Charles University

Empowering PhD Research: New Challenges and Opportunities in Medicine

21ST INTERNATIONAL MEDICAL DOCTORAL CONFERENCE 2024

Abstract Book

Content

About IMDC

Since 2005 the Faculty of Medicine in Hradec Králove has been annually organizing an international conference of PhD students from medical faculties – the International Medical Doctoral Conference in Hradec Králové.

The number of European participants is increasing every year (students as well as evaluators). The level of individual conference papers and the prestige of the conference are constantly rising.

Organizing Committee

Prof. Dr. Milan Kaška, M.D., Ph.D.

Prof. Romana Koberová Ivančaková, M.D., Ph.D.

Assoc. Prof. Ilja Tachecí, M.D., Ph.D.

Contacts

Email: conference@lfhk.cuni.cz, visit our Official website

prof. MUDr. RNDr. Milan Kaška, Ph.D., Vice-dean for doctoral study programmes
kaskam@lfhk.cuni.cz

Mgr. Tereza Silbernaglova, Project manager
silbernaglova@lfhk.cuni.cz

Support

Supported by the Special University Research Grant of Charles University no. 260546/2020

Under the Auspices of

Her Magnificence, Rector of the Charles University Milena Králíčková

and Spectabilis, dean of Medical Faculty in Hradec Králové Jiří Manďák.

Programme

The programme is subject to change.

Wednesday November 20, 2024

Lecture Hall, Educational Center FM

9:00 **Opening ceremony**

Lectures

Session 1

Chairs: **Jakub Radocha, Vladimír Koblížek**

9:15 **Stribrny, Martin (2nd Faculty of Medicine, Charles University, Czech Republic):** Assessing Trunk Muscle Function and Intra-Abdominal Pressure: Evaluations and Correlations in Clinical Practice

9:30 **Smolák, Petr (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Prediction of early postoperative morbidity and ability to rehabilitate in patience after cardiac surgery („CardioStep Study“)

9:45 **Šenkýřová, Tereza (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Profiling Methylation Changes of DNA in Lung Cancer

10:00 **Vigláš, Pavol (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Outcome of tailored antiplatelet therapy in carotid stenting: a retrospective comparative study

10:15 **Tůma, Tomáš (Military Faculty of Medicine, Hradec Králové, University of Defence, Czech Republic):** Balloon-occluded chemoembolization of hepatocellular carcinoma

10:30 **Coffee break**

Session 2

Chairs: **Hana Langrová, Romana Koberová Ivančaková**

10:50 **Awad, Omar Masoud Morshedy Mohamed (Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia):** Effect of Platelet Rich Plasma (PRP) in treatment of late stage osteoarthritis

11:05 **Broskevičová, Lucie (Faculty of Medicine, University of Ostrava, Czech Republic):** Optimization of cytokine profiling in multiple myeloma patients undergoing modern immunotherapy

11:20 **Kałuska, Barbara (Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland):** Diagnostic and Therapeutic Challenges in Patients with Congenital Anomalies of the Kidney and Urinary Tract (CAKUT)

11:35 **Kukučka, Tomáš (Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia):** Fluoxetine in adolescent depression: acute effects of monotherapy vs. quetiapine augmentation on body composition and autonomic stress response

11:50 **Short break**

Session 3

Chairs: **Jakub Radocha, Ilja Tachecí**

11:55 **Bartoš, Michal (Faculty of Medicine, Masaryk University, Czech Republic):** Microbiome of the middle ear in children with chronic otitis media: a preliminary study

12:10 **Ibrahim, Rana (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** The Effect of Gestational Diabetes Mellitus on Infant Development: Umbilical Cord Blood Level of C-peptide and Glycemia

12:25 **Košťálová, Kateřina (Military Faculty of Medicine, Hradec Králové, University of Defence, Czech Republic):** Interim Analysis: Endoscopic Treatment of Gastroesophageal Reflux Disease by Applying Radiofrequency Energy to the Lower Esophageal Sphincter

12:40 **Kolesár, Dushan Michael (3rd Faculty of Medicine, Charles University, Czech Republic):** Sex-Linked Differences in Cardiac Atrophy After Mechanical Unloading Induced by Heterotopic Heart Transplantation

12:55 **Lunch**

Session 4

Chairs: **Martina Řezáčová, Emil Rudolf**

13:45 **Kápičková, Kateřina (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Testing the effect of temozolomide and flubendazole in glioma primary cell cultures and 3D cell cultures

14:00 **Chebernina, Inna (Medical, SE Lugansk State Medical University, Ukraine):** Effects Of Colon Cancer On Hippocampal Astrocytes In Dental Gyrus: Neuroinflammation And Astrogliosis

14:15 **Geryková, Lucie (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** α-internexin (INA) as a possible effective diagnostic marker of sparsely granulated somatotroph PitNETs: proposition of INA-based classification system

14:30 **Kuchyňka, Jiří (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** A Novel Multi-Modal Approach to Polysomnographic Data Analysis Using Tailored Machine Learning Techniques

14:45 **Németh, Adél Ágnes (Medical Faculty, Dental School, University of Pécs, Hungary):** In vitro study of YD-38 epithelial and MRC-5 fibroblast cells attachment and proliferation on anodized surfaces compared with turned surfaces

15:00 **Coffee break**

Posters

15:15 – 16:45 **Poster presentation 1 - 9**

Chairs: **Milan Kaška, Romana Koberová Ivančaková, Hana Langrová**

16:45 **Short break**

17:00 **Mgr. Martin Schwarzer, Ph.D.: Gnotobiology for a medical practice**
Invited speaker (Czech Academy of Sciences, Czech Republic)

Mgr. Martin Schwarzer, Ph.D., leads the Integrative Physiology of Gnotobionts research group at the Laboratory of Gnotobiology in the Institute of Microbiology of the Czech Academy of Sciences in Nový Hrádek. His three-year stay in Lyon, France, at the Institute of Functional Genomics in Dr. Leulier's group resulted in two first-authored articles in the prestigious journal Science. Among the primary scientific questions he and his colleagues address are the effects of the nutritional environment and intestinal microbial communities on mammalian host physiology, specifically in the context of mucosal immune system development and the juvenile growth. In 2023, he was awarded the Neuron Prize.

18:00 **Dinner, Social evening**

Thursday November 21, 2024

Lecture Hall, Educational Center FM

Lectures

Session 5

Chairs: **Emil Rudolf, Otto Kučera**

9:00 **Timbilla, Abdul Aziz (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Preparation and biological evaluation of novel harmine β-carboline alkaloid derivatives as potential antitumor candidate

9:15 **Adandedjan, David (Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia):** The Impact of Nephrotoxic Antibiotics on Kidney Graft Viability During Hypothermic Machine Perfusion: A Preclinical Study

9:30 **Kapustová, Veronika (Faculty of Medicine, University of Ostrava, Czech Republic):** Composition and fitness of T and NK cells in extramedullary myeloma tumor microenvironment

9:45 **Cakmakci, Riza Can (Faculty of Medicine, Masaryk University, Czech Republic):** Endoplasmic Reticulum Stress and Unfolded Protein Response Investigation in Patients with Pancreatic Adenocarcinoma

10:00 **Short break**

Session 6

Chairs: **Aleš Ryška, Jan Kremláček**

10:05 **Živná, Natálie (Military Faculty of Medicine, Hradec Králové, University of Defence, Czech Republic):** Synthesis and evaluation of novel compounds as potential active ingredients against CBRN threats

10:20 **Dobrovolskii, Mark (3rd Faculty of Medicine, Charles University, Czech Republic):** Functional properties and pharmacology of zebrafish NMDA receptors

10:35 **Portakal, Türkan (Medicine, Masaryk University, Czech Republic):** Role of LPS on EMT and ER Stress in Lung Epithelial Cells

10:50 **Navratil, Pavel (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Protection of the Endothelium and Endothelial Glycocalyx by Albumin and Sulodexide in Porcine Model of Kidney Transplantation

11:05 **Warwas, Szymon (Doctoral School, Medical University of Silesia in Katowice, Poland):** The safety and efficacy of orthotopic heart transplantation in patients aged 65 and older

11:20 **Coffee break**

Session 7

Chairs: **Aleš Ryška, Martina Řezáčová**

11:45 **Špalková, Veronika (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Changes in lipid and glucose metabolism in cells exposed to hypoxia

12:00 **Melek, Jan (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Obeticholic Acid Induces Uncoupling of Oxidative Phosphorylation in Models of Metabolic Dysfunction-Associated Steatotic Liver Disease

12:15 **Dodzi Kodzitse Lelkes, Krisztina (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Investigating the Degradation Mechanisms of Polydioxanone (PPDX) in Low pH Conditions for Gastrointestinal Applications

12:30 **Kriváková, Eva (Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia):** Role of Hyaluronic Acid Metabolism in Endometrial Receptivity

12:45 **Baďurová, Klára (Faculty of Medicine, University of Ostrava, Czech Republic):** Activation of NK cells by different interleukin combinations

13:00 **Speakers award: The best lecture (start of voting)**

13:05 **Lunch**

Posters

13:50– 15:20 **Poster presentation 10 - 17**

Chairs: **Milan Kaška, Romana Koberová Ivančaková**

15:15 **Meeting of the organizing committee / Free time**

18:00 **Evaluation of the best presentations, music performance**

19:30 **Raut, social evening**

Friday November 22, 2024

Lecture Hall, Educational Center FM

Invited speakers and workshops:

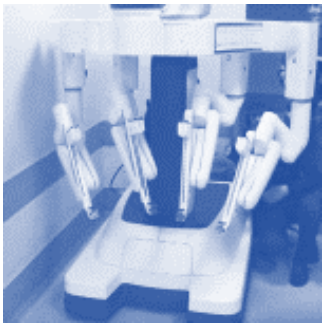
09:00 **Prof. Kurt Zatloukal M.D.: Changes in research paradigms and the impact on medicine**
Invited speaker (Medizinische Universität Graz, Austria)

Kurt Zatloukal, M.D. is a pathologist at the Medical University of Graz. Research focuses on digital pathology and biobanking, the development of molecular diagnostics as well as the molecular pathology of diseases with a focus on infectious disease and biosafety, which includes the BSL-3 laboratory at MUG. He is member of ISIDORe which provides integrated services for infectious disease outbreak research, and he is involved in diagnostics standard development. Other projects: next generation sequencing in medical diagnostics, and building a central repository for digital pathology. He has published over 277 scientific papers and was co-inventor on 26 patent applications.

10:00 **Mgr. Kristina Nešporová, Ph.D.:**
Hyaluronic acid in a research, clinical practice and cosmetics
Invited speaker (Contipro a.s., Czech Republic)

Dr. Kristina Nešporová, molecular biologist and head of the Biological Sciences research group at Contipro a.s. While working at Contipro, Kristina pursued her Ph.D. in Animal Physiology at Masaryk University, defending it in 2016. Her role involves planning research projects, learning new concepts, and sharing knowledge, all without the pressure of securing grants. Working in a private company, she presents results to clients, participates in PR activities, and adapts to strategic management decisions that may shift research priorities. Although her current position limits her lab time, Kristina remains involved in managing scientific activities, seeking new research areas, and exploring collaborations.

11:00 **Workshops**



1. **Experimental and clinical gastrointestinal endoscopy**
   Ilja Tachecí (2nd Department of Internal Medicine – Gastroenterology)
2. **Robot-assisted surgery**
   Michal Balík (Department of Urology)
3. **Digital dentistry**
   Ondřej Heneberk, Dafni Katopodi (Department of Dentistry)

For workshops please register **here**

12:30 **Lunch, Farewell**

Posters

1) **Afshari, Mohammadreza** (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic): Evaluation of newly synthesized FLT-3 inhibitors on proliferation of cancer cells
2) **Alamailess, Yasmeen Mekhled** (Medical School, University of Pécs, Hungary): Comprehensive analysis of invasive and non-invasive methods used in preimplantation genetic testing
3) **Artimovič, Peter** (Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia): Targeting PPARγ in Endometrial Receptivity: Friend or Foe?
4) **Dallmer-Zerbe, Isa Margarethe Irene** (2nd Faculty of Medicine, Charles University, Czech Republic): Data-driven computational modelling of epileptic activity
5) **Morávek, Alexander** (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic): Bone mineral density as a sex determination feature
6) **Staniszewska, Agnieszka** (International Doctoral School, Medical Science, Medical University of Lodz, Poland): Optimization of Adherent Cell Cultures: Enhancing Growth Monitoring with Automated Cell Counting
7) **Striteska, Maja** (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic): Binocular Video Head Impulse: Looking beyond the balance
8) **Vakhnovska, Khrystyna** (Medicine, State Establishment "Lugansk State Medical University", Ukraine): Structural and Morphological Changes in the Blood Vessels of the Kidney of White Rats Under Simulating Different Degrees of Dehydration
9) **Vladyka, Ondřej** (2nd Faculty of Medicine, Charles University, Czech Republic): Profound T lymphocyte and DNA repair defects characterize Schimke Immuno-Osseous Dysplasia

* * *

10) **Aleksandrenko, Hlib** (Faculty of Healthcare, Social Work and Psychology, School of Public Health of National University of Kyiv-Mohyla Academy, Ukraine): Beyond the Clinic: A Pilot Study of a Digital Health Intervention for Extending Mental Health Care in Ukraine
11) **Čermáková, Erika** (Faculty of Medicine, Masaryk University, Czech Republic): Is intermittent fasting a suitable method for the prevention and treatment of obesity?
12) **Écsiová, Dominika** (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic): Unveiling the Spectrum of Genetic Alterations in Relapsed/Refractory CLL Patients on Targeted Inhibitors: A Prospective Unicentric Study
13) **Hemerková, Pavlína** (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic): The Relationship Between Oxidative Stress Markers and the Severity of Amyotrophic Lateral Sclerosis (ALS)
14) **Husivargová Theofanidis, Alexandra** (Faculty of Medicine, PJ Safarik University in Košice, Slovakia/ University Medical Center Groningen, University of Groningen, The Netherlands): Challenges of rheumatoid arthritis and the impact of COVID-19
15) **Sabová, Jana** (Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia): Assessment of Agrimonia eupatoria L. and lipophosphonoxin combination for wound repair: Bridging the gap between phytomedicine and organic chemistry
16) **Świętek, Agata** (Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland): Assessment of serum granulin levels in patients with neuroendocrine tumors of the lung and the gastrointestinal tract
17) **Holmannova, Drahomira** (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic): Vitamin D deficiency is highly prevalent in the Czech Republic: a retrospective study of 119 925 participants

Abstracts

Abstracts are listed alphabetically by presenting author.

Title	**The Impact of Nephrotoxic Antibiotics on Kidney Graft Viability During Hypothermic Machine Perfusion: A Preclinical Study**
Author	**David Adandedjan**
Co-Authors	Hulík Š., Kalanin R., Gaľa I., Tóth Š., Čoma M.,
Affiliation(s)	University of Pavol Jozef Šafarik, Faculty of Medicine in Košice

Introduction

The shortage of donor kidneys for transplantation is a critical global issue. Marginal donors, including those with infections, present a potential solution but pose risks of donor-derived infections. This study aims to evaluate whether the use of nephrotoxic antibiotics—tigecycline and gentamicin—during hypothermic machine perfusion (HMP) damages kidney grafts.

