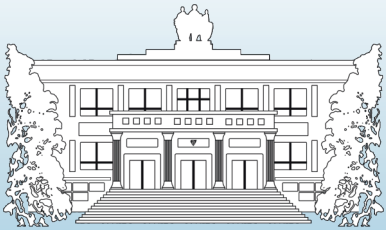
Czech Anatomical Society
Czech Society for Histochemistry and Cytochemistry
Faculty of Medicine and Dentistry, Palacký University Olomouc

MORPHOLOGY 2025

56th INTERNATIONAL CONGRESS OF CZECH ANATOMICAL SOCIETY AND 61st LOJDA SYMPOSIUM ON HISTOCHEMISTRY

BOOK OF ABSTRACTS

7th - 9th of September 2025
Olomouc
Czech republic



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Česká společnost histo- a cytochemická
Czech Society for Histochemistry and Cytochemistry

MORPHOLOGY 2025.

56th International Congress of Czech Anatomical Society and 61st Lojda Symposium on Histochemistry

Editors: doc. RNDr. Petr Mlejnek, Ph.D.
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Topics

Neurosciences, Molecular and Cell Biology, Experimental and Developmental Morphology, Histology, Clinical Anatomy, Anthropology, Teaching and Anatomical Terminology, Varia

General Information

Venue

Theoretical Institutes of the Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 976/3, 779 00 Olomouc

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Web Pages

www.morphology2025.upol.cz
www.nan.upol.cz

Registration and Information Desk

Foyer of the old building, Theoretical Institutes, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 976/3, 779 00 Olomouc

Registration of the participations: Sunday 15:30 – 17:00
Monday 7:30 – 8:30

Oral Presentations

Invited lectures are scheduled for 30 minutes, regular lectures are scheduled for 10 minutes presentation followed by 5 minutes of discussion. Windows PC with USB port are installed in lecture halls. Your own laptop can be connected via HDMI cable. Speakers with apple devices are kindly requested to bring the necessary adapter for connectivity.

Poster Presentation

The recommended size of poster panel is A0 (84.1 cm width × 118.9 cm height). Maximal size is 90 cm (width) × 120 cm (height). Tapes and clips will be available. Posters will be presented during poster session, on Monday, September 8th. Authors are asked to be present at posters during the poster session.

Social events

- **Welcome Evening**
  will be held immediately after the Opening ceremony on Sunday, September 7th, at the Faculty of Medicine and Dentistry, in the foyer of old building Theoretical institutes. It will include buffet and drinks free of charge.
- **Concert of Women's Choir DUHA**
  will take place in the Corpus Christi Chapel, Univerzitní 3, 779 00, Olomouc on Monday, September 8th from 18:00.
- **Social Evening**
  will take place at Long Story Short, Koželužská 945/31, 779 00 Olomouc on Monday, September 8th from 19:00. Please buy a ticket at the registration desk (price 800 CZK).

Coffee Breaks

Refreshments will be served free of charge.

Lunches

will be served at the bistro in the new building of the Theoretical Institutes. Payment will be made on site.

The Meeting of the Society Committee of the CAS

will take place on Monday, September 8th in the Right Lecture Hall from 15:30 to 16:30.

The Meeting of the Society Committee of the CSHC

will take place on Monday, September 8th in the Left Lecture Hall from 15:30 to 16:30.

City Transportation

Transport to the Faculty of Medicine and Dentistry from the Main Railway station:

- Bus No. 21 – bus stop "Lékařská fakulta", approx. 2 min walk
- Buses No. 12, 19 – bus stop "Dvořákova", approx. 5 min walk
- Trams No. 1, 4, 6 – tram stop "Výstaviště Flora", approx. 12 min walk

Contents

Abstracts

Radiation-induced bystander effect on the rat brain after fractionated spinal cord irradiation

Bálentová S.[1], Hnilicová P.[2], Baranovičová E.[2], Kalenská D.[3], Muríň P.[4], Hajtmanová E.[4]

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We investigated the influence of the so-called bystander effect on metabolic and histopathological changes in the rat brain after fractionated spinal cord irradiation. The study was initiated with adult Wistar male rats (n=20) at the age of 9 months. The group designated to irradiation (n=10) and the age-matched control animals (n=10) were subjected to an initial measurement using in vivo proton magnetic resonance spectroscopy (1H MRS) and magnetic resonance imaging (MRI). After allowing the animals to survive until 12 months, they received fractionated spinal cord irradiation with a total dose of 24 Gy administered in 3 fractions (8 Gy per fraction) once a week on the same day for 3 consecutive weeks. 1H MRS and MRI of brain metabolites were performed in the hippocampus, corpus striatum, and olfactory bulb (OB) before irradiation (9-month-old rats) and subsequently 48 hours (12-month-old) and 2 months (14-month-old) after the completion of irradiation. After the animals were sacrificed at the age of 14 months, brain tissue changes were investigated in two neurogenic regions: the hippocampal dentate gyrus (DG) and the rostral migratory stream (RMS). By comparing the group of 9-month-old rats and individuals measured 48 hours (at the age of 12 months) after irradiation, we found a significant decrease in the ratio of total N-acetyl aspartate to total creatine (tNAA/tCr) and gamma-aminobutyric acid to tCr (GABA/tCr) in OB and hippocampus. A significant increase in myoinositol to tCr (mIns/tCr) in the OB persisted up to 14 months of age. Proton nuclear magnetic resonance (1H NMR)-based plasma metabolomics showed a significant increase in keto acids and decreased tyrosine and tricarboxylic cycle enzymes. Morphometric analysis of neurogenic regions of 14-month-old rats showed well-preserved stem cells, neuroblasts, and increased neurodegeneration. The radiation-induced bystander effect more significantly affected metabolite concentration than the distribution of selected cell types.

Acknowledgement: This study was supported by the Ministry of Education, Slovak Republic (VEGA) 1/0131/22 grant.

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Johannes Jessenius: vel plagiator vel propagator anatomiae?

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Johannes Jessenius is an important person in the history of Czech anatomy because he performed the first public anatomical dissection in Prague in 1600. A year later he published a description of this dissection in print in monographic form. In the following centuries, however, many authors evaluated this publication rather negatively, and Jessenius was mainly reproached with the fact that the text did not bring anything new from the anatomical point of view and was mostly just copied from Vesalius' Fabrica (1543). The authors critically analysed Jessenius' publication and came to the following conclusions: 1. There is no doubt that the autopsy was performed in the then usual order, from the abdominal organs through the thorax to the extremities and head. The anatomical terminology used is, with few exceptions, consistent with Vesalius and contains no factual errors. There are also many extensive passages describing the functional meanings of the various organs and their systems. In these explanations the author relies mainly on the views of the ancient classics (Galen, Aristotle, Hippocrates), while Vesalius is cited only twice. 2. The evaluation made can be concluded as follows: it was certainly quite correct to use Vesalius' terminology in anatomical descriptions, because no other terminology so exact and correct existed at that time. On the other hand, at the time of the book's publication, the teachings and philosophy of the ancient classics were still in full force in the natural sciences and in medicine. 3. Jessenius's work may therefore be regarded not as a plagiarism, but as a useful and important work of popular science which, on the one hand, presents to the general public a simple description of the structure of the human body, and, on the other hand, thus prepares them for the imminent arrival of many revolutionary fundamental discoveries.

Acknowledgement: The work was supported by Charles University projects COOP 33 a COOP 36.

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Interdisciplinary cooperation of faculties in teaching students – an example of long-established good practice

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We consider the complex (or holistic) approach, which perceives the human being as a bio-psycho-social-spiritual complex, to be very useful in medicine and psychology. The need for knowledge of basic anatomy and physiology is important for psychology students and the work of psychologists in general. At the Department of Psychology, two courses of medical specialization are reserved for students of single-discipline psychology: Basics of Anatomy and Physiology and Introduction to Neuropsychology. In the framework of both courses, many years of cooperation includes excursions to the Institute of Normal Anatomy, where psychology students learn about individual organs and organ systems and the whole body in the autopsy laboratory and the anatomical museum. Students (full-time form of study) have these excursions planned as part of their curriculum. Combined (distance form of study) students participate voluntarily beyond the curriculum. The practical experience in anatomy is generally evaluated very positively by all. As a part of the presentation given at the conference, we also present authentic statements from students. The second output of the interdisciplinary cooperation will be a shared publication – specifically, the 2nd expanded and revised edition of Anatomy and Physiology of the Human Body – for humanities disciplines. In this publication photographs of organs and parts of the human body prepared by the staff of the Institute of Normal Anatomy are also newly introduced. All the objects are photographed in the hands, so that students of humanities can imagine the real size and proportionality of body parts. We are convinced that this concept will enrich the publication and will be a meaningful appreciation of the decisions of voluntary donors of bodies. Thus, we consider the long-established cooperation between the Faculty of Arts and the Faculty of Medicine as an effective example of good practice with an outreaching value.

Acknowledgement: The Department of Psychology, Faculty of Arts, Palacký University Olomouc

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The effect of different gluten concentrates and gluten-free diet on ovarian, testicular, and thyroid gland morphology

Makovický P.[1,2], Šťastná M.[1,3], Jabandžiev P.[4], Hrunka M.[4], Jeklová E.[1], Norek A.[1], Straková P.[3], Makovická M.[2], Kráľová K.[5], Janda L.[3]

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It is well known that gluten intake is harmful for celiac disease patients, but there are several discussions about his effects, including gluten-free diet effects on organ morphology in healthy people. The objective of this study is to compare the effects of cereal concentrates and gluten-free diet on ovarian, testicular, and thyroid gland morphology in an experimental mouse model. Forty-eight (n=48) laboratory mice of the BALB/c line were included in the experiment, divided into 4 groups, and maintained on special diets for 5 weeks. The control group, (6♀, 6♂) was fed a gluten-free diet. The first (E1), second (E2) and third (E3) experimental group, (6♀, 6♂) was fed a mixture of casein hydrolysate combined with E1: pure extracted gluten in a 30%:70% ratio. E2: gliadins at a ratio of 30%:70% and E3: avenin at a ratio of 30%:70%. At the end of the experiment, the mice were euthanized, and ovaries, testes, and thyroid glands were sampled. The samples were fixed in a 10% formalin solution and processed into hematoxylin-eosin-stained slides. The oocyte and follicle widths of the ovaries were measured; as well as the germinal epithelium and the width of the seminiferous tubules of the testes; as well as the follicle epithelium width and the follicle width of the thyroid gland. The results showed significant differences in the width of oocytes, follicles, testicular seminiferous tubule epithelium, testicular tubules, thyroid follicle epithelium as well as differences in the width of thyroid follicles. Concentrated gluten and gliadin-based diets showed positive results compared to concentrated avenin and gluten-free diets. Gluten may not be harmful and is recommended for healthy people.

Acknowledgement: Supported: NU23-06-00553.

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Histological and functional adaptations of tooth healing

Křivánek J.

Ústav histologie a embryologie – Lékařská fakulta, MUNI

Continuously growing mouse incisors, have for decades served as a model system for investigating the mechanisms of regeneration and studying the stem cell niche. It has been shown that the growth rate of these teeth does not always proceed at the same pace, but can be accelerated following injury. However, the dynamics of growth acceleration, the mechanisms responsible for damage detection and subsequent process of stem cell niche activation remains entirely unresolved. Here, we provide new insights into the dynamics of mouse incisor regeneration, both in healthy (non-damaged) tooth and after its injury. To quantify incisor growth, we have invented a new method (BEE-ST: Bones and tEEth Spatio-Temporal growth monitoring approach) that allows to monitor the growth and healing dynamics of any hard tissue on micrometre scale in both space and time. Subsequently, using an interdisciplinary approach involving mathematical modelling, single-cell RNA-sequencing, spatial transcriptomics and lineage tracing, we reveal new mechanisms responsible for the detection of injury and we provide new insights into the mechanical, cellular and molecular mechanisms responsible for the acceleration of tooth growth. Our research approaches the study of continuously growing teeth from a new interdisciplinary perspective. These results shed light on the large complexity of biological and mechanical processes standing behind the regulation of continuous tooth growth and suggest a new insight into stem cell niche activation from a more general perspective.

Acknowledgement: GA25-18087S

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Expression of Sod1-3 and Sod4/Ccs proteins in rat uterine tube during early period of pregnancy

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AIM. The aim of this study was to qualitatively detect the presence of superoxide dismutase enzymes (SOD1–3) and the chaperone SOD4/CCS in the rat oviduct during the preimplantation period of pregnancy using immunohistochemistry, and to quantitatively assess their levels using Western blot analysis.

MATERIAL AND METHODS. On the first (D1), third (D3), and fifth (D5) days of pregnancy, six female Sprague-Dawley rats were euthanized via anesthetic overdose. The left ovaries, oviducts, and uterine horns were collected for immunohistochemical detection of the target proteins, while the right ones were used for Western blot analysis.

RESULTS. With the exception of SOD3, all monitored proteins were detected in the epithelial cells of the oviduct throughout the examined stages of pregnancy. SOD1 was mainly present as granules in the luminal regions, SOD2 was diffusely distributed throughout the cytoplasm, and SOD4/CCS was primarily localized in the nuclei. SOD3 was observed only on D1, appearing as granules in the apical regions of epithelial cells near ovulated oocytes. No significant differences were found in the expression levels of the target proteins between the evaluated days of pregnancy.

CONCLUSION. Our results suggest that SODs may be crucial role in enabling sperm fertilization capacity and/or protecting the early embryo and epithelial cells from oxidative stress during the preimplantation period of development.

Acknowledgement: ACKNOWLEDGEMENTS. This work was funded by a VEGA grants 1/0173/19, 1/0500/23, and 1/0074/24.

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Basiliensia Nomina Anatomica – 130th anniversary of the official anatomical terminology

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The contemporary development of anatomical terminology began with the release of the Basiliensia Nomina Anatomica in 1895 in Basel, Switzerland, by the Anatomische Gesellschaft. This was followed by nine revisions from 1935 to 1998, with the latest being the Terminologia Anatomica, which is the most widely adopted version today. TA has also been enhanced with frequently used English synonyms. While eponyms play a crucial role in the terminology, they were excluded from the official Latin anatomical nomenclature in 1955. This paper aims to honour the 130th anniversary of the BNA, explore its origins, highlight key elements that have remained unchanged in TA, and address aspects that have seen modifications or remain ambiguous, unclear, or frustrating. Despite some minor issues, the BNA continues to be a foundational element of the anatomical nomenclature and represents significant progress, deserving of respect and serving as a model for future practices. The knowledge of its origin and heritage should belong to basic education of any morphologist.

Acknowledgement: The work was supported by the Charles University project COOP 33.

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Distinct phenotypes of primary ciliary dyskinesia in monozygotic twins

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Primary ciliary dyskinesia (PCD) is a chronic autosomal recessive disorder that is both genetically and phenotypically heterogeneous. Patients typically suffer from recurrent respiratory infections, which may lead to respiratory failure, otitis media, infertility, and laterality defects. The global prevalence is approximately 1 in 7,500, and over 50 genes have been identified as causative. Specific genetic variants generally correspond to particular phenotypes. This study reports on monozygotic twins with biallelic variants in the HYDIN gene, resulting in PCD with normal ciliary ultrastructure. Transmission electron microscopy (TEM) revealed a significantly higher percentage of secondary ciliary ultrastructural defects in patient 1, who also presented with a higher clinical index and distinct ciliary motion during high-speed video microscopy compared to patient 2. Genetic testing results were confirmed by immunocytochemistry and electron tomography in both patients. Electron tomography enabled visualization of defects that could not be detected in the two-dimensional space of TEM analysis. In this case, morphological diagnostics is essential due to the existence of HYDIN2, a paralogous copy of the HYDIN gene. Additionally, the twins in this study are compound heterozygotes: one variant is of uncertain significance, and the other is likely pathogenic. Given that approximately 30% of PCD cases still have an unknown genetic cause, incorporating morphological diagnostics into the PCD diagnostic toolkit remains crucial. Although the twins share the same genotype, patient 1's ciliary axonemes exhibit a significantly higher proportion of numerical defects, correlating with altered ciliary motion and more severe clinical symptoms. This case demonstrates that, despite identical genotypes, the PCD phenotype can vary even in monozygotic twins, highlighting the overall heterogeneity and complexity of the disease.

Acknowledgement: Charles University Grant Agency, grant No. 239123; Ministry of Health of the Czech Republic, grant No. NW25-07-00214

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Engaging and motivating students in histology and embryology through social media content

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Establishing good study habits is an essential step for incoming medical students. Our experience with early-semester teaching suggests we cannot rely on students to become effective facilitators of their own learning. At the same time, we as teachers compete for students' attention with the constant pull of social media. Emerging studies provide empirical evidence that academic integration and a sense of connection to peers and the institution are key factors in student success. Thoughtfully designed social media content can support this integration by building motivation, identity, and a sense of belonging — as seen on many medical faculties' platforms worldwide. We aimed to explore this potential by creating content for our department's YouTube and Instagram accounts. We produced a series of YouTube videos, Instagram posts, and reels (@histologie.embryologie, @histology.embryology). YouTube videos delivered educational content such as explanations of slides and developmental schemes, demonstrations of histological slide descriptions, presentations of learning tools, and interviews with clinical experts. Instagram posts provided weekly lecture reviews and quizzes after practical classes. Our Instagram reels were created with student demonstrators, who had creative freedom to address issues such as students' passive approach to practicals, lack of engagement, poor planning, procrastination, and lack of responsibility. The reels were scripted and directed by student assistants to ensure they spoke the students' language. Our experience suggests that content which may seem superficial at first glance can help reduce maladaptive coping and inefficient stress while fostering engagement, motivation, academic connection, and the foundations of self-directed lifelong learning. We also aim to address other aspects of the hidden curriculum — such as modeling empathy, inclusion, and respectful communication in medical education.

Acknowledgement: This work was partially supported by Charles University, Project No. SVV 260 773.

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Modeling human alveolar architecture using expandable lung progenitors in 3D and ALI cultures: A platform for investigation of alveolar differentiation and fibrosis-related pathologies

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The regeneration of human alveolar epithelium is a dynamic and tightly regulated process. Culturing distal lung epithelial cells capable of forming functional alveolar structures remains a challenge due to their architectural complexity and tendency to rapidly differentiate or enter senescence in vitro. Recent protocols employing Wnt/Yap activation and TGFβ inhibition have enabled the differentiation of lung progenitors into alveolar type 2 (AT2) and type 1 (AT1) cells, yet the mechanisms governing alveolar development and fibrotic transformation remain incompletely understood. Moreover, there is a pressing need for robust and reproducible in vitro models that reflect the physiological and pathological features of the human lung. Our lab previously developed a protocol to generate expandable lung epithelial progenitors (ELEPs) from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) characterized by stable expression of NKX2.1 and prosurfactant proteins B and C, and capability to differentiate to airway and alveolar structures in 3D and in vivo contexts. In this study, we aimed to differentiate ELEPs into more mature alveolar phenotypes under air-liquid interface (ALI) and 3D culture conditions using defined modulators of signaling pathways. Under ALI conditions, ELEPs demonstrated morphological and protein-level hallmarks of differentiation, including SPC processing, caveolin-1 expression, and early extracellular matrix production (e.g., collagen I). The prolonged differentiation activated ER stress responses linked to transitional AT2/AT1 states and cellular senescence—features often associated with fibrotic lung diseases. In 3D cultures, ELEPs maintained prolonged viability and exhibited structural and molecular features closely resembling alveolar networks. Therefore, ELEPs can recapitulate key aspects of alveolar maturation and pathological remodeling. ELEPs represent a powerful in vitro platform for modeling alveolar differentiation and provide a promising tool for investigating mechanisms underlying pulmonary fibrosis, including epithelial responses, matrix remodeling, and aberrant differentiation. This system may also support preclinical testing of antifibrotic therapies.

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Quantification of tumor-infiltrating immune cells as predictive biomarkers of immunotherapy response and toxicity in metastatic melanoma

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Background: Immunotherapy by immune checkpoint inhibitors (ICI) revolutionized treatment of melanoma patients. Tumor-infiltrating immune cells (TIICs) play a crucial role in antitumor immunity activated by ICI. Although ICI treatment improved survival outcomes, efficacy varies widely among patients. Additionally, ICI treatment may be associated with immune-related adverse events (irAEs). The aim of the study was to identify the key immune cells of the tumor microenvironment responsible for the treatment effects and risk of irAEs.

Methods: We retrospectively analyzed melanoma metastases tissue samples (FFPE) of 28 patients treated with ICI. Multilevel sampling and stereological quantification as area fractions were used to assess TIICs identified by IHC markers CD1a, CD1d, CD3, CD4, CD8, CD20, CD56, CD68, FOXP3, including immune checkpoint molecules LAG3, PD1, PD-L1.

Results: Higher expression of PD-L1 positive cells in tumor tissue was significant predictor of response to immunotherapy (P<0.05). Lower PD-L1 ($P \leq 0.05$) and CD3 ($P \leq 0.001$) expression were associated with irAEs occurence. Furthermore, in subanalysis of lymph node metastases, higher expression of PD-L1, CD8 and lower expression of CD1a cellular markers predicted response to ICI treatment ($P \leq 0.05$, $P \leq 0.05$, $P \leq 0.05$, resp.). In relation to the prognosis, higher CD8 expressions in tissue of metastases were associated with patient´s longer progression-free survival ($P = 0.0166$) as well as overall survival ($P = 0.0454$).

