate cellularity with neuronal and glial components, as well as microcystic myxoid, well-vascularized stroma. Neoplastic glial component was positive for GFAP, neuronal component was diffusely positive for synaptophysin and neurofilament. There was mild nuclear positivity for p53 (15%). Proliferative activity (Ki-67) was low (2%). At the age of 5, prophylactic MRI examination revealed recurrent tumour. Second surgery was performed. Histological examination disclosed the same tumour- ganglioglioma with slightly higher proliferative activity (8%).

The second patient presented at the Hospital with complex partial seizures at the age of 3 years. MRI revealed arachnoid cyst. Treatment of epileptic seizures was ineffective. At the age of 8 years, MRI showed focal cortical dysplasia of the right hippocampus. Seizure frequency increased and at the age of 10, right temporal lobe resection was performed. Histological examination revealed well-differentiated glioneuronal tumour, infiltrating the amygdala and hippocampus. Tumour cells were positive for GFAP, MAP2, synaptophysin and CD34; proliferative activity did not exceed 4%. Histologically, focal cortical dysplasia was not found.

Discussion. Ganglioglioma is well-differentiated, slow-growing glioneuronal neoplasm of children and young adults with recurrence-free survival rate 97% and the mean duration of symptoms before the diagnosis 1.4 years (WHO, 2016). One of our cases showed recurrent tumour growth. In first of our cases symptoms lasted longer than 2 years till the surgical therapy and in the second case there were no MRI changes for 5 years. Low-grade gangliogliomas can be treated surgically, and complete tumour resection is the most effective treatment. Epilepsy associated with ganglioglioma, even if it is medically refractory, can be controlled with tumor resection.

Conclusions. Symptoms of ganglioglioma can last for years till the first radiological changes are found and surgical treatment performed.

Histopathological Heterogeneity and Location Variations Exhibited by Basal Cell Carcinoma of Head and Neck

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Study Highlights. The study highlights practical issues appearing when diagnosing basal cell carcinoma (BCC) of head and neck.

Background. BCC is the most common malignant cutaneous neoplasm. Manifesting as a heterogeneous tumor, BCC commonly presents as a nodular, mixed, infiltrating, adenoid, micronodular, superficial, and basosquamous histopathological subtype. The recurrence rates of BCC reported by the literature are greatly varying – from 10 up to 67% (Lara, 2017), whereas the distinguished rates of residual tumors vary between 7% and 45% (Ríos-Buceta, 2007). It has been pointed out that despite sufficient progress achieved in diagnostics of BCC and progress made in its treatment options, recurrent, aggressive, and metastatic variants of the tumor still pose a significant challenge for the healthcare system. The study brings evidence of the histopathological heterogeneity, recurrence, and location variations of BCC of head and neck.

Material and Methods. Seventy nine patients treated prospectively in Department of Maxillofacial Surgery presented with BCC of head and neck. In total 46 (58.2%) female and 33 (41.7%) male patients were enrolled. The age range was 32-95 years. The clinical data of patients were obtained with respect to duration and type of the lesion at the time of presentation, clinical features, anatomic location, and course of the tumor. Sixty one of 79 (77.2%) were primary tumors whereas 18 (22.8%) – recurrent BCC. The disease relapse was monitored over a 2-year follow-up period. Surgically removed tumor masses were fixed and processed conventionally.

Results. Among 79 patients, 15 (19%) presented with the nodular histopathological subtype of the tumor, 18 (23%) – superficial, 10 (12%) – infiltrative, 7 (9%) – micronodular subtype, and 29 (37%) – mixed subtype. The most frequent combinations of the mixed BCCs included nodular-infiltrative, superficial-nodular, and nodular-micronodular subtype. No statistical differences in sex distribution were found among histological subtypes ($p=0.102$). Analysis of the anatomical location of the lesion confirmed that the nose and cheek were predominant regions affected by both primary and recurrent tumors constituting 36.7 and 29.1%, respectively.

Discussion. We found that the gender distribution and the age at the time of diagnosis were similar comparing this study to former ones. Furthermore, according to our study, the nose area was very susceptible to tumor recurrence – nine of 18 cases (50%), and the prevalence of the mixed subtype in nasal recurrent BCC was very high – seven of 9 cases (77.8%).

Conclusion. In the present study, we assessed histopathologically the occurrence and distribution of 79 primary and recurrent BCCs developed in the head and neck region. Histopathological characteristics of BCC of the head and neck have changed over time, and new studies deepening our knowledge about the biology of BCC are acknowledged.

Levelling of Bisphosphonate Therapy-Associated Jaw Osteonecrosis by Trilon B

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Background. Up to now, the optimal strategies of non-surgical treatment for the patients with bisphosphonate therapy-associated jaw osteonecrosis caused by clandestine methamphetamine have not been developed. In this aspect, the use of a chelate compound Trilon B is of interest. This substance is known as the antidote for heavy metals poisoning.

The goal of this study was to correct the osteosclerotic changes in the mandibular bones of white rats by chelating compounds (Trilon B). The rats were under influence of non-narcotic impurity that has been formed during the clandestine production of the meta-amphetamine and had antiresorptive properties.