Methods

First, HEK-293 cells were exposed to varying concentrations of gentamicin and tigecycline, and cytotoxicity was measured using MTT assays. A minipig model was used for kidney procurement and machine perfusion. Kidneys were perfused with or without antibiotics and then analyzed histopathologically. Immunohistochemical markers (MCM2, MPO,iNOS) were evaluated to assess proliferation and inflammation. Gentamicin and tigecycline concentrations in perfusate were analyzed using high-performance liquid chromatography.

Results

The MTT assay revealed dose-dependent cytotoxicity of tigecycline but not gentamicin. Histopathological analysis showed no significant tissue damage between the antibiotic-treated and control groups. Immunohistochemical markers indicated only a mild increase in cell proliferation and inflammation. Antibiotic concentrations in the perfusate decreased minimally after 4 hours of perfusion.

Discussion

The results suggest that HMP with high doses of gentamicin and tigecycline does not cause significant kidney graft damage. The minimal reduction in antibiotic concentration during perfusion highlights the potential efficacy of this approach for marginal donors. Additionally, HMP seems to mitigate the nephrotoxic effects of these antibiotics.

Conclusions

The combination of nephrotoxic antibiotics with HMP appears safe for kidney grafts, showing no significant histopathological damage. Further research is needed to confirm these findings and assess graft function post-transplantation.

This work was supported by the Slovak Research and Development Agency under contract no. APVV-23-0594 and Early stage grant – UPJŠ under contract no. VVGS-2023-2976.

Title	Evaluation of newly synthesized FLT-3 inhibitors on proliferation of cancer cells
Author	**Mohammadreza Afshari[1]**
Co-Authors	D. Muthna[1], M. Rezacova[1], L. Gorecki[2], J. Korabecny[2]
Affiliation(s)	1- Department of Medical Biochemistry, Charles University Faculty of Medicine in Hradec Kralove, Czech Republic 2- Biomedical Research Center, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic

Introduction

Despite advancements in cancer therapy, the search for new approaches continues due to the resistance and side effects associated with current treatments. As part of this effort, the antiproliferative effects of 30 newly synthesized FLT-3 inhibitors were tested, aiming to assess their potential as antileukemic agents as well as their off-target therapeutic applications.

Methods:

A one-dose cytotoxicity screening was performed on 10 human cell lines with various histotypes, exposing each to 10 μM of the compounds for 48 hours. The WST-1 assay was used to assess cell proliferation. Subsequently, IC_{50} of selected compounds values were determined.

Results and Discussion:

Compounds 21 and 29 are position isomers derived from 7-azaindole. They proved distinct effects on acute lymphoblastic leukemia cells with various p53 status. Compound 21 reduced MOLT-4 (functional P53) cell proliferation to 19%, with an IC_{50} of 6.9 μM, but maintained 80% proliferation in Jurkat (P53 mutated) cells with an IC_{50} of 34.7 μM. Conversely, compound 29 left MOLT-4 at 80% (IC_{50} 15.6 μM), while decreasing Jurkat cell proliferation to 36% (IC_{50} 7.5 μM). As tested compounds are FLT-3 inhibitors with the primary target in acute myeloid leukemia therapy, we further established the IC_{50} values on MV4-11 cells (p53 WT), which were 2.3 μM for compound 21 and 0.5 μM for compound 29. Further evaluation of IC_{50} values on an AML cell line bearing FLT-3 ITD and p53 mutation will be processed.

Conclusion:

Our results indicate that 7-azaindole derivatives have the potential as novel pharmacophores, with structural modifications that could enhance selective cytotoxicity.

Title	**Comprehensive analysis of invasive and non-invasive methods used in preimplantation genetic testing**
Author	**Yasmeen Mekhled Alamailess[2]**
Co-Authors	Katalin Gombos[1,2], Henrietta Orsolya Gellén[1,2], Salma Alsalti[2], Bence Gálik[1], Péter Urbán[1], Ákos Várnagy[1,3], József Bódis[1,3], Attila Gyenesei[1], and Gábor L. Kovács[1,2]
Affiliation(s)	[1]National Laboratory on Human Reproduction, University of Pécs; [2]Department of Laboratory Medicine, Medical School, University of Pécs, Ifjúság útja 13., 7624 Pécs, Hungary; [3]Department of Obstetrics and Gynaecology, Medical School, University of Pécs, Édesanyák útja 17, 7624 Pécs, Hungary;

Introduction

PGT (preimplantation genetic testing) is the standard for the conventional embryonic genetic assessment method. The invasiveness of the biopsies raised concerns, and non-invasive methods were introduced. Our research group carried out concise research to compare the effectiveness of non-invasive methods to invasive methods with an analysis of embryonic spent culture media (SCM) for developing a clinically applicable workflow.

Methods

Data were gathered from in silico databases and articles which compared invasive methods (whole blastocyst (WB) and trophectoderm biopsy (TE)) with non-invasive methods (SCM) were then analysed according to sensitivity, specificity and concordance. The experimental study was developed to analyse embryonic cell-free DNA found in SCM. The Multiple Annealing and Looping-based amplification (MALBAC) method was used and then NGS libraries were prepared to detect CNV (copy number variation).

Results

The review showed an average concordance of 71% and an average sensitivity of 87% between invasive and non-invasive methods. In our developed workflow, a low embryonal genomic DNA (gDNA) copy number characterizes a healthy embryo while a higher gDNA was associated with miscarriages. We found an autosomal gain in the 18q22.3-q3 region, which is often involved in Edwards syndrome.

Discussion

The traditional embryo-selection method, based on morphology and time-lapse technology, produced inconsistent results. Invasive PGT methods raised concerns about potential harm to embryos, leading an intensive research in non-invasive approaches.

Conclusions

With optimised procedures and standardized protocols, non-invasive PGT can be as effective as the invasive approaches.

Title	**Beyond the Clinic: A Pilot Study of a Digital Health Intervention for Extending Mental Health Care in Ukraine**
Author	**Hlib Aleksandrenko, MPH, MS, PhD candidate**
Co-Authors	Maksim Perepelitsa, GP
Affiliation(s)	School of Public Health of National University of Kyiv-Mohyla Academy

Introduction: It is well documented that excessive stress can lead to several adverse health outcomes. Digital health interventions (DHIs) offer a promising approach to extend physician care beyond clinical settings to patients facing high-stress challenges such as pandemics or war. This study aimed to evaluate the effectiveness of a DHI as a mental health chatbot-physician assistant.

Methods: A study was conducted with 16 participants from Ukraine (six male, 10 female, aged 21-45) within a workplace health program. The intervention, based on an original author-developed DHI framework, comprised a chatbot that provided stress management recommendations, mental health monitoring, and facilitated communication with the physician. The Perceived Stress Scale (PSS) was used to assess stress levels before and after the 3-month intervention. Statistical analysis included paired t-tests, effect size calculations (Cohen's d).

Results: A statistically significant decrease in mean PSS score from 26.06 to 22.88 ($p = 0.036$, $d = -0.64$) was observed post-intervention. Stress coping levels, based on reverse PSS items, showed significant improvement (6.25 to 7.49, $p = 0.014$, $d = 0.69$). 75% of participants demonstrated a reduction in overall stress levels, and 69% showed improved stress coping.

Discussion: The integration of continuous mental health monitoring and facilitated access to professional help appears to be a valuable intervention feature. Further research should focus on larger-scale implementation and personalization to optimize outcomes for all participants.

Conclusions: This study provides preliminary evidence of the potential of DHI to assist physicians in extending mental health care beyond clinical settings by reducing stress and improving coping.

Title	**Targeting PPARγ in Endometrial Receptivity: Friend or Foe?**
Author	**Peter Artimovič [1]**
Co-Authors	Mária Mareková [1], Miroslava Rabajdová [1]
Affiliation(s)	[1] Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia

Introduction

Endometrial receptivity is crucial for embryo implantation, yet its regulation is not fully understood. This study explores the emerging role of PPARγ in reproductive physiology, aiming to uncover novel regulatory mechanisms to enhance endometrial receptivity and improve outcomes in assisted reproductive technologies.

Methods

The expression of *PPARG* and 6 genes associated with endometrial receptivity was measured in receptive (RL95-2) and non-receptive (AN3CA) endometrial epithelial cell lines using RT-qPCR. Cells were treated with an agonist and antagonist of PPARγ at varying concentrations to assess the effects of PPARγ's activity on gene expression.

Results

AN3CA cells did not express *PPARG*, in contrast to RL95-2 cells. Treatment of RL95-2 cells with a PPARγ agonist (120 μM) increased the expression of *HOXA10* ($p < 0{,}001$), while treatment with a PPARγ antagonist (30 μM) increased the expression of *BMP2* ($p < 0{,}01$), *ITGB3* ($p < 0{,}01$) and *CD44* ($p < 0{,}0001$). The expression of *LIF* and *IGFBP1* did not change significantly during treatments.

Discussion

The study's findings indicate that *PPARG*'s expression is necessary to maintain the initial receptivity of selected endometrial epithelial cell lines. Modulating PPARγ's activity significantly affected the expression of receptivity-related genes, with inhibition showing a more favourable effect.

Conclusions

In our previous studies, we found significantly elevated *PPARG* levels in the endometrium of patients undergoing IVF with recurrent implantation failure. The presented research suggests that inhibiting PPARγ's activity may mitigate the negative impact of its overexpression on endometrial receptivity. Further *ex vivo* studies are needed to confirm these findings.

Title	**Effect of Platelet Rich Plasma (PRP) in treatment of late stage osteoarthritis**
Author	MUDr. Omar Masoud Morshedy Mohamed Awad
Co-Authors	prof. MUDr. Marek Lacko, PhD., MUDr. Martin Matúška, Ing. Denisa Harvanová, PhD., RNDr. Lucia Slovinská, PhD.
Affiliation(s)	Department of Orthopaedics and Traumatology of Locomotory Apparatus, Associated Tissue Bank

Introduction

The study aims to compare the efficacy and safety of intra-articular PRP injections with corticosteroid and anesthetic (CSA) injections for pain relief and functional improvement in late-stage knee osteoarthritis (KOA). A secondary objective is to evaluate the effect of PRP injections on specific osteoarthritis biomarkers in serum and synovial fluid.

Methods

This randomized, controlled study will involve 90 patients awaiting total knee replacement, divided into two groups. One group will receive two weekly doses of autologous PRP injections, the second group will receive one dose corticosteroid injection. Clinical assessments, including pain, stiffness, and function, will be conducted at baseline and at 1, 3, and 6 months using the WOMAC questionnaire and the Visual Analog Scale for pain.

Results

In this preliminary analysis of 23 patients (11 PRP, 12 DIPRO), VAS and WOMAC scores were compared over 3 months. PRP demonstrated significantly greater improvements in pain reduction (VAS) (45.01%) compared to DIPRO (9.63%), with a p-value of 0.027. Functional improvement (WOMAC) was also higher for PRP (27.18%) versus DIPRO (-0.78%), with a p-value of 0.0179. Although functional changes within each group were not significant.

Discussion

These are preliminary results of the study which will continue with also the comparison of NSAID's as a third group, and will involve the analysis of biomarkers.

Conclusions

PRP showed superior efficacy in reducing pain and enhancing function.

Acknowledgment

This study was supported by VEGA grant No.1/0686/24.

Title	**Activation of NK cells by different interleukin combinations**
Author	**Mgr. Klára Baďurová [1, 2]**
Co-Authors	Bc. Maxima Warmuzová [2, 3], Mgr. Jana Kotulová, Ph.D. [1, 2], Mgr. Zuzana Chyra, Ph.D. [1, 2]
Affiliation(s)	[1]University of Ostrava, Faculty of Medicine [2]University Hospital Ostrava, Department of Hematooncology [3]University of Ostrava, Faculty of Science

Introduction

Cultivation of NK cells *in vitro* is dependent on supplied interleukins. In this study, we activated primary NK cells with various combinations of interleukins and subsequently analysed their metabolic parameters and markers related to their phenotype and function. We also assessed expression of surface molecules, that suggest their exhaustion.

Methods

Primary NK cells were cultivated with various interleukin combinations for 24 hours. Subsequently, flow cytometry analysis of phenotype, functional and exhaustion markers was performed. For metabolic functions we detected ATP levels by luminometric method.

Results

Following cultivation, each combination demonstrated significant cell expansion and viability. Analysis of phenotype markers didn't show any striking differences between cultivation conditions. We observed varying expression profile of proteins that are associated with NK cell cytotoxic or modulatory functions (e.g. CD107a, CD336) as well as exhaustion markers. Based on ATP levels, we selected combinations with the most promising outcomes.

Discussion

We detected specific marker expressions that correlate with NK cell activation and cytotoxic potential, evaluated exhaustion markers and ATP levels, providing further insights into the condition of NK cells following specific interleukin stimulation. Building upon these observations, we selected candidate combinations for culture optimization, that need to be further investigated.

Conclusions

We aimed to identify the ideal interleukin combination that would yield the most desirable effects. This cultivation strategy lays the groundwork for optimizing NK cell-based immunotherapies, where sustaining strong functionality and preventing exhaustion are critical for clinical success.

This work was supported by the student's grant system of the University of Ostrava (SGS03/LF/2024).

Title	**Microbiome of the middle ear in children with chronic otitis media: a preliminary study**
Author	**MUDr. Michal Bartos[1,2]**
Co-Authors	Milan Urik[1,2], Lucie Buresova[3], Pavla Holochova[3], Eva Budinska[3], Petra Borilova Linhartova[3]
Affiliation(s)	[1]Department of Pediatric Otorhinolaryngology, University Hospital Brno, Cernopolni 9, Brno, Czech Republic [2]Department of Pediatric Otorhinolaryngology, Faculty of Medicine, Masaryk University, Cernopolni 9, Brno, Czech Republic [3]RECETOX, Faculty of Science, Masaryk University, Kotlarska 2, Brno, Czech Republic

Introduction

Chronic otitis media (COM), a common middle ear disease in children, could be associated with bacterial infection. Here we aimed to describe and compare microbiomes of the middle ear in children with serious forms of COM, such as cholesteatoma and retraction pocket of the tympanic membrane, with microbiomes in healthy middle ears.

Methods

In this case-control study, swabs from middle ears were obtained during surgery from children with cholesteatoma (N=23) or retraction pocket (N=26) and from children indicated for cochlear implant (N=15, controls). Extraction of DNA was followed by 16S rRNA amplicon sequencing on a MiSeq instrument. Samples with relative abundance of at least one bacterial genus >20% were considered positive for the specific genus.