Conclusion: Our findings suggest that stereological quantification of specific tumor-infiltrating immune cells in tissue of melanoma metastases, such as T-lymphocytes (CD3), cytotoxic T-lymphocytes (CD8), dendritic cells (CD1a) and PD-L1 positive cells, may serve as biomarkers for predicting treatment efficacy or the risk of immune-related toxicity. Moreover, high infiltration of metastatic tissue by CD8-positive T cells is important for a long-term favorable therapeutic response of

ICIs. Characterizing the immune microenvironment could help refine therapeutic strategies and improve patient outcomes.

Acknowledgement: This study was supported by the Charles University Cooperatio Program, Research Areas MED/DIAG. L. V. was also supported by Charles University SVV – 2025 No 260 773.

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The history of the term substantia alba

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The earliest macro-anatomical terms for parts of the human body were often motivated by the shape of the anatomical structure, and one of the key features was the expression of the structure's color. This appeared as a defining characteristic in brain anatomy long before anatomists began distinguishing brain structures based on theirstructure and function. There is no consensus in historical medical dictionaries about the origin, or rather. about the authors of the term substantia alba. The first to refer to the brain as a white body was the Persian physician Ali Abbas, in his work Al-Malaki (The Royal Book) from the year 980. This was translated by Constantine Africanus into the Pantegni, the basic medieval medical work of Western Europe.

As a result, the idea that the brain is white is also found in some of the four known anatomical works of the Salernitan medical school, as well as in the writings of some pre-Vesalian anatomists. Andreas Vesalius was the first to distinguish two types of brain substance based on color. Marcello Malpighi was the first to discover that the white matter is composed of fibers, and over time, more characteristics of these two types of brain substance were identified. By the end of the 17th century, the idea emerged in brain anatomy that there are more than just two types of brain substance, and other "colored" substances were identified. The concept of substantia alba gradually developed and its meaning became more specific. However, the term—originally reflecting an external feature, color—has remained in use.

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Classification, morphology and prevalence of the external occipital protuberance variants in the paediatric population

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INTRODUCTION: Despite the external occipital protuberance (EOP) being a characteristic landmark, little is known about its variants (EOPVs). Recent reports reveal their clinical significance highlighting a possible association to cranial pain disorders or pressure ulcer formation. EOPVs might also be subject to trauma and require surgical intervention, in both the adult and paediatric population. There is also a pronounced inconsistency in anatomical and craniometric terminology regarding the EOPVs, possibly resulting from the poor recognition and understanding of the Variant morphology and formation process. In the light of current research this issue requires a clarification to establish a coherent and comprehensive classification and EOPV definition to be used in clinical practice further on and this was the aim of the study. METHODOLOGY: Retrospective analysis of 345 (M-184, F-161) anonymized head CT examinations of patients aged 0-18 years. Definitions previously utilised in the literature have been compared, along with criteria that could establish a new classification. Morphometric evaluation was performed on axial and sagittal slices using the bone window as well as 3D reconstruction to complement the EOPV morphological description. RESULTS: Analysis enabled the introduction of a coherent EOPV definition and classification. Two types (Type I – 26.19%; Type II – 73.81%) have been distinguished based on their morphological appearance. A pronounced increase in prevalence at the age of 12-13 years has been noted, reaching 40.3% after this leap, which also corresponds with structural changes and delamination of the occipital bone. DISCUSSION: Contrary to what was previously believed, this study proved that the EOPVs develop in children and not in young adults. Prevalence of the EOPVs above the age of 13 matches the one previously reported in adults. New definition and classification reduce the observer bias and establish a coherent framework for clinical and scientific use.

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Clinical anatomy in facial reconstruction

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Reconstruction of facial defects, whether due to oncological resections, trauma, or inflammatory complications, represents a challenging area within maxillofacial surgery where detailed clinical anatomical knowledge is crucial. The aim of this presentation is to demonstrate how the application of anatomical knowledge influences the planning and execution of reconstructive procedures in the facial and jaw region. The lecture focuses on the vascular anatomy of the face and jaws, including the most common anatomical variations that can affect the safe perfusion of flaps or access to vascular anastomoses. Additionally, key anatomical aspects of free flaps commonly used in reconstructions of this area—particularly free fibula flap and radial forearm free flap—will be discussed, emphasizing their vascular pedicles, shaping capabilities, and integration into three-dimensional defects. The practical part will address specific clinical scenarios where anatomical insights have guided the selection of particular flap types or surgical strategies. Thus, the presentation integrates theoretical knowledge with practical surgical decision-making and illustrates how precise anatomical understanding can improve reconstruction outcomes and minimize complications.

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Novel soluble Bazedoxifene formulation with IL-6 receptor affinity influences the properties of squamous cell carcinoma cells

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The IL-6 signalling plays a significant role in the progression and development of squamous cell carcinoma. Side effect of specific estrogen receptor modulator Bazedoxifene is also a potent inhibitor of IL-6R signalisation. Unfortunately, this substance is almost insoluble in water. We tested a new bazedoxifene formulation BAZE-X1, with enhanced solubility in water concerning its biological properties under in vitro conditions. BAZE-X1 demonstrated no toxicity measured by the MTT procedure toward squamous cell carcinoma cell lines (SCC13, LLSCC1, and FaDu). It reduced phosphorylation of STAT3, a downstream protein of IL-6R, which is good evidence of its inhibitory effect on IL-6 signalisation. BAZE-X1 has an inhibitory effect on the proliferation and migration of the studied cells in 2D and 3D conditions. It also strongly reduced their clonogenic potential. These unique properties are conditioned by the combination of affinity to IL-6 and estrogen receptor. The presented data represent an example of the repurposing of a drug.

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Morphology of Gantzer's muscle: A case report and review of the literature

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Introduction: The accessory head of the flexor pollicis longus (FPL), commonly known as Gantzer's muscle (GM) may originate from the medial epicondyle of the humerus, the coronoid process of the ulna or the flexor digitorum superficialis muscle (FDS). The GM has an oblique trajectory from medial to lateral aspect of the forearm below the FDS and then joins the FPL muscle. Today it is still debated whether the Ganzer´s muscle is a normal anatomical structure or variation.

Case Report: During a routine dissection of a 75-year-old male cadaver, the GM was identified in both forearms. The GM originated from the undersurface of the FDS. It was passing obliquely from superomedial to inferolateral. The insertion was at the ulnar border of the FPL tendon. It coursed posterior to the median nerve (MN) and anterior to anterior interosseous nerve (AIN), potentially compressing these neurovascular structures. It was a thin spindle shaped muscle. Muscle was length 8 cm, tendon length 3 cm, muscle width 2 cm. No other anomalies were observed in the upper limbs.

Results and Literature Review: According to the meta-analysis reported by Ballestretos (2018) the prevalence of the GM varies between 3-62%. Misidentification and misclassification of GM may be one of the reasons why the results are so different from each other. The GM can be inserted to the flexor digitorum profundus or pronator teres muscles and in these cases the GM is considered as additional head of these muscles. This may cause the prevalence of GM can be falsely lower.

Conclusion: The GM is a common anatomical variation with significant clinical implications, particularly in the context of unexplained median or anterior interosseous nerve compression. The radiologists and reconstructive surgeons should be accustomed to the incosistent presence of GM for precise interpretation of CT and MRI scans.

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Photographic atlas of the human body as a modern tool in anatomy education

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A photographic atlas of the human body represents a modern and effective tool in anatomy education that significantly enriches traditional forms of medical training. With its realistic depiction of anatomical structures based on photographs of actual human bodies, it enables students to better understand the spatial relationships between organs, tissues and systems. This type of atlas overcomes the limitations of classical illustrations by providing visually accurate and detailed views of the human body in its natural form. The present paper discusses the advantages and possibilities of using a modern educational tool called Anatomical Photographic Atlas for practical exercises, which will be prepared in electronic form. The benefits of a photographic atlas are multidimensional. First, it supports visual learning, which is more effective for many students than textual or abstract forms of instruction. It also enhances information retention due to its realistic representation, which positively impacts performance in practical exams and clinical diagnostics. The atlas also serves as a bridge between theory and practice, as students learn to recognize structures as they appear during dissections or surgical procedures. Moreover, in settings where access to cadavers is limited, the photographic atlas serves as a valuable alternative or supplement to hands-on learning. Although it has advantages, the photographic atlas is not intended to replace traditional dissection-based teaching, but rather to complement it.

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The epigenetic role of Chokeberry in a rat model of breast cancer: A natural chemopreventive study

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Introduction: The increasing number of new breast cancer cases in the female population worldwide requires alternative preventive and therapeutic strategies as the supplement to conventional clinical approaches. Chemoprevention is characterized by the use of naturally occurring phytosubstances that exert many beneficial effects on human health. Therefore, this research aimed to analyze the chemopreventive and antitumor effects of chokeberry (Aronia melanocarpa L.) based on epigenetic changes in chemically-induced breast cancer in vivo.

Methods: The chemopreventive and antitumor effects of chokeberry were evaluated in N-methyl-N-nitrosourea-induced breast cancer in female rats (Sprague-Dawley). Chokeberry (A. melanocarpa L.) was dietary administered at two concentrations: group ARO 0.3 (3 g/kg of food) and group ARO 3 (30 g/kg of food) during 14 weeks. As a control group (CONT) was used rats with normal diet without chokeberry. After decapitation and obtaining the tumor samples, we analyzed the modulation of epigenetic mechanisms such as histone modifications (immunohistochemical analysis), DNA methylation (pyrosequencing), and miRNA expression (real-time PCR).

Results: The epigenetic analyses of rat tumor tissues revealed that administration of chokeberry significantly and positively altered histone modifications (H3K4m3 and H3K9m3), DNA methylation in promoter regions of tumor suppressor genes (PTEN and TIMP3), and miRNA expression (miR155, miR210, and miR34a) when compared with control group. Generally, the higher dose of chokeberry (ARO 3) was slightly more effective than the lower dose (ARO 0.3).

Conclusion: In our experiment, the chokeberry demonstrated chemopreventive and antitumor effects through the modulation of epigenetic mechanisms in a rat model of breast cancer but the utilization of these effects in clinical practice is still in its infancy and requires long-term clinical evaluations.

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Integration of educational tools in the histology teaching leads to a higher level of interactivity

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Over the decades, we have modified the tuition of histology by computer-based technologies including interactive slides and virtual microscopy. Interactive slides are based on digitized microscopic images taken at different magnifications and endowed with a dynamic element that allows the image to be overlaid with a coloured mask when the cursor is hovered over, in which important structures are highlighted. This way students are alerted to the presence of microscopic structures that need attention. Interactive slides are a part of e-learning courses prepared on a free platform Moodle. The e-courses include the necessary theoretical explanation and quizzes. For virtual microscopy we use Smart Zoom e-learning platform to access the collection of histological slides scanned at high-power magnification. Students can view digitized microscopic images at different zooms in a similar way like under the light microscope with the help of the computer or the mobile phone via a high-speed internet connection. Another favourite feature of Smart Zoom is a possibility to annotate the slides. Although both of the above innovations brought huge improvements to traditional teaching, they remained more or less separate and unconnected tools. Stdents are supplied with handbooks used in practical teaching provided descriptions of slides with URL links to find the access to virtual microscopy and interactive slides of relevant tissues. However, finding a link by typing URL coordinates into iPads was tedious. Currently we entered QR codes of URL locations for both virtual microscopy and interactive slides by placing these codes next to the text describing the corresponding tissue. By scanning the code, the student can immediately access the required interactive tool. Thus, our new textbook integrates previously separate tools to a new level of interactivity.

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Application of geometric morphometry in the evaluation of the foramen supratrochleare from the Early Bronze Age site

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This research was focused on the size variability of the foramen supratrochleare of skeletal remains from the Early Bronze Age site Košice-Krásna. This anatomical variation is located on the distal part of the humerus between the fossa olecrani and fossa coronoidea. This structure is not present in every individual and the etiology is unknown. The aim was to use geometric morphometry to accurately assess shape characteristics and verify whether there are statistically significant differences between males and females, as well as between the right and left sides. Together, 25 humeri were used for the evaluation. Measurements with 7 fixed landmarks were performed using digital tools – ImageJ (with Fiji add-on), TPSUtil, and TPSdig. Statistical analysis was performed in the RStudio using methods such as Generalized Procrustes analysis with Thin-Plate Spline warping of 2D mesh and Principal Component analysis. Visualization of the data indicated larger aperture dimensions in females, while male bones were more robust. Mean shape configurations for the left and right humerus were also calculated. In both, GPA showed slight shape variability, particularly at landmarks E, F, and G, located at the lower part of the bone. Significant sex-based differences in the left humerus were observed at point E. For the right humerus, GPA revealed lateral differences at points B, E, F, and G; sex-based differences were found at E, F, and G. TPS analysis revealed greater distortion on the right side of the foramen, possibly due to different biomechanical loading of the dominant hand as an important factor in morphological variation. These results showed the occurrence of this anatomical variation also in the prehistoric population, and statistical analysis confirmed significant differences between sexes and sides, which can help explain the etiology.

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Comparative analysis of the Conjunctiva-Associated Lymphoid Tissue (CALT) and the eyelids in the selected birds of prey from Accipitridae family

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The goal of current study was the morphological analysis of the upper, lower and third eyelids as well as the CALT in the representatives of the Accipitridae family. The eyelid samples of Accipiter gentilis, Accipiter nisus, Aquila nipalensis, Buteo buteo, Circus cyaneus, Haliaeetus albicilla, Pernis apivorus were collected. The Movat pentachrome, Masson-Goldner trichrome, and picro-Mallory trichrome staining methods were used for the histological analysis. The upper and lower eyelids were composed of an anterior palpebral surface and a posterior palpebral surface, while the third eyelid was composed of palpebral and bulbar surface (both with folds), marginal plait and leading edge. The keratinized stratified squamous epithelium covered the anterior surface of the upper and lower eyelids, while the posterior surface of the both eyelids contained non-keratinized stratified squamous epithelium with goblet cells. The palpebral surface of the third eyelid was lined with non-keratinized stratified squamous epithelium with presence of numerous goblet cells. Aquila nipalensis and the Haliaeetus albicilla had less numerous muscle fibers in the stroma of both eyelids in comparison to the rest analyzed birds of prey. The number of palpebral and conjunctival folds was from 6 to 30 and 29 to 45, respectively. The CALT was organized in the form of lymphoid follicle, diffuse lymphocytes and high endothelial venules (HEV) in all examined species. Ethical statement: The studies on tissues obtained post-mortem do not require the approval of the Ethics Committee (Journal of Laws of the Republic of Poland, the Act of January 15, 2015, on the protection of animals used for scientific or educational purposes (Directive 2010/63/EU of the European Parliament and of the Council of 22nd September 2010 on the protection of animals used for scientific purposes).

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Immunohistochemical analysis of changes in intestinal epithelium of germ-free piglets after Escherichia coli infection

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Germ-free (GF) piglets are considered as relevant model for studying of gastrointestinal diseases, because of their anatomical, physiological and also immunological similarities to humans. In our actual work, GF piglets were infected orally by culture of Escherichia coli O149:K88 on day 5 (ECK group) and samples from jejunum and colon were harvested for quantitative and semi-quantitative histological analyses. Our presented study was focused on investigating the intestinal epithelium integrity and changes in distribution and secretory activity of goblet cells and enteroendocrine serotonin producing cells of GF piglets after E. coli infection compared to a healthy control group (HC group). For this aim, the immunohistochemistry was used to detect ZO-3 (zonulla ocludens protein 3), MUC1/anti-EMA (mucin 1) and 5HT/Serotonin in intestinal epithelium. Our results showed increase in ZO-3, MUC1/anti-EMA and 5HT/Serotonin positivity in jejunum in ECK group vs. jejunum in HC group. Similar changes were observed in colon of HC group vs. ECK group except 5HT/Serotonin immunohistochemistry, which showed significant decrease ($p<0.001$) of the number in 5HT/Serotonin positive cells in ECK group vs. HC group. Our immunohistochemical analyses also showed significant increase ($p<0.05$) of MUC1/anti-EMA and ZO-3 positivity as well as significant decrease ($p<0.001$) in 5HT/Serotonin positive cells in colon in ECK group vs. jejunum in ECK group. The results suggest that the colon of the GF piglets is more resistant and less susceptible to E. coli infection compared to the jejunum. Detailed understanding of all mechanisms in intestinal segments after infection may lead to the development of more effective strategies for prevention as well as for treatment of intestinal diseases.

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Quantitative profiling of DOR immunofluorescence at the plasma membrane and in the cytoplasm of primary sensory neurons in the mouse spared nerve injury model of neuropathic pain

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Delta opioid receptor (DOR) plays a crucial role in pain perception. Under physiological conditions, its intraneuronal localization predominates over its presence at the plasma membrane of primary sensory neurons (PSNs) in the dorsal root ganglia (DRG). However, the regulation of DOR intraneuronal trafficking remains controversial across different models of neuropathic pain. We compared the quantitative profiling of DOR immunofluorescence (IF) at the plasma membrane and in the cytoplasm of DRG neurons in the naïve and sham-operated mice, and mice subjected to unilateral spared nerve injury (SNIt) of the sciatic nerve at 7- and 21-days post-operation (POD7, POD21). Cryostat sections of L3 DRG from both the ipsilateral and contralateral sides were immunostained for DOR. The intensity profiles of DOR-IF were analyzed across individual somata of large, medium and small DRG neurons. In Sham POD7 group, the DOR-IF intensity was significantly reduced bilaterally in the cytoplasm of all subtypes of DRG neurons compared to the naive control group, while same reduction on the plasma membrane was observed exclusively in ipsilateral DRG neurons. In contrast, SNIt POD7 compared to the sham group induced an increase in DOR-IF on the plasma membrane and in the cytoplasm especially in large DRG neurons. Comparing SNIt POD7 to naïve, there was predominantly decrease in DOR-IF bilaterally. At POD21, both Sham and SNIt surgeries induced an increase in DOR-IF located to the plasma membrane of large DRG neurons in ipsilateral side. Cytoplasmic levels in large neurons, as well as both cytoplasmic and plasma membrane levels in medium and small neurons, were either nearly equal to those in naive group (in sham-operated mice) or predominantly reduced bilaterally (in the SNIt-group). These results indicate that DOR trafficking is a dynamic and nerve injury-responsive process that changes over time among DRG neuronal subpopulations.

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Comprehensive histological examination of the renal corpuscle

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Comprehensive histological examination of the renal corpuscle is essential in the study of the basic physiological architecture of the nephron and it is also crucial for the histopathological diagnosis of various renal diseases. This examination is usually performed on a specimen obtained by renal biopsy and involves three main areas: light microscopy, immunofluorescence and electron microscopy. Due to the extreme complexity of the histological structure of the renal corpuscle, a single staining method cannot highlight the complete structure under physiological or pathological conditions. A basic overview of the architecture of the individual components of the renal corpuscle (Bowman's capsule layers, glomerulus, mesangium, juxtaglomerular apparatus and vessels) can only be obtained by combining different appropriate histological staining methods. Therefore, histological practice uses an algorithm involving different staining methods: routine methods (basic morphological orientation), selective methods (highlighting of selected structures), histochemical methods (detection of the distribution of components according to their chemical composition), and immunohistochemistry (detection of selected cell/tissue markers according to the primary intent of the histological analysis). Based on the obtained methods, the structure of a healthy renal corpuscle can be described in terms of its normal configuration and cellularity. Histopathological analysis can determine the exact type of glomerulopathy (e.g. focal/segmental glomerulosclerosis or IgA mesangial nephropathy), the overall degree of activity and chronicity, and the presence of any systemic disease. The following can be assessed and analysed in a targeted manner: the histomorphometry of the glomeruli and Bowman's capsule; the presence of hypercellularity (e.g. membranous, mesangial or endocapillary); the presence of sclerotisation and hyaline membranes; and changes to the interstitium.

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Glutathione peroxidase 6 in rat oocytes and preimplantation embryos

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Background: Antioxidant enzymes, such as glutathione peroxidases, were developed by the cell to defend it against high levels of reactive oxygen species, which are capable to damage proteins, lipids or DNA of cells. If oocytes or preimplantation embryos are damaged, this may lead to the impaired fertility. That is the reason, why we decided to detect glutathione peroxidase 6 (GPx6) in oocytes and preimplantation embryos (O/PEs) of rat.

Materials and methods: Female Sprague Dawley rats were mated with males of the same strain. Females were euthanized after successful mating proving by the presence of the vaginal plug. Oviducts and uterine horns were removed and flushed out to obtain oocytes and preimplantation embryos. GPx6 in O/PEs was visualized by the antibody conjugated with FITC and DAPI was used for DNA visualization. Confocal microscope was used to observe isolated O/PEs.

