CHARLES UNIVERSITY
Faculty of Medicine
in Hradec Králové

INTERNATIONAL CONFERENCE
OF PHD STUDENTS

FROM BENCH TO BEDSIDE IN PHD RESEARCH PROJECTS

Abstract Book

19th International Medical Postgraduate Conference

Content

About IPMC

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

FROM BENCH TO BEDSIDE IN PHD RESEARCH PROJECTS

19th International Medical Postgraduate Conference

November 24 – 25, 2022

Organized by

Faculty of Medicine in Hradec Králové

Venue

Educational Centre (Výukové centrum)

Faculty of Medicine - Charles University
in the University Hospital Hradec Králové

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Programme

SCIENTIFIC PROGRAMME

Thursday November 24, 2022

Great Lecture Hall

9:00 **Opening ceremony**

Lectures

Block 1

Chairs: **Romana Koberová Ivančaková, Jakub Radocha**

9:30 **Balík M. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** The effect of prophylactic use of tranexamic acid in the robotic-assisted radical prostatectomy - the results of double-blind prospective controlled study (RARPEX)

9:45 **Heneberk O. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Periodontitis and nonsurgical periodontal therapy. Impact on local and systemic neopterin levels

10:00 **Sun Y. (Maastricht UMC+, The Netherlands):** Tumor metabolic activity is associated with myosteatosis and reduced survival in patients with non-small cell lung cancer

10:15 **Švejdová A. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Vocal fold leukoplakia – value of intraoperative use of enhanced contact endoscopy (ECE) for assessment of malignant potential

10:30 **Trávníček P. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** The effect of the use of nanomaterials in the prevention of epidural scar formation after laminectomy. Experimental study in rabbits.

10:45 **Break**

Block 2

Chairs: **Milan Kaška, Pavel Žák**

11:00 **Hrečko J. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Comparison of six decision aid rules for diagnosis of acute myocardial infarction in elderly patients presenting to the emergency department with acute chest pain

11:15 **Nejtek T. (Faculty of Military Health Science, University of Defence, Hradec Králové, Czech Republic):** Comparison of procalcitonin levels in septic patients with blood culture results and foci of infection in first 24 hours

11:30 **Ramishvili A. (David Tvildiani Medical University, Tbilisi, Georgia):** Investigation of the effects of geomagnetic storms on healthy males heart rate autonomic regulation, in natural and simulated conditions, using geomagnetic field compensation technology.

11:45 **Stafford A. (Hull York Medical School, University of Hull, United Kingdom):** Application of long-read sequencing to profile chronic wound microbiota

12:00 **Lunch**

Block 3

Chairs: **Romana Koberová Ivančaková, Jakub Radocha**

12:45 **Přibíková M. (First Faculty of Medicine, Charles University, Prague, Czech Republic):** CMTM4 is a subunit of the IL-17 receptor and mediates autoimmune pathology

13:00 **Deng M. (Maastricht UMC+, The Netherlands):** Intramuscular lipid alterations in human pancreatic cancer cachexia

13:15 **Ibrahim R. (Institute of Medicine, University of Gothenburg, Sweden):** Personalized circulating tumour DNA analysis as a biomarker of treatment response in paediatric acute lymphoblastic leukaemia

13:30 **Kiss K. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Imaging the spatial distribution of selected elements in various cutaneous tumours using complementary analytical methods

13:45 **Łabędź N. (Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Science, Poland):** Vitamin D and breast cancer associated fibroblasts

14:00 **Break**

Block 4

Chairs: **Hana Langrová, Ilja Tachecí**

14:05 **Gellén H. O. (University of Pécs Medical School, Hungary):** Non-invasive preimplantation genetic diagnostics

14:20 **Grayson K. (Hull York Medical School, University of Hull, United Kingdom):** A novel role for the synovial sarcoma, x breakpoint family in ovarian cancer

14:35 **Kolářová K. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** History of cervical excisional treatment increases the risk of the presence of microorganisms in the amniotic fluid and the development of early-onset neonatal sepsis in pregnancies with preterm prelabor rupture of membranes: a retrospective cohort study

14:50 **Matulová J. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Acute histological chorioamnionitis and birth weight in pregnancies with preterm prelabor rupture of membranes: a retrospective cohort study

15:05 **Break**

15:20 **Presentation of posters 1 – 10**
1st floor of the Educational centre
Chairs: **Aleš Ryška, Milan Kaška, Martina Řezáčová, Jan Kremláček, Ilja Tachecí**

18:00 Invited speaker **Prof. Steven Olde Damink, MD, PhD (Maastricht UMC+, Netherlands):**
Theory and practice in translational research

19:00 **Social evening**

Friday November 25, 2022

Great Lecture Hall

Lectures

Block 5

Chairs: **Aleš Ryška, Milan Kaška**

9:00 **Fürstová E. (Second Faculty of Medicine, Charles University, Prague, Czech Republic):** ELX/TEZ/IVA vs. TEZ/IVA in intestinal organoids: analysis of 63 individuals with CF

9:15 **Groborz O. (First Faculty of Medicine, Charles University, Prague, Czech Republic):** Chelating Polymers for Hereditary Hemochromatosis Treatment

9:30 **Klásková E. (Masaryk University Faculty of Medicine, Brno, Czech Republic):** In vivo and in vitro effects of salidroside on liver cytochrome p450

9:45 **Krbec M. (Third Faculty of Medicine, Charles University, Prague, Czech Republic):** Experimental determination of PKA and ATOT of weak non-volatile acids in plasma of healthy volunteers and its application in critically ill patients

10:00 **Uher M. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** A new approach in the development and validation of a method for the quantification of bile acids using liquid chromatography – mass spectrometry

10:15 **Break**

Block 6

Chairs: **Jan Kremláček, Aleš Ryška**

10:30 **Azevedo C. (University of Copenhagen, Faculty of Health and Medical Sciences, Denmark):** Multiparametric and accurate functional analysis of genetic sequence variants using crispr-select

10:45 **Bencúrová M. (Jessenius Faculty of Medicine in Martin, Comenius University Bratislava, Slovakia):** Ischemia-reperfusion effect on the aged heart and changes in proteins of mitochondria-associated membranes

11:00 **Holý P. (Third Faculty of Medicine, Charles University, Prague, Czech Republic):** Somatic DNA variation and expression of oxysterol-related genes and the full miRNome in early-stage luminal breast cancer – a multiomic analysis

11:15 **Hrala M. (Masaryk University Faculty of Medicine, Brno, Czech Republic):** Therapeutic potential of bacteriocin producing Escherichia coli in collibacteriosis treatment

11:30 **Vítovcová B. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Microscopic evaluation of flubendazole effect on microtubule organization, distribution of posttranslational modifications and cell shrinkage in glioblastoma cells

11:45 Invited speaker **Ing. Pavla Hubálková, PhD (Charles University, Prague, Czech Republic): Promotion of own research**

12:45 **Lunch**

Block 7

Chairs: **Pavel Žák, Ilja Tachecí**

13:30 **Birknerová N. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Circulating cell-free DNA-based methylation pattern in saliva for early diagnosis of head and neck cancer

13:45 **Revendová Ž. K. (Faculty of Medicine, University of Ostrava, Czech Republic):** Demographic and disease-related factors impacting on cerebrospinal fluid neurofilament light chain levels in multiple sclerosis

14:00 **Sauerzopf L. (Faculty of Medicine, University of Zurich, Switzerland):** Implementation of tools for technology-based teleassessment of sensorimotor recovery after stroke

14:15 **Šoštarič P. (School of Medicine, University of Zagreb, Croatia):** Dare we look beyond muscular effects; lasting central action of botulinum toxin rising us to possible clinical implications?

14:30 **Break**

Block 8

Chairs: **Hana Langrová, Vladimír Koblížek**

14:35 **Bobčáková A. (Jessenius Faculty of Medicine in Martin, Comenius University Bratislava, Slovakia):** Immune dysregulation in hospitalized covid-19 patients

14:50 **Hamar Á. (University of Pécs Medical School, Hungary):** Sars-cov-2 PCR diagnostics and epidemiologic study depicted on a regional dynamic map

15:05 **Skála M. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Post-covid symptoms and respiratory impairment: analysis of one-year prospective study

15:20 **Smaha J. (Medical faculty, Comenius University, Bratislava, Slovakia):** Patients with covid-19 pneumonia with 25(oh)d levels lower than 12 ng/ml are at increased risk of death

15:35 **Break**

15:50 **Presentation of posters 11 – 20**
1st floor of the Educational centre
Chairs: **Martina Řezáčová, Aleš Ryška, Jan Kremláček, Ilja Tachecí, Milan Kaška**

17:00 **Meeting of the organizing committee**

19:00 **Evaluation of the best presentations** (*Great Lecture Hall*)

19:30 **Social evening with a musical performance**

Posters

1) **Almesmary B. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Comparison of visual quality after femtosecond-lasik, transepithelial PRK and conventional PRK in patients with mild and moderate myopia

2) **Boudková P. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Immunophenotyping in patients with atopic dermatitis

3) **Dokoupil J. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Characteristics and Outcomes of Patients Admitted for Acute Heart Failure in Single Centre Study

4) **Krausová V. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Determination of reference values for sublingual microcirculation in healthy volunteers in the pediatric population using the sidestream dark-field imaging method

5) **Malá A. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Cardiac output and access flow measurements as useful tools for management of chronic hemodialysis patients

6) **Švarc M. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** The effect of inhaled furosemide and orally applied levodropropizin on dyspnea in patients with severe pulmonary diseases (INFURO trial)

7) **Cihlo M. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Usage of telemetric prechamber sensor reservoir® in management of normal pressure hydrocephalus in adults – our pilot study.

8) **Vodárek P. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Comprehensive assessment of immune functions in patients with chronic lymphocytic leukemia

9) **Zavřelová A. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Pharmacokinetics superiority of high dose ceftazidime during treatment of suspected or proven difficult to treat Pseudomonas aeruginosa infections in hemato - oncological patients.

10) **Zubáňová V. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Identification and validation of endogenous control micornas in visceral adipose tissue from murine model of non-alcoholic fatty liver disease

11) **Weiss V. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Brain areas predisposing to the stroke-related epilepsy development

12) **Janečková K. (Masaryk University Faculty of Medicine, Brno, Czech Republic):** Whole genome sequencing and analysis of treponema pallidum subsp. Pertenue of non-human primate origin

13) **Gugushvili M. (David Tvildiani Medical University, Tbilisi, Georgia):** Energy Homeostasis in Adolescence with Polycystic ovary syndrome

14) **Issa C. (Faculty of Medicine, University of Zurich, Switzerland):** Effect of cannabinoids on pain in Fabry disease patients: A prospective, randomized, double-blind, placebo-controlled, crossover, multicenter study

15) **McCullough L. (Medical faculty, Comenius University, Bratislava, Slovakia):** Invasive and metastatic mole treatment outcomes in the Slovak Republic in 1993-2021

16) **Bavlovič J. (Faculty of Military Health Science, University of Defence, Hradec Králové, Czech Republic):** Study of the secretion of outer membrane vesicles in the bacterium F. Tularensis, their role in host-pathogen interaction and their protective potential

17) **Sklenářová R. (Faculty of Medicine, Palacký University Olomouc, Czech Republic):** Skin aging

18) **Holub L. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Effect of changing the drug on the lower urinary tract dynamics and quality of life in patients with overactive bladder

19) **Karalko M. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Transforming growth factor serum concentrations in patients with proven non-syndromic aortopathy

20) **Halúsková S. (Faculty of Medicine in Hradec Králové, Charles University, Czech Republic):** Intravenous thrombolysis in posterior versus anterior circulation stroke: clinical outcome differs only in patients with large vessel occlusion

Oral Abstracts

The abstracts are in order according to the programme.

THE EFFECT OF PROPHYLACTIC USE OF TRANEXAMIC ACID IN THE ROBOTIC-ASSISTED RADICAL PROSTATECTOMY - THE RESULTS OF DOUBLE-BLIND PROSPECTIVE CONTROLLED STUDY (RARPEX)

Author: Michal Balík[1,2]

Supervisor: Filip Čečka[1]

Co-authors: Pavel Navrátil jr.[2], Josef Košina[2], Petr Hušek[2], Miloš Broďák[1,2]

Affiliations: [1]Academic Department of Surgery, Faculty of Medicine in Hradec Králové; [2]Urological Department, University Hospital Hradec Králové

Introduction: (the aim of the study) prophylactic administration of tranexamic acid reduces blood loss during procedures at high risk of perioperative bleeding. Several studies in cardiac surgery and orthopaedics confirmed this finding. The aim of this prospective, double-blind, randomized study was to evaluate the effect of tranexamic acid on peri-and postoperative blood loss and on the incidence and severity of complications after robotic-assisted radical prostatectomy.

Methods: Based on the results from our pilot study, we decided to conduct the trial to confirm the preliminary data. From February 2021 to April 2022 a total of 200n undergoing robotic assisted radical prostatectomy were included in the trial after meeting eligibility criteria. The subjects were randomized to either 20mg/kg tranexamic acid or placebo at the beginning of the procedure clinical significance was set to 10 g decrease in haemoglobin level. The trial was registered on ClinicalTrials.gov NCT04319614 on 25 March 2020. The design of the study was published in Trials. 2022 Jun 18;23(1):508

Results: The characteristics (BMI, age, weight of the prostate, PSA level) of the treatment and control group were similar. Statistically significant differences were found in haemoglobin level drop 3 hours after the procedure, on POD1 (post-operative day), POD2 and POD7 (11.9 vs. 17.7; 15.3 vs. 21.0; 12.0 vs. 19.9; 7.6 vs. 12.7), but it didn't meet the level of clinical significance.

Discussion: Our data from long term follow-up accord with those from other studies and reviews that have not recorded an increased number of complications (including non-fatal thromboembolism) associated with tranexamic acid use. However, the number of complications was low and do not have sufficient power to make a definitive conclusion about safety. A decrease in haemoglobin level by 30 % or 40 g/L or need for blood transfusion was reported in the literature as clinically significant blood loss. Considering these data, the difference of 10 g/L can seem insufficient. However, on the other hand, a similar change in haemoglobin level was found after administration of one unit of packed red blood cells.

Conclusions: Despite the enormous development in robot-assisted radical prostatectomy over 25 years, improvement is still needed. The prophylactic use of tranexamic acid at the beginning of robotic-assisted prostatectomy could be another piece of this mosaic.

PERIODONTITIS AND NONSURGICAL PERIODONTAL THERAPY. IMPACT ON LOCAL AND SYSTEMIC NEOPTERIN LEVELS

Author: Ondřej Heneberk[1]

Supervisor: Vladimíra Radochová[1]

Co-authors: Andrea Vernerová[2,3], Lenka Kujovská Krčmová[2,3]

Affiliations: [1]Department of Dentistry, Faculty of Medicine in Hradec Králové, University Hospital in Hradec Králové, Charles University, Czech Republic; [2]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic; [3]Department of Clinical Biochemistry and Diagnostics, University Hospital in Hradec Králové, Czech Republic

Introduction: Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilm and characterized by destruction of the tooth-supporting apparatus1. Neopterin (Np) is a biomarker of cell-mediated immune response that is secreted via activated macrophages. 2 The aim of the study is to evaluate the impact of periodontitis and nonsurgical periodontal therapy on neopterin levels in gingival crevicular fluid (GCF), oral fluid (OF) serum and urine. The second aim is to evaluate the relation among neopterin levels in these biological fluids using correlation analysis.

Methods: 25 subjects with periodontitis and 25 individuals with healthy periodontium were recruited to the study. GCF, OF, serum and urine samples were collected. In study group, samples were collected before nonsurgical periodontal therapy (BT) and after 3 months after treatment (AT). Results are presented as Np concentration (GCF, OF, serum), GCF total amount (amount of substance in whole sample, TA) or neopterin to creatinine ratio (NC) and expressed as median (inter-quartile range).

Results: GCF Np concentration in study group (BT) and in control group were not significantly different ($p=0.32$). TA GCF were significantly higher in study group BT to control group ($p=0.001$)]. No significant difference was found between OF levels study group BT and control group ($p=0.21$). Serum Np levels in study group BT were not significantly different ($p=0.23$). Urinary NC in study group BT were significantly higher to control group ($p=0.020$). Np GCF concentration in study group (AT) were not significantly different to BT levels ($p=0.31$) but significantly higher to control group ($p=0.038$). Np TA in study group AT were significantly lower to BT levels ($p=0.024$), but not significantly different to control group ($p=0.11$). OF Np concentration in study group AT were significantly higher to BT concentration ($p=0.020$) but not significantly different to control group ($p=0.11$). Serum Np levels in study group AT were not significantly different to BT concentration ($p=0.72$) and to control group (0.14). Urinary NC in study group AT were not significantly different to BT levels ($p=0.36$) but significantly lower to control group ($p=0.001$). The only significant correlation ($p<0.05$) was found in serum and oral fluid concentration ($p=0.001$, $\rho=0.40$).

Discussion: Macrophages produce hydrolytic enzymes such as collagenase and reactive oxygen species that may contribute to tissue damage in periodontitis3,4. Macrophages play also an important role in resolution of periodontal inflammation5. TA Np was suggested as more valuable than Np concentration3. Main Np source in OF is probably serum, but it is influenced also by GCF and other ongoing processes in oral cavity and oropharynx6.

Conclusion: Periodontitis was associated with increased GCF TA Np levels and urinary UC. Nonsurgical periodontal therapy led to decrease in Np TA. OF and serum levels were found to be significantly correlating.

Acknowledgements: The study was supported by Project SVV 260 548, by Cooperatio Program, research area Dental Medicine and by University Hospital Hradec Kralove, MZO 00179906.

References:

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TUMOR METABOLIC ACTIVITY IS ASSOCIATED WITH MYOSTEATOSIS AND REDUCED SURVIVAL IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Co-authors: Min Deng[1], Olivier Gevaert[2], Shaimaa Hesham Bakr[2], Merel Aberle[1], Steven W.M.Olde Damink[1,3], Sander S. Rensen[1]

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Introduction: Cancer cachexia and tumor metabolic activity are both associated with poor survival, but it is unclear how tumors promote development of cachexia. We hypothesized that tumors with higher metabolic activity instigate peripheral metabolic alterations that lead to cachexia and investigated whether tumor metabolic activity is associated with cachexia-related body composition changes and survival in non-small cell lung cancer (NSCLC).

Methods: A retrospective analysis was performed on a cohort of 121 patients with NSCLC. 18F-fluorodeoxyglucose PET/CT-scans obtained before treatment were used to analyze tumor metabolic activity (standardized uptake value (SUV) and SUV normalized by lean body mass (SUL)) and body composition (skeletal muscle index (SMI), visceral/subcutaneous adipose tissue index (VATI/SATI), myosteatosis) at the L3 level. Subjects were divided into groups with or without myosteatosis based on age- and sex-specific thresholds of the mean Hounsfield units (HU) of muscle area. Mann-Whitney tests, Kaplan-Meier, Cox-regression, and Receiver Operator Characteristics (ROC) curves were used to analyze associations between tumor metabolic activity, myosteatosis, and survival.

Results: The overall prevalence of myosteatosis was 43.0% (52/121). Patients with myosteatosis had shorter survival compared with patients without myosteatosis (median: 25.8 vs. 42.7 months, p=0.03). Myosteatosis was independently associated with shorter overall survival (univariate Cox regression HR=0.482, 95% CI: 0.249-0.933, p=0.03). Muscle radiation attenuation correlated with tumor metabolic activity (SULpeak r_s=0.444, p=0.02; SUVpeak r_s=0.362, p=0.05), and patients in the myosteatosis group had higher tumor metabolic activity (SULpeak median 8.0, IQR 4.5-12.9, SUVpeak 11.4, 5.0-16.8) than those in the non-myosteatosis group (SULpeak 5.0, 2.0-7.6, p=0.03, SUVpeak 5.8 2.7-9.1, p=0.03, respectively). Tumor metabolic activity parameters predicted the occurrence of myosteatosis in ROC analysis (SULpeak: AUC=0.763, p=0.034, sensitivity/specificity: 80%/75%; SUVpeak: AUC=0.744, p=0.038, sensitivity/specificity:85%67%). SMI and VATI were not associated with tumor metabolic activity or survival.

Conclusions: Higher tumor metabolic activity is associated with myosteatosis and shorter survival in NSCLC.

VOCAL FOLD LEUKOPLAKIA – VALUE OF INTRAOPERATIVE USE OF ENHANCED CONTACT ENDOSCOPY (ECE) FOR ASSESSMENT OF MALIGNANT POTENTIAL

Author: Anna Švejdová[1,2]

Supervisor: Viktor Chrobok[1,2]

Co-authors: Jana Šatanková[1,2], Michal Černý[1,2], Lucie Zeinerová[1], Michal Homoláč[1,2]

Affiliations: [1]Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Kralove; [2]Charles University, Faculty of Medicine in Hradec Kralove

Introduction: Management of vocal fold leukoplakia - white plaque covering the surface of vocal folds, remains a challenge for laryngologists. Leukoplakias form a nonhomogeneous group of lesions with a variable potential of malignant transformation from 1-40 %, histologically they can be evaluated as benign lesions, precancerous lesions – low- and high-grade dysplasia, in situ carcinoma or invasive carcinoma. So far there is no consensus on timing of the treatment of leukoplakia. Therefore, we aim to distinguish between low- and high-risk leukoplakia to choose optimal treatment and minimize the number of surgeries – radicality and oncological safety on one side and voice quality on the other.

Methods: Prospective clinical trial conducted in the Department of Otorhinolaryngology and Head and Neck surgery in Hradec Kralove in the period from November 2020 to June 2022. Patients enrolled in the study were firstly examined in the outpatient department with flexible laryngoscopy in white light (WLE) and with the use of NBI (narrow band imaging). Inclusion criteria were vocal folds lesions clinically characterized as leukoplakia and accessible for enhanced contact endoscopy (ECE) examination under general anesthesia. Under general anesthesia a structured assessment of the lesion followed with the use of rigid endoscopes in WLE, NBI and ECE. Lesions were assessed as suspicious and unsuspicious according to the changes of mucosal vascular patterns. Classification of the European Laryngological Society (ELS) that divides the vascular pattern changes into longitudinal (unsuspicious) and perpendicular (suspicious) was used (Picture 1 and 2). The evaluation of the imaging methods (NBI and ECE) was correlated with histopathology.

Results: According to flexible laryngoscopic examination in the outpatient department, 25 patients with 28 lesions were enrolled in the study. Under general anesthesia in direct laryngoscopy with NBI 32.1% (9/28) lesions were assessed as benign, 32.1% (9/28) as suspicious, 39.3% (10/28) could not be assessed due to hyperkeratosis. In ECE 25.0% (7/28) lesions were assessed as benign, 50.0% (14/28) as suspicious and 25% (7/28) could not be assessed. Histologically 14.3% (4/28) lesions were benign, 10.7% (3/28) low-grade dysplasia, 21.4% (6/28) high-grade dysplasia, 10.7% (3/28) in situ carcinoma, 35.8% (10/28) invasive carcinoma, 3.6% (1/28) reactive atypia, (Table 1). In total 82.1% (23/28) of the leukoplakias were histologically malignant. Sensitivity of NBI assessment reached 52.9%, sensitivity of ECE 77.8%, in contrast to other clinical diagnoses (such as chronic laryngitis, papilloma, carcinoma etc.) the sensitivity of NBI and ECE was 77.8% and 82.4%, resp.

Discussion: Our results show that in comparison to other lesions the assessment of leukoplakias is more difficult, which is mostly due to the umbrella effect caused by hyperkeratosis and worse visibility of the changes of mucosal vascular patterns. By adding contact endoscopy into our diagnostic procedure, we reached higher sensitivity in evaluation of leukoplakias.

Conclusion: Intraoperative use of enhanced contact endoscopy (ECE) enables more accurate evaluation of leukoplakias. Due to close contact of the endoscope with the mucosa, we are able to

see the vascular patterns under the hyperkeratotic plaque and distinguish between low- and high-risk leukoplakia (example Picture 3). Beside the vascular patterns, also the margins, the surface and general image of the plaques should be evaluated. According to this evaluation, the radicality of the surgical procedure can be adjusted.

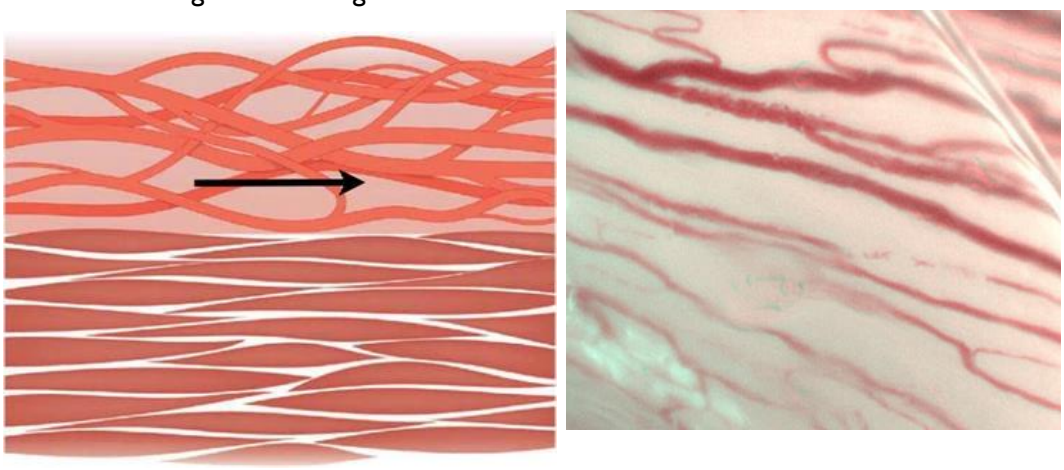
Key words: low-risk leukoplakia, high-risk leukoplakia, enhanced contact endoscopy, narrow band imaging, squamous cell carcinoma

ClinicalTrials.gov Identifier: NCT04777474

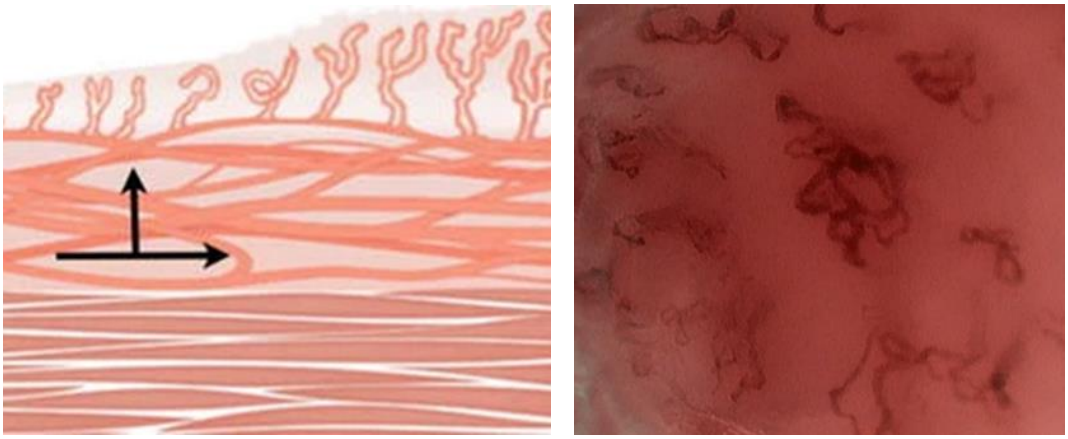
Table 1:

Evaluation of leukoplakia	NBI intraoperatively		ECE intraoperatively
unsuspicious	32.1% (9/28)		25.0% (7/28)
suspicious	32.1% (9/28)		50.0% (14/28)
not able to evaluate	35.8% (10/28)		25.0% (7/28)
sensitivity	leukoplakia	52.9%	77.8%
	other clinical diagnosis (papilloma, chronic laryngitis, carcinoma…)	77.8%	82.4%

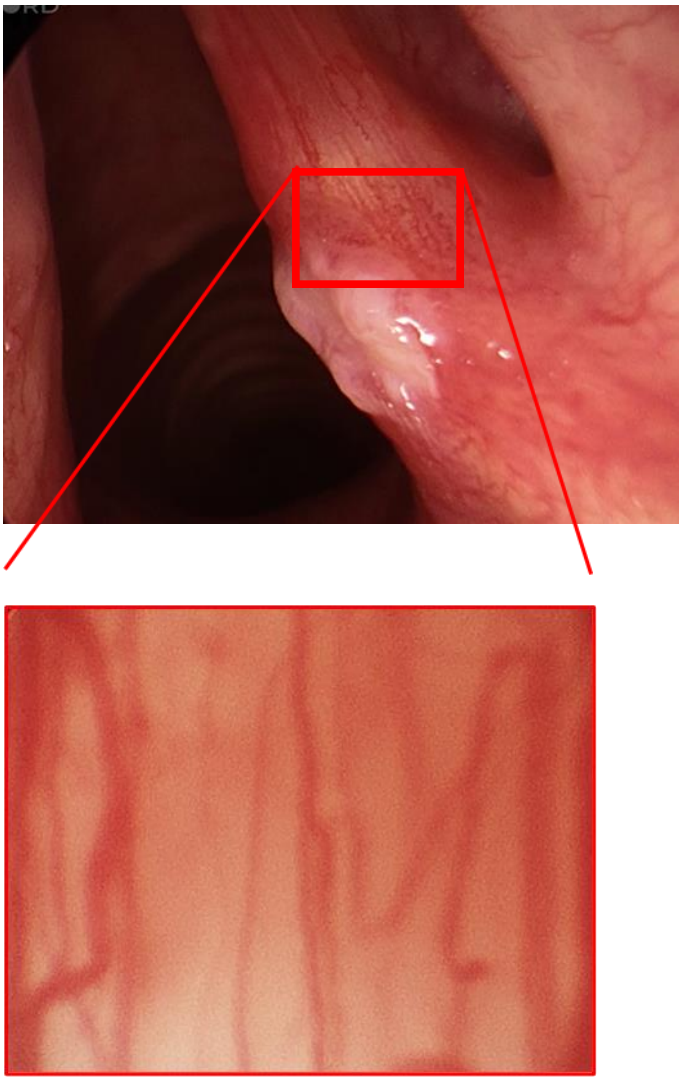
Picture 1: Longitudinal changes



Picture 2: Perpendicular changes



Picture 3: Example of contact endoscopy of leukoplakia of the posterior part of the right vocal fold: Longitudinal changes according to ELS classification type I, evaluated as low-risk leukoplakia, histologically a benign lesion was proven.



