

Dear students and colleagues,

It is my pleasure to welcome you to the 63rd Student Scientific Conference and to introduce this year's collection of abstracts. Building on the successes of last year, we continue to develop the conference as a platform that reflects both the scientific ambitions and the international character of our faculty.

Last year's decision to adopt English as the primary conference language proved highly beneficial, strengthening our integration into the global academic environment and supporting the growing presence of international master's and doctoral students, as well as the increasingly multicultural composition of our research teams. This positive experience has encouraged us to maintain the same language policy this year.

We are also pleased to introduce several enhancements to this year's program. As a new tradition, the conference opens with a lecture by one of last year's award recipients, who will share their perspective "one year later"— offering valuable insight into how their research and academic journey have progressed since their winning presentation. In the spirit of recognizing excellence, we will again award prizes for the three best presentations in both the master's and doctoral sections. We also continue the tradition of the Prof. Vladislav Třeška Prize for outstanding work in the field of surgery, and the Audience Award, chosen through online voting via QR code. New this year is a competition for the best photo related to your project, giving students the opportunity to showcase the aesthetic and creative aspects of their research and to present science in a visually compelling way.

I would like to express my sincere gratitude to all supervisors, members of the evaluation committees, and everyone involved in organizing the conference. Your long term commitment, energy, and support are what give the Student Scientific Conference its distinctive atmosphere, sense of community, and enduring quality.

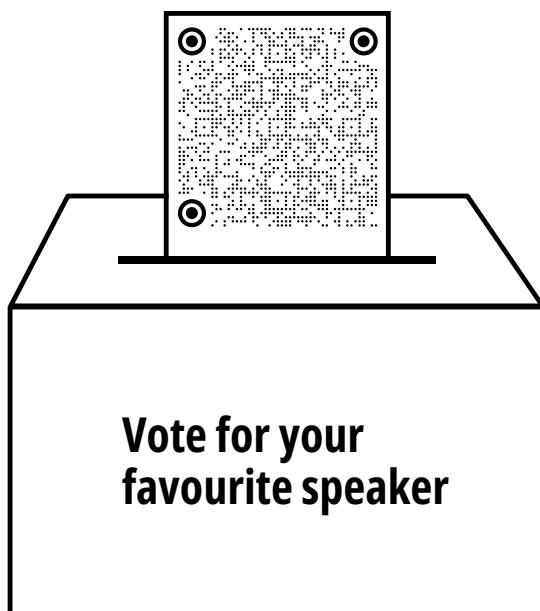
I wish all participants an inspiring and successful 63rd Student Scientific Conference, and I look forward to witnessing the scientific curiosity, innovation, and enthusiasm that each of you brings to this event.

prof. PharmDr. Radek Kučera, Ph.D.
vice-dean for doctoral study





ABSTRACTS
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The organizers reserve the right to change the program.





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MSP - Master Study Program

DSP - Doctoral Study Program

Azure lecture hall

08:30 **OPENING**

08:40 A lecture by last year's winner Eliška Jandová: Hide and seek: overcoming antigen escape in hematological malignancies

16:30 **ANNOUNCEMENT OF RESULTS**

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Comparative Study of Adult Hippocampal Neurogenesis in Lurcher and Wild-Type Mice

Running title: Neurogenesis in Adult Mice Hippocampus

Authors: Matěj Zvoneček (1), Parvathi Satheesh (1), Nilpawan Roy Choudhury (1), Jan Cendelín (1, 2), Jan Tůma (1)

Supervisor: Jan Tůma (1)

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State-of-the-Art: Cerebellar degenerations are characterized by motor deficits including ataxia, hypotonia, and nystagmus, but they are also associated with cognitive, affective, and sensory impairments. Lurcher mice (Lc) represent a well-established model of cerebellar degeneration caused by a mutation in the Grid2 gene encoding the GluR δ 2 receptor subunit, leading to progressive loss of Purkinje cells and other cerebellar neurons. Behavioral and molecular data also suggest that the hippocampus may be altered in these mice. In this study, we investigate hippocampal neurogenesis in Lc mice. Since adult hippocampal neurogenesis plays an important role in learning, memory, and brain plasticity, alterations in this process may contribute to the cognitive deficits associated with cerebellar degeneration.

Objective: The aim of this study is to quantify the rate of adult hippocampal neurogenesis in wild-type (WT) and Lc mice. Considering the reduced learning capability in Lc mice, we hypothesize that the rate of neurogenesis differs in adult hippocampus between Lc and WT mice.

Material and Methods: Adult Lc mice were used as a model of cerebellar degeneration and compared with WT mice. Brains from Lc (n = 3) and WT (n = 3) mice were sectioned on a microtome in 30 μ m sections, mounted on slides and immunostained for doublecortin (DCX), a marker of immature neurons, together with DAPI nuclear staining. Sections were imaged using confocal microscopy, and z-stack images were analyzed in Fiji software. DCX-positive immature neurons were systematically counted in the dentate gyrus of the hippocampus. All image analyses were performed as a blind study. The data were evaluated using permutational t-test with 10,000 permutations.

Results & Discussion: Preliminary analysis of Lc and WT mice revealed no significant difference between the groups. These findings suggest that the cognitive deficit observed in Lc mice in behavioral tests, such as the Morris water maze, is unlikely to be caused by alterations in hippocampal neurogenesis. Moreover, we have previously demonstrated that this cognitive deficit is not associated with activation of the hypothalamic–pituitary–adrenal axis. Therefore, other mechanisms may be involved, such as maladaptive strategies due to behavioral disinhibition or motor impairment. The optional photo captures newly formed neurons (cells stained in red) in dentate gyrus of adult mouse hippocampus. IHC staining and imaging author - Parvathi Satheesh.

Conclusion: The current study found no significant difference in hippocampal neurogenesis between Lc and WT mice. However, additional brain samples will be analyzed to increase the sample size and assesses other markers of brain plasticity.

Funding: This work was supported by the AZV grant No. NW26-08-00119, SVV262774, Cooperatio (NEUR and MED/DIAG research areas), and GAUK project No. 70124.

Gallbladder Cancer in Bohemia Exhibits a Distinct Clinicopathologic Profile Enriched in Certain Tumor Types Compared to Other Populations

Running title: Bohemian Gallbladder Carcinoma Profile

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Supervisor: Marián Švajdler (1,2)

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State-of-the-Art: The incidence of gallbladder cancer (GBC) exhibits marked geographic heterogeneity worldwide. High GBC rates are well-documented among Chilean indigenous populations, where the disease is primarily linked to cholelithiasis and obesity. Additional hotspots are recognised in Japan (associated with pancreatobiliary maljunction and biliary reflux) and India (linked to *S. typhi* infection and heavy metal exposure).

However, an unusually high incidence is also observed in the Bohemian region of Central Europe, where etiological factors remain poorly defined, and data on the incidence of GBC subtypes remain largely unavailable.

Objective: This study aimed to investigate the distinct clinicopathologic features of GBC cases from Bohemia compared to other global cohorts.

Material and Methods: A total of 102 GBC cases diagnosed at Bioptická Laboratory (Plzeň, Czech Republic) were analysed and compared with 606 cases from Chile, the USA, and Turkey.

The comparative analysis focused on a comprehensive clinicopathologic characterisation, including tumor types and precursor lesions.

Results & Discussion: 1. Hyalinizing Cholecystitis: More prevalent in the Bohemian cohort (18.6%, 19/102) compared to others (5.9%, 36/606; $p < 0.00001$) (Fig 1a). These cases might be associated with radon exposure.

2. Poorly Cohesive Carcinomas (PCCs): Represented 22.5% (23/102) of cases in Bohemia vs. 3.8% (24/606) in other populations ($p < 0.0001$). Bohemian PCCs exhibited a striking „invisible carcinoma“ pattern, resembling mammary lobular ca. with preserved mucosa and a linitis plastica-like appearance, unlike the pleomorphic/destructive PCCs observed in other cohorts (Fig 1b).

3. Intracholecystic Papillary-Tubular Neoplasm (ICPN)-associated carcinoma: Observed in 14% of Bohemian cases versus 6% in other populations (Fig 1c), suggesting an alternative chemical/reflux-based etiology.

Conclusion: The Bohemian GBC cohort shows a distinct landscape enriched in hyalinizing cholecystitis, diffuse-type PCC, and ICPN-associated GBC. These findings suggest unique pathogenetic pathways in this population, offering a novel model to explore underrecognized biliary carcinogenesis.

Identification of Sexually Dimorphic Anatomical Features in Tricentennial Femora: An Osteometric Study

Running title: Whose femora were these?

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State-of-the-Art: Forensic osteometry of mass burial assemblages provides a non-invasive framework for reconstructing population composition, demographic structure. The Arthron R package was used to provide a reference for population-calibrated discriminant functions that translate continuous skeletal measurements into probabilistic sex, ethnicity and age estimates.

Objective: We aim to evaluate all discernible anatomical features of the 380-year-old femora in order to estimate the sex and age category of the individuals to whom these bones belonged.

Material and Methods: Departmental femora purchased from Stahl.cz (stěhovací služba, spol. s r.o.), with data enabling cross-referral to a Swedish mass grave assemblage in Bohemia (ante 1648), were assessed for sex, age, and skeletal morphometry using manual osteometric measurements and automated 3D landmark detection. The arthron R package reference dataset provided population-level benchmarks. Variables recorded were femoral head diameter (FHD), subtrochanteric diameter (SubD), vertical femoral head diameter (VD-FH), intertrochanteric crest width (IT Crest), linea aspera prominence, neck-shaft angle (NSA), shaft length, and shaft contour, to determine sex and approximate age of the remains.

Results & Discussion: Sex was estimated by two independent observers (Cohen's $\kappa = 0.811$; 18/20 agreement). Of the studied specimens, the cohort comprised males, females and juveniles (60%, 30%, and 10%). Female femora showed femoral head diameter ratio of 0.91 (83.3 ± 9.8 mm in females versus 91.6 ± 8.5 mm in males; $p = 0.083$), shaft length ratio 0.93 ($p = 0.034$), intertrochanteric crest ratio 0.89 ($p = 0.050$), NSA 105° – 125° (mean $115^\circ \pm 7.1^\circ$). Only one femur showed a distal shaft fracture consistent with low-energy axial loading. No specimen exhibited cortical entry defects, stellate fracture patterns, or lead deformation traces of musket-ball injuries or other incapacitating injuries. The statistical significance seems underpowered owing to the small samples obtained from historical context.

Conclusion: The heterogeneous sex composition, the representation of adult females, the inclusion of juvenile individuals, and indications of weapon-related trauma collectively render the assemblage inconsistent with an interpretation as a burial of military combatants or physically depleted.

Genes with generally higher activity across multiple cell populations and tissues contain fewer upstream start and stop codons in their leader sequences

Running title: Genes higher activity fewer upstream starts stops

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Supervisor: *Pavel Dvořák (1, 2)*

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State-of-the-Art: The 5' untranslated region (5' UTR), also called leader sequence, regulates translation by shaping how ribosomes bind and scan mRNA. Its structures—such as hairpins, stemloops, or G-quadruplexes—can slow or enhance initiation. Functional motifs like IRES, TOP sequences, or Kozak elements further tune translation efficiency. Many 5' UTRs also contain upstream AUGs, uORFs, and stop codons that modulate ribosome scanning and influence expression of the main coding sequence.

Objective: The aim was to test the hypothesis that genes that have different numbers of upstream start and stop codons in their 5' UTR regions also have specifically different expression at the protein level, either in general or in specific tissues.

Material and Methods: 1) Downloading sequences of 5'UTR regions of all human protein-coding genes from the Ensembl database in FASTA format. 2) Selection of Group 1 - genes in whose sequences there are no upstream start or stop codons. 3) Selection of Groups 2 and 3 - genes whose 5'UTRs are approximately the same length as Group 1 (i.e. in the range of 120 to 400 bp) and contain fewer (i.e. 1 to 3) and more motifs of interest (i.e. 6 and more), respectively. 4) Downloading data on protein expression in tissues from The Human Protein Atlas database. 5) Assignment of protein expression data to genes from Groups 1, 2 and 3. 6) Statistical comparison of selected data and interpretation of results.

Results & Discussion: Statistical comparison of the three tested gene groups showed that the individual groups differ significantly in the level of expression assessed using the IHC technique (categories „Not detected“, „Low“, „Medium“ and „High“) as well as in expression in different cell types and tissues ($\chi^2 = 217.4$, $p \approx 3.7 \times 10^{-41}$). The most striking difference is the behavior of Group 2, which differs from the others mainly in the higher representation of „Medium“ and „High“ expression levels. At the „High“ level, all three pairwise differences between the groups were statistically significant. In contrast, Group 1 has a dominant level of „Not detected“ in most tissues and cell types. Group 3 can be characterized as in between Groups 1 and 2 with more extremities.

Conclusion: Group 1 (no upstream start and/or stop codons) represents genes with low or no expression; Group 2 (1 to 3 upstream starts and/or stops) genes with higher activity and significant variability; Group 3 (6 or more upstream starts and/or stops) is in between, but with clear tissue specializations.

Funding: Supported by the project „Integration of biomedical research and health care in the Pilsen metropolitan area“ (reg. no. CZ.02.01.01/00/23_021/0008828) - co-funded by the European Regional Development Fund, European Union, and State Budget of the Czech Republic.

The Interplay Between YAP, Sox2, and the Hippo Signaling Pathway in Human Fibrosarcoma Stem-Like Cells

Running title: YAP and Sox2 in Fibrosarcoma Stem Cells

Authors: Adam Gryč (1), Matěj Laub (1), Martin Prchal (1)

Supervisor: Jiří Hatina (1)

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State-of-the-Art: Fibrosarcoma is an uncommon but highly aggressive malignant tumor. HT1080 is a permanent human fibrosarcoma cell line frequently used to model complex fibrosarcoma biology. According to the cancer stem cell (CSC) hypothesis, tumor aggressiveness and drug resistance are sustained by a minority subpopulation of undifferentiated stem-like cells. There are many approaches how to identify putative CSCs, such as side population or aldehyde dehydrogenase – based cell sorting, selection of anchorage-independently growing cells, using of specific cell surface markers or fluorescent reporter constructs, such as the YAP-activated mCherry used in our study.

Objective: This study investigates YAP oncogenic activity in HT1080 fibrosarcoma cells in relation to the expression of a battery of stemness-related genes and anchorage independent clonogenicity as a canonical stemness-related trait.

Material and Methods: We used HT1080 cells expressing an mCherry reporter driven by a YAP-responsive promoter. Cells were cultured adherently with serum. Loss of contact inhibition was assessed via fluorescence microscopy in confluent cultures. Cells were then sorted (FACS) into mCherry-high (YAP-active) and mCherry-low subpopulations for two purposes: 1) evaluating clonogenicity in methylcellulose with serum (anchorage-independent conditions), and 2) isolating RNA for subsequent qRT-PCR of stemness-related genes. These include Sox2, ABCG2, ZFP57, IGF2 and ALDH1A1.

Results & Discussion: Fluorescence microscopy revealed that YAP activity in HT1080 cells does not undergo contact inhibition. Even in fully confluent cultures, a significant proportion of cells remained strongly mCherry-positive, indicating sustained YAP activation. In anchorage-independent assays, the YAP-active (mCherry-high) subpopulation exhibited a significantly higher capacity to form spherical colonies compared to mCherry-low cells. Although final quantification of specific genes is ongoing, the overall qPCR gene expression profile strongly indicates that mCherry-high cells possess a pronounced stem-like character.

Conclusion: The Hippo pathway acts as a tumor suppressor, degrading YAP upon cell contact (contact inhibition). When dysregulated, YAP accumulates and drives downstream targets like CTGF, promoting tumor aggressiveness, and the CSC phenotype.

Cerebellar and hippocampal morphology in a mouse model of spinocerebellar ataxia type 1

Running title: Cerebellar and hippocampal morphology in SCA1 mice

Authors: *Patricie Klossé (1), Jan Látal (1), Štěpán Kápl (1), Nilpawan Roy Choudhury (1), Parvathi Satheesh (1)*

Supervisor: *Jan Cendelín (1)*

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State-of-the-Art: Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant cerebellar ataxia caused by expanded CAG repeat in Ataxin 1 gene. Besides adult-onset progressive cerebellar degeneration resulting in cerebellar motor and cognitive affective syndromes, there are also changes in the hippocampus contributing to pathological cognitive and behavioral phenotypes. The disease leads to severe disability of the patients and even premature death. SCA1 mice have been used as a knock-in model of this disease for about 20 years. They have been considered replicating development and symptoms of SCA1 with well-described severe neurological and neuropathological manifestations in advanced age. However, in the last two years, the phenotype appears to be alleviating.

Objective: The aim of the study was to verify presence of cerebellar and hippocampal neuropathology SCA1 mice.

Material and Methods: Paraformaldehyde-fixed brains of heterozygous SCA1 mice aged 7-8 months and strain- and age-matched healthy control mice were cryosliced and stained with hematoxylin-eosin. The volumes of the hippocampus (dentate gyrus, pyramidal layer and stratum oriens) and the cerebellum (total volume and molecular layer separately) were estimated using Cavalieri principle. Heterozygous mice and mutation non-carriers were distinguished by genotyping identifying presence of normal length allele as well as extended allele, or normal length allele only, respectively.

Results & Discussion: We did not find any significant reduction in the volume of individual parts of the hippocampus or the cerebellum as a whole or its molecular layer selectively in SCA1 mice. It is in accordance with our previous observation of non-increased density of glial markers in the hippocampus of SCA1 mice. However, it is in contrast with marked cerebellar and hippocampal neuropathology described earlier in our laboratory in mice from the same colony even at a younger age. It suggests that reduction of pathological functional phenotypes during several years is accompanied by alleviation of neuropathology, although the mutation itself persists.

Conclusion: The results suggest instability of phenotype of SCA1 mice. Elucidation of the mechanisms of this phenomenon requires sequencing of the allele, examination of its expression, searching for potential disease-modifying gene mutations, and more detailed analysis of the phenotype.

Funding: The work was supported by Cooperatio (NEUR, DIAG) and SVV 262774.

Enhancing cognitive performance in the first minutes after napping through gamma binaural beat stimulation

Running title: Gamma binaural beats and sleep inertia

Authors: *Natália Poláková (1)*

Supervisor: Karel Blahna (1)

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State-of-the-Art: Sleep inertia is a transitional state occurring immediately after awakening, characterized by reduced alertness, slower reaction times, impaired cognitive performance, and increased subjective sleepiness. These effects can persist for several minutes and may negatively influence activities that require rapid decision-making and sustained attention. Various approaches have been investigated to mitigate sleep inertia and accelerate the recovery of cognitive functioning. Auditory stimulation using binaural beats has been shown to influence brain activity and cognitive processes, with gamma-frequency stimulation being associated with increased cortical activation and attention-related processes.

Objective: The aim of this pilot study was to investigate whether gamma-frequency (40 Hz) binaural beat stimulation can influence vigilance and cognitive performance during the early post-nap period characterized by sleep inertia.

Material and Methods: Ten healthy participants aged 18–30 took part in the study. The experiment was conducted over two testing days in a within-subject design: one day without auditory stimulation (control) and one day with 40 Hz binaural beat stimulation. Participants completed a 30-minute nap, followed immediately by a 10-minute Psychomotor Vigilance Task (PVT) assessing reaction time and sustained attention. After the task, subjective sleepiness was evaluated using the Stanford Sleepiness Scale (SSS) and the Karolinska Sleepiness Scale (KSS). Reaction time data were analyzed using vigilance-related metrics reflecting temporal performance dynamics, including optimal-performance episodes and the AUC_{good} metric. Linear mixed-effects models were used for statistical comparison.

Results & Discussion: Preliminary analyses revealed differences in the temporal dynamics of vigilance between the stimulation and control conditions. The overall aggregated vigilance energy metric (AUC_{good}) did not show a statistically significant effect of binaural beat stimulation. However, exploratory analyses of performance across the task suggested differences in the temporal structure of vigilance between conditions, indicating that the stimulation may influence short-term vigilance dynamics during the post-nap period. These findings suggest that while the overall level of vigilance did not significantly differ between conditions, binaural beat stimulation may affect how vigilance fluctuates over time.

Conclusion: This pilot study found no significant overall improvement in vigilance after gamma-frequency binaural beat stimulation during the early post-nap period. However, differences in vigilance dynamics suggest subtle effects that should be examined in future studies with larger samples.

Quantitative Evaluation of Skeletal Muscle Structure in a TFAM-Deficient Mouse Model of Mitochondrial Myopathy

Running title: Tfam knock-out mice muscle morphometry

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State-of-the-Art: Mitochondrial myopathies cause progressive skeletal-muscle weakness and remodeling, but linking genotype to quantitative tissue changes remains challenging. Muscle-specific disruption of mitochondrial transcription factor A (Tfam) is an established model of mtDNA depletion and myopathy. Recent studies have proposed reproducible, TFAM-associated remodeling patterns in skeletal muscle. Nevertheless, these reports rely largely on qualitative evaluation of histological sections and lack quantitative assessment of the analyzed material. This prompted us to verify these reported changes and determine whether the observed differences are consistent in the available mice with mitochondrial disease or reflect inter-individual biological variability.

Objective: The aim of this pilot study was to perform a quantitative analysis of histological sections of the quadriceps muscle in TFAM-deficient and wild-type control mice. For this purpose, design-based stereological approaches were applied to analyze images obtained from the tissue sections.

Material and Methods: Fifteen male mice were included in the study. Six animals served as healthy controls, while nine had mitochondrial myopathy induced by skeletal muscle-specific disruption of the Tfam-gene. At six months, animals were euthanized and the quadriceps femoris muscle was dissected for histological evaluation. Samples were fixed in formaldehyde, routinely processed, and sectioned in random orientation. Sections were stained with hematoxylin and eosin (H&E) and green trichrome. Quantitative analysis was performed using the Cavalieri method in Steriologer 11 software. The following parameters were assessed: fascicle area, myofibril number and volume fractions, and the volume fractions of connective tissue and myonuclei.

Results & Discussion: Histological examination of Tfam mice revealed a higher number of blood vessels of varying diameters compared with WT controls. Quantitative analysis showed a mild 3.5% increase in myofibril area fraction (WT $79.9 \pm 12.7\%$; KO $82.7 \pm 1.5\%$). The total connective tissue area increased by 22% (WT $2.5 \pm 0.875 \text{ mm}^2$; KO $3.12 \pm 1.599 \text{ mm}^2$), although this difference was likely influenced by variation in sample size distribution. In contrast, the connective tissue area fraction per 1 mm^2 slightly decreased (WT $0.041 \pm 0.009 \text{ mm}^2/1 \text{ mm}^2$; KO $0.038 \pm 0.009 \text{ mm}^2/1 \text{ mm}^2$). The overall myofibril amount remained within biological variability, while the myonuclear volume fraction decreased by 16% (WT $0.019 \pm 0.006 \text{ mm}^2/1 \text{ mm}^2$; KO $0.016 \pm 0.003 \text{ mm}^2/1 \text{ mm}^2$).

Conclusion: Tfam mice showed increased vascularity compared with WT. However, no significant differences in major morphometric parameters were detected. These findings suggest that more advanced methods and biochemical or organelle-level analyses are needed. Trichrome staining may improve structural assessment.

Funding: Cooperatio Program, research areas MED/DIAG.

Assessing Muscle-to-Muscle Augmentation and Impairment in the Brachium Using Anatomage Virtual Dissection and OpenSim Simulation

Running title: Morphometric Virtual Dissection of the Brachium

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State-of-the-Art: Computational musculoskeletal modelling of the upper limb has relied mainly on Delp's upper extremity (1995) and Holzbaur's (2005) models, and their cadaveric derivatives, to assess many parameters. These parameters include force-generating capacity, pennation geometry, and moment arm profiles. We propose an Anatomage-based model that evaluates inter-individual and inter-sex variation in brachial muscle architecture.

Objective: We aim to determine which muscular neighbourhood configuration most can indicate augmentation and functional impairment patterns within the anterior and posterior brachial compartments.

Material and Methods: First, morphometric measurements of bilateral muscle belly radii and lengths for biceps brachii, brachialis, triceps, coracobrachialis, and anconeus were retrieved from Anatomage data, stratified by sex and laterality. Second, these measurements were exported into OpenSim 4.5 software program to estimate maximum isometric force at a specific tension, inverse kinematics, static optimisation, and musculature performance of the brachium.

Results & Discussion: Maximum isometric forces in the anterior compartment revealed a bilateral asymmetry. The brachialis demonstrated an 8.31-fold right-to-left force asymmetry with a right optimal fibre length. In the posterior compartment, the triceps produced identical forces bilaterally, with a greater optimal fibre lengths of 0.241 m on the right. The coracobrachialis demonstrated a 3.13-fold right-to-left force differential. The anconeus showed moderate bilateral asymmetry. The biceps-brachialis pairing exhibited the greatest combined force asymmetry. All tonicity readings characterise co-augmentation on the right side.

Conclusion: Anatomage virtual dissection provided morphometric fidelity sufficient to reveal clinically and biomechanically meaningful inter-limb and inter-sex asymmetry in the brachial muscle.