Results: GPx6 was detected in all O/PEs. In oocytes, GPx6 was detected in the whole cytoplasm homogeneously. In 4-cell embryos, this enzyme was mostly not detected, only few embryos had homogeneously positive cytoplasm, but the signal intensity was very weak. Slightly stronger signal intensity was observed in the cytoplasm of 8-cell embryos and an even stronger intensity was detected in the morula and blastocyst stages.

Conclusion: GPx6 was detected in all examined O/PEs. The weakest signal was detected in 4-cell embryos, but in 8-cell embryos, morula and blastocyst stages, the signal was stronger. This may be related to the embryonal-maternal shift of the genome. The role of the embryonal genome in 4-cell embryos is to be taken over by the maternal genome in 8-cell embryos. Besides that, one can assume, that the role of GPx6 may be important for oocytes and embryos during the preimplantation period of pregnancy.

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Arteriolar density in myocardia of patients with the right ventricular dysfunction

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Background: Pathophysiological processes during the progress of heart failure affect the morphology of the heart on the macroscopic and microscopic level. An important factor for proper functioning of myocardium is efficient blood circulation in the microvascular bed, which also includes arterioles. Right ventricular dysfunction (RVD) is a situation that can affect the progress of heart failure. We evaluated density of arterioles (AD) in the hearts of patients with terminal heart failure with respect to concomitant RVD.

Methods: For analysis, we used the myocardial tissue from explanted hearts of heart transplant recipients with heart failure. Therefore, we obtained the samples of left (n=23) and right ventricles (n=35). In addition, we obtained samples of right ventricles from hearts of transplant donors (n=15). The samples were immediately fixed with 4% formaldehyde, embedded into paraffin and then cut for the purpose of immunohistochemical labeling. An antibody against smooth muscle actin was used to visualize vascular smooth muscle cells to better identify arterioles in the myocardium. Histomorphometric measurements were performed using the program ImageJ.

Results: In the left ventricular myocardium of patients with heart failure, the AD was 1,70±0,53/mm2, while in the right ventricular myocardium the AD was 1,5±0,83/mm2. When we divided the samples of right ventricles according to presence of RVD, the AD values were 1,70±0,86/mm2 for the RVD group and 1,40±0,82/mm2 for the non-RVD group. In the donor group, the AD value was 2,35±1,1/mm2. The differences between the groups were not statistically significant.

Conclusion: Samples of left and right myocardia from patients with terminal heart failure had similar AD. Furthermore, in our set of patients we did not observe a significant difference of AD between RVD and non-RVD groups. This corresponds with our findings of capillary density. To provide more conclusive data, additional samples from donors should be evaluated.

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Shank3 deficiency induces region-specific alterations in presynaptic protein levels in dopaminergic brain areas

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Presynaptic proteins are essential for synapse formation and maintenance throughout the brain. Alterations in their expression, organization, and interaction with postsynaptic elements can result in synaptic dysfunction, contributing to neurodevelopmental disorders such as autism spectrum disorders (ASD). ASD is characterized by heterogeneous symptomatology, with core symptoms including impairments in social interaction and the presence of repetitive behaviors. Although disruptions in dopaminergic pathways are thought to play a significant role in ASD pathophysiology, the molecular mechanisms underlying these alterations remain poorly understood. The aim of this study was to evaluate changes in presynaptic protein expression and distribution in Shank3-deficient mice – an established animal model of ASD – focusing on dopaminergic brain regions, specifically the ventral midbrain and frontal cortex. Gene expression analysis revealed a significant upregulation of Synaptophysin and Synaptic Vesicular Glycoprotein 2A (SV2A) mRNA in the midbrain of Shank3-deficient mice, while no such changes were detected in the frontal cortex. Furthermore, immunofluorescence analysis of primary neuronal cultures isolated from these regions in Shank3-deficient mice showed a reduced Bassoon signal in midbrain neurons, while cortical neurons exhibited a decreased Synapsin I signal. However, cortical neurons exhibited an increase in Bassoon levels. These findings suggest that Shank3 deficiency leads to region-specific dysregulation of presynaptic proteins, which may disturb synaptic architecture and contribute to the pathogenesis of ASD.

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Odontometric analysis in the Early Bronze Age population

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Background: Tooth dimensions offer key insights into biological variation, population history, and lifestyle in ancient human groups. Because teeth preserve well, dental measurements are widely used in bioarchaeology to study sexual dimorphism, population relationships, and adaptations to environmental and cultural changes. Changes in diet can affect tooth size and shape, revealing information about past environmental pressures and cultural habits.

Aim: The main objective of the study was to observe the buccolingual and mesiodistal dimensions of permanent teeth in an adult population from the Early Bronze Age (Ottoman culture, 1600-1450 BCE) and then to determine sexual dimorphism in each dimension of each permanent tooth.

Material and Methods: This study included a set of tooth analyses of 48 individuals (17 females and 31 males) whose skeletal remains were discovered at the archaeological site of Košice-Krásna. The maximum width of the MD (mesiodistal) and the maximum width of the BL (buccolingual) of all preserved maxillary and mandibular permanent teeth were evaluated using a digital caliper. Significant results were obtained at a level of p < 0.05.

Results: The odontometric analysis revealed statistically significant sex-related differences, with 11 teeth showing differences in the mesiodistal dimension and 18 teeth in the buccolingual dimension. The MD dimension of the maxillary first molars and mandibular canines and the BL dimension of the maxillary and mandibular canines and first molars represented reliable indicators for sex determination. The second premolars showed the smallest inter-sex differences in the measured dimensions.

Conclusion: Measurements of adult teeth from the Early Bronze Age varied by size and sex, and these differences may be useful for comparison with findings from other ancient populations. The results will provide valuable information about their dietary habits and health status, and will contribute to our understanding of biological and cultural diversity in the past.

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Workshop: AI in ImageJ – Modern approaches to image analysis

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This hands-on workshop will introduce participants to the practical use of AI-powered plug-ins for image segmentation and analysis in Fiji (ImageJ). The session will focus on three widely used tools: Trainable WEKA Segmentation, StarDist and Cellpose. These plugins utilise machine learning and deep learning techniques to enable accurate, efficient and reproducible image analysis. Participants will learn how to apply each tool in real-world scenarios. This includes: (1) training and applying custom models for pixel classification using Trainable WEKA, (2) utilising StarDist for the precise detection of star-convex shaped nuclei and other cell types, (3) applying Cellpose, a generalist deep learning-based segmentation tool, for versatile cell shape analysis. The workshop is designed for individuals interested in integrating AI-based methods into their workflows.

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Sex-specific modulation of Hofbauer cell phenotype and apoptotic body accumulation in diabetic pregnancies

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Hofbauer cells (HBCs) are macrophages of fetal origin located in the stroma of placental villi. Their function is quite complex, including ensuring immunological tolerance at the feto-maternal interface. They are considered as M2 macrophages expressing a variety of antigens, including CD206. HBCs are the only cells responsible for efferocytosis in villi stroma of placenta. Efficient clearance of apoptotic bodies is essential for maintaining placental homeostasis. Their accumulation can disrupt the local immune environment, damage tissue architecture, and contribute to the development of placental pathologies. Diabetes is a common pregnancy complication known to affect placental function. Using immunohistochemistry and morphometric analysis we investigated the numbers of HBCs, the relationship between CD206 intensity and inflammation and their morphology in human placenta samples. We also examined the accumulation of apoptotic bodies in the stroma of placental villi. The assessed parameters were compared across pregnancies complicated by type 1 diabetes mellitus (T1DM), gestational diabetes mellitus (GDM), and normoglycemic controls, as well as between male and female fetuses. We analyzed 54 formalin-fixed, paraffin-embedded term placental samples, including those from healthy pregnancies (n = 18), patients with type 1 diabetes mellitus (T1DM, n = 22), and patients with gestational diabetes mellitus (GDM, n = 14). Our findings demonstrate that both the presence of T1DM and GDM, as well as fetal sex significantly influence the investigated parameters in the placental villous stroma. These results may enhance our understanding of the mechanisms underlying pregnancy complications.

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Assessment of pro-angiogenic properties of polyhydroxybutyrate/chitosan-based scaffolds using an ex ovo quail CAM model

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The chorioallantoic membrane (CAM) of avian embryos serves as a well-established in vivo model for investigating angiogenesis and its modulation under various experimental conditions. The present study aims to assess the pro-angiogenic potential of a composite scaffold consisting of polyhydroxybutyrate/chitosan (PCHLY), either alone or combined with an agarose/gelatin gel (AG), utilizing an ex ovo quail CAM model. On embryonic day (ED) 6, the PCHLY and AG-PCHLY scaffolds were placed onto the surface of the quail CAM. After 72 hours (ED9), the scaffold-CAM complexes were excised for histological and immunohistochemical analyses. The angiogenic response was quantitatively assessed by calculating the vascular index, which reflects the difference in the number of blood vessels in the surrounding CAM area between ED6 and ED9. The neovascularization around the implants was examined using the IKOSA software. The formation of blood vessels in the surrounding area of the PCHLY and AG-PCHLY scaffold, and cell invasion into the implants, were evaluated using the markers of endothelial cells and hemangioblasts (QH1), myofibroblasts (α-SMA), and the proliferative activity of the cells (PHH3) with immunohistochemical staining. The in vivo evaluation of angiogenic activity demonstrated that both scaffolds possess pro-angiogenic potential. However, the quantitative analysis of the vascular index revealed a significantly enhanced angiogenic response of AG-PCHLY (81.59% ± 3.42%) compared to PCHLY (74.12% ± 4.90%). Histological examination revealed the formation of new CAM villi and their integration into both scaffolds, indicating favourable biocompatibility. Immunohistochemical analysis further confirmed the occurrence of angiogenesis and vascular ingrowth through the expression of immunomarkers, including α-SMA, QH1, and PHH3. These findings underscore the potential of the tested scaffolds to support neovascularization without the need for exogenous pro-angiogenic agents such as growth factors or bioactive molecules. Their acellular composition, coupled with the ease of their fabrication and cost-effectiveness, highlights their potential as biomaterials for regenerative applications.

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TPPU-induced changes in p38 expression and nuclear translocation in intestinal cell lines

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Soluble epoxide hydrolase (sEH) inhibitors, such as TPPU, are currently being investigated in clinical trials due to their potential therapeutic benefits. However, their impact on intracellular signaling pathways remains poorly understood. We investigated the effects of TPPU on the activation and subcellular localisation of the stress-activated protein kinase p38 in the colon cancer cell lines Caco-2 and HT-29. The In-Cell ELISA method revealed that the expression of phosphorylated p38 (p-p38) was increased in untreated differentiated cells. Similarly, TPPU treatment increased the levels of p-p38. Immunocytochemical staining further demonstrated enhanced nuclear localisation of p-p38 in both, differentiated and undifferentiated, cells exposed to the inhibitor. To evaluate whether these changes reflect differentiation-related events, we performed an immunohistochemical staining of human tissue samples, including normal colon tissue, grade 2 colorectal tumors, and embryonic/foetal tissue. During normal differentiation, p-p38 translocates from the cytoplasm to the nucleus, however, tumor samples exhibited persistent cytoplasmic localisation of p-p38, resembling undifferentiated tissue. These findings suggest that sEH inhibition alters p38 MAPK signaling dynamics and may interfere with differentiation processes in intestinal cells. This raises concerns about the potential adverse effects of sEH inhibitors on epithelial cell maturation and stress response regulation.

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Nestin and connexin43 co-expression in cardiomyocytes of spontaneously hypertensive rats

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Nestin, a cytoskeletal intermediate filament, is typically expressed in developing and regenerating tissues. In the myocardium, its expression is limited to fetal development; however, in adults, nestin-positive (nestin$^+$) cardiomyocytes appear under pathological conditions such as hypertrophy and myocardial infarction. This study aimed to contribute to the clarification of the mechanisms behind nestin re-expression in the adult myocardium by performing double immunofluorescence detection of nestin and connexin 43 (Cx43), the main component of gap junctions in the intercalated discs, in the left ventricles of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Cryosections from 5-month-old SHR and WKY rats and 15-month-old SHR rats were examined. Nestin and Cx43 were detected by an indirect two-step immunofluorescence method. In WKY rats, no nestin$^+$ cardiomyocytes were identified, and Cx43 was regularly localized in the intercalated discs. In 5-month-old SHR rats, rare nestin$^+$ cardiac muscle cells were found, with Cx43 distribution similar to controls. In 15-month-old SHR rats, more nestin$^+$ cardiomyocytes were observed. Although their Cx43 expression resembled that of surrounding nestin$^-$ cells, some nestin$^+$ cardiomyocytes showed Cx43 immunoreactivity also on lateral membranes, indicating remodelling. The number of nestin$^+$ cardiomyocytes increased with the progression of hypertension and myocardial hypertrophy, accompanied by structural remodelling of cardiac tissue, evidenced by hemichannel lateralization in both nestin$^+$ and nestin$^-$ cells. These findings suggest that nestin$^+$ cardiomyocytes are regulated by similar mechanisms of Cx43 expression and maintain comparable electrical conduction properties to nestin$^-$ cells, even during pathological remodelling.

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Effects of E. coli infection on intestinal mucosal renewal in germ-free piglets – histological analysis

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We focused on histological investigation the impact of E. coli infection influence on intestinal mucosal turnover and apoptosis. We have verified immunohistochemical changes in proliferation and apoptosis in epithelial lining as well as in lamina propria of jejunum and colon of germ-free (GF) piglets as healthy control group and GF piglets in which at 5th day their gut was colonized with E. coli bacteria (ECK group). According to our results we detected significant increase in proliferation of the epithelial cells only in the jejunum of the ECK group, indicating a higher sensitivity to colonization with E. coli. Significant changes in the TUNEL assay and immunohistochemistry of other studied markers (TNF-α, Caspase-3 and HSP-70) were noted only in the lamina propria mucosae of both intestinal segments in the ECK group. We finally found that the commensal gut microbiota plays a role in regulation of the turnover rate in the epithelial lining, but also in the cells in the lamina propria mucosae in both intestinal segments, and that the host response is dependent on the colonising bacteria. Open access funding provided by The Ministry of Education, Science, Research and Sport of the Slovak Republic in cooperation with Centre for Scientific and Technical Information of the Slovak Republic. This work was supported by EÚ project “Replace” at University of Veterinary medicine and Pharmacy in Košice (FOOD CT-2003-506487), and by the Cultural and Educational Grant Agency Ministry of Education, Science, Research and Sport of the Slovak Republic under contract no. KEGA 008UPJŠ-4/2024.

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Proteomic analysis of chick embryonic heart in experimental hypoxia

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Investigating prenatal hypoxia is difficult in mammals, as there are confounding factors stemming from maternal adaptations and compensatory mechanisms. We have thus established an avian model of hypoxic incubation (starting after 2 days of normoxia, 15% O2, normobaric, until the time of sampling at embryonic day 8) to study embryonic reactions to low oxygen concentration. Our previous studies have shown increased vascularization, oedema, and ventricular wall thinning preceding the lethality at mid-gestation. Analysis of the cardiac proteome after 6 days of hypoxic incubation showed strong upregulation of enzymes involved in anaerobic glycolysis as well as an increase in apoptosis-related proteins, cell adhesion proteins, and secretory activity.

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Expression of superoxide dismutase 3 in malignant pancreatic tumors: an immunohistochemical study

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Background: Malignant pancreatic tumors represent one of the most aggressive oncological diseases. The most prevalent type is pancreatic ductal adenocarcinoma (PDAC). Our study aimed to investigate the relationship between the expression of superoxide dismutase 3 (SOD3) in PDAC cells and selected clinicopathological parameters, as classified according to the 8th edition of the AJCC/UICC TNM system. These parameters included tumor size (T1, T2, T3), tumor location (head, body, and tail of the pancreas), extent of lymph node metastasis (N0, N1, N2), degree of tumor differentiation (G1, G2, G3), and patient sex (male, female).

Material and Methods: The study included 60 formalin-fixed, paraffin-embedded tissue samples of PDAC. SOD3 immunoreactivity was evaluated using a three-step indirect immunohistochemical method under light microscopy.

Results: Among the total sample set (n = 60), 42 samples (70%) were negative for SOD3 expression, while 18 samples (30%) were positive. The results of the immunohistochemical analysis were correlated with the aforementioned clinical and pathological parameters. To assess statistical associations, Fisher's exact test and the chi-square test were used. A statistically significant correlation was found between SOD3 expression and the degree of tumor differentiation.

Conclusion: The findings of our study suggest that SOD3 may serve as a marker related to tumor differentiation in PDAC, warranting further investigation into its prognostic and biological relevance.

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BeWo cells as an in vitro model of trophoblast for studying diabetes mellitus

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There are three main types of Diabetes Mellitus: Type 1, Type 2, and Gestational Diabetes. Gestational Diabetes Mellitus (GDM) is a transient metabolic disorder characterized by an inadequate insulin response to hyperglycemia and the development of insulin resistance. Hyperglycaemia induces a systemic inflammatory state that affects several tissues, including the placenta. However, the molecular mechanisms underlying placental dysfunction due to diabetic complications are not yet clear and will require further research. GLUT1 has been identified as the primary transporter responsible for glucose transfer in the placenta and its expression is significantly higher in placentas of women with GDM compared to those from normoglycemic pregnancies. The trophoblast-derived choriocarcinoma cell line BeWo is commonly used as an in vitro model of trophoblast and can be used to study the effects of hyperglycemia because it has been shown to be sensitive to changes in the glucose concentration in culture medium. Despites its common use, current literature is inconsistent regarding the different types of culture media and glucose concentrations used to study the effect of hyperglycemia on BeWo cells. Furthermore, BeWo cells can undergo syncytialization upon treatment with forskolin, making them a suitable model for studying syncytiotrophoblast formation. In our study, we investigated the effects of different culture media and varying glucose levels on GLUT1 expression and syncytialization, using PP13 as a marker, in BeWo cells. Our data suggest that GLUT1 expression is primarily influenced by the total glucose concentration in the culture medium, rather than the type of medium itself.

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Monitoring the effects of geographical location on the embryotoxicity of Vipera ammodytes venom originating from different regions of the Balkan peninsula

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Vipera ammodytes is annually responsible for a significant number of human envenomations in the Balkan Peninsula. In this study, we compared the embryotoxicity of venom from individuals of this species collected from three distinct localities: Skadar (Montenegro), Saint Helena (Romania), and Biser (Bulgaria), using the Chicken Embryotoxicity Screening Test (CHEST). We focused on mortality rates, changes in overall embryo and organ weights, specifically the liver and heart, as well as the occurrence of morphological alterations. According to our findings, the venom exhibiting the highest toxicity as indicated by embryonic mortality, originated from V. ammodytes specimens collected at the Skadar locality, with an LD_{50} value of 3.9 µg/egg. The venom from snakes originating in Biser exhibited comparatively lower toxicity, with an LD_{50} of 4.1 µg/egg, whereas the weakest embryotoxic effect was observed following the administration of venom from Saint Helena specimens, which exhibited an LD_{50} of 6.9 µg/egg. Morphological alterations were observed in two cases: cyclopia was detected following exposure to venom from Saint Helena specimen, while unilateral microphthalmia was found after exposure to venom obtained from Skadar specimen. Our findings indicate that geographic origin may play a significant role in modulating venom composition and toxicity. The observed variability in venom profiles likely results from a combination of factors across the compared regions, including climate and meteorological conditions, altitude, differences in atmospheric pressure, and diversity in prey species, to which the snakes have adapted their predatory hunting strategies. Differences in venom composition and effects among snakes of the same species from various locations raise concerns regarding the efficacy of currently available antivenoms, thereby representing a significant medical challenge. To better understand these

observed differences, further proteomic analysis and validation of antivenom effectiveness are necessary.

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Changes in C3 and C3aR in glia limitans superficialis astrocytes in the medial prefrontal cortex after sciatic nerve compression

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The glia limitans superficialis (GLS), a superficial barrier formed by astrocyte bodies and their cytoplasmic processes underlying the pia mater, serves as an interface between the subpial space and the brain parenchyma. The complement component C3 and its receptor C3aR play pivotal roles in neuroinflammatory processes within the central nervous system. Activation of the complement system leads to the generation of C3a, which binds to C3aR expressed on astrocytes and microglia, initiating pro-inflammatory signaling cascades and cytokine release. The objective of our study was to investigate and compare levels of C3 and C3aR in the GLS of naïve, sham-operated, and sciatic nerve compression (SNC)-operated animals on post-operative days (POD) 3 and 7. Immunohistochemical detection of C3 and C3aR was performed on coronal cryostat sections of the medial prefrontal cortex. To localize C3 and C3aR specifically within GLS astrocytes, double immunostaining with a GFAP antibody was employed. In naive rats, C3 immunofluorescence (IF) intensity in GLS astrocytes was low. On POD3, C3-IF intensity increased in SNC-operated rats. By POD7, both sham- and SNC-operated animals showed elevated C3-IF levels compared to naïve controls. In contrast, C3aR-IF intensity levels were increased only in SNC-operated animals at both POD3 and POD7 compared to both naïve and sham-operated animals. The increase in C3 and C3aR levels in GLS astrocytes of SNC-operated rats on POD3 and POD7 suggests an autocrine signaling role of C3/C3aR in sustained inflammatory activation of this astrocyte subtype following nerve injury.