THE EFFECT OF THE USE OF NANOMATERIALS IN THE PREVENTION OF EPIDURAL SCAR FORMATION AFTER LAMINECTOMY. EXPERIMENTAL STUDY IN RABBITS

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Introduction: During the surgical approach to the spinal canal, the natural barriers between the dural sac and the paravertebral muscles are removed. After the operation, the dural sac with the spinal roots and the paravertebral muscles are in direct contact. The formation of an epidural scar is a natural part of healing, but the scar may compress the dural sac and spinal roots and reduces its mobility in the spinal canal. It can cause significant clinical problems. The topic of the project is the insertion of a nanofiber layer in the form of a sheet into the epidural space in order to prevent the formation of an epidural scar after operations in the spinal canal. In the present project, histological analysis is performed using an animal model (Oryctolagus cuniculus f. domesticus)

Methods: In the first year we evaluate the non-adhesive materials PVDF and PCL. The basic assumption was the same histological evaluability of the materials, which was verified. The basic surgical procedure is bone decompression to activate bone cells in the L1 and L5 area (2nd year L3+5) and expose the dural sac. In the first, a PVDF or PCL nanomaterial was applied to the dura mater and fixed in loco by the physical properties of the material. The stable model was monitored for 12 weeks in basic parameters. In the 13th week, the experiment was terminated and samples were taken. The samples were fixed and examined histologically, due to these results we withdrew from electron microscopy.

Results: After the histological evaluation of samples, we found that both materials, PVDF and PCL, lead to a reduction in scar thickness from the measured natural values of 0.8-1mm to approx. 0.5-0.7mm for PCL and 0.4-0 .6mm for PVDF. However, the PCL nanomaterial dissolves too quickly and, on the contrary, leads to new blood vessel formation (neovascularization) and the formation of adhesive bridges between the dura mater and the surrounding tissue. The PVDF material leads to the already mentioned greater reduction of the scar than PCL, but it is still colonized by cells. Because it does not completely degrade, it can also be found in samples 12 weeks after implantation. PVDF does not provoke the surrounding tissue to form adhesive bridges.

Discussion: The main finding compared to the original assumption is that both materials are populated by cells and are resorbed faster in the in vivo model compared to the in vitro model.

PVDF

By limiting the action of cells and agents capable of forming a scar (e. g. by placing it under the bone), the material in vivo seems relatively inert to scar formation. Due to ongoing thematically the same work, but with a different location of the implant, PVDF can be thought of as a separation barrier.

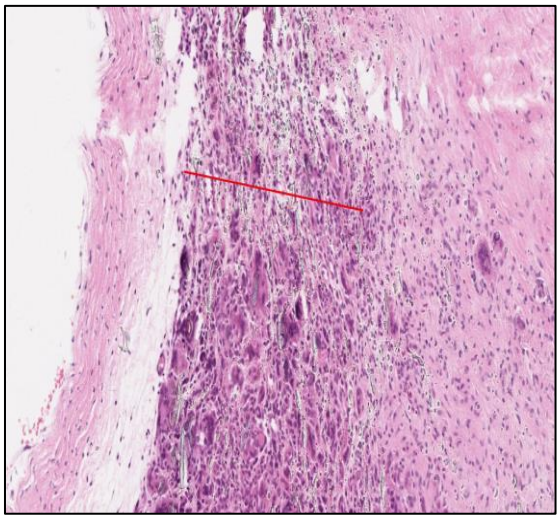
PCL

The material placed separately on the dura mater in the region of the spinal canal is not a suitable barrier although in vitro its degradability is more than a year. We have shown that its in vivo degradability increases significantly and is no longer detectable after 3 months. The condition for this behavior is the presence of cells and agents capable of forming a scar. Although the scar is thinner than a natural scar, the formation of adhesions certainly leads to the loss of the natural sliding of the individual layers. However, PCL could work well as a dura repair layer (replacement/patch) provided it is combined with another inert material in the spinal canal area.

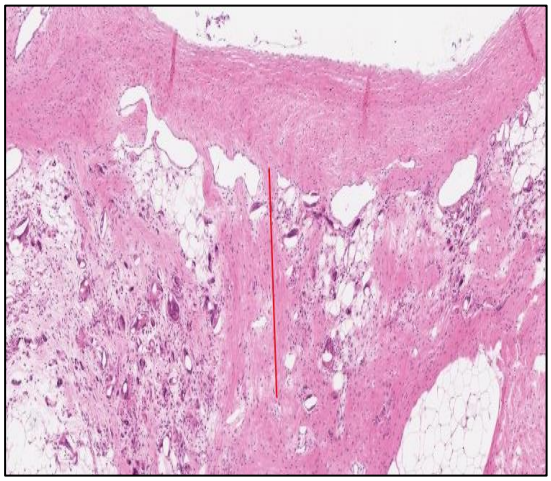
Therefore, our study continues in the second year with combined nanomaterials – PVDF/PVA and PVDF/PCL.

Grant Agency: GA UK Project Number: 341021

Pic.1 – PVDF scar, red line 427um



Pic.2 – PCL scar, red line is adhezive bridge



COMPARISON OF SIX DECISION AID RULES FOR DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION IN ELDERLY PATIENTS PRESENTING TO THE EMERGENCY DEPARTMENT WITH ACUTE CHEST PAIN

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Objective: This study aimed to evaluate the accuracy and effectiveness of different strategies for the diagnosis of acute myocardial infarction (AMI) in the elderly patients in real-life clinical practice.

Methods: Patients older than 70 years presenting to the emergency department with chest pain were included. The performance of six decision aid rules (T-MACS, HEART, EDACS, TIMI, GRACE, and ADAPT) and solo troponin T strategy for diagnosing AMI was evaluated by calculating sensitivity, specificity, odds ratios, negative and positive predictive values.

Results: A total of 250 patients, with a mean age of 78.5 years, were enrolled. Forty-eight patients (19.2%) had an acute myocardial infarction in a 30 day follow-up period. The sensitivity for ruling-out AMI was 100% for T-MACS, HEART, and ADAPT; 97.9% for EDACS, 93.8% for TIMI, and 81.3% for GRACE and solo TnT strategy. For ruling-in AMI, the specificity was 97.5% for T-MACS, 95% for TIMI, 83.2% for HEART, 81.7% for GRACE, and 46% for ADAPT.

Discussion: As far as we know, there are not enough studies that evaluated the use of decision aids in the selected population of the elderly and compared these protocols in-between. Our results suggest that these protocols can be used even in this group with great accuracy. To our knowledge, this study is unique because of the mean age of participants included and the number of decision protocols used.

Conclusion: T-MACS decision aid rule had the best performance for rule-out and rule-in diagnostics of AMI. Risk stratification of patients with suspected acute coronary syndrome based on decision aid rules can be used in real-life practice, even in the population of the elderly.

COMPARISON OF PROCALCITONIN LEVELS IN SEPTIC PATIENTS WITH BLOOD CULTURE RESULTS AND FOCI OF INFECTION IN FIRST 24 HOURS

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Introduction: Potential of procalcitonin (PCT) to predict blood culture results according to Gram staining, different types of pathogens and foci of infection is discussed lately. The primary aim of the study was to compare the PCT levels between Gram-positive (GP) and Gram-negative (GN) bacteraemia in the time of sepsis diagnosis and after 24 hours in septic patients. We compared the PCT levels between different foci of sepsis and between different types of pathogens.

Methods: The retrospective analysis of hospital patient dataset from December 2012 to July 2020 in the Department of Anesthesiology and Intensive Care, First Faculty of Medicine, Charles University and Thomayer Hospital, Prague was performed. Following criteria were required for study inclusion: 1) Fulfilled criteria according to the Sepsis-3 definition. 2) Results of blood cultures (BC) - at least 1 aerobic and 1 anaerobic bottle drawn in the time of sepsis diagnosis. 3) Procalcitonin levels evaluated in the time of sepsis diagnosis (PCT1) and after 24 hours (PCT2). A total number of 258 patients met the inclusion criteria.

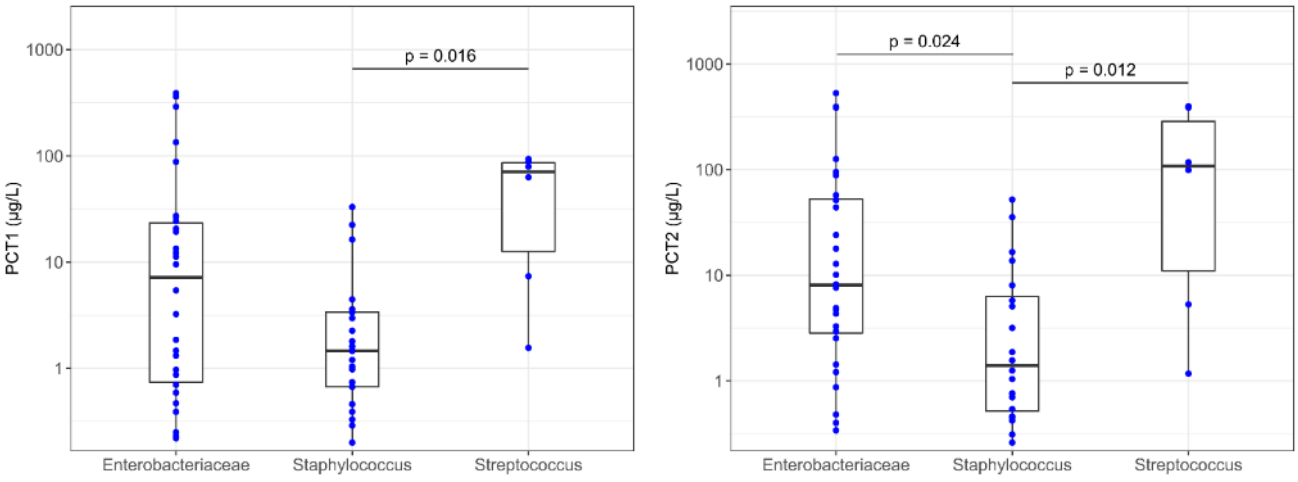
Results: Out of 258 samples were 157 (60,85%) BC negative and 23 (8,91%) contaminants. Out of 78 (30,23%) positive blood cultures 34 (13,18%) were GP, 36 (13,95%) GN and 4 (1,55%) with mixed flora. In four (1,55%) positive BC fungi were found, and these were not further evaluated. Other selected patient characteristics are shown in Table 1. Difference in PCT1 and PCT2 levels in BC negative and contaminants group compared to BC positive group with or without previous antibiotic treatment was not statistically significant.

In BC positive samples levels of PCT1 in GN group were higher (2,81; 0,68 – 20,56 ug/L) than concentrations of PCT1 in GP group (1,92; 0,92 – 9,6 ug/L), but the difference was not statistically significant. Similarly, PCT2 levels in GN group (7,79; 1,38 – 45,79 ug/L) were also higher than concentrations of PCT2 in GP group (2,54; 0,77 – 15,88 ug/L), but the difference was not statistically significant neither.

Mostly represented group of pathogens according to species type out of 74 positive BC were: *Enterobacteriaceae* (n=31), *Staphylococci* (n=21) and *Streptococci* (n=6). Figure 1. illustrates PCT levels between group of pathogens. The highest PCT1 also PCT2 concentrations were recorded in group Urosepsis. PCT1 and PCT2 levels between individual groups according to foci of infection are shown in Figure 2.

Table 1. Patient characteristics. Data are displayed as number (percentage) or median (Q1 – Q3).

Characteristic	All patients (N = 254)	Blood culture results		Previous antibiotics	
		BC negative or contamination (N = 180)	BC positive (N = 74)	Yes (N = 150)	No (N = 104)
Age (years)	66 (58 – 73)	66 (59 – 72)	66.5 (56 – 73)	64 (52.5 – 72)	67.5 (60.8 – 73)
Sex – male	167 (65.7)	119 (66.1)	48 (64.9)	103 (68.7)	64 (61.5)
Sex – female	87 (34.3)	61 (33.9)	26 (35.1)	47 (31.3)	40 (38.5)
Initial SOFA (points)	11 (8 – 13)	11 (8 – 13)	11 (8 – 14)	11 (8 – 13)	10.5 (9 – 13)
Lactate (mmol/L)	1.8 (1.1 – 2.9)	1.75 (1.2 – 2.8)	1.75 (1.0 – 3.2)	1.6 (1.1 – 2.4)	2,2 (1.3 – 3.7)
Septic shock	94 (37.0)	65 (36.1)	29 (39.2)	44 (29.3)	50 (48.1)
1-day mortality	8 (3.1)	4 (1.6)	4 (1.6)	3 (1.2)	5 (1.9)
7-day mortality	37 (14.6)	19 (10.6)	18 (24.3)	17 (11.3)	20 (19.2)
30-day mortality	74 (29.1)	49 (27.2)	25 (33.8)	42 (28.0)	32 (30.8)
90-day mortality	95 (37.4)	65 (36.1)	30 (40.5)	55 (36.7)	40 (38.5)
ICU length of stay (days)	21 (11 – 42.5)	20.5 (10.25 – 36.75)	29 (12 – 62)	25 (12 – 49.75)	20.5 (9 – 35)
Focus of infection					
Respiratory	105 (41.3)	90 (50.0)	15 (20.3)	65 (43.3)	40 (38.5)
Abdominal	66 (26.0)	51 (28.3)	15 (20.3)	46 (30.7)	20 (19.2)
Soft tissues	20 (7.9)	9 (5.0)	11 (14.9)	10 (6.7)	10 (9.6)
Urosepsis	19 (7.5)	9 (5.0)	10 (13.5)	7 (4.7)	12 (11.5)
Other	33 (13.0)	11 (6.1)	22 (29.7)	16 (10.7)	17 (16.3)
Unknown	11 (4.3)	10 (5.6)	1 (1.4)	6 (4.0)	5 (4.8)
Blood culture positive	74 (29.0)			31 (20.7)	43 (41.3)
Previous antibiotics	150 (59.1)	119 (66.1)	31 (41.9)		



(a) (b)

Figure 1. PCT1 (a) and PCT2 (b) concentrations (ug/L) in different groups of pathogens. *Y* axis is in logarithmic scale. *P* value(s) are demonstrated with horizontal line(s), if significant.

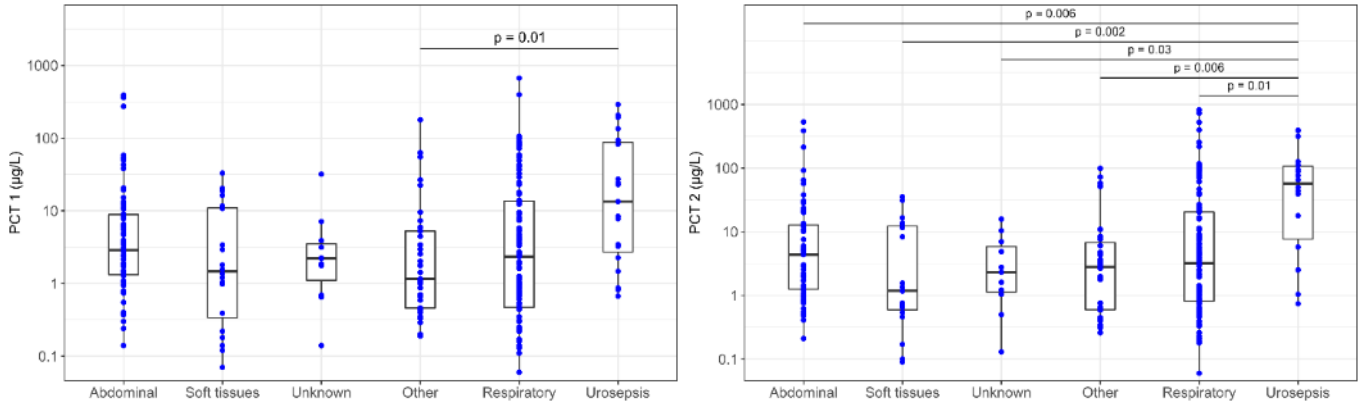


Figure 2. PCT1 (a) and PCT2 (b) concentrations (ug/L) according to foci of infection. *Y* axis is in logarithmic scale. *P* value(s) are demonstrated by horizontal line(s), if significant.

Discussion: PCT discriminatory power to differentiate between GN and GP bacteraemia in septic patients appears to be low. PCT concentrations correlates probably more closely to foci of infection and different pathogens rather than result of the Gram stain.

Conclusion: In our study population, urosepsis showed statistically significant higher PCT concentrations 24 hours following sepsis diagnosis when compared to other site of infection.

Acknowledgement: This research was supported by the Ministry of Health, Czech Republic – Conceptual Development of Research Organization (Thomayer University Hospital – TUH, 00064190).

INVESTIGATION OF THE EFFECTS OF GEOMAGNETIC STORMS ON HEALTHY MALES HEART RATE AUTONOMIC REGULATION, IN NATURAL AND SIMULATED CONDITIONS, USING GEOMAGNETIC FIELD COMPENSATION TECHNOLOGY

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Introduction: During geomagnetic storms (GMSs), the strength of the disturbed geomagnetic field (GMF) sharply increased hundreds of times and can pose a serious threat to people with cardiovascular diseases (CVD) (1-3). The alternative way of protecting people during GMSs, which administer beta-blockers and anti-aggregates is the use of new technology to compensate for the disturbing time-varying components of the GMF, that block GMSs negative impact (4).

Methods: Using such technology, we compared the effects of GMSs on healthy young males autonomic nervous system (ANS), in natural and simulated conditions. At different strengths of outdoor GMF, 1h lab measurement of heart rate variability (HRV) (5, 6) was carried out. The geomagnetic K-index was used to characterize the magnitude of GMSs. Testing was performed on n=8 volunteers during quiet magnetic days (K=1-3), and on n=17 volunteers on days with GMSs (K≥4=5) (7).

For statistical analysis of the data obtained the "Primer of Biostatistics" software by Stanton A. Glantz (seventh edition) was used based on a one-way ANOVA and Bonferroni adjusted t-test, taking the significant level as 0.05.

Results: During quiet magnetic days, the comparison between HRV initial values with values measured under GMF time-varying components compensation mode (CM) did not reveal any changes (table 1). On days with GMSs, statistically significant changes in SDNN and RMSSD were observed in and after GMS CM compared to their initial values, to indicate a significant intensification of the parasympathetic part of ANS (8, 9).

Conclusion: Our experiments showed that a specific stress reaction of the ANS caused by GMSs, can be canceled in the GMS CM. To the results of this study, in in-hospital conditions, to prevent CVD complications and to protect patients during GMSs, the use of new technology for compensation of disturbed GMF, can be considered as an alternative way.

Table 1

Subgroup A	K	HR	SDNN	RMSSD	VLF%	LF%	HF%
I group	1-3	68.9±8.4	79.4±24.2	29.9±22.7	30.7±5.1	37±3	32.42±3.6
II group	CM	68.1±8.8	70.3±36	40.1±21	30.4±3.3	37.7±3.8	31.9±4.5
III group	1-3	67.8±8.2	77.5±34.9	33.5±20.6	32.2±5	37.6±3.3	30.22±3.9
Between groups	SS	5.25	371.6	432.2	13.73	1.818	21.28

Between groups	DF	2	2	2	2	2	2
Between groups	MS	2.625	185.8	216.1	6.863	0.9088	10.64
Within groups	DF	21	21	21	21	21	21
	F	0.04	0.18	0.47	0.33	0.08	0.67
	P	0.964	0.837	0.631	0.72	0.923	0.524
t	I to II	0	0.792	1.32	0	0	0
t	II to III	0	0.616	0.792	0.539	0	0.708
t	I to III	0.335	0.088	0.396	0.539	0	1.416

Remarks: * Indicates statistically significant differences; HR-heart rate, heart beats in min.; SDNN-Standard deviation of all Normal-to-Normal RR intervals; RMSSD-The square root of the arithmetical mean of the sum of the squares of differences between adjacent NN intervals; VLF- very low-frequency band; LF-low-frequency band; HF-high-frequency band; SS-Sum of squares; DF-Degrees of freedom; MS-Mean squares; F-F value, P-P value, t-t test.

Table 2

Subgroup B	days	HR	SDNN	RMSSD	VLF%	LF%	HF%
I group	4-5	76.4±10.6	56.9±20.9	26.7±17.6	30.8±7.7	36.16±4.1	33.1±7.9
II group	CM	73.2±7.9	70.7±24.2	38.7±23.3	31.6±6.9	36.5±4.8	31.8±7.7
III group	4-5	72.8±8.4	73.9±26.7	27.6±16.2	33.2±5.6	37.3±3.7	28.2±9.2
Between groups	SS	133	2791	1514	49.98	11.62	219
Between groups	DF	2	2	2	2	2	2
Between groups	MS	66.49	1396	757	24.99	5.812	109.5
Within groups	DF	48	48	48	48	48	48
	F	0.81	2.41	2.03	0.55	0.33	1.59
	P	0.449	0.101	0.142	0.583	0.719	0.214
t	I to II	1.369	2.226	2.564*	0	0	0.497
t	II to III	0	0.514	2.35	0.609	0	1.492
t	I to III	1.369	2.911*	0	1.218	0.985	1.99

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APPLICATION OF LONG-READ SEQUENCING TO PROFILE CHRONIC WOUND MICROBIOTA

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Introduction: Chronic, non-healing skin wounds represent a substantial area of unmet clinical need, leading to debilitating morbidity and mortality in affected individuals. Due to their high prevalence and recurrence, chronic wounds pose a significant economic burden, with wound management estimated to cost >€50Bn per annum across Europe and £5Bn in the UK alone [1]. Wound infection is a major component of healing pathology, with up to 70% of wound-associated lower limb amputation preceded by infection [1]. Despite this, the wound microbiome remains poorly understood. Therefore, the aim of this research was to characterise the wound microbiome and explore the complex interactions that occur in the wound environment.

Methods: Clinical wound samples were analysed using a novel long-read nanopore sequencing-based approach that delivers quantitative species-level taxonomic identification. Microbial community composition and host tissue transcriptional (RNAseq) profiles were correlated to clinical parameters and markers of favourable clinical outcomes.

Results: This study identified that specific commensal and pathogenic organisms are mechanistically linked to healing, eliciting unique host response signatures. Patient- and site-specific shifts in microbial abundance and community composition were observed, linked to chronic wounds versus healthy skin. Abundant species present in the wounds included *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Corynebacterium striatum* and *Morganella morganii* (Figure 1). Tandem transcriptional profiling (RNAseq) of the corresponding wound tissue revealed important insight into functional elements of host-microbe interaction, focused around host defense protein transcripts for calprotectin, psoriasin, RNAase 7 and beta-defensin 2. Intriguingly, the study revealed that patient diabetic status was associated with the level of commensal species, such as *S. epidermis*, whilst uncontrolled participant glycaemic control was associated with the presence of the poorly characterised species, *Peptoniphilus harei*. High-resolution long-read sequencing offers clinically important genomic insights, including rapid wide-spectrum pathogen identification and antimicrobial resistance profiling, which is not possible using current culture-based diagnostic approaches.

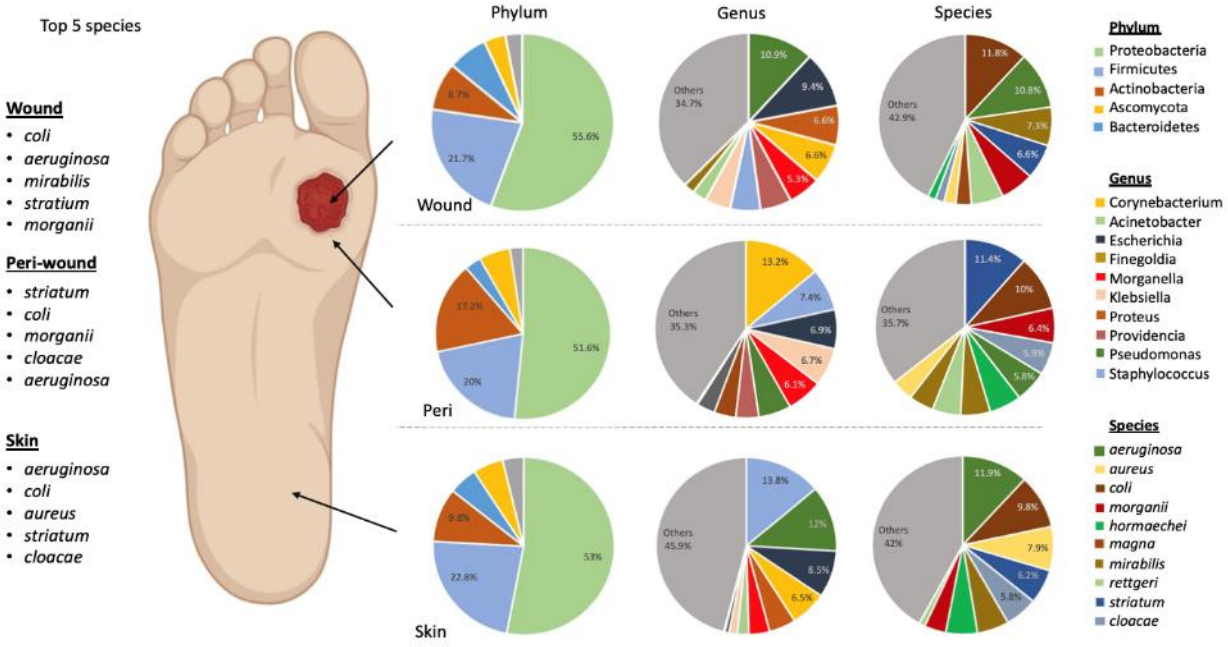

Figure 1. Topographical distribution of most abundant bacterial phyla, genera and species present at the wound, peri-wound and intact skin of subjects with an active foot ulcer.

Conclusion: In summary, combining long-read sequencing technology, clinical and host transcriptome data has provided unprecedented insight into the complex host-microbe interactions within the wound microbiome. These data highlight the power of integrating complex datasets to understand host-microbe interactions in clinical samples

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CMTM4 IS A SUBUNIT OF THE IL-17 RECEPTOR AND MEDIATES AUTOIMMUNE PATHOLOGY

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Interleukin-17A (IL-17A) is a key mediator of protective immunity to yeast and bacterial infections but also drives the pathogenesis of several autoimmune diseases, such as psoriasis or psoriatic arthritis. We show that the tetra-transmembrane protein CMTM4 is a subunit of the IL-17 receptor (IL-17R). CMTM4 constitutively associated with IL-17R subunit C (IL-17RC) to mediate its stability, glycosylation and plasma membrane localization. Both mouse and human cell lines deficient in CMTM4 were largely unresponsive to IL-17A, due to their inability to assemble the IL-17 receptor signaling complex. Accordingly, CMTM4-deficient mice had a severe defect in the recruitment of immune cells following IL-17A administration and were largely resistant to experimental psoriasis, but not to experimental autoimmune encephalomyelitis. Collectively, our data identified CMTM4 as an essential component of the IL-17 receptor and a potential therapeutic target for treating IL-17-mediated autoimmune diseases.

INTRAMUSCULAR LIPID ALTERATIONS IN HUMAN PANCREATIC CANCER CACHEXIA

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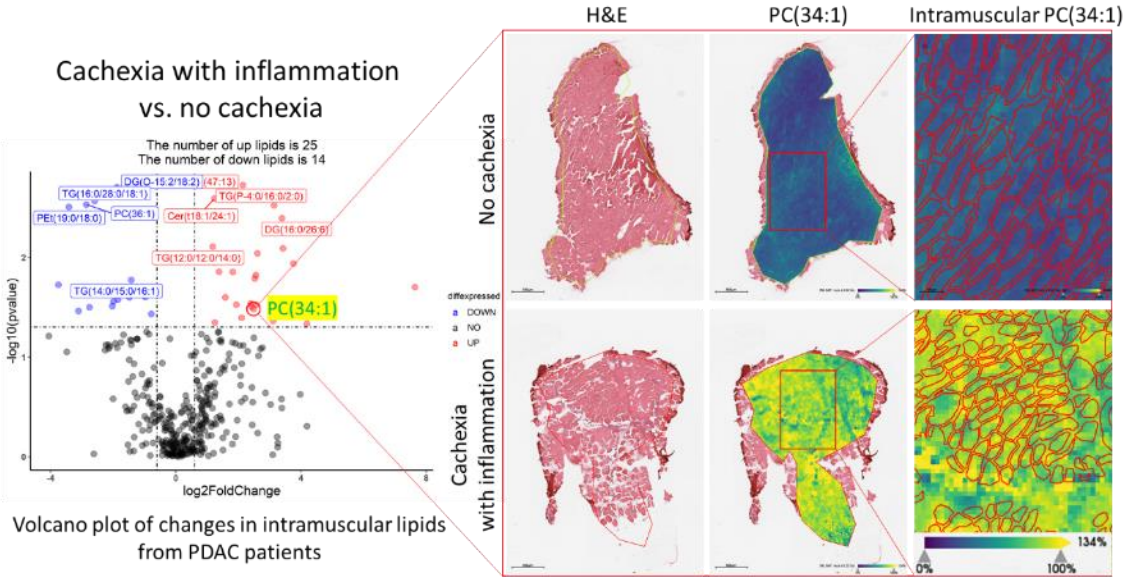
Introduction: Cancer cachexia is a multifactorial metabolic syndrome characterized by ongoing skeletal muscle loss resulting in weakness, poor quality of life, and decreased survival. Lipid accumulation in skeletal muscle is increasingly recognized as a defining cachexia feature that is promoted by inflammation and independently associated with muscle function and long-term survival. However, surprisingly little is known about the nature of the lipids that accumulate in skeletal muscle during cancer cachexia. We aimed to identify the types and distribution of intramuscular lipids in patients with cancer cachexia.

Methods: Rectus abdominis muscle biopsies were collected during surgery from pancreatic ductal adenocarcinoma patients (n=12 weight-stable, n=24 with cachexia but without inflammation (CRP<10 mg/L), n=12 with cachexia with inflammation (CRP≥10 mg/L). L3-CT scans were analyzed to assess body composition. Muscle sections were stained with Oil-Red-O and H&E. Untargeted lipidomic analyses were performed on laser-micro-dissected lipid-rich muscle tissue areas using LC-MS/MS. Intramuscular lipid distribution was visualized by MALDI-MS-Imaging. Genes coding for enzymes involved in de novo ceramides synthesis were studied by qPCR.

Results: Muscle radiation attenuation was lower in cachectic patients with inflammation (median 24.3 HU, IQR 18.6-30.8) as compared to those without inflammation (34.2 HU, 29.3-38.7, p=0.033) or weight loss (37.4 HU, 33.9-42.9, p=0.012). Accordingly, intramuscular lipid content was lower in weight-stable patients (1.8%, 1.5-2.0) as compared to those with cachexia with inflammation (5.5%, 4.5-7.3, p=0.005) or without inflammation (4.8%, 2.6-6.1, p=0.046). Compared to weight-stable patients, cachectic patients had a higher relative abundance of intramuscular glycerophospholipids and a lower relative abundance of glycero-lipids. Furthermore, increases in several intramuscular sphingolipids including Cer(m10:0/16:0), Cer(d18:0/17:3), Cer(m10:0/18:0) and sphingomyelin(d36:1) were found in cachectic patients with inflammation and correlated with cachexia features. Genes related to ceramide syntheses such as SPT1/2, KDSR, Cers1-6, and DEGS1 showed higher expression in cachectic patients with inflammation. Additionally, the altered intramuscular lipids species such as PC (34:1) (see figure below), PC (33:2), and TG (48:1) were capable of visualization by MALDI-MSI.