Results

A significant difference in positivity for one or more bacterial genera was observed between patients with cholesteatoma or retraction pocket (38.8%) versus patients indicated for cochlear implant (6.7%). The relative abundance of *Staphylococcus* exceeded 20% in samples from six patients with cholesteatoma and seven patients with retraction pocket compared to none in the group of patients indicated for cochlear implant.

Discussion

The similarity of the bacteria present in both forms of COM is consistent with our hypothesis and adds to the existing knowledge that potential otopathogens may influence the course of severe forms of COM.

Conclusions

While middle ear bacteriomes in cases of cholesteatoma and retraction pocket differed from those of controls, findings in the two pathological conditions were similar. These results support the statement that retraction pocket could be a pre-cholesteatoma stage.

Title	**Optimization of cytokine profiling in multiple myeloma patients undergoing modern immunotherapy**
Author	**Lucie Broskevičová[1,2]**
Co-Authors	Jana Klepáčová[2], Hana Križňanská[2], David Žíhala[1,2], Tereza Ševčíková[1,2], Jana Mihályová[1,2], Roman Hájek[1,2]
Affiliation(s)	[1] Department of Hematooncology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic [2] Department of Hematooncology, University Hospital Ostrava, Ostrava, Czech Republic

Introduction

Modern immunotherapy, including T cell redirected therapy with T cell engagers (TCEs) and CAR T cell therapy, activates the immune system to destroy malignant cells, with cytokines playing a crucial role. This project seeks to optimize the analysis of changes in cytokine profiles in multiple myeloma (MM) patients undergoing different therapies.

Methods

Peripheral blood was collected before treatment and on days 7, 14, and 21, as well as at months 1, 2, 3, 6, 9, 12, and 24, and at progression. Samples were centrifuged to obtain serum and plasma, aliquoted, and frozen. Plasma samples from patients were analysed using multiplex immunoassay to profile 26 cytokines after one year of monitoring or upon progression.

Results

Forty-seven patients were enrolled, receiving the following treatments: 6 patients with CAR T (Cilta-cel) and 31 patients treated with TCE (16 Teclistamab, 13 Talquetamab, 7 Elranatamab, and 5 Cevostamab). Analysis of 7 patients (Teclistamab: n=3, Talquetamab: n=3, Cilta-cel: n=2) revealed significant variations in cytokine profiles among treatment subgroups.

Discussion

Our preliminary findings indicated a significant increase in specific cytokines (IL-6, IP-10, MCP-1) associated with cytokine release syndrome during episodes of this adverse effect, while other cytokines showed no changes. To enhance our analysis, we expanded the number of cytokines to 47, with analysis performed on an additional 12 patients who completed follow-up.

Conclusion

We established a cytokine profiling approach and biobanked a substantial cohort for further analysis, which will help confirm our preliminary results and may be valuable for identifying cytokines predictive of treatment outcomes.

Title	**Endoplasmic Reticulum Stress and Unfolded Protein Response Investigation in Patients with Pancreatic Adenocarcinoma**
Author	**Riza Can Cakmakci[1]**
Co-Authors	Ivana Acimovic[1], Lukáš Jan[1], Türkan Portakal[1], Michal Eid[2], Jakub Vlažný[3], Petr Moravčík[4], Zdeněk Kala[4], Petr Vaňhara[1,5]
Affiliation(s)	[1]Department of Histology and Embryology, Faulty of Medicine, Masaryk University, Brno, Czech Republic [2]Internal Hematology and Oncology Clinic, University Hospital Brno, Brno, Czech Republic [3]Department of Pathology, University Hospital Brno, Brno, Czech Republic [4]Surgery Clinic, University Hospital Brno, Brno, Czech Republic [5]International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

Introduction: Pancreatic adenocarcinoma (PDAC) is a lethal cancer that is frequently diagnosed at later stages. Rapid growth of PDAC cells causes accumulation of misfolded proteins within endoplasmic reticulum (ER), leading to stress. This study aims to explore patient-specific alterations in proteins associated with ER stress and progression of PDAC, with the goal of identifying novel molecular markers. To better represent diversity of PDAC and develop targeted therapies, A three-dimensional (3D) culture model was used.

Methods: Surgical resections from patients were transferred into *in vitro* culture. Intracellular levels of BIP, CHOP, TUSC3, TGF-βR, E-cadherin, N-cadherin, and EGFR expressions were investigated. Cells were treated with ER modulators to achieve response to ER stress. 3D culture model based on hanging drop, agarose coating and agarose molds was used.

Results: Basal levels of ER stress markers differed in individual patients and differed with ER stress. EGFR was upregulated while non-canonical ER stress signaling or epithelial to mesenchymal transition molecules, TUSC3 and E-cadherin, respectively, were downregulated.

Discussion: When we compared the patient cells, we observed different BIP, CHOP and ATF6 expression in each patient, which suggests that UPR differs between patients. The different expressions of N-cad, E-cad, TUSC3 and TGFβR indicates that PDAC cells may have different invasion and migration potential. Increased EGFR expression by ER stress supports therapeutic approaches aimed at EGFR inhibition.

Conclusion: This study highlights patient-specific differences in antibody expression in PDAC cells, creating a path for unique treatment approaches. Different ER stress responses may contribute to phenotypic differences in patient cells.

Title	**Is intermittent fasting a suitable method for the prevention and treatment of obesity?**
Author	**Erika Čermáková[1]**
Co-Authors	Martin Forejt[1], Martin Čermák[2]
Affiliation(s)	[1] Department of Public Health, Faculty of Medicine, Masaryk University [2]Independent Researcher, Czech Republic

Introduction

In the prevention and treatment of obesity, the goal is to maintain or reduce the amount of adipose tissue. Intermittent fasting (IF) is a widely used intervention, but the effect of IF on body composition is still unclear. The aim of this study was to assess the effect of IF on body fat loss.

Methods

A total of 75 healthy adult participants completed the three-week intervention phase of the study. The participants were divided into three groups: IG1 (IF with 75% energy intake), IG2 (75% continuous energy intake - no time restriction) and IG3 (IF with 100% energy intake). Body composition was determined by BIA and meal plans were generated using NutriPro software. Adverse events were continuously monitored throughout the intervention.

Results

The highest mean fat mass loss was observed in the IG2 group: 1.5±1.2 kg compared to the IG1 group: 1.3±1.1 kg ($p>0.99$). The lowest fat mass loss of 0.9±1.1 kg was observed in the IG3 group (IG1×IG3: $p=0.73$; IG2×IG3: $p=0.17$). Fatigue rates were statistically significantly highest in IG1: n=14 (51.9%) versus IG2: n=6 (22.2%) ($p=0.0473$) and IG3: n=9 (42.9%) versus IG1 ($p=0.5734$) and IG2 ($p=0.2089$).

Discussion and Conclusions

IF with energy restriction causes excessive fatigue in some individuals, which may lead to a spontaneous reduction in physical activity, negatively affecting the amount of adipose tissue loss. The appropriateness of the timing of food intake will depend on the incidence of adverse events, which will have a significant impact on long-term adherence to the chosen dietary intervention.

Title	**Data-driven Computational Modeling of Epileptic Activity**
Author	**Isa Dallmer-Zerbe**
Co-Authors	supervisor: Ing. Mgr. Jaroslav Hlinka, Ph.D. (advisor: prof. MUDr. Přemysl Jiruška, Ph.D.)
Affiliation(s)	Charles University, 2 LF UK

Computational modeling is a powerful tool for exploring brain mechanisms underlying neuroimaging data. Nowadays, it is increasingly used to design and evaluate the effect of therapeutic interventions, such as brain surgery or stimulation. In this contribution, the computational approach is used in the context of epilepsy treatment, to identify and characterize epileptic brain states in EEG data.

Building on the modeling work of Wendling et al. [1], we replicate rat in vitro and human in vivo hippocampal EEG recordings. In a data-driven manner, we fit the simulated and real data segments, which provides us with an automatized classification of the observed type of epileptic activity (interictal, pre-onset, onset, ictal) and with estimates of the underlying levels of excitatory and inhibitory synaptic gains at a given time.

I will present evidence, how the modeling environment can 1) correctly classify epilepsy brain states, both in rat and human data samples, 2) predict the effects of brain stimulation on the epileptic tissue in vitro, and 3) characterize the differences between brain location of seizure onset and healthy brain areas in vivo intracranial EEG recorded during the presurgical evaluation of epilepsy patients. We find that a slow build-up of dendritic inhibition could be linked to seizure build-up, opposing effects of brain stimulation (seizure facilitating or suppressing) could be associated with characteristic changes in synaptic transmission, and that clinically marked seizure onset areas could be marked by higher increases in excitation and inhibition during seizure transitions than healthy brain areas.

In conclusion, this contribution exemplifies the promising potential of computational modeling in the context of epilepsy research and treatment planning, i.e. for the classification of epileptic brain states, prediction of therapeutic outcomes, and the differentiation between pathological and healthy brain regions.

Title	**Functional properties and pharmacology of zebrafish NMDA receptors**
Author	**Mark Dobrovolskii** [1,2]
Co-Authors	B. Kysilov [1], V. Abramova [1], L. Vyklicky[1, 2]
Affiliation(s)	[1] Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic [2] Third Faculty of Medicine, Charles University, Prague, Czech Republic

Introduction

This project aimed to establish the electrophysiological and pharmacological properties of zebrafish N-Methyl-D-Aspartate Receptors. Zebrafish are increasingly viewed as a feasible model of disorders linked to NMDARs' dysfunction, therefore understanding their functional characteristics and responses to agonists and antagonists would be necessary for a great range of subsequent studies.

Methods

Zebrafish NMDARs with various subunit combinations (GluN1a/b; GluN2A/B;a/b) were expressed in HEK293 cells patched using whole-cell voltage-clamp recording. Deactivation rates were measured in response to synapse-like fast agonist application. The channel open probability was determined by measuring the inhibition of agonist responses in the presence of a blocker. Lastly, we measured the affinity for both agonists of NMDARs, four inhibitors, and two positive modulators.

Results

The affinity for glutamate was lower in GluN1a/GluN2Aa receptors; open probability was lower and deactivation slower in Glun2Bb receptors; and the affinity for glycine was higher in GluN2Aa and GluN1b/GluN2Bb receptors. Compared to rat NMDARs, zebrafish receptors had slightly lower affinity to antagonists, and a similar affinity for positive modulators.

Discussion

Our experiments demonstrated that zebrafish NMDARs have properties largely similar to mammalian NMDARs with statistically significant but modest variation, however, there are cases in which this strongly depends on the subunit combination.

Conclusions

These findings will allow us to connect morphological and behavioural changes in zebrafish with NMDA mutations to the changes in the functional properties of the mutant receptors; they also validate using established NMDAR modulators in zebrafish experiments by demonstrati
ng that they act on zebrafish and mammalian receptors similarly.

Title	**Investigating the Degradation Mechanisms of Polydioxanone (PPDX) in Low pH Conditions for Gastrointestinal Applications**
Author	**Krisztina Dodzi Kodzitse Lelkes**
Co-Authors	Daniel Jezbera, Roman Svoboda, Štěpán Podzimek, Jan Loskot, Martina Nalezinková, Petr Voda, Piotr Duda, Alena Myslivcová Fučíková, Tomáš Hosszú, Dino Alferi, Aleš Bezrouk
Affiliation(s)	Charles University, Faculty of Medicine in Hradec Králové

Introduction

Polydioxanone (PPDX) has gained significant attention as a biocompatible and absorbable polymer used in various medical applications, such as sutures and tissue scaffolds. This research presents a thorough investigation into the degradation mechanisms of PPDX under low pH conditions, simulating physiological environments like the esophagus and stomach. It mainly focuses on the dependence of the PPDX degradation rate on various ambient pH values (7.4 and below), which is crucial for successful gastrointestinal treatment.

Methods

The PPDX suture samples were degraded for up to 6 weeks and analyzed using size exclusion chromatography, differential scanning calorimetry, Raman spectroscopy, scanning electron microscopy, X-ray microtomography, and mechanical property measurements.

Results

The results show that the PPDX degradation is significantly accelerated at pH below 1.67. Correlations of the molecular weight, crystallinity, glass transition temperature, Young's modulus, shear modulus, tensile strength, and the 1733 cm^{-1} Raman peak shoulder area (RPSA1733) indicate that the degradation mechanism does not change with increasing acidity.

Discussion

Measurements of tensile strength, shear modulus, and RPSA1733 were found to be the most suitable parameters for characterizing the PPDX filament's macroscopic integrity. Raman spectroscopy is of particular interest due to its rapidity and minimal sample preparation requirements.

Conclusions

This study provides substantial knowledge for the successful application of PPDX in gastrointestinal treatments, highlighting the importance of understanding its degradation under varying pH.

Title	**Unveiling the Spectrum of Genetic Alterations in Relapsed/Refractory CLL Patients on Targeted Inhibitors: A Prospective Unicentric Study**
Author	**Dominika Écsiová[1]**
Co-Authors	Pavel Vodárek 1,Lukáš Smolej 1, Filip Vrbacký 1, Kateřina Hrochová 2, Michaela Řehounková 3, Martin Šimkovič 1
Affiliation(s)	(1) 4th Department of Internal Medicine – Hematology, University Hospital and Charles University Faculty of Medicine in Hradec Králové (Czech Republic), (2) The Fingerland Department of Pathology, University Hospital Hradec Králové (Czech Republic), (3) Department of Clinical Biochemistry and Diagnostics, University Hospital and Charles University Faculty of Medicine in Hradec Králové (Czech Republic)

Introduction:

Next-generation sequencing (NGS) has advanced the understanding of the genomic landscape in Chronic Lymphocytic Leukemia (CLL). However, interpreting the mutational landscape remains challenging due to variants of uncertain significance (VUS) and minor clone presence. This study aimed to map genetic mutations, track clonal evolution, and assess their clinical significance.

Patients and Methods:

This prospective unicentric study included patients with relapsed/refractory CLL initiating treatment with oral kinase inhibitors between 2019 and 2023. CLL cells were isolated using the RosetteSep method, and NGS analysis was performed using the Illumina MiSeq platform. Sample collection occurred after 12 months of therapy and at disease progression.

Results:

We analyzed 45 patients (median age 72 years, 71% male). Of these, 76% had unmutated IGHV, 40% had TP53 mutation/del17p, and 33% had del11q. Median follow-up was 22 months. In terms of treatment, 56% received BTK inhibitors, 31% venetoclax, and 13% idelalisib. Molecular profiling revealed frequent mutations in TP53 (40%) and SF3B1 (37%). NOTCH1 and ATM mutations appeared in 19% of patients. VUS were detected in several genes, including ATM, KIT, and TP53. Clonal evolution was observed in 15 of 16 patients, with 69% showing new mutations and 31% experiencing mutation disappearance.

Discussion and Conclusion:

This interim analysis points to the dynamic and complex nature of genomic alterations in CLL, with implications for disease progression and therapeutic responses. Our findings highlight the importance of longitudinal genomic monitoring to uncover evolving mutational profiles and their clinical implications.