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Modeling the ocular toxicity of Spitting Cobra venom on the chorioallantoic membrane of chicken embryos

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The aim of our study was to monitor and evaluate the effects of the oculotoxic venom from Hemachatus haemachatus on the vascular network of the chorioallantoic membrane (CAM). ‚Spitting cobras' are known for their unique defence mechanism, the ability to eject venom over several meters, primarily targeting the eyes of potential threats. The venom components of these snakes induce severe ophthalmia upon contact with the eye surface, potentially leading to permanent blindness without prompt and appropriate medical intervention. Following the administration of H. haemachatus venom onto the exposed CAM vasculature of chicken embryos (embryonic day 9), all treated groups exhibited rapid vascular reactions. These responses were characterized by an initial phase of hyperaemia and vasodilation, which subsequently progressed to a "blanching" of the blood vessels. Vessels that were initially well-perfused and visually bright red became intensely hyperaemic within the first minute, subsequently becoming pale or almost translucent by five minutes post-application. This effect was consistently observed following the administration of both tested doses (3 µl and 2 µl) across all eight venom-treated CAMs. At the site of venom application, blood vessels exhibited a loss of perfusion while remaining structurally intact, with only minimal evidence of haemorrhage. The progressive discoloration facilitated the visual tracking of venom diffusion and its subsequent impact on more distant vasculature. In certain instances, coagulation and thrombus formation were also documented. The vascular response was assessed using the HET-CAM assay, in accordance with the protocol established by Luepke (1985). The resulting cumulative scores were 13.5 for the cohort treated with 3 µl of venom and 11.7 for the cohort treated with 2 µl, with both values falling within the range indicative of strong irritation potential as delineated by the Luepke grading system.

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Interaction of FTO rs9939609 polymorphism and physical activity on body composition in women: A bioimpedance-based study

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Background: The FTO gene is the most well-established genetic factor associated with obesity. The rs9939609 variant is strongly linked to increased body mass index (BMI) and body fat. However, its effect on obesity risk can be reduced by up to 40% through physical activity or a healthy lifestyle. Since traditional anthropometric measures cannot distinguish between fat and lean mass, this study employed bioelectrical impedance analysis (BIA) for a more accurate assessment. We examined whether physical activity modifies the association between the FTO rs9939609 variant and body composition in healthy adult women.

Methods: This cross-sectional study included a total of 361 women between the ages of 18 and 69. The FTO rs9939609 polymorphism was genotyped using real-time PCR (Applied Biosystems™ 7500 Fast). Body composition was assessed using anthropometry and bioelectrical impedance analysis (BIA) (Bodystat QuadScan 4000).

Results: The frequency of the FTO risk allele A was 45.3%, and the most common genotype was AT (43.9%). Women carrying at least one A allele had lower skeletal muscle mass and a higher body fat percentage. This resulted in reduced fat-free mass and total body water. Among women with risk genotypes (AA or AT), those who were recreationally active showed significantly lower body weight and fat mass and significantly higher fat-free mass, total body water, and muscle mass compared to non-athletes. The waist-to-height ratio (WHtR) was the most predictive anthropometric indicator of adiposity, while the fat mass-to-fat-free mass ratio and body fat percentage were the strongest predictors in bioimpedance analysis.

Conclusion: Regular physical activity is key to managing obesity, especially for women who are genetically predisposed. Combining anthropometric and bioelectrical impedance analysis (BIA) methods offers a more accurate assessment of body composition. Standardized body fat measurement should be included in obesity screening protocols. Physical activity may mitigate the negative impact of the FTO risk allele on body composition.

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MRI morphological detection of basal ganglia abnormalities in epileptic patients:

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Background: Epileptic activity is highly integrated phenomena of the brain, involving cortical and subcortical structures. Experimental studies indicate that status epilepticus caused neurodegenerative changes in the hippocampus, limbic structures (amygdala, parahippocampal cortices, piriform cortex, claustrum), thalamus and neocortical areas. However, basal ganglia were rarely mentioned. Several clinical studies have reported brain abnormalities (atrophy, metabolic changes) in structures remote from the seizure focus as well as in the basal ganglia. Based on large body of experimental data, it has been proposed that the basal ganglia are involved in controlling cortical seizure activity.

Materials and Methods: This is a preliminary study based on narrative review of the reports in the literature concerning the potential detectable by MRI morphological changes.

Results: Two imaging modalities had reported detected changes in the basal ganglia. 1) MRI diffusion tensor imaging and voxel-based morphometry. Volumetric studies had demonstrated loss of volume in putamen and pallidum while another study had illustrated reduction in caudate in addition to the putamen. Increased mean diffusivity value accompanied with increased fraction anisotropy value was detected in putamen and caudate. The observed increase in fractional anisotropy correlates with an observed decreased volume of the putamen. Progressive DWI-T2 hyperintensity also observed in putamen. Conclusion On the contrary of imaging studies focusing on epileptic changed in the hippocampus, few studies had demonstrated detectable changes in extrahippocampal regions including the basal ganglia.

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Imunohistochemical detection of GPx5 and GPx7 in human colorectal adenocarcinoma

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Background: Understanding the specific functions and regulatory mechanisms of the glutathione peroxidase (GPx) enzyme family in colorectal cancer (CRC) holds great potential for advancing cancer research and treatment strategies. GPx enzymes are essential antioxidants that maintain redox homeostasis by reducing harmful reactive oxygen species (ROS). In CRC, oxidative stress—resulting from an imbalance between ROS production and antioxidant defenses—plays a critical role in tumor initiation, progression, and therapy resistance. Investigating the roles of individual GPx enzymes in CRC could provide valuable insights into redox biology and identify novel therapeutic targets. Among these enzymes, GPx5 and GPx7 have shown particular relevance. GPx5, known for its ability to reduce hydrogen peroxide and lipid hydroperoxides, may influence tumor behavior and response to treatment, despite its role in CRC being less understood. GPx7 helps sustain redox balance in normal colon tissue, and its dysregulated expression in tumors may reflect underlying biological heterogeneity.

Materials and Methods: This study employed a three-step indirect immunohistochemical technique to assess GPx5 and GPx7 expression in 62 colorectal adenocarcinoma samples and 6 healthy colon tissue samples. All specimens were obtained from the Institute of Pathology, Louis Pasteur University Hospital Košice, Slovak Republic.

Results: All healthy colon samples (100%) exhibited high GPx5 and GPx7 expression in the basal cells and columnar epithelium. In colorectal cancer specimens, GPx5 was highly expressed in 37 cases (59.68%) and absent in 25 (40.32%). GPx7 showed more variability: 38 samples (61%) demonstrated strong positivity in atypical epithelial cells, while 24 samples (39%) lacked expression entirely.

Conclusions: A detailed characterization of GPx enzymes in CRC could improve our understanding of redox regulation in cancer and support the development of antioxidant-based therapies and prognostic biomarkers.

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Peripheral nerve regeneration using biocompatible 3D conduits: In vivo evaluation in a murine model

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Peripheral nerve regeneration following injury is a complex process involving molecular, cellular, and tissue-level changes. Restoration of function requires reconnection of the proximal and distal nerve stumps. Biocompatible polymer-based 3D conduits offer a promising strategy to guide and support this process. Our project focuses on developing novel, reversibly compressible, structured porous 3D conduits from biocompatible polymers that promote cell adhesion, proliferation, and differentiation. These conduits are designed to be flexible, accommodating physiological nerve length changes, and biodegradable with minimal inflammatory response. We conducted three experimental series using male C57BL/6 mice (25 g) in a sciatic nerve transection model. A 5 mm nerve segment was excised, and a 5 mm conduit (with a 1 mm overlap for nerve fixation) was implanted. After 4 or 8 weeks, nerve samples were harvested and processed immunohistochemically using GAP43, a key marker of axonal regeneration. In the first set, conduits made from pure poly(ε-caprolactone) (PCL) and PCL coated with collagen were tested. Both materials were user-friendly during surgery and tissue processing. GAP43 staining confirmed axonal regrowth in both, with better results observed for pure PCL. The second set tested NG-CON (PCL collagen), NG-RGD (PCL with RGD peptide), and pure PCL. These conduits presented technical challenges, including rigidity and delamination of outer layers during implantation. GAP43 expression showed minimal or no axonal penetration. The third set introduced improved nanolayer surface structures for PCL, PCL collagen, and PCL RGD. All conduits were easily implantable and stable throughout the experiment. IHC analysis confirmed axonal regeneration in all three variants, with the best outcomes again seen in the pure PCL group. Our findings demonstrate that pure PCL conduits provide optimal handling properties and the most consistent support for peripheral nerve regeneration in vivo after the first experimental phase.

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"The crypt project": Interdisciplinary research of the human remains from 17th and 18th century from Chodov in the Bohemia anthropology – radiology – histology – history – archaeology

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The inglorious end of a famous local knight, the founder of the Church of St. Lawrence in Chodov near Karlovy Vary in western Bohemia, led us to research his crypt, where he was buried together with members of his family. The post-war period in the 1940s and 1950s in the Sudetenland brought grave robbing and desecration. As part of „The crypt project", an anthropological, medical, archaeological and historical research of this crypt was carried out in 2017, where we found the partially mummified remains of many young children who were no more than a year or two old. Our task was not only to identify the individuals, but also to examine them, and rebury them reverently in new coffins with identification. Historical records were largely well preserved. Twenty-two individuals ranging in age from a few hours to 86 years were found in the crypt in the wooden coffins. Three of these individuals, Franz Flamin von Plankenheim – the founder and builder of the church, his daughter Maria Anna von Braunsdorf and her husband Johann Ferdinand Braun von Braunsdorf, were also examined by CT scan. It turned out that Maria Anna suffered from osseous osteolytic lesions that resembled Kahler's disease. Multiple myeloma was also confirmed by histological examination.

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The role of LGR5 in sequential tooth development: Insights from single-cell transcriptomics

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Tooth replacement in vertebrates is governed by the persistence and activity of the dental lamina, yet the molecular basis for species-specific differences in tooth generation remains unclear. Here, we use single-cell RNA sequencing to generate a cellular atlas of mouse molar development at the stage of maximal successional dental lamina (SDL) projection. We identify distinct populations of LGR5$^+$ cells in both epithelial and mesenchymal compartments, with epithelial LGR5$^+$ cells localized to the dental stalk and SDL, and mesenchymal LGR5$^+$ cells residing in quiescent progenitor-like zones outside the tooth germ. Lineage tracing reveals that epithelial LGR5$^+$ cells contribute to second molar formation but not to the first, suggesting a spatially restricted stem cell niche. In Lgr5-deficient mice, sequential molar development is disrupted, with shortened stalks, disorganized epithelia, reduced SOX2 expression, and compromised basal membrane integrity. These defects are accompanied by altered Wnt signaling and downregulation of PTK7. Organoid cultures from first and second molars confirm differential contributions of LGR5$^+$ cells and support a niche-stabilizing, rather than proliferative, role in sequential tooth formation. Comparative analysis in diphyodont minipigs further links Lgr5 expression with active tooth replacement. Our findings uncover an epithelial LGR5$^+$ stem cell niche essential for successional molar development and reveal a broader regulatory network governing dental regeneration potential in mammals.

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The retrocochlear recess of the medial retrotympanum – radioanatomical investigation in children under five years old

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Introduction: The medial retrotympanum is a bony area containing a number of recesses, located at the posteromedial aspect of the tympanic cavity. Sinus subtympanicus (SS) is a bony recess, located at the inferior aspect of the medial retrotympanum, housing the round window niche. The subcochlear canaliculus (SC) occurs within the sinus extending to the petrous apex of the temporal bone. The retrocochlear recess (RR) is a previously undescribed bony space, present within the SS. It extends medially, posterior to the basal turn of the cochlea, with its place of origin close to the round window niche. Aim of the study: To assess the presence and the morphology of the retrocochlear recess in relation to the medial retrotympanum and surrounding structures.

Materials and methods: For the study we used an anonymized group consisting of 140 sets of CT-scans (280 temporal bones). We divided them into two smaller groups: children from 4 to 24 months and from 25 to 60 months. The analysis involved assessing the presence of the RR as well as the presence of the SC. In case of the present RR, we measured its depth in relation to the promontory and the medial pillar of the round window niche. We also assessed whether the jugular bulb was adjusting the space of the recess. Basic descriptive statistics analysis was provided for the study.

Results: We found the RR in 192 out of 280 temporal bones assessed (68,57%). Its mean depth regarding the cochlear promontory was 6,56 mm, while it was 3,91 mm deep on average regarding the posterior pillar of the round window niche.

Conclusions: The retrocochlear recess is a common variation of the medial retrotympanum in children under five. Its presence should be considered important regarding the surgical procedures and pathologies occurring within or near the SS.

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Potential of new plausible biomarkers in the differential diagnosis of cervical lesions

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Metaplastic changes of cervical epithelial cells, called precancerous cervical lesions, represent a critical stage in the development of cervical carcinoma. Despite extensive primary and secondary prevention strategies, the global burden of cervical malignancy persists as a significant public health concern. In the context of molecular pathogenesis, particular attention is paid to protein arginine methyltransferases (PRMTs), primarily PRMT5, catalyzing post-translational protein modifications and interactively influencing a vast network of signaling pathways. These enzymes play a pivotal role in epithelial-mesenchymal transition (EMT), fundamental for the progression of tumorigenic processes. In synergy with desmoglein-2 (DSG2), a key adhesion protein of cell junctions, both hypothesized to be associated with the progressive development of dysplastic changes leading to a malignant phenotype. The presented study utilized archival paraffin-embedded tissue blocks obtained from adult female patients. Samples were classified as low-grade and high-grade squamous intraepithelial lesions. Subcellular localization, immunoreaction intensity and percentage of PRMT5- and DSG2- expressing cells, were statistically evaluated. Preliminary results demonstrated statistically significant differences between the expression and subcellular distribution of the analyzed proteins between groups of low-grade and high-grade squamous intraepithelial lesions. The primary objective of this study was to elucidate the involvement of PRMT5 and DSG2 in the initiation and progression of cervical lesions. Our findings suggest significant potential of evaluated proteins as diagnostic and prognostic biomarkers in cervical oncology. Nevertheless, for a comprehensive understanding of their precise mechanisms and implications in cervical carcinogenesis, more extensive prospective studies of PRMT5 and DSG2 are imperative. Key words: squamous intraepithelial lesions, PRMT5, DSG2, prognosis, carcinogenesis.

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Altered morphology of dopaminergic neurons in an autism-like animal model: the potential role of oxytocin in neurite outgrowth

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Alterations in dopaminergic pathway development and neuronal morphology have been implicated in the pathogenesis of neuropsychiatric disorders characterized by social deficits, including autism spectrum disorder. Although midbrain and striatal dopaminergic neurons are important components of dopaminergic pathways, it remains unclear whether they show altered neurite growth and dendritic arborization during early development in autism conditions, or if these changes can be modified. Oxytocin, a well-known brain neuropeptide and neuromodulator, may influence neurite outgrowth and morphology in brain regions involved in social behavior, including dopaminergic areas. Therefore, the aim of this study was to investigate the effects of prenatal valproate (VPA) exposure, an established autism-like animal model, on dopaminergic neuron morphology; measure dopamine receptor and synthesis enzyme levels in the midbrain and striatum; and assess the effect of oxytocin on primary striatal neuron morphology. It was found that primary neurons from the ventral tegmental area of the midbrain of prenatally VPA-exposed rats exhibited a significant reduction in both neurite number and length. Similar, though less significant, reductions in neurite number were observed in neurons isolated from the striatum. Additionally, tyrosine hydroxylase-positive dopaminergic neurons from the striatum showed overall morphological changes, although no significant differences were detected at specific distances from the nucleus of prenatally VPA-exposed rats. A significant increase in neurite arborization was also observed in tyrosine hydroxylase-positive dopaminergic striatal neurons after incubation with oxytocin. A significant increase in dopamine receptor D4 gene expression was observed at postnatal day 30 in the midbrain of male rats prenatally exposed to VPA. In conclusion, altered neuron morphology in dopaminergic regions may contribute to autism-like behaviors both in this model and more broadly in patients with autistic symptoms. Oxytocin may promote neurite outgrowth in striatal dopamine neurons, which requires further studies.

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Pancreatic lipase secretion in the pancreas and its production in duodenal enterocytes following fecal microbiota transplantation, probiotics, and prebiotic administration in a pseudo germ-free animal model

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The influence of gut microbiota (GM) on systemic health and metabolism is gaining increasing attention in scientific research. Alterations in GM composition have also been reported in children with autism spectrum disorder (ASD), potentially associated with inappropriate dietary habits and the presence of gastrointestinal symptoms that further complicate the health and cognitive status of individuals with ASD. Our research focused on changes in the secretion of pancreatic lipase from the pancreas and intestinal pancreatic lipase production within duodenal enterocytes in a pseudo germ-free (PGF) animal model with reduced GM, following the administration of fecal microbiota transplantation (FMT) from neurotypical and ASD donors during the I. phase of the experiment. The II. phase involved treatment with probiotics (Lactobacillus reuteri, Lactiplantibacillus plantarum) and a prebiotic (flaxseed). Donors for FMT were selected from two groups: neurotypical, otherwise healthy individuals and children with ASD aged 4–6 years. All donors were screened using the Autism Diagnostic Interview–Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS-2). Additionally, all donors were at least two months post-antibiotic therapy, tested negative for microbiological pathogens, and exhibited normal levels of the calprotectin. The results indicated a significant decrease (p < 0.001) in pancreatic lipase production in the pancreas of the ASD group during the I. phase of the experiment. After therapy in the II. phase, the ASD pro pre group showed a significant increase (p < 0.001) in pancreatic lipase production compared to the untreated ASD group. Pancreatic lipase production in duodenal enterocytes was significantly elevated (p < 0.001) in the ASD group relative to the control group and further increased after therapy in the ASD pro pre group during the II. phase. Our results suggest a strong interplay within the microbiota–gut–pancreas axis, mediated by GM, influencing multiple metabolic pathways, including cholecystokinin and serotonin secretion.

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Sprouty2 /4 deficiency disrupts AER integrity and ZPA cell migration impacting chondrogenesis of the mouse forelimb

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FGF signaling pathway plays an important role in the regulation of limb development, controlling cell migration, proliferation, differentiation, and apoptosis. Sprouty proteins as FGF pathway antagonist control FGF signaling as part of a negative feedback loop. Alterations in Sprouty expression has been shown to lead to limb defects in both mouse and chick embryos. Sprouty2/4 deficient mice evince defects in endochondral bone formation and digit patterning in their forelimbs, with pathogenesis recently related to ciliopathies. To understand the mechanisms behind these pathologies, the limb defects in Sprouty2/4 deficient male and female mice were characterized and correlated to the expression patterns of Sprouty2 and Sprouty4. The impact of Sprouty2/4 deficiency on the main signaling centers of the limb bud was assessed. Our results showed that, despite similar Sprouty2/4 expression patterns in all limbs, the hindlimbs did not evince any obvious alterations in development, while the forelimbs showed a broad variety of pathologies of variable severity in the autopodium of the forelimb, including changes in digit number, size, shape. The left forelimb was significantly more affected than the right one. This was shownprenatally as well as postnatally. Changes in bone numbers, hand clefts, and digit fusions were detected by micro-CT scans. Ectopic ossification of bones and abnormal bone fusions were frequently observed in the digital as well as in the carpal and metacarpal areas. Sprouty2/4 deficient mouse limb buds showed patchy loss of Fgf8 expression in the apical ectodermal ridge, and a loss of tissue underlying these regions. The zone of polarizing activity was also impacted, showing a change in the contribution of Sonic hedgehog descendant cells

documented using cell lineage tracing analysis. These results support the connection between Sproutys and Hedgehog signaling during limb development and highlight the important role of Sprouty2 and Sprouty4 controlling early signaling centers in the limb development.

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Potential prognostic value of Galectin-3 and Rac1 in cervical lesions

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Aim: Cervical lesions, ranging from low-grade squamous intraepithelial lesions (LSIL) to high-grade (HSIL) and invasive squamous cell carcinoma (SCCa), represent a progressive spectrum of epithelial transformation. Accurate biomarkers that reflect disease progression and malignant potential are essential for improving diagnostic precision and patient stratification. The presented immunohistochemical study investigates the subcellular distribution of two regulatory proteins Galectin-3 and Rac1, using immunohistochemistry in cervical tissue specimens.

Methods: A total of 35 lesions were evaluated, including 11 LSIL, 10 HSIL, and 14 SCCa cases. The immunohistochemical localization of Galectin-3 revealed distinct patterns across lesion grades. In LSIL, staining was evenly distributed among nuclear, cytoplasmic, and combined localizations. In HSIL, Galectin-3 was predominantly nuclear (72.73%), while in SCCa, expression shifted significantly to the cytoplasm (66.67%). The differences were statistically significant, respectively (χ^2 = 10.02, p = 0.040).