Conclusion: Patients with cachexia exhibit intramuscular accumulation of specific lipid species that may be partly related to elevated ceramide synthesis.



Volcano plot of changes in intramuscular lipids from PDAC patients (left), PC(34:1) visualization with SCiLS™ Lab (right)

PERSONALIZED CIRCULATING TUMOUR DNA ANALYSIS AS A BIOMARKER OF TREATMENT RESPONSE IN PAEDIATRIC ACUTE LYMPHOBLASTIC LEUKAEMIA

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Leukaemia is the most common childhood malignancy, accounting for ~30% of all cancers diagnosed in children. As per gold standard a bone marrow (BM) biopsy is required for diagnosis and evaluation of treatment response. BM biopsies are an invasive procedure that requires anaesthesia, which in turn, is associated with a significant risk of complications. In contrast, blood samples are less invasive and entail less risks than a BM biopsy.

In this study, circulating tumour DNA (ctDNA) analysis using SimSenSeq is presented as a candidate for minimal residual disease (MRD) evaluations during treatment. Where personalised multiplex assays are used to track patient specific single nucleotide variants (SNVs) over the course of treatment in acute lymphoblastic leukaemia (ALL) paediatric patients.

Preliminary results for 5 patients show that the targeted SNVs levels in ctDNA are similar to those in bone marrow. In addition, the classification of MRD positivity based on the ctDNA analysis resembled the classification assessed in the clinic.

Though further analyses are needed to determine a final cut-off value for MRD classification, these results show the potential use of patient-specific ctDNA analysis in peripheral blood as a marker for treatment response in childhood ALL.

IMAGING THE SPATIAL DISTRIBUTION OF SELECTED ELEMENTS IN VARIOUS CUTANEOUS TUMOURS USING COMPLEMENTARY ANALYTICAL METHODS

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Introduction (aim of the study): The continuously increasing incidence of cutaneous malignancies leads to a heavy personal and economic burden of the healthcare systems worldwide. Moreover, early and precise diagnosis, which is the major aspect in overall prognosis, seems to be constantly challenging. Despite that histopathological examination is the leading diagnostic tool for skin cancer, there is an effort to discover a novel method suitable for preliminary screening.

Our research team investigates the analytical methods of Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS), and their complementarity in providing maps of distribution of the chemical elements in the skin samples. The aim of this study is to show the potential of illustrating the differences in chemical composition between malignant and healthy tissue.

Methods: Totally 6 skin lesions were surgically removed and consequently histologically examined according to standard practice. Chosen samples of benign (pigmented nevus, human skin) and malignant tumours (basal cell carcinoma, squamous cell carcinoma, malignant melanoma, and epithelioid angiosarcoma) were embedded in paraffin blocks and presented for spectroscopic observation. Both techniques (LIBS and LA-ICP-MS) were used to analyse all these samples, and spatial distributions of selected elements (Mg, Ca, C, P, S, Fe, Cu and Zn) were completed. Comparison of sample element maps with the histological picture of the tumour was created, in which the cancerous area had been marked by a pathologist.

Results: Clear differences in chemical composition were found when healthy and malignant tissue was compared. Emphasis was placed primarily on magnesium, which showed significantly higher intensities of spectral lines in malignant areas than in healthy areas, which is demonstrated also when compared to histology specimen.

Discussion: We confirmed different concentrations of selected biogenic elements in all the above selected skin cancers and their surroundings, e. g. healthy tissue and benign lesions. This proof of concept suggests the opportunity to diagnose a tumour using the analytical method of spectroscopic analysis. The LIBS method, characterized by its speed, has limits in the detection of some trace elements that occur only in very small quantities. These trace elements can be captured using LA-ICP-MS technology, which is more time-consuming and more costly.

Conclusions: Different spatial distribution of selected chemical elements was found between malignant and benign skin lesions using LIBS and LA-ICP-MS methods.

Despite the need to continue with further measurements, these analytical methods appear to be an option for complementary method to standard histological examination.

Acknowledgements: Financially supported by the Czech Grant Agency (20-19526Y), Brno University of Technology (FSI-S-20-6353), Charles University Grant Agency (No. 1193819).

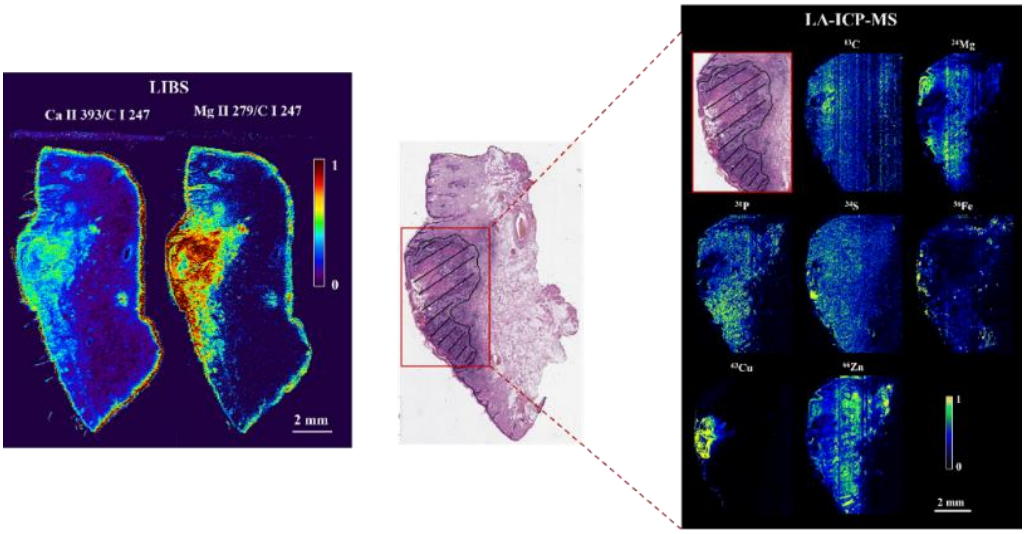

Figure 1: Histology sample of Squamous Cell Carcinoma with LIBS elemental map on the left and LA-ICP-MS on the right.

VITAMIN D AND BREAST CANCER ASSOCIATED FIBROBLASTS

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Introduction: Cancer-Associated-Fibroblasts (CAFs) promote tumor progression *via* extracellular matrix remodeling, cancer stemness, blood vessel formation, cancer cell proliferation, migration, invasion, epithelial to mesenchymal transition (EMT), drug resistance, immunosuppression or metastasis formation. While Vitamin D, exactly its active form – calcitriol, is known of its, *inter alia*, anti-proliferative, anti-metastatic, pro-apoptotic, pro-differentiation and immune-modulating capacities in cancer. Despite growing knowledge concerning vitamin D influence on epithelial compartment, little is known about its impact on CAFs especially in breast cancer, where CAFs constitute major population among tumor microenvironment (TME). This study aims to examine if calcitriol treatment counteract CAF's driven tumor progression.

Methods: Primary breast CAFs (from 51 patients) were cultured with or without calcitriol. Sensitivity to calcitriol was assessed using SRB proliferation assay with calcitriol' concentration ranging from 1 to 1000nM. After short-term (72 hours) treatment with calcitriol (1nM or 10nM), mRNA expression of selected genes was examined using Real-time PCR, protein level in cell lysates using Western blot, protein secretion using ELISA kits and gelatinases activity in cell lysates using gelatin zymography assay.

Results: Calcitriol treatment decreased CAFs proliferation (except 1nM), reduced the expression *CCL2*, *MMP9*, *TNC* and enhanced *PDPN*, *SPP1* or *TIMP1*. Secretion of chemokines – CCL2 or CXCL12 as well as extracellular protein TNC was decreased. No influence was observed on TGFβ and MMP9 secretion. Calcitriol diminished protein level of IDO1 (only 10nM), increased TIMP1 and did not change OPN or TGFβ. Activity of MMP2 and MMP9 in cell lysates decreased after calcitriol treatment.

Discussion: Results presented here are not uniform: upregulation of OPN, PDPN and TIMP1 reflects tumor-supportive function of calcitriol, when downregulation of CCL2, CXCL12, TNC, MMPs or IDO1 suggests that calcitriol affects CAFs in tumor-restraining way. Calcitriol limits CCL2, CXCL12 and TNC secretion as well as IDO1 protein level in CAFs, which are engaged in monocyte/neutrophile recruitment and differentiation or T cell anergy – suggesting that calcitriol hamper immunosuppressive activity of CAFs. Through reducing the production of MMP9, TNC, CXCL12 calcitriol impedes pro-cancerous CAFs actions in tumor cell migration, EMT, invasion, metastasis or angiogenesis. While, increase in expression of TIMP1, PDPN and OPN lean toward tumor-supporting effects of calcitriol in CAFs.

Conclusions: Through modulation of variety of CAFs' produced factors, calcitriol reduce its immunosuppressive activity. However calcitriol-impact on other tumor-supporting CAFs functions remain to be fully elucidated.

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NON-INVASIVE PREIMPLANTATION GENETIC DIAGNOSTICS

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The number of assisted reproductive procedures is steadily increasing because of the increasing infertility rates developing in the highly industrialized countries. The risk of fetal aneuploidity raises with the advanced maternal age, which consequently reduces the chances of implantation and successful pregnancy. Although non-invasive pre-implantation genetic testing for aneuploidity (NIPGT-A) is potentially appropriate to assess chromosomal ploidity of the embryo, practical application of it in a routine IVF center has not been started in the absence of recommendation. Our work group developed a workflow for a clinically applicable NIPGT-A strategy using the spent culture media (SCM) of the transferred embryos for testing. The proof-of concept workflow is based on next-generation sequencing (NGS) technology.

For this experiment we used SCM of Day 3 embryos fertilized with intracytoplasmic sperm injection (ICSI) and the corresponding blank culture media as a background control. The multiple annealing and looping-based amplification (MALBAC) WGA method was used to amplify DNA from the culture media samples and the blank control media as well. After a quality control of the amplified embryonal genomic DNA (gDNA), NGS libraries were prepared. Chromosomal abnormalities were identified by an optimized bioinformatics pipeline applying a copy number variation (CNV) detecting algorithm.

Analysis of DNA profiles of Day 3 embryonic SCM demonstrated that higher gDNA copy number is associated with impaired intrauterine development and indicated miscarriage outcomes, while low gDNA of embryonic origin in the culture medium was found to be characteristic of healthy pregnancy and live birth. We found clinically significant autosomal ploidy alterations only among the aborted embryos—this affected 75% of the studied SCMs, which later correlated with failed pregnancy outcome. In some cases, the chromosomal ploidy aberration was found to be multiple, which can be irreconcilable with healthy embryonic development and embryonic viability.

In the recent presentation we aim to demonstrate the comprehensive workflow covering both wet- and dry-lab procedures supporting a clinically applicable strategy for NIPGT-A. It can be carried out within 48 h which is critical for the same-cycle blastocyst transfer, but also suitable for "freeze all" and "elective frozen embryo" strategies. The described integrated approach of non-invasive evaluation of embryonic DNA content of the culture media can potentially supplement existing pre-implantation genetic screening methods.

Keywords: in vitro fertilization, next-generation sequencing, spent culture medium, copy number variation analysis,

A NOVEL ROLE FOR THE SYNOVIAL SARCOMA, X BREAKPOINT FAMILY IN OVARIAN CANCER

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Introduction: Ovarian cancer (OC) is considered the deadliest gynaecological malignancy, and, unlike other carcinomas, the mortality rate of OC has improved only marginally in the past 50 years. The asymptotic nature of OC delays diagnosis, with almost half of patients diagnosed with late stage disease, where the five-year survival rate is < 40%. Due to the limitations of current diagnostic techniques and the importance of detecting OC in the early stages, the search for new biomarkers and understanding of OC disease progression is paramount. Our previous studies identified cancer-testis antigen (CTA), SSX2, as more frequently expressed in stage I and II OC than current gold-standard clinical biomarkers. SSX2 has previously been linked to the progression of melanoma, breast and prostate cancer, however, the role of the SSX family in OC remains to be determined.

Methods: SSX2, 3 and 4 were profiled (immunohistochemistry) in OC (*n* = 90) and healthy adjacent tissue (*n* = 10). SSX family members were overexpressed (plasmid-based) in immortalised OC cells to investigate their function *in vitro*, using RNA-Sequencing, real-time PCR and Western blotting. Finally, positron emission tomography/computed tomography (PET/CT) studies evaluated the effect of SSX overexpression on tumour growth *in vivo*.

Results: SSX family members were observed at a higher intensity in OC samples than normal adjacent tissue. Following the generation of overexpression systems of SSX2A, 2B, 3 and 4, all showed increased cell proliferation. SSX family members were also shown to cause an increase in the transcript levels of genes that contribute to the epithelial-to-mesenchymal transition. SSX3 overexpression had the greatest effect on cell proliferation and invasiveness. The consequence of these effects was assessed using wound closure and invasion assays which confirmed an increase in the migratory ability of cells that overexpress SSX3. *In vivo* PET/CT imaging of tumour xenografts overexpressing SSX3 indicated an increase in uptake of Fluorine-18 fluorothymidine ([^{18}F]FLT) demonstrating an increase in proliferation (**Figure 1**). Overexpression models were also analysed by RNA-Sequencing to identify downstream pathways. The results show that SSX family members perform both unique and common functions.

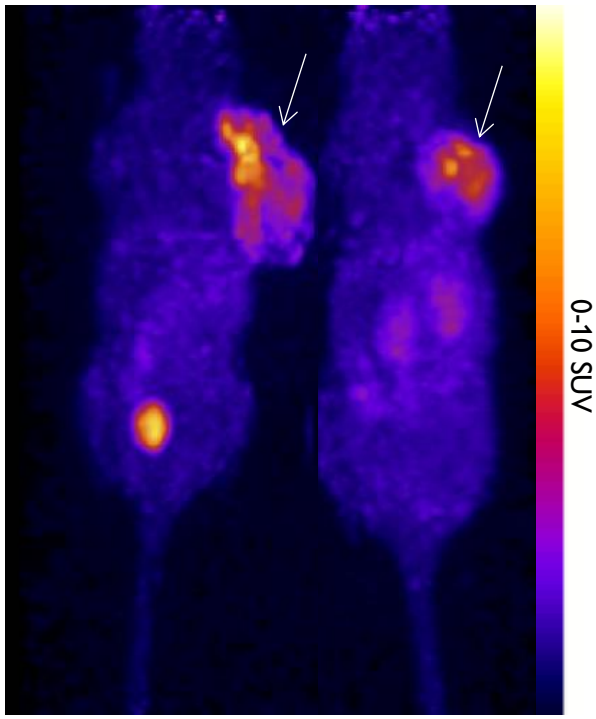

Figure 1 – PET scans of mice implanted on the right shoulder with OVCAR-3 overexpressing SSX3 (left) and vehicle control (right) and injected with Fluorine-18 fluorothymidine.

Discussion: There is a strong rationale for investigating the role of SSX family members in OC. The observation of SSX proteins in OC patient samples supports the need for further clinical investigation. *In vivo* and *in vitro* validation of SSX family members as contributors to OC progression has shown that they increase cell proliferation. SSX expression is restricted to the testis in healthy patients, while aberrant expression of SSX in cancer means they could be promising targets for immunotherapy.

Conclusion: This study reveals SSX family members may be novel OC biomarkers, which likely directly contribute to OC disease progression. Our data supports further translational studies and clinical investigation of SSX in OC.

HISTORY OF CERVICAL EXCISIONAL TREATMENT INCREASES THE RISK OF THE PRESENCE OF MICROORGANISMS IN THE AMNIOTIC FLUID AND THE DEVELOPMENT OF EARLY-ONSET NEONATAL SEPSIS IN PREGNANCIES WITH PRETERM PRELABOR RUPTURE OF MEMBRANES: A RETROSPECTIVE COHORT STUDY

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Introduction: Preterm prelabor rupture of membranes (PPROM), a phenotype of spontaneous preterm delivery, is characterized by the leakage of amniotic fluid owing to the rupture of fetal membranes before the onset of regular uterine activity prior to 37 weeks of gestational age [1, 2]. Due to breaching of the barrier between the vaginal/cervical and intra-amniotic environments, the ascension of microorganisms might follow from these niches to the choriodecidual space and amniotic cavity [1-9]. The presence of microorganisms and/or their nucleic acids in amniotic fluid, called microbial invasion of the amniotic cavity (MIAC), is relatively common in PPROM pregnancies, and the frequency may reach up to 23%-63% [13, 14].

There is a plethora of evidence that cervical excisional treatment of cervical intraepithelial neoplasia (CIN) or very early stages of cervical cancer increases the risk of developing PPROM in subsequent pregnancies [12-15]. Furthermore, the risk of PPROM after cervical excisional treatment has been shown to increase with an increase in the excised cone length [15]. The following mechanisms have been proposed to explain this association: i) impairment of antimicrobial defence mechanisms (cervical mucus and antimicrobial peptides/proteins) that prevent the ascension of bacteria from the vagina or cervix to the higher parts of the genital tract owing to the removal of endocervical columnar epithelium; ii) alteration of cervical/vaginal microbiota; and iii) lack of mechanical support of the cervix owing to its shortening [15-17].

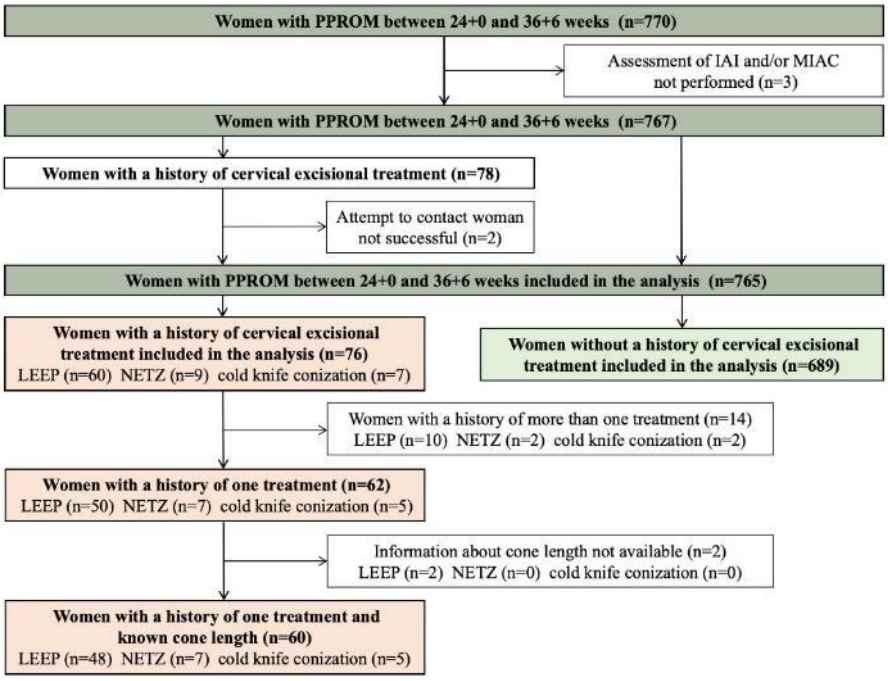
Given the diminishing cervical antimicrobial defence mechanisms and alteration of cervical/vaginal microbiota as possible mechanisms underlying PPROM development in women with a history of cervical excisional treatment, we can hypothesize that this subset of PPROM could also be jeopardized by a higher risk of MIAC and associated intra-amniotic complications compared with PPROM without a history of this treatment. However, there is a paucity of relevant information on this field.

Aims: To assess the differences in the rates of MIAC and/or intra-amniotic inflammation and short-term neonatal morbidity between singleton PPROM pregnancies with and without a history of cervical

excisional treatment and identify an association between the complications of PPROM and the excised cone length.

Methods: This retrospective cohort study included 770 PPROM pregnancies. Transabdominal amniocentesis was performed as part of standard clinical management in all pregnancies to determine the presence of MIAC (through culturing and molecular biology methods) and intra-amniotic inflammation (through amniotic fluid interleukin-6 level evaluation). According to the presence of MIAC and/or intra-amniotic inflammation, participants were divided into four subgroups: intra-amniotic infection (presence of both), sterile intra-amniotic inflammation (intra-amniotic inflammation alone), colonization of the amniotic cavity (MIAC alone), and negative amniotic fluid (absence of both). Information on the history of cervical excisional treatment, the cone length and neonatal outcomes was collected from hospital records. The normality of the data was tested using the Anderson–Darling test. Continuous variables were compared using the nonparametric Mann–Whitney U or Kruskal–Wallis test, as appropriate, and are presented as medians [interquartile ranges]. Categorical variables were compared using Fisher's exact or the chi-square test, as appropriate, and are presented as numbers (%). Odds ratios (OR) with 95% confidence intervals were calculated. Binary logistic regression analyses were used to adjust the results and ORs for potential confounders.

Results: A flow of the women throughout the study is shown on the Figure. A history of cervical excisional treatment was found in 10% (76/765) of the women. Of these, 82% (62/76) had a history of only one treatment, and information on the cone length was available for 97% (60/62) of them (Figure 1).



The presence of a history of cervical excisional treatment in PPROM pregnancies was related to higher rates of MIAC [with: 50% (38/76) vs. without: 23% (159/689), adjusted (adj.) odds ratio (OR): 3.5, adj. $p < 0.0001$], intra-amniotic infection [with: 25% (19/76) vs. without: 12% (85/689), adj. OR: 2.5, adj. $p = 0.004$], colonization of the amniotic cavity [with: 25% (19/76) vs. without: 11% (74/689), adj. OR: 3.1, adj. $p < 0.0001$], and early-onset neonatal sepsis [with: 8% (11/76) vs. without: 3% (23/689), adj. OR: 2.9, adj. $p = 0.02$] than the absence of such.

A cone length of ≥ 18 mm in women with history of one treatment was associated with higher rates of MIAC [with: 73% (11/15) vs. without: 23% (159/689), adj. OR: 7.8, adjusted $p = 0.001$], colonization

of the amniotic cavity [with: 40% (6/15) vs. without: 11% (74/689), adj. OR: 6.1, adj. p = 0.003), and early-onset neonatal sepsis [with: 20% (3/15) vs. without: 3% (23/689), adj. OR: 5.7, adj. p = 0.02] than the absence of a history of cervical excisional treatment.

Discussion: The findings of this study show that every second and fourth PPROM pregnancies with a history of cervical excisional treatment are complicated by MIAC or intra-amniotic infection, respectively. This clinically vital and relevant information suggests that this subset of PPROM cases should be treated cautiously. In addition, it raises the question of whether this subset might benefit from personalized PPROM management driven by knowledge of the intra-amniotic environment based on the assessment of invasively or non-invasively obtained amniotic fluid samples. Identification of MIAC or intra-amniotic infection is of utmost importance because of the accumulating evidence that such complications in PPROM can be successfully treated using a combination of ceftriaxone, clarithromycin, and metronidazole, as well as with monotherapy with intravenous clarithromycin. Next, the observations in this study further support the utmost importance of preserving as much endocervical epithelium as possible while performing cervical excisional treatment for CIN in women of childbearing age.

Conclusions: The presence of a history of cervical excisional treatment was associated with a higher risk of ascension of microorganisms to the amniotic cavity and subsequent development of early-onset neonatal sepsis than the absence of such.

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ACUTE HISTOLOGICAL CHORIOAMNIONITIS AND BIRTH WEIGHT IN PREGNANCIES WITH PRETERM PRELABOR RUPTURE OF MAMBRANES: A RETROSPECTIVE COHORT STUDY

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Introduction: Preterm prelabor rupture of the membranes (PPROM) is defined as the rupture of fetal membranes with leakage of amniotic fluid before the onset of regular uterine activity prior to 37 weeks of gestational age. PPROM might be complicated by the presence of acute inflammatory lesions of the placenta, characterized by diffuse infiltration of neutrophils in any of the structures of the placenta, called acute histological chorioamnionitis (HCA). Depending on the primary source of infiltrating neutrophils, HCA can be divided into the maternal and fetal inflammatory responses. It is obvious that placental lesions other than HCA (maternal and fetal vascular malperfusion, placental hemorrhage, and chronic villitis) mainly lead to impaired placental functions, which can be followed by an alteration of fetal growth. Nevertheless, some studies have provided evidence for the relationship between impaired fetal growth and HCA. However, there is a shortage of information on whether the presence of HCA is related to impaired fetal growth in PPROM pregnancies.

Aim: To assess the association between the birth weight of newborns from pregnancies with PPROM and the presence of HCA with respect to the: i) fetal and maternal inflammatory responses and ii) acute inflammation of the amnion.

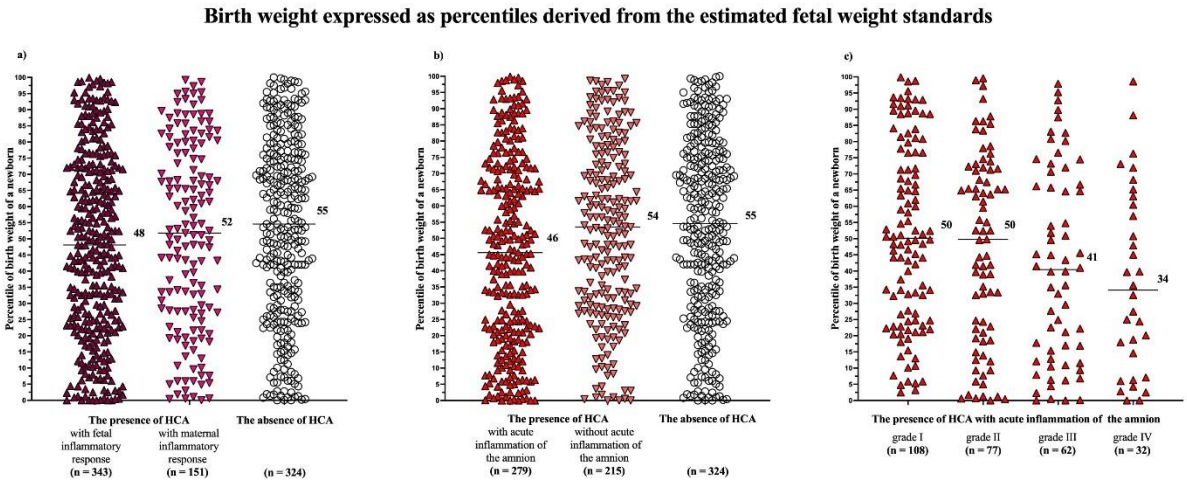
Methods: This retrospective cohort study included 818 women with PPROM. A thorough histopathological examination of the placenta was performed. Birth weights of newborns were expressed as percentiles derived from INTERGROWTH-21st standards for the estimated fetal weight. Continuous variables were compared using the nonparametric Jonckheere-Terpstra test for trend. Categorical variables were compared using the Cochran-Armitage test for trend. Spearman's partial correlation was used to adjust the results for potential confounders.

Results: In total, HCA was observed in 494 (60%) women. Among women with HCA, fetal and maternal inflammatory responses were identified in 343 (69%) and 151 (31%) women, respectively. Among women with HCA, 279 (57%) and 215 (43%) women were with and without acute inflammation of the amnion. The women with HCA with acute inflammation of the amnion were divided into four subgroups based on the severity of acute inflammation of the amnion.

A difference in the percentiles of birth weights of newborns was identified among the women with HCA with fetal (median 48) and maternal (median 52) inflammatory responses and those without HCA (median 55) in the crude analysis ($p = 0.02$; Figure 1A) but not in the adjusted analysis ($p = 0.14$). Women with HCA with acute inflammation of the amnion had lower percentiles of birth weights of newborns (median 46) than women with HCA without acute inflammation of the amnion (median 54) and those without HCA (median 55) in the crude ($p = 0.004$; Figure 1B) and adjusted ($p = 0.02$)

analyses. A difference in the percentiles of birth weights of newborns was found among the women with HCA with acute inflammation of the amnion, when the women were divided into four subgroups based on the severity of acute inflammation of the amnion [grade I (median 50), grade II (median 50), grade III (median 41), and grade IV (median 37)] in the crude (p = 0.004; Figure 1C) and adjusted (p = 0.03) analyses.

Figure 1



Discussion: In this study, newborns from pregnancies with HCA with acute inflammation of the amnion had the lowest birth weight. The alteration of fetal growth was dependent on the severity of acute inflammation of the amnion. The mechanistic explanation for this observation is unclear. It can be hypothesized that the placenta is affected by various lesions (maternal or fetal vascular malperfusion or chronic villitis), which are responsible for impaired fetal growth, might produce endogenous "danger signals" (alarmins) These signals lead to the production of chemotactic stimuli such as interleukin-8, which is followed by the migration of maternal neutrophils into the placenta and/or fetal membranes.

Conclusion: Acute inflammation of the amnion was associated with a lower birth weight in PPROM pregnancies.

ELX/TEZ/IVA VS. TEZ/IVA IN INTESTINAL ORGANOIDS: ANALYSIS OF 63 INDIVIDUALS WITH CF

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Objectives: To compare in vitro treatment response of CFTR modulators (CFTRm) ELX/TEZ/IVA and TEZ/IVA using forskolin induced swelling (FIS) assay in 63 CF subjects with uniform CFTR genotype F508del/F508del. To identify possible additional genetic variants responsible for variable in vitro treatment responses.

Methods: We used intestinal organoids and performed FIS assays to compare ELX/TEZ/IVA and TEZ/IVA. The rescue of the CFTR function was expressed as AUC at 0.128 μM concentration of forskolin. We used median and interquartile range (IQR) to quantify the response; low response = AUC bellow 413 for TEZ/IVA and 2311 for ELX/TEZ/IVA; high response = AUC above 1029 and 2998, respectively. CFTR genotypes and the presence of extra CFTR variants were analyzed by the NGS.

Results: The AUC for ELX/TEZ/IVA was 2655; 2311 – 2998 (median; IQR), and 721; 413 – 1029 for TEZ/IVA. 54% of subjects had treatment response for both CFTRm within the IQR, 27% had low treatment response for ELX/TEZ/IVA and 21% for TEZ/IVA; high treatment response was found in 19% and 25% of subjects, respectively. We discovered following CFTR variants beyond F508del in 5 subjects: L467F (2 subjects), c.3079A>G (variant of unknown significance, VUS), c.2988+33G>T (VUS), c.2658-37T>C (VUS). Subjects with complex allele F508del;L467F had noticeably low response to TEZ/IVA and exceptionally high response to ELX/TEZ/IVA. One subject with CFTR variant c.2658-37T>C had similar treatment responses to both CFTRm.

