Abstracts from 20th International Medical Doctoral Conference (2023) – New Horizons for PhD Students in Medical Research

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Al Akkad Marwan: Are Restorations Manufactured by Cad/Cam Ready to Fixation without Adjustments?

Alferi Dino: Solution to Protect Esophageal NiTi Stent from the Corrosive Environment of the Human Body and New Approaches in Their Testing

Andreew Martyna: The Relationship between Nutritional Status and the Occurrence of Frailty and Quality of Life in Patients with Chronic Kidney Disease Undergoing Hemodialysis – A Pilot Study

Banni Aml Mustafa: Aspirin Therapy Increases Likelihood of Operability and Reduces Metastatic Risk in Pancreatic Adenocarcinoma

Barry Antonia: Hull's Magic Box: Arginine Methylation Inhibitors as a Potential Novel Therapy for GBM

Chebirnina Inna: Changes in Mucin Secretion in the Rats' Stomach with Type 2 Diabetes and under Correction

Dvořáková Kateřina: The Effect of the Combination of Temozolomide and Flubendazole on Glioblastoma Cells

Giuashvili Tinatin: Cerebral Palsy Risk Factors, Comorbid Conditions and Types in Georgian Infants

Gregušová Alexandra: Gene Expression Profiling of Primary Low- and High-Grade Bladder Cancer in Relation to Circulating Tumor Cell Dissemination

Groborz Ondřej: Pharmacokinetics of Intramuscularly Administered Thermoresponsive Polymers

Hříbek Petr: Spectroscopy of Blood Plasma as a Tool for Early Diagnostics of Hepatocellular Carcinoma

Imani Atefe: In Vitro Studies Using Collagen Membranes: A Systematic Review

Jaźwiec Anna: Miraculous Fat – How Lipotransfer Can Alter the Periprosthetic Tissue. In Vivo Animal Model

Jenča Dominik: Remote Heart Failure Symptom Assessment after Myocardial Infarction Identifies Patients at Risk of Death

Jirásko Michal: Glycoprofiling of Proteins Applicable as Biomarkers of Prostate Cancer

Knížek Zdeněk: Outcome of Continuous Positive Airway Pressure Adherence Based on Nasal Endoscopy and Measurement of Nasal Patency

Kovářová Petra: Prediction of Treatment Response Using PET/MRI Examination Performed During Treatment in Patients with Locally Advanced Cervical Cancer Treated with Concurrent Chemoradiotherapy

Krtičková Jana: Validation of the Czech Version of the Voice Handicap Index

Lásková Pavlína: Validation of Francisella Tularensis Peptides as Immunogenic T-Cell Epitopes

Leško Peter: Plasma Vitamin D Level in Germ Cell Tumour Patients Is Associated with Survival Outcome and Disease Characteristics

Leugner Ella: Transcription-Factor Ap-1 in Colorectal Cancer

Matyáš David: Computer Analysis of Gait Disorders

Mokrejšová Magdaléna: Sequenation of DNA Methylation of HMGCR Gene Could Be Predictor of Morbidity and Mortality in Patients on Hemodialysis

Mortada Mohamad Mahdi: Assessment of Allergic Reaction and Specific Immunoglobulin E Level in Nasal Secretion of Local Allergic Rhinitis Patients

Navrátil Pavel: Syndecan-1 Levels in Organ Donors and Kidney Transplant Recipients

Pakizer David: Diagnostic Accuracy of Carotid Plaque Instability by Noninvasive Imaging: A Systematic Review and Meta-analysis

Péč Jozef: Rotational Thromboelastometry and Psoriasis

ARE RESTORATIONS MANUFACTURED BY CAD/CAM READY TO FIXATION WITHOUT ADJUSTMENTS?

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Introduction (aim of the study): The master cast is the gold standard for the control and eventual adjustment of restorations produced by conventional procedures. Some digital workflow bypasses the master cast and relies completely on the precision of the CAD/CAM restoration.

The study aims to examine the reproducibility of the margins of CAD/CAM restorations generated from a single digital scan. Also, to check the readiness of these restorations for delivery directly after fabrication without adjustment on a master cast and thereby eliminate the need for the master cast.

Methods: A total of 18 metal substructures made from cobalt chrome alloy were fabricated utilizing a single STL file. The circumference was divided into eight zones. The vertical marginal discrepancy (VMD) was measured at each zone of each metal substructure, with optical microscopy at ×200 magnification.

Results: Measurements of vertical marginal discrepancy were in a range of (−94: 300) with a mean of 62 ± 60 μm. A one-way ANOVA test revealed that the mean VMD is significantly different among the 18 substructures ($F_{17, 1,134} = 63.948$, $p < 0.001$).

Discussion: Although all the received substructures were fabricated from the same scan file, they were not identical and varied widely, and they were going outside the acceptable range in some zones.

Conclusions: Within the limitations of this study, the marginal fit can be improved by extraoral adjustments on the master cast. Thus, skipping the master cast deprives the dentist of delivering a restoration of higher quality.

SOLUTION TO PROTECT ESOPHAGEAL NITI STENT FROM THE CORROSIVE ENVIRONMENT OF THE HUMAN BODY AND NEW APPROACHES IN THEIR TESTING

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Introduction: Due to its properties such as mechanical properties, biocompatibility or corrosion resistance, NiTi alloy is used in a wide range of medical fields from orthodontic wires to spinal clips and stents. A typical use of NiTi stents is to mitigate the impact of esophageal cancer, where the stent causes patency of the esophagus. Corrosion resistance is crucial to ensuring the safety and efficacy of the stent. The key to obtaining relevant information on corrosion resistance is to establish an electrolyte that better reflects the actual environment of esophagus. There is currently no standard that defines the environment for measuring the corrosion resistance of esophageal stents.

Methods:

1. Comparison of different environment. NiTi esophageal stents from 4 anonymized manufacturers (A-D) using 3 types of wires from different suppliers (1-3) were used for the measurements. The samples were exposed in an environment SGF and PBS. Cyclic polarization according to ASTM F2129-19a standard.

2. Influence of input material and production processes on corrosion resistance. The 6-week immersion of stents in SGF was performed. With a subsequent assessment of the surface condition by X-Ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). The amount of ions released from the stents was monitored by atomic absorption spectroscopy (AAS).

Results:

Ad 1

- Breakdown potentials (E_b) decrease in SGF and at the same time to an increase in the corrosion current density (j_{corr}).
- The integrity of the stents was compromised in the SGF.

Ad 2

- Corrosion initiation occurs on the free surface of the stents and not in the wire crossing
- There was a decrease in nickel concentration from the surface of the stents.
- Stents with a higher concentration of nickel on the surface before exposure release more nickel ions.

Discussion: Based on the comparison of cyclic polarizations, the environment determined by the above-mentioned standard can give distorted results about the corrosion resistance of stents in the esophagus (higher Eb values and lower jcorr values in PBS).

The 6-week exposure of the stents shows that the production process significantly affects the corrosion resistance. An inappropriate combination of input material and production process, a significant reduction in corrosion resistance can occur.

Conclusion: From current research, a corrosion testing environment has been established that provides relevant information on corrosion resistance.

From the 6-week immersion, it was found that a poorly chosen combination of wire and production process can rapidly deteriorate corrosion resistance, but also that there is a production process in which the quality of the input material has a negligible effect on the final corrosion resistance of the stent.