Results: Rac1 also demonstrated marked subcellular redistribution. In LSIL, Rac1 was primarily cytoplasmic (81.8%). In HSIL, a notable shift occurred toward combined nuclear/cytoplasmic expression (60%), whereas in SCCa, Rac1 showed dominant membranous localization (71.4%). These differences were highly significant (χ^2 = 23.63, p < 0.001). Our results indicate that the subcellular localization of Galectin-3 and Rac1 changes dynamically along the histological spectrum of cervical neoplasia. The nuclear predominance of Galectin-3 in HSIL may be associated with transcriptional regulation during dysplasia, while its cytoplasmic localization in SCCa could reflect roles in cell motility or survival. Similarly, the progressive membranous translocation of Rac1 may reflect its role in cytoskeletal remodeling and invasive behavior in carcinoma.

Conclusion: These localization patterns suggest that Galectin-3 and Rac1 are not only involved in the pathophysiology of cervical lesion progression but also hold promise as supplementary biomarkers for assessing lesion severity and invasive potential.

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Experimentally induced histological and cognitive changes in the brain after exposure to ionizing radiation

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Ionizing radiation used in radiotherapy is known to cause delayed cognitive deficits. This study investigated the long-term consequences of fractionated whole-brain irradiation (fWBI) on behavior and adult neurogenesis in a rat model. Four-month-old male Wistar rats received a clinically relevant fWBI protocol of 32 Gy (4 × 8 Gy/week). Four months post-irradiation, locomotor activity and anxiety-like behaviors were evaluated using the open-field and elevated plus maze tests. Histological changes in the subventricular zone (SVZ) and hippocampal dentate gyrus (DG) were assessed 5–6 months post-irradiation on cryosections via immunofluorescence and confocal microscopy. The irradiated cohort exhibited significantly reduced locomotor activity compared to controls. Furthermore, histological analysis revealed a profound depletion of neural stem/progenitor cells (nestin) and immature neurons (DCX) within both neurogenic niches. These findings demonstrate that clinically relevant fWBI induces persistent behavioral alterations and a substantial loss of key cell populations in adult neurogenic regions. This impairment of neurogenesis likely contributes to the long-term cognitive deficits observed in patients following radiotherapy.

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Immunohistochemical methods to demonstrate the structure of enteric nervous system in intestinal wall of germ-free piglets

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The wall of digestive tube is inervated by autonomic nervous system with a separate branch – enteric nervous system (ENS), located throughout the whole length of digestive tube. Enteric nervous system regulates physiological functions of gastrointestinal tract, predominantly small and large intestine. The ENS is getting more in the focus of researchers, who were mainly interested in the structure of ENS under physiological conditions, while the ENS in germ-free (GF) piglets, as relevant models to study human diseases, have not yet been the point of interest. GF piglets have clear microbiological background, are reared in sterile environment, and they manifest similar clinical symptoms to humans. In this study we tried to demonstrate components of ENS and study its structure by using several different antibodies such as anti-S100 primary antibody, anti-PGP 9.5 primary antibody, anti-synaptophysin primary antibody, anti-serotonin monoclonal antibody in histological sections from small and large intestine obtained from germ-free piglets. Immunoreactivity was evaluated in 4 randomly selected intestinal wall areas on a standard histological scale: 0-negative (-), 1- weak ( ), 2-moderate ( ), 3-severe ( ). Based on a series of histological analyses of the small and large intestinal walls in gnotobiotic piglets, we found a difference between the distribution of nerve cells/fibers and enteric glial cells of nerve plexuses. According to the results of the analysis, all used immunohistochemical methods are suitable for detection of ENS structures. The S 100 and PGP 9.5 showed the most pronounced immunopositivity of enteric plexuses, and PGP 9.5 and synaptophysin seem to be suitable for detection of fine nerve fibers up to their terminal ends. The detailed quantitative parameters need further investigation.

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Modeling congenital heart disease: Insights from Jagged1 and Notch1 single point variants in mice

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Jagged1 (Jag1) plays a pivotal role in cardiac development and is strongly implicated in Alagille syndrome. Even partial loss or single nucleotide variants (SNV) in Jag1 can result in congenital heart defects (CHDs) in human patients. This study explores the impact of two patient-derived Jag1 SNVs (R1097W – RW; R1213Q – RQ) and Notch1 variants (C1549Y and an 11 bp deletion) in the context of Tetralogy of Fallot (TOF). Embryonic and postnatal hearts were analyzed from these models, using histology, micro-CT, and functional ultrasound (VEVO). Jag1^RQ/RQ hearts showed a thinner compact myocardium and smaller size at postnatal day 2 (P2), correlating with reduced end-systolic volume. By P10–P30, cardiac size normalized, but thickened valve leaflets persisted (50% penetrance). In contrast, Jag1^RW/RW mice developed enlarged hearts with ventricular wall thickening at P2, progressing toward trends of dilated cardiomyopathy (increased end-systolic volume, decreased ejection fraction) by P10. These changes are largely resolved by P30, with minimal residual valve anomalies. Notch1 point and deletion variants produced only mild morphological defects, milder than the typical human TOF phenotype. Our findings confirm that even subtle changes in Jag1 and Notch1 significantly affect heart development, particularly myocardial compaction, valve morphology, and contractile function. These humanized mouse models provide crucial insights into the mechanisms behind Jag-Notch related CHDs and the genetic contribution to TOF pathogenesis.

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Effect of high methionine diet on testicular morphology in experimental conditions

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A high intake of methionine (Met) from dietary sources can elevate homocysteine (Hcy) levels in the body, which has been associated with an increased risk of cardiovascular diseases, oxidative stress, and inflammation. However, its role in testicular dysfunction remains insufficiently understood. This pilot study aimed to evaluate the impact of a mild form of hyperhomocysteinemia (hHcy) induced by a high Met-rich diet on testicular structure. Ten adult Wistar male rats were randomly assigned to two groups: a control group and a Met-treated group. The latter received an oral dose of L-methionine (2 g/kg/day) for a period of 28 days to induce mild hHcy. Blood and testicular tissue samples were collected for biochemical and histomorphological analyses. Serum concentrations of Hcy were measured. After sacrifice, the testes were excised, weighed, and fixed in formalin. Histomorphological analysis was performed using H&E staining, PAS reaction, and an immunohistochemical procedure. The Met-treated group exhibited reductions in both body and testicular weights compared to the control group. Histopathological evaluation revealed structural disruption of the seminiferous tubules, characterized by impairment of their basement membranes, accompanied by affected junctional complexes between cells. These pilot findings suggest that elevated Hcy levels exert detrimental effects on testicular morphology and function, potentially interfering with spermatogenesis.

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Effect of hyperhomocysteinemia on heart-brain axis

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Modifying risk factors remains a crucial strategy in preventing cardiovascular and cerebrovascular diseases (CVD). Despite the expansion of treatment options targeting modifiable factors, the effective implementation of these options in clinical practice remains a challenge. Increasing evidence suggests a link between Alzheimer's disease (AD) and CVD, particularly through shared manifestations such as hypertension, as well as intra- and extracranial atherosclerosis and arteriosclerosis. Elevated plasma homocysteine (Hcy) levels are recognized as a risk factor for CVD, with even mild elevations contributing to endothelial dysfunction. Although hyperhomocysteinemia (hHcy) is associated with increased cardiovascular risk, the precise role of Hcy in the pathophysiology of CVD remains unclear. Furthermore, neurodegeneration in AD, characterized by impaired neural signaling and potential peripheral amyloid accumulation, may exert adverse effects on other organs, including the heart. In this study, adult Wistar rats received daily subcutaneous injections of Hcy (0.6 μmol/g/twice a day) for three weeks. Following treatment, the animals were sacrificed, and their hearts were isolated and perfused using the Langendorff system with Krebs-Henseleit solution. The hearts were then fixed in formalin and subjected to histological and immunohistochemical analyses. In the hHcy group, perfusion pressure was elevated, while left ventricular end-diastolic pressure (LVDP) and indices of myocardial contraction and relaxation (LVdP/dt and –LVdP/dt) were significantly reduced. Histological examination revealed structural alterations including cardiomyocyte disintegration, increased cellular volume, and heightened incidence of programmed cell death. Immunohistochemical analysis confirmed the presence of β-amyloid aggregates in the myocardium of Hcy-treated animals. These findings suggest that hHcy impairs cardiac microcirculation and disrupts cardiomyocyte integrity and function, potentially linking cardiac alterations to neurodegenerative processes observed in AD.

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The game element and its place in anatomy teaching

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Since its beginnings, anatomy teaching has been based on theoretical lectures, practical teaching and work with specimens. However, with the rapid development of virtual tools and AI, this traditional approach is being sidelined. How to engage the students in traditional learning of anatomy? Does the gaming element have a place in teaching? How specifically can it be used? Does its use have any impact? The game element in anatomy teaching is an innovative approach that allows students to be more actively involved during the traditional approach. Instead of passively receiving information, the student becomes an active participant in the learning process—a "player" who navigates a designed scenario. It also brings in essential reflection, encouraging students to discuss and analyze the steps they´ve taken. The game elements thus draw the student into the problems of anatomy, increasing his intrinsic motivation and engagement in teaching and learning. We might use simple structure-finding tasks framed as individual competitions, construct a clinical case to demonstrate the anatomical basis of a problem, or even develop a comprehensive anatomy-themed game. In this aspect, only our imagination is a limitation. It's crucial to identify appropriate elements that can be seamlessly incorporated into teaching and used effectively, striking a balance between fun and academic rigor. The outcome should be a dynamic, engaging environment that ignites a genuine thirst for knowledge. The goal should always be to better understand and connect the context of anatomical structures and their functions.

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Using ChatGPT and AI as an anatomical assistant in medical education

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Artificial intelligence (AI) and large language models (LLMs) such as ChatGPT offer extensive possibilities for supporting medical education. Interactive chatbots enable instant access to information, explanations of anatomical structures, and practical usage scenarios for both self-study and classroom teaching. The aim of this presentation is to introduce the "Anatomical Assistant" module based on ChatGPT and demonstrate its applications in medical curricula. This specific module integrates the scripts and all teaching texts from our Department of Anatomy, along with model questions from various credit exams. Students can pose any questions in natural language—from basic concepts to detailed descriptions of functions and topography—and can also generate quizzes and practice questions to aid their preparation for final tests and examinations. The "Anatomical Assistant" module represents a practical tool that enriches anatomy teaching with interactive AI support, providing students and instructors with more efficient access to educational materials, fostering flexible learning, and paving the way for future expansion with multimodal content (3D models, histological sections, and integrated clinical cases).

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Immunofluorescence distribution of atypical chemokine receptor ACKR3/CXCR7 in dorsal root ganglion neurons of a mouse model of neuropathic pain

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Under physiological conditions, primary sensory neurons of the dorsal root ganglia (DRG) display a balanced expression of endogenous opioid peptides and receptors, including the delta opioid receptor (DOR). The chemokine receptor CXCR7, also known as the atypical chemokine receptor ACKR3, was recently identified as an atypical opioid receptor. However, its intraneuronal localization and dynamics in DRG neurons remain unexplored in experimental models of neuropathic pain. We used a mouse spared nerve injury model with unilateral tibial nerve sparing (SNIt) to study the intraneuronal immunodetection of the CXCR7 protein in DRG neurons, compared to naïve and sham-operated animals. Double immunofluorescence staining for DOR and CXCR7, combined with confocal microscope analysis, was utilized to investigate their potential intraneuronal interactions. Immunofluorescence and confocal microscopy analyses revealed CXCR7 immunofluorescence (IF) predominantly in the neuronal cytoplasm of the DRG in naïve, sham- and SNIt-operated mice. On post-operation day (POD) 7 and 21, both sham and SNIt operations induced a bilateral increase in cytoplasmic CXCR7-IF intensity in small- and medium-sized neurons, accompanied by a shift of CXCR7-IF toward the neuronal surface. In contrast, large-sized neurons exhibited only an increase in intraneuronal CXCR7-IF intensity. CXCR7-IF was colocalized with DOR-IF in vesicular structures within the neuronal cytoplasm, as observed by confocal microscopy. These results suggest a mechanism of modulation in endogenous and exogenous potential DOR ligands in DRG neurons in an experimental neuropathic pain model.

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The significance of vascular system visualisation in the clinical anatomy

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Teaching the anatomy of the vascular system is challenging due to interspecies differences and a high degree of variability. Today, various methods using different polymers are available for vascular system visualisation. The aim of this study was to visualise the vascular system in foetuses and young animals—including the domestic horse (n=3), cat (n=3), and dog (n=3)—using different polymer injection techniques. The quality of the injections and microvascular penetration was evaluated using scanning electron microscopy (SEM). We used body parts (limbs, heads) and organs (kidney, liver, spleen, tongue, and digital corium). The specimens were perfused with an anticoagulant solution and injected with one of the following: acrylic resin, epoxy resin, or silicone. The samples were then macerated in a potassium hydroxide solution at a constant temperature for 3 to 70 days and subsequently dried in a laboratory oven. Representative casts for SEM analysis were coated with gold-palladium in the 15 nm layer and examined using a scanning electron microscope Zeiss EVO LS 15. The best macroscopic results were obtained with acrylic resin, although it had limited penetration into the microvasculature and was prone to fragility. Epoxy resin proved suitable for microvascular injection in organs but did not produce high-quality imaging for larger vessels. Silicone was most appropriate for macroscopic preparations but not for corrosion casting techniques. Based on SEM imaging, epoxy resin provided the best results for visualising microvascular structures. However, even a perfect macroscopic 3D rendering does not guarantee clear angioarchitectonic visualisation under SEM. Injected specimens of body parts and organs serve as valuable tools for teaching vascular anatomy. Additionally, such preparations can be utilized in research and in the study of the vascular system in relation to clinical applications.

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Do we have four or eight Egyptian eyes?

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Introduction: The superficial fascia of the limbs (fascia superficialis; stratum membranosum telae subcutaneae) is called the saphenous fascia (fascia saphena) or Sherman's fascia in the lower extremities. It is relatively weak and is well expressed only in the area of the superficial venous trunks. It can be visualised on the lower extremities by ultrasound and is described in the image as the "upper eyelid of the Egyptian eye". But does it also exist on the upper extremities?Methods: Ultrasound examination of the superficial compartment of the upper extremities of 20 volunteers. Further, we harvested subcutaneous samples from five body donors and processed them for routine histological examination and staining for connective tissue components.

Results: In all cases of ultrasound and histological examinations, we found the trunk of the cephalic and basilic veins (vena cewphalica et basilica) and described it as a vein that always runs within its own compartment, from the wrist to the site of its submerging through the deep/muscular fascia (trigonum deltopectorale and hiatus basilicus), although in the distal part of the forearm the superficial thickening of the fascia is more difficult to identify. The appearance of the own compartment does not always have the typical appearance of an Egyptian eye as in the case of the saphenous veins of the lower limb. This phenomenon can only be observed in the proximal half of the forearm in the cephalic vein, otherwise it is blurred. However, the compartment is demonstrable and serves to clearly show the course of the trunks of the superficial veins of the upper limb.

Conclusion: We have demonstrated the existence of a separate, so far unnamed compartment for the superficial venous trunks of the upper limb. The clinical significance lies in the clear demonstration of these veins using ultrasound, e.g. for graft harvest.

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The HOX code of human adult fibroblasts reflects their ectomesenchymal or mesodermal origin

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Fibroblasts, the most abundant cell type in the human body, play crucial roles in biological processes such as inflammationand cancer progression. They originate from the mesoderm or neural-crest-derived ectomesenchyme. Ectomesenchymederivedfibroblasts contribute to facial formation and do not express HOX genes during development. The expression androle of the HOX genes in adult fibroblasts is not known. We investigated whether the developmental pattern persists intoadulthood and under pathological conditions, such as cancer. We collected adult fibroblasts of ectomesenchymal and mesodermalorigins from distinct body parts. The isolated fibroblasts were characterised by immunocytochemistry, and theirtranscriptome was analysed by whole genome profiling. Significant differences were observed between normal fibroblastsfrom the face (ectomesenchyme) and upper limb (mesoderm), particularly in genes associated with limb development, includingHOX genes, e.g., HOXA9 and HOXD9. Notably, the pattern of HOX gene expression remained consistent postnatally, even in fibroblasts from pathological tissues, including inflammatory states and cancer-associated fibroblasts from primaryand metastatic tumours. Therefore, the distinctive HOX gene expression pattern can serve as an indicator of the topologicalorigin of fibroblasts. The influence of

cell position and HOX gene expression in fibroblasts on disease progression warrantsfurther investigation.

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Testing the inhibitory efficacy of synthesized mPTP inhibitors

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Introduction and ObjectivesCyclophilin D (CypD) is a mitochondrial enzyme that regulates the mitochondrial permeability transition pore (mPTP), a critical component in mitochondrial membrane permeability. mPTP opening is primarily triggered by elevated intracellular calcium levels and oxidative stress. The sensitivity of the mPTP to Ca^{2+} ions varies with age, and its dysregulation has been linked to the development of various diseases. Therefore, mPTP inhibitors are a promising therapeutic strategy for treating conditions associated with mitochondrial dysfunction. The aim of this study was to evaluate the inhibitory efficacy of newly synthesized CypD/mPTP inhibitors based on the structural scaffold of N-4-aminobenzyl-N'-(2-(2-phenylpyrrolidine)-2-oxoethyl)urea, designed to modulate mPTP activity and potentially offer therapeutic benefits.

Materials and Methods: To assess the ability of the compounds to inhibit mPTP opening in isolated mitochondria, nine CypD inhibitors were selected. mPTP opening was evaluated by measuring mitochondrial calcium retention capacity (CRC) and mitochondrial swelling. The inhibitory potential of each compound was tested at various concentrations under in vitro conditions, using calcium-induced mPTP opening. Results were compared to those obtained with the reference inhibitor, cyclosporin A (CsA).

Results and Discussion: All tested compounds significantly influenced mitochondrial CRC and swelling at a concentration of 5 μM. Among them, compounds 3 and 13 were the most effective, showing comparable efficacy to CsA. Since compounds 3, 13, and CsA completely blocked mitochondrial swelling at 5 μM, they were further tested at lower concentrations to evaluate dose-dependent effects. All three compounds exhibited concentration-dependent inhibition of mPTP opening. While compound 13 and CsA maintained similar levels of inhibition across the tested range, compound 3 was less effective at lower concentrations. At 0.1 μM, compound 3 showed no effect, whereas compound 13 and CsA still significantly inhibited mitochondrial swelling. Interestingly, at very low concentrations (0.01–0.03 μM), compound 13 appeared slightly more effective than CsA.

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Choroid plexus in Alzheimer's disease and neuroinflammation

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive deterioration. The main pathological features include the accumulation of amyloid-beta (Aβ), hyperphosphorylation of tau protein, and chronic neuroinflammation. The choroid plexus, which forms the blood–cerebrospinal fluid (CSF) barrier, has emerged as a potential contributor to AD pathogenesis by influencing and mediating immune activity within the brain. In this study, we investigated how AD-related changes affect the choroid plexus. An in vitro model was established using Z310 choroid plexus epithelial cells treated with Aβ(42) peptide across multiple time points. We evaluated both gene and protein expression, with an emphasis on inflammatory markers. Notable alterations were identified as early as 24 hours following Aβ exposure, including disruptions in key proteins such as phosphorylated tau, amyloid precursor protein, and inflammation-associated markers. Our results show that Aβ alters choroid plexus function early in the disease process.

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Analysis of caspase-3 non-apoptotic functions in astrocytes using a neurosphere-derived in vitro model

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Although caspase-3 is considered the pivotal protease involved in apoptosis, its activity has also been observed in non-apoptotic processes, including differentiation or neuroplasticity. Our previous analyses revealed that population of non-apoptotic cleaved caspase-3 positive cells (cC3), especially astrocytes, is present in the spinal cord of postnatal and adult rats. Suggesting its potential role in astrocyte differentiation, we prepared in vitro cell culture model formed from neurospheres, which were derived from neonatal rat spinal cord. In the selected time points 3DIV, 6DIV, 9DIV, 12DIV (Days In Vitro), we analysed a) phenotypes of cells formed from neurospheres and mRNA expression of specific cell markers, b) percentage of cC3 cells, and c) colocalised cC3 with markers of astrocytes. Within the 12-day differentiation period, the culture exhibited consistent proportion of the studied cell phenotypes in each studied time point: Olig2 oligodendrocytes (approx. 25%), TUJ1 neurons (approx. 3%), Vimentin or GFAP astrocytes, representing predominant population (65-70%), including a subpopulation of Nestin cells. Early presence of astrocytic markers corresponds to the results of gene expression analysis. These data indicate that differentiated astrocytes are present in cell culture soon after the initiation of differentiation of neurosphere cells. Interestingly, a significant increase of cC3 population occurs in the period between 3DIV (35,75% ± 14,73%) and 6DIV (69,5% ± 3,11%) and remains constant until 12DIV. Furthermore, approximately 50% of analysed cells correspond to cC3 /GFAP astrocytes. These preliminary data indicate that GFAP positive astrocytes emerge early after the initiation of differentiation, and thus, a shorter time window has to be investigated to verify cC3 involvement in astrocyte differentiation. Since the number of cC3 cells with non-apoptotic morphology significantly increases between 3DIV and 6DIV, we assume that another unknown function of cC3 should also be considered.