Materials and Methods. Experimental study was performed on 40 white outbred male rats weighing up to 200 grams. The animals were divided into 4 equal groups. The first and second groups consisted of the animals that received amino phosphonic impurity at a dose of 63 mg/kg 1 time per day for 30 and 90 days intragastrically. Then, these animals received Trilon B at a dose of 250 mg/kg once a day for a month intragastrically. Mandibles of rats were fixed for more than 72 hours in 10% neutral buffered formalin, decalcified in 14% water solution of trilon B, buffered to pH 7.0 with sodium hydroxide. Sections were stained with hematoxylin and eosin, according to Romanowsky-Giemsa, Masson-Goldner and alcian blue (pH 2.5) with iodic acid and Schiff's reagent (AC + Schiff-reaction). The morphometric study was carried out by a computerized morphometric complex "Olympus". Statistics were carried out by the parametric methods with STATISTICA 6.0 (Stat Soft, USA) in order to identify differences of the groups. The significance of differences was assessed by the parametric Student's t-test.

Results. Use of Trilon B after non-narcotic impurity normalized bone remodeling processes and, consequently, decreased formation of excessive and sclerosed bone substance. Myelofibrotic processes in bone marrow cavities underwent involution. Ample vascularization also took place.

For a long-term influence of non-narcotic impurity (90 days), bone resorption was carried out with a predominance of non-cellular mechanisms caused by high density of the bone matrix and avascular areas (osteosclerotic changes) took place.

Conclusions. Trilon B at a dose of 250 mg/kg after 1- and 3-months influence of non-narcotic impurity at a dose of 63 mg/kg normalized remodeling processes of the mandible bone tissue and activated resorptive mechanisms in white rats. It led to levelling of osteosclerosis changes, involution zones of myelofibrosis and the restoration of microcirculation of the target affected organ.

Morphological Study of Bovine Pelvic Bone and Osteocytes: A Pilot Study

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Background. Animal bones are in the process of remodelling throughout their life, which allows the skeleton to adjust dynamically to the new conditions according to changing load, while maintaining the mechanical strength of the bones.

Adaptation of bone tissue is driven by bone cells or osteocytes, which form over 90% of all the cells in the bones of an adult animal. Osteocytes in the mineralized bone matrix produce various signal molecules regulating formation and absorption of bone tissue. The sensitivity of osteocytes is strongly affected by their morphology and orientation in the bone tissue and it varies in different parts of the pelvic floor.

Material. The pelvic floor sawn from the fresh carcass of 3 heifers (age 1.5 years) and 5 older cows (age 6.5–11.5 years) was investigated. Samples for histological examination were taken from four areas of the pelvic symphysis: the pubic tubercles, the junction of the pubic caudal rami and rami of the ischium, the symphysial eminence, the body of the interischial bone. Histological slides were stained with H&E. In each preparation, two examiners counted osteocytes under 20X magnification in 20 fields of view.

Results. The preparations contained both round and elongated osteocytes which were aligned in different directions. In cows, due to more advanced ossification of the pelvic symphysis, the total number of osteocytes was higher than in heifers. In heifers, a relatively large amount of cartilage tissue was found in the examined areas, and the bone tissue contained statistically more elongated as compared to round osteocytes (49.4±4.50 and 18.4±3.48 respectively). The number of elongated osteocytes was higher in the connection point of the pubic bone, the body of the interischial bone and in the symphysial eminence. In older animals, however, the amount of elongated osteocytes was also increased in the pubic tubercle.

Discussion. Different orientation of osteocytes indicates different mechanical loads which the bone tissue in the pelvis has endured. Round osteocytes were more abundant in areas which had not yet completely ossified and where the load on bones was apparently smaller. In terms of giving birth, however, partially ossified areas are important, enabling expansion of the pelvic cavity while expelling the foetus. Round cells are more sensitive than elongated cells to load and pull forces. On the other hand, the abundance of round osteocytes may also be an indication of the decrease of bone density. Elongated osteocytes were more dominant in the ossified areas of the pelvic floor, such as the symphysial eminence and the body of the interischial bone; these areas have endured more mechanical stress and have functionally adapted to carrying the mass of the udder but are immobile during giving birth.

Conclusion. Based on the results obtained, we can conclude that the presence of round and elongated osteocytes in heifers and cows pelvic is dependent on the age and physiological condition of an animal.

Expression of Pax Proteins During Embryogenesis of the Human Spinal Cord Development

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Recent evidence from experiments made on mouse and chicken embryos indicate that Pax genes play important roles in the early spinal cord embryogenesis. Only limited results exist about the developmental role of Pax proteins in the human spinal cord development, which act as transcriptional regulators that bind to specific DNA. The expression of Pax2, Pax6 and Pax7 was examined in 29 human embryos by immunohistochemistry. The embryos were collected after legal abortions, fixed in 4% paraformaldehyde, embedded in paraffin and tissue blocks were serially cut in transversal direction. The embryos were classified according to Carnegie stages (CS). For immunohistochemistry the slices were incubated with primary antibodies of Pax2, Pax6, and Pax7 and with a universal secondary antibody.

The results demonstrated spatially and temporally restricted pattern of the expression of Pax2, Pax6 and Pax7 in the developing spinal cord of human embryos at CS 10-20. Although the studied proteins expression at CS 10-14 was relatively weaker in the forming spinal cord, it was found stronger in later stages. In the embryos of CS 16-20, the Pax2 expression was detected at the essential level in the ventricular, the mantel and also in the marginal layer of the developing spinal cord. In the same embryos the stronger expression of Pax6 and Pax7 was noticed in the ventricular layer while the weaker expression characterized the mantel and marginal layers of the forming spinal cord. Comparing Pax2, Pax6 and Pax7 expression in the dorsal and ventral parts, varying signal intensity was seen in the embryos of CS 14-20. Pax2 and Pax7 proteins expression was more intensive in the dorsal part of the developing spinal cord. The dorsal-ventral boundary of Pax6 expression was very vague compared to that of Pax2 and Pax7.