Characterization of Stem-Like and Differentiated Urothelial Carcinoma Cells Using GFP/FACS Separation and Transcriptomic Profiling

Running title: Characterization of Bladder Cancer Stem-Like Cells

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State-of-the-Art: Invasive urothelial carcinoma of the bladder is treated with radical cystectomy combined with neoadjuvant cisplatin-based chemotherapy to improve survival or adjuvant chemotherapy for high-risk patients. Despite these treatments, local recurrence and metastasis remain common and clinically significant. Recurrence is driven by tumor cell heterogeneity, which includes chemosensitive differentiated cells and stem-like, chemoresistant cells. This intrinsic heterogeneity limits efficacy of chemotherapy, and stem-like cells are associated with aggressive, high-grade tumors. These findings highlight the need to characterize the molecular and genetic profiles of stem-like cells as a basis for potential targeted therapies.

Objective: This study aimed to characterize stem-like and well-differentiated bladder cancer cells using a stem-cell-specific GFP reporter and fluorescence-activated cell sorting (FACS), and subsequently analyze gene expression profiles of the sorted populations.

Material and Methods: Urothelial carcinoma cell lines derived from both low-grade and high-grade tumors were included and maintained under standard culture conditions.

To distinguish stem-like from differentiated tumor cell populations, cells were labeled with a doxorubicin-responsive GFP reporter and physically separated using FACS into GFP-positive (GFP⁺) and GFP^{low} fractions. The stem-like GFP pattern was validated by mutually exclusive GFP and CK-14 expression.

Transcriptomic profiling was performed using DNA microarray analysis to assess global gene expression patterns and identify genes associated with each cell population.

Subsequent immunofluorescence analysis was performed using a primary antibody against the pluripotency factor DPPA3 (Stella) and a secondary TRITC-conjugated antibody.

Results & Discussion: Putative stem cells show constitutive ABC-efflux pump activity, preventing doxorubicin-activated GFP expression; GFP^{low} cells therefore exhibited stem-like characteristics, whereas GFP⁺ cells represented differentiated populations. Upon doxorubicin treatment, GFP⁺ cells were mostly eliminated, while GFP^{low} cells survived, expanded, and partially differentiated, restoring the original GFP pattern and demonstrating stem-like plasticity.

Transcriptomic analysis revealed higher DPPA3 expression in GFP^{low} cells and higher GRHL3 expression in GFP⁺ cells, suggesting DPPA3 as a stemness marker. Future work will quantify DPPA3 expression in both populations by RT-qPCR to clarify its role in stemness and plasticity. This may aid strategies to overcome chemoresistance and limit tumor relapse.

Conclusion: We successfully distinguished stem-like (GFP^{low}) and differentiated (GFP⁺) bladder cancer cells. Microarray analysis identified distinct gene expression signatures in each population, including DPPA3 in GFP^{low} cells and GRHL3 in GFP⁺ cells, suggesting targets for preventing tumor recurrence.

Funding: This work was supported by the Specific University Research program: SVV 261773.

Automatic AI-powered analysis of experimental and clinical progression of urothelial carcinoma based on cell and nuclear morphometry

Running title: AI-Powered Analysis of Urocarcinoma Progression

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State-of-the-Art: The progression of bladder cancer, both in clinic and experimental, is characterized by substantial morphological alterations, with tumor cells transitioning from early, well-differentiated states to advanced, poorly differentiated, and invasive phenotypes. Conventional histopathological grading predominantly rely on subjective visual assessment performed by pathologists. Recent advances in digital pathology and AI-based image analysis offer new opportunities to integrate objective and quantitative metrics into morphological evaluation. Establishing a robust link between traditional qualitative assessment and quantitative morphological characterization is therefore critical for improving diagnostic accuracy, reproducibility, and the broader clinical implementation of morphometric data.

Objective: The aim of this study is to develop and validate an objective, AI-driven methodology for the morphometric analysis of bladder cancer cells in experimental and clinical progression. This approach is evaluated using both theoretical models and clinical samples to assess its general applicability.

Material and Methods: Our experimental model comprised five human bladder cancer cell lines: RT4, RT112, RT112H, RT112D21, and UMUC-3. RT4 and RT112 represent low grade urothelial carcinoma, RT112H represents grade- and potentially also stage-progression, RT112D21 is high-grade chemoresistant derivative of RT112H, and UMUC-3 corresponds to high-grade advanced stage cancer cell line. The clinical model comprised bladder carcinoma pathological slides and urine cytologies of tumours of defined grades and stages; samples were stained with Papanicolaou and hematoxylin-eosin (H&E) and analyzed from microscopic images and whole-slide scans. Image processing and quantitative analysis of morphological parameters were performed in QuPath with AI-based advanced cell detection based on custom Python script and Cellpose.

Results & Discussion: Our complex and robust approach allows for highly accurate cell detection and morphometric measurements, even within the complex microenvironment of clinical samples. The resulting objective data revealed clear differences in morphometric parameters among cancer cell lines representing distinct levels of differentiation and aggressiveness. Quantifiable cellular morphological profiles were also found to correlate reliably with tumor grading and staging of clinical samples. Our findings thus demonstrate that this advanced and optimized AI-based detection approach can be successfully applied not only in controlled in vitro environments using established cell lines but is also fully functional and applicable to real-world solid tumor samples derived from clinical practice.

Conclusion: The integration of QuPath with advanced AI-based cell detection provides a robust

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platform for objective morphometric analysis of bladder cancer cells. Quantitative morphometric profiling can thus significantly contribute to accurate pathological diagnosis in both experimental and clinical setting.

Funding: This study was supported by SVV-2025-260773 and SVV-2026-206773, Academic Migrant 4EU+ MA/25/F1/0/018 and by the League Against Cancer Prague.

Immunoexpression and Amplification of MDM2 gene as a Potential Modifier of Behavior in PLAG1::LIFR-Fused Salivary Gland Tumors (SGT)

Running title: MDM2 in PLAG1::LIFR-Fused SGTs

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State-of-the-Art: Recently, immunohistochemical (IHC) expression of MDM2, including MDM2 amplification, was proposed to play a role in malignant progression in the group of HMGA2-altered pleomorphic adenomas. The MDM2 is associated with tumor aggressiveness in various neoplasms, often through amplification but also via protein overexpression. Its clinicopathologic relevance in PLAG1::LIFR fusion-positive SGTs has not been yet systematically investigated.

Objective: To explore the potential impact of recurrent MDM2 expression and amplification on tumor behavior in different subtypes of PLAG1::LIFR fusion-positive SGTs.

Material and Methods: A retrospective search in the authors' registry identified 12 cases of SGTs with PLAG1::LIFR fusion. All tumors were stained for anti-MDM2 antibodies. All slides were scanned and IHC positivity was assessed using semi-quantitative scoring system, incorporating both nuclear staining intensity and the extend of positive tumor nuclei.

Results & Discussion: 12 PLAG1::LIFR-fused SGTs were analyzed (2 males and 10 females; ages 35-79). The anatomical distribution showed a predominance of tumors arising in palate (7/12), followed by the parotid gland (2/12), submandibular gland (2/12) and jaw (1/12). Diagnoses varied, including pleomorphic adenoma (PA) and its variants (n=5), myoepithelioma (n=1) and malignant myoepithelial neoplasms (n=6). Weak immunopositivity was seen in four tumors comprising both benign and malignant SGTs. Moderate immunoexpression was detected in four cases, predominantly in malignant myoepithelial tumors. Negative staining was observed in three cases including benign PA-related tumors and one carcinoma ex-PA. One case was not analyzed due to insufficient material.

Conclusion: MDM2 IHC was expressed in eight PLAG1::LIFR-fused SGTs, while FISH did not reveal a MDM2 gene amplification in any case. These findings suggest that MDM2 alterations do not play a significant role in malignant progression, and that the PLAG1::LIFR fusion itself is the major oncogenic driver.

Diagnostic Significance of HRAS gene mutation in Salivary Gland Tumors: A Histological, Molecular and Immunohistochemical Correlation

Running title: Significance of HRAS gene in SGTs

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State-of-the-Art: HRAS gene mutation has been discovered as primary driver event in epithelial-myoepithelial carcinoma (EMC). We propose that HRAS-driven salivary neoplasia is not confined to EMC, but may possibly relate to multiple lines of ductal and acinar differentiation in Salivary Gland Tumors (SGTs).

Objective: SGTs show tumor type-specific recurrent fusions and/or mutations. HRAS mutation was defined as a diagnostic marker of EMC. We aimed to (a) examine the specificity of HRAS mutation in EMC and (b) correlate an HRAS gene mutation to immunohistochemical (IHC) expression.

Material and Methods: A retrospective search in the authors' registry identified 16 cases of SGTs with HRAS mutation. All tumors were stained for anti-RAS Q61R antibodies. All slides were scanned and IHC positivity was assessed.

Results & Discussion: 16 HRAS-mutated SGTs were analyzed (9 males and 7 females; age 43–92). Most arose in parotid gland (N=15); one involved a lymph node with metastatic salivary ductal carcinoma (SDC). All cases showed HRAS codon 182A mutation on next generation sequencing (NGS). RAS Q61R was positive in 13 cases (81%). Two major phenotypes of SGTs were encountered: biphasic ductal–myoepithelial neoplasms diagnosed as EMC (N=10) and apocrine ductal carcinomas with tubular/cribriform and solid architecture characterized by androgen receptor-positive and myoepithelial markers-negative, diagnosed as SDC (N=4). Two additional cases showed variable phenotypes, including metastatic squamous cell carcinoma (mSCC) and intraductal carcinoma (IDC) arising in sclerosing polycystic adenoma (SPA).

Conclusion: RAS Q61R was expressed in 13 HRAS-mutated carcinomas including strong staining in 9/10 cases of EMC, mild or focal staining in all four SDCs, while IDC ex SPA and mSCC were negative. Our short series compiled immunophenotypic and genotypic assessment of HRAS, which was strongly correlated.

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Salivary Gland-like Tumors (SGT) of the Breast: Histopathologic and Genetic features

Running title: Salivary Gland-like Tumors of the Breast

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State-of-the-Art: There are several special types of breast carcinomas that share morphologic, immunophenotypic, and molecular genetic features of salivary gland tumors (SGT). These breast carcinomas are typically negative for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu), referred to as “triple-negative,” yet generally have a much better prognosis than triple-negative breast carcinomas of no special type.

Objective: To discuss the histologic, immunophenotypic, molecular, and clinical features of salivary gland-like carcinomas of the breast.

Material and Methods: A retrospective search in the authors’ registry identified 71 cases of SGT of the breast. Most of the tumors were stained for ER, PR, and HER-2/neu. All slides were scanned, and IHC positivity was assessed. Molecular testing was performed in 28 cases using next-generation sequencing (NGS) and/or fluorescence in situ hybridization (FISH).

Results & Discussion: 50 SGTs of breast were analyzed (3 males and 47 females; age 3-87). All tested cases were triple negative (ER/PR and HER2/neu). Three major phenotypes of SGTs of the breast were encountered: adenoid cystic carcinoma (AdCC) (n = 17), secretory carcinoma (SC) (n = 21), and mucoepidermoid carcinoma (n = 1), characterized by MYB::NFIB, ETV6::NTRK3, and CRTC1::MAML2 gene fusion, respectively. The other SGT of the breast included acinic cell carcinoma (AcicC) (n = 2), polymorphous adenocarcinoma (n = 2), and myoepithelial carcinoma (n = 7).

Conclusion: Salivary gland-like breast tumors represent a subset of triple-negative breast carcinomas. Identifying characteristic genetic alterations and/or immunohistochemical surrogates in these tumors has practical applications for establishing an accurate diagnosis and directing clinical management.

Evaluation of the therapeutic potential of lipophosphonoxins DR-6180 & DR-7072 in biodegradable nanofibrous carrier systems for treatment of murine skin wounds experimentally infected with *S. aureus*

Running title: Lipophosphonoxin in Nanofibers for *S. aureus* Wounds

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State-of-the-Art: The presence of infection in wounds is a common cause of complications of healing and in most cases antimicrobial therapy is necessary. In view of the development of resistance, it is desirable to develop new antimicrobial agents useful for the control of localised bacterial infections of soft tissues. Lipophosphonoxins (LPPOs), unique molecules synthesized at the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, appear to be a very promising group of agents suitable for such an indication. The aim of the study was to test the efficacy of LPPOs (molecules DR-6180 and DR-7072) in a biodegradable nanofibrous sandwich carrier system consisting of PCL and PVA layers for antimicrobial therapy of the wounds experimentally infected with *Staphylococcus aureus*.

Objective: To evaluate the antimicrobial efficacy of lipophosphonoxins DR-6180 and DR-7072 incorporated in biodegradable nanofibrous carrier systems for the treatment of murine skin wounds experimentally infected with *Staphylococcus aureus*.

Material and Methods: An *in vivo* murine wound infection model was used. Male CB6F1 mice immunosuppressed with cyclophosphamide were inoculated with *Staphylococcus aureus* in two full-thickness dorsal skin wounds. After infection establishment, wounds were covered with experimental biodegradable nanofibrous dressings consisting of PCL/PVA layers incorporating lipophosphonoxins DR-6180 or DR-7072. Control groups included nanofibers without active compound and standard antimicrobial treatment. Wounds were covered with an occlusive membrane dressing. Treatment efficacy was evaluated after four days using microbiological examination of wound tissue and visual wound scoring assessing infection severity and healing

Results & Discussion: Both tested lipophosphonoxins demonstrated antimicrobial activity against *S. aureus* in infected wounds. The biodegradable PCL/PVA nanofibrous sandwich structure proved to be a suitable carrier system enabling local delivery of the compounds. In several cases, substantial reduction of bacterial burden and improvement of wound healing were observed. However, variability in treatment efficacy was noted, suggesting that the current concentration of lipophosphonoxins in the nanofibrous dressing may be close to the minimal effective dose. These findings indicate that optimization of drug loading and release kinetics will likely improve therapeutic performance

Conclusion: Lipophosphonoxins incorporated in biodegradable nanofibrous dressings show

promising antimicrobial activity in a murine model of *S. aureus* wound infection. Further optimization of drug concentration and release properties is required to enhance therapeutic efficacy

HPLC-Guided Purification of Antibacterial Metabolites from UV-Stressed *Agaricus bisporus*

Running title: Mushroom Bioactive Compounds

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State-of-the-Art: Ultraviolet (UV) irradiation modulates the metabolisms of certain mushrooms by inducing oxidative stress, which in turn stimulates the production of secondary „stress-response“ metabolites, such as of ergosterol into Vitamin D₂, or anti-oxidants like flavinoids and ascorbic acid. Although this exposure to UV radiation is known to enhance antioxidant capacities and alter the metabolite profiles of many mushrooms, the isolation of UV-induced compounds with antibacterial properties in *Agaricus bisporus* remains limited. In this study, an HPLC-guided bioassay fractionation approach was employed in order to purify metabolites from UV-stressed *Agaricus bisporus* mushrooms, which were then screened for their antibacterial activity.

Objective: To purify and concentrate the bioactive metabolites of *Agaricus bisporus* responsible for antibacterial activity against Gram-positive and Gram-negative bacteria.

Material and Methods: For this study, we developed a method of extracting and purifying antibacterial compounds from UV-stressed *Agaricus bisporus* through an HPLC system. Mushroom samples were acquired through AGARICUS, spol s.r.o. (Czech Republic), which were then washed, lyophilized, and stored at -80°C. 1 gram of sample was dissolved in 15ml of distilled water along with 0,1 mM PMSF. The sample was then sonicated for 30 seconds, followed by 30 seconds of rest on ice. After sonication, the sample was centrifuged at 10 000 RPM for 20 minutes at 4°C. Debris was removed and the supernatant collected. Prior to HPLC, the sample was ultrafiltrated using a Amicon Ultra 3 kDa filter. A C18 reversed-phase column was used to separate the bioactive compound from the *Agaricus bisporus* extract.

Results & Discussion: HPLC purification of the aqueous extract derived from UV-stressed *Agaricus bisporus* allowed for the collection of multiple fractions, each of which were individually evaluated for antibacterial activity. One of these fractions exhibited significant inhibitory activity against *E. coli* and *S. aureus*, while the other fractions showed none. The zone of inhibition of this active fraction is equivalent to that of the pre-HPLC purified extract, about 11mm, indicating successful isolation of its antibacterial metabolites. The metabolite's activity is broad spectrum, acting against both Gram positive and negative bacteria, and should further be tested against clinical isolates. Further goals are to determine its minimum inhibitory concentration, and to characterise the HPLC-purified compound.

Conclusion: In conclusion, this study demonstrates that HPLC-guided bioassay fractionation has enabled us to identify and purify the antibacterial metabolites of UV-stressed *Agaricus bisporus*. Future work will be focused on the characterisation of the HPLC-purified molecule using NMR and mass spectrometry.

Susceptible but Not Eradicated: Persistence in Low-MIC OXA-48-Producing *Escherichia coli* and *Klebsiella pneumoniae*

Running title: Persistence in Low-MIC OXA-48 isolates

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State-of-the-Art: Bacterial persistence represents a reversible phenotypic state in which a small subpopulation of cells survives antibiotic exposure without apparent resistance mechanisms. Unlike resistant mutants, persister cells remain genetically susceptible but enter a transient dormant or slow-growing state that reduces the efficacy of antibiotics targeting active cellular processes. This phenomenon is particularly relevant in chronic and relapsing infections, where persisters can repopulate the niche once antimicrobial pressure is removed.

Objective: The aim of the study was to identify low-MIC carbapenemase-producing isolates, particularly those harboring OXA-48-like enzymes, and to assess their persistence levels.

Material and Methods: The detection of bacterial persisters was performed using the Tolerance Disk (TD) test, a modified version of the standard disk diffusion assay. In this method, bacteria are first exposed to an antibiotic disk (meropenem or amikacin) under routine susceptibility testing conditions. Following overnight incubation, susceptibility is evaluated, and the assay is continued only with isolates categorized as susceptible (S) or susceptible, increased exposure (I). In these cases, the antibiotic disk is replaced with a glucose disk. In the presence of persister cells, glucose supplementation induces metabolic resuscitation, resulting in visible colony growth within the original inhibition zone.

Results & Discussion: Total 185 bacterial strains were screened, of which 141 were susceptible to at least one of the antibiotics - meropenem and/or amikacin. In 49 strains, we detected persistent subpopulations, with strains susceptible to meropenem showing persistent subpopulations in 34.8% of cases, while for amikacin it was less than 1%. Our study shows that a significant proportion of antibiotic-susceptible clinical isolates exhibit persistent subpopulations, despite the absence of inherited resistance mechanisms. Persistence was significantly more common in meropenem-susceptible strains than in amikacin-susceptible strains, suggesting that the induction or survival of persistent cells may depend on the class of antibiotic and its mechanism of action.

Conclusion: Persistence in OXA-48-producing *E. coli* and *K. pneumoniae* is clinically relevant, especially during carbapenem therapy and relapse risk. Distinguishing persistence from resistance and understanding its mechanisms is essential to improve treatment and prevent recurrent infections.

Neuron-Specific Enolase as a Prognostic Marker in Patients with Metastatic Castration-Resistant Prostate Cancer Treated with Androgen Receptor Signaling Inhibitors

Running title: NSE in mCRPC patients

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State-of-the-Art: Prostate cancer is the most commonly diagnosed cancer in men. Its progressive form, metastatic castration-resistant prostate cancer (mCRPC), is characterized by resistance to androgen-deprivation therapy. Androgen receptor signaling inhibitors (ARSIs) are used in this advanced stage, and the response to treatment varies according to the biology of the tumor. Currently, the most widely used prognostic marker is prostatic-specific antigen (PSA). However, its role in patients with mCRPC is limited. NSE is an enzyme produced as a result of neuroendocrine differentiation of prostate cancer and may therefore play a significant role in predicting the behavior of mCRPC.

Objective: The aim of this retrospective study was to evaluate the prognostic significance of serum NSE at baseline and its early on-treatment dynamics in patients with mCRPC treated with ARSI.

Material and Methods: We retrospectively studied ARSI-treated mCRPC patients with assessed baseline serum NSE (n=120). Radiographic progression-free survival (rPFS) and overall survival (OS) were estimated with Kaplan–Meier curves and compared using the log-rank test. Multivariable Cox models adjusted for age, baseline PSA, therapy line, Gleason score, metastasis timing, and visceral involvement. NSE dynamics were assessed at a 28-day landmark in patients with baseline and week-4 measurements, modeled as $\log_2(\text{NSE}_{4w}/\text{NSE}_0)$ per doubling (rPFS n=47; OS n=48).

Results & Discussion: The median follow-up duration was 44.7 months (95% CI 35.7–48.3). Baseline NSE levels above the upper limit of normal (ULN) were associated with shorter rPFS (9.4 vs 20.8 months) and overall survival (OS; 23.5 vs 38.2 months). In the multivariable analysis, baseline NSE >ULN remained an independent predictor of poor survival outcomes (rPFS HR 1.81, 95% CI 1.15–2.85, p=0.011; OS HR 1.85, 95% CI 1.12–3.05, p=0.017).

Conclusion: The results of this study suggest that the serum NSE levels are associated with treatment outcomes in patients with mCRPC undergoing ARSI therapy.

Altered inhibitory killer cell immunoglobulin-like receptors expression on peripheral NK cells in women with endometrioma: a case-control study

Running title: Endometriosis and NK cells

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State-of-the-Art: Natural killer (NK) cells play a crucial role in immune surveillance and clearance of ectopic endometrial cells. Dysregulation of inhibitory killer cell immunoglobulin-like receptors (KIRs) has been implicated in endometriosis, but data on their surface expression in well-defined disease phenotypes remain limited.

Objective: The aim of this study was to characterise the expression of KIRs on peripheral blood NK cells in women with endometrioma and to compare their distribution with that in healthy controls.

Material and Methods: This case-control observational study included 23 treatment-naïve women with surgically and histologically confirmed endometrioma and 23 age-matched healthy controls. Peripheral blood mononuclear cells were analysed by multiparametric flow cytometry using two KIR-specific antibody panels. NK cells were defined as CD45⁺CD3⁻CD56⁺ lymphocytes, and the expression of inhibitory KIR receptors was quantified. Correlations between clinical parameters and KIR expression were assessed to evaluate potential confounding effects.

Results & Discussion: The proportion of NK cells within the lymphocyte compartment did not differ between groups. However, women with endometrioma exhibited a significantly reduced proportion of lymphocytes and a selective enrichment of KIR2DL2⁺ and KIR2DL5⁺ NK-cell subsets compared with controls. No significant associations were identified between demographic or gynaecologic variables and KIR receptor expression.

Conclusion: Women with endometrioma exhibit a distinct inhibitory KIR profile on peripheral NK cells, with increased KIR2DL2⁺ and KIR2DL5⁺ subsets. These findings suggest altered NK-cell inhibition contributes to systemic immune dysregulation in endometriosis and identify KIR2DL5 as a potential research target.

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Evaluation of early ECHO parameters in patients with intermediate-high risk acute pulmonary embolism treated with catheter-directed thrombolysis or standard anticoagulation

Running title: ECHO findings in intermediate-high risk PE

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State-of-the-Art: Acute pulmonary embolism (PE) is a potentially life-threatening condition and represents one of the leading causes of cardiovascular mortality. Intermediate-high risk pulmonary embolism is characterised by hemodynamic stability in the presence of right ventricular dysfunction, elevated cardiac biomarkers and class III-V according to the PESI (Pulmonary Embolism Severity Index) score or sPESI (simplified PESI) ≥ 1 . The first-line therapy is anticoagulation. Among newer therapeutic approaches is catheter-directed thrombolysis (CDT), which enables local administration of a thrombolytic agent.

Objective: To compare the effect of CDT versus standard anticoagulation therapy by assessing selected echocardiographic parameters at 24 hours after randomisation.

Material and Methods: A retrospective analysis was performed on 30 patients hospitalised for acute intermediate-high risk PE. This analysis constitutes part of the larger PRAGUE-26 study. PRAGUE-26 is a multicentre, randomised, open-label, actively controlled trial designed to evaluate clinical outcomes in patients with acute intermediate-high risk PE undergoing CDT vs. standard anticoagulation therapy. The CDT was performed using the Cragg-McNamara valved infusion catheter. Follow-up focused on the development of selected echocardiographic parameters (RV diameter, RV/LV ratio, TAPSE, s'TDI, and sPAP) at 24 hours after randomisation to either the standard anticoagulation or the CDT. Statistical analysis was conducted using the nonparametric Mann-Whitney U test.

Results & Discussion: Baseline echocardiographic parameters did not differ significantly between the groups. At 24 hours, comparison of outcomes demonstrated significant improvement in TAPSE ($+4.6 \pm 3.6$ mm vs. $+1.8 \pm 2.4$ mm, $p = 0.014$) and sPAP (-14.3 ± 8.2 mmHg vs. -3.8 ± 7.9 mmHg, $p = 0.004$) in the CDT group compared with the standard anticoagulation. Other parameters (RV diameter and RV/LV ratio) showed a trend toward greater improvement in the CDT group; however, these differences did not reach statistical significance (all $p > 0.05$). No statistically significant improvement or trend was observed for s'TDI in the CDT group. No significant correlation was found between the BMI or the selected comorbidities and the treatment response in either group.