Title	**α-internexin (INA) as a possible effective diagnostic marker of sparsely granulated somatotroph PitNETs: proposition of INA-based classification system**
Author	**Lucie Gerykova[1]**
Co-Authors	**Filip Gabalec[2], David Netuka[3], Jiri Soukup[4]**
Affiliation(s)	[1] The Fingerland Department of Pathology, Faculty of Medicine Hradec Kralove, University Hospital, Charles University, Hradec Kralove, Czech Republic. [2] 4th Department of Internal Medicine, Faculty of Medicine Hradec Kralove, University Hospital, Charles University, Hradec Kralove, Czech Republic [3] Department of Neurosurgery and Neurooncology, 1st Medical Faculty, Charles University, Military University Hospital Prague, Prague, Czech Republic [4] Department of Pathology, Military University Hospital Prague, Prague, Czech Republic.

Introduction: Pituitary neuroendocrine tumours (PitNETs) arise from hormone-producing cells of hypophysis. Distinguishing SGST (sparsely granulated somatotroph tumours) from other types of Pit1+ PitNETs has clinical impact. The diagnostic feature of SGST is cytokeratin expression pattern, which may sometimes be challenging. The aim of our study was assessment of α-internexin (INA) as a possible diagnostic marker of SGST.

Methods: Expression of INA and other markers (CK18, SSTR2A, SSTR5, E-cadherin,…) was detected immunohistochemically in 101 PitNETs of acromegalic patients. SGST (CK18-based): CK18+ fibrous bodies in more than 70% of cells (same cut off used for INA). Several tests were used for analysis, P<.05 were considered significant.

Results: A strong positive correlation was found between expression of CK18 and INA (P<.0001, Spearman r=.9014, Spearman nonparametric correlation). Cases were classified by CK18: SGST (n=41), non-SGST (n=60) and subsequently by INA: SGST (n=31), non-SGST (n=70). 12 cases were discordant: 1 case non-SGST (CK18-based), SGST (INA-based); 11 cases SGST (CK18-based), non-SGST (INA-based). Both SGST groups showed no differences between expression of E-cadherin, SSTR2A or SSTR5. The same results were attained for non-SGST groups.

Discussion: Because of the positive correlation between expression of CK18 and INA, INA could be a part of fibrous bodies alongside with CK18. When both systems (CK18-based, INA-based) were compared, majority of cases was classified in the same way and no statistically significant difference was found between the subgroups.

Conclusions: Our findings suggest that INA could be used as a diagnostic marker of SGST, in cases with difficult interpretation of cytokeratin immunohistochemistry.

Title	**The Relationship Between Oxidative Stress Markers and the Progression of Amyotrophic Lateral Sclerosis (ALS)**
Author	**Pavlína Malá (Hemerková)**
Co-Authors	Oldřich Vyšata, David Matyáš
Affiliation(s)	Department of Neurology, University Hospital Hradec Králové and Faculty of Medicine in Hradec Králové

Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease leading to the loss of motor functions and eventually respiratory failure. One of the key mechanisms involved in the pathogenesis of ALS is oxidative stress, though its exact relationship to the progression of the disease remains unclear. The aim of this study was to evaluate the correlation between levels of oxidative stress markers in blood, urine, and cerebrospinal fluid (CSF) and the progression of ALS, assessed using the ALS Functional Rating Scale-Revised (ALSFRS-R).

Methods: The study included 28 patients with confirmed ALS diagnosis. Samples of blood, urine, and CSF were analyzed, with oxidative stress marker levels measured using advanced chromatographic methods, specifically high-performance liquid chromatography (HPLC). The progression of ALS was assessed using the ALSFRS-R scale, which provides a comprehensive evaluation of physical functions and symptom severity.

Results: The results of this study will be presented at the upcoming conference. It is expected to include an analysis of oxidative stress marker concentrations across all biological sample types (blood, urine, CSF) and their correlation with ALSFRS-R scores. These findings may provide new insights into the relationship between oxidative stress levels and ALS progression.

Discussion: Although the results are not yet available, our hypothesis suggests that patients with more advanced ALS, as evaluated by the ALSFRS-R, will have elevated levels of oxidative stress markers. If this correlation is confirmed, it may indicate that oxidative stress is closely linked to ALS progression. These findings could open new avenues for therapeutic approaches - patients might benefit from more targeted interventions aimed at reducing oxidative stress, including the use of natural antioxidants with potential neuroprotective effects. This strategy could slow disease progression and improve patients' quality of life.

Conclusions: This study has the potential to contribute to a better understanding of the relationship between oxidative stress and ALS severity. If a significant correlation between oxidative stress markers and disease progression is demonstrated, it could lead to the identification of new therapeutic targets and the development of treatment strategies focused on reducing oxidative stress, such as natural antioxidants with potential neuroprotective properties. This could enhance care for ALS patients.

Title	**Vitamin D deficiency is highly prevalent in the Czech Republic: a retrospective study of 119 925 participants**
Author	**Drahomíra Holmannová[1]**
Co-Authors	Pavel Borský[1], Jan Kremláček[2], Jak Krejsek[3], Lenka Hodacova[1], Lenka Borská[1]
Affiliation(s)	[1]Institute of Preventive Medicine, Faculty of Medicine in Hradec Kralove, Charles University, 500 03 Hradec Kralove, Czech Republic [2] Institute of Medical Biophysics, Faculty of Medicine in Hradec Kralove, Charles University, 500 03 Hradec Kralove, Czech Republic [3] Institute of Clinical Immunology and Allergology, University Hospital Hradec Kralove and Faculty of Medicine in Hradec Kralove, Charles University, 500 03 Hradec Kralove, Czech Republic

Introduction

Vitamin D is essential for a wide range of body processes, and deficiency is associated with increased susceptibility to many disorders. Studies show that vitamin D deficiency is a global problem. We designed a study that examined vitamin D levels in the Czech Republic because there is a lack of such studies.

Methods

We collected data from 119,925 people aged 0 to 100 years. Vitamin D status, (sufficiency 75-250 nmol/l, insufficiency 50-75 nmol/l, deficiency < 50 nmol/l), CRP and homocysteine levels and correlations between vitamin D and selected parameters (age, sex, months of the year, cumulative exposure to the sun, influenza positivity, CRP, homocysteine)

Results

The prevalence of vitamin D inadequacy was very high, except for infants aged 0-12 months, when sufficient levels reached 65.6% infants. The prevalence of vitamin D sufficiency was lowest in the 6-15 years (19.2%) and 16-30 years (22.1%) groups. The prevalence of vitamin D deficiency was highest in the 91-100 years group (51.8%). Vitamin D levels were negatively correlated with less sun exposure, higher flu positivity, male gender, homocysteine, and CRP.

Discussion

The prevalence of vitamin D inadequacy in the Czech population is high, even among children and young adults. The highest prevalence of vitamin D deficiency was among the oldest participants.

Conclusions

There is a high prevalence of vitamin D inadequacy in the Czech Republic, which may be risky for the health of the population. Based on our results, recommendations on vitamin D monitoring and supplementation in primary care can be formulated.

Title	**Challenges of rheumatoid arthritis and the impact of COVID-19**
Author	**Alexandra Husivargova Theofanidis[1,2]**
Co-Authors	Vladimira Timkova[1], Zelmira Macejova[3], Zuzana Kotradyova[3], Dagmar Breznoscakova[1], Robbert Sanderman[2,4], Iveta Nagyova[1]
Affiliation(s)	[1]Department of Social and Behavioural Medicine, Faculty of Medicine, PJ Safarik University in Kosice, Slovakia [2] Department of Health Psychology, University Medical Center Groningen, University of Groningen, The Netherlands [3] 1st Department of Internal Medicine, Faculty of Medicine, PJ Safarik University in Kosice and UNLP in Kosice, Slovakia [4] Department of Health Psychology and Technology, University of Twente, Enschede, The Netherlands

Introduction

Despite advances in biologic therapy, patients suffering from rheumatoid arthritis (RA) still face many challenges that persist and are often overlooked when establishing a treatment plan. In addition, crises such as the COVID-19 pandemic may negatively impact physical and mental health, especially for vulnerable groups such as RA patients. Therefore, we focused on investigating RA-related problems and changes in clinical, physical, and psychological variables before and after the pandemic.

Methods

In a study on self-reported RA-related problems, 187 patients were interviewed by phone (81.5% women; mean age 55.9±13.6 years; mean disease duration 19.3±9 years). Of these, 103 patients completed questionnaires (GAD-7, PHQ-9, VAS-pain and fatigue, HAQ, DAS-28) before and after the pandemic. We used paired sample t-tests to analyse the data.

Results

The findings showed a high prevalence of persistent physical problems (97.3%), such as pain (91.3%), physical limitations (89.7%), and fatigue (82.1%). A lower prevalence was reported in psychological problems (33.7%), including feeling sad (27.1%), depressed (25%), and lonely (7.4%). The pandemic led to a worsening of pain ($p \leq 0.05$) and anxiety ($p \leq 0.05$), but no significant differences were found in depression, fatigue, and clinical variables.

Discussion

Our findings highlight the high importance of addressing pain, fatigue, and psychological distress in RA patients, especially in challenging times such as pandemics.

Conclusions

The study results are consistent with the new EULAR recommendations for regular screening and management of pain, fatigue, and mental health through non-pharmacological interventions, which are still not considered standard care for RA patients in Slovakia.

Title	**Effects Of Colon Cancer On Hippocampal Astrocytes In Dental Gyrus: Neuroinflammation And Astrogliosis**
Author	**Inna Chebernina**
Affiliation(s)	State Establishment "Lugansk state medical university", Rivne, Ukraine Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine

Introduction

Astrocytes in the hippocampus are activated under various pathological conditions, but there is limited information about their response to colon cancer.

Methods

The experiment was conducted on 48 white laboratory rats (190±20 g) injected with dimethylhydrazine (7.2 mg/kg). Hippocampal samples with GFAP antibodies were analyzed using light and confocal microscopy, with statistical processing in Microsoft Excel and STATISTICA 12.0.

Results

In the control group, the average number of astrocytes in the dentate gyrus (DG) of the hippocampus was 150.4±9.1 per 1 mm². After 5 months of dimethylhydrazine exposure, this number doubled to 300.6±15.3 cells ($p<0.001$). By the 7th month, it returned to control levels. The number of astrocyte processes in the DG was 2.9±0.3 initially, with significant increases at 3, 4, 5, and 6 months compared to controls ($p<0.001$). Electron microscopy showed a decrease in the number of astroglial synapses. In the 2nd month, their number decreased by 13.07% (17.3±0.87; $p<0.05$), reaching the maximum decrease in the 5th month — by 27.64% (14.4±0.45; $p< 0.001$). By the 7th month, a partial restoration of synapses was observed.

Discussion

Astrocytes in colon carcinogenesis cause neuroinflammation in the hippocampus, similar to reactions in colitis and other pathologies. This disrupts GFAP expression, leads to astrogliosis, and can cause anxiety, depression, and chronic pain.

Conclusions

The highest expression of GFAP and the doubling of the number of astrocytes in the dentate gyrus of the hippocampus occurs at the 5th month of carcinogenesis. Reduction of astrogliosis and normalization of indicators is observed after 7 months.

Title	**The Effect of Gestational Diabetes Mellitus on Infant Development: Umbilical Cord Blood Levels of C-peptide and Glycemia**
Author	**Rana Ibrahim**
Co-Authors	Kateřina Krylová, Alžběta Dubská, Jaroslav Stráník, David Neumann
Affiliation(s)	Charles University, Faculty of Medicine in Hradec Králové

Background:

Gestational Diabetes Mellitus (GDM) is the most common pregnancy associated complication. Gestational hyperglycaemia appears to have a strong continuous association with negative foetal and neonatal effects such as increased birth weight, birth trauma, risk of caesarean delivery, and neonatal hypoglycaemia. The long-term effect of GDM on the offspring is however still unclear. Several studies demonstrated an adverse effect on neurodevelopment of the infants of GDM mothers. In the perinatal phase of this year-long study, we assess cord blood levels of C-peptide and glycemia in GDM and healthy controls.

Methods:

A prospective observational study including 60 mothers was launched August 2023. The cohort includes mothers with high-risk GDM managed by medications (insulin and or oral antidiabetics) (n=19: GDM insulin n=9, GDM PAD n=10), mothers with low-risk diet-controlled GDM (n=19) and healthy controls (n=22). Glycemia measurement took place using the hexokinase method and C-peptide via a microparticle-based chemiluminescent immunoassay method. GraphPad Prism 6.07 was used for statistical evaluation.

Results:

Glycemia was highest in the group of GDM mothers treated with insulin (mean: 5,0; SD: 1,2), followed by PAD (mean: 4,8; SD: 1,0), diet (mean: 4,4; SD: 1,0) and finally the control group (mean: 4,3; SD: 0,9). C-peptide was highest in high-risk GDM group on PAD (mean: 414; SD: 173) followed the group of mothers with GDM treated by insulin (mean: 385; SD: 147), those compensated by diet (mean: 355; SD: 133) and lastly the control group (mean: 329; SD: 114).

Conclusion:

The cord blood levels of c-peptide and glycemia were highest in high-risk GDM followed by low-risk GDM as compared with healthy controls.

Discussion:

We plan to further follow-up the infants to determine whether these results may be correlated with worse neurodevelopment outcome at 12-months of age and hypothesise that cord levels of C-peptide and glycemia may serve as useful markers for assessing the severity of long-term adverse effects of GDM.

Title	**Diagnostic and Therapeutic Challenges in Patients with Congenital Anomalies of the Kidney and Urinary Tract (CAKUT)**
Author	**Barbara Kałuska[1]**
Co-Authors	Maria Szczepańska[2], Jakub Behrendt[1]
Affiliation(s)	[1]Clinic of Intensive Care and Neonatal Pathology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland [2]Department of Pediatrics, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland

Introduction

The natural history of congenital anomalies of the kidney and urinary tract (CAKUT) has shown that they could develop asymptomatically. Diagnosis frequently occurring incidentally during routine ultrasound examinations. A key challenge for pediatricians and neonatologists is to identify children who require closer monitoring before development of complications.

Methods

A retrospective pilot study was conducted on infants hospitalized between 2018-2022 at the Clinic of Intensive Care and Neonatal Pathology, Medical University of Silesia in Katowice. The primary inclusion criterion was the diagnosis of CAKUT. Data collection encompassed maternal and labour history, clinical course with the analysis of selected anthropometric, blood chemistry parameters, treatment and intervention.

Results

The general prevalence of CAKUT among all hospitalized newborns was 8,25% (128 cases). The study group was characterized by male predominance (87:41). Just over a quarter of cohort had a diagnosed urinary tract infections (UTIs), with UTIs occurring more frequently in children with complex anomalies ($p = .007$). Significant relationship was found between the type of anomaly and length of hospitalization. No statistically significant relationship was found between the type of defect and the occurrence of proteinuria or arterial hypertension.

Discussion

The incidence of CAKUT in this study exceeded the global population prevalence reported in other studies. Consistent with findings from other researchers, CAKUT was identified as a common risk factor for UTIs in neonates.