Conclusion: Significantly larger swelling was observed in organoids exposed to ELX/TEZ/IVA compared to TEZ/IVA. We noted differences in the response between individual organoids, which indicates that the CFTR variants beyond F508del, and/or CFTR modifying genes, might alter the individual efficacy of CFTRmtherapies.

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CHELATING POLYMERS FOR HEREDITARY HEMOCHROMATOSIS TREATMENT

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Aim of the study: Hereditary hemochromatosis (iron overload) encompasses a group of diseases characterized by a toxic hyper-accumulation of iron in parenchymal organs. Currently, only few treatments for hereditary hemochromatosis have been approved; however, all these treatments exhibit severe side effects. In this study, we investigate a new paradigm for maintenance/preventive therapy of hereditary hemochromatosis. We aimed to prepare polymers with negligible systemic biological availability which form stable complexes with iron ions in the gastrointestinal tract (GIT), and thus prevent iron from being absorbed (reducing the biological availability of iron).

Methods: We synthesized macroporous polymer beads are synthesized with three different iron-chelating moieties (benzene-1,2-diol, benzene-1,2,3-triol, and 1,10-phenanthroline) and characterized their properties in vitro (selectivity of chelation, chelation kinetics, anti-oxidative behaviour, cytotoxicity) and in silico (quantum chemistry calculations). Afterwards, we traced the polymers with radioisotope (125I) and investigated their biodistribution in mice after peroral administration using SPECT/CT and ex vivo analysis. Subsequently, we fed mice with diet that contained our polymers and monitored markers of iron metabolism in mice to detect any decrease of iron in their system. Finally, we performed histopathological examination to detect any pathologies.

Results: Our polymers rapidly and selectively chelate iron ions from in vitro aqueous solutions in the course of minutes and are noncytotoxic and anti-oxidant. Subsequently, our in vivo and ex vivo studies showed that our polymers were non-resorbable from GIT. Finally, we showed that our polymers significantly lowered the biological availability of iron. Polymers in our study showed no signs of toxicity, irritation, or any other side effect.

Discussion: We demonstrated that non-resorbable materials can alter the metabolism of iron even without being absorbed, which can be utilized in the treatment of hereditary hemochromatosis. Importantly, because the chelating materials are non-resorbable, these materials are not in contact with most organs (apart from GIT), thus avoiding any side effects of this treatment on parenchymal organ.

Conclusion: We prepared non-resorbable polymers that can selectively chelate iron in vitro and in vivo. Then, we demonstrated that our non-resorbable polymers can decrease the biological availability

of iron, which can be utilized in the long-term treatment of hereditary hemochromatosis. Moreover, such polymers avoid typical side-effects of common treatment (e.g., liver toxicity). Thus, our paradigm of treatment can be applied to the next-generation maintenance/preventive treatment for hemochromatosis and/or other diseases of similar pathophysiology.

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IN VIVO AND IN VITRO EFFECTS OF SALIDROSIDE ON LIVER CYTOCHROME P450

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Salidroside (SAL) is one of the main active compounds of Rhodiola rosea, a herb with antidepressant and anxiolytic potential proven by clinical studies. Cytochromes P450 (CYP) are enzymes responsible for the metabolism of 70-80 % of medicaments in clinical practice. Since R. rosea is the ingredient of various over-the-counter food supplements, we have focused on its potential for pharmacokinetic interactions with the most important CYP isoforms.

SAL was administered intragastrically to Wistar Albino rats for 7 days at the doses of 5, 15 and 45 mg/kg/day. The control group was administered with water (vehicle). 24 hours after the last dose, animals were sacrificed, and liver samples were collected. Rat liver microsomes (RLMs) were isolated and total protein and total CYP content were measured. To evaluate the effect of SAL on the metabolic activity of various CYP isoforms, marker reactions of specific substrates were performed (CYP1A2 – phenacetine, CYP2C6 – diclofenac, CYP2D – dextromethorphan, CYP2A, CYP2B, CYP2C11, CYP3A1/2 – testosterone). The concentration of metabolites was analysed via HPLC. In those isoforms with altered metabolic activity, their content and expression were assessed via western blot or PCR methods. The inhibitory potential of SAL to CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 was evaluated in human liver microsomes (HLMs) and intact RLMs (orthologous of the abovementioned CYP isoforms). All experiments were performed in triplicates.

The total protein and total CYP content were consistent between animal groups. The in vivo experiment revealed statistically significant but weak induction of the metabolic activity of CYP1A2 and CYP2C6 in 5 mg/kg/day group. The increased content of CYP1A2 and CYP2C6 correlated with metabolic activity; however, it was not statistically significant. SAL did not exhibit inhibitory effects either in intact RLMs or in HLMs.

In accordance with our results, a previous in vivo study by Wei et al. revealed the induction of CYP1A2 and CYP2C9 as a result of SAL (30 mg/kg) administration. The effect differed with the frequency of the intake. This study used a cocktail method for the metabolic activity evaluation [1]. However, the clinical study did not prove the effect on CYP3A4, CYP2D6, CYP1A2 and CYP2C19 but revealed the weak inhibitory effect on CYP2C9 after administration of commercial R. rosea extract [2].

In conclusion, our data show that SAL has a low interaction potential with rat and human CYP isoforms investigated. It is necessary to perform a clinical study to assess the risk of pharmacokinetic interactions of SAL in clinical practice.

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EXPERIMENTAL DETERMINATION OF PK_A AND A_{TOT} OF WEAK NON-VOLATILE ACIDS IN PLASMA OF HEALTHY VOLUNTEERS AND ITS APPLICATION IN CRITICALLY ILL PATIENTS

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Introduction: In Stewart's physicochemical theory, the acid-base impact of weak nonvolatile acids (i.e., proteins and phosphates) in plasma is represented by a single monoprotic acid [1]. This hypothetical acid is characterized by its dissociation constant (pK_a) and concentration (A_{tot}), which is often expressed as a product of albumin concentration and a coefficient (c). In human, pK_a and c have only been experimentally determined once in a group of 8 healthy volunteers [2].

In certain populations of critically ill patients, various gap calculations have repeatedly indicated the presence of unmeasured anions [3–5], which were, despite vigorous attempts, never reliably identified [6,7]. A crucial part of these calculations is the estimation of the net negative charge carried by plasma proteins. This process employs several constants that were often determined in healthy volunteers only. However, critical illness is associated with an altered spectrum of plasma proteins and different representation of albumin proteoforms [8]. If the overall buffer action of nonvolatile weak acids in plasma is altered during critical illness, pK_a or c may need to be modified accordingly.

Our objective was to experimentally determine pK_a and c in healthy volunteers and two distinct groups of critically ill. Furthermore, our aim was to estimate the magnitude of error that can occur when the values obtained from healthy volunteers are applied to the critically ill.

Methods: The partial pressure of carbon dioxide (pCO_2) was artificially manipulated (2 to 16 kPa) in heparinized plasma samples from healthy volunteers (Control, n=30), critically ill patients with sepsis (Septic, n=30) and patients who underwent major abdominal surgery (Postoperative, n=27). During this process, repeated measurements of pH, pCO2, and strong ion concentration (including lactate) were performed using a conventional blood gas analyzer. We used nonlinear regression of the experimental data and Stewart's equations to simultaneously solve for the mean pK_a and c in each group.

In all individuals, the predicted charge carried by weak nonvolatile acids at a pH of 7.4 ($[A^-]_{pred}$), calculated using the mean pK_a and c of the Control group, was compared with the actual charge present ($[A^-]_{act}$), calculated from the measured concentration of strong ions and bicarbonate at this pH. The resulting error ($\Delta[A^-] = [A^-]_{act} - [A^-]_{pred}$) is reported.

Results: The mean pK_a and c are presented in Table 1. The resulting $\Delta[A^-]$ is shown in Figure 1.

Conclusions: In the Controls, the mean pK_a and c agree perfectly with their previous estimate (pK_a = 6.64, c = 0.52 mmol/g) [2,tab.5]. In the postoperative and septic groups, pK_a and c were significantly different, leading to an increase in $\Delta[A^-]$. Because of the magnitude of this elevation, we believe that it is unlikely to be fully explained by circulating unmeasured anions and that the altered behavior of plasma weak nonvolatile acids also contributes.

	Controls	Postoperative	Septic	p (one-way ANOVA)
pK_a	6.62 ± 0.02	6.32 ± 0.06	6.02 ± 0.11	<0.0001
c (mmol/g)	0.510 ± 0.004	0.577 ± 0.009	0.842 ± 0.015	<0.0001

Table 1.

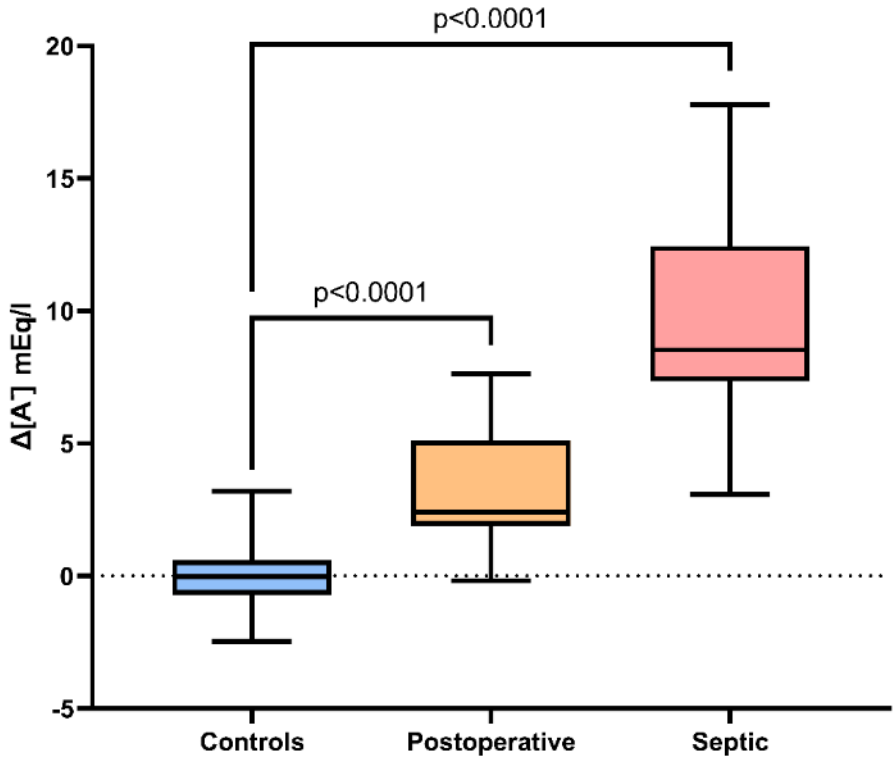

Figure 1.

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A NEW APPROACH IN THE DEVELOPMENT AND VALIDATION OF A METHOD FOR THE QUANTIFICATION OF BILE ACIDS USING LIQUID CHROMATGRAPHY – MASS SPECTROMETRY

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Introduction: Numerous metabolomic studies have identified endogenous compounds that are eligible for the translation to a clinical practice as markers of diseases. Change in bile acids pool is associated with several disorders and liver injury and they may serve as biomarkers. The inherent presence of bile acids in the biological matrices used to prepare calibrators and quality control samples for quantification can complicate validation study and compromise analysis. Finding authentic matrices free from these endogenous compounds or choosing the correct procedure to purify the matrix of bile acids is challenging. Several approaches have been published to overcome this problem. In general, we could divide them into procedures affecting and not affecting the biological matrix. The purpose of this study was to design and validate a new approach for the preparation of quality control samples while preserving the natural composition of the biological matrix and solving the problem with presence of endogenous compounds in these samples. This procedure is based on the standard-addition type procedure. The volumes of added standard solution were calculated according to the measured concentration of endogenous bile acids. The resulting stock solutions with concentrations consisting of endogenous and spiked bile acids were then used to prepare quality control samples according to European Medicines Agency guideline (EMA).

Methods and results: We developed, optimized, and validated LC-MS/MS method for quantification of 19 unconjugated, taurine conjugated and glycine conjugated bile acids in serum. Separation was carried out on C18 reverse phase with MS detection in negative ion mode using electrospray ionization. Total analysis runtime was 12 minutes per sample. The method was validated according to the EMA guideline with linear range 0.005-2 µmol/L. The quantification limits 5.0 nmol/L was defined as the lowest point on the calibration curve. Intra-day and inter-day variation coefficients were <12%.

Discussion: The new validated method covers the spectrum of major bile acids in human and rodents. A great advantage of our method is the use of an authentic biological matrix in the validation study. The procedure in which the biological matrix is used during the preparation of quality control samples is also recommended in the latest edition of the Food and Drug Administration (FDA) and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for endogenous compounds.

Conclusion: In conclusion, an accurate, sensitive, and rapid analytical method which includes correct sample preparation, is vital for the reliable quantification of bile acids in various biological matrices. Using the method with the original matrix for quality control samples can reduce possible errors introduced by use of surrogate matrix instead of native serum or plasma.

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MULTIPARAMETRIC AND ACCURATE FUNCTIONAL ANALYSIS OF GENETIC SEQUENCE VARIANTS USING CRISPR-SELECT

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Introduction: Most sequence variants revealed by next-generation sequencing (NGS) in genetic diseases are variants-of-uncertain-significance (VUS), as opposed to benign or pathogenic variants. VUS represent a huge medical problem by precluding diagnosis, risk prediction and patient treatment. We therefore developed a multiparametric functional assay for variant evaluation that accommodates the strengths of previous approaches while eliminating shortcomings: CRISPR-Select.

Methods: CRISPR-Select uses CRISPR-Cas9 technology to knock in engineer cells with a variant-of-interest or a synonymous mutation (WT´) in a cell population relevant for the disease (Fig. 1a). Changes in absolute frequencies of variant cells are then tracked relative to WT´ cells in the cell population by genomic PCR and NGS of the target site as a function of: 1) time, to determine variant effect on cell growth (Fig. 1b), 2) space, to determine variant effect on cell motility/invasiveness (Fig. 1c) or 3) a FACS marker for any cell process, to determine variant effect on the process (Fig. 1d).

Results: CRISPR-SELECT demonstrated driver function of variants in tumor suppressors and oncogenes and validated candidate drug targets. The method proved useful for classification of VUS as benign or pathogenic, providing a quantitative measure of variant effects ranging from small to large (Fig. 2a,b). The method revealed whether a variant confers drug responsiveness or resistance (Fig. 2c,e) and if a drug acts through the intended target. The method enabled mechanistic dissection of variant roles in disease, for instance, demonstrating if variants confer cancer hallmarks like sustained proliferation, resistance to apoptosis or invasiveness (Fig. 3). The method worked in any human cell type tested (organoids, immortalized and non-transformed cell lines or cancer cell lines). Finally, the method is usable in single dish format, high throughput 96-well format and *in vivo*.

Discussion: The multiparametric readout allows tailoring of a CRISPR-Select assay to most genetic diseases. The absolute variant:WT´ quantitation allows accurate and quantitative determination of variant effects. The cell population-based design provides cost-efficiency and fast turn-around (2 weeks).

Conclusions: CRISPR-Select provides a versatile functional variant assay for assessing variant pathogenicity, drug responsiveness or resistance and mechanistic role in disease.

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Figures

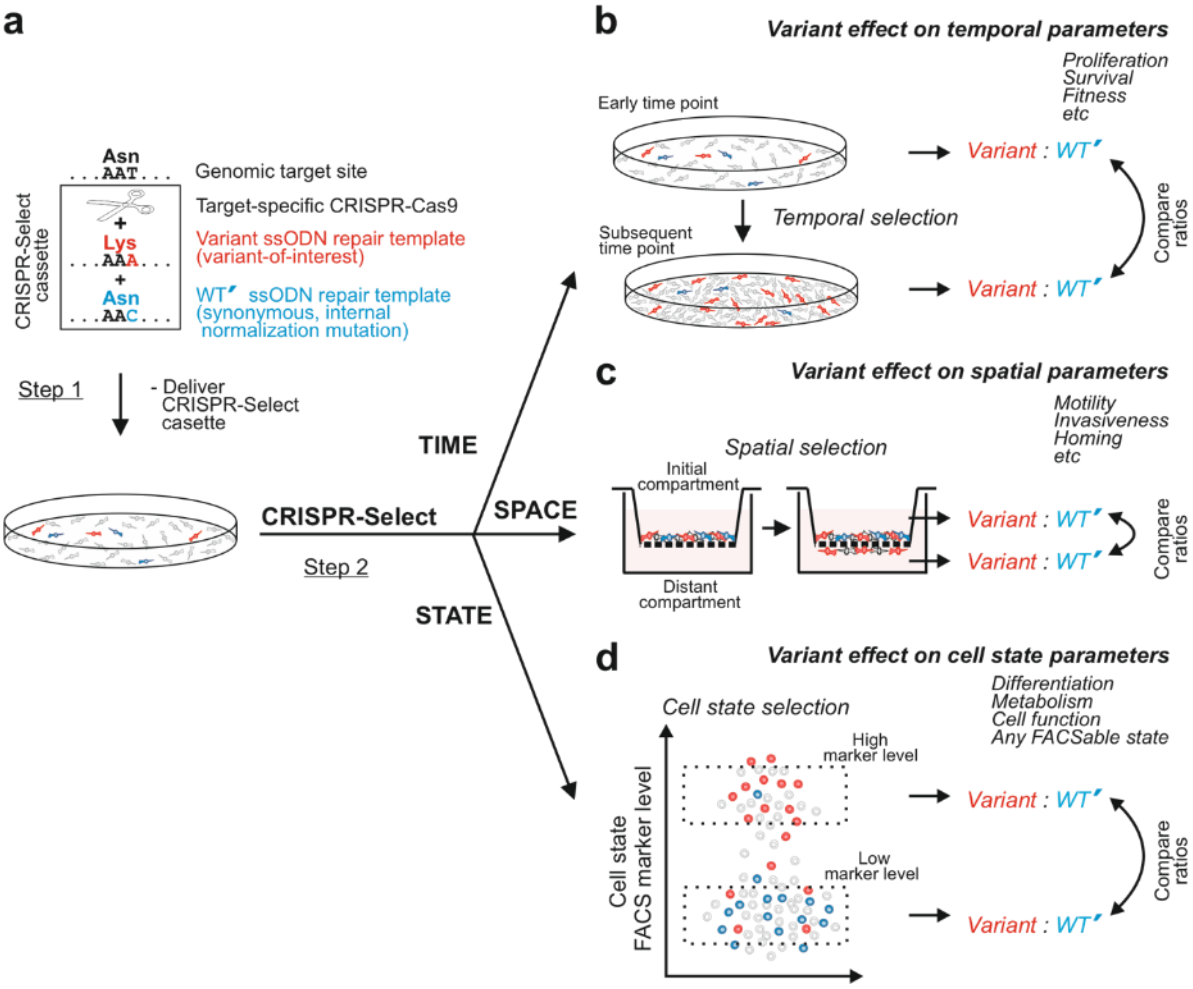

Fig. 1 | Principle of CRISPR-Select multiparametric and accurate functional analysis of genetic sequence variants. a, In step 1, a cell population of interest is transfected with a CRISPR-Select cassette composed of target-specific CRISPR-Cas9 and two ssODN repair templates that are identical, except that one harbors the variant-of-interest and the other a synonymous, internal normalization mutation (WT′). In step 2, the difference in the ratios of cells with knockin of variant relative to WT′ are determined as a function of either a temporal parameter (CRISPR-Select^{TIME}), a spatial parameter (CRISPR-Select^{SPACE}) or a cell state parameter (CRISPR-Select^{STATE}). **b**, For CRISPR-Select^{TIME}, comparison of variant:WT′ ratios at an early and a subsequent time point determines selection for or against the variant, which is a readout of variant effect on cell proliferation, survival or fitness. **c**, For CRISPR-Select^{SPACE}, comparison of variant:WT′ ratios in an initial compartment and a spatially distant compartment determines the selective effect of the variant on cell motile/invasive/homing or similar properties. **d**, For CRISPR-Select^{STATE}, comparison of variant:WT′ ratios in two cell populations FACS-isolated according to different levels of a marker for any cell-state-of-interest determines the effect of the variant on that cell state.

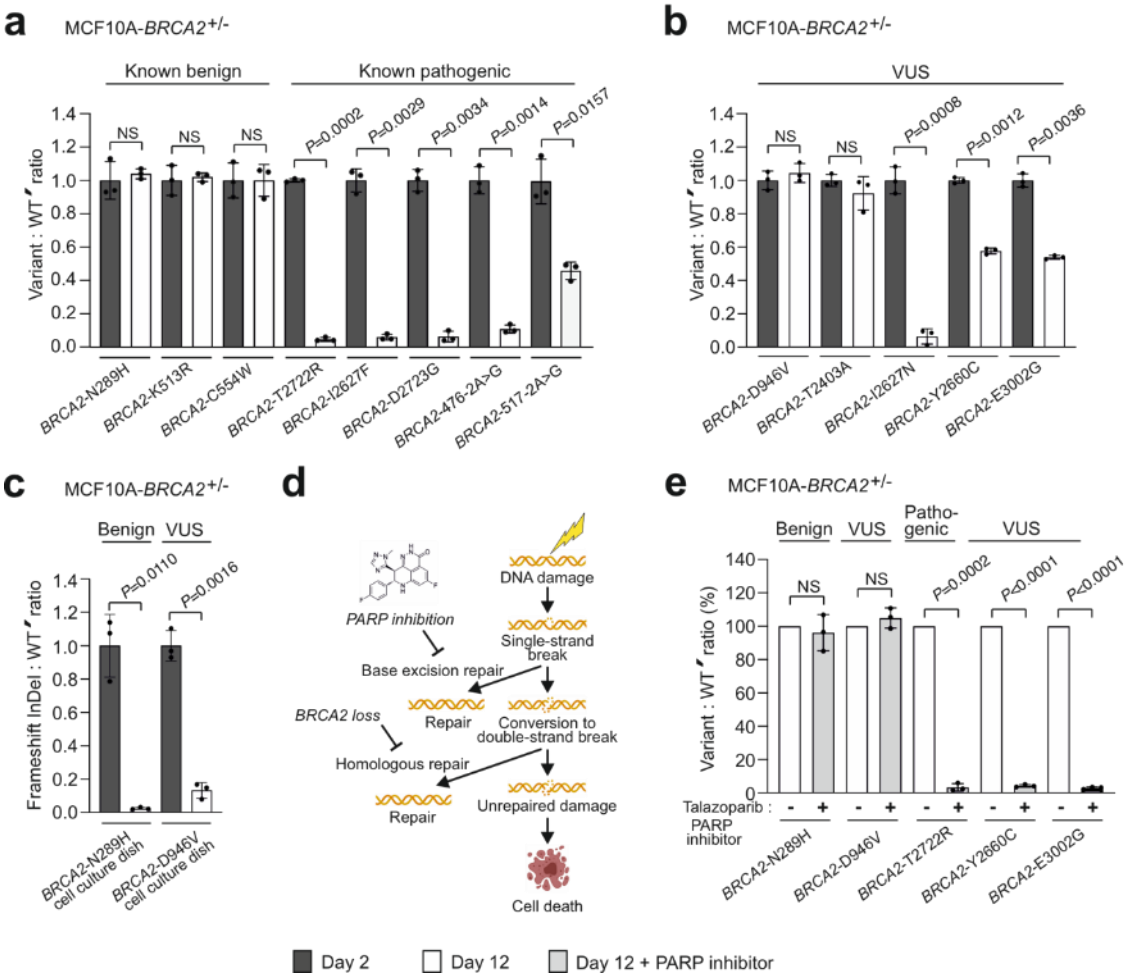

Fig. 2 | CRISPR-Select^{TIME} classification of *BRCA2* variants and prediction of PARP inhibitor response. Cassettes for various *BRCA2* variants were delivered to iCas9-MCF10A-*BRCA2*$^{+/-}$ cells and variant:WT´ ratios were determined at time points, as indicated. **a**, Correct classification of known benign and pathogenic *BRCA2* variants as being neutral and negatively selected for, respectively. **b**, Selection effect of *BRCA2* variants-of-uncertain-significance (VUS). **c**, Internal frameshift InDel control for neutral variants. Frameshift InDel:WT´ ratios were determined in the samples from the cell culture dishes for CRISPR-Select analysis of *BRCA2*-N289H in (a) or *BRCA2*-D946V in (b). **d**, Schematic illustrating that blockade of base excision repair by PARP inhibition and homologous repair by *BRCA2* loss can cause synthetic lethality after DNA damage. **e**, Correct classification of *BRCA2* variants as being resistant or sensitive to PARP inhibition. On day 2 after delivery of cassettes for neutral (N289H, D946V) or loss-of-function (T2722R, Y2660C, E3002G) *BRCA2* variants, cells were split and cultured from day 3-12 in the presence of vehicle (-) or Talazoparib (+). All variant:WT´ or frameshift:WT´ ratios were normalized to the day 2 value, except for (**e**), where ratios on day 12 were normalized to the values obtained with vehicle set to 100%. Data are means +/- s.d. of $n = 3$ independent biological replicates. *P* values are from two-tailed paired *t*-tests. NS, not significant ($P > 0.05$).

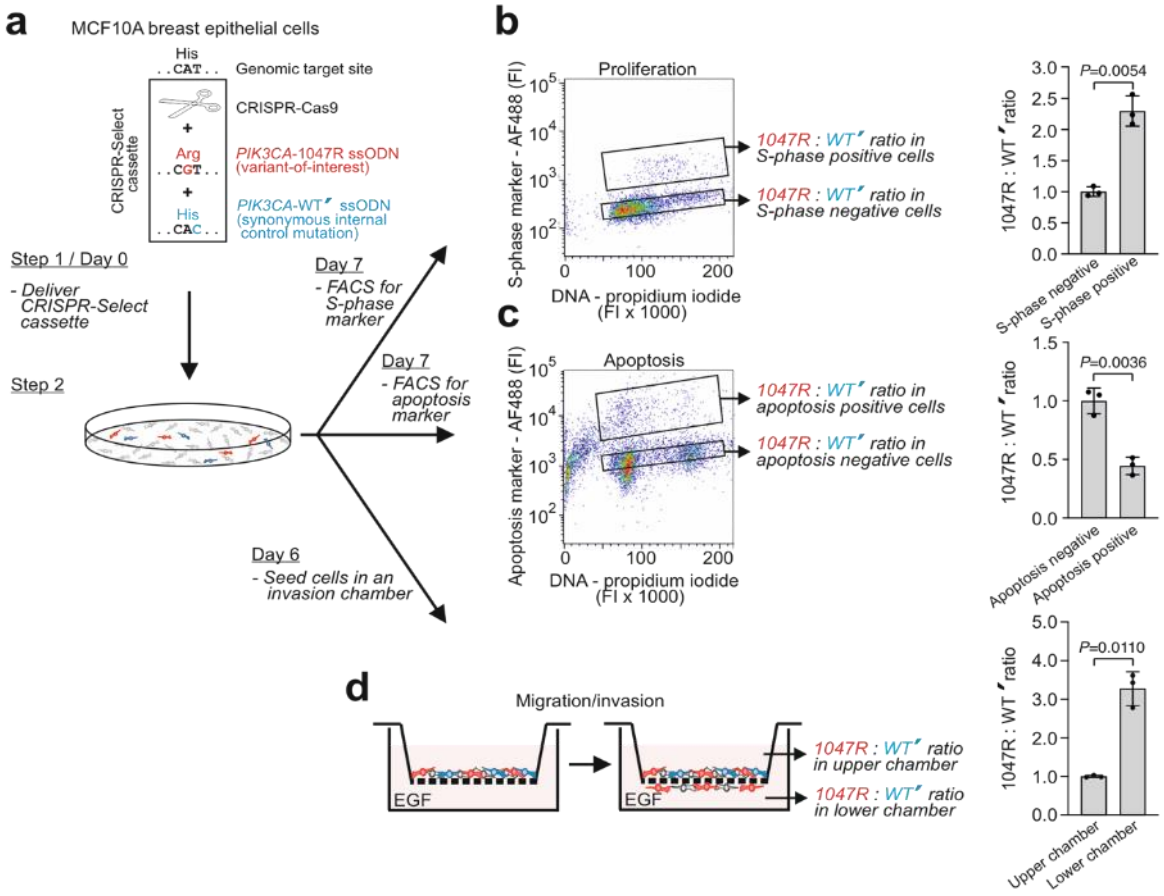

Fig. 3 | CRISPR-Select^{STATE} and CRISPR-Select^{SPACE} dissection of *PIK3CA*-H1047R effects on cancer hallmarks. a, A cassette for *PIK3CA*-H1047R mutation was delivered to iCas9-MCF10A cells. On day 7, cells were subjected to FACS for **b**, an S-phase cell state marker or **c**, an apoptosis cell state marker. Cell populations positive or negative for the respective cell-state markers were FACS-isolated and 1047R:WT′ ratios were determined in the various cell populations. Representative FACS profiles with gating for the sorted S-phase (b) or apoptosis (c) negative/positive populations are shown. **d**, Alternatively, cells were seeded on day 6 in the upper chamber of a Transwell filter insert coated with basement membrane. On day 7, 1047R:WT′ ratios were determined as a function of a spatial dimension, i.e. in the cell populations in the upper and the lower chambers. 1047R:WT′ ratios were normalized to the values of (**b**) S-phase negative cells, (**c**) apoptosis negative cells or (**d**) upper chamber cells. Data are means +/- s.d. of *n* = 3 independent biological replicates. *P* values are from two-tailed paired *t*-tests. Af488; Alexa Fluor 488. FI, arbitrary fluorescence intensity units.