Conclusion: Significant improvement in TAPSE and sPAP, along with a trend toward greater improvement in RV diameter and RV/LV ratio, suggests a promising potential for the routine use of CDT therapy in acute intermediate-high risk PE. Further large-scale prospective research is required.

Preprocedural Comparison of Left Atrial Volume by 2D and 3D Echocardiography in Patients with Atrial Fibrillation Undergoing Pulsed-Field Ablation

Running title: 2D vs 3D LA volume in AF before PFA

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State-of-the-Art: Atrial fibrillation (AF) is the most common sustained arrhythmia and is associated with atrial remodeling and left atrial (LA) enlargement. LA volume (LAV) and indexed LA volume (LAVI) are clinically relevant markers used in patient characteristics and outcome assessment in rhythm-control strategies, including catheter ablation. Three-dimensional (3D) echocardiography is often considered more accurate than conventional two-dimensional (2D) methods; however, real-world measurements may vary depending on acquisition quality and rhythm during imaging.

Objective: To compare LAV and LAVI obtained by 2D TTE using Simpson's method and by 3D TTE in AF patients evaluated prior to pulsed-field ablation (PFA; catheter ablation using pulsed electric fields), and to assess whether left ventricular ejection fraction (LVEF) differs between 2D and 3D measurements.

Material and Methods: We performed a single-center observational analysis of 58 consecutive AF patients undergoing TTE as part of the pre-procedural work-up before PFA between 03/2023 and 09/2025. Examinations were performed using a Philips EPIQ 7 ultrasound system. LAV and LAVI were measured by both 2D Simpson's method and 3D methods during the same exam. For categorical comparison, an absolute difference of ≤ 5 mL between 2D and 3D LAV was considered clinically negligible ("agreement"); differences > 5 mL were categorized as "2D higher" or "3D higher". Descriptive statistics were used. Paired 2D vs 3D comparisons were performed using a two-sided Wilcoxon signed-rank test.

Results & Discussion: The cohort included 58 patients (62.1% male) with a mean age of 64.8 ± 9.6 years. Hypertension was present in 57.9%, dyslipidemia in 49.1%, type 2 diabetes in 10.5%, and dysglycemia in 10.5%. Mean 2D LAV was 84.3 ± 23.4 mL, whereas mean 3D LAV was 71.7 ± 23.3 mL, with an average difference (3D – 2D) of -12.6 ± 16.0 mL; this 2D vs 3D difference was statistically significant ($p < 0.001$). In relative terms, 2D LAV was 17.6% higher than 3D LAV. Using the predefined 5 mL threshold, 2D exceeded 3D in 67.2%, agreement was observed in 20.7%, and 3D exceeded 2D in 12.1%. Contrary to the commonly expected tendency toward larger LA volumes with 3D TTE, our cohort demonstrated a reverse trend. LVEF assessed by 2D and 3D methods did not differ significantly.

Conclusion: In AF patients assessed prior to PFA, 2D TTE using Simpson's method yielded higher LAV estimates than 3D in the majority of cases. These findings highlight the importance of standardized acquisition and analysis when comparing 2D and 3D LAV. LVEF values derived from 2D and 3D TTE were comparable.

Analysis of clinical parameters and risk stratification in patients with pulmonary embolism

Running title: Risk Stratification and BMI Paradox in PE

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State-of-the-Art: Risk stratification of patients with pulmonary embolism (PE) is crucial for selecting the optimal therapeutic strategy. The Pulmonary Embolism Severity Index (PESI) is an established tool for assessing 30-day mortality risk across different patient populations. However, clinical stability defined by PESI may not always reflect the underlying hemodynamic stress. Integration of cardiac biomarkers and imaging methods is therefore essential to refine risk assessment, particularly in hemodynamically stable patients with potential right ventricular (RV) dysfunction.

Objective: The aim of this study was to evaluate the demographic, clinical, and laboratory characteristics of the patient group and to analyse the relationships between Pulmonary Embolism Severity Index (PESI) scores, biomarkers, and the presence of RV dilatation.

Material and Methods: A retrospective analysis included 203 patients with PE hospitalised at the Department of Cardiology, University Hospital Pilsen. We evaluated PESI scores, cardiac biomarker levels (hsTnT, NT-proBNP, D-dimer), and RV dilatation on CT and echocardiography. Statistical analysis was performed using non-parametric tests (Spearman correlation, Mann-Whitney U test, Kruskal-Wallis analysis) and Cohen's kappa (κ) to assess the agreement between imaging modalities.

Results & Discussion: Mean age was 65.9 ± 17.9 years, with a mean BMI of 30.8 ± 7.1 kg/m². The largest group consisted of intermediate-high risk patients (56.2%). A significant negative correlation was found between BMI and PESI score ($\rho = -0.252$; $p < 0.001$), being strongest in low ($\rho = -0.670$; $p = 0.004$) and intermediate-low ($\rho = -0.625$; $p < 0.001$) groups. hsTnT levels were significantly associated with RV dilatation (median 72 vs 19.5 ng/l; $p < 0.001$) and served as a predictor of in-hospital mortality ($p = 0.033$). High agreement for RV dilatation was found between CT and echocardiography ($\kappa = 0.893$). Notably, despite comparable PESI scores (mean 101.6 ± 23.2 vs 100.1 ± 31.0), a fundamental difference in RV dilatation prevalence exists between intermediate-high and intermediate-low groups (99.0% vs 2.3%; $p < 0.001$).

Conclusion: The registry confirms the current ESC scheme's validity. A negative BMI-PESI correlation suggests an obesity paradox. Significant PESI limitations in intermediate-risk groups show necessity of biomarker and echo assessment for the accurate identification of patients at higher risk of mortality.

Quantitative Cerebrospinal Fluid Cytology: A Comparison of Leukocyte Differentiation Using the Fuchs-Rosenthal Chamber Versus Permanent Cytological Preparation

Running title: Methods for CSF Leukocyte Differentiation

Authors: *Veronika Pechová (1), Pavel Brož (1), Simona Kukrálová (1), Klára Koldušková (1), Eliška Naušová (1), Daniel Rajdl (1)*

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State-of-the-Art: Cerebrospinal fluid (CSF) examination plays an indispensable role in diagnosing diseases of the central nervous system. Accurate quantitative cytology is essential for appropriate clinical decision-making; however, this examination remains highly dependent on the expertise of trained personnel. Furthermore, routine laboratory practice lacks rigorously validated procedures for standardizing comparisons between basic leukocyte differentiation performed in a Fuchs-Rosenthal (F-R) chamber and differentiation using permanent cytological preparations (CP). Addressing this methodological gap is important for improving diagnostic reliability.

Objective: The primary aim was to compare leukocyte differentiation in a F-R chamber with that obtained from stained permanent CP. The secondary aim was to assess inter-rater agreement between an experienced laboratory professional and a trained medical student.

Material and Methods: The study included CSF samples from 145 patients aged 10 days to 84 years at the Faculty Hospital in Pilsen. Manual cell counting and differentiation were performed using the F-R chamber for all samples per routine protocols. Subsequently, permanent CP were prepared from the same samples and stained using May-Grünwald-Giemsa staining. Leukocyte differentiation was then evaluated from the CP.

In a subset of 83 samples, leukocyte differentiation was independently evaluated by an experienced professional and a trained medical student. Statistical analysis was performed using MedCalc software (Shapiro-Wilk, Wilcoxon signed-rank test, and Bland-Altman plots). Cohen's kappa was used to evaluate the agreement for oligocytosis vs. pleocytosis classification. Significance was set at $p < 0.05$.

Results & Discussion: Statistically significant differences were observed between leukocyte differentiation in the F-R chamber and permanent CP. For mononuclear cells (MNC), median (min-max) was 4 (1-724)/ μL in the F-R chamber vs. 4 (1-1356)/ μL in CP ($p = 0.004$). For polymorphonuclear cells (PMNC), the values were 0 (0-6400)/ μL in the F-R chamber vs. 0 (0-5471)/ μL in CP ($p < 0.001$).

In the subset evaluated by both raters, significant differences were found between the professional and the student: MNC 7 (1-1356)/ μL vs. 7 (1-793)/ μL ($p = 0.016$) and PMNC 0 (0-5471)/ μL vs. 0 (0-6034)/ μL ($p = 0.002$).

Cohen's kappa showed excellent agreement in the classification of oligocytosis vs. pleocytosis between the F-R chamber and the student ($\kappa = 0.95$) as well as between the professional and the student ($\kappa = 0.95$).

Conclusion: Leukocyte differentiation by the student significantly differed from both the F-R chamber and the professional. However, from a clinical perspective, agreement between raters remained excellent, and the classification of oligocytosis vs. pleocytosis was highly consistent.

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search area Medical Diagnostics and Basic Medical Sciences.

β-Blocker administration in Septic Shock: A Retrospective Study of Hemodynamic Safety and Clinical Outcomes

Running title: β-Blocker administration in Septic Shock

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State-of-the-Art: Septic shock is a life-threatening complication of sepsis characterized by microcirculatory dysfunction and hemodynamic instability, including persistent hypotension, tachycardia, and sympathetic overactivation despite adequate fluid resuscitation. β-blocker therapy has been proposed as an adjunctive treatment to counteract the hyperadrenergic state. β₁-receptor blockade reduces heart rate, myocardial contractility, and oxygen consumption, improving cardiac efficiency. Studies also suggest β-blockers may reduce cytokine release, limit hypermetabolism, and protect against catecholamine-induced myocardial toxicity. However, evidence regarding their immunomodulatory effects and safety in septic shock remains inconclusive, requiring further investigation in critically ill patients.

Objective: To evaluate the hemodynamic safety of β-blocker therapy in septic shock and assess its potential immunomodulatory effects through analysis of inflammatory markers.

Material and Methods: A retrospective observational study included 50 adult patients with septic shock admitted to the ICU in 2024. Patients were stratified by β-blockers exposure to those receiving parenteral cardio selective B-blockers (BB group) and those without, β-blockers (NB group). Cardio selective agents included atenolol, acebutolol, bisoprolol, esmolol, and metoprolol, used in patients with persistent tachycardia despite standard management. Clinical and laboratory data were obtained from electronic medical records. Hemodynamic parameters were recorded daily during the first seven days after diagnosis.

Comorbidities, interventions, and 30- and 90-day mortality were also documented. Continuous variables were expressed as mean ± SEM or median with quartiles, and group differences were presented in graphs.

Results & Discussion: Heart rate decreased progressively during the 7-day observation period. In the NB group, mean HR declined from approximately 90 bpm on day 1 to about 72 bpm by days 6-7, while patients receiving β-blockers maintained more stable values ranging between 78-87 bpm without clinically significant bradycardia. Systolic blood pressure increased modestly in both groups, while diastolic pressure remained relatively stable around 58-61 mmHg throughout the observation period. No episodes of treatment-related hypotension were observed. Electrolyte levels and markers of organ function remained within acceptable ranges. These findings suggest that the benefits of B-Blocker therapy primarily relate to cardiovascular and hemodynamic modulation rather than direct suppression of systemic inflammatory activity.

Conclusion: β-blocker therapy in septic shock was well tolerated and effectively reduced heart rate without compromising arterial pressure or systemic perfusion. These findings support β-blockers as a safe adjunctive therapy to improve cardiovascular stability in critically ill patients.

Creation of genetic pedigrees in patients with hereditary angioedema

Running title: Genetic pedigrees in hereditary angioedema

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State-of-the-Art: Hereditary angioedema (HAE) is a rare genetic disorder characterized by recurrent attacks of pale, non-itchy swelling of subcutaneous tissue and mucous membranes. The most common cause of hereditary angioedema is deficiency or dysfunction of C1 inhibitor (C1-INH), leading to excessive bradykinin production. Bradykinin contributes to increased vascular permeability and angioedema development by acting on B2 receptors on surface of endothelial cells and smooth muscle cells of blood vessels. Angioedema attacks cause significant discomfort to patients and, depending on location, may have a serious clinical course. The Department of Immunology and Allergology at the University Hospital in Pilsen is one of four specialized centers for the diagnosis and treatment of HAE in the Czech Republic.

Objective: The aim of this study is to create family trees of patients with HAE using retrospective data and medical history. It tracks disease in family lines and summarizes the diagnostic and therapeutic approach, including patient care organization at the Pilsen center.

Material and Methods: The diagnosis of hereditary angioedema is based on a combination of typical clinical presentation and laboratory findings (changes in C4 complement levels, C1 inhibitor levels and functional activity). Molecular genetic testing focused primarily on the SERPING1 gene (and other genes in rare forms) is used to confirm the diagnosis and specify the genetic basis.

The work is presented retrospectively in the form of case studies based on the analysis of medical records and targeted family history. The data obtained were used to compile family trees in the draw.io tool, which graphically illustrate the occurrence of the disease in family lines and describe the diagnostic and therapeutic procedure for selected patients at the University Hospital in Pilsen.

Results & Discussion: Based on targeted family history, detailed multigenerational family trees were compiled in draw.io for two families of patients with HAE. The graphical representation confirmed autosomal dominant inheritance with both sexes affected across generations. Analysis of the family trees showed that the disease occurs in at least three consecutive generations in both families.

Conclusion: The occurrence of HAE was mapped in two families from analysis of medical history and available laboratory data. This visualization of family lines can serve as a basis for further analysis of clinical data from medical records and for observed families to streamline long-term dispensary care.

The impact of obesity on birth injuries

Running title: Obesity and Birth Injury

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State-of-the-Art: Birth injury refers to tissue damage in the perineal, vaginal, or cervical areas caused by extreme pressure and stretching during the passage of the fetus through the birth canal. This may involve spontaneous tearing of the skin and muscles or a controlled surgical incision performed by a physician. The number of obese parturients is rising continually, hence the increased interest in the effect of BMI on labor outcome. Large cohort studies have suggested obesity as a protective factor for maternal perineal injury. As this conclusion is rather surprising and comes from countries with a different obstetric care, we wanted to find out whether it is valid also among Czech parturients that delivered in our institution.

Objective: The objective of this study is to test the hypothesis that women with a higher BMI have a significantly reduced risk of sustaining severe birth injuries compared to ones with normal weight. This study focuses on whether BMI can be considered a protective factor for child-birth trauma in our setting.

Material and Methods: The methodology employed a retrospective cohort analysis, comparing a group of parturient women divided by BMI into a normal weight group (BMI under 25) and an obese group (BMI over 30). The study included all women who gave birth at term in the department of Gynecology and Obstetrics, Faculty of Medicine in Pilsen between 1/2023-12/2025 the necessary data was extracted from clinical records and the hospital information system. Monitored parameters included age, parity, delivery mechanism, and BMI. Furthermore, the analysis accounted for the duration of labor, neonatal gender, and specific interventions, such as oxytocin administration for labor augmentation, mode of labor induction, vaginal birth after cesarean (VBAC), or operative deliveries using vacuum extraction (VEX) and forceps.

Results & Discussion: The results of the analysis demonstrated that parturient women with a normal body weight (BMI under 25) show a statistically higher probability of delivery without perineal injury compared to obese patients (39,4 vs 32,3%, $p=0,0002$). While a higher overall incidence of ruptures was recorded in the group with a BMI over 30, the data did not demonstrate an increased risk of the most severe injuries involving the anal sphincter (rpt. per III.st. 1,5 vs 1,2 %, $p=0,5677$).

Conclusion: The final analysis shows that a normal BMI represents a significant protective factor in reducing the risk of birth injuries. In obese women, a higher incidence of ruptures was demonstrated. These results are in contradiction to previously published data from different countries.

Optimizing Dosage of the Maurten Bicarbonate System for Peak Performance in Hyrox-Specific FTP Testing

Running title: Optimizing Dosage of the Maurten Bicarb System

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State-of-the-Art: Sodium bicarbonate (NaHCO_3) is a well-known ergogenic aid, but its effectiveness is often limited by gastrointestinal side effects. Traditional soda is long-established but reportedly fails to reach an effective ergogenic threshold in approximately half of all cases. The Maurten Bicarb System is a newer hydrogel-based delivery system designed to minimize these side effects. However, data on its absorption kinetics compared to traditional soda is still limited, necessitating pilot testing to establish baseline dosing protocols for future performance research.

Objective: The primary aim of this pilot study was to track individual venous blood bicarbonate (HCO_3^-) changes at 1 and 2 hours post-ingestion to determine how many subjects reach the ergogenic threshold (increase of ≥ 5 mmol/L) using both delivery methods. This pilot serves as a foundation for a future study.

Material and Methods: Four male subjects (aged 26–53) participated in this crossover pilot. In two separate sessions, they received a dose of 0.25 g/kg of NaHCO_3 via either the Maurten Bicarb System or traditional soda. Acid-base balance parameters were measured from venous blood samples at baseline (T0), 1 hour (T1), and 2 hours (T2) post-ingestion.

Results & Discussion: In the pilot study, blood bicarbonate (HCO_3^-) levels were tracked across eight observations (4 subjects, each testing both systems). The results demonstrated significant inter-individual variability: under the Maurten Bicarb System, two subjects reached the ergogenic threshold of +5 mmol/L by the 2-hour mark, while the other two peaked at +4.2 mmol/L. Similarly, when using traditional sodium bicarbonate, two subjects successfully crossed the +5 mmol/L threshold, while the remaining two showed lower increases. These findings indicate that a standard weight-based dose of 0.25 g/kg results in an effective ergogenic blood profile in only 50% of cases within the initial two-hour window, regardless of the delivery method.

Conclusion: Both the Maurten Bicarb System and traditional soda showed a 50% success rate (2 out of 4 subjects) in reaching the ergogenic threshold within the first two hours. These results highlight the high inter-individual variability in bicarb kinetics and underscore the necessity of our planned research.

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Single-cell Transcriptomics Reveals a Pro-coagulant B-cell Phenotype and ERO1A-driven Metabolic Remodelling in Metastatic Lung Adenocarcinoma

Running title: B-cell Coagulation and ERO1A in LUAD

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State-of-the-Art: Lung adenocarcinoma (LUAD) metastasis is a complex process driven by the interplay between tumour cells and the immune microenvironment. While cancer-associated hypercoagulability is a known facilitator of systemic spread, the specific role of the adaptive immune system—particularly B cells—in modulating haemostatic pathways remains poorly characterized. Most studies focus on platelet-driven coagulation, leaving a gap in our understanding of how immune cells actively contribute to pro-thrombotic states within the tumour role. Furthermore, metabolic adaptations, such as those regulated by ERO1A, are essential for tumour cell survival during dissemination. Identifying these cell-specific signatures is essential for developing targeted therapies to intercept the metastatic cascade.

Objective: The aim is to characterize the transcriptomic landscape of B cells and epithelial cells in primary versus metastatic LUAD, with a focus on the expression of coagulation-related markers (S100A1, PF4) and metabolic drivers (ERO1A, ADGRL2).

Material and Methods: We analysed single-cell RNA sequencing (scRNA-seq) datasets from primary and metastatic LUAD. Data integration and batch effect removal were performed using the Harmony package in R. To ensure statistical robustness and account for biological variation between samples, we employed a pseudo bulk differential expression analysis. Raw counts were aggregated by sample and cell type, and differentially expressed genes (DEGs) were identified using a Quasi-Likelihood F-test via the edgeR package. Functional enrichment was conducted using MSigDB Hallmark 2020 and Oncogenic Signatures to identify pathway-level shifts associated with metastasis.

Results & Discussion: Pseudobulk analysis shows the Coagulation hallmark is enriched in B cells from metastatic sites versus primary tumours. This is driven by S100A1 and PF4 upregulation. PF4 expression in B cells suggests a specialized immune-haemostatic role, potentially facilitating tumour-cell-platelet aggregates that shield metastatic cells. In the epithelial compartment, ERO1A is upregulated DEG ($\{\log_{2}FC\} > 1.5$), correlating with Hypoxia and Glycolysis enrichment. This indicates metabolic reprogramming for metastatic adaptation. Conversely, ADGRL2 expression decreased during progression. Together, these results provide evidence for a coordinated shift where B cells adopt a pro-coagulant phenotype alongside epithelial metabolic remodelling potentially promoting LUAD dissemination.

Conclusion: We identified a novel pro-coagulant B-cell signature (S100A1+/PF4+) and confirmed ERO1A and ADGRL2 as key markers of metastatic remodelling in LUAD which had not previously been discussed. These findings highlight the immune-coagulation interface as a potential therapeutic target.

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Diazepam treatment and stress reactivity in Lurcher mouse, a model of cerebellar motor and cognitive affective syndrome

Running title: Treatment of stress reactivity in Lurcher mice

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State-of-the-Art: The cerebellum is involved in non-motor functions, beyond motor coordination. Lurcher mice are one of the best models for studying cerebellar ataxia and behavioral and cognitive impairments resulting from selective olivocerebellar degeneration. Altered behavior in these mice is hypothesized to be caused in part by stress-induced behavioral disinhibition. Such behavioral traits potentially impair the performance not only in behavioral but also in cognitive and motor tests and abnormal stress response might lead to a different stress-related gene expression profile in Lurcher mice. We have hypothesized that pharmacological suppression of anxiety may improve performance on cognitive and motor tests. However, low diazepam doses (0.5 & 1.0 mg/kg) were not effective in Lurcher mice.

Objective: This study aimed to investigate whether a higher dose of Diazepam could influence stress-related gene expression profile, reduce signs of behavioral disinhibition, and thereby improve pathological behavioral phenotypes in Lurcher mice.

Material and Methods: Lurcher and Wild-type B6CBA mice aged 4-5 months were used. Mice were treated with saline (control) or diazepam at a dose of 2 mg/kg, 30 mins prior to the tests. The tests were arranged in a three-week protocol that included the open-field test, elevated plus maze test, pre-pulse inhibition and startle response, grip strength measurement, Morris water maze test with a hidden and visible goal task, and rotarod test. To evaluate gene expression, brains were collected from Lurcher and Wild-type mice 60 minutes after exposure to a stressor, represented by 5 minutes of the forced swimming test, or from undisturbed naïve controls. For this experiment, the mice were also injected with diazepam (2.0 mg/kg) or saline.

Results & Discussion: Saline-treated Lurcher mice exhibited impaired behavior compared to Wild-type mice in the open field, grip strength, Morris water maze with a visual platform, and rotarod tests, and didn't show any significant differences compared to Wild-type mice in the elevated plus maze and pre-pulse inhibition test. The effect of diazepam was observed in a scattered manner across all tests. Gene expression analysis showed that stress exposure down-regulated *Avpr1* expression in the hippocampus of Wild-type mice, but not in Lurcher mice. While diazepam up-regulated *Crhr1* and *Avpr1* expression in the hippocampus of Wild-type mice, it down-regulated these genes in Lurcher mice. Diazepam upregulated *Crhr1* expression in the amygdala of Wild-type mice and downregulated *Gabra1* in the cortex of Lurcher mice.

Conclusion: The effect of diazepam was observed in a scattered manner with different tests. The higher dose of Diazepam treatment also didn't improve overall behavior in Lurcher mice. Stressor response and diazepam treatment were predominantly observed in the hippocampus.

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Preoperative inflammatory profiling and mitochondrial DNA copy number predict postoperative complications in colorectal cancer

Running title: Inflammatory and mitochondrial markers in CRC

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State-of-the-Art: Postoperative complications (POCs) after colorectal cancer (CRC) surgery remain a major clinical problem, as they may negatively affect recovery and overall patient outcomes. However, reliable preoperative biomarkers for identifying patients at higher risk of POCs are still lacking. Surgical trauma triggers a systemic inflammatory response and may also disrupt mitochondrial homeostasis. Therefore, inflammatory cytokines such as IL-6, IL-10, IL-1 β , TGF- β 1, and TNF- α , together with mitochondrial DNA copy number (mtDNA-CN), represent promising candidate biomarkers of systemic inflammation and mitochondrial stress. Early identification of patients at increased risk could improve perioperative risk stratification, postoperative monitoring, and individualized care.

Objective: The aim of this study was to evaluate perioperative changes in selected cytokines and mtDNA-CN in patients undergoing CRC surgery and to assess their potential, individually and in combination, for predicting POCs.

Material and Methods: This prospective study enrolled 75 patients with newly diagnosed CRC undergoing surgical resection. Based on predefined inclusion and exclusion criteria, relative mtDNA-CN was measured in whole blood collected preoperatively and ten days after surgery in a subset of 51 patients using multiplex qPCR. In a clinically homogeneous subcohort of 20 patients, plasma levels of IL-6, IL-10, TNF- α , IL-1 β , and TGF- β 1 were determined by ELISA. Statistical analyses included paired comparisons between time points and receiver operating characteristic (ROC) analyses to evaluate the predictive performance of individual biomarkers and their combinations for POCs. To account for multiple testing, p-values were adjusted using the false discovery rate (FDR) method and are reported as q-values.