THE RELATIONSHIP BETWEEN NUTRITIONAL STATUS AND THE OCCURRENCE OF FRAILTY AND QUALITY OF LIFE IN PATIENTS WITH CHRONIC KIDNEY DISEASE UNDERGOING HEMODIALYSIS – A PILOT STUDY

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Introduction: Chronic Kidney Disease (CKD) is often in Poland disease syndrome classified as a chronic non-communicable disease. Hemodialysis is one of the methods of renal replacement therapy. People with CKD often have abnormal body weight, protein-calorie malnutrition, muscular sarcopenia, and frailty syndrome. The specificity of hemodialysis treatment requires regular visits to the dialysis center, which worsens the quality of life of patients. The main purpose of the study was to define the relationship between nutritional status and the risk of frailty syndrome and quality of life of patients with CKD undergoing hemodialysis (HD) treatment.

Methods: The study was conducted at a dialysis station at a hospital in Katowice with the participation of chronic hemodialysis patients and consisted of a total of 34 patients aged 30 to 90. Men constituted the majority (N = 22) in comparison with women (N = 12). Body Mass Index (BMI), muscle strength, arm circumference, lean body mass after dialysis, adipose tissue mass after dialysis, % of adipose tissue after dialysis and phase angle after dialysis, were measured. Standardized questionnaires KDQOL 1.3, Tilburg, were used. An additional research tool was a self-constructed questionnaire containing questions on age, education, place of residence, vascular access.

Results: The study involved 34 patients aged 30 to 90 (M = 63.06; SD = 14.70), treated with HD. As strength increases muscle quality of the examined persons, the quality of life index in the physical area increased moderately functioning and symptom and problem list ($p < 0.05$). Positive associations of moderate strength occurred also between lean body mass after dialysis and physical functioning, sf 12 mental composite, symptom and problem list, cognitive function, energy/fatigue and role physical ($p < 0.05$).

Discussion: Other authors also cite the relationship between muscle strength and the quality of life and patient satisfaction (Hoshino, 2021). Malnutrition, in turn, is perceived as the most significant determinant of the quality of life domains of the KDQOL-SF questionnaire (Visiedo et al., 2022). Saitoh et al. (2020) suggested that a lower phase angle value was associated with a higher risk of protein-calorie malnutrition and frailty in HD patients.

Conclusions: The strongest relationship between the nutritional status assessment parameters used in the study and the quality of life domains was obtained between: muscle strength and the quality of the quality of life index in the physical area and functioning and symptom and problem list, between lean body mass after dialysis and physical functioning, sf 12 mental composite, symptom and problem list, cognitive function, energy/fatigue and role physical.

ASPIRIN THERAPY INCREASES LIKELIHOOD OF OPERABILITY AND REDUCES METASTATIC RISK IN PANCREATIC ADENOCARCINOMA

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Introduction: Blood platelets (PLT) are known to support tumorigenesis, angiogenesis and metastasis. Acetylsalicylic acid (ASA) is a known PLT inhibitor and has been shown to reduce cancer incidence, metastatic rates and improve

survival in some cancer types. This study aims to assess the effect of ASA on the clinical outcome in pancreatic ductal adenocarcinoma (PDAC) and determine whether use of ASA may offer survival benefits or therapeutic advantages.

Methods and patients: Retrospective analysis of data collected from a cohort of 182 patients with PDAC in a 6-year period was performed. The effects of ASA use for two or more years on operability, TNM stage, overall survival, disease-free survival and time to progression was assessed. Furthermore, the effect of ASA on metastasis in subgroups of patients was evaluated.

Results: According to our data, in the group of patients without ASA therapy (non-ASA), 75% presented with inoperable tumours and 57% had metastasis upon diagnosis. We observed a significant difference in the ASA group, where 46% patients presented with inoperable tumours and 21% had metastases upon diagnosis. Among the patients with tumours localized in the head of the pancreas, 49% in comparison to 21% had metastases when diagnosed, in non-ASA and ASA group respectively. Similarly, in the subgroup of patients with tumours localized outside the head of the pancreas, 74% in comparison to 33% of patients presented with metastasis at diagnosis in non-ASA and ASA group, respectively. These findings were shown to be statistically significant using logistic regression and age was found to have no correlation with operability or risk of metastasis. We observed no significant difference in either T or N stage between both groups, as well as no significant effect on over-all survival, disease free survival or time to progression using log-rank test.

Discussion: In this study, we demonstrate that ASA use is associated with a higher probability of operability. The reason for this finding could be reduction of metastatic spread through the hematogenous route rather than the lymphatic route for tumours involving the head of the pancreas, or outside of the head (body, tail and uncinate process). Although ASA treatment did not influence survival endpoints, it may offer clinical and therapeutic advantages and allow a higher probability for presenting in curable or treatable stages of PDAC. Limitation of this study, however, is its single-centred character and relatively small sample size.

Conclusion: Our retrospective analysis shows that patients treated by ASA for two or more years were nearly twice more likely to present in operable stages upon diagnosis of PDAC and over two times less likely to present with metastasis.

HULL'S MAGIC BOX: ARGININE METHYLATION INHIBITORS AS A POTENTIAL NOVEL THERAPY FOR GBM

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Introduction: GBM tumours are diffuse, necrotic and the most common and aggressive form of glioma in the central nervous system (Louis et al. 2021). Patients undergo the Stupp regimen of surgical resection, ionizing radiation and chemotherapy using temozolomide (TMZ) (Stupp et al. 2009). This has remained unchanged for 15 years and median life expectancy for these patients is around 14 months from diagnosis (Zhu et al. 2017), highlighting the urgent requirement for novel therapeutics. Research into the effect of arginine methylation in GBM has provided new therapeutic targets (Samuel et al. 2021) and protein arginine methyltransferase (PRMT) inhibitors, such as GSK3368715 have recently entered clinical trials for a range of other cancers (Fedoriw et al. 2019) and could be promising new therapies for GBM.

Methods: GBM biopsies from Hull Royal Infirmary, or healthy mouse brain tissues, were maintained in a novel fluidics system, pioneered in Hull, for 8-days and perfused with GSK3368715-treated media, at 3 µl/min, mimicking the *in vivo* environment and synergising with personalised patient care and precision medicine.

Results and Discussion: GSK3368715 causes apoptosis of GBM tissue *ex vivo* but not of healthy tissue. Immunohistochemistry using apoptotic inhibitor cleaved-PARP indicated a 2.17 ± 1.1-fold increase in apoptosis in GSK3368715-treated GBM samples, showing that PRMT inhibition causes cell death in GBM *ex vivo.*

RNA-sequencing determined thousands of differentially expressed genes in GBM tissues resulting from GSK3368715 treatment, showing highly significant GO-term-enrichment in ribosome and translation pathways, suggesting decreased protein synthesis capacity after GSK3368715 treatment. My data also indicated a reduction in variation of differentially expressed genes upon PRMT inhibition, from more aggressive to less aggressive phenotypes in principal component analysis. Additionally, several hundreds of genes were found to be undergoing alternative splicing (AS), compatible with a mechanism where changes in the arginine methylation pattern of spliceosome member fused in sarcoma (FUS), upon GSK3368715 treatment, contribute to AS. GO-term-enrichment of these AS events was found in DNA damage and cell death, giving credence to my results which highlight GSK3368715 as a cause of apoptosis in GBM maintained *ex vivo.*

Conclusion: My results highlight an exciting new potential therapeutic target for GBM, *via* induction of alternative splicing pathways, through arginine methylation inhibition, which may underly the initiation of apoptotic pathways and tumour cell death of a disease with clear clinical needs.