ISCHEMIA-REPERFUSION EFFECT ON THE AGED HEART AND CHANGES IN PROTEINS OF MITOCHONDRIA-ASSOCIATED MEMBRANES

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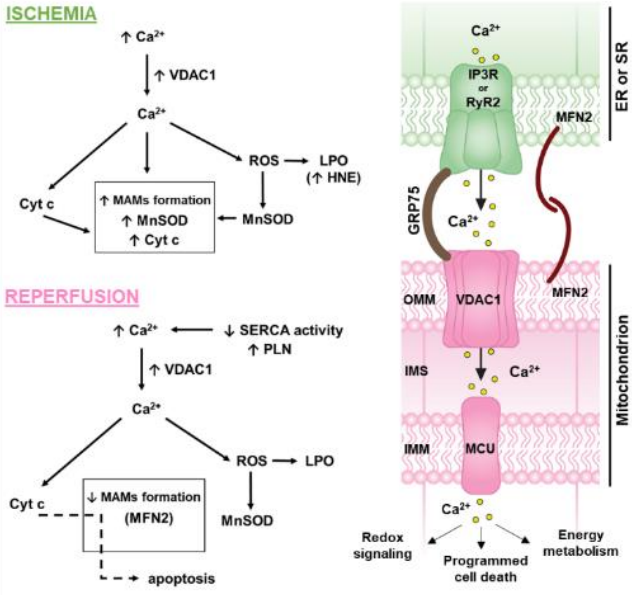
Cardiovascular diseases remain one of the leading causes of death worldwide. Insufficient knowledge of molecular mechanisms and unknown age-associated changes lead to the failure of the therapy. Although mitochondria and sarcoplasmic reticulum (SR) are important for normal heart function, inter-communication between these organelles maintained by mitochondria-associated membranes (MAMs) seems to have a crucial impact on various myocardial processes. In the heart, MAMs mediate the regulation of Ca^{2+} homeostasis, thus contraction. However, the exact protein composition of MAMs in the heart and changes during the pathological condition, like ischemia-reperfusion (IR) injury, remains to be accomplished. Here we present the effect of IR on the physiological function parameters, lipid peroxidation, antioxidant capacity, and MAMs protein content in aged (14-month-old) rat hearts. For the study were used 30 male Wistar rats. IR was induced using Langerdorff's perfusion model followed by tissue homogenization and isolation of mitochondria, SR, and MAMs. Fractions were then used for measuring SR/ER Ca^{2+}-ATPase and antioxidant enzymes activity, detection of proteins (MFN2, GRP75, VDAC1, MnSOD, Cyt c), and the evaluation of lipid peroxidation.



We found that aged hearts exposed to IR exhibited less efficient Ca^{2+} uptake by SR depleting to half of the Ca^{2+}-ATPase control protein level with activity reaching 71.5% of maximal saturation at higher free Ca^{2+} (0.5-5.0 M). This has implications for Ca^{2+} handling among the SR and mitochondria. Upon ischemia, Ca^{2+} accesses the mitochondria through the upregulated (3.9-fold) in MAMs-enriched

voltage-dependent anion channel 1 (VDAC1) and promotes the production of energy, but at the price of increased lipid peroxidation. Furthermore, overproduced 4-hydroxynonenal may potentiate the release of Cyt c from mitochondria and reciprocally modify Ca^{2+} release from SR, thereby altering the contraction. Loss of mitofusin 2 (MFN2), combined with retained MnSOD abundance and activity, possibly preserves mitochondria under stress induced by lipid peroxidation during ischemia. Importantly, the presence of MnSOD in MAMs in the heart, for the first time shown in our work, could have a prolonged protective role for reperfused aged hearts, which have a stabilized antioxidant capacity and hence a better ability to respond to IR stress conditions. All in all, our work illustrates the value of MAMs in the development and progression of age-related pathologies, in particular IR myocardial damage.

This work was supported by VEGA 1/0004/19 and UK/34/2022.

SOMATIC DNA VARIATION AND EXPRESSION OF OXYSTEROL-RELATED GENES AND THE FULL MIRNOME IN EARLY-STAGE LUMINAL BREAST CANCER – A MULTIOMIC ANALYSIS

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Introduction: Oxysterols (OxS) are oxidized derivatives of cholesterol. In breast cancer (BC), OxS can act as selective estrogen receptor modulators, but various effects on cholesterol homeostasis, drug transport, nuclear and cell receptors, and other signaling proteins mean that OxS have been implicated in a range of cancer types and can also affect efficacy of therapy. To date, research has focused primarily on OxS effects on cancer cells *in vitro* or on OxS blood levels *in vivo*. A dedicated study of OxS-related genes (OGs) and pathways in patients has been missing. Our aim was to investigate a cohort of BC patients in terms of interactions between DNA variation, mRNA and miRNA expression and clinical factors in an integrated multi-omic study.

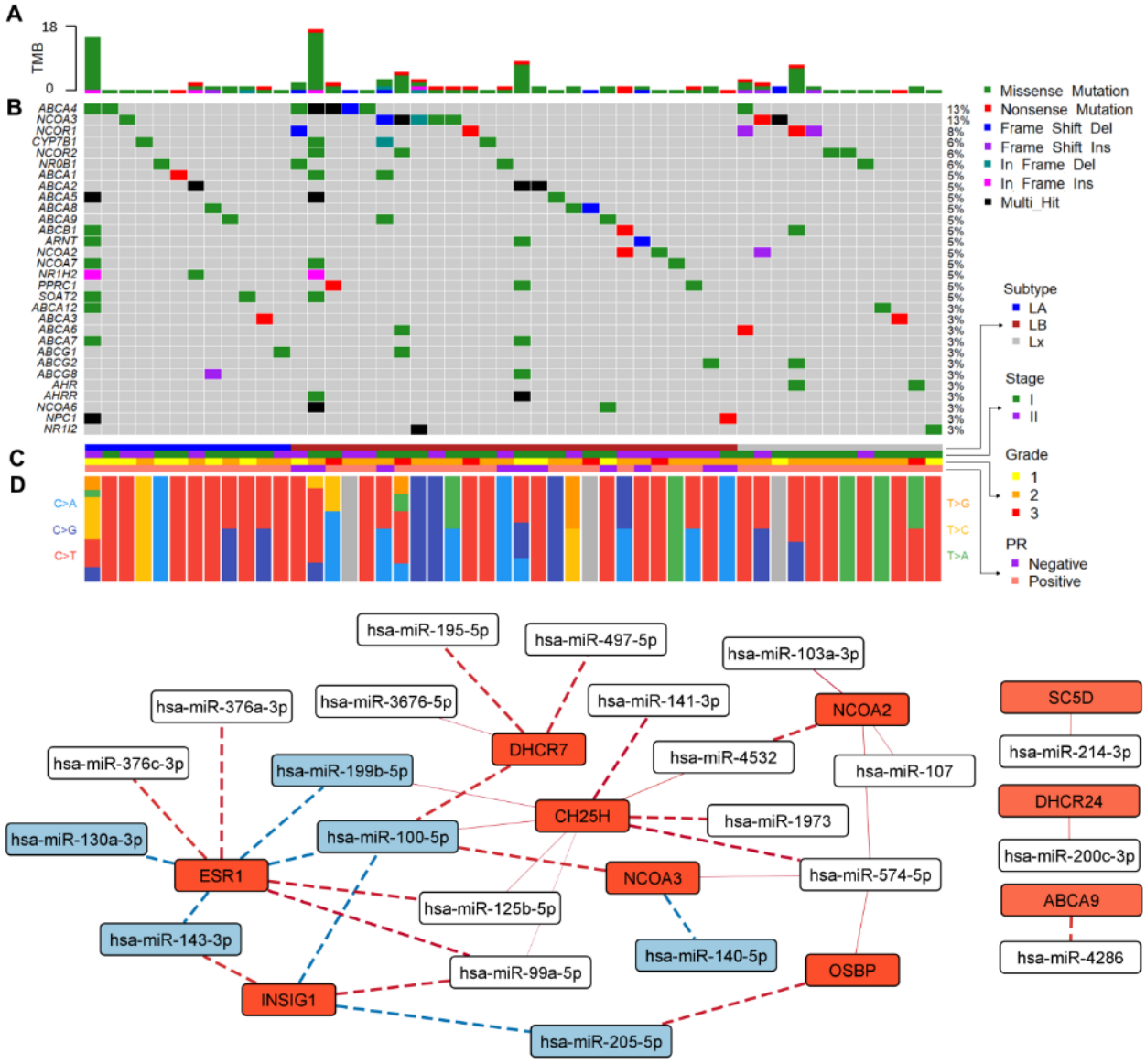
Methods: In total, 162 early luminal BC patients were included with 15+ clinical parameters measured and complete follow-up. Targeted DNA-seq (n=101) of 113 OGs with very high coverage (>800x per tumor-normal pair) to discover short variants. Transcriptome and miRNome of tumors measured by mRNA-seq (n=67) and by microarray (n=125), respectively. All three datasets overlapped in 56 patients. Survival analysis used the Kaplan-Meier method with the log rank test and the Cox proportional hazard model. Analyses conducted using custom bioinformatic pipelines programmed in *Bash* and *R*, in combination with *SPSS*, *GeneSpring* and *Cytoscape*. mRNA-miRNA and miRNA-miRNA interactions validated using The Cancer Genome Atlas (TCGA) BC data (1218 patients before curation). The Benjamini-Hochberg method (FDR) used for p-value adjustment.

Results: There were 96 411 germline and 1 177 somatic variants found, in 101 and 99 patients, respectively. Twelve germline variants associated with poor survival, and three with good survival, but none passed correction for FDR. Somatic mutation status of *CYP46A1* and a functionally related set of nine genes associated with poor survival after FDR correction and led also to differential expression of *EBP*, *PPARGC1B* and *DHCR7*, among others. miRNA-miRNA correlation analysis revealed several co-expressed clusters, with 92 out of 230 interactions confirmed by TCGA validation. mRNA-miRNA interaction networks were constructed, with 123 significant interactions (19 validated by at least one

of 14 queried databases and 55 by TCGA data (14 by both)). *CH25H*, *ESR1* and *INSIG1* were most prominent.

Discussion and conclusions: Our results suggest that there may be interactions of mutation status and expression of oxysterol-related genes, as well as with patient survival, with *CYP46A1* being the highlight. We also confirm that miRNAs are indeed co-expressed in clusters, and document potentially important oxysterol-related mRNA-miRNA interactions. Further insight could be provided by integration of other datasets, e.g. proteomics or oxysterol-focused blood/tissue metabolomics.

Acknowledgements: Grant Agency of Charles University, no. 698119 and the Czech Health Research Council, no. NU22-08-00281.



THERAPEUTIC POTENTIAL OF BACTERIOCIN PRODUCING *ESCHERICHIA COLI* IN COLIBACTERIOSIS TREATMENT

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Introduction: Instestinal colibacterioses caused by *Escherichia coli* belong to diarrheal illnesses spreading in countries with low hygienic standards. Moreover, colibacterioses are one of the most common cause of diarrhea in livestock.

The aim of this study was to characterize set of pathogenic and commensal *E. coli* and test their susceptibility to bacteriocins. Afterwards, we wanted to select potentially probiotic *E. coli* strains with production of active bacteriocins and test their potential *in vitro* and *in vivo*.

Methods: The set of pathogenic *E. coli* (n=277) was isolated in years 1996-2016 from piglets with clinical symptoms of colibacteriosis. For comparison to pathogens, the set of commensal *E. coli* (n=188) was isolated from healthy piglets. Afterwards, all isolates were screened for the presence of genetic determinants for phylogenetic groups (n=4), virulence factors (n=18) and bacteriocinogeny (n=31). Every isolate was also tested for its susceptibility to 31 different bacteriocin monoproducers.

From previously characterized set of human *E. coli* (n=695), three *E. coli* strains were selected based on their production of bacteriocins and presence of virulence factors. Afterwards, the inhibition activity of selected potentially probiotic strains was studied under *in vitro* conditions. Finally, the promising combination of potentially probiotic strains was tested during experimental colibacteriosis in piglets (n=25).

Results: The average susceptibility of both, pathogens and commensals, was tested to select the most effective bacteriocins. The activity of bacteriocins against pathogens and commensals is shown in Fig. 1A. The broadest effect was found for microcin B17 which inhibited 90,6% of pathogenic strains. In general, the bacteriocin activity was significantly higher against pathogenic strains compared to commensals (Fig 1B). Based on activity of various bacteriocins, three strains with combination of produced bacteriocins were selected with emphasis on bacteriocins with higher activity towards pathogens. Their activity was verified by cocultivation with pathogen in liquid medium and *in vivo* during experimental infection. The combination of selected potentially probiotic *E. coli* was able to decrease the number of pathogen in feces of infected piglets and lower duration and severity of diarrhea (Fig. 2).

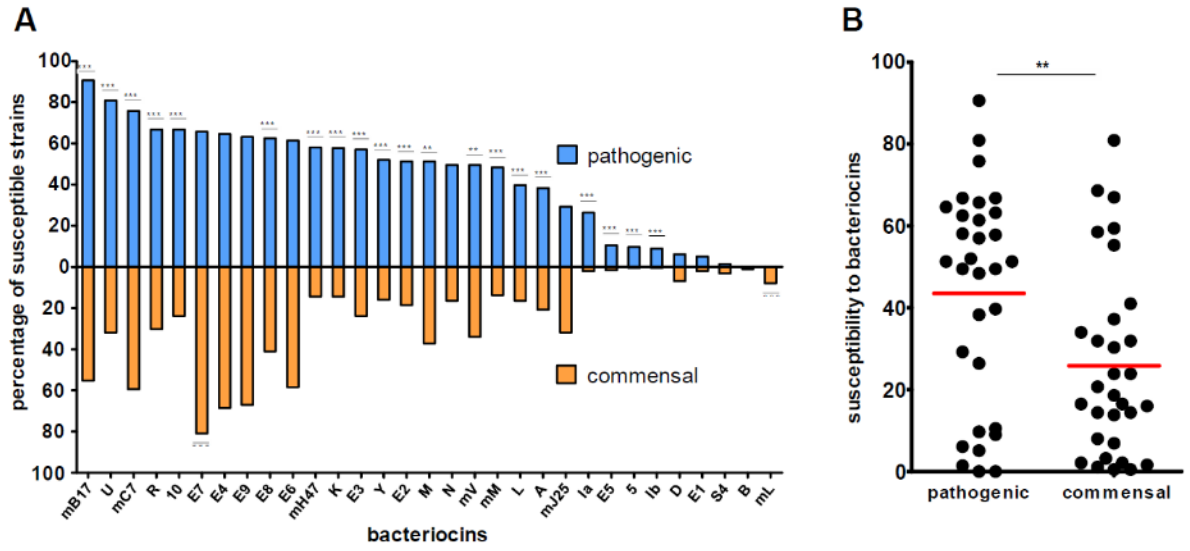

Fig. 1: Susceptibility of pathogenic and commensal *E. coli* strains to bacteriocins; susceptibility to different bacteriocin types (2A; **p<0,01, p<0,001); overall susceptibility, each dot represents one bacteriocin type inhibiting certain perecentage of strains (2B; **p<0,01; red line, mean)

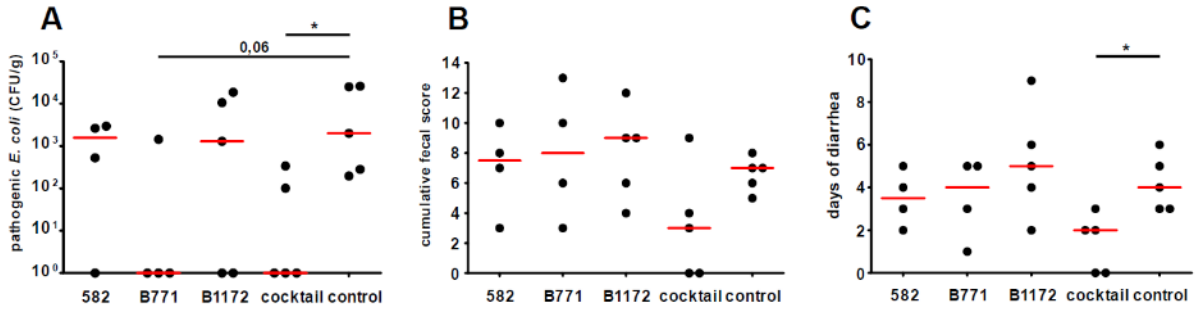

Fig. 2: Probiotic activity of bacteriocinogenic *E. coli* during experimental infection in piglets; (**A**) CFU of pathogen by the last day of experiment (day 14); (B) cumulative fecal score; (C) number of days when piglet suffer from any type of diarrhea

Discussion: Probiotic effect of three selected *E. coli* strains was shown during *in vitro* and *in vivo* experiments. These strains may contribute to welfare of livestock as they often suffer from colibacterioses. Since selected strains are of human origin, these findings may have applications in human medicine. However, more supplementary experiments are needed in future.

Conclusion: Obtained results show that prophylactic administration of selected bacteriocinogenic *E. coli* strains may improve clinical conditions during post-weaning diarrhea in piglets. Moreover, pathogenic bacteria were found to be more susceptible to bacteriocin activity than commensal ones.

MICROSCOPIC EVALUATION OF FLUBENDAZOLE EFFECT ON MICROTUBULE ORGANIZATION, DISTRIBUTION OF POSTTRANSLATIONAL MODIFICATIONS AND CELL SHRINKAGE IN GLIOBLASTOMA CELLS

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Introduction: Glioblastoma multiforme (GBM) is characterized by complex biological changes and extensive heterogeneity. These include alterations in GBM cytoskeleton network, especially βIII-tubulin overexpression and dysregulation of vimentin expression.

Microtubule targeting belongs to basic approaches to cancer treatment. Apart from clinically approved compounds, other drugs, such as repurposed drug flubendazole (FLU), previously showed activity against glioma and glioblastoma cells.

The aim of this study was to 1) assess the effect of FLU on microtubule polymerization in GBM cells; 2) evaluate the effect of FLU on GBM microtubule appearance, expression, and its posttranslational modifications; and 3) determine the overall GBM cell shape, size, and appearance of cytoskeletal markers after FLU treatment.

Methods: Three stabilized GBM cell lines were used for this study – A172, T98G, U87MG. The growing microtubule ends were detected by EB1/EB3-specific immunostaining. The selected cytoskeletal markers were studied using fluorescent microscopy, followed by image processing and analysis (ImageJ). Expression of selected cytoskeletal markers was further evaluated by Western blotting.

Results: In all tested cell lines, FLU decreased EB1/EB3 particles in concentration dependent manner, altered microtubule organization and appearance, and lowered the expression of acetylated-α-tubulin. In addition, low concentration FLU caused a significant shrinkage of cells. This effect was however not observed after high concentration FLU treatment.

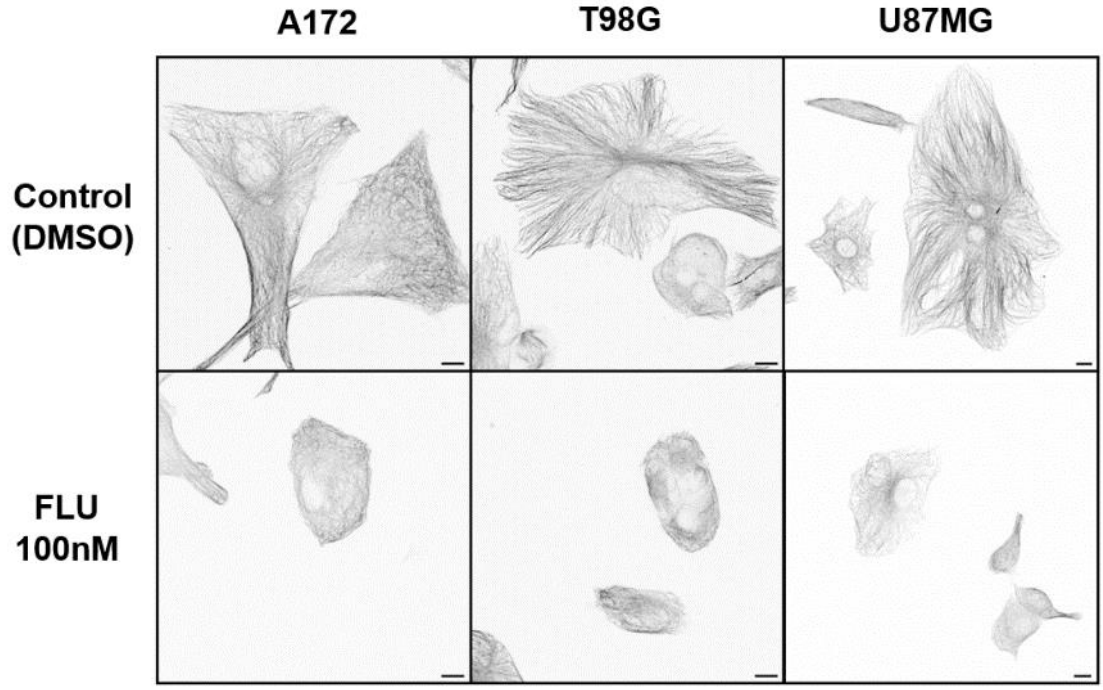

Fig. The effect of 100nM FLU on α-tubulin appearance in GBM cell after 2h exposition. Magnification 630x, scale bar 10 μm.

Discussion: FLU inhibits microtubule polymerization in targeted cells, but various models differ in sensitivity to this blockage. Still, effects of FLU towards cell cytoskeleton, including microfilaments and intermediate filaments are broader and our results also suggest possible changes in distribution of posttranslational modifications. These changes lead to significant cell shrinking after administration of lower concentrations of FLU, while higher FLU concentrations produce microtubule disassembly without other marked changes to the cell. This might be sign of resistance caused by stress response, which should be further investigated.

Conclusion: Our results demonstrate that low concentration FLU potently targets microtubule cytoskeleton, with resulting significant cell morphological changes (shape and volume). These new aspects of FLU activity might offer strategic advantage towards GBM therapy and should be further investigated.

Acknowledgement: This study was supported by Ministry of Health, Czech Republic, project No. NU20-03-00360.

CIRCULATING CELL-FREE DNA-BASED METHYLATION PATTERN IN SALIVA FOR EARLY DIAGNOSIS OF HEAD AND NECK CANCER

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Head and neck cancer (HNC) remains one of the leading causes of mortality worldwide due to late-stage diagnosis, loco-regional aggression, and distant metastases development. A standardized diagnostic procedure for HNC and a tissue biopsy cannot faithfully portray the in-depth tumor dynamics. In the "precision oncology" era the characterization of tumor genetic features (especially early cancer detection) is a pivotal step in cancer patient management. Liquid biopsy approaches, such as analysis of cell-free DNA from plasma or saliva, represent a powerful and non-invasive strategy to obtain information about the genomic status of the head and neck tumors.

The Oxford Nanopore (ONT) platform provides portable and rapid genome sequencing, its ability to natively profile DNA methylation without complex sample processing is attractive for point-of-care real-time sequencing. We recently demonstrated ONT shallow whole-genome sequencing to detect the methylation profile of circulating tumor DNA (ctDNA) of cancer patients. Compared to Illumina sequencing, Nanopore represents a reliable alternative with the advantages of minute instrumentation costs and extremely short analysis time.

Here, we present the first successful use of a customized Nanopore sequencing workflow for the DNA methylation detection changes from saliva of cancer patients. Methylation analysis of 7 saliva samples of HNC patients and 6 healthy subjects was successfully obtained and provided proof of suitability of Nanopore sequencing for new biomarker identification.

Utilization of non-invasive liquid biopsy approaches significantly simplifies the sample collection process, and the diagnostic results are easier to obtain and generally more reliable. Although it has been extensively studied and discussed in many published studies, validated clinical trials are urgently needed to demonstrate the extent of feasibility and effectiveness of earlier detection technologies in combination with standard-of-care screening modalities. Regarding validity, safety, and minimal costs, the future widespread of this technology in preventive care may provide a significant advancement in early cancer detection.

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DEMOGRAPHIC AND DISEASE-RELATED FACTORS IMPACTING ON CEREBROSPINAL FLUID NEUROFILAMENT LIGHT CHAIN LEVELS IN MULTIPLE SCLEROSIS

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Supervisor: Fredrik Piehl[1,2], Michal Bar[3]

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Introduction: Neurofilament light (NfL) levels reflect inflammatory disease activity in multiple sclerosis (MS), but it is less clear if NfL also can serve as a biomarker for MS progression in treated patients without relapses and focal lesion accrual. In addition, it has not been well established if clinically effective treatment re-establishes an age and sex-pattern for cerebrospinal fluid NfL (cNfL) as seen in controls, and to what degree levels are affected by disability level and magnetic resonance imaging (MRI) atrophy metrics.

Methods: We included subjects for whom cNfL levels had been determined as per clinical routine or in clinical research, classified as healthy controls (HCs; n=89), MS-free disease controls (DCs; n=251), untreated MS patients (uMS; n=296), relapse-free treated MS patients (tMS; n=78) and ProTEct-MS clinical trial participants (pMS; n=41).

Results: Using linear regression, we found a positive association between cNfL and age, as well as lower concentrations among women, in all groups, except for uMS patients. In contrast, disability level in the entire MS cohort, or T1 and T2 lesion volumes, brain parenchymal fraction, thalamic fraction, and cortical thickness in the pMS trial cohort, did not correlate with cNfL concentrations. Furthermore, the cNfL levels in tMS and pMS groups did not differ.

Discussion and conclusions: In participants with MS lacking signs of inflammatory disease activity, disease modulatory therapy reinstates an age and sex cNfL pattern similar to that of control subjects. No significant association was found between cNfL levels and clinical worsening, disability level or MRI metrics. Therefore, while cNfL remains a valid biomarker for reflecting the inflammatory aspects of MS, additional biomarkers that can better capture neurodegenerative disease mechanisms must be investigated.

Acknowledgements: We would like to thank all the patients and staff contributing to data collection.

IMPLEMENTATION OF TOOLS FOR TECHNOLOGY-BASED TELEASSESSMENT OF SENSORIMOTOR RECOVERY AFTER STROKE

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Introduction: Up to 75% of stroke survivors are affected by upper limb hemiparesis. To cope with these deficits those affected compensate with available motor strategies. Compensation movements are considered limiting factors for sensorimotor recovery after stroke. They may be beneficial in the short term to perform a task but lead to long-term problems such as increased muscle contractures. Therefore, there is an effort to detect and correct compensation strategies in rehabilitation after stroke. Telerehabilitation including technology-based services are an appropriate and effective way of providing therapeutic services.

The presented project aims to summarise the evidence of existing technology-based teleassessments and to provide implications for practical and research use. A contribution will be made through the development of a tele-assessment of motor compensation, which will provide gold standards for the measurement of arm motor compensation after stroke.

Methods: The first study is a systematic review on observational tele-assessments for clients after stroke. The literature search was conducted in the databases Embase, Medline, CINAHL, Cochrane, Scopus, Web of Science and IEEE. Studies looking at function-based or task-orientated assessments are assessed.

In the second study we develop a webcam-based tele-assessment to detect motor compensation within a drinking task. Therefore, we include up to 50 patients with sensorimotor deficits after stroke who will perform up to 2000 repetitions of a drinking task. The technology is based on machine learning algorithms. We use kinematic metrics of movements, therapist visual assessments based on the data of webcam-based body key points (webcam and depth cameras) and sensors (IMUs).

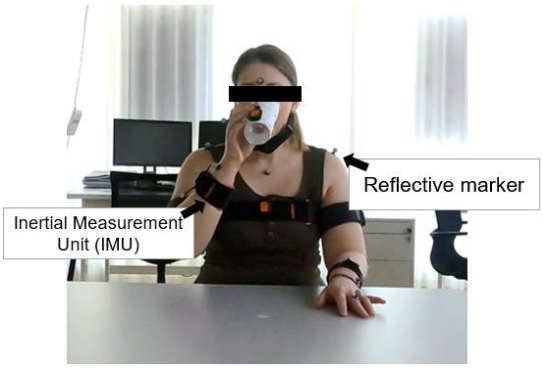

Fig. 1. Setup

Results: The literature search resulted in 3318 hits which are currently being screened by two authors.

The preliminary results of the development of the webcam-based tele-assessment show that the chosen set-up and data collection procedure work in the test run and are now ready to be used with participants after stroke (Fig. 1.).

Discussion: The use of technology-based systems provides objective, reliable, valid, and cost-effective solutions of assessing the impact of stroke. With the literature review we will capture the state of research regarding objective teleassessments in stroke rehabilitation. The webcam-based assessment will take therapist-independent personalized training a step further.

Conclusions: The research will contribute to the evidence for technology-based assessments in research and practice and provide standards for the detection of motor compensation and motor recovery after stroke.

Acknowledgments: I thank Prof. Dr. med. Andreas Luft and Dr. Martina Spiess for supervising my PhD project, Prof. Dr. med. Armin Curt and Prof. Susanne Guidetti for valuable discussions and professional input as PhD committee members, and Prof. Dr. med Verena Klamroth-Marganska for supervising my first PhD year.

DARE WE LOOK BEYOND MUSCULAR EFFECTS; LASTING CENTRAL ACTION OF BOTULINUM TOXIN RISING US TO POSSIBLE CLINICAL IMPLICATIONS?

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Introduction: Botulinum toxin type A (BoNT-A) is a potent neurotoxin with anticholinergic effect. It is a standard therapy in various movement disorders, presumably due to action on local neuromuscular terminals. However, clinical findings and recent experimental data, points to the possible central effects. Moreover, BoNT-A non-local actions have been reported after BoNT-A treatments as effects on distant muscles located far from treated neuromuscular junctions or muscle spindle zone. Herein, after toxin's peripheral injection, the aim was to examine the contribution of the transcytosis-dependent central toxin action, on normal tone muscle function and recovery, as well as tetanus neurotoxin (TeNT) evoked spasticity

Methods: Rats were bilaterally injected with BoNT-A into the gastrocnemius muscle (2 U/kg) or sciatic nerve (5 U/kg). To stop the toxin central transcytosis, BoNT-A-neutralizing antitoxin was intrathecally (i.t.) administered after 24 hours. After recovery from flaccid paralysis, TeNT was intramuscularly (i.m.) injected to animals on day 62, and animals were followed for next 14 days, until they recovered from evoked spastic paralysis.

Results: In different motor tests (gait ability score, digit abduction score, rota-rod, beam walking, catwalk), i.t. antitoxin significantly accelerated the flaccid paralysis and motor performance recovery. TeNT-evoked increase in muscle tone was reduced by BoNT-A dependently on its central effect. However, the H-reflex, when corrected for reduced muscle size or reduced compound muscle action potential (CMAP), was not affected by the toxin treatment, suggestive of the lack of the toxin's direct effect on monosynaptic reflex. The toxin enzymatic activity examined by cleaved synaptosomal-associated protein 25 (cSNAP-25) immunohistochemistry, was still present in neuromuscular junctions and spinal cord. cSNAP-25, presence in second order spinal cord cholinergic neurons, depended on the toxin's central transcytosis.

Conclusion: Long term motor effects of BoNT-A both on normal motor performance (day 1-62), as well as the spastic paralysis (days 62-78), are influenced by the toxin's ongoing central action mediated by retrograde transport and transcytosis. These data suggest that clinically relevant beneficial effect of BoNT-A result from toxin's combined peripheral and central effects.

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IMMUNE DYSREGULATION IN HOSPITALIZED COVID-19 PATIENTS

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Introduction: Several risk factors are associated with unfavorable COVID-19 outcome and various clinical and laboratory parameters were shown to be promising predictive markers. Immune system dysregulation is closely related to disease progression, organ damage and late complications. Monitoring of selected immune parameters might be beneficial for prediction of the disease progression and therapeutic strategy optimization. Our aim was to evaluate the immune profile and its changes over time in 823 COVID-19 patients hospitalized in Martin University Hospital from March 2020 to August 2021 with respect to the disease course.