Results & Discussion: Relative mtDNA-CN decreased significantly between the preoperative and postoperative time points ($p = 0.004$). Preoperatively, obese patients had higher mtDNA-CN and were also at greater risk of developing POCs ($p = 0.006$ and $p = 0.009$, respectively). After surgery, plasma levels of IL-6, IL-10, and TNF- α increased significantly, indicating a sustained postoperative inflammatory response ($q = 0.022$, $q = 0.034$, and $q = 0.003$, respectively). ROC analyses further showed that combined biomarker models integrating cytokines and mtDNA-CN had good discriminatory ability for identifying patients who developed POCs (AUC up to 0.91). Together, these findings support a link between mitochondrial alterations, postoperative inflammation, and the risk of POCs in CRC.

Conclusion: Combined assessment of inflammatory cytokines and mtDNA-CN may improve the identification of CRC patients at higher risk of POCs. However, further validation in larger cohorts is needed.

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Chromatographic separation of bioactive molecules from edible mushroom species and their evaluation for the anti-inflammatory potential using nitric oxide production in LPS-stimulated macrophages

Running title: Identification of mushroom bioactive compounds

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State-of-the-Art: In recent decades, bioactive molecules from natural sources have attracted increasing attention due to their health-promoting properties as alternatives to synthetic drugs. Various biological activities, including anti-inflammatory have been reported. Mushrooms represent a rich source of biologically active compounds such as peptides and polysaccharides with immunomodulatory potential. To isolate these molecules, chromatographic techniques are commonly used to separate complex mixtures into further fractions that can be screened for the pharmacological activity. To complement necessary information for further biomedical research, tested molecules are commonly analyzed by nuclear magnetic resonance (NMR) spectroscopy to characterize functional groups within the molecules.

Objective: The aim of this study was to optimize chromatographic separation of complex mixtures from edible mushroom species and evaluate fractions for their ability to modulate nitric oxide production in LPS-stimulated RAW 264.7 cells. The most promising fractions were further analyzed by NMR spectroscopy.

Material and Methods: Extracts from three edible mushroom species were prepared using Milli-Q water. Ultracentrifugation was used to separate molecules by their molecular weight. Two chromatographic methods, solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC), were then applied independently to fractionate the extracts. Murine RAW 264.7 macrophages were cultured as an in vitro inflammation model. Cells were stimulated with lipopolysaccharide (LPS) to induce nitric oxide (NO) production. Fractions from both methods were applied to LPS-stimulated cells, and nitrite levels in the supernatant were measured using the Griess reaction. This NO assay enabled comparison of fractionation efficiency and bioassay compatibility. Selected fractions were further analyzed by NMR spectroscopy.

Results & Discussion: RAW 264.7 macrophages were treated with fractions obtained from both SPE and HPLC methods. Results were compared with positive and negative controls. HPLC showed to be a non-invasive method compatible with the NO assay. Fractions showed different NO inhibition levels. Some fractions reduced nitric oxide production in activated macrophages up to 32 %, suggesting the presence of compounds that modulate inflammatory processes. Therefore, HPLC fractions were further subjected to NMR spectroscopy to identify the functional groups responsible for the observed activity.

Conclusion: Immunomodulatory differences among fractions suggest certain molecules reduce NO levels. Findings support mushroom-derived bioactive molecules as anti-inflammatory agents. Future work will focus on screening additional pharmacological effects of promising fractions.

Methylation Analysis of Sialdenoma Papilliferum of the Salivary Glands, and Syringocystadenoma Papilliferum and Tubular adenoma of the Skin: Are They Similar Entities?

Running title: SP, SCAP, TA: are they similar entities?

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State-of-the-Art: Sialadenoma papilliferum (SP) of the salivary gland, syringocystadenoma papilliferum (SCAP), and tubular adenoma (TA) of the skin are distinguished as separate entities. The pathogenesis of SP and SCAP, however, partially overlaps: the BRAF p.V600E mutation is consistently reported in SP and in a subset of SCAP. TAs carry mutations in the MAPK signalling pathway, affecting BRAF, KRAS, and HRAS genes. Despite their different locations, the morphology of these three lesions is comparable. They are characterised by exo-/endophytic squamous proliferation and inverted papillary/tubular/ductal proliferations, with a predominance of tubular growth in TA.

Objective: To investigate potential underlying biological similarities among sialadenoma papilliferum, syringocystadenoma papilliferum, and tubular adenoma of the skin by comparing their DNA methylation profiles and BRAF expression, using selected adnexal and follicular tumors as controls.

Material and Methods: We reviewed the authors' archives and selected three groups of tumors for study: cohort 1, comprising 14 cases of SP (including 4 recurrences); cohort 2, comprising 17 cases of SCAP; and cohort 3, comprising 15 cases of TA. All groups underwent classification analysis based on methylation profiles and were investigated for BRAF immunohistochemistry (IHC) using the VE1 antibody clone (ready-to-use, Ventana). The control cohort consisted of cylindromas (n=23), basal cell carcinomas of the skin (n=32) and trichoblastomas (n=22).

Results & Discussion: The methylation analysis revealed partially overlapping clustering among the predefined labels SCAP and TA categories, while SP was separated. Control cohorts were distinctly separated from each other and from the test groups. All tumor types showed BRAF IHC expression mostly in moderate to strong intensity.

Methylation profiles of SCAP and TA were not unambiguously separated indicating similarity of underlying biology, while the SP seem to be more distinguishable. The three tested entities are characterized by similar morphology with variable phenotype of subepithelial portion of the tumor with predominance of papillary architecture in SCAP, tubular architecture in TA and both in SP, however this was not recognizable in methylation analysis.

Conclusion: SCAP and TA showed overlapping methylation profiles, suggesting shared underlying biology, whereas SP was more distinct. Despite characteristic papillary, tubular, or mixed architecture on morphology, these structural differences were not reflected in methylation-based classification.

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From Hamartoma to Carcinoma: Molecular Genetic Landscape of Seromucinous and Respiratory Epithelial Adenomatoid Hamartomas as Precursor Lesions

Running title: Adenocarcinomas ex REAH/SH

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State-of-the-Art: Since the first identification of seromucinous hamartomas (SH) and respiratory epithelial adenomatoid hamartomas (REAH), there have been speculations about whether these entities truly represent hamartomas or if they are true preneoplastic lesions. The subsequent identification of an entity named atypical sinonasal glands arising in seromucinous hamartoma (ASGSH) led to multiple studies to assess their morphological, immunohistochemical and molecular genetic profile.

Objective: The aim of the study was to determine the correlation of ASGSH to several types of malignant sinonasal tumors and to further warrant the importance of the detection of ASGSH component in these tumors.

Material and Methods: Out of the authors' archives 69 cases of varying lesions were selected and subsequently subjected to histomorphological, immunohistochemical and molecular genetic analysis (Next Generation Sequencing RNA and DNA panel). The histomorphological evaluation focused mainly on the presence of SH, REAH and the true precancerous lesion ASGSH. The antibodies tested in the immunohistochemical analysis were among others SOX10, S100 protein, p63, p40, lysozyme and CK7. The correlation of all research methods was done subsequently.

Results & Discussion: A subset of tumors was found to show an apparent association with SH, REAH or ASGSH, suggesting a possible histogenetic relationship and raising the hypothesis that ASGSH may represent a precursor lesion in some cases. These findings indicate that certain tumors may arise in this setting although such a pathway is unlikely to be universal. In instances where ASGSH is not identified within the tumor mass, it may have been overgrown by the neoplasm or may persist only focally within the lesion. Careful histologic evaluation is therefore important to detect any preneoplastic component, even when present only in a limited area. Molecular findings indicate a heterogeneous genetic background that at present shows no consistent association with specific histologic or immunohistochemical features.

Conclusion: ASGSH may be a precursor to some sinonasal malignancies. Detection of ASGSH at tumor periphery indicates potential developmental relationship which highlights the diagnostic

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value of morphologic evaluation in head and neck tumors. When ASGSH can't be found, immunohistochemistry may prove useful.

Funding: This study was supported by study grant SVV 260652 from the Ministry of Education.

Spatial profiling and prognostic value of CD66+ neutrophils in colorectal cancer patients with synchronous and metachronous liver metastases

Running title: Spatial & prognostic analysis of CD66+ neutrophils

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State-of-the-Art: Colorectal cancer (CRC) is among the most common and lethal cancers globally. The development of distant metastases—either synchronous or metachronous—significantly worsens prognosis, with each type displaying distinct biological and clinicopathological features. Neutrophils have been shown to play dual roles in CRC progression, acting as either pro- or anti-tumor effectors.

Objective: This study investigated the spatial distribution and prognostic significance of neutrophils within primary tumor (pCRC) and paired liver metastases (LM).

Material and Methods: Formalin-fixed paraffin-embedded (FFPE) tissue sections from pCRC and paired synchronous (N=55) or metachronous (N=44) LM were analyzed. CD66+ neutrophils were detected via immunoperoxidase staining. Whole-slide images were analyzed using QuPath software to quantify cell densities across four spatial regions: tumor center (TC), inner margin (IM), outer margin (OM) and peritumor (PT) region of pCRC and LM. Cell densities were stratified by the 25th percentile into low and high groups. Prognostic associations with disease-free survival (DFS) after resection of LM in both groups and time to occurrence (TTO) of metachronous LM were assessed using Cox regression.

Results & Discussion: In LM, higher cell densities of CD66+ neutrophils were observed in IM and OM compared to TC and PT in both synchronous and metachronous groups. In pCRC, no regional differences were found in the synchronous group, whereas in the metachronous group, higher densities were found in TC, IM and OM compared to PT. Compared with pCRC, higher CD66+ cell densities were observed in the OM and PT of LM in both groups. Additionally, a higher cell density in TC of metachronous LM than that of synchronous LM was observed (Fig A).

A high IM/OM ratio in pCRC was associated with shorter TTO (Fig B). High neutrophil densities in OM and PT of synchronous LM and OM of metachronous pCRC predicted shorter DFS (Fig C,D,E), whereas high densities in TC of metachronous LM correlated with longer DFS (Fig F).

Conclusion: Neutrophils exhibited protumor prognostic associations in metachronous pCRC and synchronous LM, and anti-tumor associations in metachronous LM. These findings suggest that CD66 may serve as a potential biomarker for predicting metastatic progression and clinical outcomes in CRC.

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CRISPR/Cas9-mediated editing of the pathogenic CAG repeat in the ATXN1 gene for spinocerebellar ataxia type 1.

Running title: CRISPR Editing of ATXN1 CAG Repeat in SCA1

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State-of-the-Art: Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disorder characterized by cerebellar atrophy, progressive motor impairment, dysarthria, and cognitive decline. The disease is caused by an expanded CAG trinucleotide repeat in the ATXN1 gene, which produces a polyglutamine-expanded ataxin-1 protein that accumulates in neuronal cells and leads to cellular stress and neurodegeneration. Despite the well-defined genetic cause, there is currently no disease-modifying therapy for SCA1. CRISPR/Cas9 genome editing has emerged as a promising strategy for targeting pathogenic repeat expansions and correcting underlying genetic mutations. Precise targeting of the expanded CAG tract in ATXN1 could therefore represent a potential therapeutic approach for SCA1.

Objective: To develop and validate a CRISPR/Cas9 genome-editing strategy targeting the expanded CAG repeat in the ATXN1 gene and evaluate its ability to generate precise DNA breaks and enable removal of the pathogenic repeat in cellular models of SCA1.

Material and Methods: In vitro cellular models were established from SCA1^{Δ154Q/2Q} transgenic mice and wild-type controls, such as primary fibroblasts, embryonic neural stem cells (eNSCs), and eNSC-derived neurons. Candidate single-guide RNAs (sgRNAs) targeting sequences flanking the expanded CAG tract within the ATXN1 gene were designed and evaluated in silico for specificity. Four sgRNAs were selected for in vitro validation. CRISPR/Cas9 components were delivered into SCA1 fibroblasts and eNSCs as ribonucleoprotein (RNP) complexes and plasmids. The CRISPR machineries were delivered into the cells by chemical transfection and electroporation protocols, followed by genomic DNA isolation and analysis by cleavage assay to assess editing activity and detect CRISPR-induced double-strand breaks at the target locus.

Results & Discussion: In vitro validation in SCA1 fibroblasts showed that two sgRNAs could induce some double-strand breaks (DSBs) at the target locus, as confirmed by cleavage assay. These results provide initial proof-of-concept that the expanded CAG tract can be targeted using CRISPR/Cas9 tools. The two most effective sgRNAs are now being evaluated in combination to excise the expanded repeat segment in SCA1 mouse eNSCs and differentiated neuronal cells to assess editing efficiency and potential rescue of disease-associated cellular phenotypes. We are also optimising the electroporation protocol for eNSC cells for better transformation efficiency. The image attached shows eNSCs with primary cilia (green basal body, yellow actin filament) and mitotic cells with actin filaments separating pink chromatids.

Conclusion: The site-specific DSB induction by CRISPR potentially enables excision of the CAG repeat. This is a promising step toward developing therapeutic strategies for SCA1. These studies will also help evaluate the feasibility and safety of repeat-targeting CRISPR strategies for polyglutamine disorders.

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Mathematical Modelling of the Carotid–Ophthalmic Arterial Network from Anatomage Virtual Dissection: Proof-of-Concept

Running title: Modelling Carotid–Ophthalmic Arterial Network

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State-of-the-Art: Ophthalmic artery pathology arises from atherosclerotic stenosis, embolic occlusion, vasospasm, inflammatory arteritis, compressive mass, traumatic dissection, fibromuscular dysplasia, or iatrogenic injury. While mathematical models of intracranial vascular networks exist for stroke and aneurysm management, quantitative analysis of full extracranial-to-intracranial collateral supply remains lacking. Virtual dissection enables precise geometric extraction of vessel radii, lengths, and bifurcation angles across the entire arterial tree. Our Anatomage-based mathematical model focuses on intracranial-extracranial segmentations, with a particular emphasis on orbital revascularization.

Objective: We aim to develop a cadaver-adaptable mathematical network model of the cerebrovascular tree, derived from Anatomage virtual dissection, to rank extracranial routes suggestive of ophthalmic artery pressure relief.

Material and Methods: Geometric measurements were extracted from virtual dissection of 65 arterial segments across cerebrovascular, vertebrobasilar, craniofacial, and cervical vessels bilaterally using Anatomage Table data, assuming steady-state, rigid-wall Newtonian flow. Unlike synthetic Lindenmayer-system or constructive constrained optimization methods, we assessed every bifurcation using directly measured Anatomage morphometry, which was computationally enhanced by processing the VPF segment. Hemodynamic resistance was modeled proportionally using the simplified Poiseuille relationship. The network was assembled as a Kirchhoff admittance matrix and solved across 65 nodes via Gaussian elimination. Possible stenotic foci were imposed as a resistance penalty scaling to simulate plausible branch occlusion.

Results & Discussion: Our model identified the inferolateral trunk, the meningohipophyseal trunk branches, and the sphenopalatine artery as collateral steal conduits that reduce ophthalmic perfusion, highlighting extracranial therapeutic targets. Among 28 tested extracranial bypass configurations, angular artery-to-dorsal nasal anastomosis ranked first (18–24% pressure reduction), followed by superficial temporal-to-supraorbital and infraorbital-to-lacrimal anastomoses. Internal carotid artery cavernous segment stenting produced the largest single-vessel conductance gain. Murray's law deviation concentrated at the ophthalmic origin and carotid-cotympanic bifurcation, predilection sites for turbulence and embolic lodgment. A user-friendly interface for the model is shown in Fig 1.

Conclusion: Mathematical modelling of the full cerebrovascular network from Anatomage virtual dissection morphometry may provide quantifiable alternative scenarios for cerebrovascular diseases, potentially obviating the need for a craniotomy.

Anatamage-Derived Morphometric Model for Coronary Arterial Reconstruction

Running title: Morphometric Model for Coronary Grafting

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State-of-the-Art: Coronary heart disease is often arising secondary to the build-up of plaque in the endothelial lumen rendering it stenotic. This morbid vascular dysfunction is highly prevalent worldwide, especially in geriatric population. Contemporary computational models of coronary arterial anatomy rely predominantly on L-system branching algorithms, three-dimensional Navier–Stokes solvers, synthetic tree generators, and constrained constructive optimisation. These models flatten segmental tortuosity, bifurcation angles, and collateral topology. Our study therefore characterise the broader permutational range of coronary anastomotic configurations using Anatamage data.

Objective: Using virtual cadaveric dissections, we aim to characterise the luminal radius, taper rate, cross-sectional area, segment length, relative hydraulic resistance, and branching hierarchy of coronary arteries to improve grafting strategies of tortuous coronary arteries with maximal efficiency.

Material and Methods: The right coronary artery (RCA), left coronary artery (LCA), left anterior descending artery (LAD), left circumflex artery (LCX) and their branches were digitally dissected and precisely measured using the Anatamage measurement tools. For each segment, the following parameters were measured: proximal radius (R_u), distal radius (R_d), segment length, mean radius, cross-sectional area, taper rate, and relative hydraulic resistance. Branching hierarchy and parent-segment relationships were charted across 37 discrete arterial segments from the aortic arch down to terminal coronary vessels. Tortuosity indices and ramification patterns were assessed at each bifurcation point.

Results & Discussion: Morphometric data were derived from four virtual cadavers (2F/2M; Asian and Caucasian; adult and geriatric). Proximal radii ranged from 84.6 μm (RCA proximal) to 3.3 μm (AV nodal branch, right), with taper rates spanning 0.057 (LCX proximal) to 1.008 (Acute Marginal 1). Relative hydraulic resistance varied by four orders of magnitude, from 3.3×10^{-6} (RCA proximal) to 4.99×10^{-2} (AV nodal branch, right). Septal perforators, ciliary branches, and nodal vessels were resolved at sub-millimetre radii. The dataset further identified deviations from Murray’s law at multiple bifurcation nodes, with area ratios and asymmetry indices incompatible with synthetic tree assumptions.

Conclusion: The Anatamage virtual dissection platform provided calibration data of sufficient fidelity to resolve the visualisation of individual septal perforators, ciliary branches, and nodal vessels at sub-millimetre resolution. Limitations included undisclosed medical histories and variable segmentation details among the cadavers.

Lymphatic Segmentation and Regional Architectural Patterns Using Anatomage: A Pilot Study

Running title: Lymphatic Segmentation and Architectural Patterns

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State-of-the-Art: Nodal size and shape have been considered determinants of neoplastic metastasis, with open questions raised on lymphotropic routing and anatomic predilection sites for neoplastic progression. Moreover, inadequate attention has been paid to anatomical morphometric characteristics of lymphatic vessels, nodal clustering and geometric architecture. This study postulates that lymphatic vessel segmentation and regional architectural patterns may test theoretically grounded insights.

Objective: To segment lymphatic vessels in Anatomage and characterize lymphatic architecture patterns—including vessel continuity, regional node clustering, and vessel-node spatial relationships—in cancer, non-cancer, and morphologically indeterminate cases.

Material and Methods: Anatomage Table lymphatic data retrieved from three representative cadavers was used with color-coded segmentation. They included a female with confirmed GIT malignancy (Cancer_1), a cancer-free female (No_Cancer_2), and a male with unknown clinical status (Unknown_3). Lymphatic vessels and lymph nodes in head/neck, thorax, abdomen/pelvis, and extremities were labelled with different channel dominance. Manual and automatic measurements assessed regional node count, node density, vessel density, mean node size, lymphotropic routing and vessel tapering gradient. Manual measures for all nodes was performed using Anatomage's toolkit. The automatically calibrated parameters were assessed computationally after exporting VPF files for the lymphatic maps and corresponding imaging.

Results & Discussion: GIT Cancer cadaver demonstrated higher overall node burden (39.93% concentrated in abdomen and thorax) and elevated vessel density. In contrast, Cancer-free cadaver showed inverse predominance, with abdominal/pelvic nodes comprising 31.18%. Male cadaver with unknown data presented a generalized node distribution accounting for 71.07% of all nodes, with minimal extremity involvement (3.21%). Exceeding even Cancer_1, node pixel concentration and lymphotropic routing in Unknown_3 were similarly skewed toward the thorax and neck with whose sizes nodes exceeded 3 cm in largest diameter across multiple regions (Fig. 1). All cadavers showed free-end saddles of lymphatic vessels in the head/scalp, inferring tapering connection with the glymphatic system.

Conclusion: Lymphatic architectural analysis can be used as a complementary tool in the morphometric classification of nodal disease and mapping functional lymphatic system. Limitations of the study include a few representative cadavers for sampling.

Funding: None

Management of Chronic Subdural Hematoma – Retrospective Single-Center Study

Running title: Management of Chronic Subdural Hematoma

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State-of-the-Art: Chronic subdural hematoma (CSDH) is one of the most common neurosurgical diagnoses in the elderly population, with incidence increasing due to population aging and widespread use of antithrombotic therapy. Burr-hole evacuation with subdural drainage represents the current standard of surgical treatment. However, recurrence rates reported in the literature vary between 5–30%. Several perioperative factors have been suggested to influence recurrence and complications, including the experience of the surgeon, operative time, subdural drainage, corticosteroid therapy, and antithrombotic medication. The role of these factors remains debated in current literature

Objective: To retrospectively analyze patients operated for chronic subdural hematoma and evaluate whether recurrence correlates with surgeon experience, operative time, subdural drainage, corticosteroid therapy, and antithrombotic treatment, and whether these factors influence postoperative complications.

Material and Methods: A retrospective single-center study included 100 consecutive patients surgically treated for chronic subdural hematoma at the Department of Neurosurgery, University Hospital Pilsen, from November 2023 to September 2025. Evaluated parameters included recurrence requiring reoperation, perioperative complications, operative time, surgeon experience, use of corticosteroids, subdural drainage, and antithrombotic therapy. Functional outcome at discharge was assessed using the Glasgow Outcome Scale Extended (GOS-E). Statistical analysis included descriptive statistics and appropriate non-parametric tests for group comparisons, with a significance level of $p < 0.05$.

Results & Discussion: The cohort included 100 patients with a mean age of 73.7 years; 80% were male. Recurrence occurred in 3 patients (3%) and postoperative complications in 2 patients (2%). Functional outcome at discharge was excellent with median GOS-E of 8. No statistically significant association was found between recurrence and surgeon experience ($p = 0.244$), corticosteroid therapy ($p = 1.000$), or antithrombotic therapy ($p = 0.500$). Similarly, complications were not associated with surgeon experience or corticosteroid therapy. Operative time showed a non-significant trend toward association with recurrence ($p = 0.074$). A statistically significant difference in operative time was observed between surgeon experience levels (Kruskal-Wallis $p = 0.00037$).

Conclusion: Treatment of chronic subdural hematoma in our cohort was associated with low recurrence and complication rates and excellent functional outcome. However, due to the limited cohort size and low number of events, the statistical power of the analysis was limited.

Polypropylene in Ophthalmology: Biomechanical Properties, Degradation, and Clinical Use of Monofilament Fibers

Running title: Polypropylene in Ophthalmology

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State-of-the-Art: Polypropylene is one of the most widely used synthetic suture materials in surgery. In ophthalmology it plays an important role in microsurgical procedures requiring stable and biologically well-tolerated materials. Due to its chemical stability, low tissue reactivity and favorable mechanical properties, polypropylene has become a standard material. Despite its long clinical use, increasing attention has recently been paid to the biomechanical properties of polypropylene sutures and their long-term behavior in ocular tissues.

Objective: The aim of this work is to summarize current knowledge on the biomechanical properties, biocompatibility and degradation of polypropylene sutures used in ophthalmology, with particular focus on their role in scleral fixation of intraocular lenses.

Material and Methods: This work is based on a review of current scientific literature focusing on the use of polypropylene sutures in ophthalmology. In addition, ongoing experimental research investigating tissue reactions to different scleral suspension materials in an in vivo rabbit model was considered. Histological analysis of scleral tissue was used to evaluate inflammatory response and collagen formation around implanted fibers.

Results & Discussion: Available experimental and clinical studies indicate that polypropylene sutures are generally well tolerated by ocular tissues and provide stable fixation of intraocular implants. However, long-term implantation may lead to gradual structural changes of the polymer fiber, including surface microcracks and mechanical weakening, which in some cases may result in late suture breakage and implant dislocation. These findings have led to modifications in surgical techniques and to the use of thicker polypropylene sutures in order to increase mechanical stability. Modern fixation techniques and modified suture configurations may improve long-term clinical outcomes.

Conclusion: Polypropylene remains an important suture material in ophthalmic surgery due to its chemical stability, favorable mechanical properties and good tissue tolerance. However, the optimal suture diameter and surgical technique for long-term implantation have not yet been clearly defined.

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Biological augmentation of different types of meniscus repair using fibrin clot

Running title: Fibrin clot in meniscus repair

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State-of-the-Art: Menisci are essential structures of the knee joint and are crucial for proper knee function and biomechanics. Degenerative lesions and traumatic ruptures are found in almost every patient who undergoes knee arthroscopy, and in recent years there has been a significant shift toward meniscal preservation, particularly in younger patients, rather than performing a simple meniscectomy. However, some of these lesions are difficult to repair and have limited healing potential, mainly due to the poor blood supply in certain regions of the menisci. Therefore, biological augmentation is often required to ensure a satisfactory clinical outcome; however, many of these methods are expensive or not readily available to all patients.