CHANGES IN MUCIN SECRETION IN THE RATS' STOMACH WITH TYPE 2 DIABETES AND UNDER CORRECTION

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Introduction: Type 2 diabetes mellitus (T2DM) is one of the most common diseases in the world. The disease affects all organ systems and, in particular, gastrointestinal. There are some studies, that display such changes in morphology and the functional activity of the gastrointestinal tract. For instance, atrophy of the gastric mucosa, antibodies to parietal cells, a decrease in tone and motility, abnormalities in the vessels of the villi, and microbiome misbalance. The influence of T2DM on the gastrointestinal tract is generally studied, although, the changes in the functional activity of stomach granulocytes are insufficiently studied. Therefore, the aim of the study is to observe the changes in the mucin secretion of the gastric mucosa of rats against the background of the course of diabetes mellitus 2 and its correction by the combination of metformin with propionic acid.

Methods: T2DM was simulated by streptozocin injections and food challenge in 24 white laboratory rats, which were divided into 4 groups: the 1st group – T2DM without treatment, the 2nd group – T2DM+metformin, the 3rd group – T2DM + propionate, and the 4th group – T2DM and metformin + propionate. The group received drugs for 14 days. For histological examination, the stomach was taken for histochemical examination. Statistical data processing was done using the StatPlus program ver. 7.3.0. The difference between groups was considered significant at $p \leq 0.05$.

Results: The gastric glands of rats from the control group had the localization of mucin both on the surface and in the cells. There are mucopolysaccharides in all parts of the fundal glands. The 1st and the 2nd groups had similar changes, which included the reduction of mucin in the cells by 2.6 times compared to the control, and mucopolysaccharides were not found in the deep parts of the glands. In the 3rd group against the background of treatment by propionate are increase in mucin synthesis by 25% ($p \leq 0.05$) was observed in comparison with the indicators of the 1st, 2nd, and 4th groups. However, the amount of mucus-producing cells also increased in the 4th group that used the combination of metformin and propionic acid. It is believed that a sharp decrease in the amount of mucin occurs as a result of inhibition of the activity of synthetic processes and, in particular, glycoproteins, against the background of type 2 diabetes.

Conclusions: A significant decrease in mucin production in the base of the gastric glands of rats by 2.6 times was established, which is associated with a decrease in the number of cells producing mucopolysaccharides. Metformin did not affect the increase in the production of mucin in the stomach in patients with type 2 diabetes, but the use of propionate both alone and in combination with metformin increased the production of mucopolysaccharides.

THE EFFECT OF THE COMBINATION OF TEMOZOLOMIDE AND FLUBENDAZOLE ON GLIOBLASTOMA CELLS

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Introduction: Glioblastoma multiforme (GBM) is the most common and malignant primary brain tumor. Infiltrative growth, many different genetic and epigenetic variants as well as low plasma concentration of the alkylating drug temozolomide (TMZ) in the tumor and quickly developing chemoresistance are the reasons of therapy failure.

One of the strategies of increasing the treatment effectiveness is to combine drugs with different mechanisms of action. Flubendazole (FLU), the member of benzimidazoles group, exhibits anticancer effect based on its ability to interact with cell microtubules. Cell microtubules play important role in proliferation and invasion of cancer cells. Higher expression of βIII-tubulin found in higher grade gliomas indicates possible treatment target.

The aim of this study was to investigate the effect of the combination of TMZ and FLU on the viability and survival of stabilized GBM cell lines (A172, T98G and U118MG) and to verify this effect in an *in vivo* model.

Methods: Cell viability and proliferation were evaluated biochemically (WST-1 assay) and cytometrically (phase contrast microscopy). Drug synergism was determined using CompuSyn software. Changes in microtubule morphology were examined by fluorescence microscopy. Quantification of TMZ, FLU and their metabolites in cells was carried out using LC/MS analysis, while expression of selected cell cycle markers was determined by RT-PCR and Western blot.

Results: The combination of TMZ and FLU decreased cell viability and proliferation in exposed cell lines more effectively than with individual drug. Furthermore, changes in cell morphology and microtubule structure were noted as well as differently expressed cell cycle markers, such as cdc2 or cyclin B. Use of this combination also increased the amount of TMZ and FLU accumulated in tested GBM cell lines as well as in the tumor and brain of *in vivo* model organism.

Discussion: In our study, we combined the commonly used chemotherapeutic drug TMZ and a candidate anticancer drug FLU, which fits in a currently used concept aiming the improvement of the treatment efficiency in various types of cancers. Our obtained data clearly demonstrate improved efficiency of our drug combination in *in vitro* and *in vivo* GBM models. Since the use of tested drug combination also resulted in an increase of drug accumulation in GBM cells, this strategy should be the subject of further investigation.

Conclusions: Our results demonstrate that FLU improves TMZ antiproliferative effect in all tested GBM cell lines. Moreover, this drug combination caused significant changes in cell morphology, microtubule cytoskeleton and drug accumulation in cells. Obtained results indicate

possible strategic advantage of combination therapy in GBM and should be further investigated.

CEREBRAL PALSY RISK FACTORS, COMORBID CONDITIONS AND TYPES IN GEORGIAN INFANTS

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Introduction: Cerebral palsy is the most common motor disability among children and has many comorbidities. Diagnosing cerebral palsy is not easy in some cases. According to available literature data and guidelines, cerebral palsy is diagnosed before 6 months of age in only 25% of cases. Furthermore, looking for new methods to improve the early diagnosis of the disease is ongoing. Early diagnosis leads to early intervention, thus provides a better outcome. The study aims to develop appropriate diagnostic tools and effective measures for improving the early diagnosis of children under 9 months of age with cerebral palsy risk factors in the Georgian population for better outcomes.

Methods: Prospective/Longitudinal Cohort Study – At least 80 infants at high risk of cerebral palsy (extremely premature, extremely low birth weight, HIE, other neurological risk factors) are being screened and followed for 24 months. The infants at high risk of CP are assessed with Prechtl's general movements, Hammersmith Infant Neurological Evaluation, MRI. Additionally, with BSID III-IV or ASQ and GMFCS.

Results: The research is ongoing, at this stage 114 children have been evaluated, and have 12-month data in 53.5%. The most common risk factor for the development of CP in our research group is birth asphyxia – 43, prematurity – 48, infections – 23, seizures – 19, NICU administration – 79.

According to HINE assessment, study group was divided into non-walkers 23.8% (<40 score) and walkers 49.5% (40–62 score). Percentage distribution according GMFCS levels: I – 32.8%, II – 22.9%, III – 11.5%, IV – 26.2%, V – 6.6%. According to motor limitation, uni-, 3 limb-spasticity, and bi-spasticity are the most common 29.5%, 26.2%, and 22.9% important portion has mixed form and hypotonicity – 13.2% and 6.6%. Vision and cognitive dysfunction are predominant among comorbidities, also the detectable part has epilepsy and hearing problems.

Discussion: Modern scientific literature shows us very disturbing statistics and the difference between developed and developing countries in relation to the early diagnosis of the disease. The average age of diagnosis of cerebral palsy is about 18 months in high-income countries. The diagnosis of cerebral palsy is further delayed in low- and middle-income countries, for several reasons that include cultural, historical, geographic, and economic challenges.

The mean age of diagnosis of cerebral palsy in low and middle-income countries varies from 2.5 years to 6.5 years and most children are described as GMFCS levels III to V.

Conclusions: Among the 12-month-old patients included in the Georgian population study, the proportion of the ambulant type of cerebral palsy is high, which indicates that early diagnosis and appropriate intervention significantly improve the clinical outcome and lessen the burden of CP in patients and their families.