Methods: We examined differential blood cell count; serum immunoglobulin, C3 and C4 concentration; basic lymphocyte subpopulations ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+56^+$) and expression of selected activation (CD38, HLA-DR) and inhibition markers (PD-1, Tim-3, CD159) using flow cytometry on admission and after the first week.

Results: In survivors, significantly lower proportion of $CD8^+CD38^+$ cells, significantly higher proportion of $CD8^+NKG2A^+$ and NK-$NKG2A^+$ cells on admission and a significant increase in the expression on HLA-DR on $CD3^+$ and $CD8^+$ cells over the first week was found. Promising AUC values were obtained for the total number of lymphocytes, neutrophils, $CD4^+$, $CD8^+$ and $CD19^+$ cells after one week of hospitalization, various combinations of immune parameters could predict the outcome even on admission.

Discussion and Conclusions: COVID-19 is associated with immune system dysregulation. Examination of the immune profile may be helpful in identifying patients prone to clinical deterioration requiring more intensive monitoring and therapeutic approach. Understanding COVID-19 immunology may also contribute to a more targetted indication of immunomodulatory treatment.

Acknowledgements: The study was supported by KEGA 048UK-4/2021 and the Integrated Infrastructure Operational Program for the project: Creation of a Digital Biobank to support the systemic public research infrastructure, ITMS: 313011AFG4, co-financed by the European Regional Development Fund.

SARS-COV-2 PCR DIAGNOSTICS AND EPIDEMIOLOGIC STUDY DEPICTED ON A REGIONAL DYNAMIC MAP

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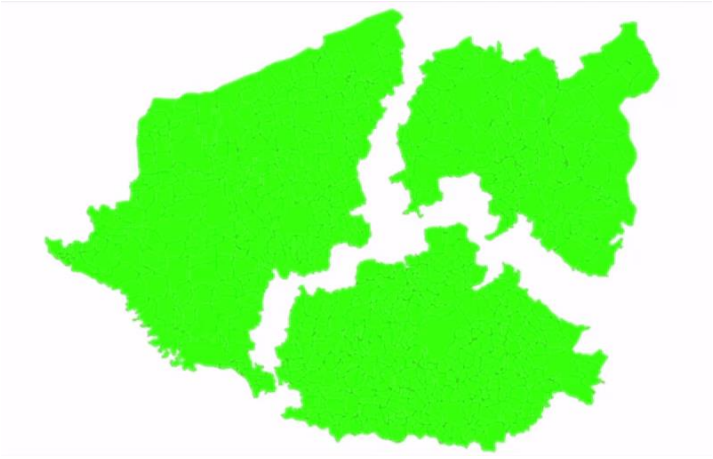
Introduction: Covid-19 burdens humankind with an evolving challenge on a global scale regarding infection prevention. In our region (Southern Transdanubia of Hungary), diagnostic PCR testing was first initiated on 17 March 2020. Currently, 199,935 positive cases have been confirmed throughout our region. The different waves of the pandemic were followed by PCR and rapid antigen testing. In our diagnostic site we constructed a digitalized dynamic map, which reflects crude and population density stratified data regarding the test sum, highlighting positive case ratios.

Methods: We generated a database and listed 271,849 tested cases between 19 August 2020 and 13 February 2022. In addition to PCR test results for SARS-CoV-2 positivity, we collected epidemiological, demographic, clinical, and outcome data, including age, presence of symptoms, cycle threshold values of the SARS-CoV-2 PCR targets and lineages of SARS-CoV-2 detected during the epidemic period. All PCR tested cases were marked on a geographic map representing the Southern Transdanubia region according to the residence of recognized subjects. Color coding was used to visualize proportionate to the number of cases found in the settlement. Two vector layers (originally from geofabrik.de and openstreetmap.hu) were merged to create our map with the aid of mapshaper.org. Data were reshaped and processed in JSON, the timeline and graphical results are based on AmCharts.

Results: In the use of registration and digitalization regarding demographic and healthcare data of tested individuals in our region, we were able to demonstrate a dynamic, well-detailed analysis of the epidemic transmission. Reproduction rate of SARS-CoV-2 was 1.7 at the onset of our study period and showed an increase with the appearance of the new viral variants. Comparative statistical analysis confirmed significant differences in presence of clinical symptoms and age distribution among the positive tested cases in different waves of the pandemic.



Conclusions: Detailed and precious visualization and data analysis can uncover and drive the attention towards key factors which maintain disease transmission dynamics. Aggregated data reflects the phase of the epidemic curve and prove helpful in suggesting the quarantine period and effective primary preventive measurements.

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POST-COVID SYMPTOMS AND RESPIRATORY IMPAIRMENT: ONE-YEAR PROSPECTIVE STUDY

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Introduction: The COVID-19 is manifested primarily by the involvement of the respiratory tract, but the disease can also attack other organ systems.

Aims: 12 months prospective study of consecutive individuals recovered from COVID-19.

Methods: All survivors >18 years from Hradec Kralove area, who had COVID-19 from Mar to Jul 20 were approached to be included in our prospective study. 102 patients (pts) were included (group A2 out-pts, A3 in-pts). Then we offered participation in study to in-pts during hospitalization with COVID-19 in University Hospital Hradec Kralove from Nov 21 to Mar 22 and we included 212 pts (group A1). **In total we analysed 314 COVID individuals**. The follow-ups were established to 3, 6 and 12 months after the COVID-19 diagnosis. Each follow-up visit included symptom assessment, lung function testing, 6-MWT, HRCT, ECG and blood tests.

Results: Three months from acute phase of COVID-19 dyspnoea reported 23% (A2+3) and 55% (A1), cough 14% and 15%, chest pain 13% and 16%. Pulmonary examination most frequently showed reduced pulmonary diffusion – after 3 months 40% (A2+3) and 67% (A1). Desaturation during 6-MWT after 3 months was seen in 11% of pts A2+3 and 36% A1. Chest HRCTs showed a pathology with causational association to previous COVID-19 in 47% (A2+3) and 86% (A1). As we looked to development during one-year observation we could observe persistent regression in all observed symptoms (fig. 2). Evolution of pulmonary impairment were in a same trend. After 12 months reduced diffusion in 40% (A2+3), respectively 57% (A1) and desaturation in 3% (A2+3), respectively 14% (A1). We stratified post-COVID pts into four groups based on the presence or absence of at least one subjective respiratory symptom and at least one objective sign of PI. The stratification and its application to observed cohort A2+3 is detailed in the Fig. 1

Discussion: Recent meta-analysis from C. Fernandez et al., comparing symptoms after mild and severe disease, say that most frequent symptoms are fatigue and dyspnea, and there is significant difference between mild/severe disease after 90 days (for dyspnea 33 % vs 19 %), but there are no data about next evolution of recovery, and what more there is no corelation these symptoms with objective measure pulmonary function. If we compere our data whit these results, we observe the same difference between mild/severe, but we have a significant discrepancy in the frequency of reported symptoms. In our cohort after 90 days reported dyspnea 55 %, respectively 33 % pts. This may be due

to the individual face to face examination of the symptoms by the doctor in our study compared with some studies based on only phone inquiry of symptoms. However, assessing post-COVID syndrome based on symptoms alone as defined by NICE, is still insufficient in our opinion, and objective measuring pulmonary functions after respiratory symptomatic COVID-19 is needed.

Conclusions: Our data show a detailed overview of the evolution of symptoms, even more so the evolution of lung functions in pts after mild and severe COVID-19 and point to persistence of reduced pulmonary diffusion and walking desaturation. In both cases, regarding symptoms and lung function evolution in the post-COVID period, there is a clear trend towards improvement during the one-year follow-up, in our opinion further ongoing pulmonary regeneration is likely. **Finally, we newly established and published the unique tool for stratification.**

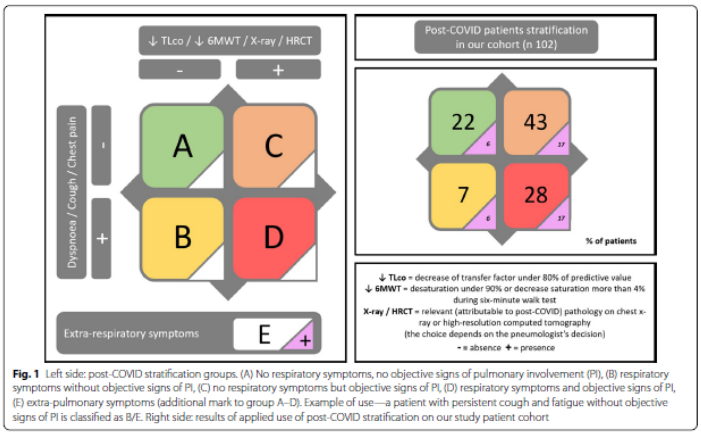

Fig. 1 Left side: post-COVID stratification groups. (A) No respiratory symptoms, no objective signs of pulmonary involvement (PI), (B) respiratory symptoms without objective signs of PI, (C) no respiratory symptoms but objective signs of PI, (D) respiratory symptoms and objective signs of PI, (E) extra-pulmonary symptoms (additional mark to group A–D). Example of use—a patient with persistent cough and fatigue without objective signs of PI is classified as B/E. Right side: results of applied use of post-COVID stratification on our study patient cohort

Part of this data has been published in Virology Journal (Skala *et al. Virol J (2021) 18:73,* https://doi.org/10.1186/s12985-021-01546-8)

References: will be mentioned in presentation

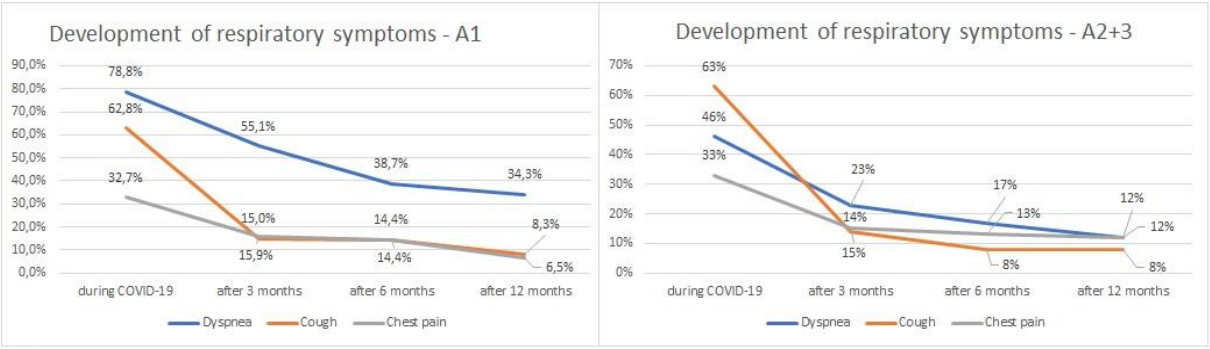

fig. 2

PATIENTS WITH COVID-19 PNEUMONIA WITH 25(OH)D LEVELS LOWER THAN 12 NG/ML ARE AT INCREASED RISK OF DEATH

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Introduction: The immunomodulatory effects of vitamin D could influence the excessive inflammatory response in the severe form of COVID-19 (1). There is a high degree of agreement that a value of 25(OH)D < 12 ng/ml represents an absolute vitamin D deficiency (2). It is possible that in patients who are severely vitamin D deficient, the peripheral tissues, including lung parenchyma, are depleted of 25(OH)D. In this scenario, the immune system is unable to exert a proper lung-protective immune response, which ultimately leads to a severe course and higher mortality in patients with COVID-19. Our aim was to evaluate the mortality of patients hospitalized with COVID-19 pneumonia in the context of absolute 25(OH)D deficiency.

Methods: This longitudinal single-center cohort study included all consecutive patients who were admitted to the internal medicine department between November 2020, and April 2021. The inclusion criteria were as follows:

• COVID-19 pneumonia was the primary diagnosis at admission;

• positive RT-PCR test for SARS-CoV-2 on nasopharyngeal swab; and

• specimen for serum 25(OH) D level obtained on admission.

The patients were monitored until discharge or in-hospital death. Serum 25(OH) D concentrations (ng/mL) were obtained at admission using an automated electrochemiluminescence system (Eclesys Vitamin D Total II, 2019, Roche Diagnostics GmBH, Mannheim, Germany). To assess predictors of mortality, multivariate logistic regression with death as a dependent variable was used. P values <0.05 were considered statistically significant.

Results: In total, 558 patients were hospitalized during the monitored period, 201 patients did not meet the inclusion criteria. 357 patients (198 men and 159 women) were analyzed. The differences between selected parameters in the survivors and non-survivors are shown in Table 1. 80% of patients had either insufficient or deficient 25(OH)D values on admission (<30 ng/ml). 74 (21%) patients had severe 25(OH)D deficiency (<12 ng/ml). Serum 25(OH)D concentration at admission was independently associated with mortality (p=0.0398). The results of the multivariate linear regression analysis are presented in Table 2. Patients with a value of 25(OH)D < 12 ng/ml had an 11% higher mortality compared to patients with 25(OH)D > 12 ng/ml (p<0.05). A comparison of clinical parameters regarding the 12 ng/ml cut-off of 25(OH)D is shown in Table 3.

Table 1: Baseline demographic, clinical and laboratory characteristics of survivors and nonsurvivors.

Variable	Survivors (n=189)	Non-survivors (n=168)	*p* value
Age (years)	63.5±13.9	73.5 ±10.5	<0.0001
BMI (kg/m2)	29.8±7.6	30.4 ±7.1	0.45
Males/females, n (%)	104(55)/85(45)	94(56) /74(44)	0.86
Arterial hypertension, n (%)	130 (69)	132 (78.5)	0.05
Chronic heart failure, n (%)	17 (9)	35(21)	0.002
Diabetes mellitus w/o complications, n (%)	48 (26)	27(16)	0.03
Diabetes mellitus w/complications, n (%)	23(12)	40(24)	0.003
Chronic kidney disease, n (%)	34 (17)	65(39)	<0.0001
Charlson comorbidity index	3.6 ±2.7	5.7±2.6	<0.0001
Use of vitamin D supplements prior hospitalization, n (%)	31(16)	32 (19)	0.51
High-flow nasal cannula, n (%)	44 (24)	132 (79)	<0.0001
Invasive mechanical ventilation, n (%)	2 (1)	22 (13)	<0.0001
White blood cells (10x9/L)	7.4 ±3.3	9.0±5.1	0.0006
Neutrophils (10x9/L)	6.0±3.1	7.7±4.6	0.0001
Lymphocytes (10x9/L)	1.0 ±0.8	0.8 ±0.9	0.08
Platelets (10x9/L)	263.2 ±110.5	231.2 ±97.0	0.0042
CRP (mg/L)	107.3 ±88.1	153.6 ±90.9	<0.0001
IL-6 (ng/L)	122.7±435.5	215.5±476.5	0.07
D-dimer (mg/L)	2.7±4.6	3.6±5.1	0.09
Procalcitonin (ug/L)	1.5±10.4	2.6±9.7	0.30
25(OH)D (ng/ml)	24.6±14.6	20.8±11.1	0.007
Viral load (CT e gene)	24.2±5.0	21.0±4.6	<0.0001
Oxygen saturation (%)	89.6±7.47	86.1±10.3	0.0003

Table 2: Results of multivariate linear regression analysis. Only findings with p <0.05 are displayed.

Independent variables	Coefficient	Std. Error	t	p	$r_{partial}$	$r_{semipartial}$	VIF
	0,2628						
Age (years)	0,01049	0,002462	4,258	<0.0001	0,2365	0,2075	1,815
BMI (kg/m2)	0,007869	0,003524	2,233	0,0263	0,1266	0,1088	1,063
CRP (mg/L)	0,001071	0,000275	3,899	0,0001	0,2176	0,19	1,086
Charlson Comorbidity Index	0,02327	0,01136	2,048	0,0414	0,1163	0,09976	1,797
Platelets (10x9/L)	-0,0007356	0,000239	-3,077	0,0023	-0,1732	0,1499	1,031
Oxygen saturation (%)	-0,008144	0,002905	-2,803	0,0054	-0,1582	0,1366	1,09
25(OH)D (ng/mL)	-0,00395	0,001913	-2,065	0,0398	-0,1172	0,1006	1,028

Table 3: Comparison of clinical and laboratory parameters according to the absolute 25(OH)D deficiency (< 12 ng/ml vs > 12 ng/ml).

Variable	25(OH)D ≥12 ng/ml (n=283)	25(OH)D <12 ng/ml (n=74)	*p* value
Age (years)	67.9±13	69.4±14.7	0.39
BMI (kg/m2)	30.5±7.4	28.7±7.2	0.08
Males/females, n (%)	164(58) / 119 (42)	34 (46) /40 (54)	0.07
Arterial hypertension, n (%)	210 (74)	52 (70)	0.60
Chronic heart failure, n (%)	38 (13)	14 (19)	0.41
Diabetes mellitus w/o complications, n (%)	62 (22)	13 (17)	0.41
Diabetes mellitus w/complications, n (%)	50 (18)	13(18)	0.8
Chronic kidney disease, n (%)	70 (25)	29 (40)	0.007
Charlson Comorbidity Index	4.4±2.8	5.3±3.09	0.022
Use of vitamin D supplements prior hospitalization, n (%)	50(18)	13 (17)	0.81
High-flow nasal cannula, n (%)	141 (49)	35(46)	0.56
Invasive mechanical ventilation, n (%)	18 (6)	4 (5)	0.61
White blood cells (10x9/L)	8.4±4.6	8.1±3.6	0.65
Neutrophils (10x9/L)	7±4.2	6.8±3.5	0.73
Lymphocytes (10x9/L)	0.97±0.8	1.07±1.1	0.43

Platelets (10x9/L)	251±108	246±100	0.75
CRP (mg/l)	131.6±93	125.15±91	0.59
IL-6 (ng/l)	171.1±475	143.6±330	0.65
D-dimer (mg/L)	3.4±5.2	3.2±4.8	0.79
Prokalcitonin	1.7 ±9.3	2.8±12.1	0.41
25(OH)D (ng/ml)	26.7±12	8.2±2.6	<0.0001
Oxygen saturation (%)	87.7±9.3	87.6±10.5	0.9
Viral load (CT e gene)	23.2±5.2	21.5±4.9	0.04
Non-survivors/Survivors, n (%)	127(44)/166(54)	41(55)/31(46)	0.05

Discussion and conclusions: Our study found that the 25(OH)D value at admission is independently associated with mortality. Absolute 25(OH)D deficiency (<12 ng/ml) is associated with an 11% increase in mortality. Even though patients with absolute 25(OH)D deficiency had significantly higher mortality, we did not observe significant differences in the values of inflammatory markers between the groups (<12 ng/ml vs. >12 ng/ml). This finding could support the claim that 25(OH)D deficiency is not just a by-product of the inflammatory response but rather a potentially modifiable risk factor in the severe form of COVID-19 pneumonia.

Acknowledgements: None.

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Poster Abstracts

The abstracts are in order according to the programme.

COMPARISON OF VISUAL QUALITY AFTER FEMTOSECOND-LASIK, TRANSEPITHELIAL PRK AND CONVENTIONAL PRK IN PATIENTS WITH MILD AND MODERATE MYOPIA

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Introduction: Refractive surgery has greatly replaced the need of corneal transplantation when conventional PRK (photorefractive keratectomy) was introduced utilizing excimer laser, which was developed in the early 1970s and modified for ophthalmic use in the early 1980s. TPRK (transepithelial photorefractive keratectomy) was proposed in the late 1990s as an alternative to conventional PRK. Although PRK is an established procedure for refractive correction of myopia, its popularity was downsized in favor for FS-LASIK (Femtosecond laser in situ keratomileusis), it was approved for use in the United States in 1999. Yet PRK is still needed in conditions where LASIK is not possible. The femtosecond laser has been used in wide variety of ophthalmological procedures, allowing customization of the corneal flap parameters which lessen the risk of flap related complications when using the classic keratome blade. Refractive ophthalmic surgery allows refractive errors to be corrected permanently in a safe, effective, and reliable way with few complications. The aim of this study is to compare the visual quality of each method in patients with low and moderate myopia. Our study was mainly focused on the early clinical outcomes.

Methods: We performed a prospective study of 80 eyes in 40 patients with mild and moderate myopia with or without astigmatism that underwent either PRK (n=10), TPRK (n=14) or FS-LASIK (n=16) at Ophthalmology clinic of faculty university hospital in Hradec Králové, Czech Republic. Treatment was done using SCHWIND AMARIS excimer laser and or LenSx femtosecond laser. Visual acuity (Decimal) was assessed, wound healing (hours between surgery and complete epithelial closure) was monitored at the slit lamp. At day 4, patients subjectively rated the maximum pain intensity within the last 4 days using a Visual Analogue Scale (VAS). Patient satisfaction was gauged at the time of chart review by contacting the patient. The follow up period was 6 months. Patient consent was sought before carrying out the procedure.

Results: In short -term postoperative period (1week postoperatively), visual recovery was significantly faster in the FS-LASIK group compared to TPRK and PRK groups. Complete epithelial closure was achieved faster in TPRK group compared to patients who underwent PRK. Pain score was highest in those who underwent PRK. No adverse complications were observed. [1]

Discussion: PRK has been compared with a variety of LASIK techniques; for example, LASIK performed with a microkeratome or LASIK. However, no consensus has been established on the superior procedure between PRK and LASIK. Although previous studies have demonstrated a quicker and less painful recovery in patients undergoing FAL, the final postoperative outcome has been found to be similar among the two modalities [1]

Conclusions: FS-LASIK gives a faster visual recovery and is a less painful technique than PRK and TRPK, however, the three techniques appear to give similar outcomes 6 months postoperatively.

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IMMUNOPHENOTYPING IN PATIENTS WITH ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a common inflammatory skin disorder characterized by recurrent eczematous skin lesions and intense itch. Skin barrier abnormalities have been suggested to play an essential role in initiation of early AD. Antigen penetration through a compromised barrier likely leads to increased innate immune responses, antigen-presenting cell stimulation, and priming of overt cutaneous disease. There are a lot of studies in science, which describe the change in quantity of T cells in patients with atopic dermatitis compared to heatlh subjects. Other components of lymhocytes such as B cells are not examined as well as T cells. For this reason we focus on immunophenotyping of B cells and their subsets for example memory B cells, switched and non-switched B cells. The expression of marker CD200 and CD23 on B cells and their subsets compared with other white blood cells could be of great importance to differentiation in severity of AD and to the better understanding of immunity reaction to biological treatment. Complete examination of immunophenotype in patients with AD could be a benefit to additional treatment. Three subject groups as follows were examined: thirty-two patients suffering from AD without dupilumab treatment, thirteen patients with dupilumab and thirty subjects as a control groups. Immunophenotype was examined by flow cytometry in which monoclonal antibodies with fluorescent molecules were used. For assessment Non-parametric Kruskal-Wallis one-factor analysis of variance with post-hoc (follow-up multiple comparison) Dunn's test with Bonferroni modification of significance level was used. The significance level is chosen at 5 %. The results of analysis showed different count of subsets white blood cells compared with the control group. However, the count of lymphocytes was decreased. It could be a significant indicator of ongoing inflammation in patients with AD. Analysis also showed some difference between expression of CD23 and CD200 on B cells. The reason is in fact, that AD patients are in majority sensitised to different allergens. Our results demonstrated the difference in the count of subsets white blood cells in patients suffering from atopic dermatitis compared with the control group. There were a difference in expression of activation markers as CD23 and CD200 on subsets of B cells (memory, switched and non-switched) at all examined groups. This research was conducted at the Institute of Clinical Imunology and Alergology, Faculty Hospital and Medical Faculty of Charles University Hradec Králové in collaboration with Department of Dermatology and Venerealogy, Faculty Hospital and Medical Faculty of Charles University, Hradec Králové. This work was supported by the Cooperatio Program, research are IMMU and INDI.

CHARACTERISTICS AND OUTCOMES OF PATIENTS ADMITTED FOR ACUTE HEART FAILURE IN SINGLE CENTRE STUDY

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Introduction: Acute heart failure represents a medical condition with very high mortality. Accurate risk stratification can help physicians to improve the health care about these patients. The aim of our study was to characterise real-life patients admitted for acute heart failure in a specific region with one tertiary medical centre and to describe risk factors of short-term and long-term mortality.

Methods and results: We performed a retrospective analysis of patients admitted from January 2017 to December 2017 to Department of cardiology of the tertiary medical centre University Hospital in Hradec Kralove. We identified 385 patients admitted for acute heart failure to the standard care and intensive care unit. The median of age was 74 years (IQR 67.5 - 80) and 34% of patients were females. Hospital admission was due to de novo heart failure in 222 (57.7%) patients. The most common comorbidities were arterial hypertension (77.7%), dyslipidaemia (67.3%) and coronary artery disease (63.1%). Coronary artery disease (52.7% of cases) and valve disease (28.1% of cases) were the most common etiologies of heart failure. The all-cause in-hospital mortality was 12.7%, 30-day mortality was 14.6% and 1-year mortality was 34%. Among risk factors of in-hospital mortality, the most significant factors were haemodialysis during the hospitalization (OR 15.82, 95% CI 2.96 – 84.57, p = 0.0008), chronic heart failure (OR 4.27, 95% CI 1.66 – 11.03, p = 0.001) and STEMI as a precipitating factor of heart failure (OR 4.19, 95% CI 1.23 – 14.25, p = 0.023). Haemodialysis during the hospitalization (OR 4.28, 95% CI 1.17 – 15.61, p = 0.025) and the comorbidity depression and anxiety (OR 3.49, 95% CI 1.45 – 8.39, p = 0.005) were the most significant risk factors of long-term mortality.

Conclusion: Our study confirms very high mortality rates among patients with acute heart failure underlying poor prognosis of these patients. Comorbidities (peripheral artery disease, atrial fibrillation, chronic heart failure and depression and anxiety), precipitating factors of heart failure (myocardial infarction with ST segment elevation), complications occurring during the hospitalization (acute kidney injury, pulmonary ventilation for respiratory failure and haemodialysis) and the age of patients should be included in the risk stratification of in-hospital, 30-day and 1-year mortality.

Acknowledgement: This study was supported by the Cooperatio Program, area CARD.

DETERMINATION OF REFERENCE VALUES FOR SUBLINGUAL MICROCIRCULATION IN HEALTHY VOLUNTEERS IN THE PEDIATRIC POPULATION USING THE SIDESTREAM DARK-FIELD IMAGING METHOD

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Introduction: The main goal of the study is to determine the physiological parameters of sublingual microcirculation in children in different age categories using the Sidestream Dark-Field Imaging Method. Microcirculation is defined as a network of small vessels (arterioles, capillaries and venules) with a diameter of less than 100 µm. It plays a role in the control of vascular resistance, coagulation, inflammatory and immune processes. Devices using sidestream dark field imaging (SDF) provide high-contrast microcirculation imaging. The device emits green light, which illuminates the tissue to a depth (up to 3 mm, according to the manufacturer) and the scattered green light is absorbed by the hemoglobin of red blood cells in the superficial vessels. In this way, it is possible to visualize arterioles, capillaries and venules because they contain red blood cells.

Methods: After recording the basic anthropometric parameters, pressure, pulse and O_2 saturation, each volunteer who met the inclusion criteria had their microcirculation measured using a Sidestream Dark-Field (SDF) probe placed sublingually by one examiner using the SDF method. The measurement was performed in the supine position in a disease-free period, with normal diets, at least 2 hours after the last meal in the afternoon, for girls outside the menses period. Premedication or analgesia was not used. A total of 3 video clips were recorded from different parts of the sublingual area with a minimum length of 20 seconds. The recorded videos were then processed offline by one evaluator who is trained and experienced in microcirculation evaluation, three best and most stable parts of each video clip were analysed. MedCalc 7.6.0 statistical software was used. (MedCalc Software, Ostend, Belgium). ANOVA test was used to compare multiple groups with normal distribution according to D'Agostino-Pearson test, analogously a Kruscal-Wallis test for results whose distribution was not normal. To obtain reference intervals, double sided or left sided 95% reference interval calculation was used.

Results: A total of 40 children were measured, 10 preschool healthy children aged 3-5,9 years, 10 children of younger school age aged 6-10,9 years, 10 children in puberty aged 11-14,9 years and 10 postpubertal adolescents aged 15-18,9 years. There were no differences in Total Vascular Density, Small Vessel Density, Proportion of Perfused Vessels, Proportion of Perfused Small Vessels, Perfused Vessel Density, Perfused Small Vessel Density and DeBacker's score. Proposed reference intervals are shown in Table 1.

Discussion: To our knowledge, this is the first study focusing on microcirculation in healthy children. The method was successful in measuring even in 3-year-old children without any complications, all children were able to tolerate measurement in a satisfactory way. These results may be used in further research on sublingual microcirculation in children.

Conclusion: In children aged 3-18 separated into different age groups, the age of the child showed no impact on evaluated microcirculatory parameters.

Table 1: Proposed reference intervals for sublingual microcirculation in children aged 3-18

	Mean	Standard deviation	Lower limit	Upper limit
Total Vascular Density (mm/mm^2)	12,80	2,24	8,40	17,20
Small Vessel Density (mm/mm2)	10,08	2,51	5,16	15,00
Proportion of Perfused Vessels (%)	98,12	1,66	95,38	100
Proportion of Perfused Small Vessels (%)	76,16	10,24	59,31	100
Perfused Vessel Density (mm/mm2)	12,37	2,29	7,87	16,87
Perfused Small Vessel Density (mm/mm2)	9,90	2,52	4,96	14,83
DeBacker's score (1/mm)	8,19	1,47	5,31	11,06

CARDIAC OUTPUT AND ACCESS FLOW MEASUREMENTS AS USEFUL TOOLS FOR MANAGEMENT OF CHRONIC HEMODIALYSIS PATIENTS

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Introduction: Cardiovascular disease is the leading cause of morbidity and mortality in patients whose lives depend on hemodialysis. High vascular access flow (QVA) may lead to increase in cardiac output and cause high-output cardiac failure. This condition is usually under-diagnosed in chronic dialysis patients. The aim of the study is: (i) to compare QVA values measured by routinely used thermodilution (BTM module, Fresenius, Germany) and ultrasonic dilution (HD03 device. Transonic System, USA) (ii) to present pilot results of measuring cardiac output (CO, ml/min) during dialysis and determine their possible relationship to QVA values and other indicators.

Methods: Cardiac output, cardiac index (CI, cardiac output converted to body surface area) and vascular access flow (QVA) were measured by blood dilution using ultrasound (Transonic HD03) in all stable chronic dialyzed patients with a native arterio-venous fistula. Vascular access blood flow vas compared to routinely used method in our hemodialysis unit – dilution technique by reversal of the blood dialysis lines with the venous outlet facing the access stream. For this technique was used Blood Temperature Monitor (BTM) in Fresenius dialysis machines.