In human embryos Pax2, Pax6 and Pax7 were identified as signaling molecules that involved in the formation of the early spinal cord. It can be said that studied proteins are associated with the establishment of neuroepithelial cells within the developing spinal cord and with the migration and the differentiation of specific neural cell populations. In particular, Pax2, Pax6 and Pax7 proteins play an essentially important role in the determination of the dorsal-ventral axis of the developing spinal cord.

Different Proliferation of Endometrial Tissues in Postmenopausal Women as Detected With Ki-67 Marker

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Study Highlights. Present study of endometrial polyps in postmenopausal women demonstrated that the proliferation index of endometrial epithelial cells is higher than that of connective tissue, and that they have positive correlation.

Background. Endometrial polyps are a common finding in general population, and in postmenopausal women can be even more prevalent. Scientific literature lacks firm conclusions, which tissue component's proliferation is responsible for the regional growth resulting in endometrial polyps, and what clinical data is associated with it. The aim of the study was to determine and to compare the proliferation indices of endometrial epithelial and connective tissues' cells in postmenopausal women and to correlate them with patients' clinical data.

Material and Methods. Histological sections of endometrial polyps, obtained from asymptomatic postmenopausal women (n=50), were immunohistochemically stained with mouse monoclonal antibodies anti-Ki-67 (Hu), (MIB-1, Dako, 1:100) and using EnVision visualization system. A digital camera was used to capture ten vision fields in highest magnification (x40). Ki-67 positive-stained and unstained epithelial as well as connective tissue cells were counted, and total proliferation index (TPI) as well as proliferation index of epithelial and connective tissue cells was calculated. Correlation of the obtained results with the clinical data of the patients was tested. A level of $p<0.05$ was chosen as statistically significant.

Results. A total of 500 vision fields were investigated morphologically, and mitotically active cells were counted. It was determined that proliferation index of epithelial cells (EPI) is greater than proliferation index of stromal cells (SPI), and their median values are, correspondingly, 4.8 and 0.9, $p<0.05$. Medium strong correlation was determined between EPI and SPI, $r=0.613$, $p<0.01$. A tendency of a greater TPI as well as EPI in early postmenopausal age was observed.

Discussion. The findings of the present study demonstrated that the proliferation index of endometrial epithelial cells is higher than of connective tissue cells. In some women the EPI was several-fold greater than the median value. This fact raises the question, what factors could have had influence on this difference. From scientific literature it is known, that proliferation of endometrial epithelial cells may be associated with age, lifestyle, hormonal status, obesity and type 2 diabetes. Patients in this study did not take any steroidal hormones.

Conclusions. Although proliferation of both – the endometrial epithelial and stromal cells – in the postmenopausal period was detected, the epithelial cells proliferate more intensively, than the stromal cells. A tendency of greater proliferation of epithelial cells in younger postmenopausal women was observed.

Different Interleukins and Proliferation in Cleft Affected Lip

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Study Highlights. The appearance of pro- and anti-inflammatory cytokines and proliferation marker Ki67, and their inter-correlations in cleft affected lip (CAL) of children was studied as the inflammation and proliferation of the cells are connected processes.

Background. Cleft lip palate takes the stable second place among anomalies worldwide. The complex appearance of cytokines and proliferation markers was still not clarified despite their possible crucial role in tissue. Materials and Methods. The lip material was obtained from 16 children aged before primary dentition during plastic surgery. Control was obtained from 7 non-CAL oral tissue. Tissues were stained for IL-1, IL-4, IL-6, IL-8, IL-10 and Ki67 immunohistochemically. Non-parametric statistic, Mann-Whitney and Spearman coefficient were used to evaluate results.

Results. All cytokines positive cells were observed more into the epithelium. However, statistically significant difference was seen between epithelial IL-1, IL-10, IL-8 and Ki67 positive cells and IL-10-, IL-4-containing connective tissue cells in comparison to the control. Strong positive correlation was detected in CAL epithelium between IL-10 and IL-8, IL-10 and IL-4, IL-10 and IL-1, IL-1 and IL-8, IL-1 and IL-4, IL-4 and IL-8, IL-8 and Ki67, IL-10 and Ki67, but moderate – in connective tissue between IL-1 and IL-10, IL-1 and IL-4.

Discussion. We suggest the specific role of the most expressed cytokines IL-1, IL-8 and IL-10 in the cleft affected epithelial barrier, which remarkably stimulates the proliferation of cells. IL-1 is the main pro-inflammatory cytokine, but its expression is also necessary for healing and delayed wound repair. All these effects take place in CAL. As the patchy inflammation was detected in subepithelium in CAL, we speculate of common compensatory inflammation-suppressing expression of IL-10 in long term persisting inflammation. The inter-correlations of factors indicate the common self-protection compensatory mechanism in the CAL epithelium. We speculate on the triggered influence of some interleukins on the Ki67 expression in CAL and to those belong IL-8 and IL-10 due to their strong and moderate inter-correlations in the epithelium. From all cytokines in case of cleft triggered inflammation, the IL-1, -4 and -10 are those, which are the result of the local connective tissue response.

Conclusion. Rich in IL-1, IL-10 and IL-8 cleft affected lip epithelium proves the balance between pro- and anti-inflammatory tissue responses. Common increase of cytokines in the epithelium and their correlations suggest the specific mutual increase of local inflammatory-immune response, while correlations of Ki67 and cytokines indicate the involvement of IL-8 and IL-10 in proliferation of cells. IL-4 and IL-10 expression from CAL connective tissue simultaneously to IL-1, IL-4 and IL-10 inter-correlations there suggests the mutual local tissue immune response regulated by main pro-inflammatory cytokine – IL-1.