Conclusions

Newborns diagnosed with CAKUT should undergo postnatal evaluation for potential nephrological complications. Infants displaying signs of renal damage require multidisciplinary care in specialized centers.

Title	**Testing the effect of temozolomide and flubendazole in glioma primary cell cultures and 3D cell cultures**
Author	**RNDr. Kateřina Kápičková**
Co-Authors	RNDr. Veronika Skarková, Ph.D.[1]; PharmDr. Barbora Vítovcová, Ph.D.[2]; prof. PharmDr. Emil Rudolf, Ph.D.[1]
Affiliation(s)	[1] Department of Medical Biology and Genetics, Faculty of Medicine in Hradec Králové, Charles University [2] Department of Biology, Faculty of Medicine in Pilsen, Charles University

Introduction

Glioblastoma multiforme is an aggressive primary brain tumor with rapidly developing chemoresistance leading to shortened survival of patients. One of the potential treatment strategies to overcome chemoresistance involves using the combination of DNA-damaging agents and microtubule-targeting drugs.

The aim of this study was to prepare primary cell lines and 3D cultures of high-grade gliomas for cell viability and morphology evaluation after combined treatment with temozolomide (TMZ) and flubendazole (FLU). Further, changes in tubulin expression and other markers were investigated.

Methods

Changes in cell viability were determined by WST-1 Assay (primary cell cultures) or CellTiter-Glo® 3D Cell Viability Assay (3D cultures). Morphological changes were determined by phase contrast microscopy. The expression of selected markers was determined by RT-PCR.

Results

FLU increased TMZ effect in tested biological models, in comparison with TMZ alone as evidenced by altered microtubule structure as well as by the changes in morphology of co-treated cells.

Discussion

Drug repurposing is one of the concepts aimed at improving the efficacy of treatment for various types of cancer. Therefore, TMZ, a commonly used chemotherapeutic drug, was combined with FLU, a promising anticancer drug. Our previous results demonstrated the significant inhibitory effect of TMZ and FLU in glioma stabilised cell lines. Primary cells and 3D cell models confirmed this observed effect and thus this strategy merits further investigation.

Conclusions

Our findings indicate that FLU can improve the effect of TMZ in primary glioma cells and 3D cell cultures as well, making this drug combination an interesting subject for further research.

Title	**Composition and fitness of T and NK cells in extramedullary myeloma tumor microenvironment**
Author	**Mgr. Veronika Kapustová**
Co-Authors	Anjana Anilkumar Sithara[1,3], David Zihala[1,2], Ondrej Venglar[1,2], Eva Radova[1,2], Lucie Broskevicova[1,2], Jan Vrana[1,2], Serafim Nenarokov[1,2], Dan Bilek[1,3], Ludmila Muronova[1,2], Tereza Popkova[1,2], Jana Mihalyova[1,2], Hana Plonkova[1], Sandra Charvatova[1,2], Kamlesh Bisht[4], Donald Jackson[4], Hongfang Wang[4], Helgi van de Velde[4], Michal Simicek[1,2], Tereza Sevcikova[1,3], Roman Hajek[1,2], Tomas Jelinek[1,2]
Affiliation(s)	[1] Department of Hematooncology, University Hospital Ostrava, Ostrava, Czech Republic [2] Department of Hematooncology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic [3] Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic [4]Sanofi, Research and Development, Cambridge, Massachusetts, USA

Introduction: Extramedullary disease (EMD) is an aggressive manifestation of multiple myeloma (MM) characterized by the independence of clonal plasma cells (PCs) from the bone marrow (BM), leading to their invasion of distant tissues. Modern immunotherapy is dependent on patient's immune system. However, there is limited knowledge about EMD microenvironment and immune cell fitness. In this study, we aimed to investigate the composition and fitness of immune cells in EMD.

Methods: Single-cell RNA sequencing was performed from 7 EMD tumors, 5 BM from time of EMD (EMD_BM), and 5 RRMM_BM without EMD. For flow cytometry (FC), four 8-color panels were used for dissection of immune cell subsets.

Results: Single-cell RNA-seq analysis revealed that EMD tumor consists of lower number of CD4+ T cells compared to RRMM_BM and EMD_BM, being supported by FC. The CD8+ T cells in EMD displayed lower cytotoxicity and increased dysfunction.

Analysis of NK cells revealed higher proportion of CD16- NK cells in EMD compared to CD16+ NK cells, being confirmed by FC. CD16- NK cells from EMD exhibited higher expression of NKG2A, suggesting potential therapeutic opportunity.

Discussion: In this study, we revealed that majority of T cells in EMD are dysfunctional CD8+ T cells with lower cytotoxicity. NK cells in EMD predominantly consist of CD16- NK cells, being a well-known phenomenon in solid cancers.

Conclusions: This study provided first insights into the composition and fitness of immune cells in EMD in single-cell resolution, showing that the tumor's immune cells consist mostly of CD8+ T cells and NK-cells.

Title	**Sex-Linked Differences in Cardiac Atrophy After Mechanical Unloading Induced by Heterotopic Heart Transplantation**
Author	**Dushan Michael Kolesár 1,2**
Co-Authors	Petr Kujal 3, Iveta Mrázová 4, Martin Pokorný 1, Petra Škaroupková 3, Janusz Sadowski 5, Luděk Červenka 4, Ivan Netuka 1
Affiliation(s)	1 Department of Cardiovascular Surgery, Institute for Clinical and Experimental Medicine, Prague, Czech Republic, 2 Third Faculty of Medicine, Charles University, Prague, Czech Republic, 3 Department of Pathology, Third Faculty of Medicine, Charles University, Prague, Czech Republic, 4 Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic, 5 Department of Renal and Body Fluid Physiology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Republic of Poland

Heart failure (HF) is a global problem with at least 38 million patients worldwide, with the number of new HF patients estimated to increase by 50 % per year and it is accepted that there is an urgent need for studies elucidating biological mechanisms underlying sex-related differences in the pathophysiology of HF and in responses to new therapeutic measures

The classical heterotopic HTx, originally described by Ono and Lindsey and 2024 Sex-Linked Differences in Cardiac Atrophy 11 employed and validated by many investigators was used as the model to simulate the effect of full mechanical unloading of the heart; its modification was established in our laboratory and is routinely employed.

The first important set of findings of the present study relates to our observation that the development of unloading-induced cardiac atrophy is substantially less significant in female than in male rats. We observed smaller decreases in whole heart weight, left ventricular weight, and right ventriuclar weight of the transplanted heart in the females.

The second important set of findings relates to the potential role of sex hormones on the differences in the process of unloading-induced cardiac atrophy. We found that gonadectomy did not alter the course of HTx-induced cardiac atrophy in male or female rats.

Our present data clearly show that the development of unloading-induced cardiac atrophy and myocardial fibrosis is substantially attenuated in females as compared with male rats, and these differences cannot be simply ascribed to the presence of sex steroid hormones.

Title	**Interim Analysis: Endoscopic Treatment of Gastroesophageal Reflux Disease by Applying Radiofrequency Energy to the Lower Esophageal Sphincter**
Author	**Kateřina Košťálová [1,2,3]**
Co-Authors	Ivan Kuzko [1], Miroslav Zavoral [1,2], Petr Urbánek [1], Štěpán Suchánek [1,2]
Affiliation(s)	[1] Department of Medicine, Charles University, 1st Faculty of Medicine and Military University Hospital Prague, Czech Republic [2] Department of Gastrointestinal Oncology, Military University Hospital Prague, Czech Republic [3] University of Defence, Military Faculty of Medicine, Department of Military Internal Medicine, Hradec Kralove, Czech Republic

Introduction: Gastroesophageal reflux disease (GERD) is common, and many patients do not fully respond to lifestyle changes or medication. The Stretta procedure, using non-ablative radiofrequency energy to strengthen the anti-reflux barrier, is a new treatment in the Czech Republic. This study compares the effectiveness of Stretta versus conservative treatment in reducing GERD symptoms and medication use.

Methods: This prospective, randomized 1:1 study evaluates primary outcomes of symptom relief (GERD-HRQL and RSI questionnaires) and daily proton pump inhibitors (PPI) use. Secondary outcomes include objective measures like pH-metry and manometry. Interim analysis was performed at 6 months.

Results: Of 152 screened patients, 30 were enrolled, with one dropout per group. The interim analysis included 10 Stretta and 13 control patients. Stretta reduced daily PPI use from 40 mg to 0 mg ($p = 0.049$) and improved GERD-HRQL ($p = 0.049$) and RSI ($p = 0.012$). However, objective measures like DeMeester score, acid exposure time (AET), and LES pressure showed no significant changes. Conservative treatment reduced PPI to 20 mg ($p = 0.010$) but did not significantly improve subjective or objective outcomes, except for a drop in LES pressure.

Discussion: Stretta led to a notable reduction in PPI use and improved subjective symptoms, though objective parameters remained unchanged. These findings suggest that Stretta may benefit patients with persistent symptoms despite conservative treatment.

Conclusions

The Stretta procedure shows potential for reducing PPI use and improving GERD symptoms. Larger, long-term studies are needed to confirm these interim findings.

Title	**Role of Hyaluronic Acid Metabolism in Endometrial Receptivity**
Author	**Eva Kriváková[1]**
Co-Authors	Daniela Rubanová [3,4], Petra Raptová[3], Zuzana Badovská [1], Peter Artimovič[1], Lukáš Kubala [2,3,4], Miroslava Rabajdová [1]
Affiliation(s)	[1]Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Safarik University, Košice, Slovakia [2] International Clinical Research Center – Centre of Biomolecular and Cellular Engineering, St. Anne's University Hospital, Brno, Czech Republic [3]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic [4]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Introduction: Despite extensive research, the success rate of embryo implantation during in vitro fertilization (IVF) remains low, approximately 30%, leading to a significant number of patients experiencing non-receptive endometrium. The extracellular matrix (ECM) of the endometrium plays a crucial role in this process, particularly through its adhesive molecules, with hyaluronic acid (HA) identified as critical for embryo implantation.

Methods: This study investigates HA metabolism's role in endometrial receptivity by analysing RNA sequencing datasets from the GEO database. We employed two endometrial epithelial cell lines: RL95-2, which resembles receptive endometrium, and AN3CA, representing non-receptive endometrium. Various techniques, including PCR and flow cytometry, were utilized to assess differences in HA metabolism between these cell lines.

Results: Our findings revealed significant downregulation of HA metabolism in samples from women with repeated IVF failures compared to those with successful pregnancies. In vitro experiments showed altered expression levels of HA-related genes and proteins consistent with the differences observed in human samples.

Discussion: The findings suggest that impaired hyaluronic acid (HA) metabolism may significantly contribute to the non-receptive state of the endometrium, which could hinder successful embryo implantation.

By identifying the downregulation of HA-related gene expression and protein levels in patients with repeated IVF failures, this study underscores the importance of HA in endometrial receptivity. Targeting HA metabolism may offer a promising therapeutic approach to enhance implantation success in assisted reproductive technologies (ART).

Conclusions: Altered HA metabolism may play a significant role in endometrial receptivity, highlighting its potential as a therapeutic target to improve ART outcomes.

Title	**A Novel Multi-Modal Approach to Polysomnographic Data Analysis Using Tailored Machine Learning Techniques**
Author	**Jiří Kuchyňka**
Co-Authors	Oldřich Vyšata
Affiliation(s)	Charles University, Faculty of Medicine in Hradec Králové

Introduction

Polysomnography (PSG) provides complex, multi-modal data essential for sleep disorder diagnosis. This study aims to develop an innovative framework for PSG data analysis using tailored machine learning approaches for different sleep-related events.

Methods

Methods: We developed a program for processing and analysing PSG data, incorporating separate pipelines for snoring, leg movements, respiratory events, and sleep stages. Our study utilized approximately 200 polysomnographic recordings. The approach features:

1. Integration of multiple modalities for comprehensive analysis.
2. Overlapping segmentation for training data, allowing for more precise event detection.
3. Multi-length segmental analysis for respiratory events to capture varying durations.
4. Optimized machine learning methods specific to each modality's characteristics.

Results

Initial analyses demonstrate high accuracy rates across modalities. For leg movement detection, our model achieved 96.96% accuracy on test data, with precision of 96.55% and recall of 96.96%. The ROC AUC of 0.9591 indicates excellent discriminative ability. Similar high performance was observed for other modalities, supporting the effectiveness of our approach.

Discussion

Our multi-modal, segmented approach shows promise in improving sleep event detection accuracy compared to traditional methods. The framework's flexibility allows for continuous incorporation of new improvements and techniques as they are developed.

Conclusions

This novel framework offers a comprehensive solution for PSG data analysis, potentially enhancing the accuracy and efficiency of sleep disorder diagnostics. Future work will focus on validating the approach across different clinical settings, various PSG devices, and alternative methods such as sleep polygraphy, further demonstrating its robustness and applicability.

Title	**Fluoxetine in adolescent depression: acute effects of monotherapy vs. quetiapine augmentation on body composition and autonomic stress response**
Author	**Kukučka Tomáš [1]**
Co-Authors	Ondrejka Igor [1], Višňovcová Zuzana [2], Ferencová Nika [2,3], Kováčová Veronika [1], Mačejová Andrea [1], Tonhajzerová Ingrid 1,[3]
Affiliation(s)	[1] Psychiatrická klinika, Jesseniova lekárska fakulta UK v Martine, Univerzitná nemocnica Martin, Kollárova 2, 036 59 Martin [2] Martinské centrum pre biomedicínu, Jesseniova lekárska fakulta UK v Martine, Malá hora 11161/4D, 036 01 Martin [3] Ústav fyziológie, Jesseniova lekárska fakulta UK v Martine, Malá Hora 11161/4C, 036 01 Martin

Introduction: Antidepressant treatment effects in adolescents are typically first assessed after 3-4 weeks. This study focuses on the impact of fluoxetine monotherapy vs. fluoxetine plus quetiapine augmentation on body composition and complex stress response using analysis of anthropometric and autonomic parameters.

Methods: Sixteen adolescent depressive patients were divided into monotherapy and augmented groups, measured on days 0 and 21 of treatment. Body composition was measured using bioelectrical impedance analysis, and dynamic sympathovagal balance in response to cognitive stressors was evaluated using heart rate variability (HRV) and electrodermal activity (EDA) analysis.

Results: The anthropometric measures - weight, muscle mass, BMI - significantly decreased in the fluoxetine group, but not in the augmented group. The HRV parameters HF % power mean and LF % power mean significantly increased in the monotherapy group, and increases of HF total power in the augmented group.

Discussion: This pilot study revealed expected decreases in anthropometric indices associated with increased autonomic coactivation indexed by HRV parameters in response to cognitive stress in the fluoxetine group, while the augmented group exhibited decreased sympathetic cholinergic activity indexed by EDA. According to neurovisceral theory, we suggest that fluoxetine monotherapy significantly improved cognitive and emotional regulation. In contrast, augmented treatment had a greater impact on anxiety reduction.