Results: One hundred and six chronic hemodialysis patients were included in the study. Data are expressed as median (interquartile range). Cardiac index was byl 2,51 (2,10; 2,98) l/min/m^2. Arterio-venous fistula blood flow measured by Transonic HD03 and Fresenius BTM monitor was 720 (530; 1030) ml/min and BTM 541 (425; 670) ml/min.

Discussion: There was significant difference between arterio-venous fistula blood flow measured by Transonic HD03 and Fresenius BTM monitor. When we compare these methods, 28 % difference in blood flow was in fistulas with the blood flow lower than 500 ml/min. In fistulas with blood flow over 500 ml/min the difference between the two methods was even higher – 59%. We also analyzed effect of fistula blood flow on CI. In patients with fistula blood flow below 1l/min, only 21 % of patients had CI over 3 l/min/m2. In patients with fistula blood flow over 1l/min, the number of patients with CI over 3 l/min/m2 increased to 48 %.

Conclusions: Arterio-venous fistula blood flow may be measured by different methods that may yield significantly different results and cannot be used interchangeably.

Cardiac index measurement might be useful to identify increased cardiac burden due to increased blood flow in venous access and help in management of vascular access in chronic hemodialysis patients.

Acknowledgements: Supported by the Faculty of Medicine in Hradec Kralove, Charles University.

THE EFFECT OF INHALED FUROSEMIDE AND ORALLY APPLIED LEVODROPROPIZIN ON DYSPNEA IN PATIENTS WITH SEVERE PULMONARY DISEASES (INFURO TRIAL) MONOCENTRIC PROSPECTIVE RANDOMIZED DOUBLE BLINDED CROSS OVER STUDY

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Introduction: Numbers of patients suffering of dyspnea especially in palliative care worldwide grow annually with lack of effective and safe treatment of dyspnea, especially in terminally ill patients. Inhaled furosemide showed promising results in various studies worldwide acting supposedly by affecting the C fibers in lungs and bronchi which are also affected by levodropropizine. The aim of this study is to prove the effect of furosemide on alleviation of dyspnea regardless underlying disease and test whether there is an additive effect of levodropropizin.

Methods: 102 patients with dyspnea and signed consent will be randomized to obtain inhalations of furosemide or placebo in a double blinded regim. After cross over the patients who obtained placebo will be given inhaled furosemide and vice versa still double blinded. At the end of the study procedures all the patients obtain oral levodropropizine not blinded . Vital functions and dyspnea will be measured before and after each step. See Fig 1

Results: our team is currently dealing with local regulatory authorities for two solid years expecting approval for commencing the study. The amount of demanded data about pharmacokinetics and pharmacodynamics of inhaled furosemide is enormous despite many trials with inhaled furosemide worldwide with no major adverse events observed. Furthermore the process of approving was seriously slowed down during the COVID19 pandemic.

Discussion: There is quite a long evolution of the study design. We started with a simple and cheap study design, being later précised due to advices of the scientific board to cross over study. Lot of changes in the protocol was forced by regulating authorities. Lot of time consumed fund raising and several applications for grants that changed the study protocol and budget several times. Actually the study is prepared to be launched.

Conclusion: the results of similar studies on specific populations worldwide suggest the positive effect of inhaled furosemide to alleviate dyspnea especially in patients with no causal treatment available. There is no evidence of major adverse effects of this treatment unlike opioids for instance. The possible dual affection of dyspnea in combination with levodropropizine was never studied before. According to the results of our study, there could be a shift in treatment of dyspnea regardless of causative disease.

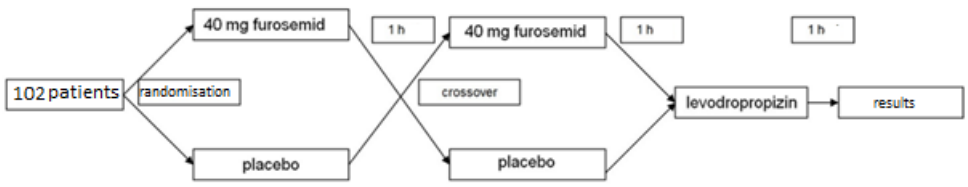

Fig1: study design

USAGE OF TELEMETRIC PRECHAMBER SENSOR RESERVOIR® IN MANAGEMENT OF NORMAL PRESSURE HYDROCEPHALUS IN ADULTS – OUR PILOT STUDY.

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Supervisor: Svatopluk Řehák[1]

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Introduction: Normal pressure hydrocephalus is a type of communicating hydrocephalus of unknown etiology in most cases, typically in elderly people. "Classical" symptoms are dementia, gait disturbance and incontinence. Normal pressure of cerebrospinal fluid (CSF) is found in lumbar puncture (LP). Natural course has been described by many authors. Andrén et al. found out that early surgery in symptomatic patients lead to better outcome. Because of unknown etiology of NPH many biomarkers and parameters (CT, MR findings, specific proteins, etc.) are investigated as a specific marker for NPH. The aim of this study was to assess whether usage of telemetric prechamber as a part of ventriculo-peritoneal drainage (VPD) could be beneficial for patients with hydrocephalus. We compared our results with literature.

Methods: We compared two groups of patients with hydrocephalus after VPD placement. Group A was without implanted telemetric prechamber and group B was with implanted telemetric prechamber. The patients have been included according to inclusion criterions and randomly divided in these groups. Each patient underwent neurological examination, brain MR examination, gain examination prior to the 1st CSF examination (lumbar puncture or lumbar infusion test) and 4 hours after this test and then CT examination 3, 6, 9, 12, 18 and 24 months after surgery, in patients with placed telemetric prechamber also telemetric CSF pressure measurement. Then they were retrospectively assessed in several parameters (gain quality, MMSE score, quality of memory, self-assessment, brain CT characteristics, valve settings, number of valve-pressure changes, number of complications).

Results: We included 6 patients into group A - and 3 males and 3 females, 45-76 years old (mean 63,8) - and 8 patients into group B - 6 males and 2 females, 61-74 years old (mean 68,5. MMSE score were 18-30 points (mean 25) in group A and 22-30 points (mean 27) in group B. For other characteristics see Figure 1. The changes in gait and step length prior and after CSF examination are shown in Fig. 2. According to our expectations there was significant difference between initial (perioperative) and final (after 24month follow-up) valve setting. The difference was 0-30 mm H_2O in group A vs. 0-90 mm H2O in group B. Surprisingly there were more changes of valve setting in group B compared to group A. The outcome of the patients in group B was better than in group A.

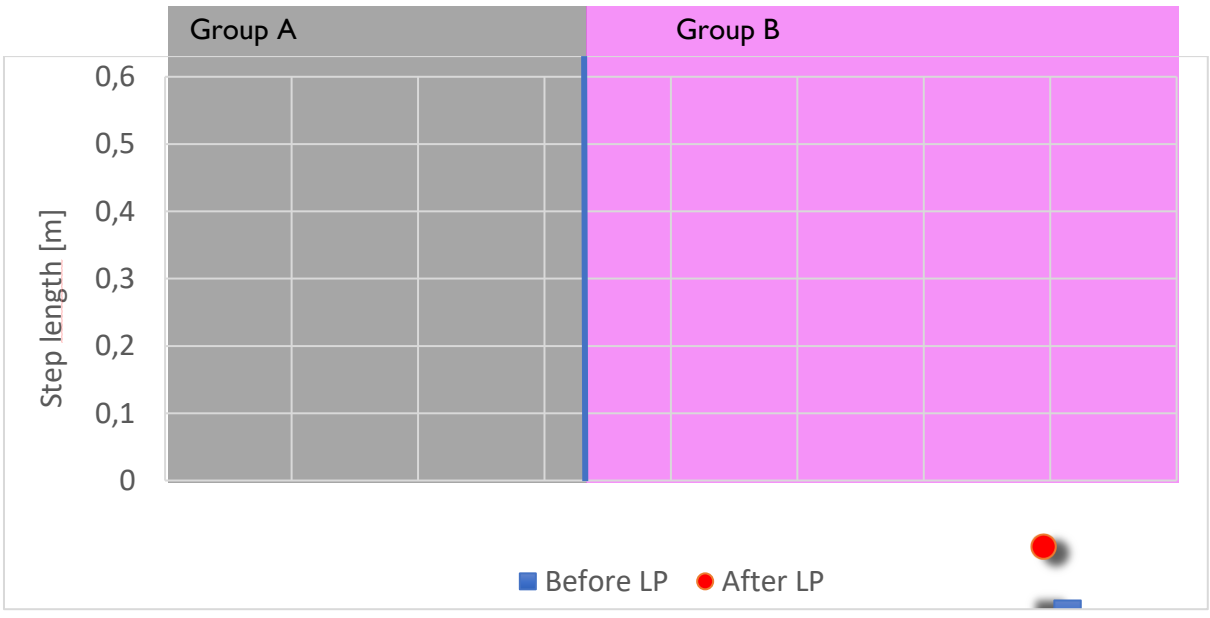
Discussion: As mentioned in several similar studies, telemetric prechamber seems to be a beneficial tool in management in patient with hydrocephalus. In our pilot study we confirmed similar results. But honestly said, there was only small number of patients included in our study. The next issue is also the heterogeneity of hydrocephalus disease.

Conclusion: Telemetric prechamber seems to be a beneficial tool in management in patient with hydrocephalus leading to better outcome of patients, early detection of malfunction and individualized valve setting.

Figure 1. Other patient's characteristics

	Group A: Without Sensor Reservoir	Group B: With Sensor Reservoir
Gender (F/M)	3 F + 3 M	6 F + 2 M
Age	45-76 years (mean 63,8)	61-74 yrs (mean 68,5)
Diabetes mellitus	1 (16,7 %)	5 (62,5 %)
Hypertension	3 (50 %)	8 (100 %)
M. Parkinson	0	1 (12,5 %)
Stroke in history	0	2 (25 %)
Other dementia	0	0
MMSE	18-30 points (mean 25)	22-30 points (mean 27)

Figure 2. Changes in gait and step length before and after lumbar puncture and CSF evacuation.



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COMPREHENSIVE ASSESSMENT OF IMMUNE FUNCTIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: chronic lymphocytic leukemia (CLL) is associated with a significant combined immune deficiency which plays an important role in disease progression and infectious complications, the leading cause of death of CLL patients (pts). The impact of chemoimmunotherapy (CIT) on these changes has not been extensively studied.

Methods: parameters of immune function were prospectively evaluated in 34 healthy controls and 125 pts with CLL, both indolent and progressive disease. In 55 pts, we analyzed the impact of CIT. Serum concentrations of immunoglobulin (Ig) classes IgG, IgA and IgM and subclasses IgG1-4 and IgA1-2 were measured using immunonephelometry. Major lymphocyte populations (CLL cells, T cells, Th and Tc cells and their functional subsets, polyclonal B cells and NK cells) were quantified by flow cytometry.

Results: Pts with progressive disease had significantly lower concentrations of all Ig classes and subclasses than pts with stable CLL. After treatment, there was significant increase in IgA ($p=0.0031$), while other Ig levels remained unchanged. CLL pts had a significant increase of most cell populations in comparison to the controls. The progression of CLL was characterized by significantly elevated cell counts with the exception of a lower percentage of naïve T-cells (Figure 1). After treatment, the percentage of naïve T-cells further decreased at the expense of the effector memory T-cells (TEM). In pts with indolent CLL, higher percentages of naïve CD4+ ($p=0.0026$) and naïve CD8+ ($p=0.023$) T-cells were associated with a longer time to first treatment (TTFT) (Figure 2). The elevation of CD4+ central memory T-cells (TCM) ($p=0.27$) and the TEM ($p=0.003$) counts and a higher percentage of CD4+ TEM ($p=0.0047$), were linked with shorter TTFT. In treated pts, the increased regulatory T-cells count was associated with a shorter time to next treatment (TTNT) ($p=0.042$), while higher CD4+ TCM count with shorter TTNT ($p=0.035$) and a shorter overall survival ($p=0.041$).

Discussion: We hypothesize that decrease in the relative number of naïve cells at the expense of the more mature subsets reduces the responsiveness of the immune system to new stimuli, and thus the ability to control infection or CLL progression.

Conclusions: CIT may lead to increase in IgA level. Naïve cells depletion and the CD4+ TCM and TEM increases are detrimental to the CLL patients' prognosis.

Acknowledgements: This work was supported by MH CZ - DRO (UHHK, 00179906) from the Ministry of Health, Czech Republic and by program Cooperatio, research area ONCO.

Figure 1. Box and whisker plots of the changes in absolute numbers of the selected cell populations during disease progression and treatment. The numbers are given in 109/L. The boxes represent the interquartile range and the whiskers are drawn at a distance of 1.5 times the interquartile range from the first and third quartiles.

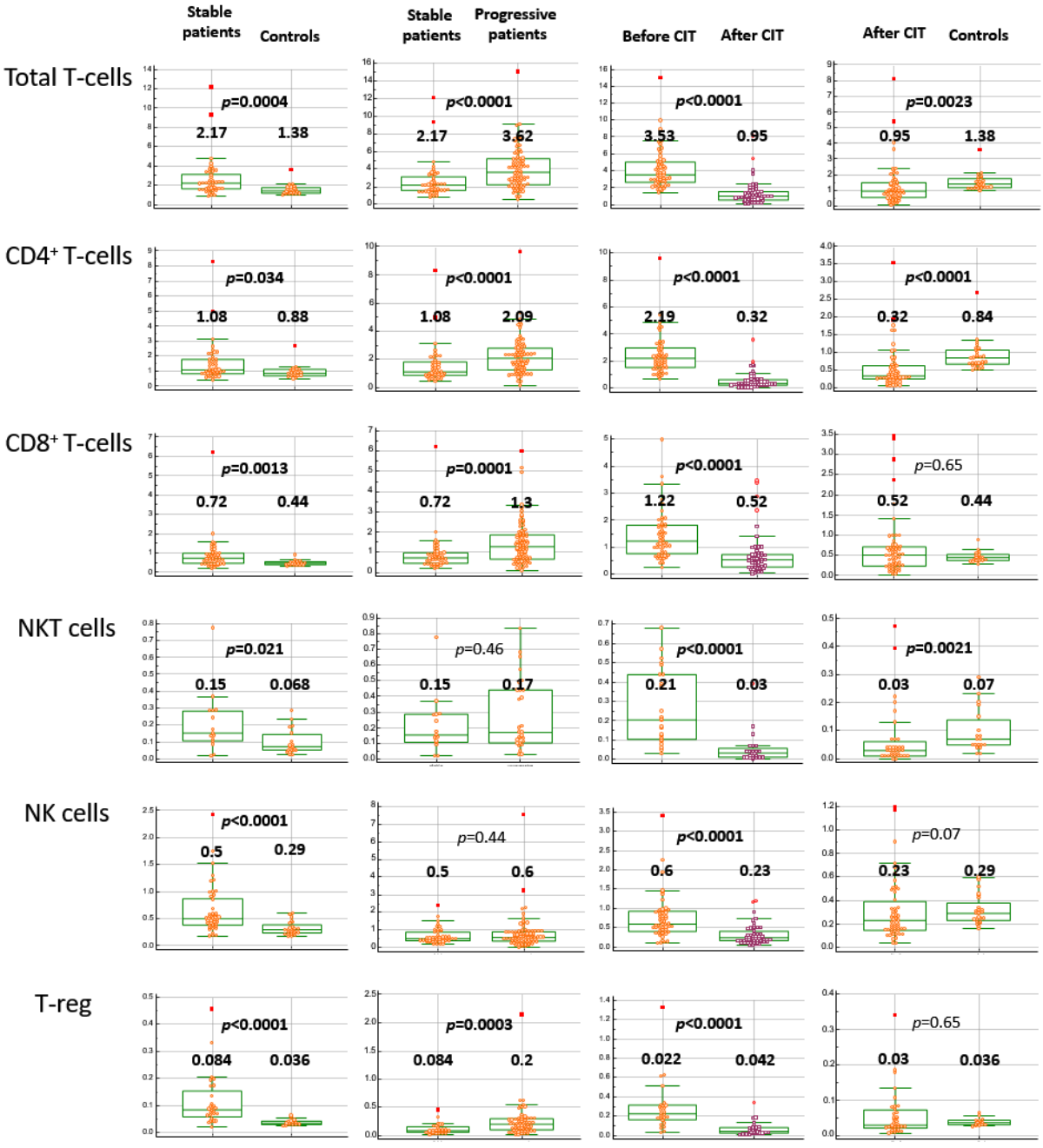
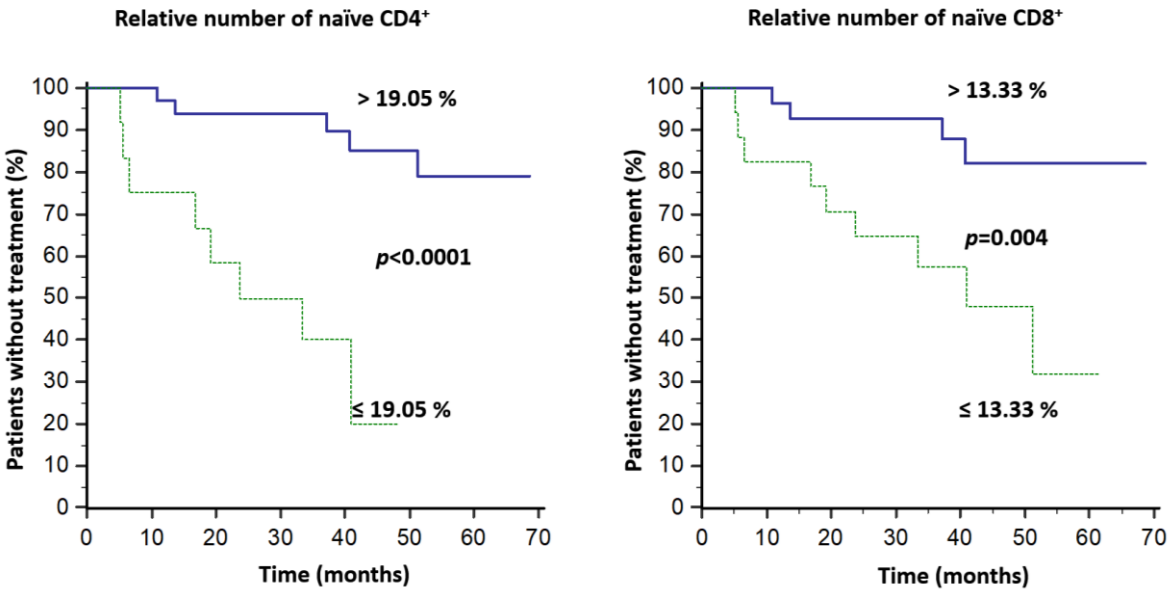


Figure 2. Differences between TTFT of patients based on the relative numbers of naïve $CD4^+$ cells (left) and naïve $CD8^+$ cells (right). ROC analysis set a cut-off level for the best separation of curves as 19.05% for naïve $CD4^+$ cells and 13.33% for naïve $CD8^+$ cells.



PHARMACOKINETICS SUPERIORITY OF HIGH DOSE CEFTAZIDIME DURING TREATMENT OF SUSPECTED OR PROVEN DIFFICULT TO TREAT PSEUDOMONAS AERUGINOSA INFECTIONS IN HEMATO - ONCOLOGICAL PATIENTS

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Objectives: To evaluate pharmacokinetics of high dose ceftazidime in the treatment of serious infection caused by XDR P. aeruginosa in the cohort of hemato - oncological patients.

Methods: Pharmacokinetic model was calculated using nonlinear mixed-effects modeling approach and Monte Carlo simulation was than used to prove pharmacokinetics superiority for high dose ceftazidime. Clinical data were collected from all patients to evaluate safety and efficacy.

Results: 14 hemato - oncological patients with serious infection suspicious of XDR P. aeruginosa etiology were treated with high dose ceftazidime and blood levels were evaluated in all of them. XDR P. aeruginosa was confirmed in 10 pts as causative pathogen. Pharmacokinetics superiority was proved with high dose regimen as compared to standard dosing. Probability of reaching 100% time above MIC 8mg/L for high dose and standard dose regimen was 95,5% and 83,9%, respectively and it was statistically significant. Differences were even bigger for higher MICs, for MIC 32mg/L, which was mostly present at our pts population it was 85,9% and 60,5%, respectively. This was translated into very low mortality rate of 20%. Toxicity of high dose regimen was not frequent and in one present case was completely reversible.

Conclusion: High dose ceftazidime is clinically effective and not excessively toxic in the treatment of XDR P. aeruginosa infections and may be used to optimize treatment of this difficult to treat infection.

IDENTIFICATION AND VALIDATION OF ENDOGENOUS CONTROL MICORNAS IN VISCERAL ADIPOSE TISSUE FROM MURINE MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction: MicroRNAs (miRNAs) belong to an evolutionarily conserved class of small noncoding RNAs (about 18-22 nt long) playing crucial role in post-transcriptional regulation of eukaryotic gene expression. Successful implementation of miRNAs detection in diagnostic and treatment-stratification strategies is influenced by many factors, including the right choice of method/analysis approach and optimal strategy for miRNA expression normalization. Proper selection of reference genes and materials is an essential procedure in the design of quantitative PCR (qPCR) experiments, especially in differential expression analyses. Here, we report the strategy to identify optimal control miRNAs for qPCR profiling of miRNA expressed from visceral adipose tissue of mice with experimentally induced non-alcoholic fatty liver disease.

Methods: Visceral adipose tissues dissected from 36 male C57BL/6J mice alternately fed by control (PicoLab RD 20, LabDiet) or Western diet (AIN-76A WD, TestDiet, glucose - 18.1 g/L, and fructose -24 g/L) provided in water over 36 weeks were homogenized and miRNAs were isolated. TaqMan Human Endogenous Control Card and Small RNA Sequencing were used for the primary expression study. Control miRNA candidates were then validated by qPCR with specific TaqMan Advanced miRNA probes.

Results: From TaqMan Card containing 32 endogenous control miRNAs, miR-30b-5p and miR-331-3p were chosen as a normalizer candidate. Although, RNA sequencing results were not in compliance to TaqMan Card data, and different three miRNAs (miR-1839-5p together with let-7d-5p and let-7a-5p) were chosen as best performed.

Discussion: None of our identified miRNAs have yet been selected as normalizer candidates. Additionally, miR-331-3p, let-7d and let-7a were previously found to be down-regulated in visceral adipose tissue of patients with different stages of fatty liver disease. The disagreement in the results may also be caused by the choice of normalizer, when nuclear RNAs such as U6 and RNU6 were widely used, but they do not belong to miRNAs and thus may not accurately represent miRNA fraction. Another reason for the discrepancy between ours and previous scientific studies may also be the difference in the algorithms of choice and the general absence of a uniform workflow.

Conclusions: Currently, there are no definitive guidelines ruling data normalization in miRNA expression analysis although it is clear that different normalization strategies give rise to very different results, with the high risk of generating confusion. Universal endogenous controls are lacking due to tissue heterogeneity; thus, normalizers must be evaluated individually for each experiment. Here, we identified our set of miRNA normalizers for next analyses.

Acknowledgments: This work was supported by the Cooperatio Program, research area DIAG and by MH CZ - DRO (UHHK, 00179906).

BRAIN AREAS PREDISPOSING TO THE STROKE-RELATED EPILEPSY DEVELOPMENT

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Introduction: Stroke-related epilepsy (STRE) represents a significant health problem (50% of newly diagnosed epilepsy in the elderly). We focused on identifying brain areas, in which involvement in ischemia predisposes a patient to STRE development.

Methods: We retrospectively identified a group of patients with STRE. The STRE group was subdivided into three subgroups based on the involved large vessel territory: middle cerebral artery (MCA), posterior cerebral artery (PCA), and anterior cerebral artery (ACA). STRE group consisted of 29 patients (median age 70 years, minimum 52, maximum 88 years). Subsequently, age- and gender-matched controls who underwent stroke but did not develop STRE at least 5 years after the stroke (Control group - CG) were identified. The CG was composed of 37 patients (median age 64 years, minimum 34, maximum 92 years). The ischemic borders were marked on CT scans in both STRE and Control groups. The total volume of ischemia was compared between STRE and CG. Their distribution was compared to reveal differences in affected areas between STRE and CG.

Results: The patients with STRE exhibited a bigger volume of ischemia in comparison to CG (average volume of ischemia in STRE 60.8 cm^3, in CG 42.4cm^3, p=0.029). When compared STRE and CG, there were differences in the distribution of ischemic changes in the temporal lobe (transverse temporal gyri, superior temporal gyrus, middle temporal gyrus), and parieto-occipital region (lingual gyrus, post-central gyrus, supramarginal gyrus, superior occipital gyrus, angular gyrus, and parietal operculum). After the correction for the multiple comparisons, only the differences in the parietal operculum were significant. There were no statistically significant differences in the level of individual large vessel territories.

Conclusions: The higher volume of ischemia correlates with a higher risk of STRE development. Some areas, in particular in the temporal and parietal neocortex, predispose the brain to generate epileptic activity. The involvement of the parietal operculum seems to play a pivotal role when analyzed on the level of all patients.

Figure 1. The differences in the distribution of ischemic changes between stroke-related epilepsy patients' group (STRE group) and the Control group.

1A. In the STRE group, the ischemic changes were more expressed in marked areas localized in (1) temporal lobe, and (2) parieto-occipital region.

1B. After the correction for multiple comparisons, the ischemic changes in the STRE subgroup were more often present only in the parietal operculum.

Left-side results were flipped to the right side, so the results are only in the right hemisphere. The differences are marked by color.

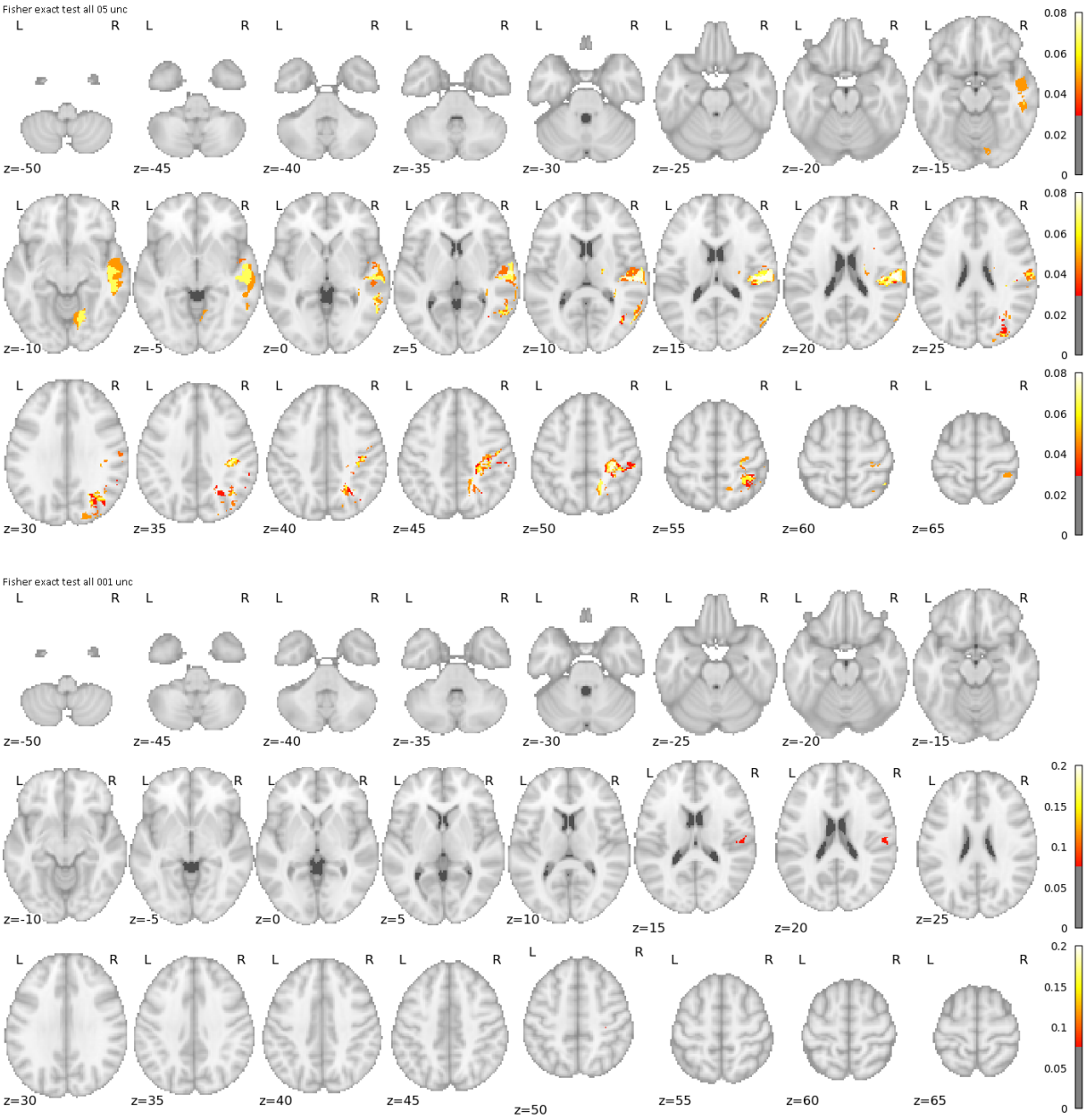
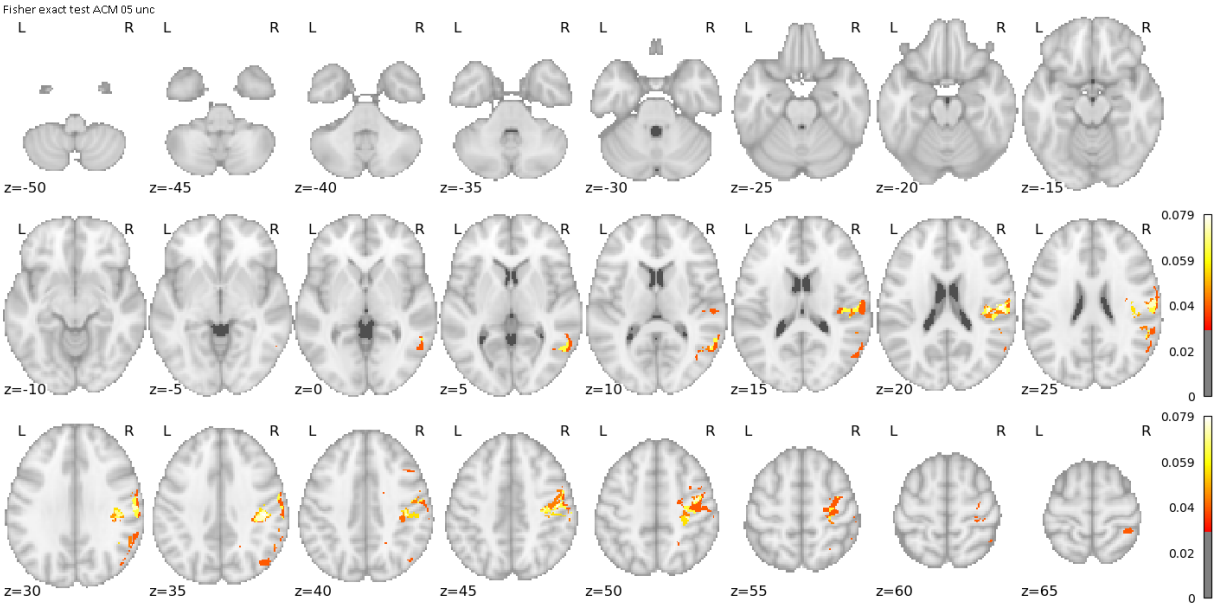


Figure 2. The differences in the distribution of ischemic changes in the territory of the middle cerebral artery between the stroke-related epilepsy patients' subgroup (MCA-STRE subgroup) and the Control subgroup (MCA-Control subgroup).