GENE EXPRESSION PROFILING OF PRIMARY LOW- AND HIGH-GRADE BLADDER CANCER IN RELATION TO CIRCULATING TUMOR CELL DISSEMINATION

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Introduction: Evaluation of the presence of circulating tumor cells (CTCs) in the peripheral blood (PB) of non muscle-invasive bladder cancer patients and analysis of gene expression of both primary tumor (PT) cells and CTCs isolated from peripheral blood (PB) could help define the aggressiveness of tumours and could be prognostic marker and marker assessing the efficacy of therapy (e.g. BCG vaccine). The aim of this study is to test the presence of CTCs in the blood of patients prior to transurethral resection (TUR) of primo-resected disease and to evaluate the expression of selected genes in the resected PT and correlating CTCs by quantitative PCR (qPCR).

Methods: Patients with primo-finding of BC were included in the study (n = 12, mean age = 72.5). Before TUR, blood sample (2x 8mL, EDTA) was collected. During TUR, a small sample of pathological tissue is collected for gene expression analysis (GEA). Blood samples are examined for the presence of CTC using a cell-size-base separation method (MetaCell®). Subsequently, enriched CTC are stained with vital fluorescent dyes for cytomorphological analysis, including assessment of CTC metabolism and their quantity. RNA isolated from the PT and CTC is subsequently transcribed into cDNA. The expression of 16 selected genes related to BC pathophysiology was measured in the tested cDNA samples. Taqman® chemistry and Taqman® gene probes were used for qPCR for the following genes: ACTB, CD274, CK20, GLUT1, TWIST, ZEB1, TRPM4, EGFR, HER2, SOX2, POUSF1, NANOG, ALDH1, AURKA, CD24, CD44).

Results: A total number of 12 PT samples and 30 CTC samples are currently being evaluated . There was no difference in numbers of CTCs in compared groups (LG vs. HG). GEA data were compared for both LG and HG groups and genes with elevated expression were identified for HG/LG.

In PT samples, there is increased expression of EGFR, TRPM4 and GLUT1 in LG group, and increased expression of HER2, AURKA, NANOG and CD24 in HG group. In CTCs

samples there is increased expression of EGFR, TRPM4 and GLUT1in LG and increased expression of POUSF1, NANOG, TWIST and CD274 (PD-L1) in HG group.

HG groups displayed significantly increased expression of stemness-associated genes.

Discussion: The data indicate that there is a significant portion of stem cells present in HG patient blood and primary tumor, which might cause the aggressive behavior of tumor cells. CTCs enriched from LG patients are more differentiated as seen by the gene expression profile.

Conclusion: There is a difference in gene expression in LG and HG group. HG group has significantly increased expression of stemness-associated genes. Changes in the expression profile of CTCs over time could mirror the effect of administered therapy (BCG-vaccine) in HG patients.

PHARMACOKINETICS OF INTRAMUSCULARLY ADMINISTERED THERMORESPONSIVE POLYMERS

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Introduction: Aqueous solutions of some polymers exhibit a lower critical solution temperature (LCST); that is, they form phase-separated aggregates when heated above a threshold temperature. Such polymers found many promising medical applications, including in situ thermogelling with controlled drug release, polymer-supported radiotherapy (brachytherapy), immunotherapy, and wound dressing, among others. Yet, despite the extensive research on medicinal applications of thermoresponsive polymers, their biodistribution and fate after administration remained unknown. Thus, herein, we studied the pharmacokinetics of four different thermoresponsive polyacrylamides after intramuscular administration in mice.

Method: We synthesized 4 different thermoresponsive polymers, each with two different molar masses, and labelled them with a covalently bound fluorescent dye. Next, we administered solutions of these polymers into hind legs of BALB/c mice and observed their dissolution over time with *in vivo* fluorescence imaging. Finally, we evaluated the dissolution kinetics of these polymers.

Results and discussion: In vivo, these thermoresponsive polymers formed depots that subsequently dissolved with a two-phase kinetics (depot maturation, and slow redissolution) with half-lives 2 weeks to 5 months, as depot vitrification prolonged their half-lives. Additionally, the decrease of T_{CP} of a polymer solution increased the density of the intramuscular depot. Moreover, we detected secondary polymer depots in the kidneys and liver; these secondary depots also followed two-phase kinetics (depot maturation and slow dissolution), with half-lives 8 to 38 days (kidneys) and 15 to 22 days (liver).

Conclusions: In our study, we elucidated the fate or thermoresponsive polymers in body and our head-to-tail study compared the dissolution kinetics of 8 different polymers. Overall, these findings may be used to tailor the properties of thermoresponsive polymers to meet the demands of their medicinal applications. Our methods for assessing polymers distribution and dissolution may become a benchmark for future studies of polymer biodistribution.

Further reading at https://doi.org/10.1002/adhm .202201344.

SPECTROSCOPY OF BLOOD PLASMA AS A TOOL FOR EARLY DIAGNOSTICS OF HEPATOCELLULAR CARCINOMA

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Introduction (Aim of the Study): Hepatocellular carcinoma (HCC) is the most common primary liver malignancy. The main risk factor for its development is liver cirrhosis of different etiologies or chronic HBV infection even in pre-cirrhotic stages. None of the biomarkers studied so far in the HCC area has yielded higher sensitivity than the liver ultrasonography examination in the early stage diagnosis (approx. 60%). There is an urgent clinical need for establishing a laboratory marker for HCC that meets the high sensitivity and specificity requirements for the screening and early diagnosis of at-risk patients. As various pathological processes, including carcinogenesis, may cause changes in both the concentration and the structure and spatial arrangement of body biomolecules, the spectroscopic analysis of blood-based derivatives appears to be an appropriate tool for early detection. In our research, the focus is on the identification of novel biomarkers in blood plasma that would exhibit sufficient sensitivity and specificity to detect early and potentially curable HCC stages, and that would be potentially useful for routine screening of this disease in well-defined at-risk groups. We hypothesized that spectroscopic methods can reach sensitivity of 65%, and specificity of 75%, and thus will be more efficient than liver ultrasound.

Methods: We included 60 subjects in the study (20 with liver cirrhosis, 20 with proven HCC, and 20 healthy volunteers). The blood plasma of the subjects was examined by Raman optic activity, Raman spectroscopy, infra-red spectroscopy, and electronic circular dichroism. To reduce the high computational time of classification while using whole spectra, mean-centered data from individual methods were subjected to a dimensionality reduction

method, namely principal component analysis (PCA). The classification was performed using partial least square discriminant analysis. Then we made models for sample discrimination.

Results: The combined model involving all spectroscopic methods reached for discrimination samples between HCC patients and cirrhotics with a sensitivity of 85%, and specificity of 95% (AUC-ROC = 0.958).

Conclusion: Based on our data the combination of advanced spectroscopic analysis of blood plasma could be a promising tool in HCC diagnosis.

IN VITRO STUDIES USING COLLAGEN MEMBRANES: A SYSTEMATIC REVIEW

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Introduction: Collagen membranes are widely used in regenerative dentistry to stabilize defect areas and to shield them from the soft tissue. Apart from serving as a barrier membrane, collagen membranes become a temporary scaffold for immigrating local cells. It is thus reasonable to assume that the immigrating cells respond to the collagen membranes.

Materials: This clinical situation is simulated under laboratory conditions aiming to understand the direction the cells response takes once they get into contact with the collagen membranes. Our aim was therefore to summarize current knowledge based on laboratory-based in vitro research using cell culture models.

Results: Based on the search terms "collagen membranes" and "in vitro" we could identify 170 abstracts, however, only 42 abstracts were related to "dentistry". Based on our systematic search, we identified collagen membranes tested were typically of xenogenic origin and either remained native or were cross-linked. We identified various cell types to respond to the collagen membranes including oral fibroblasts, osteogenic cells, epithelial cells and cells of the haematopoietic lineage such as macrophages and osteoclasts.