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Database of digital radiological images of all modalities for teaching and testing anatomy in SmartZoom

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Objective: The aim of the project was to develop a large, systematically organized digital database of radiological images from various modalities, designed for students' independent study, self-assessment, and knowledge testing in topographic and clinical anatomy. The database includes both normal anatomy and common anatomical variants as well as selected pathological findings.

Methods: The database was created in collaboration with the Radiology Department of the University Hospital in Hradec Králové. Approximately 300 images were collected across a broad range of imaging methods, including native and contrast X-rays, CT and HRCT, MRI (T1, T2, STIR), digital subtraction angiography (DSA), and ERCP. Images were categorized by organ system and imaging modality. All files were saved in JPG format and uploaded to the SmartZoom e-learning platform, which supports interactive functions such as hiding labels, creating custom annotations, and enabling self-testing. Each image is accompanied by descriptive text, imaging modality details, and annotations on skeletal development and bone age. The database is available in both Czech and English.

Results: The database is actively used in undergraduate teaching of systematic and clinical anatomy for medical and dental students. It also serves for self-study and exam preparation. A dedicated section is used for "flag tests" focused on the topography of the musculoskeletal system, splanchnology, and the central nervous system. Ongoing collaboration with the Radiology Department ensures continuous updates and the inclusion of didactically valuable cases.

Conclusion: The interactive radiological image database is a modern and effective teaching tool that integrates traditional morphological education with clinically relevant imaging. SmartZoom enables individualized learning and supports the development of spatial and topographic understanding. Future plans include linking the repository with clinical case studies and expanding it with DICOM files for dynamic image viewing.

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Incidence of dental pathologies on skeletal remains from Middle Age population, Eastern Slovakia

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Pathological changes in teeth are an important indicator of the health status, dietary habits and living conditions of historical populations. They are deviations from the normal healthy state of the teeth, which can be caused by a variety of factors. Analysis of the teeth of medieval skeletal remains provides valuable information on the frequency of dental caries, periodontal disease, dental wear, calculus and another dental lesions. The aim of the research was to observe pathological changes on teeth of skeletal remains from the Middle Age locality Vyšná Myšľa – Koscelek in Eastern Slovakia. At the archaeological site were exhumed 13 (43.33%) female and 17 (56.66%) male skeletal remains of adults from the Middle Age locality Vyšná Myšľa – Koscelek. However, female individuals had a total of 18 (8.21%) teeth retained, and male individuals had a total of 112 (51.14%) teeth retained. Dental caries was present on 10 (55.55%) teeth belonging to female individuals and on 22 (19.64%) teeth belonging to male individuals. Calculus was present on 3 (16.66%) teeth belonging to female individuals and on 19 (16.96%) teeth belonging to male individuals. Enamel hypoplasia was present on 22 (19.64%) teeth belonging to male subjects and was not observed at all on the teeth of female subjects. Dental wear was present on 18 (100%) teeth of female and on 93 (83.04%) teeth of male individuals. In the analyzed population, dental wear was the most frequently occurring pathological change manifestated on 111 (85.38%) teeth. Dental wear and abrasive defects may have been caused by ingestion of hard food such as grain, but also sand present from mill processing, as well as the use of teeth as a tool in daily life. The teeth were worn down rapidly, with molars in some individuals being ground down almost to the marrow.

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Guiding students beyond university walls: Implementing flipped classroom in histology teaching

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Encouraging students to arrive well-prepared for class remains a persistent challenge in medical education. Despite various strategies, many students struggle with consistent independent study. One effective approach to address this issue is the flipped classroom model, which shifts the initial phase of learning to an individual, pre-class setting. In-class time is then dedicated to the application and reinforcement of acquired knowledge. In the General Histology course for dentistry students at the Faculty of Medicine in Pilsen, a flipped classroom structure was implemented to improve student preparation and outcomes. Asynchronous learning was supported through Moodle, which provided clearly defined learning outcomes, chapter-specific materials, and formative quizzes. Students completed Reading Guides prior to each class—structured assignments directing their study of textbooks, videos, and digital texts, and requiring completion of defined tasks. Each face-to-face session began with a short written test focused on histological scheme drawing, followed by guided application activities. Additionally, students completed three mandatory online tests during the semester. These assessments evaluated not only factual knowledge but also higher-order cognitive skills aligned with Bloom's taxonomy, such as clinical reasoning and microscopic structure identification. The course concluded with a credit examination consisting of histological drawing and tissue identification under the microscope. Of the 26 enrolled students, 24 successfully completed the course, and 19 fulfilled all requirements within the regular term. This high success rate reflects the continuous interaction between students and instructor through regular feedback, which enabled early identification of at-risk students and provision of targeted academic support. Beyond improved academic performance, the flipped classroom yielded several benefits: enhanced student responsibility for their own learning, continuous feedback, and a more accurate estimation of student workload. Based on detailed analysis, fulfilling the intended learning outcomes corresponds more realistically to 150 hours (6 ECTS), rather than the accredited 75 hours (3 ECTS).

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Mapping the abdominal vasculature of the Prestice Black Pied pig: Implications for human translational research

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Introduction: Porcine models are widely used in translational research due to cardiovascular and abdominal anatomical similarities with humans. However, differences in vascular branching patterns can affect the reliability of preclinical testing. This study maps the abdominal vasculature of the Prestice Black Pied pig and compares it to human reference anatomy, to guide experimental designs and AI-based vessel segmentation.

Methods: Twelve Prestice Black Pied piglets (3–4 months, 24–36 kg) underwent arterial-phase CT angiography. The abdominal aorta and its branches (celiac, renal, cranial and caudal mesenteric arteries) were segmented using 3D Slicer. Vessel measurements were conducted in Syngovia (Siemens Healthineers). Diameters and cross-sectional areas were obtained at supra- and infra-branching segments, as well as at the origin of each branch. Branching angles were also recorded. Each measurement was performed three times and averaged. Arterial origins were referenced to vertebral levels. The Kruskal–Wallis test with post-hoc analysis and correction for multiple comparisons was used for statistical analysis.

Results: Celiac artery: Origin at T13–T14, trifurcation pattern preserved; left gastric artery arose from splenic artery (vs. direct origin in humans). Renal arteries: Origin at L1; 8/12 symmetric origin, variability similar to humans. Cranial mesenteric artery: Origin at T15–T16; no arcades present. Caudal mesenteric artery: Origin at L4–L5 (vs. L3 in humans); variable course, absent in 1 case. Quantitatively, the celiac artery had the largest diameter and area at both supra- and infra-branching levels. A gradual decline in aortic cross-sectional area was observed across all pigs (mean above the celiac a.: 1.17 ± 1.66 cm^2; below the caudal mesenteric a.: 0.78 ± 1.5 cm^2).

Conclusion: General vascular architecture does not completely align with human anatomy. The data acquired supports the development of AI-assisted segmentation tools and underlines the value of breed-specific vascular atlases in preclinical modelling.

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Immunohistochemical detection of GPX7 in normal kidney tissue and clear cell renal cell carcinoma: A preliminary study

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GPx7 is a member of the glutathione peroxidase family-enzymes that protect cells from oxidative damage by reducing peroxides, particularly in the endoplasmic reticulum. Recent studies have reported that GPx7 gene expression is upregulated in renal cell carcinoma (RCC), potentially influencing cell survival, proliferation, migration, and invasion. This upregulation may also be associated with poor prognosis and reduced overall survival, especially in clear cell and papillary RCC subtypes. However, to date, no study has demonstrated increased GPx7 expression in clear cell RCC using immunohistochemical detection. Therefore, in this preliminary study, we aimed to visualize GPx7 expression immunohistochemically in normal kidney tissue and in specimens from patients with RCC. We observed GPx7 positivity in normal kidney tissue, particularly in the cytoplasm and nuclei of epithelial cells in the proximal and distal convoluted tubules, as well as in Bowman's capsule and glomeruli. In contrast, only very faint GPx7 positivity was observed in the cytoplasm and nuclei of clear cell RCC specimens, which contradicts previous studies reporting GPx7 overexpression in cancer cells. Hence, further research is necessary to elucidate the functional role of GPx7 in the development and progression of RCC.

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Compression-related transient ischemia of the foot during sport climbing

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Sport climbing is gaining popularity worldwide, accompanied by an increasing number of complaints related to the use of climbing shoes. These shoes are typically worn 0.5 to 2.5 sizes smaller than normal footwear, which can affect both foot biomechanics and blood circulation. Common issues include ulcers, toe deformities, fungal infections, and neurological symptoms. This study aimed to determine whether wearing tight climbing shoes can lead to local ischemia. Measurements were conducted using the Masimo Radical-7 pulse oximeter, which assesses hemoglobin oxygen saturation (SpO_2) and the perfusion index (PI). Values were recorded on the big toe before climbing and three minutes after climbing. A total of 72 climbers were examined, along with a control group of 72 non-climbers who underwent only resting measurements. In ten climbers, additional measurements were taken immediately after climbing—once after climbing with shoes on, and once after climbing barefoot. SpO_2 values remained stable throughout the activity. However, significant changes were observed in PI. The average PI before climbing was 1.12 in climbers, compared to 1.67 in the control group. Immediately after climbing, PI dropped sharply to as low as 0.11, but three minutes later it rose above baseline. When climbing was done without shoes, the drop in PI was significantly milder. These findings suggest that while oxygen saturation is not notably affected, the perfusion index is strongly influenced by climbing activity, especially when tight footwear is used. Climbers showed lower resting PI and a marked drop post-exercise, followed by reactive hyperemia. The post-climb increase in PI indicates compensatory mechanisms restoring blood flow after temporary compression, pointing to a state of transient ischemia caused by tight climbing shoes. Prolonged use of such footwear may contribute to the development of chronic ischemic symptoms.

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Structural remodeling of the atria during atrial fibrillation

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Background: Atrial fibrillation (AF) is closely linked to structural remodeling of the atria, primarily through activation of fibroblasts and subsequent production of extracellular matrix (ECM) proteins. These processes are not only markers of disease progression but also actively contribute to the formation of an arrhythmogenic substrate, disrupting normal tissue architecture, impairing electrical conduction, and promoting the persistence of AF.

Methods: We analyzed histological sections of the left and right atria from 20 patients (75% male) undergoing cardiopulmonary bypass, with a high prevalence of AF in this group. Our focus was on fibroelastosis, presence of myofibroblasts, and deposition of ECM components (collagen, fibronectin, and periostin).

Results: Histological analysis (Picrosirius Red staining) revealed variable collagen accumulation, predominantly in perivascular and subendocardial regions. This pattern is characteristic of atrial fibrosis and aligns with literature describing increased interstitial and perivascular collagen as a key element of AF-associated remodeling. Immunohistochemical analysis revealed a continuous subendocardial layer of myofibroblasts in some samples, as determined by smooth muscle actin staining. No such positivity was present in healthy controls (N=3). Myofibroblasts are the main effector cells in cardiac fibrosis, producing ECM proteins and increasing tissue stiffness. Subendocardial regions were also positive for periostin and fibronectin, with staining intensity varying among patients. Both proteins are implicated in the pathogenesis of fibrosis and are elevated in AF.

Conclusion: AF is a condition induced or promoted by ECM remodeling and fibrosis. This is a complex, multifactorial process that varies in severity and distribution between the left and right atria. Further quantitative and clinical correlation studies are needed to fully elucidate the relationship between fibrosis severity and atrial function, and to develop targeted interventions. ECM proteins such as collagen, fibronectin, and periostin are being investigated as biomarkers of atrial remodeling and may aid in risk stratification and therapeutic decision-making.

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Hydroxyapatite cylinders in the bone regeneration process

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Calcium phosphate based biomaterials have composition comparable to bone minerals and they are able to induce biological responses similar to those found in bone healing. Calcium phosphate based bioceramics present an appropriate choice in the field of bone tissue engineering and appear to be a suitable alternative to bone grafts in surgical bone replacement. The aim of this study was to investigate the effect of hydroxyapatite ceramic implants (hydroxyapatite cylinders) on bone defect healing with respect to biocompatibility, biodegradability, osteoconductivity, osteoinductivity, and osteointegration with the surrounding bone tissue. Hydroxyapatite ceramic implants were prepared by the Institute of Materials Research of SAS using tape-casting method which allows shape variation in samples after packing hydroxyapatite paste to 3D printed plastic forms. A sheep animal model was used to perform in vivo experiments with bone defects created in long bones, where histological and macroscopical tissue analysis as well as X-ray and CT images were applied. After 6 months, all implants showed excellent biocompatibility with the surrounding bone tissue with no observed signs of inflammatory reaction. We did not observe anomalous bone formation, fibrous encapsulation, focal osteolytic and osteosclerotic changes or specific degenerative changes in the surrounding tissue. Treatment of bone defects had a positive impact on new bone formation and resulted in efficient bone regeneration. The histomorphological findings revealed bone growth immediately over and around the implants, indicating excellent osteoconductivity of implants. A number of islands of bone tissue were observed towards the centres of the hydroxyapatite cylinders. The bioceramics was more or less degraded, however, incomplete biodegradation and bioresorption were observed. Integration of the biomaterial with adjacent bone was confirmed, and the material was firmly attached to the bone. We consider hydroxyapatite cylinders to be suitable biomaterials for further research and use in human medicine.

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3D anatomical models for medical education

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This project expands a virtual collection of high-resolution 3D anatomical models based on real human specimens, now including additional organs and cadaveric parts. Each model, labeled with Latin anatomical terminology, is created using photogrammetry and 3D scanning, allowing students to study anatomy outside the dissection room. To ensure clarity and usability, the models undergo post-processing in Blender, including mesh cleaning and texture correction. This facilitates smooth integration into online platforms such as Moodle and Sketchfab, supporting interactive learning and better access to anatomically accurate content.

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Comparative analysis of 3D reconstruction technologies for anatomical models

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Introduction: Human anatomy education is affected by limited availability of cadavers. Modern 3D reconstruction technologies offer promising alternatives for teaching, diagnostics, and surgical planning. While 3D scanning, modeling, and printing are increasingly established, a systematic comparison of diverse methodological approaches for anatomical model creation remains not fully utilized.

Material and methods: Presented study aimed to bridge this gap by systematically evaluating the quality, practicality, and limitations of smartphone-based photogrammetry / LiDAR scanning (Kiri Engine app), blue light encoded structured light technology (3DMakerpro Seal 3D Scanner) and CT-based reconstruction methods (manual and AI-assisted segmentation via TotalSegmentator plugin); using two anatomical structures (scapula and skull) from our depositary. Model quality was visually assessed for precision, detail, and morphological accuracy. No other artificial alteration of the skeletal model has been performed.

Results: Manual CT segmentation consistently yielded the most precise and detailed models, capturing anatomical features but demanding significant resources and post-processing. Automated CT segmentation via TotalSegmentator resulted in excessive smoothing and simplification, proving insufficient for accurate representation of complex details. Photogrammetry / LiDAR with Kiri Engine, as well as blue light scanning technology (3DMakerpro) offered time and cost-effective solutions, excelling in visualizing external structures and general morphology, yet lacking the detail required for complex internal anatomy.

Conclusion: Each 3D reconstruction method presents distinct advantages and limitations. While both direct-scanning are suitable for macroscopic digitalization, CT-based models remain essential for specialized use. Further research is important to optimize model's digitalization for greater accuracy and to establish objective quality assessment criteria, enhancing the overall applicability of these technologies in anatomical education.

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Evaluation of novel mitochondrial permeability transition pore inhibitors in liver mitochondria

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Introduction: Cyclophilin D (CypD) is a mitochondrial enzyme widely recognized as a regulator of the mitochondrial permeability transition pore (MPTP). Alterations in calcium homeostasis, increased frequency, and prolonged opening of the MPTP are associated with mitochondrial dysfunction, aging, and the development of numerous diseases, including ischemia-reperfusion injury of the heart, liver, brain, and kidneys. Suppression of calcium/CypD-induced MPTP opening represents a promising therapeutic approach for the treatment of these conditions. Currently, only a limited number of selective CypD inhibitors are available. The aim of our study was to evaluate the effects of newly synthesized potential CypD/MPTP inhibitors on isolated liver mitochondria for their possible future clinical application.

Methods: To assess the newly synthesized potential MPTP inhibitors, we used two methods: mitochondrial swelling evaluation and determination of mitochondrial calcium retention capacity (CRC). All experiments were performed on liver mitochondria isolated from male Wistar rats. CRC was evaluated using the membrane-impermeable fluorescent probe Calcium Green-5N. This probe enables tracking of Ca^{2+} ions movements across the mitochondrial membrane and indicates the amount of Ca^{2+} ions required to accumulate in the mitochondria to trigger MPTP opening. To study the activating effect of Ca^{2+} ions on MPTP following the action of potential inhibitors, we used mitochondrial swelling.

Conclusion: In our study, we tested a total of 19 newly synthesized CypD inhibitors based on N-4-aminobenzyl-N'-(2-(2-phenylpyrrolidin)-2-oxoethyl)urea and 2-(benzyloxy)arylurea. Their inhibitory effect was also compared with that of cyclosporin A (CsA), an immunosuppressant used in vitro as a CypD/MPTP inhibitor. Our results indicate that the synthesized 2-(benzyloxy)arylurea derivatives are only weak inhibitors of CypD and have no effect on calcium-induced MPTP opening. In contrast, the N-4-aminobenzyl-N'-(2-(2-phenylpyrrolidin)-2-oxoethyl)urea-based derivatives exhibited significant inhibitory effects on calcium-induced MPTP opening in vitro. The most effective CypD/MPTP inhibitors achieved values comparable to those of the control compound CsA.

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Bones before chromosomes: How skeletal traits reveal sex

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Aim: Sex estimation based on skeletal remains is generally preferred, as it is considered non-invasive, more-cost effective, and time-efficient compared to DNA analysis. However, sexestimation from the skeletal remains is complicated by the wide variety of available methods and the differing reliability of various bones, which may be confusing for novice researchers. The choice of method is also influenced by the preservation of the skeletal remains. Therefore, a review of methods for sex estimation based on the humerus, radius, and ulna was conducted.

Methods: A structured literature review was performed using the databases Google Scholar, PubMed, Scopus, and Web of Science. Only studies focusing on adult individuals and providing sufficiently detailed methodology and results were included. The year of publication, and a cut off of 2005 were established as another criteria.

Results: Morphological and osteometric methods were identified as the two main approaches to sex estimation from dry bones. Morphological assessment is dependent on the examiner's experience and is often considered subjective. In contrast, osteometric methods allow for both univariate and multivariate analyses and are generally regarded as more objective and reproducible. For more accurate results, population-specific methods should be selected, as the size of the upper limb bones varies by population origin.

Conclusions: For sex estimation, the pelvis is considered the most reliable skeletal element. If the pelvis is not preserved, long bones are recommended as the preferred alternative.

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Shh in the jawbone formation in mice

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Using Cre-loxP system, we documented the progeny of cells previously expressing Sonic Hedgehog (Shh) in the ossification regions of both, upper and lower mouse jaws from embryonic day 14.5 till 18.5. Interestingly, these cells were present at the tooth-bone interface of upper and lower first molar tooth germs also postnatally. Their number was higher in the lower jaw compared to the upper jaw. Using a combination of RNA scope and immunohistochemistry, we showed that these Shh descendant cells were osteoclasts involved in bone resorption, but also osteoblasts and osteocytes involved in the formation and maintenance of the bone matrix during ossification. We found a mixed population of Shh descendant and non-descendant cells participating in bone formation and resorption in the tooth-bone interface documenting that osteoblasts, osteocytes and osteoclasts in jawbones are of mixed origin. Further, we detected an active expression of Shh in the ossification regions in jaws at E18.5 using RNA scope providing evidence of the ability of bone cells to express Shh during bone formation. In conclusion, we have demonstrated that bone cells present at tooth-bone interface can express Shh, a signaling molecule that has been recently shown to play an important role in regenerative processes in postnatal healing of bone defects and in tumors. We also showed that the cells with Shh expression history were present in the bone forming area not only during jaw-bone formation but also during adulthood. From this perspective, the results bring also new insights into developmental bone forming processes and provide significant evidence of the presence of bone cells with kept potential to express Shh, which could possibly be awakened later, during regenerative healing in the bone area. Thus, our results can significantly contribute not only to understanding bone development and pathogenesis but also to the area of bone regeneration and healing.