Characterization of Morphology in Vocal Nodules: A Pilot Study

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Study Highlights. The vocal cord nodules in different ontogenetic aspect were studied to clarify the most intensive molecular tissue events.

Background. Vocal nodules together with vocal polyps are raised by vocal abuse and usually are treated surgically. The routine histological description of vocal nodules is seen mainly in the scientific literature, thus we have focused on the detection of such molecular events as proliferation, programmed cell death, growth, ischemia and inflammation.

Materials and Methods. The vocal nodules were obtained from 10 patients aged from 7-56 years with chronic hyperplastic laryngitis during the surgical treatment. Control was obtained from 7 vocal cords of the tissue collection in Institute of Anatomy and Anthropology. Tissues were stained for IL-1, VEGF, Ki67 and EGFR immunohistochemically. Apoptosis was detected by TUNEL kit. Non-parametric statistic, Mann-Whitney and Spearman coefficient were used to evaluate results.

Results. All vocal nodules were covered by stratified squamous epithelium with notable basal cell hyperplasia and vacuolized epitheliocytes. Basal membrane showed thickening, but subepithelium demonstrated patchy distribution of inflammatory cells, oedema, fibrosis and patchy neoangiogenesis. Ganglionic cells and cyst were seen in two cases. Ki67 marked moderate number of basal epitheliocytes, while controls didn't demonstrated this factor at all. Also IL1 was observed in numerous number of epithelial and connective tissue cells, while controls showed only up to few positive cells in connective tissue in some cases. Numerous number of positive cells was seen also for apoptosis, EGFR, VEGF. All factor positive structures differed statistically significant between the patients and controls.

Discussion. The routine morphology findings together with dominance of Ki67 and EGFR in vocal nodules epithelium suggest this type of tissue as more intensively affected in vocal abuse. Although, we speculate that apoptosis would be the best type of cell death in epithelium here due to the absence of scar development. Subepithelial connective tissue with rich inflammation, VEGF and IL1 expression may suggest the presence of ischemia and stimulate the development of fibrosis here and epithelial changes in vocal nodules. The age of patients seems not to show any impact on the vocal nodules structural changes.

Conclusion. The elevation of Ki67 and EGFR suggests the intensification of epithelial growth and proliferation on phone of the persistent inflammation (increase of IL1 positive structures) in vocal nodules proving the basis for self-renew under the vocal cords abuse conditions. Intensification of apoptosis in the epithelium gives the basis for the regular remove of affected vocal cord cells. Rich VEGF expression in vocal nodules proves the ischemia and compensatory involvement of epithelium for this factor production. The morphology of vocal nodules seems not to depend on the age.

Evaluation of Anthropometric Parameters and Posture Status of Participants Advance High Physical Load Exercises in Field Conditions

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Introduction. High standards of physical fitness and health standards are essential for military personnel training in field condition. Aim of the study is assessment of anthropometric characteristics of participants, analysis of posture status, foot status, and muscles` functional tests. That allows managing pre-courses training program and optimize adaptation of participants to high physical load in field condition during combat training course.

Material and Method. Study group included 59 participants of both genders (47 males and 12 females) in aged 23 to 30 years who participated in Combat training course. Sport medical doctor provided examination of posture status (in frontal and sagittal planes), foot status analysis, muscle functions` testing (balance, coordination, and elasticity tests), and assessment of anthropometric characteristics. The height was fixed by anthropometry with an accuracy of 0.001 m. The body mass was fixed by scale with an accuracy of 0.01 kg. The body mass indexes (BMI) were calculated as the quotient of body mass (kg) and the square of height (m^2).

Results and Disscussion. We provided analysis and evaluating of musculoskeletal disorders in study by using standardized Nordic Questionnaire. The most problematic region was the lower back, about 52.2% of respondents indicated pain symptoms into it in last six month, and the knee region about 45.6% of respondents had pain symptoms into it in last six month. There were revealed the combined asymmetric posture (in sagittal plane and frontal plane) was found for 67.8% of participants of study group that could be risk for musculoskeletal disorders during high physical load. The increased level of muscle tone was fixed for 91.5% of participants during examination tests. There were revealed correlations between the combined asymmetric posture that induced muscular hyper tonus ($p=0.371$). Evaluation of the balance status fixed deviations from standards for 18.6% of participants of study group. We have fixed correlations between balance dysfunction for individuals and frontal posture asymmetry ($p= 0.759$). We have fixed coordination dysfunction that correlated to the frontal posture asymmetry. Analysis of anthropometric characteristic shown that body mass index changes were in the interval from 21.34 to 33.24. BMI value for 22.4% of male participants and 6.1% of female participants corresponded to the standards recommendations' of WHO. The overweight were fixed for rest part of participants that could be risk factor for musculoskeletal disorders during high physical load.

Conclusion. Analysis of posture and foot status were topical for providing preventive measures and reduction the risk of health disorders related to high physical load in military environment. The managing pre-courses training program for reducing muscular tone, balance and coordination dysfunctions is important for optimize adaptation of participants to high physical load exercises.

Neurochemical and Morphological Characterization of Porcine Atrial Intracardiac Ganglia

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Study Highlights. 1) The majority of porcine atrial intracardiac ganglia neurons are cholinergic; 2) Few neurons are adrenergic or nitrergic; 3) Neurons of almost all phenotypes were evenly distributed across atria.