Discussion & conclusions: The in vitro cell response was related to viability, proliferation, osteogenic differentiation, osteoclastogenesis, and the adsorption of growth factors including TGF-β1 and BMP-2, as well as of antimicrobial compounds. Overall, this systematic review identifies a spectrum of in vitro bioassays helping to better understand the biological properties of collagen membranes.

MIRACULOUS FAT – HOW LIPOTRANSFER CAN ALTER THE PERIPROSTHETIC TISSUE. IN VIVO ANIMAL MODEL

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Introduction: Since the first reported successful fat transfer in 1893 by Dr Neuman, we have learned much more about the properties of this fascinating tissue and its ability to remodel and rejuvenate the environment through its surprising density of the viable stromal cells. Nowadays, after mastering fat grafting techniques and gaining the wide knowledge about its histological and physiological properties lipotransfer is known for a wide range of possible applications both in aesthetic and reconstructive surgery. The aim of this study was to investigate the histological effect of lipotransfer on the tissue surrounding the implant.

Methods: 20 male Sprague-Dowley rats underwent 3 separate surgical procedures: 2 mini-tissue expanders implantation, periprosthetic fat grafting and saline injections serving as control, and harvesting of the implant with capsule and surrounding tissue (3 and 12 weeks after the primary procedure). Subsequently, the tissues were stained and analyzed histologically. The data obtained was statistically analyzed (Stata v.15.1).

Results: Statistically higher inflammation score (P < 0.001) with edema, granulation tissue and mixed inflammatory cells was noted among study group. Fat grafting was associated with a significant reduction in capsule density (P = 0.001). Moreover, fat-grafted samples had a fiber orientation score 67-fold lower than the controls (P < 0.001). Lipotransfer led to 28.7% reduction in dense collagen (P = 0.001). Control group samples displayed dense collagen bands firmly adjacent to the implant, rather than disorganized collagen bands infiltrating into fat in study group. Synovial metaplasia occurred in 25% of periprosthetic tissue, but no significant difference was found between the groups.

Discussion: Our histological analysis supports the hypothesis that collagen beads are the preferred scaffold for the conversion of adipose stem cells into fat, because the collagen fibers in graft are entangled in a disordered manner with reduced alignment of the collagen fibers.

The observations from our experiment have direct clinical implications. The arrangement of collagen fibers is believed to be a key feature of capsular contracture. Disruption of collagen fibers is a crucial intervention to reduce the severity of capsular contracture. As demonstrated in our study, early fat grafting can prevent formation of a dense and organized capsule.

Conclusions: The successful clinical lipotransfer application in prosthetic surgery might be supported by data confirming that enhancement of peri-prosthetic tissue with fat grafting decreases collagen content, density, and fiber alignment of implant capsules.

REMOTE HEART FAILURE SYMPTOM ASSESSMENT AFTER MYOCARDIAL INFARCTION IDENTIFIES PATIENTS AT RISK OF DEATH

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Introduction: Despite improvements in the treatment of myocardial infarction (MI), heart failure (HF) remains the most common complication associated with increased mortality. Evaluation of HF symptoms early after hospital discharge is not routinely performed, and validated tools do not exist.

Whether evaluation of HF symptoms after MI hospital discharge can improve stratification of patient mortality risk is unknown. The present study aimed to evaluate the predictive value of the 23-item Kansas City Cardiomyopathy Questionnaire (KCCQ) and its components for all-cause mortality in patients after acute coronary syndrome (ACS).

Methods: Our study examined 1829 consecutive patients hospitalized for ACS at a large tertiary cardiac centre between June 2017 and September 2022. A total of 1,174 patients (aged 65 ± 12 years, aged 65 ± 12 years) completed KCCQ one month after hospital discharge. Cox regression was used to test the association of four categories of the KCCQ overall summary score (<25, 25–49, 50–74, ≥75) and the 23 individual items with all-cause mortality.

Results: KCCQ score <25 and 25-49 were independently associated with increased mortality risk compared to the reference group KCCQ ≥75. We developed a novel HF score utilizing three items of KCCQ (and their respective HR), that were independently associated with mortality risk: walking impairment, leg swelling, and change in symptoms over the last 2 weeks. Compared with reference group (HF score 3), patients with HF score 4–5, 6–7, 8–10 had significantly higher mortality risk.

Discussion: We examined the prognostic significance of patient-reported outcomes (as measured by KCCQ) one month after hospital discharge in large, prospective cohort of MI patients. To the best of our knowledge, this is the first study that evaluates the impact of KCCQ on long-term prognosis of consecutive patients with MI across the full range of left ventricular ejection fraction.

In the present study, KCCQ scores < 50 were present in approximately 13% of patients; with 68% of them were in Killip class I during hospital stay. Thus, large proportion of these patients developed HF symptoms after hospitalization, or Killip class is insensitive to HF signs. Remote assessment of patients' health status may decrease the burden on both patients and medical staff. It may identify high-risk patients who require closer follow-up and targeted therapy.

Conclusion: Evaluation of HF symptoms by KCCQ in post-MI patients after hospital discharge can identify a group at increased mortality risk. The novel HF score, which evaluates three symptom domains, is a simple tool that can further improve risk stratification.

GLYCOPROFILING OF PROTEINS APPLICABLE AS BIOMARKERS OF PROSTATE CANCER

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Introduction: Yearly incidence rate of prostate cancer (PCa) is over milion new cases worldwide with mortality rate over 300 000. In the Czech Republic in 2015 the incidence rate was 70 new cases per 100 000 people and the mortality over 12 per 100 000. Although the number of diagnosed cases is increasing, the mortality rate remains stable. This is mostly due to highly sensitive diagnostic methods and care provided to patients suffering from PCa. Prostate-specific antigen (PSA) and Zn-alpha-2-glycoprotein (ZA2G) are proteins whose glycosylated forms change significantly with the development and progression of PCa and such forms could be used to further increase the specificity of the diagnostic algorithm. The aim of our study was to conduct the glycoprofiling of those two proteins to prove their applicability as PCa biomarkers.

Methods: Changes in glycan composition were detected using lectins. Specific glycoprofiling was performed using magnetic particle (MP) modified with horseradish peroxidase and antibodies that selectively enriched fPSA and ZA2G in human serum samples. Subsequently, the proteins attached to the MP-antibodies were incubated on lectin-modified ELISA plates.

Results: Two clinical validation studies were performed, one for fPSA glycoprofiling and the other for ZA2G glycoprofiling. The results were presented in the form of a reciever operating characteristic curve. Glycoprofiling of fPSA showed better specificity and sensitivity (AUC = 0.84, n = 501) over fPSA alone (AUC = 0.76) in PCa diagnostics as well as in accompanying diagnostics, namely to differentiate between PCa patients undergoing treatment and those who do not. The analysis also revealed in a subset of serum samples (n = 215) that glycoprofiling of fPSA surpassed the prostate health index test (AUC = 0.69). Furthermore, out of 392 negative biopsies considered to be avoidable, 70-73% could have been prevented with the use of fPSA glycoprofiling compared with 52–53% in common fPSA test. No significant advantages were found in ZA2G glycoprofiling over commonly used diagnostic methods.

Discussion: PSA-based tests are quantitative methods commonly used for PCa screening and accompanying diagnostics. The false negative/positive rates of these test are high, therefore its use in screening and diagnostics is limited. In our study, we confirmed that two glycoforms of fPSA recognised by specific lectins are cancer-specific and thus useful in distinguishing malignant from benign cases and can outperform other tests applied in PCa screening and diagnostics.