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Anterior musculocapsular complex of the hip and its relevance for direct anterior approach to the hip joint

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Anterior musculocapsular complex (AMCC) of the hip represents a concept of topographical interactions between the articular hip capsule and surrounding, anteriorly located, muscles. Based on our extensive macroscopic and microscopic observations on fresh-frozen cadavers and dry bones, we present several clinically relevant nuances, which are implicated for the direct anterior approach (DAA) to the hip joint. We emphasize the importance of respecting the correct fascial planes in order to avoid vascular injury. Macroscopically, the rectus femoris muscle contributed to the articular capsule exclusively through its reflected head. The iliocapsularis and iliopsoas muscles were in direct contact with the articular capsule. Although the iliocapsularis muscle was adherent to the capsule throughout its whole course, the iliopsoas muscle was connected to the capsule through the iliopectineal bursa. Microscopically, different spatial thickness of the capsule was observed, with the thicker regions corresponding to the capsular ligaments. Osseous landmarks identified on dry hip bones, relevant to the course of the iliopsoas muscle, included the iliopsoas notch and a groove for the psoas major muscle. Furthermore, split of the anterior inferior iliac spine and the "subspine" were constant findings with respect to the origin of the direct head of the rectus femoris muscle and the iliocapsularis muscle, and attachment of the medial band of the iliofemoral ligament, respectively. The intimate relationship between the medial band of the iliofemoral ligament and the iliocapsularis muscle led to us to the developed a new technique that partially spares the articular capsule in total hip arthtroplasty, and aims to decrease the risk of postoperative iliopsoas impingement. Together with the enhanced knowledge of soft-tissue anatomy around the anterior aspect of the hip, the resultant tips and tricks are believed to improve clinical outcomes in patients undergoing total hip arthroplasty through the DAA.

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Functional replacements in nerve tissue regeneration, an in vitro study

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Injuries to the sciatic nerve present a profound therapeutic challenge, often resulting in significant motor and sensory deficits and a reduced quality of life. Advances in 3D printing technology have enabled the development of scaffolds that closely replicate the native architecture of peripheral nerve tissue, offering new possibilities for nerve regeneration. Schwann cells play a pivotal role in this process by supporting axonal regrowth and facilitating myelination. This study explores printable, polymer-based biocompatible materials designed to support Schwann cell adhesion, viability, and differentiation in vitro. Immunocytochemical staining for β-actin was used to assess cell morphology and the extension of cellular processes. Different biomaterials and fabrication patterns were systematically evaluated to identify optimal conditions for enhancing cell proliferation and neurite-like process outgrowth. The enhanced composite scaffolds demonstrated superior performance, supporting robust Schwann cell growth and structural development. These findings provide a promising basis for the design of advanced scaffold-based conduits in peripheral nerve tissue engineering.

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Relation between the onset of puberty and ossification of sesamoid bones – preliminary report

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The sesamoid bones of the hand have been known for nearly two millennia. The basic description of their anatomy was followed by functional and biomechanical findings that are still scientifically discussed. Due to fact that they are the smallest and most variable part of our musculoskeletal system the importance of their physiology and pathological states is mostly omitted, especially when it comes to the hand. Only a few of the scientific studies try to essay description of arrangement, relations to the joints of fingers, size and morphology of the sesamoid bones. Those parameters are very changeable and depend on many factors such as ethnics, sex, hormonal changes or specific types and intensity of dynamic load during a variety of movements. Moreover, to this day the golden standard that occurs in literature for imaging ossa sesamoidea is classical radiology. Thus, it is mostly used in adult populations due to methodology and possible radiation. The aim of our study is to describe morphology, size and shape of sesamoid bones in children aged between 10 to 15 with ultrasonography. All the participants were interviewed in presence and with assistance from their parents regarding the onset of puberty – here marked by menarche in girls and beginning of voice mutation in boys. In all the participants we had mapped the arrangement of the sesamoid bones and their dimensions in three axes using ultrasound imaging. Those measurements were analyzed in respect of the correlation between times of ossification and the onset of puberty. The examination is planned to be repeated yearly throughout the participants' puberty. To conclude, the objective is to describe the shaping pattern, times of ossification and morphology of sesamoid bones in correlation with stages of puberty with hope of supplementing the missing literature and giving a better insight into upper limb development.

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Chemotherapy induces epitranscriptomic changes in the choroid plexus

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More than half of chemotherapy patients develop neuropathic pain, an irreversible and debilitating medical condition that significantly impacts patient well-being and survival chances. Our recent study demonstrated that paclitaxel, a widely used and effective chemotherapy drug for solid tumors, directly modifies the inflammatory profile of the choroid plexus. This finding challenges the conventional view of the choroid plexus as a passive barrier and instead suggests it is an active contributor to neuroinflammatory responses during chemotherapy. We view a comprehensive characterization of the choroid plexus in response to chemotherapy as a unique opportunity to shed light on long-standing unresolved mechanisms behind the central side effects of chemotherapeutic agents. To investigate the effects of paclitaxel on the choroid plexus, we performed next-generation sequencing (RNA-seq) on the choroid plexus tissue of an in vivo chemo-pain rat model (male and female) to profile molecular changes at 1 day, 7 days, and 21 days post-treatment. Our initial analysis focused on differential gene expression (DEG) and alternative splicing. From DEG analysis, we observed limited transcriptional changes. Recognizing the unexpected limited transcriptional changes, we turned our attention to alternative splicing, hypothesizing that the paclitaxel might exert its effects through post-transcriptional regulation. We identified significant local splice variants (LSVs) in the choroid plexus at 1- and 21-day post-treatment. Additionally, we identified regulators of alternative splicing, including RNA-binding proteins (RBPs), that were significantly enriched downstream of the target exon in choroid plexus mRNAs exhibiting increased exon skipping. These changes may impact choroid plexus functions such as neuroinflammation, with broader implications for chemotherapy-induced toxicity. Our findings could accelerate the development of more effective therapies targeting both acute and long-term effects of chemotherapy on the brain.

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Type 2 diabetes mellitus and its influence on the distribution of volume of adipocytes in different adipose tissue compartments—preliminary result

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Type 2 diabetes is associated with functional disturbances of adipose tissue, including excessive growth of adipocytes followed by intracellular stress. Adipose tissue is not a homogenous tissue, both physiological responses and involvement in pathological processes differ across anatomical compartments. Our study aimed to compare the distribution of volumes of adipocytes in subcutaneous, visceral, and pericardial adipose tissue. Tissue samples harvested during routine autopsy were processed for routine hematoxylin-eosin staining. From each sample, representative pictures were obtained. Digital images were processed using a short script written in Python using the OpenCV2 library. Empty areas related to the main lipid droplet inside adipocytes were measured. The area of each lipid droplet was used as a number related to the volume. We used the areas as the main result for analysis of the histogram because the transformation of the areas to either diameters or volumes requires some additional assumptions and simplifications. We demonstrated the value of microscopic morphometry of adipose tissue based on image processing, especially the morphometry focused on the distribution of analyzed parameters rather than average values.

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Inflammatory reaction of the choroid plexus induced by the diabetes mellitus

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Diabetes mellitus (DM) is chronic metabolic disease affected, without exception, the whole organism including the central nervous system. One of the lesser-explored diabetic complication is diabetic encephalopathy (DE), where structural and functional alterations of the brain are evident. Pathophysiology of the DE is complex and there is certainly involvement of the choroid plexus as the blood-cerebrospinal fluid barrier (BCSFB) due to its immunological and barrier functions. In this study, we investigated the inflammatory response of the choroid plexus in a rat model of type 1 DM. Male Wistar rats (8–10-week-old males) were divided into two groups: the diabetic group (n=13) and the control group (n=8). DM was induced by a single intraperitoneal injection of streptozotocin (80 mg/kg), while controls received only vehicle. After three weeks, animals were euthanized using CO_2 inhalation, followed by perfusion with Zamboni's fixative. Brains were collected and processed for coronal sectioning. Immunohistochemical staining of the lateral ventricular choroid plexus was performed using markers of macrophage/microglial activation and polarization: CD68, CD163, CCR7, CD206, CD11b/c, and MHC-II. The number of positive cells per mm2 was examined and statistically evaluated. Our results demonstrate a significant increase in immune cell infiltration and activation in the choroid plexus of diabetic rats compared to controls. These findings support the hypothesis that inflammatory response of the choroid plexus induced by DM could lead to disruption of brain microenvironment and development of the DE. Therefore, the choroid plexus may represent a potential therapeutic target for modulating neuroinflammation in diabetes.

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BoneDat, a database of standardized bone morphology for in silico analyses

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In silico analysis is key to understanding bone structure-function relationships in orthopedics and evolutionary biology, but its potential is limited by a lack of standardized, high-quality human bone morphology datasets. This absence hinders research reproducibility and the development of reliable computational models. To overcome this, BoneDat has been developed. It is a comprehensive database containing standardized bone morphology data from 278 clinical lumbopelvic CT scans (pelvis and lower spine). The dataset includes individuals aged 16 to 91, balanced by sex across ten age groups. BoneDat provides curated segmentation masks, normalized bone geometry (volumetric meshes), and reference morphology templates organized by sex and age. By offering standardized reference geometry and enabling shape normalization, BoneDat enhances the repeatability and credibility of computational models. It also allows for integrating other open datasets, supporting the training and benchmarking of deep learning models and accelerating their path to clinical use.

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Role of Jagged1-Notch signaling in cardiac development

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The Notch signaling pathway is a conserved intercellular mechanism essential for mammalian embryonic development. Mutations in the human Jagged1 (Jag1) gene, encoding a Notch ligand, cause Alagille syndrome—an autosomal dominant disorder frequently associated with congenital heart defects (CHDs), including Tetralogy of Fallot. To investigate the role of Jag1 in cardiac development, we generated Jag1 flox/flox Islet1-cre/ mice with a conditional deletion of Jag1 in the cardiac outflow tract. These mutants exhibited severe cardiac malformations characteristic of Tetralogy of Fallot. The main defect observed was double outlet right ventricle (DORV), where both the aorta and pulmonary trunk arise from the right ventricle. This anomaly was consistently accompanied by a ventricular septal defect (VSD), present in 100% of homozygous embryos. Additional defects included atrioventricular and semilunar valve malformations, particularly myxomatous changes in the mitral valve and abnormalities in leaflet number. Since Islet1 is also expressed in the sinoatrial and atrioventricular nodes, we used optical mapping to assess the cardiac electrical activation. At embryonic day 14.5 and in adult mice, mutants showed altered activation patterns. While controls exhibited normal apex-to-base conduction via both bundle branches, mutants frequently initiated activation from the left ventricle, suggesting right bundle branch block (RBBB). In adult heterozygotes, electrical activation was asynchronous and often initiated at ectopic sites, especially in the posterior ventricular wall. Physiological analysis using Vevo ultrasound was performed on adult heterozygotes only, due to high postnatal mortality in homozygotes. Hemodynamic parameters remained largely unchanged, suggesting early cardiac compensation. However, speckle-tracking strain analysis revealed localized contractile defects and mechanical dyssynchrony, especially in the anterior wall. In conclusion, our study demonstrates that conditional deletion of Jag1 causes both morphological and electrophysiological abnormalities in the heart, disrupting normal cardiac development and function, and resulting in structural defects and impaired electrical conduction.

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Retrospective 3D segmentation of temporal bone HRCT scans in patients with chronic otitis media with cholesteatoma: Focus on morphological and functional changes

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Introduction: Chronic otitis media with cholesteatoma (CSOM) is a serious middle ear disease that can lead to the destruction of surrounding structures, hearing loss, and potentially life-threatening complications. Anatomical factors such as mastoid air cell pneumatization and the configuration of the anterior epitympanic recess (AER) appear to be potential risk factors for both the development and recurrence of cholesteatoma. Modern imaging techniques, including 3D segmentation of high-resolution computed tomography (HRCT) scans of the temporal bone, enable detailed morphometric analysis of these structures.

Objective: The aim of this project is to identify differences in anatomical parameters associated with CSOM using 3D segmentation of HRCT data and to compare them with a control group of patients without inflammatory middle ear changes.

Methods: This retrospective case–control observational study includes HRCT data from 15 patients with CSOM and 15 control subjects. Selected anatomical structures of the temporal bone are segmented and quantified using 3D Slicer software. Differences between the pathological and healthy sides in patients, as well as between groups, are statistically evaluated. Audiometric findings and the occurrence of recurrence are also assessed.

Results: Overall, patients with CSOM exhibited smaller dimensions of middle ear structures compared to controls, including on the clinically unaffected side. Incompletely developed anatomical structures and spaces, such as the crista tegmentalis transversa (COG) and limited pneumatization of the anterior epitympanic recess (AER), show a higher degree of association with disease development.

Conclusion: The project offers a new perspective on the role of middle ear anatomy in the pathogenesis of CSOM and highlights the potential of advanced 3D HRCT segmentation in clinical research.

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Liver endothelial sinusoidal cells activation and inflammatory response to liver injury in MASH and DDC animal model

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Liver sinusoidal endothelial cells (LSECs) are essential regulators of hepatic microenvironmental homeostasis and play an active role in coordinating inflammatory and fibrogenic responses to liver injury. In our study, we investigated LSECs activation and inflammation in two murine models of liver damage: intrahepatic cholestasis induced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) and metabolic-associated steatohepatitis (MASH) induced by a choline-deficient, L-amino acid-defined high-fat diet (CDAA-HFD). Histological staining revealed significant fibrosis in both models. Collagen accumulation was observed primarily in portal areas in DDC-treated mice and in periportal and perisinusoidal regions in MASH. Immunohistochemical detection of α-smooth muscle actin (α-SMA) confirmed the presence of activated myofibroblasts, especially in fibrotic zones. LSECs contribute to the activation of hepatic stellate cells (HSCs), which differentiate into α-SMA-expressing myofibroblasts and promote fibrogenesis. LSEC activation and inflammatory response were further documented by increased expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). ICAM-1 was predominantly detected in sinusoidal endothelial cells in both models. VCAM-1 showed strong expression in LSECs in MASH, whereas in DDC-treated mice, it was limited to cholangiocytes and myofibroblasts in areas of ductular reaction. Additionally, galectin-3-positive macrophages were markedly increased in periportal and fibrotic regions, indicating enhanced immune cell recruitment and local inflammation. Increased laminin deposition along sinusoidal walls indicated the LSECs capillarization and basement membrane formation, hallmark features of liver sinusoidal endothelial dysfunction (LSED). Endoglin (ENG) expression was localized to LSECs and showed reduced staining in DDC and enhanced positivity in MASH. In conclusion, activation of LSECs, inflammatory signaling, and capillarization are shared features of both cholestatic and metabolic liver injury, facilitating HSC activation and contributing to fibrosis progression.

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The intersaphenous veins in the leg: anatomical considerations

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Background: The intersaphenous veins are inconstant veins interconnecting the great and the small saphenous veins. Due to the inclination of the superficial venous system to be rather inconsistent, this topic has never been described thoroughly and adequately before. The aim of our research was to observe intersaphenous veins in the leg and give a profound description of their variable anatomy.

Methods: Sixty-three body donor limbs of Central European origin embalmed in formaldehyde were dissected. The tips of the medial and lateral malleoli were selected as suitable points of reference for measurements on the vertical axis of the leg. Additionally, each fibula was measured and related to the average length of the fibula in our examined sample. Consequently, each measurement was re-calculated by this coefficient.

Results: Five types of arrangement were described: no connection (22.22%), one connection (46.03%), two connections (19.05%), three connections (11.11%) and four connections (1.59%). The average point of communication between the great saphenous vein and the intersaphenous vein was located 21.50±9.64 cm proximal to the medial malleolus and between the small saphenous vein and the intersaphenous vein, 18.45±6.05 cm proximal to the lateral malleolus. Multiple heat-maps were created for an easier comprehension of the topic.

Conclusion: This research provides a detailed anatomy of intersaphenous veins of the leg. Yet variable in their arrangement, intersaphenous veins were found in the majority of investigated limbs. Thanks to their favorable anatomy, intersaphenous veins offer new alternatives to the traditional venous grafts from the saphenous veins, not only for heart by-passes.

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Effect of amniotic membrane on peripheral nerve regeneration using the nerve wrap technique: A rat model s7udy

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Peripheral nerve injuries significantly impact patients‘ quality of life due to the typically slow and often incomplete process of nerve regeneration, resulting in impaired action potential transmission to the neuromuscular junction and reduced muscle function. Despite advances in our understanding of nerve physiology and regeneration, current surgical techniques remain limited, allowing reconstruction at the fascicular but not the axonal level. This study focuses on the application of the Nerve Wrap technique in peripheral nerve reconstruction in rats. The method involves wrapping the suture site of the sciatic nerve with a biological membrane, aiming to enhance nerve regeneration, prevent adhesions of surrounding tissues, and reduce neuroma formation. We evaluated the effectiveness of human amniotic membrane, in both lyophilized (ALM) and cryopreserved (AMM) forms, compared to a commercially available (CM) nerve wrap product, a nerve reconstruction without membrane (CO), and a nerve transection (SNT). All animals survive for three weeks. Behavioral tests were performed during the specified survival period. Mechanoallodynia was measured. The regenerative response was assessed by monitoring the activation of satellite glial cells (as indicated by GFAP), the upregulation of GAP43, a growth-associated protein involved in axonal regeneration, and the expression of the pro-inflammatory cytokine IL-6 in dorsal root ganglia (DRG), including its colocalization with GFAP and NeuN. We observed higher activation of satellite glial cells (SGCs) following amniotic membrane application compared to the CM, CO, and SNT groups. Also, higher levels of IL-6 and GAP43 immunoreactivity were detected in the amniotic membrane groups. Our results suggest that the use of amniotic membrane positively modulates the local microenvironment and supports functional nerve regeneration more effectively than the commercial membrane and control groups.

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Morphological evaluation of experimentally created articular cartilage defects in sheep after application of composite biocement c.

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Currently, various types of biocompatible materials, including bioceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP), are used to create scaffolds for the regeneration of osteochondral defects. In our study, female Merino/Wallachian breed sheep aged 2 to 2.5 years (n=14) were used. In experimental sheep (n=6), a traumatic defect of the articular cartilage of the left knee joint in the area of the femoral trochanter was performed with a diameter of 8 mm and a depth of 10 mm. The experimental group 1 (C) had the defects filled with biocement based on a powder mixture of tetracalcium/monetite (n=6), group 2 (control) (n=2), and group 3 (spontaneously healed defect) (CYS) (n=6) did not have the cartilage defect filled with biocement. At the end of the 6-month monitoring period of the regeneration process, the sheep were humanely euthanized. Subsequently, samples were taken from the implantation site for histological processing. The group 2 showed cartilage of a hyaline nature with a regular arrangement of individual layers and the presence of a tidemark line. The group 1 showed the formation of homogeneous, morphologically similar hyaline cartilage tissue with a regular surface, lower proteoglycan content in the superficial zone, but with comparable content in the middle and deep zones compared to the control, at the interface of the deep zone and the subchondral bone tidemark line. In group 3, we observed surface erosion with the formation of horizontal cracks and the formation of cell clusters in the superficial zone, hypocellularity to acellularity in certain places in the intermediate zone and the formation of vertical cracks against the background of the fibrous structure of the cartilage matrix, and the formation of cell clusters was evident at the interface between the intermediate and deep zones. Based on histological results, we demonstrated the regenerative potential of the applied biomaterial in experimentally created osteochondral defects in sheep.

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The impact of anatomy course duration on academic performance: A comparative study of three – semester and two – semester anatomy curriculum

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Anatomy is a key theoretical subject in medical education, playing a critical role in students' understanding the human body. The knowledge obtained in anatomy are subsequently used in another theoretical subjects like physiology and pathophysiology and finally in the clinical practice. The optimal duration and structure of anatomy education remains a subject of the ongoing discussions. This study focuses to compare the academic performance of students enrolled in two different curricular models: a two-semester and a three-semester anatomy course. Academic performance was assessed through standardized written midsemester tests, oral midsemester practical examinations, and final exams, consisting of written exam test, practical exam, and oral exam. Data were collected from two groups of undergraduate students of the program General Medicine at the Jessenius Faculty of Medicine in academic years 2023/2024 and 2024/2025, one following three-semester course and the other completing two-semester course. The study compares the academic performance of both groups and highlights the benefits and disadvantages of both two- and a three-semester anatomy curricula.

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The use of micro-CT in the paleopathology

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For paleopathologists, paleoepidemiologists and clinical research specialists, understanding the pathology and epidemiology of diseases that affected human populations in the past is important. This knowledge contributes to our understanding of modern diseases. As micro-CT machines have become more accessible, paleopathological studies have begun utilising micro-computed tomography to diagnose historical diseases evident in skeletal remains. This relatively new method offers novel approaches to diagnosis and could lead to faster and more accurate assessments of individual cases. The selected bone pathologies originate from archaeological material dating from various historical periods and are caused by different aetiological processes, including tumours, metabolic diseases, infectious diseases, and trauma. Micro-CT scanning was performed using an experimental in-house CT scanner (TORATOM, European patent EP 2835631, 2016) at the Institute of Theoretical and Applied Mechanics of the Czech Academy of Sciences. The resolution of the scanned samples ranged from 8 to 30 µm. Scanning resulted in a large number of images of bone microstructure, providing the possibility of 3D imaging of samples suitable for structural analysis of bone tissue pathology. A micro-CT examination offers a detailed view of the microstructure of bone tissue, which is useful for identifying osteolytic/osteoplastic lesions, examining bone tissue cavities and their potential connections, analysing the effects of injury, and examining bone tissue density. This study aims to improve the diagnosis of bone pathologies.