Objective: To evaluate the clinical effectiveness of autologous fibrin clot augmentation in meniscal repair.

Material and Methods: In our department, we frequently use a fibrin clot to augment meniscal repairs. This is a safe and inexpensive method in which a fibrin clot is prepared directly from the patient's venous blood and then applied into the meniscal lesion together with the suture. The fibrin clot serves as a source of important growth factors such as PDGF, TGF- β , and VEGF. It also acts as a natural scaffold for fibroblasts or mesenchymal stem cells and, due to its adhesive properties, functions as a biological glue. Our indications mainly include complex meniscal tears such as bucket-handle tears, horizontal cleavage lesions, and radial ruptures.

Results & Discussion: We are conducting a prospective clinical study involving patients with various types of meniscal lesions who underwent meniscal suturing with or without augmentation using an autologous fibrin clot. The aim of the study is to evaluate the effect of fibrin clot augmentation on the healing of sutured menisci. We monitor the occurrence of repair failure, the need for reoperation, and other complications. IKDC and Lysholm scores are assessed, and follow-up MRI examinations are performed. Patients treated with fibrin clot augmentation demonstrated a statistically significant improvement in postoperative IKDC and Lysholm scores, along with a reduced rate of suture failure compared to non-augmented repairs.

Conclusion: Autologous fibrin clot augmentation is a safe, inexpensive, and effective method to enhance healing in meniscal repairs, particularly in complex tear patterns.

Regeneration of extrahepatic bile ducts after their reconstruction and its differences - experiment on large animal model

Running title: Differences in bile duct regeneration

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State-of-the-Art: Despite today's age biliary surgery still poses a significant challenge even for experienced HPB surgeons. Its complexity lies in combination of technically challenging procedures paired with its long term complications, especially tendencies to form stenosis in site of biliary reconstruction with subsequent biliary obstruction, weighing patients with additive morbidity. Current understanding behind the processes leading to this situation is limited, which complicates further research seeking possible solutions. Due to these facts we decided to compare healing of bile duct reconstructed by a) direct anastomosis, b) decellularized interponate after 4 weeks with native bile duct on domestic pig model.

Objective: To do a clinical and histological comparison of bile duct healing after different methods of extrahepatic bile duct reconstruction with native bile duct with intention to identify possible processes leading to stenosis formation.

Material and Methods: Porcine extrahepatic bile duct grafts were harvested under sterile conditions, then divided into 2 subgroups - a) native bile ducts for comparison b) bile ducts used for decellularized graft procurement. First experimental group (N = 7) of pigs underwent resection of a segment of the common bile duct, followed by anatomical reconstruction using an interposed decellularized graft. Second experimental group (N = 6) underwent direct anatomical reconstruction without an interposition graft. The comparison aimed to evaluate clinical, qualitative and quantitative histological differences after 4 weeks of healing in recipients' body with native bile duct characteristics.

Results & Discussion: No statistically significant differences were observed between the experimental and control groups in overall survival, morbidity, mortality, or complication rates. The experimental group showed a slight, statistically insignificant increase in alkaline phosphatase levels, without elevation of other markers of biliary obstruction, suggesting no relevant impairment of biliary function. Histological analysis revealed higher presence of inflammatory cells in the muscular layer of the group reconstructed by decellularized graft. A higher collagen I/III ratio in the same group may indicate ongoing graft remodeling. Lower smooth muscle actin expression in the serosa of likely reflects reduced peritoneal surface contact due to graft interposition.

Conclusion: Level of epithelial coverage integrity of the reconstructed part coupled with successful peritoneal sheets interaction may affect the healing process of bile duct reconstruction. Further study with cross-sections across individual time periods is required.

Funding: The study was supported by GA UK grant No. 434522 (Charles University) and Cooperatio project "Surgical disciplines" (COOPERATIO-207043, Charles University).

Prevention of esophageal anastomotic leak using a nanofibrous MMP-9-inhibiting patch: a large animal experimental study

Running title: Patch preventing esophageal anastomotic leaks

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State-of-the-Art: Surgical resection remains the only curative treatment for esophageal carcinoma. Esophageal anastomotic leak is a serious postoperative complication, occurring in 10–20% of cases. Despite advances in surgical techniques, perioperative care, and minimally invasive approaches, its incidence has not significantly decreased. Indicators of anastomotic healing include collagen content and mechanical strength. Experimental studies have shown that systemic inhibition of matrix metalloproteinase-9 (MMP-9) can increase collagen proportion in the anastomosis and improve its mechanical strength. In the past, our team successfully developed nanofibrous patches supporting gastrointestinal anastomotic healing. A new platform incorporating an MMP-9 inhibitor is being tested to prevent esophageal leaks.

Objective: To evaluate the feasibility and potential protective effect of a polycaprolactone nanofibrous patch containing an MMP-9 inhibitor on the healing of esophageal anastomosis in a large animal experimental model.

Material and Methods: The experiment included 24 pigs divided into four groups of six: (1) control, 1-week survival; (2) control, 3-week survival; (3) experimental, 1-week survival; and (4) experimental, 3-week survival. Control animals underwent standard esophageal reconstruction. In the experimental groups, a nanofibrous patch containing the MMP-9 inhibitor JNJ-0966 (MedChemExpress, USA) was applied around the anastomosis. The two observation periods were chosen to evaluate anastomotic healing during the inflammatory, proliferative, and remodeling phases. Clinical status was monitored daily. Laboratory tests and CT examinations were performed preoperatively and on postoperative days 3, 7, 14, and 21. After euthanasia, tissue samples were collected for macroscopic, histological, RNA, and protein analyses.

Results & Discussion: The experimental model of esophageal reconstruction in pigs using a nanofibrous patch was successfully established despite technical difficulties during patch application due to limited esophageal mobility. The postoperative course was largely uneventful. Animals tolerated soft oral intake from postoperative day 2. CT scans revealed no clinically significant esophageal anastomotic leak. Macroscopic evaluation identified three minor anastomotic dehiscences (12.5%) with localized abscess formation. The incidence of defects appeared lower in animals with the patch than in controls. Histological examination showed incomplete healing in all animals, with a purulent inflammatory infiltrate in the submucosa and suture area. The patch was not completely absorbed after three weeks.

Conclusion: Application of a nanofibrous patch containing the MMP-9 inhibitor appears feasible and may reduce minor anastomotic defects. More detailed microscopic and molecular analyses are ongoing (e.g., MMP-9 gene expression and protein activity) to further elucidate the protective effect of the patch.

Funding: Funded by Charles University Grant Agency (GAUK), project No. 204324: "Prevention of esophageal anastomotic leak using an innovative nanofibrous material inhibiting MMP-9 activity in a large animal model."

Oral microbiota as a potential therapeutic target in progression and malignant transformation of proliferative verrucous leukoplakia

Running title: The role of oral microbiota in PVL

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State-of-the-Art: Proliferative verrucous leukoplakia (PVL) is a rare oral potentially malignant disorder characterised by a high recurrence rate and up to 40% risk of malignant transformation to oral squamous cell carcinoma. Increasing evidence suggests that dysbiosis of the oral microbiota contributes to PVL pathogenesis and disease progression. In our department, a pilot study analysing 12 periodontal pathogens demonstrated significantly higher bacterial prevalence in PVL lesions compared with other oral mucosal conditions. A recent study published in 2025 further indicates that interactions between oral microbiota and oncogenic viruses, particularly human papillomavirus (HPV), may influence inflammation, immune response and carcinogenesis.

Objective: To characterise the oral microbiota in 35 patients with proliferative verrucous leukoplakia and compare bacterial composition according to radiographic findings, clinical progression and viral positivity (HPV, EBV, CMV) to identify microbial patterns associated with disease development.

Material and Methods: After establishing inclusion criteria samples were collected from 35 patients diagnosed with PVL using sterile paper points inserted into periodontal pockets. DNA was isolated and subjected to Illumina metagenomic sequencing targeting the V3–V4 region of the 16S rRNA gene. Sequencing libraries were prepared using barcoded primers and pooled prior to analysis. In parallel PCR screening for oncogenic viruses, including human papillomavirus (HPV), Epstein–Barr virus (EBV) and cytomegalovirus (CMV) is currently ongoing. Virus-positive samples will subsequently undergo sequencing for viral typing and to assess potential associations with alterations in bacterial community composition.

Results & Discussion: In our pilot study 12 aggressive periodontal pathogens were monitored in 38 PVL cases, 38 patients with oral lichen planus presenting as desquamative gingivitis and 38 with chronic periodontitis. Pathogens were relatively quantified using qPCR with TaqMan probes. PVL samples showed a threefold higher bacterial abundance compared with control groups ($p = 0.0009$). Subsequent metagenomic sequencing confirmed these findings and revealed dysbiosis of the oral microbiota associated with progression of PVL lesions. These results support a significant role of microbial imbalance in PVL pathogenesis and highlight the importance of analysing the full spectrum of oral bacteria and potential viral cofactors.

Conclusion: The findings highlight microbial dysbiosis as a potential cofactor in PVL pathogenesis. Targeting microbial communities and their products may represent a promising therapeutic approach, as current treatment options remain largely ineffective.

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Submucosal fibrosis in oral lichen planus

Running title: A common complication of oral lichen planus

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State-of-the-Art: Oral lichen planus (OLP) is the most frequent chronic non-infectious inflammatory disease of the oral mucosa with a prevalence of 0.5-2.5%. The issue of submucosal fibrosis (SMF) in OLP is rarely discussed in the literature. According to the only available study, the incidence of SMF in OLP is 30.1%. No relationship was found between SMF and erosive OLP lesions, extraoral manifestations of lichen, smoking, age, VAS pain assessment, or duration of disease. The development of SMF can lead to restricted mouth opening (RMO) with an interincisal distance (IID) of less than 35 mm, which impairs many functions of the mouth.

Objective: The aim of this study is to draw attention to the etiological and clinical context of OLP, which is neglected in the literature. Submucosal fibrosis in OLP has an impact on patients' quality of life, the possibility of dental treatment, and the examination of oral mucosa.

Material and Methods: The presence of SMF and actual interdental distance were evaluated in 400 confirmed cases of OLP. All patients were monitored at the Department of Oral Medicine of the Dental Clinic of the University Hospital in Pilsen. Correlation of SMF cofactors was performed. The one-year development of the SMF phenomenon was studied in 330 cases of OLP.

Results & Discussion: Significant associations between SMF and specific aspects of OLP, such as erosive form and desquamative gingivitis, have been confirmed. Ventrally located SMF bundles have a greater effect on RMO. Critical RMO below 35 mm was present in almost 7% of SMF positive cases. Even one year of SMF presence leads to a significant reduction in mouth opening with $p\text{-value}=0.0005$.

Conclusion: This is the first study to demonstrate significant associations with erosive lichen, desquamative gingivitis, extraoral lichen, and higher symptomatology of SMF positive cases of OLP. This comprehensive approach may improve the outcomes of OLP monitoring.

Exploring the Combined Prognostic Value of Age and miR-1972 Expression in Hepatocellular Carcinoma

Running title: Age and miR-1972 Expression in Hepatocellular Carcinoma

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State-of-the-Art: Hepatocellular carcinoma (HCC) is an aggressive malignancy with poor prognosis due to high recurrence rates and limited reliable prognostic biomarkers. MicroRNAs (miRNAs) regulate gene expression and play key roles in cancer progression. miR-1972 has been implicated in cancer biology, and our previous work identified its prognostic relevance in non-viral HCC.

Objective: This study aimed to investigate the impact of patient age and miR-1972 expression on survival outcomes in non-viral HCC.

Material and Methods: Tumour samples from 45 patients with non-viral HCC who underwent surgical resection were analysed using a miRNA expression microarray. Associations between age and miR-1972 expression were evaluated. Cox proportional hazards regression and Kaplan–Meier analyses were performed to assess the effects of age and miR-1972 expression on disease-free survival (DFS) and time to recurrence (TTR).

Results & Discussion: Older age showed a trend towards longer DFS and was significantly associated with longer TTR (HR = 0.39, 95% CI: 0.39–0.96, $p = 0.04$). Multivariable analysis confirmed that older age independently predicted longer DFS (HR = 0.42, 95% CI: 0.20–0.88, $p = 0.02$) and TTR (HR = 0.30, 95% CI: 0.12–0.77, $p = 0.01$). High miR-1972 expression was also associated with improved DFS (HR = 0.30, 95% CI: 0.13–0.69, $p = 0.005$) and TTR (HR = 0.27, 95% CI: 0.10–0.77, $p = 0.01$). The combination of older age and high miR-1972 expression was strongly associated with improved DFS (HR = 0.13, 95% CI: 0.04–0.43, $p < 0.001$) and TTR (HR = 0.08, 95% CI: 0.02–0.31, $p < 0.001$), which was further confirmed by Kaplan–Meier analysis ($p = 0.006$ and $p = 0.001$, respectively).

Conclusion: Patient age and miR-1972 expression are favourable prognostic indicators in non-viral HCC. Their combined assessment may improve patient stratification and survival prediction, highlighting potential differences in prognostic biology between viral and non-viral HCC.

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Evaluation of Proenkephalin and Uromodulin in Relation to Renal Function in Patients with Hematological Malignancies

Running title: Renal Markers in Hematologic Malignancies

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State-of-the-Art: Renal dysfunction is a frequent and clinically relevant complication in patients with hematological malignancies. Routine assessment is commonly based on serum creatinine, cystatin C, and estimated glomerular filtration rate (eGFR), but these markers may have limitations in this specific clinical setting. Proenkephalin A 119–159 (penKid) and uromodulin have emerged as promising biomarkers reflecting different aspects of kidney function and injury. Their role in patients with hematological malignancies, however, remains insufficiently characterized.

Objective: The aim of the study is to assess the relationship of proenkephalin A 119–159 (penKid) and uromodulin to parameters of renal function in hospitalized hemato-oncological patients and to evaluate their potential as complementary biomarkers in comparison with routinely used laboratory markers.

Material and Methods: The ongoing analysis includes hospitalized hemato-oncological patients with available residual plasma samples and a urea/creatinine ratio > 70. Renal function is assessed using estimated glomerular filtration rate (eGFR) calculated according to the CKD-EPI 2021 creatinine equation, the CKD-EPI cystatin C equation, and the combined creatinine–cystatin C equation. penKid concentrations are measured by immunofluorescence assay on the AFIAS-4 analyzer, while uromodulin is measured by chemiluminescent immunoassay on the Kleeeya analyzer. The evaluation includes descriptive statistics, comparison of individual eGFR estimates, correlation analysis, and assessment of biomarker changes over time.

Results & Discussion: The analysis of the cohort is currently ongoing. Preliminary observations suggest differences among individual approaches to renal function estimation and at the same time indicate a possible contribution of the studied biomarkers as complementary indicators to routine laboratory parameters. Further analysis is focused on evaluating the relationship of penKid and uromodulin to eGFR and other laboratory markers, including the assessment of their temporal dynamics in repeated sampling.

Conclusion: Proenkephalin and uromodulin may represent promising complementary biomarkers of renal function in patients with hematological malignancies. This study may help clarify their relationship to standard renal markers and their potential clinical relevance.

Effect of Virtual Reality Exercise on Range of Motion and Pain Perception in Patients with Limited Shoulder Mobility

Running title: VR Exercise and Shoulder Pain

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State-of-the-Art: Pain and limited range of motion will commonly restrict progress in the rehabilitation of shoulder disorders. Patients will often limit movement due to pain perception or fear of pain, which may reduce therapeutic effectiveness. Virtual reality (VR) will be used as a supportive rehabilitation tool providing interactive environments that promote active participation in exercise. Previous studies suggest that VR may decrease perceived pain through distraction while increasing patient engagement. However, objective evidence on the relationship between VR-assisted exercise, range of motion, and pain perception in individuals with shoulder mobility limitations will remain limited. This study will examine this interaction to help optimize rehabilitation strategies and improve functional outcomes.

Objective: To evaluate whether short-term VR-based exercise influences active range of motion and subjective pain perception in patients with limited shoulder mobility during rehabilitation.

Material and Methods: Patients with painful shoulder mobility limitations will be evaluated in a single session. Active shoulder range of motion (ROM) will first be measured using a standard goniometer. Participants will then engage in 10–15 minutes of active exercise in a virtual reality (VR) environment targeting upper limb movements. During VR exercise, movement range will be recorded via the VR tracking system. Subjective pain intensity will be assessed using the Visual Analogue Scale (VAS) before, during, and immediately after the session. Collected data will be analyzed to determine whether VR-assisted exercise will enhance movement range and modulate perceived pain during rehabilitation.

Results & Discussion: Preliminary observations suggest that patients often achieve greater active range of motion during VR-based exercise compared with baseline goniometric measurement. Many participants report lower perceived pain during the initial phase of VR activity, which may be explained by distraction from pain due to immersive visual and motor engagement. Toward the end of the exercise session, some patients report increased fatigue and a mild increase in pain intensity. Despite this, the achieved range of motion frequently remains higher than during baseline assessment. These findings indicate that VR-assisted exercise may temporarily reduce pain perception and facilitate larger movement amplitudes during rehabilitation tasks.

Conclusion: Virtual reality exercise may enable patients with painful shoulder mobility limitation to perform movements with greater range while temporarily reducing perceived pain. VR could represent a useful supportive tool in rehabilitation programs focused on improving active mobility.

Clinical Trial: Effect of Respiratory Infections on Serum 25(OH)D Levels During Vitamin D₃ Supplementation

Running title: Clinical trial: Vigantol

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State-of-the-Art: Vitamin D is primarily obtained through sunlight, which enables its synthesis in the skin. At latitudes above approximately 33°, cutaneous vitamin D production is limited to the period from May to September, resulting in seasonal declines in vitamin D levels and a high prevalence of deficiency during winter. Respiratory infections, which are more common in winter, may further contribute to reductions in vitamin D levels. Some studies have reported decreases in serum vitamin D concentrations following respiratory infections, even in individuals receiving vitamin D₃ supplementation. This clinical trial evaluates short-term changes in serum 25(OH)D levels following respiratory infection.

Objective: The aim of this clinical trial was to assess whether declines in serum 25(OH)D levels were greater in participants who experienced respiratory infection during vitamin D₃ supplementation than in those who remained healthy.

Material and Methods: A total of 130 volunteers were enrolled in the study, including 35 older adults aged 65–85 years and 95 young adults aged 20–27 years. All participants received vitamin D₃ supplementation (Vigantol®, cholecalciferol oral drops) at a dose of 1000 IU daily for 60 days during October and November 2025. Blood samples were collected and serum 25(OH)D levels were measured before the start and after the end of the supplementation period. Participants were instructed to report any respiratory infection during the supplementation period and to attend an additional study visit for blood sampling 7–10 days after symptom onset.

Results & Discussion: During the supplementation period, respiratory infection was reported in 14 participants, including 5 individuals aged 65–85 years, and additional blood samples were collected in these cases. At 7–10 days after symptom onset, serum 25(OH)D levels increased in 6 infected participants, while a mild decrease was observed in the remaining 8.

After the supplementation period, 25(OH)D levels decreased in most participants, with a mean decline of 15.9 nmol/L. The extent of decline was comparable between participants who experienced respiratory infection and those who remained healthy during the supplementation period.

Conclusion: Respiratory infections did not appear to significantly affect serum 25(OH)D levels during vitamin D₃ supplementation, although the small number of infected participants limits interpretation. A daily dose of 1000 IU was insufficient in winter. Further study phases evaluating higher doses ongoing.

Funding: This work was supported by the Cooperatio Program, research area Pharmaceutical Science

Bisphenol A and Its Analogues: Implications for Gut Microbiome Health

Running title: Bisphenols and the Gut Microbiome

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State-of-the-Art: Recent research has identified bisphenol A (BPA) and its structural analogues as widespread endocrine disruptors with emerging impacts on the gut microbiome. Experimental studies consistently demonstrate that exposure to BPA, BPS, and BPF alters microbial composition, reduces diversity, and promotes pro-inflammatory profiles linked to metabolic and immune dysregulation. However, most evidence is derived from animal and in vitro models, while human data remain limited. In addition, newer bisphenol analogues and mixture effects are still insufficiently characterized, highlighting a need for integrative microbiome-focused toxicological research.

Objective: To synthesize current evidence on the effects of bisphenol A and its analogues on the gut microbiome, identify key mechanisms of microbiome-mediated toxicity, and highlight major research gaps relevant to human health and risk assessment.

Material and Methods: This narrative review is based on a structured literature search conducted in PubMed covering the period from January 2010 to December 2025. Search terms included combinations of “bisphenol,” “BPA,” “BPS,” “BPF,” “gut microbiome,” “intestinal barrier,” and “dysbiosis.” Experimental (in vivo and in vitro), epidemiological, and mechanistic studies were included. Non-English publications, studies without microbiome-related outcomes, and inaccessible full texts were excluded.

Results & Discussion: Evidence across animal, in vitro, and limited human studies indicates that bisphenol exposure consistently disrupts gut microbial composition and function. BPA and its analogues (notably BPS and BPF) reduce microbial diversity, deplete beneficial taxa such as *Bifidobacterium* and *Akkermansia*, and promote pro-inflammatory microbial profiles. These changes are associated with impaired intestinal barrier integrity, altered short-chain fatty acid production, and downstream metabolic and immune disturbances. Importantly, several BPA substitutes demonstrate microbiome-disrupting effects comparable to or exceeding BPA, raising concerns about regrettable substitution. However, the predominance of experimental models highlights the lack of long-term, low-dose human data.

Conclusion: Growing evidence identifies bisphenols and their analogues as microbiome-disrupting chemicals, underscoring the need to integrate microbiome endpoints into risk assessment and expand human-centered research.

Expression of plasminogen activator inhibitor-1 (PAI-1) in patients recovered from COVID-19 disease

Running title: PAI expression and COVID-19 disease

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State-of-the-Art: In patients infected with the SARS-CoV-2 coronavirus causing COVID-19, overexpression of plasminogen activator inhibitor-1 (PAI-1) was observed, and this increased expression was also found to be closely correlated with the severity of the viral disease. PAI-1 is produced by endothelial cells, and its elevated levels in post-COVID patients represent a manifestation of endothelial damage induced by SARS-CoV-2 infection. PAI-1 plays a key role in fibrinolysis. During the COVID-19 pandemic, it was globally observed that this disease causes a specific type of coagulopathy (COVID-19-associated coagulopathy, CAC), in which mechanisms of the innate immune response damage endothelial cells primarily in the microcirculation, leading to thrombus formation (so-called immunothrombosis).

Objective: The aim of our study was to determine how long elevated PAI-1 concentrations persist in patients who have fully recovered from COVID-19. We were also interested in whether persistent elevated PAI-1 levels after COVID-19 correlate with the clinical course of the disease.

Material and Methods: A total of 1,261 individuals were analyzed in the study, including 377 patients who had recovered from COVID-19 and 884 healthy COVID-negative controls. We compared 377 patients who were 30 to 210 days after PCR-confirmed COVID-19 with a total of 884 control individuals who had not had COVID-19. We included only patients with mild to moderate COVID-19 in our study. Patients who required mechanical ventilation or other intensive care were excluded, as were patients with secondary complications (e.g., pulmonary embolism).

Results & Discussion: Our study showed that elevated PAI-1 levels persisted for several months (up to 1 year) after COVID-19, and it likely revealed for the first time that increased PAI-1 levels also persist in completely asymptomatic patients (fully recovered individuals). The strongest predictor of elevated PAI-1 in these patients was the β mutation of SARS-CoV-2, which was associated with approximately an 11-fold higher risk of PAI-1 ≥ 32 ng/mL. A surprising finding of our study was that we did not observe any decrease in PAI-1 among patients recovered from COVID-19; on the contrary, PAI-1 levels tended to increase. Regarding CAC we did not find an association between PAI-1 and D-dimer or other basic coagulation factors. We did not observe a relationship between PAI-1 and ferritin.

Conclusion: The key finding of our analysis was that elevated PAI-1 levels can be present in patients even several months after full recovery from COVID-19, at a time when the observed individuals were almost completely asymptomatic.

Stochastic Tractography-based Glioblastoma Invasion Modelling: Current Stage of Development and Future Prospects

Running title: Stochastic Tractography-based GBM Invasion Model

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State-of-the-Art: Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor, with the average survival time being only about 15 months. Surgical resection is the primary therapeutic option for GBM, often followed by adjuvant radiotherapy and chemotherapy. Nevertheless, determining the optimal treatment extent is extremely difficult, as magnetic resonance imaging (MRI) is unable to detect the full extent of GBM invasion. Therefore, mathematical models are used to provide insight into GBM growth patterns below MRI detectability. The most prominent approaches utilize partial differential equations (PDEs) to describe the invasion as a reaction-diffusion system on a 3D voxel grid. These models can be characterized by various levels of incorporated biological knowledge and MRI data.

Objective: The model aims to provide clinicians with probabilistic patient-specific GBM invasion simulations to support surgical planning and targeted radiotherapy. Additionally, the model should facilitate the analysis of invasion patterns related to the GBM tumor mass localization in the brain connectome.