Conclusions: While ZA2G glycoprofiling showed little promise as a potential biomarker of PCa, fPSA glycoprofiling has significant clinical potential as an early diagnosis biomarker of PCa as well as in accompanying diagnostics.

OUTCOME OF CONTINUOUS POSITIVE AIRWAY PRESSURE ADHERENCE BASED ON NASAL ENDOSCOPY AND MEASUREMENT OF NASAL PATENCY

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Introduction: The gold standard for treating obstructive sleep apnea in adults is continuous positive airway pressure (CPAP). However, it can be difficult to convince patients to adhere to therapy. The aim of this study is to assess the relationship between nasal endoscopy findings and nose patency measured by flow measurement and CPAP adherence.

Methods: A cohort of 450 consecutive patients suspected to have OSA were prospectively enrolled. For further analyses, 47 subjects with OSA being treated with CPAP treatment, were selected (13 females and 34 males, average age 65.3, BMI 34.1, apnoe-hypopnoe index AHI 51.0). Patients were divided into groups: two groups based on rhinoendoscopic findings (patient with and without endoscopically significant nasal obstruction) and two groups based on measurement of flow in nasal cavity (patients with more or less than 4.57 V of flow measured by flowmeter). The influence of nasal endoscopy and flow measurement on CPAP adherence and CPAP-related nasal complaints questionairre was explored.

Results: We found a statistical independence between CPAP compliance and rhinoendoscopic findings (p = 0.498) and nasal patency measured by flow measurement (p = 0.754). We found a statistical independence between CPAP-related nasal complaints and rhinoendoscopic findings (p = 0.588) and nasal patency measured by flow measurement (p = 0.657).

Discussion: Nasal patency improvement can be useful for reducing the level of PAP when treating OSA, resulting in better adherence to CPAP therapy. However, the positive effect of surgery on CPAP tolerance was not confirmed in every study. Therefore, it is very important to carefully select patients for possible nasal intervention before inciating CPAP. Our results were in agreement with conclusions of authors (Brimioulle 2022), but in conflict with results published by several other authors (Inoueet 2019, Park 2017, Sugiuraet 2007).

Conclusions: In our studied sample, sleep monitoring data, endoscopic findings and nose patency measured by flow measurement are not predictors for CPAP non-adherence in the first year of the CPAP treatment. Our data show that although an endoscopic finding in the nasal cavity could indicate a more severe obstruction, compliance to CPAP therapy is not reduced in these patients. Neither is compliance to CPAP therapy reduced with the decrease in nasal flow, according to our observation.

PREDICTION OF TREATMENT RESPONSE USING PET/MRI EXAMINATION PERFORMED DURING TREATMENT IN PATIENTS WITH LOCALLY ADVANCED CERVICAL CANCER TREATED WITH CONCURRENT CHEMORADIOTHERAPY

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Introduction: The standard treatment approach for patients with locally advanced cervical cancer is radical concurrent chemoradiotherapy, consisting of external beam radiotherapy and concurrent chemotherapy, followed by MRI-guided brachytherapy. PET/MRI (positron emission tomography/magnetic resonance imaging) examination is a hybrid imaging method that combines metabolic imaging with the advantages of magnetic resonance imaging, providing morphological, functional and metabolic parameters in a single examination.

Aim of the study: We aimed to find morphological, functional and metabolic characteristics of the tumour to predict failure to achieve a complete metabolic remission by PET/MRI examination performed during treatment in patients with locally advanced cervical cancer treated with concurrent chemoradiotherapy.

Methods: We evaluated 66 patients treated between August 2015 and November 2019 who underwent PET/MRI examination as part of staging (pre-PET/MRI), then at the end of external chemoradiotherapy (mid-PET/MRI) and 3 months after completion of the whole treatment (post-PET/MRI). We divided the whole group of patients into a group of responders (patients who achieved a complete metabolic remission) and a group of non-responders (patients who did not achieve a complete metabolic remission) according to RECIST (Response Evaluation Criteria in Solid Tumors) and PERCIST (PET Response Criteria in

Solid Tumors) criteria. We compared these two groups based on parameters obtained from pre-PET/MRI and mid-PET/MRI. The evaluated parameters were: pre-SUV (standard uptake value) max, pre-SUVmean, pre-MTV (metabolic tumour volume), mid-SUVmax, mid-SUV mean, mid-MTV.

Results: A statistically significant difference in the evaluated parameters between responders and non-responders was found for the parameter mid-MTV (p = 0.006).

Discussion: The number of recurrences after concurrent chemoradiotherapy for locally advanced cervical cancer remains high. Therefore, it is important to identify patients who are unlikely to achieve a complete remission. These patients could be treated more intensively based on selected predictive factors identified prior to treatment. Although posttreatment imaging parameters have been proven to predict survival, they do not allow early therapeutic adjustments. It is advisable to obtain information on whether persistence or early recurrence of the disease can be expected with high probability in the period just before the decision moment on possible therapeutic intensification, i.e. optimally before the end of the whole treatment.

Conclusion: Using a PET/MRI examination performed during treatment, we were able to identify a metabolic parameter (mid-MTV) suitable for predicting failure to achieve a complete metabolic remission.

VALIDATION OF THE CZECH VERSION OF THE VOICE HANDICAP INDEX

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Introduction: The present study aims to evaluate the reliability and construct validity of the Czech version of the Voice Handicap Index and determine the cut-off value to distinguish dysphonic from nondysphonic patients.

Methods: Prospective study, parallel group design. The study included 51 healthy subjects in the control group, and 100 adult patients with dysphonia (out of these 25 were used for test-retest analysis, 45 to determine the responsiveness to change. All 151 individuals completed the VHI-CZ and were examined with the videostroboscopy.

The internal consistency, the test-retest reliability, and the construct validity were analyzed and the normative cut-off value was determined.

Results: The internal consistency of the VHI-CZ was excellent (Cronbach α = 0.984), test-retest reliability was excellent (ICC = 0.95, P < 0.001). The correlation between the self-assessed severity of the voice disorder and the VHI-CZ score was strong (Spearman's ρ = 0.877, P < 0.001). The VHI scores differences between dysphonic and non-dysphonic were statistically significant (Mann-Whitney U test, P < 0.001). The differences among the three etiological subgroups (neurogenic, functional, and structural) were statistically significant (Kruskal-Wallis test, P < 0.001). The differences in the VHI-CZ total scores between pretreatment and posttreatment were statistically significant (Wilcoxon test, P < 0.001). The cut-off score of 13 points was found, by the analysis of the Receiver Operating Characteristic.

Discussion: According to the European Laryngological Society, comprehensive assessment of voice disorders should involve auditory-perceptual evaluation, videostroboscopy, acoustics, aerodynamics, and self-assessment of the patient.

Conclusions: The Czech VHI is a useful and valid monitoring tool for clinicians.

VALIDATION OF FRANCISELLA TULARENSIS PEPTIDES AS IMMUNOGENIC T-CELL EPITOPES

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Abstract: *F. tularensis* is a gram-negative, facultative intracellular and highly virulent bacterium. In the mouse model of infection, the survival of sublethal *F. tularensis* LVS infection is dependent on the correct functioning and cooperation of innate and adaptive immune system, and the immunity mediated by T-cells is critically required for long-term survival and clearance of *Francisella*. However, the particular T-cell antigens/epitopes are not well documented yet and we established experiments for identification of *Francisella* CD4+ T-cell targets.