Background. Intracardiac ganglia (ICG) are important component of the regulation of heart rhythm and contractile properties. Neurochemical and morphological phenotypes of ICG neurons play important role in this regulation. Porcine heart is important for cardiac translational medicine because of its high similarity with human heart. However, knowledge of porcine ICG neuronal phenotype and morphology is limited. Regional distribution of atrial ICG neurons of distinct phenotypes have not been studied. To this purpose we investigated ICG from porcine whole atria.

Material and Methods. Hearts from 11 adult pigs were used in the study. Whole-mount preparations of epicardium from atria were double labelled for choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), neuronal nitric oxide synthase (nNOS), calcitonin gene-related peptide (CGRP) and vesicular glutamate transporter 2 (VGLUT2) using immunohistochemical procedures and imaged using confocal microscope. ICG neurons of distinct phenotype were calculated and area of ICG neurons were measured.

Results. Majority of porcine ICG neurons were cholinergic. 96.3% of neurons were positive for ChAT but not for TH (n=9206), 2,7% were positive both for ChAT and TH (n=256), while 0.1% were positive only for TH, but not for ChAT (n=8). 96,2% of ICG neurons were positive for ChAT but not for nNOS (n=10.907), 3.3% were positive both for ChAT and nNOS (n=379) and 0.5% positive only for nNOS but not for ChAT (n=53). There were minor differences in abundance of neurons of particular phenotype between atrial regions. Specifically, only ChAT/nNOS biphenotypic neurons in ventral regions of atria were less abundant than in dorsal. 4.5% of ICG contained TH positive clusters of small intensely fluorescent cells.

We observed ChAT, TH and CGRP positive fibers with varicosies in intracardiac nerves and ICG. nNOS positive fibers was also observed in intracardiac nerves and ICG. VGLUT2 was found only in the nerve terminals in ICG.

Depending on morphology ICG were classified into two groups: compact round or oval and that of irregular shape with more scattered neurons. Mean ICG neuronal number was 23,8. Comparison of ICG neuronal area revealed solely ChAT and TH positive neurons being the smallest while ChAT/TH, ChAT/nNOS biphenotypic neurons and solely nNOS positive neurons are comparable in size.

Conclusions. These findings show that cholinergic neurons constitute major phenotype in porcine atrial ICG while adrenergic and nitrergic neurons comprise minor part of ICG. There are no major differences of regional distribution of neurons of particular phenotype.

Malignant Rhabdoid Tumor in a 3-Month-Old Infant: A Rare Entity

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Highlights. In infants, malignant tumors are rare. The most common among them are neuroblastoma, nephroblastoma and hepatoblastoma. Here we present a rare entity: malignant rhabdoid tumor in a 3-month-old infant.

Background. Malignant rhabdoid tumors of liver are extremely rare, aggressive embryonal tumors, diagnosed in early childhood. Median age at diagnosis varies between 8 and 16 months, a 5-year survival is up to 30%, increasing proportionally to age, and 3/4 of patients present with metastatic disease upon diagnosis.

Materials and Methods. Presentation of case report, based on clinical information, autopsy and slide analysis.

Results. 3-month-old infant was hospitalised because of distended stomach with palpable mass, yellow conjunctivas and dark-coloured urine. There is anamnesis of emesis after every feeding since birth, failure to thrive and inefficient weight gain below 10th percentile. Upon examination, the infant presents with fever, tachycardia, jaundice, oxygen saturation 88% and a palpable, hard abdominal mass. Radiological examination reveals a tumorous growth extending from the liver, 16×12.5×10 cm with lobulated borders, compressing the common bile duct, abdominal aorta, pancreas and right kidney, and infiltrating hepatic and portal veins. Metastatic, multinodular, round masses up to 2 cm in diameter are found in lungs, and single, nodular lesion in the soft tissue of left gluteal region as well as in left distal metaepiphysis of humerus is seen.

Intraoperative cytology findings are suspective of rhabdomyosarcoma, and patient is started on ex-juvantibus chemotherapy. Rapid deterioration leads to exitus letalis 5 days later.

Histology of biopsy specimen reveals medium sized rhabdoid cells with large, vesicular nuclei, prominent nucleoli and eosinophilic cytoplasm, showing diffuse positivity for cytokeratin AE1/AE3 and vimentin, focal positivity for EMA, and are negative for myogenin, myoD1, INI/BAF47, synaptophysin, CD45, CD99, Ki-67 80%, establishing diagnosis of rhabdoid tumor.

On autopsy, liver was enlarged, 1110 g, on cut surface there is a 16 cm Ø, yellow-tan, round, bulking mass with hemorrhagic, necrotic and cystic changes. There are several, nodular metastatic lesions in lungs with yellow, soft cut surface, 1-2.5 cm Ø, scattered among hemorrhagic lung tissue.

Discussion. Malignant rhabdoid tumor was first described as an aggressive variant of Wilms' tumor, and kidney is the most common primary site of origin, second comes central nervous system, followed by other organs and soft tissue. Histologic hallmark is the combination of discohesive morphology of rhabdoid cells and classical immunohistochemical panel of positive both epithelial and mesenchymal markers and negative myogenic markers, and loss of INI1/BAF47 due to deletion in respective gene.

Conclusion. As most of the primary liver tumors in infancy are hepatoblastomas, specific histological distinction is of great importance, as clinical and radiological assessment can be challenging.