Material and Methods: The proposed GBM invasion model builds on the current state-of-the-art approaches in mathematical neuro-oncology by reformulating the reaction-diffusion PDEs on an undirected graph constructed from structural MRI data. The graph's edges are weighted using patient-specific tractography obtained via constrained spherical deconvolution (CSD), enabling the anisotropic invasion along white matter tracts observed in GBM. The diffusion term includes further modulation, reflecting changes in GBM growth behavior related to tumor cell density. The proliferation is expressed using deterministic and stochastic components, allowing the evaluation of invasion uncertainty introduced by innate biological variability. The model contains various parameters that influence the simulation.

Results & Discussion: The simulations indicate that the proposed tractography-based model expresses the typical finger-like extrusions from the main GBM tumor mass into the surrounding brain tissue. Although few anisotropic approaches already exist, they do not exhibit nearly as strong preferential spread along white matter tracts, possibly due to the integration of information with very limited accuracy about true tract density, such as the diffusion tensor imaging (DTI), as well as the 3D voxel grid data structure for computations. Planned combination of the proposed model with machine learning algorithms represents a unique hybrid explainable artificial intelligence (XAI) approach, attempting to combine the interpretability of the baseline model with modern data-driven methods for improved performance.

Conclusion: Representing the discrete simulation space as a graph with directly integrated patient-specific brain connectomes represents a conceptually novel approach to GBM invasion modelling. Future work will focus on quantitative validation using clinical cases and integration with machine learning.

Identification and Characterization of Distinct Hematopoietic Stem and Progenitor Cells in Health and Disease

Running title: Developing a Computational Tool for HSC annotation

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State-of-the-Art: Hematopoietic stem cells (HSCs) are essential for lifelong blood formation and are central in rapid regeneration of immune cells following stress. However, defining HSC states at the molecular level remains difficult due to their heterogeneity and gradual transitions toward progenitor cells. Single-cell RNA sequencing (scRNA-seq) provides detailed resolution of these populations but introduces challenges, including overlapping transcriptional profiles, batch effects, and inconsistent manual annotations. There is a need for a standardized tool that allows the identification of distinct HSC subsets in a reliable manner.

Objective: To develop robust and reproducible package for accurate annotation of hematopoietic stem cells using scRNA-seq data. The framework is designed to move beyond marker-based labeling by integrating gene program, regulatory network analysis, and trajectory context to achieve biologically informed cell state.

Material and Methods: Public scRNA-seq datasets of hematopoietic stem and progenitor cells will be processed using standardized quality control, normalization, and batch correction. Dimensionality reduction and clustering, followed by identification of cluster-specific gene modules and trajectory inference to define developmental relationships. These integrated features will be used to develop a supervised classification model for accurate HSC annotation. Model performance will be evaluated through cross-validation and independent external datasets. Experimental validation will include FACS isolation and colony-forming assays to confirm functional HSC identity.

Results & Discussion: Preliminary analyses suggest that HSC subclusters identified across independent public datasets may share conserved gene modules and gene regulatory network (GRN) signatures. These shared transcriptional patterns appear to persist despite dataset-specific variation and batch effects, indicating potential biological relevance. Furthermore, several gene modules and regulatory drivers show possible correlations with trajectory-defined cell states, implying they may reflect dynamic transitions along the hematopoietic hierarchy. Ongoing analyses aim to confirm the consistency and developmental significance of these observed patterns.

Conclusion: This project establishes a comprehensive and reproducible framework for accurate annotation of hematopoietic stem cell states using data. This framework provides a reliable tool for consistent HSC identification and facilitates deeper insight into hematopoietic development and functional heterogeneity.

Contribution of cumulus cells to maturation of mtDNA-deficient oocytes

Running title: Cumulus support in mtDNA-deficient oocytes

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State-of-the-Art: Mitochondrial DNA (mtDNA) is maternally inherited and massively amplified during oocyte growth, reaching >100,000 copies in mature oocytes. This expansion relies on nuclear regulators, particularly TFAM, which maintains mtDNA and supports mitochondrial gene expression. Although mtDNA copy number was long considered a proxy for oocyte quality, mitochondrial silencing and inconsistent predictive value have challenged this view. Notably, TFAM-deficient oocytes can retain developmental competence despite severe mtDNA depletion. During maturation, however, oocytes depend on cumulus cells for metabolic substrates. In vitro denudation removes this input and may unmask mitochondrial limitations. How mtDNA depletion affects cumulus-free maturation competence remains insufficiently defined.

Objective: To determine how oocyte-specific Tfam depletion affects oocyte maturation competence and chromosomal stability.

Material and Methods: We used an oocyte-specific Tfam conditional knockout mice (TfamloxP/loxP; Zp3-Cre) to produce Tfamnull oocytes with depleted mtDNA. Female mice (8–24 weeks) were hormonally stimulated by i.p. administration of PMSG. Cumulus–oocyte complexes were collected 44–48 h later, and cumulus cells were removed after 90 min of cultivation by mechanical denudation using a fine glass capillary. Denuded oocytes were subjected to in vitro maturation at 37 °C in 5% CO₂ for 14–16 h. Maturation was assessed based on first polar body extrusion and spindle morphology. Oocytes were immunostained with anti-TFAM and anti-acetyl- α -tubulin antibodies and visualized by confocal microscopy (Nikon, Japan). Animal experiments were conducted in accordance with Act No. 246/1992 Coll. (Project No. MSMT-33798/2021-4).

Results & Discussion: Our observations showed that TFAM loss caused a marked reduction in oocyte mtDNA content and significantly impaired in vitro maturation outcomes ($p = 0.0062$). Indeed, fewer Tfamnull oocytes matured compared to the wild-type oocytes. Confocal microscopy and image analysis revealed an increased frequency of spindle abnormalities in Tfamnull oocytes. These findings are consistent with a model in which cumulus-derived metabolic support can buffer compromised oocyte mitochondrial function in intact complexes, whereas denudation during in vitro handling reveals a stronger dependence on mtDNA-supported mitochondrial capacity for normal meiotic progression.

Conclusion: In denuded oocytes, TFAM loss-driven mtDNA depletion reduces in vitro maturation rate and increases the incidence of spindle abnormalities. These findings underscore the essential role of cumulus cells in oocyte maturation, particularly for oocytes suffering from insufficiency of mtDNA content.

Funding: Supported by the Cooperatio Programme (research area MED/DIAG and MED/IMMU), and SVV 260 773.

Feasibility of ATAC-seq analysis for identifying regulatory regions in CD123-high and CD-123-low AML samples

Running title: ATAC-seq analysis of CD123-high/low AML samples

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State-of-the-Art: CD123 appears to be a promising novel therapeutic target in acute myeloid leukaemia (AML), due to its high expression on AML blasts and low expression on healthy cells. Several immunotherapeutic strategies targeting CD123 are under development, however, resistance in the form of antigen escape is still observed. The mechanisms responsible for CD123 antigen escape are still not well understood, though the ongoing research indicates a substantial role of epigenetic and transcriptional regulation. The exact regulatory molecules responsible for changes in CD123 expression need to be determined. Identifying the molecules responsible for antigen escape could help establish combined therapy for AML patients and ensure efficacy of anti-CD123 immunotherapy.

Objective: The study aimed to investigate the feasibility of using ATAC-seq data from AML patients to identify regulatory regions of CD123 gene, employing patient samples that exhibit low and high CD123 expression levels.

Material and Methods: The relative CD123 expression normalized to GAPDH as the endogenous control was established in AML patients using qPCR. Based on the relative expression levels, patients were stratified into CD123-high and CD123-low cohorts. AML blasts from 4 CD123-high and 4 CD123-low samples were isolated via FACS using backbone markers CD117, and CD45, in combination with CD34 (n=4), CD33 (n=2) or CD11b (n=2). The samples were processed using ATAC-seq kit (Diagenode, USA) and sequenced on NovaSeq 6000 (paired-end, 150 bp) (Illumina, USA). A preliminary analysis was conducted on 500 000 reads per sample. The reads were filtered (MAPQ \geq 30, paired end), and peaks called via MACS2. Differential accessibility was assessed using t-tests on CPM-normalized counts with Benjamini-Hochberg correction.

Results & Discussion: A median of 405K cells was sorted (range 200K-500K), yielding a media of 65K cells (range 40K-115K) post centrifugation (500xg, 10 min, 4°C). ATAC-seq libraries were prepared from 50K cells per sample (except the one with 40K cells only). Sequencing generated ~25M reads/sample. Sample 8 had 12M due to library quality issues. QC confirmed high-quality data free of adapter and primer contamination. Preliminary analysis of 500K reads/sample identified 2,477 consensus peaks (43 on the X chromosome), which were unevenly distributed among the samples, indicating the presence of different regulatory mechanisms. Initial analysis did not confirm a trend in the locations or numbers of peaks depending on CD123 expression. However, deeper analysis is needed to determine the CD123-related region.

Conclusion: Preliminary results confirm that combination of cell sorting with AML-specific immunophenotype is feasible to detect regulatory regions. Despite 13-23% cell loss during processing, this approach produces high-quality data. However, higher sequencing depth is required for non-essential gene loci.

○○ DSP THEORETICAL DISCIPLINES

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Reduced mitochondrial DNA content in the oocyte and the risk for neurodegenerative diseases in offspring

Running title: Maternal mtDNA and neurodegeneration

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State-of-the-Art: Mitochondrial transcription factor A (TFAM) is a key regulator of mtDNA replication, transcription, and packaging. Its levels naturally decline with age, leading to impaired mitochondrial respiration, increased oxidative stress, and cellular senescence. In oocytes, TFAM is essential for maintaining the mitochondrial pool required for early development. While a significant reduction in mtDNA content (due to TFAM depletion) can be partially compensated during embryogenesis, the long-term consequences for offspring remain unclear. It is hypothesized that impaired mitochondrial quantity in oocytes increases susceptibility to neurodegeneration in offspring.

Objective: The project aims to investigate how oocyte-specific TFAM depletion and subsequent mtDNA reduction affect health of offspring, via a) testing behavior and cognitive functions, b) measurement of mitochondrial respiration, c) assessment of hippocampus histology, and d) genetic evaluation of mtDNA.

Material and Methods: We use a transgenic mouse model with oocyte-specific inactivation of the Tfam gene. Mutant offspring and wild-type control animals in the age of twelve months undergo a battery of behavioral experiments (e.g., open field, Morris water maze) to assess cognitive performance. Brain tissue is subsequently collected for detailed analyses. We perform histological staining to detect neurodegenerative markers, qPCR to quantify mtDNA copy number, and oxygraphy to evaluate mitochondrial respiratory capacity in the cortex. This approach allows us to correlate maternal mitochondrial deficiency with the functional and structural phenotype of the offspring's brain. All animal experiments were conducted in accordance with Act No. 246/1992 Coll. (Project No. MSMT-33798/2021-4).

Results & Discussion: Contrary to our hypothesis, data shows no significant differences in cognitive tests, hippocampal mtDNA content, or mitochondrial respiration of brain cortex between wild-type and mutant offspring. Correlation analysis shows relationship between oxygen respiration in cortex and failure rate in Morris water maze. These findings suggest the presence of robust compensatory mechanisms during embryogenesis that stabilizes mitochondrial functions in key brain regions despite maternal deficiency.

Conclusion: Maternal TFAM deficiency does not result in immediate mitochondrial or functional impairment in the offspring brain, suggesting effective developmental compensation. Ongoing mtDNA sequencing and histology analyses aim to clarify the underlying mechanisms.

Funding: Supported by the Cooperatio Programme (research area MED/DIAG and MED/IMMU), and SVV 260 773.

Differences in astrocyte and microglia activity across cerebellar regions in Lurcher and Purkinje cell degeneration mouse models

Running title: Neuroglial activity in cerebellar degeneration

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State-of-the-Art: Modern research indicates that during neurodegeneration, astrocytes and microglia undergo functional reprogramming characterized by the activation of protective and adaptive mechanisms to maintain tissue homeostasis. Clarifying the activity levels of these glial cells and correlating their layer-specific responses in cerebellar neurodegenerative disorders may deepen our understanding of the mechanisms driving cerebellar degeneration and facilitate the development of targeted therapeutic strategies that support long term recovery and improve patients' quality of life.

Objective: The present study aimed to compare astrocyte and microglial activation across distinct cerebellar compartments of B6CBA and B6.BR mouse strains with neurodegeneration.

Material and Methods: Brains from B6CBA Lurcher and B6.BR Purkinje cell degeneration (pcd) mice with clinical signs of cerebellar degeneration, along with strain matched healthy controls (n = 6–10 per group), were collected and processed for immunofluorescent labeling using anti-Iba1 antibodies to identify microglia and anti-GFAP antibodies as a marker of astrocytes. To evaluate microglial and astrocyte activation, the average staining intensity for Iba1 and GFAP was quantified in the cerebellum (individual cortical layers, white matter, and cerebellar nuclei) by measuring signal intensity in each region and comparing it to wild type littermate controls using software Fiji/ImageJ. Statistical analyses were performed using permutation based ANOVA followed by post hoc tests with Benjamini–Hochberg correction.

Results & Discussion: GFAP staining intensity was increased in both Lurcher and pcd mice compared to healthy controls, indicating astrocyte activation across all examined cerebellar regions. No differences were observed between the wild type mice strains in any cerebellar compartment except the white matter, where the B6CBA mice showed higher signal intensity. Lurcher mutant exhibited higher GFAP staining than pcd mutant mice across all examined cerebellar regions except the nuclei, where no significant difference was observed. Iba1 staining intensity was increased in the molecular layer in both mutant groups, as well as in the white matter of Lurcher mice and the nuclei of pcd mice, compared to their respective healthy controls. No strain related differences in Iba1 were detected.

Conclusion: The obtained results demonstrated distinct patterns of glial activation in the two neurodegeneration models. Lurcher mice showed stronger GFAP responses than pcd mice. Despite regional differences in Iba1 staining intensity in mutant, no strain related effects were observed in wild type mice.

Funding: The work was supported by the Cooperatio Program, research areas MED/DIAG and NEUR.

The Effect of Neuropeptides B and W on Cytosolic Ca²⁺ Dynamics in Beta Cells in Acute Mouse Pancreatic Tissue Slices

Running title: NPB/W Effect on β -Cell Ca²⁺ Oscillations

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State-of-the-Art: Neuropeptides B (NPB) and W (NPW) are endogenous ligands of the G_i protein-coupled receptors NPBWR1 and NPBWR2 and participate in the central regulation of energy homeostasis. However, their direct role in pancreatic islet physiology and β -cell signaling remains largely unexplored. Calcium signaling is a key determinant of β -cell excitability and insulin secretion, and subtle modulation of intracellular Ca²⁺ dynamics may critically influence endocrine output. Acute pancreatic tissue slices preserve native cytoarchitecture and paracrine interactions, providing a physiologically relevant platform for studying β -cell signaling in situ.

Objective: The aim of this study was to determine how NPB and NPW affect cytosolic Ca²⁺ dynamics in pancreatic β cells and to explore the involvement of intracellular Ca²⁺ stores and cAMP-related signaling.

Material and Methods: Experiments were performed on acute pancreatic tissue slices (130 μ m thick) prepared from C57BL/6J mice (8–11 weeks old, n = 7). Tissue architecture was preserved by intraductal 1.9% agarose injection followed by vibratome sectioning. Slices were loaded with a fluorescent Ca²⁺ indicator Calbryte 520 AM and imaged using confocal microscopy at 37 °C. β cells were identified based on their characteristic Ca²⁺ response to stimulation with 8 mM glucose. To selectively stimulate oscillatory Ca²⁺ release via IP₃ receptors, 100 nM acetylcholine was added to this solution. Either NPB or NPW was applied in 5 increasing concentrations ranging from 1 pM to 10 nM, and Ca²⁺ oscillation frequency, duration, and area under the curve were analyzed using a custom Python-based analysis pipeline.

Results & Discussion: Data indicate that NPB and NPW modulate IP₃-dependent Ca²⁺ oscillations in glucose-activated β -cells. Under these conditions, neuropeptide application altered oscillation frequency and waveform characteristics, suggesting an effect on intracellular Ca²⁺ handling. The variability observed between islets highlights the importance of preserved microarchitecture and paracrine signaling in fresh tissue slices. These findings suggest that NPB/W signaling may fine-tune β -cell excitability rather than directly trigger activity.

Conclusion: NPB and NPW modulate cytosolic Ca²⁺ dynamics in β cells in situ, likely through mechanisms involving intracellular Ca²⁺ stores. These results provide functional evidence for a modulatory role of NPB/W signaling in β -cell excitability.

Funding: The research stay at Medical University of Vienna was supported by the AKTION Österreich–Tschechien programme, the Charles University Mobility Fund, and the PPSR programme (Programme for the Support of Scientific Research).

Skeletal muscle phenotype in TFAM-deficient mice: integrating high-resolution respirometry, histology, and motor performance

Running title: Skeletal muscle phenotype in TFAM-deficient mice

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State-of-the-Art: Mitochondrial transcription factor A (TFAM) is essential for the maintenance of mitochondrial DNA and mitochondrial gene expression. Altered TFAM dosage in skeletal muscle has been linked to mitochondrial remodeling and impaired oxidative phosphorylation. However, the functional consequences for muscle strength and mitochondrial respiratory capacity during ageing remain insufficiently characterized. We hypothesize that TFAM insufficiency leads to reduced mitochondrial respiratory capacity in skeletal muscle and may be compensated for by increased muscle strength.

Objective: To define the contribution of TFAM insufficiency to muscle strength in laboratory mice and to assess the involvement of TFAM in mitochondrial respiratory capacity in skeletal muscle.

Material and Methods: A mouse model of TFAM insufficiency (Tfam wt/ Δ) and wild-type (WT) control animals underwent longitudinal motor testing throughout life, with emphasis on muscle strength and coordination/locomotor assays (e.g., grip strength, Rotarod, Erasmus ladder, T-maze). At ~13 months of age, skeletal muscles were collected, with priority given to the quadriceps for high-resolution respirometry and parallel molecular profiling. Oxidative capacity was measured using high-resolution respirometry (Oxygraph-2k). Histological evaluation was performed in selected muscles (quadriceps/soleus/gluteus). All animal experiments were conducted in accordance with Act No. 246/1992 Coll. (Project No. MSMT-33798/2021-4).

Results & Discussion: Functional data do not suggest increased muscle strength in Tfam wt/ Δ mice compared with WT controls in grip strength testing. High-resolution respirometry of skeletal muscle showed that the muscles of Tfam wt/ Δ animals preserved substrate-supported respiratory responses. Surprisingly, we found a negative correlation between grip strength and mitochondrial respiratory parameters across both Tfam wt/ Δ and WT animals.

Conclusion: This study characterizes Tfam-insufficient mice and seeks to identify mitochondrial determinants of motor performance. The limitation is the small sample size. However, as the study is ongoing, further inclusion of animals will strengthen validation of the findings.

Funding: Cooperatio – field of science Immunity and Infection

In vitro study of Sertoli-Leydig system using human primary granulosa cells to better understand male infertility treatment

Running title: Study of Sertoli-Leydig system

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State-of-the-Art: Male infertility is a health problem with socio-demographic implications; in ~40% of cases the cause remains unknown. Spermatogenesis is regulated by gonadotropins and testosterone. In hypogonadotropic hypogonadism (HH), treatment with GnRH or exogenous gonadotropins—most commonly (human chorionic gonadotropin) hCG alone or combined with FSH—raises intratesticular testosterone and restores spermatogenesis. hCG binds LH receptors (LHCGR) on Leydig cells and stimulates testosterone production, while FSH acts on Sertoli cells, probably inducing growth factors that enhance Leydig cell responsiveness to LH/hCG. However, in idiopathic infertility LH and FSH levels are usually normal, and it remains unclear whether endogenous FSH sufficiently supports sperm production or quality.

Objective: The aim of the study was to create an in vitro model recapitulating the Sertoli–Leydig signalling to analyse the effect of FSH and hCG on testosterone production in Leydig cells. To this purpose, ovarian primary granulosa cells (hGLC) was used as a substitute for Sertoli cells (not available).

Material and Methods: The production of testosterone was evaluated in the murine Leydig tumor cell line 1 (mLTC1), expressing mouse LH receptors capable to bind human LH and hCG. mLTC1 cells were first exposed (pre-treatment) to conditioned medium from cultured hGLC maintained in the presence or in the absence of FSH. mLTC1 pre-treated with unconditioned medium served as a control.

Medium was collected and evaluated for testosterone production by Homogenous Time-Resolved Fluorescence (HTRF) assay.

Results were presented as dose-response curves for testosterone and progesterone production, as values plotted against hCG and LH concentration in a X-Y graph. The 50% effective concentrations and other pharmacological parameters will be extrapolated from the dose-response curves and statistically compared.

Results & Discussion: mLTC1 cells pre-treated with unconditioned control medium showed a gonadotropin-dependent increase in testosterone. hCG was more potent than LH, inducing a testosterone plateau about twofold higher. Pre-treatment with hGLC-conditioned medium reduced LH/hCG potency compared with control medium. The effect was stronger when the conditioned medium derived from FSH-treated hGLC, suggesting ovarian cells release unidentified factors that decrease Leydig cell responsiveness to gonadotropins. This effect may depend on Gs protein expression, as the mLTC1 response to CTX, but not to forskolin, declined together with LH/hCG-induced responses.

Conclusion: Results suggest that ovarian cells produce paracrine factors reducing gonadotropin responses in nearby LHCGR-expressing cells. Thus, hGLC cannot replace Sertoli cell

functions that enhance Leydig cell responses to LH/hCG, supporting the existence of sex-specific regulator of gonadal cell metabolism.

Funding: This work was supported by the Cooperatio Program, research area Pharmaceutical Science.

Research of Psychiatrization: The Identity of Young People in the Mirror of Psychiatric Diagnosis

Running title: The Impact of Psychiatric Treatment on Adolescents' Self-Concept and Self-Narrative

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State-of-the-Art: Current literature indicates that psychiatric diagnosis can significantly influence patients' self-perception and interpretation of life experiences. This effect is particularly strong during adolescence, a critical period for identity formation. Diagnosis can disrupt personal narrative, leading to feelings of difference, self-stigmatization, or identification with the illness, but can also facilitate understanding and integration of difficulties into one's life story. Existing research is largely retrospective or cross-sectional and focuses on selected diagnoses. Prospective qualitative studies tracking self-concept development from entry into psychiatric care across neurotic disorder diagnoses remain limited. Understanding these processes may inform more sensitive therapeutic approaches.

Objective: The study aims to explore how receiving a psychiatric diagnosis and starting treatment influence the self-narrative and self-concept of adolescents and young adults, and to understand the processes through which psychiatric care shapes interpretation of experiences and personal identity.

Material and Methods: The study employs a prospective longitudinal qualitative design. Data are collected through a series of semi-structured in-depth interviews with adolescents who have not previously received psychiatric treatment. Participants with primary diagnoses of organic disorders (F0), substance use disorders (F1), psychotic disorders (F2), or intellectual disability (F7) were excluded. Interviews focus on experiences of the diagnostic process, treatment, participants' personal life and environment, and changes in self-perception over time. Data collection occurs repeatedly over an 18-month period. Interviews are transcribed and analyzed using thematic analysis to identify patterns of change in participants' self-narrative and self-concept.

Results & Discussion: We hypothesize that initiation of psychiatric treatment in adolescents will be associated with changes in sense of self-concept and self-narrative, particularly in relation to receiving a psychiatric diagnosis and assuming the patient role. Interview analysis may reveal diverse ways in which young people interpret their treatment experiences and integrate them into their life story. We expect to identify factors that support adaptive understanding of the diagnosis, as well as risk processes leading to self-stigmatization or over-identification with the patient role. Findings may inform more sensitive therapeutic approaches and promote understanding of treatment that is motivating and empowering for patients.

Conclusion: The study may enhance understanding of how psychiatric diagnosis and treatment influence adolescents' self-perception. Findings could support more sensitive therapeutic approaches and help prevent self-stigmatization and over-identification with the patient role.

Funding: The research is conducted within the Psychiatrization Research project, supported by a grant from the Charles University Grant Agency (GA UK).

Typology and Triggers of Aggression in Organic Mental Disorders: Findings from a Prospective Study

Running title: Typology and Triggers of Aggression

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State-of-the-Art: A large proportion of patients with organic mental disorders exhibit agitated or aggressive behavior. However, in clinical practice, these manifestations are often not distinguished, and the triggers of problematic behavior (e.g., pain, frustration, change of environment, or somatic comorbidity) are not systematically investigated. This often results in inappropriate or ineffective treatment. This study shows that more accurate diagnosis and targeted differentiation of these manifestations allows for a better understanding of their causes and subsequent causal treatment.

Objective: The main objective of this study was to pilot test the systematic assessment of aggression in patients with organic mental disorders, classify incidents into impulsive, psychotic, and psychopathic domains, and analyze their immediate triggers, consequences, and coping strategies.