This study follows up immunopeptidomics experiments which identified bacterial peptides presented on MHC-II molecules after *in vitro* infection of murine BMDCs by *Francisella* LVS (accompanying abstract "MHC-II peptidome of *F. tularensis* LVS infected dendritic cells"). The design and first results of experiments verifying if these peptides also operate as CD4+ T-cell epitopes and enhance the immune response in mice are presented.

Cell suspensions or isolated CD4+ T-cells were obtained from spleens and lymph nodes of mice immunized with sublethal dose of *F. tularensis* LVS. In initial screening

experiments, these cells were stimulated with synthetic *Francisella* peptides arranged into three peptides pools (PP). By IFN-γ Elispot, the following order of reactivity, PP-3 >> PP-2 > PP-1 was found. The PP-3 was consistently reactive in most replicates and stimulated most IFN-γ producing T-cells, whereas the reactivity of peptide pools 2 and 3 was either not so consistent across replicates or the production of IFN-γ was lower. This hierarchy of reactivity was observed at 3, 4 and 5 weeks post infection. Production of selected cytokines and cell proliferation after peptides restimulation was also assessed by FACS analysis. The results were however inconclusive due to very low frequency of responding cells and interference from nonspecific spontaneous cell stimulation. The screening of immune responses following stimulation with individual peptides was therefore based on IFN-γ Elispot. The results of early experiments pointed to 5-7 reactive *Francisella* peptides as potential CD4+ T-cell targets. This results could bring more knowledge and possibilities into immune monitoring of infection and identification of possible immune protective antigens.

PLASMA VITAMIN D LEVEL IN GERM CELL TUMOUR PATIENTS IS ASSOCIATED WITH SURVIVAL OUTCOME AND DISEASE CHARACTERISTICS

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Background: Testicular cancer is the most common malignancy among young men. Vitamin D has various effect in cancer pathogenesis and plays role in metastatic cascade. The aim of this study is to assess Vitamin D and its association with clinico-pathological finding along with prognostic impact of vitamin D among certain populations in our studied group.

Methods: This study included 120 newly diagnosed and/or relapsed germ cell tumor patients treated in April 2013 to July 2020 for whom plasma was available in the biobank. Blood samples were drawn at the time before 1st chemotherapy cycle as well as before 2ndcycle. Study also included 21 age-matched healthy donors. Plasma vitamin D level was measure by ELISA and correlated with disease characteristics and patient's outcome. For survival analysis it was dichotomized to "low" and "high" based on median value.

Results: Mean ± standard error of mean [SEM] plasma level of vitamin D in GCTs patients was 15.87 ± 0.68. Vitamin D level was not associated with disease characteristics except of brain metastases, where patients with brain metastases had significantly lower D vitamin level compare to patients without brain metastases (10.9 ± 3.05 vs 15.99 ± 0.7, $p = 0.034$). Lower vitamin D level was also detected among GCT patients with unfavorable response compared to favorable responses (mean + SEM, 11.26 ± 1.84 vs 16.47 ± 0.74, $p = 0.016$) and in patients, that experienced disease recurrence. Patients with "low" vitamin D level had inferior progression free survival but not overall survival compared to patients with "high" vitamin D level (HR = 3.02, 95% CI (1.36–6.71), $p = 0.014$ for PFS and HR = 2.06, 95% CI (0.84–5.06), $p = 0.135$ for OS, respectively).

Discussion: Mean vitamin D level among all studied patients was observed in range of hypovitaminosis, thus, most of patients were characterized by vitamin D insufficiency. The observation of prognostic impact of vitamin D is consistent with studies evaluating prognostic impact of vitamin D and PFS in different types of cancer, however, prognostic significance in overall survival was not statistically significant. The reason could be change of vitamin D level over the time of treatment period.

Conclusion: Our study for the first time revealed prognostic value of pretreatment vitamin D level in GCTs patients. Moreover, low plasma vitamin D level was associated with unfavorable response to therapy and disease recurrence, however, it remains to elucidated whether it mirror biology of disease or if its supplementation could affect patient outcome.

TRANSCRIPTION-FACTOR AP-1 IN COLORECTAL CANCER

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Introduction: Colorectal cancer still is a burden for humanity as it is one of the most common cancerous diseases globally, with the second highest mortality rate across all genders. Although great progress was achieved over the past decades in treating and screening for colorectal cancer, the metastasized stage has a 5-year survival rate of only 14% in Germany. These numbers indicate the importance of further research regarding the mechanisms that lead to metastasizing, to eventually identify new therapeutical targets for advanced stages.

For tumour cells to colonize a new organ, a sequence of processes must occur that causes the tumour cells to first lose their cell-cell contacts and adhesion to the

extracellular matrix. In the next step, invasion into blood or lymphatic vessels must take place. For this the cells must be able to survive in the bloodstream. Thus, cells with previously epithelial differentiation take on a more mesenchymal phenotype. This describes the so-called epithelial to mesenchymal transition (EMT), which is an important step in the process of developing metastasis.

Different signalling pathways and transcription factors induce these changes in tumour cells, of which some are already well known, whereas others need further investigation. Among these transcription factors presumably involved in the processes of tumorigenesis and metastasis is the activator protein-1 (AP-1).

AP-1 is a multigenic transcription factor complex, containing several protein families. The most prominent being the Jun, Fos, MAF and BATF family. AP-1 members are basic leucin zippers, forming homo- or heterodimers. The protooncogenic potential of AP-1 has already been shown for several tumour entities. Some of the AP-1 factors also seem to be relevant for the development of colorectal carcinoma. CJUN for example has been shown to have a growth-promoting function in colorectal cancer, but its role in progression of colorectal cancer is not understood yet. This study's aim is to investigate the very complex but seemingly relevant transcription factor family AP-1 and its role in the metastasizing process of colorectal cancer.

Methods: To start with, it was crucial to show the expression profile of several AP-1 members in colorectal cancer. For this purpose, investigations on protein level using immunohistochemical staining and western blotting were conducted. A total of 11 matched primary tumour and liver metastasis tissue samples were used for immunohistochemical staining. The lysates of nine colorectal cancer cell lines, as well as lysates of patient derived organoids were used for western blot analysis. For biological characterization a stable CRISPR/Cas 9 knock out system was established and a stable knock out cell line created for each member of the Jun and Fos family. Different assays were performed to investigate the proliferative and migrative properties of colorectal carcinoma cells after knockout. Cell viability after treatment with an AP-1 inhibitor was also tested, as well as a global knockout of AP-1 using the dominant negative AFOS. To put the *in vitro* data into clinical context, the experimental part was followed up by a retrospective correlation between AP-1 expression profile in colorectal carcinoma and clinical outcome.

Results: As data analysis is not completed yet, only preliminary results can be presented. The expression profile of AP-1 families across nine different colorectal cancer cell lines, including two metastasis cell lines, shows an overall high expression of the Jun family, whereas expression of the Fos family members differs more. The expression of the BATF family is very low throughout all cell lines. Preliminary data of immunohistochemical stainings show the upregulation of certain AP-1 members in primary tumour and liver metastasis compared to healthy mucosa. Expression is particularly high in the more invasive properties of tumour and metastasis. The knockout of single AP-1 members led to a decrease in cell viability and the ability to form colonies. Migratory properties seem to also be affected by the knockout of single AP-1 members. The global knock out of AP-1 showed a significant decrease in cell viability and cloning ability. The pharmaceutical inhibition of AP-1 in a 2D-culture did only show a tendency to reduce cell viability. The correlation of AP-1 expression and clinical outcome is still pending but will be presented at the conference.

Discussion: The expression data generated on protein level in primary tumour coincide with findings in older publications. This study now gives further insight into the expressional patterns of the Jun and Fos family members in liver metastasis compared to the matched primary tumour. The in vitro data also supports the hypothesis regarding the involvement of AP-1 in the epithelial to mesenchymal transition.