Conclusions: Although limited by small sample sizes, we suggest that the compared antidepressant treatments can differently affect body composition and autonomic stress response regulation among depressed adolescents. This study represents a first step towards understanding the specificities of emotional regulation and body composition changes in antidepressively treated adolescents

Title	**Obeticholic Acid Induces Uncoupling of Oxidative Phosphorylation in Models of Metabolic Dysfunction-Associated Steatotic Liver Disease**
Author	**Jan Melek[1]**
Co-Authors	Pavla Staňková[1], Eva Peterová[2], Tumisang Edward Maseko[1], Jan Dušek[1], Moustafa Elkalaf [1], Zuzana Červinková[1], Otto Kučera[1]
Affiliation(s)	[1]Department of Physiology, [2]Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

Introduction: Mitochondrial dysfunction plays a pivotal role in metabolic dysfunction-associated steatotic liver disease (MASLD). Farnesoid X receptor (FXR) agonists, particularly obeticholic acid (OCA), a semisynthetic bile acid derivative, have been explored as potential treatment for MASLD. However, the specific effects of OCA on liver cells remain unclear and concerns about potential hepatotoxicity led to discontinuation of key clinical trials in MASLD. The study aims to investigate the effects of OCA on liver cells using in vitro and in vivo MASLD models.

Methods: Primary murine hepatocytes, HepaRG and HepG2 cells were treated with titrated doses of OCA (1-25 µmol/L). HepaRG cells were exposed to free fatty acids (FFA) before 10 µM OCA administration. Cellular metabolism was analyzed using the Seahorse XFe-96 extracellular flux analyzer. Male C57BL/6J mice were fed with either a control diet or a Western diet for 36 weeks. From week 33, mice received either a vehicle or OCA (5 or 10 mg/kg b.w.). Liver and adipose tissue histology, plasma biochemistry, liver triglycerides and cholesterol, expression of selected genes (qRT-PCR) were evaluated. Mitochondrial respiration was assessed in liver homogenates using high-resolution respirometry (OROBOROS Oxygraph-2k).

Results: In vitro, OCA induced a dose dependent uncoupling effect on mitochondrial respiration in HepaRG and HepG2 cells, and mouse hepatocytes following acute exposure, associated with increased proton leak and decreased ATP production. This effect was also confirmed in HepaRG cells exposed to FFAs. Preliminary *in vivo* data did not reveal any significant effects of OCA on liver changes induced by WD feeding.

Discussion: The uncoupling effect of OCA on mitochondrial respiration suggests a potential mechanism by which OCA and related bile acids may influence cellular energy metabolism in liver cells. However, the lack of significant effects in vivo model highlights the complexity of OCA's role in MASLD and suggests that its therapeutic efficacy may be limited or context-dependent.

Conclusions: Obeticholic acid uncouples oxidative phosphorylation in murine hepatocytes, HepaRG, and HepG2 cells, as well as in FFA-exposed HepaRG cells. These findings provide insights into the potential direct mechanism of OCA and other bile acids on liver mitochondria. In our experimental design, OCA did not show any beneficial effects on MASLD in an experimental mouse model.

Title	**Bone mineral density as a sex determination feature**
Author	**Mgr. Alexander Morávek (1)**
Co-Authors	doc. Ing. Petr Henyš, Ph.D. (2), Mgr. Michal Kuchař, PhD. (1), Mgr. Katarína Harnádková (3,4), prof. RNDr. Jana Velemínská, Ph.D. (3), doc. RNDr. René Endlicher, Ph.D. (1), Mgr. Kateřina Prstková (1,5)
Affiliation(s)	1) Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic 2) Institute of New Technologies and Applied Informatics, Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Czech Republic 3) Department of Anthropology and Human Genetics, Faculty of Science, Charles University in Prague, Czech Republic 4) Department of Histology and Embryology, Third Faculty of Medicine, Charles University, Czech Republic 5) Faculty of Health Studies, Technical University of Liberec, Czech Republic

Introduction: The use of bone radio-density, measured in Hounsfield units, for estimating sex and age in forensic science has significant potential. This method can complement traditional anthropological analyses or be used independently. This study presents an improvement to this approach using the hyoid bone as a case study.

Methods: Seventy-three CT scans of healthy adults (44 females, 29 males) were obtained from clinical sources. We used statistical machine learning and data reduction techniques to process the data. A hyoid bone template was created, and spatial bone density was analysed through bijective mapping. To identify sex differences, a tetrahedron-wise and global partial least square regression model was applied.

Results: The study found that sex could be classified from various regions of the hyoid bone with a maximum accuracy of 79% and an average of 69%. Extreme spatial collinearity limited the benefits of using multiple points. However, by applying dimensionality reduction techniques, such as partial least squares analysis and discriminant analysis, and considering the entire bone, sex could be predicted with an accuracy exceeding 98.5%.

Discussion: Traditional methods for estimating sex from the hyoid bone have limitations. Our approach, which incorporates bone density mapping and shape normalization, achieved more than 98.5% accuracy, providing a more reliable tool for sex classification, even for smaller or fragmented bones. This method shows promise for broader forensic and anthropological applications.

Conclusions: This study introduces an innovative approach to bone radio-density analysis, demonstrating its utility for osteobiography and potential applicability to other bones.

Title	**Protection of the Endothelium and Endothelial Glycocalyx by Albumin and Sulodexide in Porcine Model of Kidney Transplantation**
Author	**Pavel Navrátil**
Co-Authors	Alena Ticha, David Weishaupt, Dana Cizkova, Ales Bezrouk, David Astapenko
Affiliation(s)	Charles University, Faculty of Medicine in Hradec Králové University Hospital Hradec Králové

Introduction: Kidney transplantation (KT) is a life-saving procedure for patients with end-stage renal disease. The success of KT is highly dependent on maintaining the integrity of the endothelium and its protective layer, the endothelial glycocalyx (EG). Ischemia-reperfusion injury (IRI), a common challenge in KT, can disrupt the EG, leading to various post-transplant complications. This study investigates the effects of albumin and sulodexide, two therapeutic agents, on protecting the endothelium and EG in a porcine model of KT.

Methods: Fourteen female piglets were prepared for KT simulation and randomly divided into three groups: a control group, an albumin-treated group, and a sulodexide-treated group. Various physiological parameters were monitored, and samples for serum and urine were collected at baseline and at multiple time points after reperfusion. The integrity of the endothelial glycocalyx was assessed by measuring serum syndecan-1 levels and urinary glycosaminoglycan concentrations. Histological examination of the renal cortex was also performed to evaluate tissue changes following the intervention.

Results: Statistically significant differences were observed in the sulodexide-treated group, where serum syndecan-1 levels were lower compared to the control group at 5 minutes post-reperfusion ($P=0.046$), indicating a potential reduction in EG damage. Similarly, in the albumin-treated group, urinary glycosaminoglycan levels were significantly lower than in the control group at 5 minutes post-reperfusion ($P=0.041$), which may suggest a protective effect on the EG. However, these findings are preliminary, and no other significant differences were detected between the treatment groups and the control group at later time points. Histological examination of the renal cortex revealed that the changes were generally minor across all groups.

Conclusion: The findings suggest that albumin and sulodexide may offer beneficial effects in preserving endothelial function during KT. The potential for these agents to enhance graft survival and improve KT outcomes warrants further investigation.

Title	***In vitro* study of YD-38 epithelial and MRC-5 fibroblast cells attachment and proliferation on anodized surfaces compared with turned surfaces**
Author	**Adél Ágnes Németh, DMD**
Co-Authors	Kinga Turzó, Ákos Nagy, Gyula Marada, Attila Mühl, Péter Szabó, Judit Kopniczky, Beáta Polgár, Gergő Kovács, Gergely Faludi
Affiliation(s)	University of Pécs, Medical Faculty, Dental School, Pécs, Hungary

Introduction

Dental implant failure is primarily attributed to peri-implant infections which lead to bone resorption. Soft tissue closure of dental implants is often inferior to natural tooth integration due to suboptimal surface characteristics. Our aim was to compare the attachment and proliferation of YD-38 epithelial and MRC-5 fibroblast cells on anodized surfaces compared with turned ones. Our hypothesis is that these anodized surfaces will present an ideal soft tissue integration for dental implants.

Methods

Surface properties of turned and anodized Ti discs (CP grade 5: Ti6Al4V) were analyzed with scanning electron microscopy (SEM) and atomic force microscopy (AFM). Cell responses were investigated via Cell-Counting Kit-8 (CCK-8), AlamarBlue and visualized with fluorescence microscopy. Cell nuclei were labeled with bisbenzimide Hoechst 33,342 blue dye and the cytoskeleton with the red phalloidin–tetramethylrhodamine B isothiocyanate (TRITC-phalloidin).

Results

SEM and AFM showed significant differences in the morphology and roughness (R_a) of the samples. Furthermore, the anodized discs exhibited significantly higher roughness than the turned ones, but still in the range of smooth surfaces ($R_a < 200$ nm). Both CCK-8 and AlamarBlue cell viability assays showed similarity between anodized and control discs. Fluorescent staining confirmed these results.

Discussion

YD-38 epithelial and MRC-5 fibroblast cells readily adhered to and proliferated on the anodized discs, therefore the anodized surface presented ideal surface characteristics for the specific cells.

Conclusions

The *in vitro* study proved that the tested anodized samples are proper candidates to be used as abutment material for optimal soft tissue integration.

Title	**Role of LPS on EMT and ER Stress in Lung Epithelial Cells**
Author	**Turkan Portakal**
Co-Authors	Vítězslav Havlíček[1], Jarmila Herůdková[1], Vendula Pelková[1], Riza Can Cakmakci[1], Aleš Hampl[1,2], Petr Vaňhara[1,2]
Affiliation(s)	[1]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic [2]International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

Introduction

Lipopolysaccharide (LPS), an outer membrane component of Gram-negative bacteria, is known to induce inflammation. However, its impact on lung epithelial cells remains poorly understood. The initiation of epithelial to mesenchymal transition (EMT) by LPS in lung epithelial cells can contribute to the interstitial fibrosis, potentially leading to respiratory failure. Elevated endoplasmic reticulum (ER) stress activates the unfolded protein response (UPR), and excessive UPR has been linked to EMT. In this study, we aimed to explore the effects of LPS on EMT in Expandable lung-like epithelial (ELEP) cells and investigate the involvement of ER stress in alterations of lung epithelia.

Methods

ELEP cells were derived from human embryonic stem cells (hESCs). Cells were treated with LPS, ER stress activator tunicamycin, and inhibitor TUDCA, and changes were evaluated by functional and morphological analysis, WB, PCR, and IF techniques under 2D and 3D culture conditions.

Results

LPS induced morphological changes in ELEP cells indicating EMT. LPS triggered the ER stress response by increasing the expression of UPR-related proteins; TUDCA partially blocked this stress. LPS promoted EMT by increasing SNAIL and SLUG expressions and led to increased E-Cadherin and N-Cadherin, cytoplasmic E-Cadherin accumulation was observed, and these effects were reduced by TUDCA.

Discussion

LPS triggered the ER stress response and EMT. TUDCA partially blocked the effects of LPS, supporting the role of ER stress in EMT. LPS appears to be a potential trigger of EMT via ER stress in lung epithelial cells.

Conclusions

LPS triggers the ER stress response in ELEP cells and induces EMT by changing E-Cadherin and N-Cadherin.

Title	**Assessment of Agrimonia eupatoria L. and lipophosphonoxin combination for wound repair: Bridging the gap between phytomedicine and organic chemistry**
Author	**Jana Sabová[1]**
Co-Authors	Miriam Kaňuchová[1], Veronika Brindza Lachová[2], Kateřina Bogdanová[3], Petra Bonová[4], Tomáš Vasilenko[5], Ivan Kováč[6], Milan Kolář[3], Pavel Mučaji[2], Dominik Rejman[7], Peter Gál[1,2,8,9]
Affiliation(s)	[1]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia [2]Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia [3]Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic. [4]Institute of Neurobiology, Biomedical Research Center of the Slovak Academy of Sciences, Košice, Slovakia [5]Department of Surgery, AGEL Hospital Košice-Šaca and Pavol Jozef Šafárik University, Košice-Šaca, Slovak Republic [6]Second Department of Surgery, Louis Pasteur University Hospital and Pavol Jozef Šafárik University, Košice, Slovakia [7]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences v.v.i., Prague, Czech Republic [8]Department of Biomedical Research, East-Slovak Institute of Cardiovascular Diseases, Inc., Košice, Slovakia [9]Prague Burn Center, Third Faculty of Medicine, Charles University and University Hospital Královske Vinohrady, Prague, Czech Republic.

Introduction: Chronic, non-healing wounds remain a significant healthcare challenge. This study explores the synergistic effects of Agrimonia eupatoria L. (AE) and lipophosphonoxin (LPPO) in enhancing wound healing by combining traditional phytomedicine with modern organic chemistry.

Methods: Lyophilized AE extracts and second-generation lipophosphonoxin LPPO DR-6180 were tested *in vitro* on human keratinocytes, fibroblasts, and endothelial cells. MTS viability, 2D migration, western blot, and immunofluorescence assays were performed to evaluate AE/LPPO effects on fibroblast-to-myofibroblast conversion, cell proliferation and differentiation, extracellular matrix (ECM) deposition and the modulation of TGF-β1 and VEGF-A signaling. Additionally, *in vivo*, full-thickness skin excisions in Sprague-Dawley rats were treated with AE, LPPO, or their combination, and wound healing was assessed macroscopically and histologically. Antibacterial efficacy against *Staphylococcus aureus* was determined through MIC and MBC tests, with checkerboard assays examining synergistic effects.

Results: Our results indicate that AE/LPPO promotes myofibroblast-like phenotypic changes, augments ECM deposition, do not interfere with TGF-β1 and VEGF-A signaling and accelerates wound closure in rats. While both AE and LPPO individually exhibited antibacterial properties, their combination did not enhance this effect, suggesting potential antagonism in antimicrobial activity.

Discussion: These findings indicate that AE/LPPO promotes tissue repair and wound healing. However, the reduced antibacterial effect when combined requires further investigation to optimize its therapeutic use.

Conclusion: The combination of AE and LPPO offers promising benefits for wound healing by combining traditional and modern approaches. Further research is needed to explore its full therapeutic potential and refine the balance between wound repair and antimicrobial activity.

Title	**Profiling Methylation Changes of DNA in Lung Cancer**
Author	**RNDr. Tereza Šenkýřová**
Co-Authors	RNDr. Petra Šauer Mikysková[1], MUDr. Eva Kočová, Ph.D.[2], Ing. Helena Párová, Ph.D.[1], prof. MUDr. Vladimír Palička, CSc.[1]
Affiliation(s)	[1] Department of Clinical Biochemistry and Diagnostics, Charles University, Faculty of Medicine in Hradec Králové and University Hospital Hradec Králové [2] Department of Radiology, University Hospital Hradec Králové

Introduction

Lung cancer is the leading cause of cancer-related death, with most cases diagnosed at an advanced stage. Screening with low-dose computed tomography has limited specificity and often yields equivocal findings. This research focuses on the early detection of lung cancer through DNA methylation analysis in circulating cell-free DNA (cfDNA).