Material and Methods: Over a period of 14 months, incidents in the acute psychiatric ward for adults in the Czech Republic were prospectively monitored. Only patients with organic mental disorders (ICD-10 F00–F09) were included in the study. Aggressive behavior was recorded using the Modified Outward Aggression Scale (MOAS). A violent incident was defined as a physically aggressive attack against persons or property with a weighted score ≥ 3 . Self-harm was analyzed separately; autoaggression was not included. Assaults were classified into impulsive, psychotic, or psychopathic domains using the Assault Interview Checklist. The Staff Observation Aggression Scale (SOAS) was used to capture context, targets, consequences, and coping methods.

Results & Discussion: Of the 31 aggressive incidents, impulsive incidents were the most common ($n = 74.2\%$, 23/31), psychotic incidents were less common ($n = 25.8\%$, 8/31), and psychopathic incidents were not recorded (0%). SOAS documentation highlighted common triggers such as confusion, refusal of medication, hygiene-related care, and delirium. Aggression was predominantly verbal with minor physical manifestations (typically punching or kicking). Incidents were mostly directed against staff and rarely against other patients. The consequences were generally mild. Impulsive incidents usually did not require medical treatment and were resolved by verbal de-escalation, sometimes followed by brief isolation or mild restraint, while sedatives were used as needed in cases of psychotic aggression.

Conclusion: Aggression in organic mental disorders is mainly impulsive and less often psychotic; no psychopathic attacks were observed. Triggers included confusion, treatment refusal, and nursing care. Management ranged from de-escalation/brief restraint to antipsychotics in psychotic cases.

Victimization of patients with gender identity disorders

Running title: Victimization of transgenders

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State-of-the-Art: Transgender and gender-diverse (TNB) individuals do not identify with their sex assigned at birth (e.g., trans women, trans men, non-binary, or gender-fluid). In the Czech Republic, past estimates were in fractions of a per mille; current estimates may be up to ten times higher, but reliable epidemiological data are lacking. In the USA, ~150,000 youth and 1.4 million adults identify as transgender. Studies report significant healthcare barriers: 24 % experienced unequal treatment, 19 % care refusal, and 33 % did not access preventive services. In a study on healthcare experiences of TNB patients, 71 % reported at least one form of victimization. Clinical studies report PTSD prevalence of 9.8 % among transfeminine young adults.

Objective: The study aims to determine the prevalence of victimization in patients with gender identity disorders using scales translated and validated by our team. A second aim is to identify reasons for underdiagnosis of trauma consequences via qualitative focus group research.

Material and Methods: Data will be collected from 40 patients with gender identity disorders at the LFP UK Psychiatric Clinic and outpatient visits with Prof. Fiala at PK FN Plzeň. Selection, power analysis, and exclusion criteria ensure at least 28 valid participants, sufficient for the primary hypothesis (victimization in one-third of patients, $\alpha=0.05$, 90 % power, US Transgender Survey 2015: 71 %) and secondary hypothesis (lifetime PTSD 20 %, $\alpha=0.05$, 90 % power, Valentine et al. 2023: 49 %). Diagnoses of psychosis, gender identity disorders, and PTSD will be assessed via M.I.N.I. 5.0.0. and SCID modules; victimization and violence via MacArthur Interview, MOAS, and CECA.Q. Qualitative focus groups with staff and patients, moderated externally, will explore diagnostic barriers.

Results & Discussion: The project will provide the first systematic data on victimization among patients with gender identity disorders (F64.0–F64.9). Quantitatively, we will determine the prevalence of victimization and PTSD (expected in one-third and 20 % of patients). Qualitative focus groups will identify barriers to diagnosing and treating trauma consequences. Findings will support education of care users and the public via an online guide, optimize clinical procedures, improve patient–clinician communication, and inform targeted therapeutic interventions.

Conclusion: The study will provide the first data on victimization and PTSD in patients with gender identity disorders, identify diagnostic barriers, support patient and staff education, and inform targeted therapeutic interventions and care improvement.

Funding: The project is not yet funded and is conducted under the auspices of the Psychiatric Clinic, FN Plzeň.

Evaluation of detransition in transgender patients

Running title: Detransition in Transgender Patients

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State-of-the-Art: Current knowledge in transgender health care shows that detransition remains far less studied than transition itself. Available studies differ in their definitions of detransition, follow-up periods, and methodologies, which makes comparison of results and estimation of its actual frequency difficult. The literature therefore still lacks sufficiently robust data from clinical practice. The proposed project focuses on determining the number of detransitions among transgender patients and aims to contribute to a more accurate understanding of this phenomenon, a better interpretation of existing findings, and the further development of informed clinical care.

Objective: To determine the number of detransitions among transgender patients and contribute to a better understanding of their occurrence in clinical practice, providing data to support more accurate interpretation and evidence-based transgender health care.

Material and Methods: This retrospective multicenter study analyzes data from transgender patients treated at two centers: the Department of Sexology, University Hospital Pilsen, and the Sexological Institute, General University Hospital in Prague. Included were patients whose first contact took place at one of these centers and who had undergone transition-related care for at least 5 years. Medical records were reviewed to identify patients who requested detransition, defined as discontinuation of transition, and, where applicable, the documented reasons for this decision.

Results & Discussion: The study is expected to provide data on the frequency of detransition among transgender patients at two Czech specialized centers and to identify the most commonly documented reasons for this decision. The findings may help clarify how often detransition occurs in long-term clinical practice and contribute to a more nuanced interpretation of this phenomenon. Given the limited and methodologically heterogeneous evidence available, the results may support better counseling, follow-up, and evidence-based clinical care.

Conclusion: This project will provide data on detransition among transgender patients in the Czech Republic and help clarify its frequency and reasons in long-term clinical care, supporting more informed and evidence-based transgender health care.

From Sample to Signal: MALDI-TOF MS for Rapid Microbial Identification Without Cultivation

Running title: Rapid Culture-Free MALDI-TOF MS Identification

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State-of-the-Art: Despite advances in Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) for clinical microbiology, serotyping and detection from clinical samples remain limited. We recently introduced a MALDI-TOF MS method for detecting microbial cell wall polysaccharides using a self-ionizable HD ligand (doi.org/10.3389/fcimb.2025.1658802). The ligand binds saccharides and enables ligand-assisted ionization. Microbial polysaccharides are excellent targets, being abundant, stable, and specific, as they constitute some of the microbial surface antigens. Here, we demonstrate this approach for direct detection from clinical specimens without prior cultivation.

Objective: The hypothesis of the study was to demonstrate whether an innovative approach to polysaccharide ionization for analyzing bacterial polysaccharides and other external structures of the cell wall can facilitate bacterial identification and typing by MALDI-TOF MS directly from clinical specimen.

Material and Methods: Analyses were performed with mono- and polysaccharide models (glucose, lactose, β -D-glucan, galactomannan, lipopolysaccharides from G- bacteria, e.g., *Escherichia coli* O55:B5 and O111:B4) and overnight cultures of *Escherichia coli*, *Streptococcus pneumoniae*, and *Candida* spp. Standards or a 1 μ L bacteriological loop were diluted in 200 μ L serum or urine. After acid protein precipitation, samples were heated at 95 °C for 10 min, centrifuged, and then cooled to 50 °C. The pH was adjusted to 3, followed by the addition of 2 μ L of a newly designed self-ionizable HDT ligand and 20-minute incubation. Detection was carried out using LC-MS and MALDI-TOF MS.

Results & Discussion: We adapted the previously published method for direct detection of microbial polysaccharides from clinical samples. We developed a novel derivatization agent, the HDT ligand, with improved thermal stability over the original HD ligand and a simplified synthesis. The HDT enabled clear ionization of hexoses and hexose disaccharides, with characteristic signals at m/z 481 and 643, respectively. Native glucose was detected in urine and serum; however, microbial samples yielded markedly stronger glucose signals.

Conclusion: This refined MALDI-TOF MS assay allows direct identification of microbial polysaccharides in serum and urine without cultivation. The HDT ligand's stability and efficient ionization support a rapid, cultivation-free strategy for microbial detection with strong potential for clinical application.

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Comparison of Quantitative Cerebrospinal Fluid Cytology Using Manual Counting and the Sysmex XN-1000

Running title: Comparison of CSF Cytology: Manual vs XN-1000

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State-of-the-Art: Cerebrospinal fluid (CSF) cytology is critical for diagnosing neurological diseases, traditionally performed by manual cell counting in a Fuchs–Rosenthal chamber. This method is time-consuming and dependent on skilled personnel. Automated haematology analysers such as the Sysmex XN-1000 offer faster results in body fluid mode, potentially improving laboratory workflow. However, their accuracy—especially for differentiating cell types like polymorphonuclear cells (PMNc) and in samples with interfering elements—is debated. Comparing manual and automated methods helps define limitations and clinical reliability of automation in routine CSF analysis.

Objective: The study aimed to compare quantitative CSF cytology results obtained by manual counting in a Fuchs–Rosenthal chamber with those from the Sysmex XN-1000 analyser, focusing on accuracy, agreement, and limitations of both methods.

Material and Methods: A total of 89 CSF samples from patients aged 10 days to 80 years were analysed. Manual counting was performed using the F–R chamber, while the Sysmex XN-1000 measured cell counts in body fluid mode. Statistical comparisons in Medcalc software included the Shapiro–Wilk test, Wilcoxon test, Bland–Altman plots, Mann–Whitney test, and Cohen’s kappa coefficient. Statistical significance was defined as $P < 0.05$.

Results & Discussion: There were no statistically significant differences between the F–R chamber compared to Sysmex in mononuclear cells. Data presented as median [min–max]: 10 [1–724]/ μl vs. 9 [0–670]/ μl resp. ($P = 0.1286$); and in red blood cells: 36 [0–18,091]/ μl – vs. 0 [0–34,000]/ μl resp. ($P = 0.5412$). However, there were statistically significant changes in polymorphonuclear cells ($P = 0.0002$) using the F–R chamber – 0 [0–1,428]/ μl in comparison with Sysmex – 1 [0–1,008]/ μl . Cohen’s kappa analysis demonstrated a very good strength of agreement ($K = 0.89$) in the classification of oligocytosis and pleocytosis.

Conclusion: The Sysmex XN-1000 is a fast and efficient tool for CSF cytology but it has limitations, especially in differentiating PMNc in siderophage–rich neonatal samples. Although it improves workflow, manual verification remains crucial for diagnostic accuracy.

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NMDA Receptor Blockade by MK801 Disrupts CA1 Ensemble Stability Beyond Baseline Representational Drift

Running title: Enhanced representational drift in psychosis model

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State-of-the-Art: Memories emerge from coordinated activity of large neuronal populations whose collective dynamics should remain stable enough to support retrieval while flexible enough to encode new experiences. Disturbances of this balance might result in hallucinations. In hippocampus, memory patterns evolve over time through representational drift, where ensemble memory pattern gradually changes while its spatial structure is preserved. NMDA-dependent plasticity is thought to regulate this balance between stability and flexibility. MK801 -an NMDAR blocker used as a model of psychosis, offers an opportunity to test this phenomenon. However, it remains unclear to what degree NMDAR disruption primarily affects single-neuron information code, stability and organization of population-level representations.

Objective: To examine how NMDA receptor blockade affects hippocampal CA1 network dynamics by comparing natural representational drift and changes in ensemble stability and spatial coding following pharmacological perturbation.

Material and Methods: GCaMP6f-expressing mice were implanted with GRIN lens into hippocampal CA1 to visualize their single cell activity during navigation in different environments across four days. Day 0 consisted of familiar-environment recording to set the baseline spatial representation. Days 1–3 mice were exposed to familiar and multiple novel environments to quantify the physiological memory coding properties. On Day 4, the same behavioral sequence was performed, but animals received an NMDAR blocker MK-801 (0.2mg/kg). Recorded cells were sorted across all days and parameters as spatial firing maps, spatial information, including their similarity, were quantified. Data recorded with and without the MK-801 injection were compared.

Results & Discussion: Familiar-track representations exhibited gradual representational drift, with session-to-session ensemble correlations slowly decreasing across days while remaining relatively stable within each day. Novel-track exposures produced distinct representations that remained internally consistent across trials. MK-801 (day4) produced a pronounced reduction in similarity between post-injection sessions and prior familiar representations. Despite this population-level destabilization, spatial modulation and neural firing rates persisted. Place fields remained detectable but were broader and less spatially precise.

Conclusion: NMDAR blockade with MK-801 produced a pronounced destabilization of neuronal ensemble representations beyond baseline representational drift, in both familiar and novel environments. This highlights a key role for NMDA-dependent mechanisms in maintaining stable network representations.

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Neural code during memory retrieval in hippocampal networks CA3 and CA1

Running title: Neural code during memory retrieval in hippocampus

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State-of-the-Art: Hippocampus plays a central role in spatial navigation and memory. It stores a complex representation of the surrounding environment in its CA3 and CA1 subnetworks, which is considered as substrate of memory traces. Our previous experiments characterised the dynamics of transition between memory traces in the CA3. We identified a profound competition between memory patterns, resulting in rapid, frequent shifts paced by local 6-12 Hz EEG oscillations. We also discovered a transient overexpression of the newly activated CA3 memory trace, as the increased number of neurons became briefly coactive. However, it remains unclear whether these phenomena are specific to CA3 memory processing or whether similar dynamics are projected outside of the hippocampus through its output network CA1.

Objective: To compare the dynamics of memory trace recall in neuronal networks of dorsal hippocampal CA1 and CA3.

Material and Methods: Population of individual neuronal activity was recorded using multiple microelectrodes implanted into dorsal hippocampal CA1 and CA3 in rats, while performing the teleportation paradigm (Jezek et al., 2011). Animals explored an arena with two sets of light cues that were abruptly switched to induce a rapid transition between the respective spatial memory representations. Individual spikes were cell-sorted, and the respective memory patterns were identified. Dynamics of transition between the patterns were analysed using custom written MatLab scripts. Electrode placement was later confirmed using histology examination at the end of the experiment.

Results & Discussion: Recordings from CA1 and CA3 were obtained during the rapid transitions between spatial representations using the teleportation paradigm. Consistent with previous findings, CA3 activity showed overexpression. In contrast, CA1 population activity did not exhibit a comparable overexpression. The absence of this effect in CA1 may be related to its distinct circuit organisation, particularly the lack of recurrent collateral connectivity and the presence of strong feed-forward inhibitory inputs that may prevent excessive activation.

Conclusion: Transient overexpression of spatial representations was observed in CA3 but not in CA1 during recall after environmental transitions. These findings suggest region-specific mechanisms of spatial memory retrieval in the dorsal hippocampus, potentially related to differences in network architecture.

Funding: GACR-26-23770S and SVV-263774.