Comparative Stereomicroscopic Study of the Intrinsic Ganglionated Nerve Plexus Distributed in Cardiac Ventricles of the Humans, Pigs and Sheep

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Background. The knowledge about the distribution of the ganglionated nerve plexus on human cardiac ventricles is considered as containing a pivotal clinical importance for general heart surgery and interventional treatment of cardiac arrhythmias. The aim of this study was to examine the distribution and structural peculiarities of the epicardiac ventricular neural ganglionated plexus in hearts of human and large popular animal models of experimental neurocardiology – pig and sheep - highlighting the differences of this plexus in humans and models.

Methods. Five non-sectioned pressure distended whole hearts of the human newborns, five hearts of newborn German black-faced lambs and five hearts of the newborn piglets were investigated applying a histochemical method for acetylcholinesterase to stain epicardiac neural structures with their subsequent stereomicroscopic examination.

Results. Mediastinal nerves accessed the ventral surface of human and sheep cardiac ventricles between the root of ascending aorta and pulmonary trunk. The ventral surface of the sheep (in part) and pig (completely) was supplied by several epicardial nerves extending from the right ventral (RV) ganglionated nerve subplexus on the ventral right atrium. The dorsal surface of the both ventricles of all three examined species was supplied by several epicardial nerves extending from the left (LD) and middle dorsal (MD) ganglionated nerve subplexuses on the dorsal left atrium.

Human ventricles were supplied by left (LC) and right (RC) coronary and LD and MD subplexuses. LC included about 31% of all counted human ventricular epicardial nerves, RC – about 29%, while both together LD and MD – 40%. Sheep ventricles were supplied by LC, RC, LD, MD and RV subplexuses. LC involved about 12% of all counted sheep ventricular epicardial nerves, RC – about 8%, RV – 31%, both LD and MD – 50%. Pig ventricles were supplied by RV, LD and MD subplexuses. RV involved about 54% of all counted pig ventricular epicardial nerves, and both LD and MD – 46%.

The ventricular epicardium involved up to 129 ganglia in humans, up to 198 – in sheep and up to 72 – in pigs. In humans, the largest number of the ventricular neurons was determined in the region in the front of the conus arteriosus (PreCA) (48%) ($P<0.05$). In sheep, the largest number of the ventricular neurons was determined in the dorsal left ventricular region (DLV) (48%) and PreCA (24%) ($P<0.05$). In pig ventricles, the largest number of the human ganglionic neurons was determined in the region of the ventral left coronary sulcus (VLCS) (47%) and ventral left ventricular region (VLV) (29%).

Conclusions. A comparative study of the morphological patterns of the human, sheep and pig epicardiac ventricular ganglionated nerve plexuses showed that the topographical and quantitative differences between all tree species are significant, especially between humans and pigs.

Morphological Characterization of Local Antimicrobial Response, Inflammatory Markers and Tissue Remodelling in the Skin of Psoriasis Patients

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Study Highlights. Natural antimicrobial peptides and their secretion affecting factors are important in the morphopathogenesis of psoriasis, which together with interleukins and tissue degeneration and remodelling markers provide the local skin protection.

Background. Psoriasis is a chronic inflammatory skin disease with significant impact on reducing patient's quality of life. The aim of the study was to evaluate antimicrobial response, tissue degeneration reaction and remodelling capacity and distribution of inflammatory cytokines in nontreated psoriatic skin, as well as the correlations between these factors and influence on the course of the disease.

Material and Methods. We evaluated 40 psoriasis vulgaris patient skin samples obtained from routine punch biopsies. All tissue specimens were examined routinely and by immunohistochemistry for human beta defensin 2 (HBD-2), tumor necrosis factor-alpha (TNF-α), interleukin-1 alpha (IL-1α), interleukin 6 (IL-6), interleukin 8 (IL-8), matrix metalloproteinase 2 (MMP-2), tissue inhibitor of metalloproteinase-2 (TIMP-2) and tissue inhibitor of metalloproteinase-4 (TIMP-4). The staining intensity was semiquantitatively graded.

Results. We observed uneven thickening of basal and spinous layers, presence of Kogoj spongiform pustules and parakeratosis, absence of granular layer in epidermis, prominent inflammatory infiltrates surrounding sclerotic arterioles in dermis. Numerous epitheliocytes, fibroblasts and macrophages expressed HBD-2. TNF-α-positive cells varied from few to numerous. Only separate keratinocytes, few fibroblasts and moderate inflammatory cells expressed IL-1α. IL-6-positive cells varied from few to abundant in each visual field and IL-8-positive cells varied from numerous to abundant in each visual field. The number of MMP-2-positive macrophages, fibroblasts and epitheliocytes varied. TIMP-2 was found in few keratinocytes in most of our skin samples, while moderate amount of dermal fibroblasts and inflammatory cells (mainly macrophages and lymphocytes) contained TIMP-2. Simultaneously, we found moderate number of TIMP-4-containing both keratinocytes and dermal lymphocytes, macrophages, as well as fibroblasts.

Discussion. In the skin of psoriasis patients with different disease duration and progress activation of skin antimicrobial peptides, inflammatory cytokines and degeneration enzymes is essential, suggesting their involvement in the morphopathogenesis of psoriasis.