Methods

cfDNA is isolated from the blood of patients undergoing screening. Next generation sequencing (NGS) using the RRBS method will be performed to assess DNA methylation. The genes SHOX2, RASSF1A, and PTGER4 were selected due to their epigenetic role in lung cancer, with additional genes included based on bioinformatic analysis of NGS results. Selected methylation alterations will be verified by MS-PCR or HRM analysis.

Results

A number of studies have confirmed the potential of detecting methylation alterations in cfDNA as a non-invasive diagnostic tool with high sensitivity and specificity, especially in the early stages.

Discussion

The detection of cfDNA provides a non-invasive way to monitor the tumour microenvironment. It allows real-time assessment of the patient's condition and early detection of tumour changes. This method has the potential to overcome the limitations of imaging techniques. NGS technology allows detailed analysis of cfDNA even in samples with small amounts of DNA, increasing the accuracy and sensitivity of the methylation profile and allowing the identification of novel epigenetic markers.

Conclusions

DNA methylation profiling in cfDNA represents a promising non-invasive approach for early lung cancer diagnosis, potentially complementing current screening methods and reducing the need for invasive biopsies.

Title	**Prediction of early postoperative morbidity and ability to rehabilitate in patience after cardiac surgery („CardioStep Study“)**
Author	**MUDr. Petr Smolák**
Co-Authors	MUDr. Ján Gofus, PhD., MUDr. Salifu Timbilla, MUDr. Martin Voborník, PhD., Martin Maťátko, prof. MUDr. Jiří Manďák, Ph.D., prof. MUDr. Jan Vojáček, Ph.D.
Affiliation(s)	Charles University, Faculty of Medicine in Hradec Králové

Introduction

Well-known scoring systems such as the EUROSCORE II or STS score focus on the risk of early postoperative mortality. However, they do not assess the risk of postoperative morbidity and the ability to rehabilitate. None of the systems developed so far has gained more popularity and fulfilled the function of a predictive tool for use in clinical practice. The aim of our study was to evaluate different methods of predicting postoperative morbidity and ability to rehabilitate in practice, and at the same time to objectify patient rehabilitation using a pedometer.

Methods

From May 2021 to December 2024, a prospective monocentric observational study of patients undergoing elective cardiac surgery from sternotomy (myocardial revascularization, single-valve procedure, MAZE procedure, or a combination of both) was conducted. Patients underwent preoperative exercise tolerance testing (one-minute sit-to-stand test - 1MSTST, six-minute walk test - 6MWT, five-meter walk test, grip strength), body composition measurements on a bioimpedance scale, and comprehensive blood tests to determine predictors of postoperative recovery and rehabilitation capacity as defined by measurement with the ActiGraph wGT3X-BT pedometer.

Results

197 patients were enrolled and examined in the study. The early mortality rate of patients in the study was 0.51%. Patients with worse 1MSTST scores were at higher risk of prolonged ICU stay (OR 1.98, p=0.05). Patients with lower muscle mass were at higher risk of prolonged hospitalization (OR 0.93, p=0.01). During the first 84 hours in the standard ward, they walked an average of 6391 steps and had 88.8% sedentary activity.

Conclusions

Patients with worse 1MSTST (p=0.03) and 6MWT (p=0.03) scores showed objectively slower rehabilitation. Patients in better physical condition before cardiac surgery are able to rehabilitate better and faster and have a lower risk of prolonged hospitalization.

Title	**Changes in lipid and glucose metabolism in cells exposed to hypoxia**
Author	**Mgr. Špalková Veronika**
Co-Authors	doc. MUDr. Otto Kučera, Ph. D., Ph.D, MUDr. Tumisang Edward Maseko, Moustafa Elkalaf, M.B.B.Ch., Ph.D.
Affiliation(s)	Charles University, Faculty of Medicine in Hradec Králové

Introduction

Hypoxia is a condition where the body or an area of the body does not have an adequate supply of oxygen at the tissue level. It is often an undiagnosed and untreated condition that significantly increases the risk of developing type 2 diabetes mellitus, non-alcoholic fatty liver disease and subsequently steatohepatitis. The central aspect of the research is mitochondria, whose function and dynamics are significantly affected by hypoxia. The goal of the study is to understand the metabolic changes that occur in cells exposed to hypoxic conditions.

Methods

***In vitro* model of hypoxia**

HepG2 cells were exposed to normoxic or artificial hypoxic conditions, simulated using gas mixtures with different oxygen concentrations (1 % O_2, 4 % O_2 and 12 % O_2) for 72 hours. Subsequently, the metabolic profile of the cell line was assessed using the Cell Mito Stress Test and Glycolytic Rate Assay (Agilent Seahorse XFe96 Analyzer).

Results

Severe hypoxia was detrimental to mitochondrial ATP production and reduced mitochondrial respiratory capacity in HepG2 cells. Glycolytic parameters showed a various response, and the optimal mitochondrial function is maintained at oxygen level between 4 % and 12 %.

Discussion

Optimal mitochondrial function in HepG2 cells is sustained within an oxygen concentration range of 4 % and 12 %, where cells exhibit efficient energy production and homeostasis. Deviations from this range, specifically at oxygen levels of 1 % (severe hypoxia) and 21 % (atmospheric oxygen level), are suboptimal. Increasing (i.e. 21 %) or decreasing (i.e. 1 %) the oxygen concentration above or below this range leads to a deterioration of the tested mitochondrial functions.

Conclusions

The results could subsequently contribute more comprehensively to a better understanding of adaptive metabolic mechanisms that operate under hypoxic conditions, both at the systemic and subcellular levels.

Title	**Optimization of Adherent Cell Cultures: Enhancing Growth Monitoring with Automated Cell Counting**
Author	**Agnieszka Staniszewska**
Co-Authors	NA
Affiliation(s)	International Doctoral School: Medical University of Lodz, Mabion S.A.

Introduction

This project focuses on the optimization of adherent cell line cultures (MRC-5, BHK-21 [C-13], Vero, C6/36). The objective was to establish growth curves, test the stability of EMEM medium with 10% FBS, and optimize cell counting using the Vi-Cell BLU counter. The goal was to replace traditional manual counting methods, like the Thoma or Bürker hemocytometer, with automated systems.

Methods

Cells were seeded at various densities (e.g., 20,000, 15,000, 10,000 cells/cm²), and their growth, viability, and confluency were monitored every 24 hours. The results were gathered via both manual counting and automated counting using the Vi-Cell BLU. Parameters, such as the minimum cell size for automated counting, were adjusted based on measurements to optimize the results. Growth and confluency curves were generated to determine optimal passage times based on seeding density.

Results

The study showed that each cell line had a distinct growth profile depending on the seeding density. For instance, Vero cells reached 100% confluency at 48 hours, while MRC-5 took 72 hours at different densities. Comparing manual and automated counting, the coefficient of variation (CV%) was calculated. In cases where CV% exceeded acceptance criteria, adjustments were made to the automated counter.

Discussion

Seeding density directly influenced growth rates and time to confluency. Adjusting the Vi-Cell BLU's settings, particularly the minimum cell size, significantly improved the accuracy of automated counting.

Conclusions

Optimizing seeding density and automated counting parameters enabled accurate cell growth monitoring. With proper adjustments, the Vi-Cell BLU can serve as a reliable alternative to manual counting methods, improving efficiency in cell culture workflows.

Title	**Assessing Trunk Muscle Function and Intra-Abdominal Pressure: Evaluations and Correlations in Clinical Practice**
Author	**Martin Stribrny**
Co-Authors	Alena Kobesova
Affiliation(s)	Department of Rehabilitation and Sports Medicine, Second Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic

Evaluating postural trunk muscle function is crucial in the clinical assessment of patients with musculoskeletal pain or dysfunction. Both intra-abdominal pressure and the postural activation of trunk muscles can be measured through various invasive and non-invasive methods. In rehabilitation practice, the most common tool for assessing trunk stabilization is the palpation of abdominal wall tension. However, this method is subjective and, therefore, unsuitable for scientific research.

In our initial study[1], we assessed the postural activity of trunk muscles in thirty-one asymptomatic participants (mean age = 26.77 ± 3.01 years) using two simultaneous measurement techniques: the pressure sensor DNS Brace and anorectal manometry. These measurements were taken across five different postural-respiratory scenarios: resting breathing, the Valsalva maneuver, the Müller maneuver, instructed breathing, and loaded breathing while holding a dumbbell. The study found a strong correlation between the measurements from the anorectal manometry and the DNS Brace across all five scenarios. This confirms that intra-abdominal pressure can be indirectly evaluated by monitoring abdominal wall tension with pressure sensors.

In our subsequent study [2] we examined the correlation between the subjective assessment of trunk muscle stabilization and the objective measurement of abdominal wall expansion in twenty-five healthy participants (mean age = 22.4 years). The objective measurements were obtained using a trunk brace (DNS Brace) that externally measures abdominal wall pressure. Clinical assessments were conducted by two experienced Dynamic Neuromuscular Stabilization (DNS) clinicians. Correlation analysis revealed moderate to strong relationships between the clinical assessments and the DNS Brace values in several tests: the intra-abdominal pressure regulation test, the Diaphragm test, and the Hip Flexion test.

The Diaphragm Test and the Intra-Abdominal Pressure Regulation Test (IAPRT) are among the most frequently used assessments within the DNS (Dynamic Neuromuscular Stabilization) concept. These qualitative clinical tests evaluate the postural stability provided by the diaphragm. In a study assessing the inter-rater reliability of the Diaphragm Test and IAPRT, comparisons were made between an experienced and a novice DNS clinician. The study involved forty-five participants with non-specific low back pain (LBP) and neck pain. The findings indicated moderate reliability in evaluating both LBP and neck pain patients. Notably, a higher degree of reliability was observed in patients reporting more severe pain [3].

Lastly, we utilized pressure sensors (Ohmbelt device) to monitor abdominal wall tension (AWT) across five different postural positions: sitting, supine with legs raised, squat, bear, and hang positions. Each position was evaluated first without any correction and then following verbal and manual instructions based on Dynamic Neuromuscular Stabilization (DNS) principles.

The study involved thirty healthy individuals with a mean age of 22.73 ± 1.91 years. The results showed a significant increase in abdominal wall tension after applying DNS instructions, with the greatest activation observed in the bear position [4].

Title	**Vestibular asymmetry: Do we interpret it correctly?**
Author	**Maja Striteska**
Co-Authors	Prof. Erich Schneider
Affiliation(s)	Charles University, Faculty of Medicine in Hradec Králové, Czechia Institute of Medical Technology, Brandenburg University of Technology Cottbus – Senftenberg, Cottbus, Germany

Introduction

The caloric and the video head impulse tests (vHIT) are the cornerstones of instrumental balance diagnostics. The key point to understand and address the patient's complaints lies in the dys/balance – vestibular asymmetry. To calculate asymmetry, both tests use Jongkees' formula (JF), which assumes a normal strong ear (SE) and an affected weaker ear (WE) to calculate unilateral weakness.

Methods

A mathematical analysis aimed to clarify what question JF answers and to discuss a more illustrative assessment of vestibular asymmetry.

Results

The JF has three limitations. First, it has a non-linear characteristic that always underestimates the paresis. Second, JF splits the difference between the two sides into two parts, with the average response in the middle. Thus, instead of reporting unilateral weakness as clinicians understand it, JF answers two other questions: "How much is the WE response below the average" and, at the same time, "How much is the SE response above the average". A linear paresis calculation was later introduced to overcome these limitations. However, even this approach did not eliminate the third limitation of JF: The results are artificially inflated and sensitive to small changes in WE when both ears are affected.

Unlike the caloric test, the vHIT already relies on head velocity as an absolute reference to calculate gain. Therefore, the SE response is not needed as an additional reference to calculate vHIT asymmetry. Instead, we suggest using the ideal gain of 1 in the denominator, which reduces the formula to the side-to-side gain difference without a denominator. This avoids artificially inflated results in bilateral deficits and is easy for clinicians to calculate.

Conclusions

To reflect the expected outcome of the caloric test, the unilateral weakness, we suggest using a linear paresis calculation with only the stronger ear (SEF) response as the unilateral reference value in the denominator.

For vHIT, we suggest using the ideal gain of 1 as a reference, which reduces the formula to only the side-to-side gain difference. This avoids artificially inflated results in bilateral deficits and is easy for clinicians to calculate.

Title	**Assessment of serum granulin levels in patients with neuroendocrine tumors of the lung and the gastrointestinal tract**
Author	**Agata Świętek [1,2]**
Co-Authors	Janusz Strzelczyk [3], Elżbieta Chełmecka [4], Joanna Katarzyna Strzelczyk [1]
Affiliation(s)	[1] Department of Medical and Molecular Biology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poland [2] Silesia LabMed Research and Implementation Centre, Medical University of Silesia in Katowice, Poland [3] Department of Endocrinology and Neuroendocrine Tumors, Department of Pathophysiology and Endocrinology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Poland [4] Department of Medical Statistics, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Poland

Introduction

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms accounting for about 2% of all malignancies. Granulin (GRN) is a glycoprotein been described as a protein involved in proliferation, cell growth and tumorigenesis in various types of cancer. The aim of this study was to evaluate serum GRN protein concentrations in NET and to examine their association with the clinical and demographic variables.

Methods

The study included 90 patients with NET and a control group of 76 individuals. The concentrations of GRN protein (ELISA method) in serum were determined.

Results

Granulin concentrations in the study group samples were found significantly higher than in the control group (7.35±3.82 vs 5.67±2.67); ($p<0.01$). Weak positive correlation was observed between GRN and the levels of serotonin and triglycerides in study group ($p<0.05$). Weak positive correlation was observed between age and GRN ($p<0.01$) in the controls.

Discussion

As in our study, overexpression of granulin or its precursor, occurs in breast cancer, ovarian cancer, bladder cancer, hepatocellular carcinoma, gliomas, multiple myeloma and uterine leiomyosarcoma.

Conclusions

Our results of the first study on NET, added more precise information on the function of granulin in the serum of NET patients. The level of the GRN may be dependent also on the other factors, such as triglycerides, which may be related to cancer pathogenesis and serotonin, which could consequently promote stimulation of cell proliferation. The data obtained may suggest the involvement of GRN in the process of NET carcinogenesis, this however requires further analyses.

Title	**Preparation and biological evaluation of novel harmine β-carboline alkaloid derivatives as potential antitumor candidate**
Author	**MUDr. Abdul Aziz Timbilla[1]**
Co-Authors	Rudolf Vrabec[2], Radim Havelek[1], Darja Koutova[1], Karel Kralovec[3], Jakub Chlebek[2], Lucie Cahlikova[2], Martina Rezacova[1]
Affiliation(s)	[1]Department of Medical Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University, Simkova 870, Hradec Kralove 500 03, Czech Republic [2]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy, Charles University, Heyrovskeho 1203, Hradec Kralove 500 05, Czech Republic [3]Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Studentska 573, Pardubice 532 10, Czech Republic

Introduction: Harmine, a β-carboline alkaloid, holds medicinal promise as an anticancer agent. Discovered in 1847 within *Peganum harmala* seeds, it exhibits diverse health benefits beyond antitumor properties.