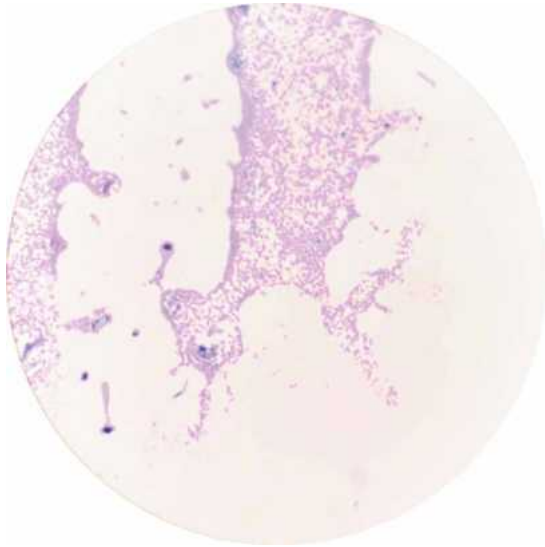


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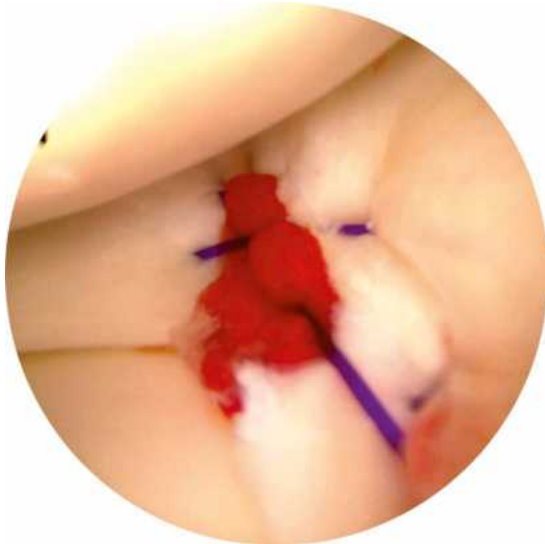
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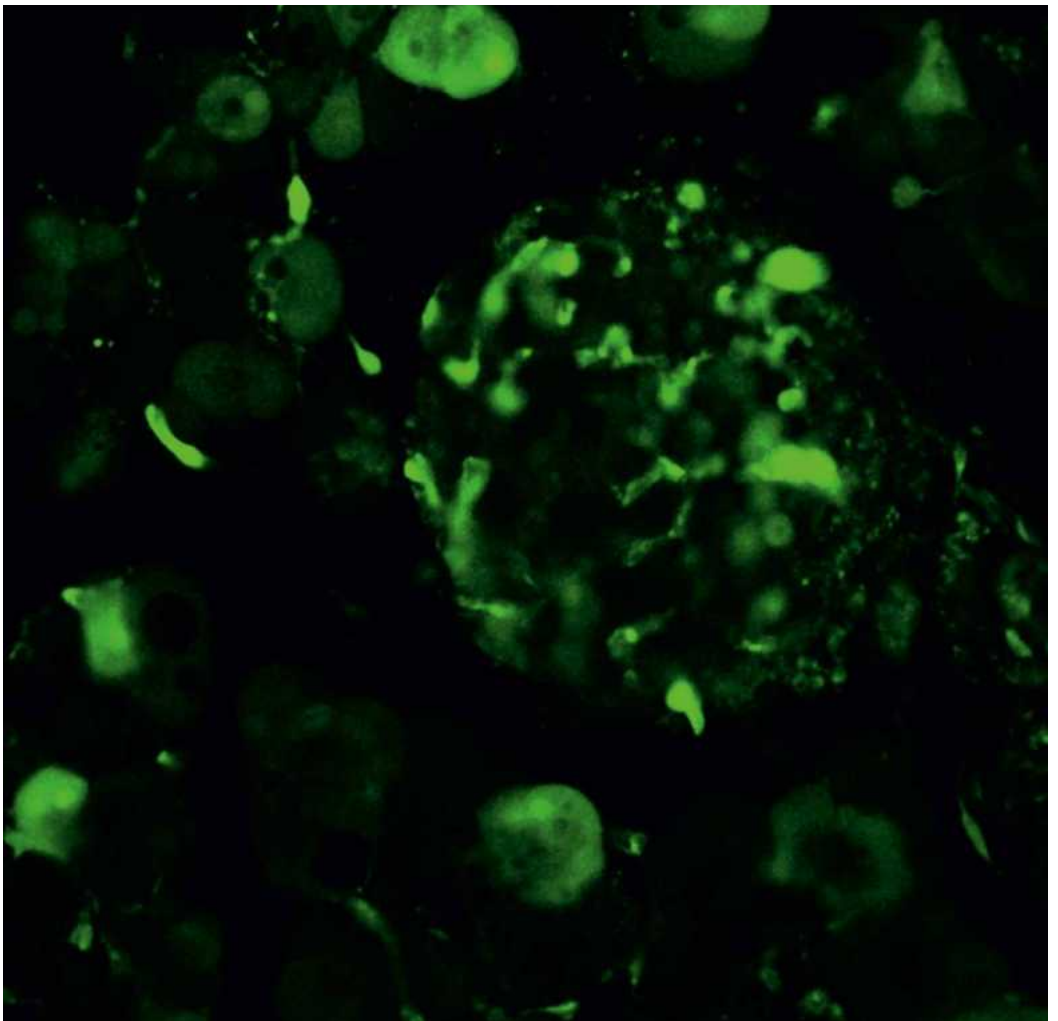
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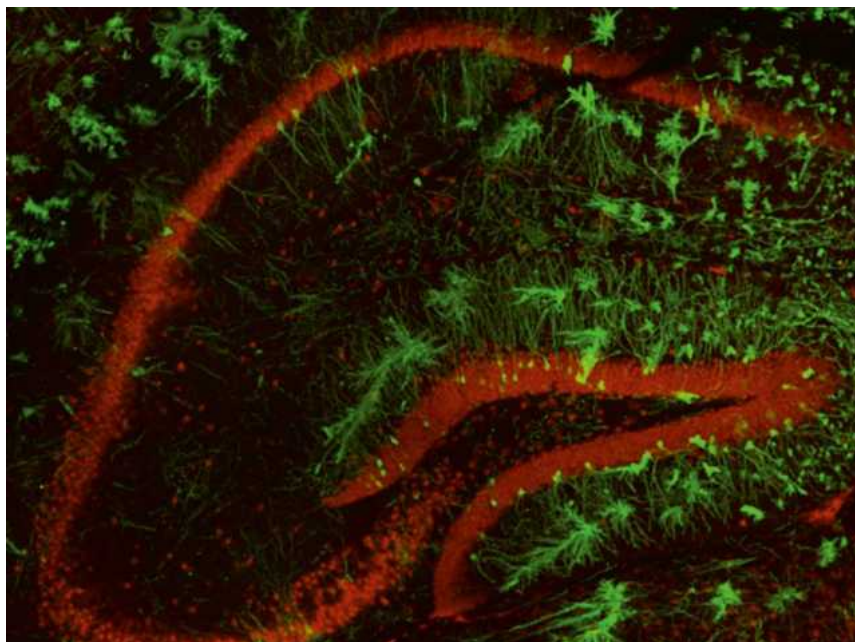
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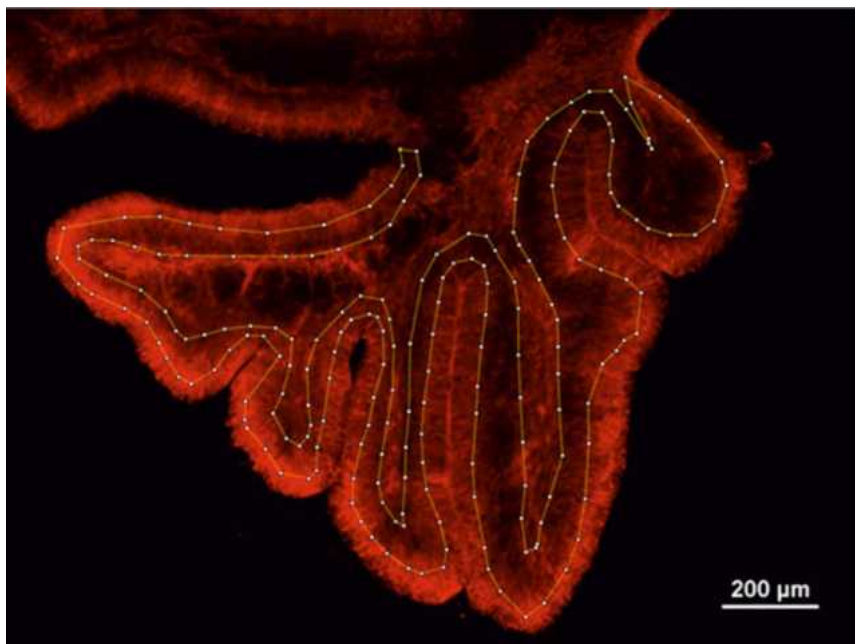
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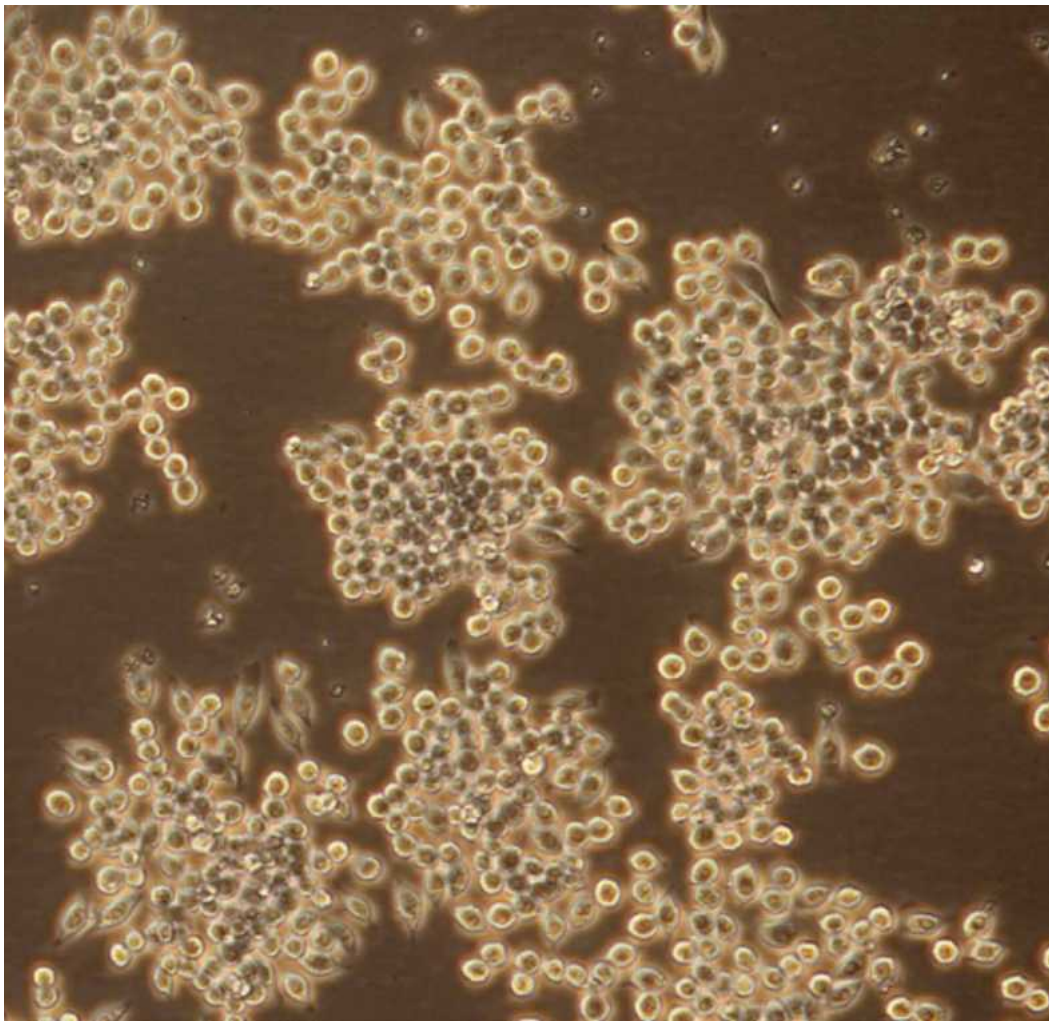
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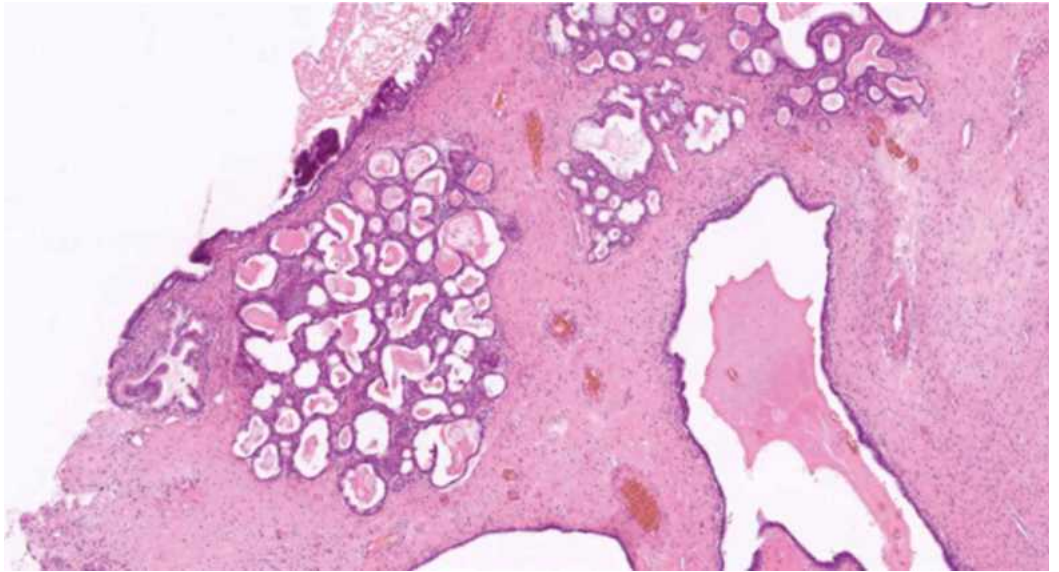
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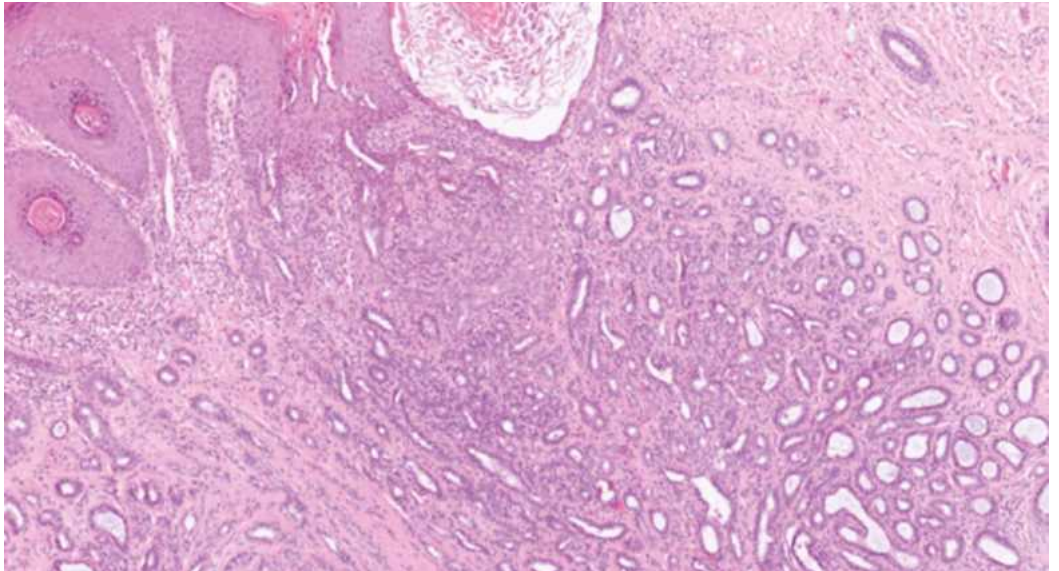
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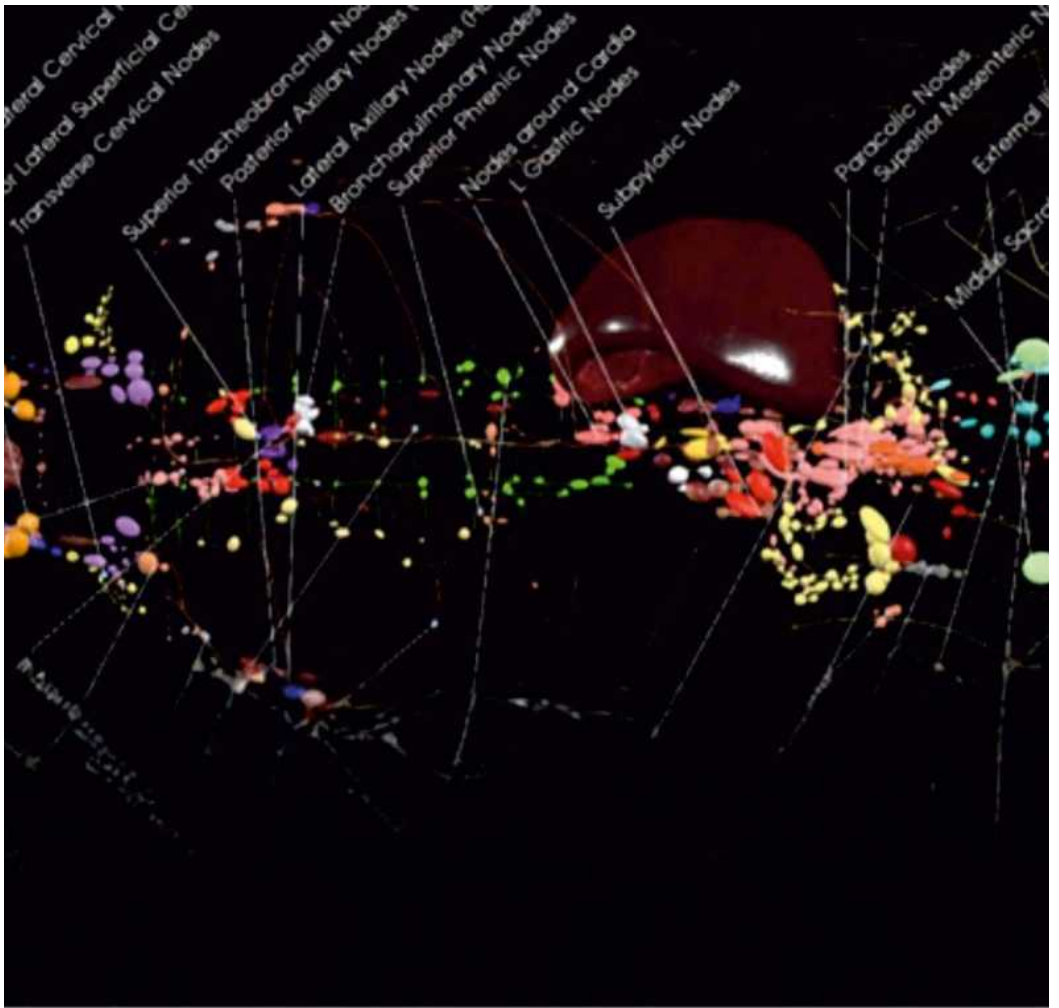
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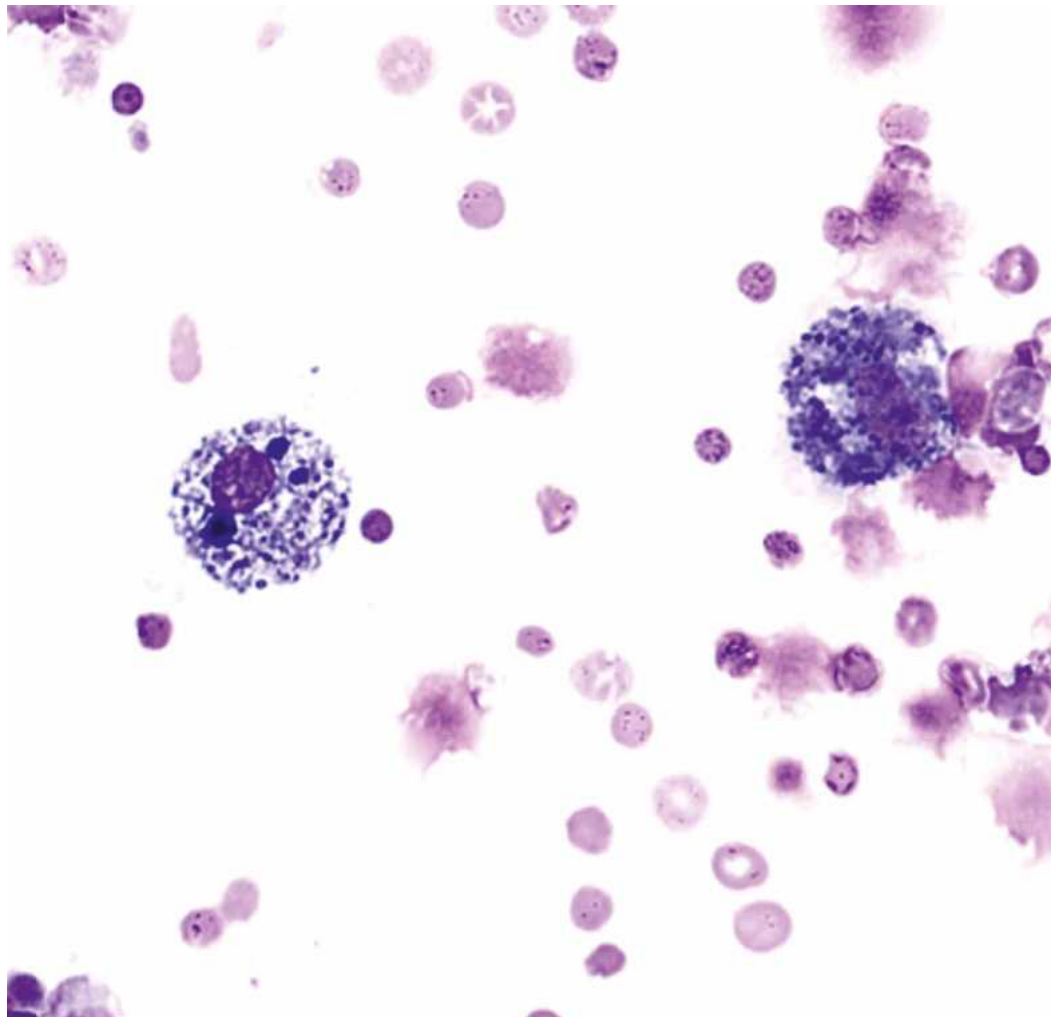
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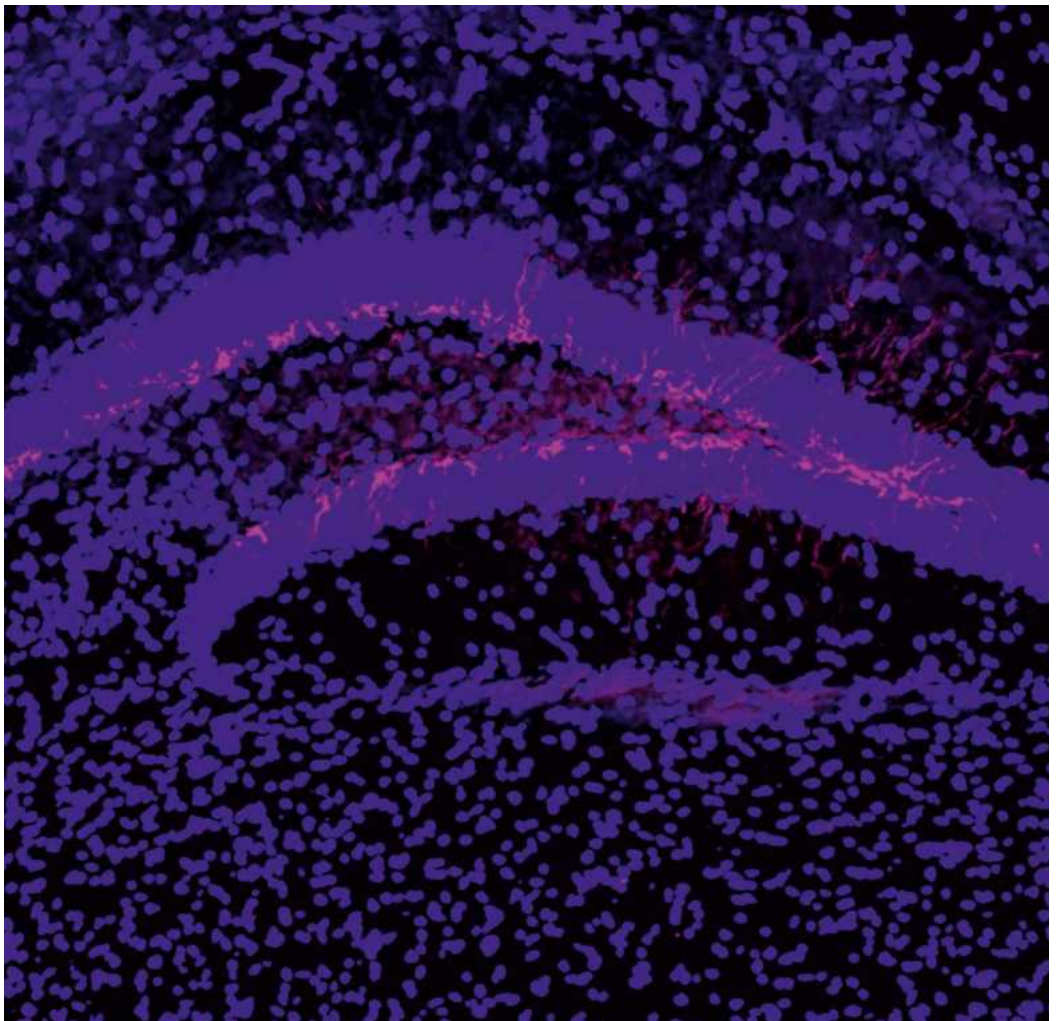
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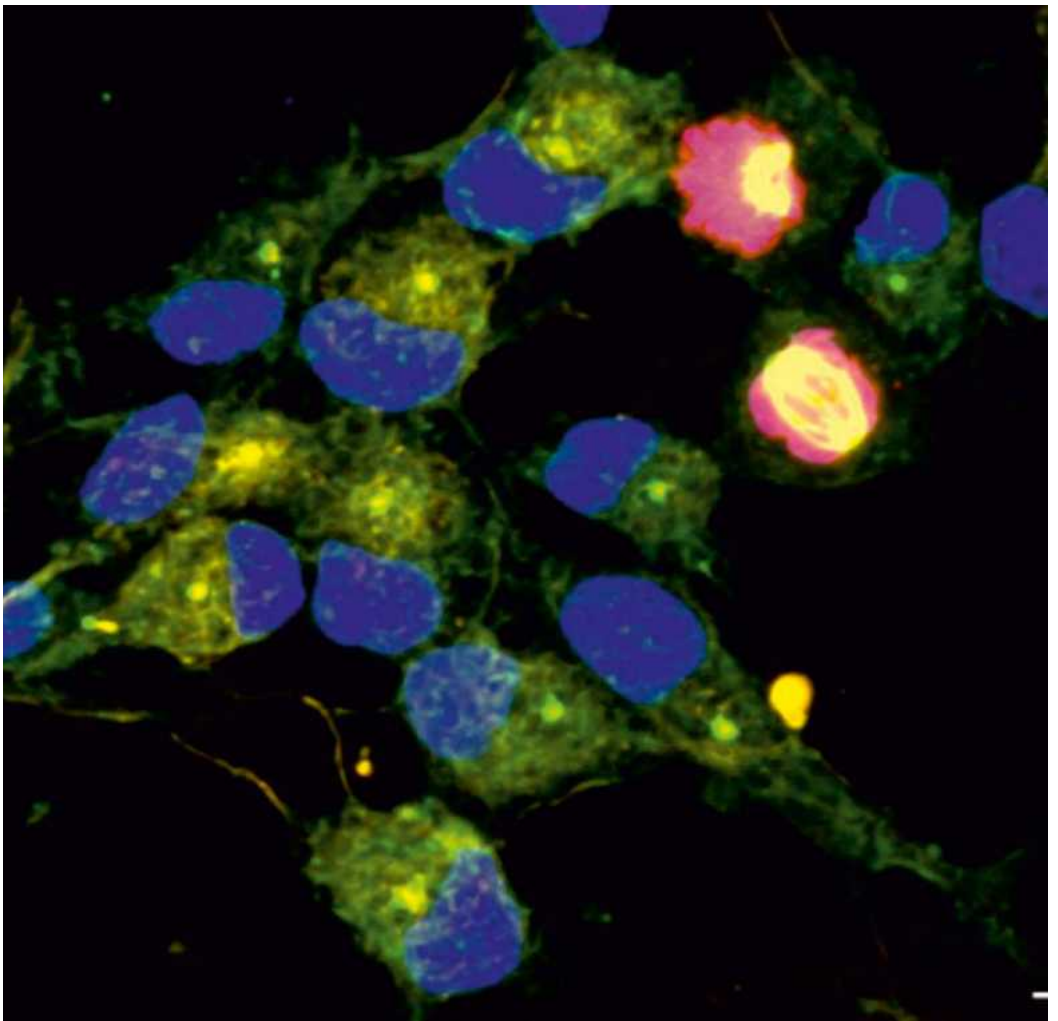
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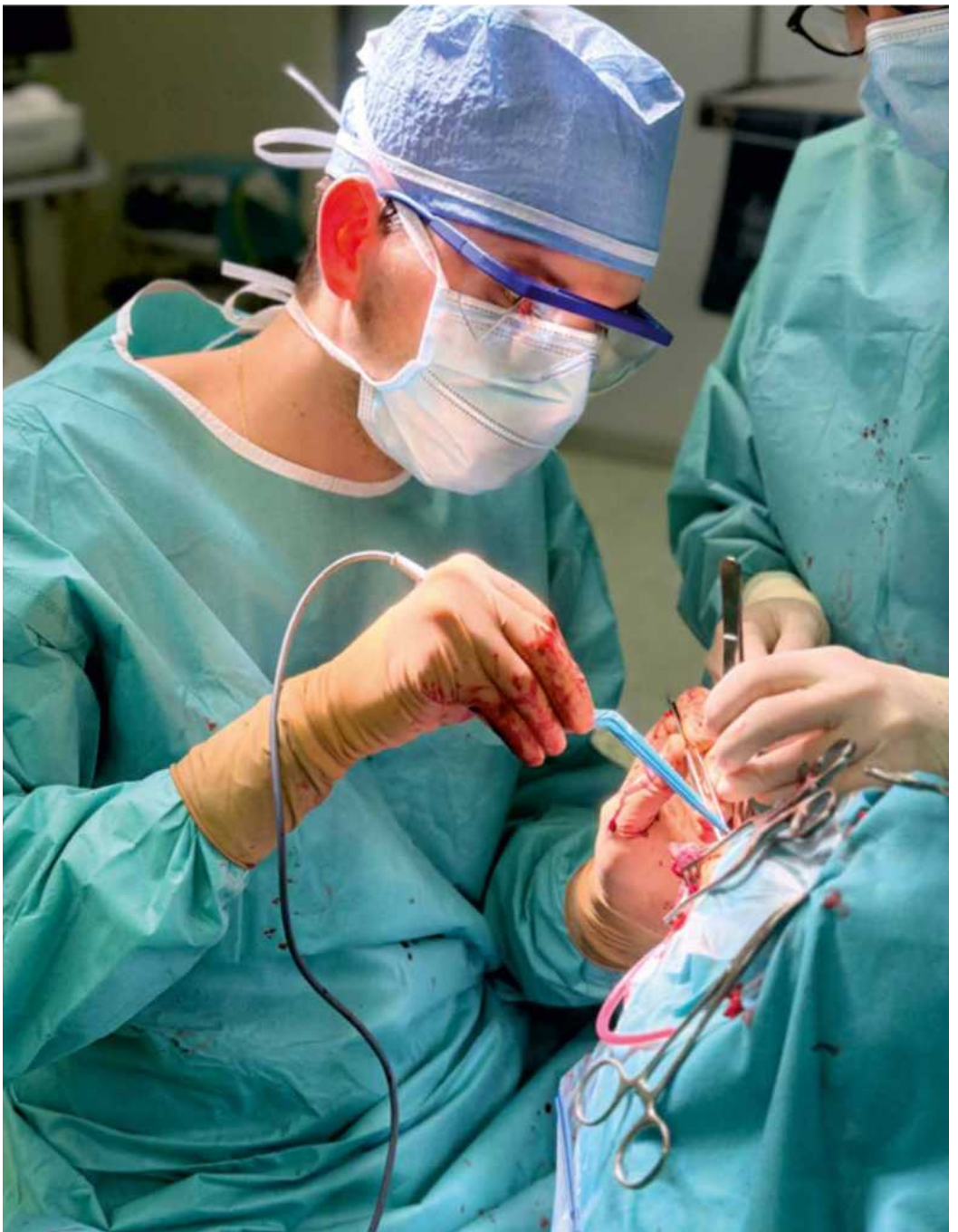
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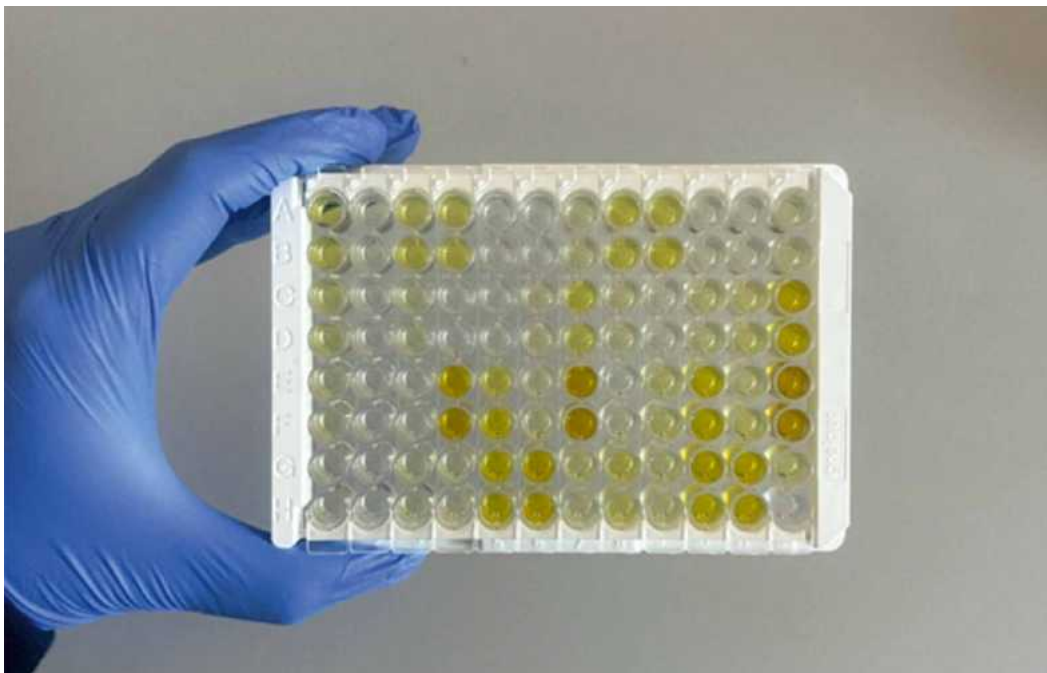
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