Conclusions. Statistically significantly increased HBD-2 points to its role in therapy unmodified psoriatic skin local protection. Main cytokines are other cytokine release inducers IL-6 and IL-8. TNF-α affecting psoriatic dermis, suggests the main inflammatory processes of the connective tissue, while the main pro-inflammatory cytokine IL-1α finding in only some patients indicates probably very individual skin response to the progress of the disease. Pronounced expression of degeneration enzyme MMP-2, while as the main MMP inhibitors significantly increase the mutually correlating TIMP-2 and TIMP-4. Overall, tissue degeneration factor and their inhibitor common (TIMP-2) and selective (TIMP-4) increase indicate intensive remodelling processes of psoriasis-affected area of the skin.

Ultrastructural Characterization of the Joint Synovium in the Case of Human Herpes Virus-6 Infection in Rheumatoid Arthritis

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Study Highlights. The findings of this study highlight a presence of beta-herpesvirus in synovial membrane. Several intriguing features are found in regard to the potential participation of herpes virus-6 in rheumatoid arthritis.

Background. Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease that primarily affects the joint synovium. The knowledge of viruses associated with RA is still growing, and includes the human herpes virus-6 (HHV-6). The morphological demonstration of HHV-6 in the synovial lining and sublining cells in the RA is currently missing.

Material and Methods. Joint tissues as exemplified by the synovial membrane obtained during joint replacement surgery were used in this study. The lining and sublining cells of the synovial membrane facing the joint cavity have been studied by scanning (SEM) and transmission (TEM) electron microscopy. Immuno-gold (IG) labelling using HHV-6 (20) mouse monoclonal antibody raised against viral lysate (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, 1:30 dilution) was performed. For ultrastructural examination, five nested PCR and immunohistochemically confirmed HHV-6-positive tissue samples were used.

Results. SEM: One to three layers of close spaced cells were distinguished in the synovial surface. The synovial membrane appeared rugged and was composed by both slender and roundish cell types. Lining cells were covered by small-sized cytoplasmic processes running in all directions. Narrow spaces between cells were filled by scanty clusters of fibrillar processes. Directly below the synovial lining cells, there was a quite regular distribution of more or less fibrous connective tissue composed of bundles of collagen fibres between vessels. TEM: Cells resembling macrophages with many finger-like cytoplasmic projections contained irregular shaped nuclei with clusters of dense-packed chromatin, a prominent Golgi apparatus, numerous vacuoles with electron-dense or fibrillary material, mitochondria, intracellular fibrils and pinocytotic vesicles. Cells appearing similar to fibroblasts contained elliptic nuclei tended to have more homogeneous nuclear chromatin, large amounts of rough endoplasmic reticulum, pinocytotic vesicles and mitochondria. Collagen fibres were observed nearby them. IG: Immunogold-positivity was detected in nuclei, perinuclear space and in cytoplasm of synoviocytes.

Discussion. An immuno-gold labelling was used to determine the presence and intracellular localization of HHV-6 in synoviocytes. Our results suggest that HHV-6 may have a role in RA, or perhaps, an alternative possibility is that RA is characterized by defects in cellular immunity and this may result in a presence of persistent HHV-6.

Conclusions. The present study has validated ultrastructural changes in the synovial membrane of RA patients by electron microscopy. Sinovial cells with morphological features of macrophage-like and fibroblast-like synoviocytes were seen to contain HHV-6.

Anthropometric Indices as Screening Tools for Obesity and Overweight in Latvian Women Older Than 40 Years of Age

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Study Highlights. The target of our study was to make the comparative analysis of obesity indices for 321 women older than 40 years of age from various institutions from Riga. We have fixed the high prevalence of overweight and obesity in the examined women groups.

Objectives. From World Health Organization data, the worldwide obesity has nearly tripled since 1975. Most of the world's populations live in countries where overweight and obesity kills more people than underweight. Approximately 3.4 million adults succumb to death every year as a result of being obese or overweight. Overweight or obese adults make up 21.3% of Latvia's population, which is the second highest figure in the European Union, according to the latest data from Eurostat. Use of simple anthropometric indices of body composition, such as body mass index (BMI), waist circumference (WC), waist hip ratio (WHR) and Weight Height index, has been considered as a practical and valuable approach to the assessment of obesity for a long time

Materials and Methods. This cross-sectional study was in Riga in various institutions from June 2016 to December 2018, and the final analysis included data obtained from 321 women older than 40 years of age. Four obesity indices, including BMI, WC, WHR and Weight Height index were investigated. The participant women were divided in five subgroups according to the age: the first - 40–44 years (n=72); the second - 45–49 years (n=45); the third - 50–54 years (n=46); the fourth - 55–59 years (n=63); the fifth - 60–65 years (n=95). In each subgroup we made the comparative analysis of anthropometric parameters. The study was approved by the Ethics Committee of Rīga Stradiņš University and the data were collected with the participant's informed consent.

Results. For the first subgroup: BMI - 25.24±0.53, WC - 78.15±1.32 cm, WHR - 0.78±0.01, Weight Height index - 415.48±8.79 g/cm. For the second subgroup: BMI - 27.51±0.93, WC - 87.79±2.45 cm, WHR - 0.82±0.01, Weight Height index - 455.61±15.66 g/cm. For the third subgroup: BMI - 29.41±0.69, WC - 91.63±1.94 cm, WHR - 0.84±0.01, Weight Height index - 483.22 g/cm. For the fourth and for the fifth subgroups results of the average values were similar.

Discussion. Analysis of investigated anthropometrics parameters, BMI and WHR for Latvian women in age after 40 revealed that the mean values of BMI in all groups have exceeded standard level (>25). Anthropometric data analysis showed that most of the examined respondents women have a moderate health risk that means that WHR is from 0.81 to 0.85. BMI and WHR was a simple, easy, inexpensive, highly reproducible, and accurate tool for prevention, control, and intervention against adults' (women) obesity.