Aim: To find semisynthetic harmine derivative which possess the most effective cytotoxic activity against cancer cell lines but shows little impact to non-cancer cells. The best derivatives will be studied further to understand its mechanism of action.

Methods: Cell viability and proliferation were assessed using Trypan blue exclusion after treating cells with harmine and most active harmine derivative HMA-16 for 24, 48, and 72 hours. IC_{50} values were determined using WST-1 assays. The cellular proliferation over time was examined using xCELLigence system. Flow cytometry with Annexin V/propidium iodide and TUNEL staining assessed apoptosis induction.

Results: HMA-16, the most promising derivative, exhibited 60% mean growth across determined cell lines, notably affecting MOLT-4, Jurkat, and A2780 (IC_{50} values: 7.68 ± 1.03, 2.90 ± 1.05, and 6.43 ± 1.13 μM, respectively). HMA-16 was able to activate apoptosis, as determined by Annexin V and TUNEL labelling. The xCELLigence system demonstrated concentration-dependent effects in cancer cells, while showing mild cytostatic activity against non-cancer lung fibroblasts MRC-5.

Discussion: HMA-16's significant cytotoxicity, especially against MOLT-4, Jurkat, and A2780, was supported by micromolar IC_{50} values. Exposure to HMA-16 caused apoptosis with phosphatidylserine externalization and DNA fragmentation. HMA-16 had a milder impact on non-cancer cells than parent harmine.

Conclusion: The result of this study reveals the potential of HMA-16 as an active anticancer drug with lower impact on non-cancer human lung fibroblast.

Title	**Balloon-occluded chemoembolization of hepatocellular carcinoma**
Author	**Lt. Col. Tomáš Tůma, MD, EDiR.**
Affiliation(s)	1 - Central Military Hospital – Military University Hospital Prague, Radiodiagnostic Department, Department of Interventional Radiology 2 - University of Defence, Faculty of Military Medicine, Hradec Králové, Department of Military Internal Medicine and Military Hygiene

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant liver tumor. In the vast majority of cases it develops in the field of pre-existing chronic liver disease. Transarterial chemoembolization (TACE) is the standard of care for intermediate stage HCC. A modern modification of this method is microballoon occlusive chemoembolization (B-TACE).

Methods

A prospective evaluation of the results of B-TACE was performed according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) 1 month after the procedure, complications of the procedure and 90-day mortality after the procedure.

Results

Totally 21 HCC nodules were treated. After the initial B-TACE a complete response was achieved in 16 nodules (76%) a partial response in 5 nodules (24%).

There was no serious post/periprocedural complication of the procedure. There was no death within 90-day follow-up.

Discussion

For standard TACE the one-month CR rate ranges from 44-60%. In the cohort presented by us B-TACE achieved a higher CR rate already after the first stage (76%).

The limitation of the presented results is the limited size of the patient cohort and the short follow-up which did not allow the evaluation of overall survival (OS) and its comparison with standard TACE which is also performed at our workplace.

Conclusions

B-TACE is a modern modification of TACE with a favorable safety profile and shows a higher rate of complete response according mRECIST. The pursuit of a complete response to treatment without the need for re-embolization is associated with longer survival and for that reason B-TACE appears to be a suitable and effective method of treatment.

Title	**Structural and Morphological Changes in the Blood Vessels of the Kidney of White Rats Under Simulating Different Degrees of Dehydration**
Author	**K. Vakhnovska[1]**
Co-Authors	Supervisor: I. Herasymiuk[2]
Affiliation(s)	[1] State Establishment «Lugansk State Medical University», Rivne, Ukraine [2] I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, Ternopil, Ukraine

Introduction: When there is a shortage of water in the body, there is a violation of the water-electrolyte balance which can significantly affect the overall homeostasis. The aim of this study was to determine the peculiarities of morphological changes in the blood vessels of the kidneys of white laboratory rats at different degrees of dehydration.

Methodology: The experiment was conducted on 36 white sexually mature male rats. They were divided into 2 groups: control and experimental. For the last dehydration was simulated by the lack of access to water for 3, 6 and 10 days and by feeding dry oats. Kidney fragments were taken for histological examination and fixed in 10% neutral formalin solution and 96% alcohol. Then paraffin sections with a thickness of 5-8 μm were stained with hematoxylin and eosin.

Results: Already after 1 day of the experiment there were changes related to the blood stream, particulary, in the arteries and veins there was an increased blood filling in comparison with the control rats. More significant changes appeared already on the 3rd day of the anhydrous diet. The tone of the walls of partial and arcuate arteries was significantly increased and in the lumen of these vessels there was an accumulation of shaped elements. The changes in the lobular arteries were somewhat different: their lumen was expanded and the content of formed blood elements was significantly lower than in the previous generation. After the 6th day of dehydration the tone of partial arteries continued to increase and their lumen narrowed which was densely filled with formed elements. In the lobular arteries and arterioles the lumen was somewhat wider and the walls were thinnerwhich indicated a decrease in the tone of the smooth muscle membrane. In the absence of water for 10 days the walls of partial arteries thickened and the lumen narrowed and was filled with shaped elements. The formation of thrombotic masses was noted in the veins that were nearby. As for the small arteries they had thinned walls a widened lumen and only insignificant accumulations of formed elements that is dilatation reactions. Adjacent veins regardless of dilation were anemic.

Discussion: With mild and moderate degrees respectively 3rd and 6th days of an anhydrous diet an increased tone narrowing of the lumen of vessels and a decrease in the permeability of extra-organ and intra-organ arteries at the level of partial and arch arteries were noted. At the same time lobular arteries and arterioles acquired the opposite changes namely a tendency to vasodilation. With a severe degree of dehydration (10 days of total dehydration) the previous changes intensified except for the arcuate arteries where there was an inversion of the smooth muscle layer which led to a change in constriction reactions to dilation reactions.

Conclusions: Changes in the vascular bed of the kidneys depend on the degree of general dehydration. Can be assumed the state of small vessels is necessary to maintain the blood supply of nephrons in the event of insufficient nutrition from the main vessels. The obtained data fully confirm the current ideas about dehydration as a cause of hypovolemia in which there are vascular reactions aimed at the centralization of blood circulation, which threatens hypoperfusion of internal organs, including the kidneys.

Title	**Outcome of tailored antiplatelet therapy in carotid stenting: a retrospective comparative study**
Author	**Pavol Vigláš [1,3]**
Co-Authors	Vojtěch Smolka[3], Jan Raupach [1,2], Aleš Hejčl [4], David Černík [5], Filip Cihlář [3]
Affiliation(s)	[1]Department of Radiology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic [2] Department of Radiology, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic [3] Department of Radiology, Masaryk hospital – J.E. Purkinje University and KZ a.s., Ústí nad Labem, Czech Republic [4] Department of Neurosurgery Masaryk Hospital – J.E. Purkinje University and KZ a.s., Ústí nad Labem, Czech Republic [5] Department of Neurology, Masaryk Hospital –, Ústí nad Labem, Czech Republic

Introduction

Carotid stenting requires dual antiplatelet therapy to effectively prevent thromboembolic complications. However, resistance to clopidogrel, a key component of this therapy, may lead to persistent risk of these complications. The aim of this study was to determine, if the implementation of routine platelet function testing and adjusting therapy was associated with lower incidence of thromboembolic complications and death.

Methods

All consecutive patients treated with carotid artery stenting in a single institution over 8 years were enlisted in a retrospective study. Platelet function testing was performed, and efficient antiplatelet therapy was set before the procedure. Incidence of procedure-related stroke or death within periprocedural period (0-30 days) was assessed. The results were evaluated in relation to the findings of six prominent randomized control trials.

Results

A total of 241 patients were treated for carotid stenosis, seven patients undergo CAS on both sides over time. There was 138 symptomatic (55,6%) and 110 asymptomatic stenoses (44,4%). Five thromboembolic complications (2,01%) occurred, four of them (1,61%) was procedure-related. Two patients died because of procedure-related stroke (0,82%). Incidence of procedure-related stroke or death was significant lower compared to the results of CREST study (2,01 % vs. 4,81 %, P=0,0243) in the entire cohorts, and to the results of ICSS study in the symptomatic cohorts (2,86 % vs. 7,37 %, P=0,0243), respectively.

Conclusions

Tailored antiplatelet therapy in carotid stenting is safe and seems to be related with lower incidence of procedure-related death or stroke rate. Larger prospective studies to assess whether platelet function testing-guided antiplatelet therapy is superior to standard dual antiplatelet should be considered.

Title	**Alterations in T cell Phenotype and Function in Schimke Immuno-osseous Dysplasia**
Author	**Ondřej Vladyka[1]**
Co-Authors	Jakub Zieg[2], Ondřej Pátek[3], Zuzana Paračková[1], Adam Klocperk[1]
Affiliation(s)	[1]Department of Immunology, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic [2]Department of Pediatrics, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic [2]Department of Internal Medicine, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic

Schimke Immuno-Osseous Dysplasia (SIOD) is a rare disease with the prevalence of 1:1-3,000,000, caused by loss-of-function mutations in the *SMARCAL1* gene, coding for a protein responsible for chromatin remodeling and DNA repair. Immunodeficiency, along with growth failure, progressive kidney failure and migraines, is one of its dominant clinical features. Cellular immunity – with T cell lymphopenia at the forefront – is most affected, but humoral immunity is also impaired due to loss of antibodies caused by nephrotic proteinuria.

In this project we compared T cells and PBMCs of four patients with SIOD to those of age and sex matched healthy donors. We employed classical and spectral flow cytometry to assess the phenotypical and functional properties of T cells, both ex vivo and in IL-7 non/enriched cell culture. Furthermore, we used the NanoString Sprint for panel RNA sequencing of genes related to the immune system and compared native RNA expression in the PBMCs and RNA expression after UV treatment. Lastly, we assessed chromatin damage related to UV treatment.

Our findings expand the existing knowledge of immunodeficiency in SIOD, specifying features of T cells in addition to the previously described reduction in naive T cells and alterations in CD127/IL7R expression. In addition to a well-documented decrease in naive T cells, we observed an increased proportion of Th1 cells and changes in the expression of several markers indicative of chronically activated/exhausted T cell phenotype in SIOD. Specifically, there was an upregulation of exhaustion and senescence-associated markers FAS, TOX, PD1, Tim3 and Ki67 in naive and a downregulation of survival/progenitor markers CD27, CD28 and TCF1 in memory CD4 T cells. In CD8 T cells, an increase in activation markers CD38 and HLA-DR was most apparent, with a further increase in Ki67 and Granzyme B.

While we saw a significant increase in UV-induced DNA damage in patients' cells, RNA sequencing analysis indicated that UV treatment did not induce a specific gene expression pattern change in SIOD. Instead, it accentuated differences already present in native samples, including a further decrease in IL7R, TCF7, and CD27 mRNA transcription.

In summary, T cells in SIOD have decreased stemness, increased expression of inhibitory and activation markers, and enhanced Granzyme B production. This altered state is further exacerbated by UV exposure. Interestingly, in vitro culture with IL-7 does not rescue nor significantly alter the differences between patient and healthy cells, despite the reduced surface expression of IL7R on T cell in SIOD.

Title	**The safety and efficacy of orthotopic heart transplantation in patients aged 65 and older**
Author	**Szymon Warwas[1]**
Co-Authors	Agnieszka Kuczaj[1], Tomasz Hrapkowicz[1]
Affiliation(s)	[1]Department of Cardiac, Vascular and Endovascular Surgery and Transplantology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland

Introduction

In recent years, the age demographic of heart transplant recipients has shifted, with an increasing number of patients over 65 with end-stage heart failure being considered for orthotopic heart transplantation (OHT). This study aimed to assess the safety and efficacy of OHT in elderly patients.

Methods:

From a total cohort of 1,509 patients who underwent OHT at the Silesian Center for Heart Diseases in Zabrze between November 1985 and December 2023, 64 patients (4.24%) aged 65 years or older were included in this analysis.

Results:

The median age of patients at the time of transplantation was 66 years (IQR: 65-67), with 11 patients (17.2%) being female. The most common cause of heart failure was ischemic cardiomyopathy, identified in 33 patients (51.6%). Mechanical circulatory support was used in 10 patients (15.6%) prior to OHT. The one-month survival rate was 76.6%, and among those who survived the first month, 83.7% lived through the first year. The median time from OHT to death was 82 days (IQR: 11-1312). Significant acute cellular rejection (ACR) occurred in 28 patients (43.8%).

Discussion:

The incidence of ACR in elderly patients was surprisingly relatively high. However, survival rates after OHT in elderly individuals were comparable to those in younger patients. Among the causes of death, cardiovascular diseases and neoplasms were the most common, aligning with the leading causes of death globally.

Conclusions:

Heart transplantation is a viable treatment option for patients aged 65 and older with end-stage heart failure.

Title	**Synthesis and evaluation of novel compounds as potential active ingredients against CBRN threats**
Author	**Natalie Zivna**
Co-Authors	
Affiliation(s)	University of Defence, Military Faculty of Medicine, Hradec Kralove

Introduction

Chemical, biological, radiological, and nuclear (CBRN) threats pose significant risks in emergencies and attacks. Protective measures are essential for mitigating these dangers. This study focused on the development of novel compounds that act as protective countermeasures against chemical and biological decontamination, as well as radioprotection.

Methods

Quaternary ammonium compounds (QACs) were synthesized and evaluated for their ability to decontaminate surfaces contaminated with nerve agents of organophosphorus origin. The degradation efficiency of the compounds was determined by measuring the rate constants of contaminant breakdown. For biological agents, similar QAC compounds were prepared with optimized solubility and reduced cytotoxicity. Additionally, piperazine-based derivatives were as radiation countermeasures, to prevent apoptosis of healthy tissue caused by ionizing radiation.

Results and Discussion

The QACs showed potent efficacy in degrading organophosphorus surrogates. Sixteen QACs derivatives were developed and published, which exhibited improved properties and strong antimicrobial efficacy. These derivatives were superior to currently used QAC-based agents in practice. The piperazine-based compounds demonstrated better radioprotective performance than amifostine, with significantly lower toxicity. While the mechanism of action remains uncertain, these compounds offer a safer and more effective profile than currently available treatments.

Conclusions

Our novel QAC-based compounds show great promise as chemical and biological decontaminants, while the piperazine derivatives outperform current treatment options. These findings highlight their potential use in protective measures against CBRN threats.

Index

This publication has undergone neither linguistic editing nor proof reading.

It is printed from the author's e-mail correspondence.

HRADEC KRÁLOVÉ, 2024

CHARLES UNIVERSITY
Faculty of Medicine
in Hradec Králové