Left-side results were flipped to the right side, so the results are only in the right hemisphere.



WHOLE GENOME SEQUENCING AND ANALYSIS OF TREPONEMA PALLIDUM SUBSP. PERTENUE OF NON-HUMAN PRIMATE ORIGIN

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Introduction: *Treponema pallidum* subsp. *pertenue* (TPE) is the causative agent of human yaws, disease endemic in tropical regions of Africa, Asia and Pacific. This bacterium affects mainly children and causes papillomas, ulcers and sometimes disfiguring lesions. During the mid-20th century, there was first eradication effort that resulted in the significant drop in prevalence of the disease, but the lack of continued surveillance led to resurgence of yaws. The disease was believed to have no animal reservoirs, allowing development of currently ongoing second yaws eradication campaign led by WHO. Currently, genetic evidence started to show that TPE strains naturally infect nonhuman primates (NHPs) in Africa. The only two available whole genome sequences of strains isolated from primates are very similar to human infecting strains.

Methods: In this study, we determined genomes of TPE isolates that originated from nonhuman primates (NHPs) and were selected based on their genetic diversity. Obtained whole genome sequences were analysed and compared to eight complete TPE sequences from human yaws patients. We performed an in-depth analysis of TPE genomes to determine if any consistent genomic differences are present between TPE genomes of human and NHP origin.

Results: In this study we obtained eight new whole genome sequences. We were able to resolve previously undetermined TPE chromosomal regions (sequencing gaps). The comparison of indels, gene alleles, repetitions, homopolymers, pseudogenes and phylogeny among completely finished genome sequences revealed no consistent genomic differences between human and NHP TPE genomes.

Discussion: As there is no consistent differences between TPE genomes of human and NHP origin, NHPs infected with TPE must be considered as a natural reservoir for human yaws infection. Although interspecies transmission seems to be rare and evidence for current spillover events are missing, the existence of TPE in NHPs is clearly demonstrated. The low risk of spillover supports the current yaws treatment campaign. Our findings highlight that yaws eradication in humans must be followed by continuous disease surveillance in areas where NHP infection is known to be present.

Conclusions: Comparison with available whole genome sequences of TPE strains isolated from humans showed that there are only minimal differences between the two groups of TPE strains and

that wild populations of NHP can serve as a reservoir of this bacterium. The existing nonhuman reservoir of yaws precludes eradication of yaws, however, yaws elimination appears to be achievable.

Acknowledgements: Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA LM2018140) provided within the program Projects of Large Research, Development, and Innovations Infrastructures. The work was partly funded by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103, Funded by the European Union - Next Generation EU) to DS.

ENERGY HOMEOSTASIS IN ADOLESCENCE WITH POLYCYSTIC OVARY SYNDROME

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Introduction: The etiopathogenesis of polycystic ovary syndrome (PCOS) has not been fully elucidated. Diagnostic criteria change frequently, due to the diversity of clinical and diagnostic markers, and confirm the scarcity and immaturity of perceptions of this pathological condition.

The classic clinical-laboratory and ultrasound features of PCOS in adolescence are not considered aberrant (1), although their persistence increases not only the risk of menstrual and reproductive dysfunction, but virtually all severe, life-threatening non-infectious, age-related diseases, chance of development (2-6). Studies in adolescents to identify currently known and probable risk factors (7-8) will be crucial both in terms of their early identification as well as the prevention of later complications and the avoidance of medical and social costs.

The aim of the study was to determine the importance of energy imbalance in the pathogenesis of PCOS in adolescents.

Methods: We conducted a case-control study enrolling adolescence girls aged age 12-19 years; with at least 1 clinical-laboratory sign of PCOS. Subjects with chronic and acute somatic pathology were excluded. We performed indirect calorimetry, total testosterone tests and identified risk factors through specially designed questionnaires.

Results: Ten patients were enrolled in the study out of the target group of 25 persons complying with the inclusion criteria. Table 1 describes the sample.

Table N1 - General characteristics of the group of patients with polycystic ovary syndrome and total testosterone in the blood.

PCOS criterion	N%	Age	BMI	Testosterone
Acne	30%	17	19.3	N (2.6 pg/ml)
Hirsutism	20%	16	22	N(3.0)pg/ml
Acne + hirsutism	20%	15.5	21	
Acne + irregular menstruation, hirsutism	30%	16.3	20.6	4.5pg/ml

No person in the group has a normal Resting Metabolic Rate. 40% of them have decreased this factor, 60% have increased it.

Table N2 - studied rate of patients in low and high Resting metabolic rate groups

	N	Age	BMI	Testosterone
Low RMR	4	16	20.25	N =2.6 pg/ml
High RMR	6	16.6	20.8	3.7 pg/ml

Discussion: The preliminary results showed variability in testosterone (total) concentration in 40%. According to literature data, it is currently believed that typical hormonal changes are not always present. It is known that during polycystic ovary syndrome testosterone concentration is slightly or moderately increased. PCOS has been studied in many ways, but the assessment of energy status has never been the subject of research. In our opinion, PCOS develops as follows: a negative energy balance is formed in the body against the background of qualitatively (proteins and fats) and energetically insufficient food intake and/or excessive physical activity (more energy expenditure than intake), which is partially confirmed by our data (see Table N2). These deviations are considered diagnostic markers of PCOS.

Conclusion: In order to develop a solid scientific basis for improving clinical diagnostics and subsequent management of patients, it is necessary to:

1) Consideration of PCOS in adolescents from the standpoint of suboptimal health status (SHS).

2) Determining the importance of energy balance in the formation of PCOS in adolescents and evaluating the developed clinical and laboratory deviations should be done in the context of adaptive syndrome. However, the need for further research is also clear.

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EFFECT OF CANNABINOIDS ON PAIN IN FABRY DISEASE PATIENTS: A PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSSOVER, MULTICENTER STUDY

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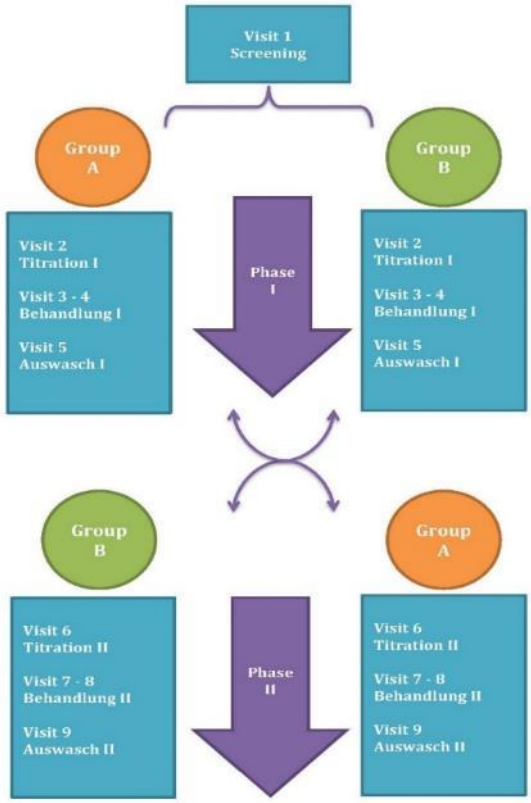
Introduction (aim of the study): Fabry Disease (FD) is a rare lysosomal storage disorder due to the absence or deficiency of hydrolase α-galactosidase A (α-Gal A) activity in lysosomes. This dysfunction results in progressive accumulation of glycosphingolipids in a wide variety of cells, resulting in major organ system damage.

Patients with Fabry disease can suffer from neuropathic pain, since lysosomal accumulation affects small unmyelinated nerve fibers. Neuropathic pain is one of the prominent and debilitating symptoms significantly interfering with life quality in FD patients. Current treatment of Fabry patients with neuropathic pain is deficient, as they respond poorly to a conventional pain therapy, often require a high-dose opioids treatment and presentation to the Emergency Department.

Sativex® has been shown to be a successful treatment option in neuropathic pain of different origin with minimal neuropsychological influence: in multiple sclerosis (MS) and chemotherapy-induced neuropathic pain. It contains Δ-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) and has recently been licensed in Switzerland for treatment of neuropathic chronic pain for MS. Sativex® is an oral spray.



Methods: 22 patients are being recruited according to specified inclusion and exclusion criteria. The clinical trial consists of two phases. Each phase includes 2 weeks titration, 4 weeks treatment, and 2 weeks washout. In each phase patients will be taking placebo or Sativex. The response to the treatment will be evaluated by:

-> Blood sample taken every two weeks to assess cannabinoid metabolites

-> Quantitative sensory testing to assess nerve conduction and neuropathic pain

-> Questionnaires and daily-recorded NRS score by patients to assess quality of life

Figure 1 represents a scheme of the study visits.

Results: The project is still ongoing so results are available as study outcomes stated below:

Primary study outcome:

Response to treatment defined as an improvement of patients' mean pain numerical rating scale (NRS) score from baseline to the last week of treatment week 4

Secondary study outcomes:

1. Change in primary afferent function assessment using the Quantitative sensory testing (QST).
2. Change in the quality of life using WHO-Quality of life-BREF (WHOQOL-BREF) and Patient Global Impression of Change Scale (PGIC).
3. Douleur Neuropathique en 4 questions (DN4-Questionnaire) and Change of the score in Short-Form McGill Pain Questionnaire (SF- MPQ)
4. Change in Profile of Mood States (POMS questionnaire)
5. Change in Insomnia severity score using Insomnia severity Index
6. Cannabinoid metabolites levels

Discussion: Not yet available (project ongoing)

Conclusions/Objective: To test the hypothesis that Sativex® reduces pain in patients with Fabry disease

Acknowledgements: The study is registered at clinicaltrials.gov, and funded by the Swiss National Science Foundation

INVASIVE AND METASTATIC MOLE TREATMENT OUTCOMES IN THE SLOVAK REPUBLIC IN 1993-2021

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Introduction: Invasive mole is a form of gestational trophoblastic neoplasia (GTN), in which a hydatiform mole invades the myometrium and/or the uterine vessels. Metastatic mole metastasizes into organs outside the pelvis. Chemotherapy is preferable for women planning pregnancy, hysterectomy in women not planning pregnancy, in chemoresistant tumors, or in cases of severe bleeding.

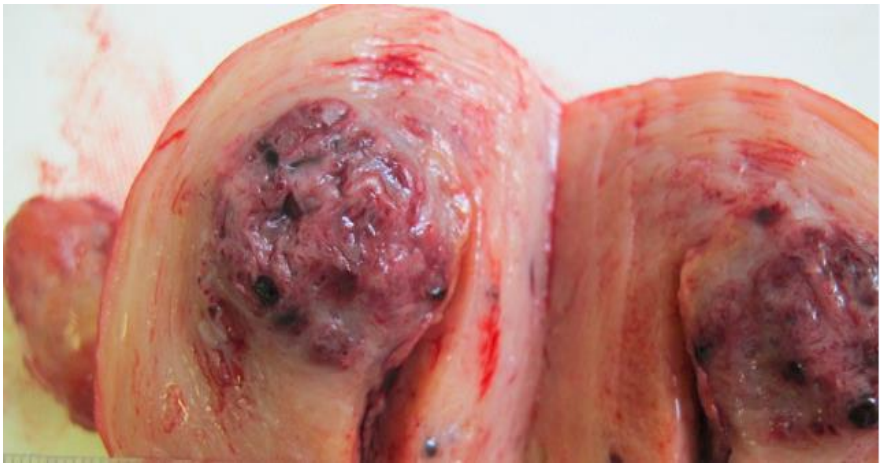

Fig. 1. Invasive mole - invasion into the uterine wall (source: Center of GTD SR)

Methods: This is a retrospective analysis of histologically confirmed 12 cases of invasive and 6 cases of metastatic mole treated in the Centre for gestational trophoblastic disease Slovak Republic (SR) in 1993-2021. Patients were divided into 2 groups according to treatment modality. MS Excel was used to analyse the data and a Pearson-Chi square test to determine significance.

Results: 18 patients were included in the analysis. The incidence of invasive and metastatic mole in SR was 1:139,814 pregnancies or 1:99,704 births. The overall curability rate was 100%, without recurrence. Primary hysterectomy without chemotherapy was performed in 8 women and 10 women were treated with chemotherapy&hysterectomy. The age average was 41 (18-53) years. 6 women had metastatic mole, all had lung metastases and 1 of them had vaginal metastasis too. 2 patients were cured by hysterectomy with spontaneous regression of metastases. The average interval to remission was 8.5 (4-19) weeks in the hysterectomy group and 12.75 (7-23) weeks in the combined therapy group. There was no statistically significant difference between the two modality treatment groups and between invasive and metastatic moles in all parameters.

Table 1. Selected parameters of invasive and metastatic mole in SR in 1993-2021

Parameter		**Treatment modality**					
		Hysterectomy		Chemotherapy & Hysterectomy		All	
		N=8	%	N=10	%	N=18	%
Antecendent pregnancy	Complete hydatidiform mole	6	75	6	60	12	66.7
	Partial hydatidiform mole	1	12.5	1	10	2	11.1
	Spontaneus misscariage	1	12.5	2	20	3	16.7
	Legally induced abortion	0	0	1	10	1	5.6
Age (years)	15-19	0	0	1	10	1	5.6
	20-29	2	25	0	0	2	11.1
	30-39	1	12.5	3	30	4	22.2
	40-49	4	50	3	30	7	38.9
	>50	1	12.5	3	30	4	22.2
hCG levels (IU/l)	$< 10^3$	2	25	3	30	5	27.8
	10^3 - 10^4	3	37.5	2	20	5	27.8
	10^4 - 10^5	2	25	4	40	6	33.3
	$> 10^5$	1	12.5	1	10	2	11.1
WHO score	Low risk	8	100	9	90	17	94.4
	High risk	0	0	1	10	1	5.6
FIGO stage	I	6	75	5	50	11	61.1
	II	0	0	1	10	1	5. 6
	III.	2	25	4	40	6	33.3
	IV.	0	0	0	0	0	0

hCG- human chorionic gonadotropin, IU – international units, WHO - World Health Organisation, FIGO – International Federation of Gynaecology & Obstetrics

Discussion: The precise incidence of invasive mole in the world is unknown, because they are mostly diagnosed as GTN. The incidence in SR presented are only histologically confirmed invasive and metastatic moles.

The reported cure rates for low-risk and high-risk GTN are 100% and 95% respectively. There was a 100% cure rate in SR.

Older age is one of the risk factors for molar pregnancy. In our study 61.1% women were over 40 years old.

Conclusion: This paper presents epidemiology and curability data of invasive and metastatic mole in SR.

Acknowledgement: I would like to thank my supervisor for his guidance in writing this article.

STUDY OF THE SECRETION OF OUTER MEMBRANE VESICLES IN THE BACTERIUM F. TULARENSIS, THEIR ROLE IN HOST-PATHOGEN INTERACTION AND THEIR PROTECTIVE POTENTIAL

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Introduction: The genus Francisella are encapsulated immobile gram-negative coccobacilli that are also intracellular pathogens of humans and many animals. Francisella is able to enter, survive and proliferate in various types of host cells, including macrophages, dendritic and polymorphonuclear cells.

Bacterial OMVs are spherical particles with a size of 20-200 nm produced by all gram-negative bacteria and are considered to be one of the major secretory pathways. In Francisella they contain many virulence factors and immunomodulatory proteins that are involved in the management of environmental stress and that they are involved in the interaction with the host cell. They also have great potential in terms of studying their protective functions.

Methods: We studied the response of the host cells (murine bone marrow macrophages) to the treatment by OMVs isolated from Francisella. We focused on the cytokine release and the ability to suppress the subsequent Francisella infection. Then we tested the protective effect of OMVs in vivo on mice and finally we characterized the vesiculation in a group of Francisella mutants with disrupted lipopolysaccharide.

Results: OMVs induced massive production of proinflammatory cytokines in BMDM, while the bacterium alone elicited no proinflammatory response. The strong proinflammatory activation of macrophages by OMVs was neither suppressed by their pretreatment by the bacterium, nor was completely reverted by the bacterium added post-OMVs. OMVs treatment also suppressed the ability of the bacterium to proliferate inside BMDM. In the in vivo experiments, the OMVs extended the life of infected mice by only a few days despite a significant increase in pro-inflammatory cytokine response in the vaccinated mice. In the LPS mutants of Francisella we discovered some hypervesiculating strains and it seems that the intact LPS is important for the stability and shape of the OMVs.

Discussion: OMVs in Francisella are expected to play some role in the host-pathogen interaction and they are also interesting from the point of view of the potential subunit vaccine development. Our results suggest that while the bacterium has some effective mechanisms to suppress the natural response of the host cell leading to inflammation and killing of the bacterium, the isolated OMVs show exactly the opposite reaction. Vaccination results are currently not providing satisfactory results regarding the protection against bacterial infection. Nevertheless, we aim to further study the protective potential of the discovered hypervesiculating mutants.

Conclusions: OMVs in Francisella represent an interesting material to be studied as subunit vaccine. As they emerge from the bacterial surface they contain a complex mixture of the bacterial antigens mimicking very effectively the whole bacterium while having all the advantages of non-viable vaccine. For future studies we would like to focus on the modification the immunization scheme to increase the protection.

SKIN AGING

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Introduction: Skin aging is a complex physiological process caused by internal and external factors. Chronological aging is associated with the accumulation of modified biomolecules by non-enzymatic glycation and the formation of advanced glycation end products (AGEs), that cause tissue damage. This is accompanied by activation of matrix metalloproteinases (MMPs) that degrade extracellular matrix components such as collagen, elastin and hyaluronic acid. One of the ways to prevent skin aging is the application of natural substances that have an antiglycation effect and suppress MMPs released by senescent fibroblasts.

Methods: The cytotoxicity of test substances was evaluated using the MTT assay on normal human dermal fibroblast (NHDF). The antisenescent activity of the test compounds was evaluated using young and physiologically old NHDF (cultivated in low or high glucose level) by measuring MMPs level by ELISA. The modulation of the activity of elastase, hyaluronidase and collagenase by tested substances was assessed spectrophotometrically.

Results: Rutinose and rhamnose inhibited the activity of isolated elastase, hyaluronidase, and collagenase. Hesperidin and hesperetin inhibited only elastase and hyaluronidase activity. Subtoxic concentrations of hesperidin, hesperetin (1-25 μM), rhamnose and rutinose (1-25 mM) were tested in skin aging models. Levels of MMP-1 and MMP-2 were reduced after the application of all tested substances.

Discussion: Rutinose was found to be the most effective inhibitor of elastase, hyaluronidase and collagenase activity of all tested substances. In case of rhamnose, the highest effect was found in the inhibition of hyaluronidase and about half the inhibition of collagenase and elastase compared to rutinose. As for hesperidin and hesperetin, they showed the greatest effect in inhibiting hyaluronidase. The levels of MMP-1 and MMP-2 were reduced after the application of the tested substances both in young NHDF and in physiologically old NHDF.

Conclusions: In our study, the anti-aging potential of hesperidin, hesperetin, rutinose and rhamnose was demonstrated.

Acknowledgements: This work was supported by grant: Skin anti-aging study of hesperidin, hesperetin, rutinose, and rhamnose: Comparative study (DSGC-2021-0065), a part of the OP VVV Project No. CZ.02.2.69/0.0/0.0/19_073/0016713, Improving Schematics of Doctoral Student Grant Competition and their Pilot Implementation. Project MEYS LTC20069 is acknowledged as well.

EFFECT OF CHANGING THE DRUG ON THE LOWER URINARY TRACT DYNAMICS AND QUALITY OF LIFE IN PATIENTS WITH OVERACTIVE BLADDER

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Introduction: To evaluate the effect of Solifenacine on quality of life of patients previously treated with antimuscarinics – measured by Perception of Treatment Satisfaction (TS-VAS)

To evaluate the effect of Solifenacine on quality of life of patients previously treated with antimuscarinics – Over Active Bladder-q Short Form (OAB-q SF) + EuroQol Group scoring system (EQ-5D-5L)

Methods and patients: This was a multi-centre, prospective non - interventional study of patients who were treated with Solifenacine as part of standard care. Enrolment into this study closed after 2000 patients have successfully completed Vesicare treatment. The decision to treat a patient with Solifenacine was made by the treating physician prior to enrolment into this NIS. The patient´s eligibility for treatment with Solifenacine was not affected by their decision to participate in the study. All patients were treated according to standard of care.

The duration of participation for each patient was estimated at approximately 12 months following commencement of Solifenacine. Any changes to concomitant medication or incidences of adverse drug reactions were recorded and the patient diaries, OAB-q SF, TS-VAS and EQ-5D-5L were collected. As this is a NIS, normal clinical care and follow up of the patient was followed. Repeated measures ANOVA were performed.

Results: A representative sample of the OAB population was evaluated in the study (2000 patients included in 60 centres in the Czech Republic, 1481 patients completed all 5 evaluations and 1,892 patients had baseline and at least 1 post-treatment). Improvements in QoL and OAB symptoms were observed over a period of 12 months in population of patients treated with Solifenacine 5-10 mg and previously treated with other antimuscarinic agents. OAB-q SF scores, including Total HRQoL Score, all HRQoL sub-scores and Symptom Severity Score, all improved over the study period.

Discussion: Changes in TS-VAS and ES-VAS scores also demonstrated improvements in overall QoL over the study period. The EQ-5D-5L showed shifts towards improved QoL for all dimensions. Data from 3-day bladder diaries demonstrated improvements in OAB symptoms over the course of the study, including frequency, urgency, nocturia and incontinence. Solifenacin was well tolerated in the current study with only 15 non-serious, mild adverse events reported, mainly dry mouth (n=12). Solifenacin did not have any notable effect on postvoid residuum suggestive of urinary retention or any effect on urinary flow rate.

Conclusion: The study shows clear improvements in all parameters evaluated. According to the results, solifenacin is a suitable drug for patients who fail to treat other antimuscarinics. There is no indication that overall OAB is affected by previous treatment.

TRANSFORMING GROWTH FACTOR SERUM CONCENTRATIONS IN PATIENTS WITH PROVEN NON-SYNDROMIC AORTOPATHY

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Introduction (aim of the study): The mechanism underlying aortic dilatation is still unknown. Vascular dilatation is thought to be the result of progressive aortic media degeneration caused by defective vascular matrix haemostasis, including TGF-b1 dysregulation. The goal of this study is to draw attention to the potential utility of TGF-b1 as a diagnostic marker in non-syndromic patients with aortic dilatation.

Methods: TGF-b1 levels in plasma were measured in 50 patients who had undergone surgery and had a tricuspid or bicuspid aortic valve as well as a normal or dilated ascending aorta. A pathologist also examined thirty resected aorta samples. To specify the reference range of TGF-b1, a control group of 40 volunteers was enrolled in this study.

Results: We discovered a significant difference in TGF-b1 levels between patients with aortic dilatation and the control group (32.5 vs. 63.92; $P < 0.001$), as well as between patients with non-dilated aorta but with aortic valve disease, and the control group (27.68 vs. 63.92; $P < 0.001$). There was no difference between the dilated ascending aorta group and the nondilated ascending aorta group. We found a poor correlation between TGF-b1 levels and ascending aorta diameter as well as the grade of ascending aorta histopathological abnormalities.

Discussion: Our findings as well as the literature suggest that TGF-b1 concentrations are significantly changed not only in patients with syndromic thoracic aortic aneurysm but also in non-syndromic aortic dilatation, as well as in patients with aortic valve pathology. TGF-b1 could not be used as a direct diagnostic marker of isolated aortic wall dilatation, at least in this series. TGF-b1 plasma levels did not correlate with the severity of TAA dimension or histopathological wall changes.

Conclusions: TGF-b1 concentration does not meet the criteria to be a specific marker of aortic dilatation, but it is sensitive to aortic valvulopathy - aortopathy. A larger patient cohort study is needed to confirm these findings.

Acknowledgements: Thanks to Dr Eva Cermakova for her assistance in the statistical analysis.

INTRAVENOUS THROMBOLYSIS IN POSTERIOR VERSUS ANTERIOR CIRCULATION STROKE: CLINICAL OUTCOME DIFFERS ONLY IN PATIENTS WITH LARGE VESSEL OCCLUSION

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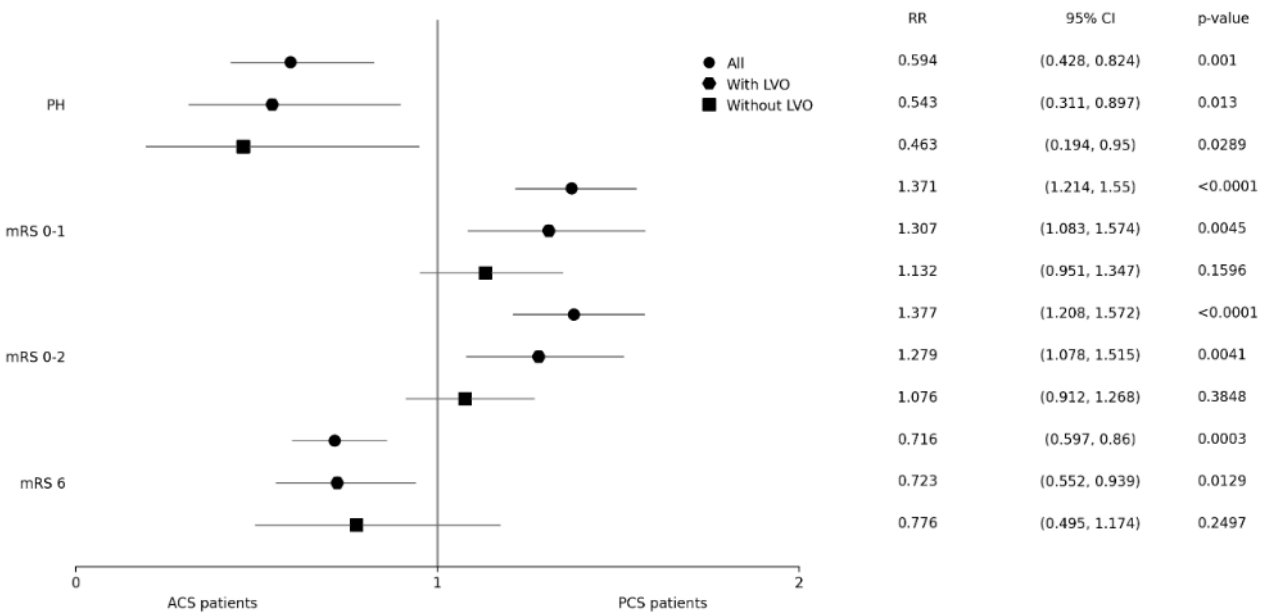
Introduction: The safety and efficacy of intravenous thrombolysis (IVT) are well established in the anterior (ACS) but are much less clear for posterior circulation stroke (PCS). The present study aimed to assess differences between PCS and ACS patients after IVT overall, as well as in the particular subgroups with and without large vessel occlusion (LVO), in terms of the occurrence of intracerebral parenchymal hematoma (PH) and 3-month clinical outcome.

Methods: In an observational, cohort multicenter study, we analyzed data of ischemic stroke patients treated with IVT, prospectively collected in the SITS registry in the Czech Republic between 2004–2018.

Results: Out of 10211 patients, 1166 (11.4%) had PCS and 9045 (88.6%) ACS. PH was less frequent in PCS versus ACS patients: 3.6 vs. 5.9%, odds ratio (OR)=0.594 in the whole set, 4.4 vs. 7.8%, OR=0.543 with LVO and 2.2 vs. 4.7%, OR=0.463 without LVO, resp. At 3 months, PCS versus ACS patients achieved more frequently excellent clinical outcome (modified Rankin scale [mRS] 0–1: 55.5 vs. 47.6%, OR=1.371 in the whole set and 49.2 vs. 37.6%, OR=1.307 with LVO, resp.), good clinical outcome (mRS 0–2: 69.9 vs. 62.8%, OR=1.377 in the whole set and 64.5 vs. 50.5%, OR=1.279 with LVO, resp.) and had lower mortality (12.4 vs. 16.6%, OR=0.716 in the whole set and 18.4 vs. 25.5%, OR=0.723 with LVO, resp.) (p<0.05 in all cases) (*Fig. 1 and Tab. 1*).

Conclusion: Data on thrombolysed patients collected in the SITS registry showed, that PCS was associated with a significantly lower risk of PH occurrence and, in patients with LVO, also with higher rates of excellent and good 3-month clinical outcome and decreased mortality at 3 months as compared with ACS.

Figure 1. Primary and secondary outcomes



ACS anterior circulation stroke, CI confidence interval, LVO large vessel occlusion, mRS modified Rankin scale, OR odds ratio, PCS posterior circulation stroke, PH parenchymal hematom

Table 1. Primary and secondary outcomes

Observed parameter n (%)	n-used	PCS group all 1166 (11.4)	ACS group all 9045 (88.6)	p	n-used	PCS group with LVO 423 (36.3)	ACS group with LVO 2451 (27.1)	p <0.0001	PCS group without LVO 377 (32.3)	ACS group without LVO 2913 (32.2)	p 0.9498
PH	9695	40 (3.6)	505 (5.9)	0.001	5854	18 (4.4)	178 (7.8)	0.013	8 (2.2)	131 (4.7)	0.0289
mRS 0–1	10211	647 (55.5)	4307 (47.6)	<0.0001	6164	208 (49.2)	922 (37.6)	0.0045	251 (66.6)	1713 (58.8)	0.1596
mRS 0–2	10211	815 (69.9)	5676 (62.8)	<0.0001	6164	273 (64.5)	1237 (50.5)	0.0041	299 (79.3)	2148 (73.7)	0.3848
mRS 6	10211	145 (12.4)	1497 (16.6)	0.0003	6164	78 (18.4)	625 (25.5)	0.0129	27 (7.2)	269 (9.2)	0.2497

ACS anterior circulation stroke, LVO large vessel occlusion, mRS modified Rankin scale, n number, PCS posterior circulation stroke, PH parenchymal hematoma

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