Conclusions. BMI, WC, WHR and Weight Height index may be useful tools to screen for adiposity using their optimum values for sex and ethnicity.

Effect of Sodium Valproate on Neoangiogensis in Small Cell Lung Cancer Cell Line Tumors

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Small cell lung cancer (SCLC) accounts for approximately up to 15% of all lung cancers. After combined therapy treatment, only a small proportion of SCLC patients survive for more than 5 years, while most patients relapse within the 1st year of treatment. Histone deacetylase (HDAC) inhibitors represent a new class of anticancer agents. Sodium valproate (VPA) is a HDAC inhibitor, and in recent years it attracted the attention of investigators as an anticancer medication. VPA was found to induce cell growth arrest and down-regulation of Notch signaling. We have reported that VPA suppresses glioma U-87 cell tumor growth and tumor cell apoptosis. The study aim was to investigate the VPA effect on SCLC (NCI-H146 cell line) tumor-induced neoangiogenesis, using the chicken embryo chorioallantoic membrane (CAM) as a 3D model. Method and Material. CAM serves as a highly vascular, naturally immunodeficient biological membrane suitabe for human cells xenogrft studies. Fertilized Cobb 500 chicken eggs were incubated at a constant temperature and humidity. The eggs were being rotated for three consecutive days until the opening of the window on the 3rd embryonic day of development (EDD3). An opened window in the eggshell was covered with a sterile transparent tape for the further grafting of VPA-treated NCI-H146 cells tumor on EDD7. The xenografts were investigated in tumor groups treated with 2, 3, 4, 6 and 8 mM NaVP concentrations and in the control. Eggs with formed tumors were visualized in ovo daily from the 9th to 12th day of EDD (2–5th day post-grafting) under an OLYMPUS SZX16 stereo microscope. Fluorescein isothiocyanate-dextran (Sigma-Aldrich, Sweden) was injected into the largest blood vessel of the CAM. The CAM with tumor at EDD12 was harvested, fixed in neutral buffered formalin, embedded in paraffin and cut into 3 μm sections. Angiogenesis induced by the NCI-H146 cells tumor was evaluated using an injection of fluorescinating dextran and in H+E stained slides; the number and mean summarized area of blood vessels was calculated. Results. Strong angiogenesis induced in NCI-H146 tumors on CAM revealed the increased number and area of blood vessels. The fluorescinating dextran assay visualized blood vessels in the CAM around and inside the tumor. The control NCI-H146 tumor group showed intensive fluorescence with clear blood vessels inside a tumor, visibly attracted blood vessels around the tumor and formed a clear "spoked-wheel" pattern. Starting from 3 mM of VPA concentration „spoked-wheel" pattern became not clear, and the fluorescence of the tumor was less expressed. Neoangiogenesis was inhibited by VPA in a dose-dependent manner.

Expression of CILP-2 in the Articular Cartilage of Patients With Knee Osteoarthritis: a Pilot Morphological Study

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Background. Osteoarthritis (OA) is a common joint disorder involving degeneration of the articular cartilage, but also affecting surrounding tissues. OA disables often weight-bearing joints including the knee. One of the important determinants of cartilage function is the structure and composition of extracellular matrix (ECM). CILP (cartilage intermediate layer protein) is one of the relatively recently described ECM components, originally found to be localized in the intermediate layer of the articular cartilage. However, accumulating data suggest that CILP in OA patients may have wider distribution in the cartilage and in the whole joint with possible involvement in the disease process. Therefore, the aim of this study was to detect CILP-2 expression in the articular cartilage of patients with knee OA.

Material and Methods. Material was obtained from 12 patients (age from 46 to 65 years) undergoing total knee arthroplasty (Tartu University Hospital, Estonia). Biopsy for routine histological analysis were embedded in paraffin, cut at four-µm thickness sections and stained with H&E for general orientation.

For transmission electron microscopy, specimens were fixed in 2.5% glutaraldehyde and embedded in Epon-812 according to standard methods. Semithin sections were analysed using a Zeiss Axiophot 2 microscope to select the region of interest for the following procedures. The ultrathin (80 nm) sections were investigated by Philips Tecnai-10 microscope.

For immunocytochemistry ultrathin sections were blocking with blocking solution and then incubated with the primary CILP-2 antibody (abcam, ab 107419) diluted 1:100 for 1h. Next the sections were incubated with the secondary antibody labelled with 20 nm colloidal gold particles (British Biocell International, UK) in 1:100 dilution.

Results. Cartilage samples obtained from OA patients contained few singly located chondrocytes, which characteristically had short branching processes and contained many vacuoles. In the perinuclear areas large bundles of filaments were seen. Nuclei were enlarged with partially disrupted chromatin. The number of distorted and round mitochondria was reduced. CILP-2 expression was detected in the cytoplasm of chondrocytes and in the interterritorial matrix.

Discussion. Despite extensive studies the molecular mechanisms involved in OA initiation remain poorly understood. Detecting relations between structural molecules (CILP and collagen II) and ECM degrading molecules (e.g. MMP-13) may help to understand critical events leading to irreversible changes of the articular cartilage. CILP-2 as a promising serum marker was recently shown to be related to cartilage thickness loss in individuals with developing knee OA (Boeth et al, 2019).

Conclusions. CILP-2 expression seen in the articular cartilage of OA patients may reflect the pathogenic steps of the disease process and thus CILP-2 may be a possible biomarker for the detection of early cartilage damage.

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