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Traditional vs. digital anatomy education: Slovak medical students' perspectives

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A thorough knowledge of anatomy is essential for medical students as they prepare for the transition into clinical practice. The understanding of human anatomy enables them to develop the necessary skills for safe and effective patient management. Anatomy, as foundational subject in medical education, is traditionally taught through formalin-fixed cadaver dissection, formalin-fixed cadaveric organ specimens, dry bone specimens, plastic anatomical models of structures and organs, textbooks, and anatomy atlases among others. However, the rapid advancement of technology in recent years reflects also a global trend toward the modernization of anatomy education, including the increasing integration of digital tools such as virtual dissection tables, three-dimensional models, animations, and other interactive resources. Therefore, we aimed to explore Slovak medical students' experiences and preferences regarding traditional and digital approaches in anatomy education. A cross-sectional questionnaire-based survey was conducted among medical students at Jessenius Faculty of Medicine in Martin, Comenius University Bratislava. The survey included predominantly quantitative items assessing students' exposure to various teaching methods, perceived effectiveness, learning preferences, and attitudes toward the traditional and digital tools in anatomy education. The findings of our survey indicate a clear preference among students for traditional anatomy teaching methods, particularly during practical and dissection sessions. Cadaveric dissection and anatomical models remain the most valued educational resources. Digital tools, such as the digital dissection table and 3D applications, are recognized as useful complementary aids but are not yet viewed as adequate substitutes for traditional approaches. As a conclusion, although digitalization has brought significant advances, traditional anatomy education continues to offer irreplaceable educational value.

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Serum markers of bone regeneration process – a review

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Bone remodelling is a dynamic phenomenon involving the simultaneous resorption and formation of new bone tissue. All bone tissue cells participate in this process, which is regulated not only by hormones but also by various signalling molecules, proteins, and enzymes. Currently, there is a growing need to assess bone regeneration using additional indicators—biomarkers. Biomarkers are biological molecules found in blood, tissues, and other body fluids. They reflect normal physiological processes but can also be valuable in monitoring pathological changes. In particular, attention should be given to the dynamics of osteogenic markers such as calcium and phosphorus, as well as the enzymes alkaline phosphatase (ALP) and bone-specific alkaline phosphatase (BSAP). Other markers, such as osteocalcin and tartrate-resistant acid phosphatase (TRAP), indicate increased osteoclastic activity. Measuring serum markers of bone metabolism may assist in evaluating cellular function during bone fracture healing. These markers can provide near real-time insights into the biological response to injury and the effectiveness of selected treatments. In the future, such tests could offer a simple, accessible, and accurate diagnostic tool. However, as is well established in various medical disciplines, no single diagnostic method is sufficient on its own. Therefore, laboratory findings should be interpreted in correlation with imaging and histological examinations.

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Ectopic bone in the aortic valve in a mare – accidental finding

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The presence of ossified foci (bone) in the myocardium and associated cardiac tissues is physiological in certain animal species, such as ruminants. However, in horses, the formation of solitary or multifocal ossified foci is typically associated with aging or pathological processes. In our case, we discovered hard, sharp, and creaking deposits in the area of the aortic valve during a routine cardiac autopsy of a 21-year-old warmblood mare. Samples were collected for histopathological examination, fixed in 4% formaldehyde, decalcified in EDTA, and stained with standard haematoxylin-eosin and Masson's trichrome. Microscopic analysis revealed the presence of all major tissue types: muscle, fibrous, cartilage, and bone. The metaplastic bone tissue was well-organized, exhibiting a typical lamellar structure with osteons and Haversian canals, as well as the characteristic cellular components of bone tissue. Similar findings, including comparably structured bone tissue, have been reported by other authors. The occurrence—and more specifically, the formation—of bone tissue within the valve is believed to be associated with active repair processes of the valvular endothelium following damage due to inflammation. In this case, it was not possible to determine the exact cause of the condition with certainty. Nevertheless, the case is notable due to the extremely rare occurrence of such findings.

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Horse as an animal model for research and therapy of temporomandibular joint osteoarthritis – a review

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Temporomandibular joint osteoarthritis (TMJ OA) is a chronic disease characterized by degenerative changes in the cartilage, accompanied by reparative processes in the surrounding tissues. As the disease causes severe pain, swelling and stiffness of the joints, limitation of mandibular movement and subsequent reduction in quality of life can be expected. The etiology of TMJ OA is complex and multifactorial, generally involving factors such as mechanical overload, abnormal occlusion, trauma and stress. However, the specific causes of cartilage and subchondral bone damage in the TMJ remain unclear, indicating the need for further research. Osteoarthritis (OA) of the temporomandibular joint (TMJ) is a disease that occurs spontaneously in humans and animal species, including horses. Genetically modified, induced, and naturally occurring animal models play a key role in understanding the pathogenesis and evaluating potential therapeutic interventions for TMJ OA, as tissue collection in humans is difficult and clinical signs appear late in the progression of the disease. The equine model of TMJ OA is characterized by a slow, age-related progression, a wide range of clinical examinations and imaging techniques that can be performed in horses, and the ease of tissue and synovial fluid collection. The morphological and functional similarities of TMJ structures in both species make the equine model of TMJ OA an excellent opportunity to monitor disease progression and response to treatment. However, the utility of biomarkers of human TMJ OA in horses remains to be determined. The main biomarkers of TMJ OA recently investigated in an equine model include IL-1, IL-6, TGF-β, TNF-α, and PGE2. The six major signaling pathways in TMJ osteoarthritis include transforming growth factor β (TGFβ)/bone morphogenic protein (BMP) signaling, nuclear factor kappaB (NFκB) signaling, fibroblast growth factor (FGF) signaling, Wnt/β-catenin signaling, Indian hedgehog (Ihh) signaling, and Notch signaling.

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The relationship of the great saphenous vein and the saphenous nerve: anatomical considerations

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Background: The superficial neurovascular bundle of the medial aspect of the leg consists of the great saphenous vein and the saphenous nerve. In clinical practice, procedures on the great saphenous vein include autologous venous graft harvesting or varicose stripping. Due to the intimate relationship between the vein and the nerve, the latter may be easily damaged during such procedures.

Methods: One hundred and fifty-five lower limbs from body donors embalmed by the traditional formaldehyde method were dissected. Dissection was performed in the medial aspect of the leg. The medial condyle of the femur and the apex of the medial malleolus were selected as adequate points of reference.

Results: Totally, four variants/types of mutual relationship between the great saphenous vein and the saphenous nerve were investigated. Two intersections between the structures of interest were present in 14.84% of limbs (Type I), one intersection among 40.00% of lower limbs (Type II). Additionally, in 27.74% of limbs, a substantial distance between the vein and the nerve was detected (0.5-30 mm; Type III). Lastly, an intimate relationship (below 0.5 mm in horizontal axis) between the structures of interest was found among 17.42% of lower limbs.

Conclusion: This paper clarifies that the tight association of the vein and the nerve was found in most legs and thus confirms the substantial tendency of the nerve to be damaged. Accordingly, an ultrasonographic examination of the medial aspect of the leg before the surgery is highly suggested.

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Lectin based detection method used for quantitative analysis of endothelial glycocalyx in renal glomeruli

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The endothelial glycocalyx (eGCX) is a thin layer of proteoglycans and carbohydrates crucial for vascular homeostasis, fluid balance, immune-endothelial interactions, and regulation of hemostasis and hemodynamics. It is present throughout the vasculature and shows morphological variability across tissues.

This study aimed to optimize a laboratory protocol and data analysis method for evaluating the relative amount of eGCX by image analysis of lectin fluorescence in renal glomeruli. Porcine renal cortex samples from an experiment examining the effect of sulodexide on ischemia-reperfusion injury to eGCX were analyzed and compared with other laboratory findings from the same study.

We processed 26 renal cortex samples from 13 pigs collected before 30 minutes of ischemia (induced by clamping the suprarenal aorta) and after 2 hours of reperfusion. Sixteen samples were control and eight were sulodexide-treated (two samples were excluded due to renal hydronephrosis). Samples were fixed in 4% paraformaldehyde and frozen without cryoprotection. Cryosections were stained with LEL lectin conjugated with DyLight® 594, targeting eGCX proteoglycans. Fluorescence intensity of renal glomeruli was measured in two independent analyses and statistically evaluated.

A higher median difference in fluorescence intensity was observed in the sulodexide group between pre- and post-ischemia samples (0.08 ± 0.19; CI: -0.38 to 0.53) compared to controls (-0.04 ± 0.08; CI: -0.21 to 0.13), but the difference was not statistically significant ($p = 0.28$).

Although no statistically significant difference in eGCX fluorescence was found between groups, the trend aligned with other experiment data (serum syndecan-1, albuminuria, urinary glycosaminoglycans). The optimized method enables visualization, quantification, and comparison of eGCX in routinely prepared cryosections using affordable lectin-based fluorescence detection.

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A rare course of the superior trunk of the brachial plexus through the anterior scalene muscle: A case report

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Knowledge of brachial plexus variations is essential across medical fields such as radiology, anaesthesia, and surgery. During the Spring Dissection Course 2025 at the Department of Anatomy, Faculty of Medicine, Masaryk University in Brno, we identified a rare unilateral variation in an 89-year-old male cadaver. The superior trunk of the brachial plexus (C5) was found to pass through the belly of the anterior scalene muscle—an atypical course not commonly described in textbooks.

A well-defined muscular slip, measuring 6 mm in thickness and 36 mm in length, formed a bridge over the nerve trunk. Other components of the plexus followed their usual route through the interscalene space.

Although the individual's clinical history was unavailable, this variation may be clinically relevant. It could increase susceptibility to neurogenic thoracic outlet syndrome and complicate the administration of interscalene brachial plexus blocks.

Keywords: brachial plexus, scalenus anterior, fissura scalenorum, anatomical variation

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The Anatomyka App and 3D E-Posters: A new tool for students and anatomists

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The aim of this presentation is to introduce the innovative integration of the Anatomyka application and newly developed 3D e-posters into anatomy education. These tools have significantly enhanced the learning experience for medical and physiotherapy students, as well as teaching efficiency for anatomists.

Over the past five years, more than 700 students of general medicine and physiotherapy at our faculty, all with access to a university license for the Anatomyka application, completed an anonymous questionnaire at the end of the anatomy course. Their feedback was analysed to assess the impact of the application on their understanding of anatomy. In addition, a new set of over 450 e-posters was developed based on 3D anatomical models, aligned with traditional medical curricula.

Student responses strongly support the effectiveness of Anatomyka. The application's intuitive interface and high-detail 3D model allow users to explore the human body layer by layer, by body region, or by system. Based on the textbook Memorix Anatomy, the application features over 13,000 labelled anatomical structures and topographical relationships, enabling users to study anatomy with clinical-level precision.

The latest addition of more than 450 ready-to-use e-posters – illustrating bones, joints, muscles, organs, vessels, nerves, and topographic spaces – provides focused, curriculum-based visual materials. These posters are directly linked to the 6th edition of Memorix Anatomy through integrated QR codes, allowing students and anatomists to instantly access them on their smartphones, tablets, or computers.

The combination of the Anatomyka application and interactive 3D e-posters represents another leap in anatomy education. By merging the clarity of a structured textbook with the power of dynamic 3D visualisation, this system significantly accelerates and deepens anatomical understanding for both students and educators.

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Variations of the extrapsoas course of the lumbar plexus with implications for the lateral transpsoas approach to the lumbar spine: a cadaveric study

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Increased interest in minimally invasive lateral transpsoas approach to the lumbar spine necessitates detailed anatomical descriptions of the lumbar plexus. Although definitions of safe zones and essential descriptions of topographical anatomy have been presented, variations in the approach pathway were omitted. Therefore, the aim of this study was to investigate the variability of the extrapsoas portion of the lumbar plexus. A total of 260 lumbar regions from embalmed cadavers were utilized in this study. The specimens were dissected as per protocol and all nerves from the lumbar plexus were morphologically evaluated. The most common variation of the iliohypogastric and ilioinguinal nerves was fusion of these two nerves (9.6%). Nearly in the half of the cases (48.1%) the genitofemoral nerve left the psoas major muscle already divided into the femoral and genital branches. The lateral femoral cutaneous nerve was the least variable one as it resembled its normal morphology in 95.0% of cases. Regarding the variant origins of the femoral nerve, there was a low formation outside the psoas major muscle in 3.8% of cases. The obturator nerve was not variable at its emergence point but frequently branched (40.4%) before entering the obturator canal. In addition to the proper femoral and obturator nerves, accessory nerves were present in 12.3% and 9.2% of cases, respectively. In conclusion, nerves of the lumbar plexus frequently show atypical anatomy outside the psoas major muscle. Surgeons must consider the possibility of neural variations during retroperitoneal access to different segments of the lumbar spine.

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Crossroads – an encounter

Šín L.

Intersections, by definition, are places where various transport routes converge, leading in different directions. Yet, beyond their practical function, intersections represent an ancient cultural phenomenon. Their mystical significance likely stems from the symbolism of the cross, which embodies the number four – representing cardinal directions, seasons, elements, or lunar phases. Prehistoric agricultural societies often imbued intersections with sacred and protective qualities. They were seen as places offering safety during encounters, and mourners frequently buried their dead at these crossing points for generations.

One example is found north of Olomouc, near what is now "exit 261" on the D35 motorway, within the cadastral area of Křelov. Over four millennia ago, earthen mounds (tumuli) were erected over some graves here, visually dominating the landscape for an extended period. This burial site demonstrates remarkable historical memory and functional continuity spanning over three millennia. Various populations consistently chose this location to inter their deceased, showing respect for the graves of previous generations, even those laid to rest centuries earlier. In doing so, these mourners legitimized the landscape across generations and simultaneously created a prominent landmark.

Another remarkable site is the intersection of today's Náves and Na Hrázi streets in the village of Majetín, south of Olomouc. This location became a burial ground during the first half of the 19th century. While some individuals interred here were members of the local community, others lacked the right to be buried in consecrated ground. Conversely, some had the right but no ties to the local community. This was not a formal cemetery but rather an ad hoc solution for unforeseen circumstances involving individuals not connected temporally or spatially during their lives. Thus, it is a place profoundly linked to the phenomenon of historical memory. Furthermore, analysis of one individual's lower jaw incisors revealed traces of trauma, offering insights into their profession and aiding in reconstructing the origins of this particular burial site.

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Beyond treatment: Paclitaxel's impact on blood-cerebrospinal fluid barrier integrity

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Paclitaxel is a common chemotherapeutic drug with notable neurotoxicity. The blood–CSF barrier, primarily formed by tight junctions between choroid plexus epithelial cells, protects the nervous system from toxins. Paclitaxel disrupts cytoskeletal dynamics, impairing tight junction function and compromising barrier integrity.

This study aimed to analyze the effect of paclitaxel on the blood–CSF barrier by assessing morphological changes in epithelial cells and quantifying ZO-1 protein expression, a key tight junction component.

Z310 cells were exposed to 20 nM paclitaxel for 6, 24, and 72 hours, while controls received solvent only. ZO-1 expression was detected by indirect immunofluorescence. Morphological changes and fluorescence intensity in tight junctions were evaluated using fluorescence microscopy and quantified in ImageJ. Statistical analysis was performed using Statistica 9.0.

After exposure to paclitaxel, there was a significant decrease in ZO-1 protein expression after 6 hours, indicating a disruption in the tight junction function of choroid plexus epithelial cells. However, a partial recovery of this protein expression was observed with increasing time, with expression reaching 85% of control levels after 72 hours. This suggests that choroid plexus epithelial cells have the ability to partially regenerate tight junctions at longer intervals following paclitaxel-induced toxicity.

This study confirms that paclitaxel disrupts tight junctions in choroid plexus epithelial cells and exerts toxic effects on other cellular processes, potentially leading to the breakdown of the blood–CSF barrier. However, the results also suggest the existence of possible reparative mechanisms within these tissues.

These findings contribute to a better understanding of the mechanisms by which chemotherapeutic agents affect the central nervous system and may help in developing strategies to protect the blood–CSF barrier during chemotherapy.

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The asymmetry of bone mineral density in human mandibles

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Introduction Bone mineral density asymmetry in the human mandible may reflect functional lateralization and biomechanical adaptation. While morphological directional asymmetry in the face has been documented (Martiková et al., 2024), less is known about asymmetry in bone mineral radio-density. This study aimed to quantify voxel-wise directional asymmetry (DA) in mandibular bone mineral density, controlling for shape variation.

Material and Methods Sex-specific 3D templates of the mandible were created from computed tomography data of adult males and females. Each mandible was warped to the corresponding sex-specific template using shape registration to remove morphological variability. Hounsfield Unit (HU) values were sampled voxel-wise from the warped models. For each voxel, DA was computed as the difference between mirrored voxel values on the left and right hemimandible. Positive values indicated greater density on the right side.

Results Both male and female datasets demonstrated a consistent pattern of higher HU values on the right hemimandible. This right-side predominance was statistically significant.

Discussion These findings reveal a reproducible directional asymmetry in mandibular bone mineral density favoring the right side, paralleling previously reported right-side bias in facial morphological asymmetry. This suggests a potential shared developmental or functional origin linking shape and tissue density asymmetry in the craniofacial complex.

Conclusion: Quantifying voxel-wise density asymmetry provides new insights into skeletal functional adaptation beyond shape analysis. These results may inform future research on the relationship between mandibular biomechanics, functional loading, and craniofacial asymmetry.

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Cancer microenvironment: key factor of tumor aggressiveness

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Tumor Microenvironment – The stroma constitutes a heterogeneous population of diverse cell types and extracellular matrix components that establish the specific conditions required for tumor growth and progression. The organization of stromal elements varies among neoplasms, and the mechanisms underlying local mesenchymal activation, as well as the bidirectional communication between tumor cells and their supportive stroma, remain incompletely understood. Cancer-associated fibroblasts (CAFs) represent a predominant stromal cell population within the tumor tissue of pancreatic ductal adenocarcinoma (PDAC), prompting questions about their role in driving tumor aggressiveness and tumorigenesis. Soluble factors secreted by CAFs and present in enriched conditioned media exerted heterogeneous effects on the phenotypes of tumor cell lines. Comparative transcriptomic analysis of control dermal fibroblasts and CAFs revealed marked differences in mRNA expression levels for IL-6, IL-8, MFGE-8, VEGFA, and periostin. Paracrine signaling by stromal-derived molecules represents a key communication pathway between tumor cells and the stroma, facilitating the establishment of a malignant ecosystem. Exosomes—small, membrane-bound vesicles of endocytic origin—can deliver biologically active cargo, including microRNAs (miRNAs). Hypoxic conditions, commonly observed in tumors and healing wounds, stimulate the synthesis of miR-21 and miR-210, which, in this study, were highly expressed compared with paraffin-embedded sections of healthy pancreas. Reduced expression of miR-21 and miR-210 correlated with decreased cellular migration. miR-217, typically present in healthy pancreatic tissue, showed a pronounced reduction in expression, particularly in CAFs and tumor cell lines. Collectively, miR-21, miR-210, and miR-217 appear to contribute to the formation of a reactive tumor microenvironment by upregulating genes involved in angiogenesis and collagen fiber cross-linking, while downregulating mRNA transcription of certain matrix metalloproteinases. The enhanced biological stability of CAFs in the presence of tumor

cells, combined with the therapeutic potential of strategies targeting the tumor microenvironment, may offer promising avenues for the future expansion of anticancer treatment modalities.

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Shh during anorectum morphogenesis and in enteric nervous system distribution

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During early development, craniocaudal and lateral demarcation folding incorporates part of the endoderm-lined yolk sac into the embryo, forming the primitive gut, which divides into foregut, midgut, and hindgut. The hindgut forms the distal third of the colon, rectum, and cranial part of the anal canal (endoderm-derived), while the caudal part of the anal canal originates from ectoderm. The hindgut opens into the cloaca, from which the anorectal canal arises. Abnormal morphogenesis in this region can result in anorectal malformations (ARMs).

Sonic hedgehog (*Shh*), expressed in endoderm, plays a crucial role in gastrointestinal (GIT) and anorectal development. *Shh* signals to adjacent mesenchyme, ensuring site-specific gene expression related to differentiation of GIT mesenchyme along the anteroposterior axis, forming the longitudinal and circular muscle, submucosa and mucosa with its muscular layer. *Shh* is also crucial for postnatal stem cell maintenance and tissue regeneration. In mice, *Shh* deficiency leads to ectopic or enlarged enteric ganglia, while overexpression causes aganglionosis.

However, the role of Shh in anorectal development remains not fully understood. Using TdTomato/ShhEGFPCre mouse embryos, we traced descendant cells with *Shh* expression history to map their fates in the developing and adult anal canal. We found a mixed cell population in the anal canal transitional zone, derived from both endodermal and non-endodermal tissues. It seems that proper formation and length of this transitional zone is critical for normal anorectal morphogenesis.

To evaluate Shh role in ARMs, we generated *Shh* null mutant embryos and studied enteric nervous system (ENS) formation in *Shh* absence. We also analysed perioperative samples from patients with perineal (PF) and vestibular fistula (VF) and compared them with patients with Hirschsprung disease (HSCR), where aganglionic bowel is the main pathology. Altered Shh and GLI1 (Shh target) expression in PF and VF patients supported abnormal Shh pathway involvement in ARMs.

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