To be able to make a more differentiated statement on AP-1's role in tumour progression double knock outs should be performed in future studies, as the function of single AP-1 members differs, depending on their dimerization partner.

As this study only generated *in vitro* data, it can show tendencies and give direction for further investigation. The correlation of clinical outcome of colorectal cancer patients and the expression profile on cDNA level could give deeper insight into the clinical relevance of AP-1. To round of this study *in vivo* experiments would be necessary. A mouse model using a highly invasive murine colorectal cancer cell line to induce tumour growth and metastasizing is planned for future projects.

Conclusion: AP-1's expression profile in primary tumour and liver metastasis indicates the importance of this transcription factor complex in the progression of colorectal cancer. In *vitro data* confirms AP-1's protooncogenic properties and suggests an involvement in the process of metastasizing. Pharmaceutical inhibition of AP-1 did not show significant effects, further investigation using 3D culture models might be insightful. AP-1 seems to have the potential to transpire as a future therapeutical target for colorectal cancer, but further investigations are necessary.

COMPUTER ANALYSIS OF GAIT DISORDERS

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Introduction: Gait disorders are common manifestations of many neurological diseases. Diagnosis, determination of its gravity, and the monitoring of its progression or regression in time are burdened with interpersonal differences. Our aim is to use objective data provided by commercially available accelerometer for effective diagnosis and treatment. To do this, it is necessary to identify key features that could be used for reliable classification.

Methods: The Random Forest method with 100 trees was used for analysis. The dataset consisted of 605 samples with 15 different features. Samples were taken from

50 healthy controls and 100 patients with neurological gait disorders.

Results: Certain features, such as RMS ML Accel, RMS AP Accel, RMS Pitch Velocity, and RMS Roll Velocity, were identified as significantly important for classification.

Discussion: While some features were identified as key, future research might explore additional potential features and their combinations for even more accurate classification.

Conclusions: The analysis confirmed the significance of certain kinematic features in distinguishing healthy subjects from patients with gait disorders.

SEQUENATION OF DNA METHYLATION OF HMGCR GENE COULD BE PREDICTOR OF MORBIDITY AND MORTALITY IN PATIENTS ON HEMODIALYSIS

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Introduction: Chronic kidney disease [CKD] is a one of the fastest growing health problem. Patients with CKD exhibit a pronounced risk for cardiovascular mortality; the most vulnerable group enrolls diabetic patients on hemodialysis.

Although a risk of cardiovascular events in general population has significantly decreased after launching of cholesterol lowering medication – inhibitors of 3-hydroxymethyl-3-glutaryl Coenzyme A reductase [HMGCR], unfortunately in patients undergoing dialysis no meaningful benefit of this therapy was demonstrated.

The incidence of complications in CKD patients correlates with changed epigenetic mechanisms, the most studied epigenetic phenomenon is DNA methylation. The principle of DNA methylation is modification of DNA structure through binding of methyl group at the cytosine. This makes change in chromatin conformation: methylated parts are more compact and no readily accessible to the transcriptional. On the contrary unmethylated genes are easily transcribed, e.g. unmethylated HMGCR gene is more expressed and cholesterol synthesis is enhanced.

The aim of this pilot study was to elucidate the methylation status of HMGCR gene and evaluate its impact on cardiovascular morbidity and mortality in patients receiving hemodialysis.

Methods: We analyzed the plasma of 12 dialyzed patients suffering from type 2 diabetes. DNA was isolated from samples before and after dialysis, then bisulfite conversion was carried out to distinguish methylated and unmethylated form of DNA. The primers of HMGCR genes promotors were designed, consequently methylation specific PCR and sequential analysis of PCR products were performed.

Results: Although the methylation rate of HMGCR gene dropped in part during the hemodialysis session, no statistical significance was confirmed.

Hypomethylated promotor of HMGCR gene was found in 6 patients, who either underwent amputation of lower extremity due to arterial ischemia or has deceased due to cardiovascular event during 6 month of follow-up period.

Discussion: These results claim for the importance of early recognition of dialyzed patients in high cardiovascular risk, since hypomethylation of HMGCR gene has consequence in higher rate of cardiovascular morbidity and mortality. The impact of dyslipidemia in hemodialysis patients has not been adequately elucidated yet and underestimation of HMGCR activity has been presumable and imminent.

The limitation of our pilot study is a low number of participants, nevertheless the ongoing investigations are performed to expand the range.

Conclusions: This pilot study suggests that both DNA demethylation and consecutive increased expression of HMGCR gene are predictors of high risk of cardiovascular morbidity and mortality in hemodialyzed diabetic patients.

ASSESSMENT OF ALLERGIC REACTION AND SPECIFIC IMMUNOGLOBULIN E LEVEL IN NASAL SECRETION OF LOCAL ALLERGIC RHINITIS PATIENTS

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Introduction: Local allergic rhinitis (LAR) is characterized by similar symptoms as allergic rhinitis (AR): nasal itching, sneezing, rhinorrhea, and nasal congestion. However, specific Immunoglobulin E (sIgE) sensitization cannot be ascertained neither through skin prick test nor through serum sIgE assessment. Unfortunately, the delayed LAR diagnosis leads to prolonged suffering of the patients. Our study aims to assess the allergic inflammation processes and IgE-mediated reaction that may occur only at the level of the local nasal mucosa.

Methods: Suspected LAR subjects (n = 23) are included in the study. Subjects are assessed through rhinoscopy for nasal symptoms. TNSS/VAS questionnaires were completed during the period of increased exposure to mites allergens (Fall/Winter) and before/after nasal provocation test (NPT) with HDM allergens (Dermatophagoides pteronyssinus; *D. p* and Dermatophagoides farinae; *D. f*) and then analyzed. Nasal secretions were collected from the subjects via the nasal lavage method and sIgE levels to D. *p* and *D. f* were measured during the exposure period (n = 12) and before and after NPT (n = 15).

Results: TNSS/VAS scores were higher in subjects during the exposure period when compared to the same subjects during the off-season. For patients who underwent NPT, TNSS/VAS scores were higher after provocation in

subjects with positive NPT, lower after provocation in subjects with negative NPT, and the same or slightly higher after provocation in subjects with doubtful NPT results. 3 sIgE measurements were excluded due to technical errors. During the exposure period, 80% of the subjects showed higher levels of sIgE for *D. p* when compared to *D. f* and the remaining 20% showed the same concentration. The level of sIgE for *D. p* was higher after NPT in 8 subjects (57.14%), the same in 2 subjects (21.42%), and lower in 2 subjects (21.42%). On the contrary, levels of sIgE for *D. f* showed less variation with 7 subjects (57.3%) having the same level, 2 subjects (15.38%) with a lower concentration, and 3 subjects (23.07%) with increased levels after NPT.

Discussion: NPT is the gold standard in LAR diagnosis; however, results may be inconclusive or inconsistent, and combining the clinical assessment and TNSS/VAS may provide more specificity in the evaluation of LAR subjects. More information can be acquired through sIgE assessment in nasal secretions as we saw in our results with the increase noted in the level of sIgE for *D. p*.

Conclusion: Albeit our study showed promised results, increasing the studied population and assessing sIgE and additional inflammatory mediators levels in nasal secretions acquired through other methods (e.g. Nasosorption) and in serum would possibly provide a more conclusive and consistent assessment of LAR subjects.

SYNDECAN-1 LEVELS IN ORGAN DONORS AND KIDNEY TRANSPLANT RECIPIENTS

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Introduction: Kidney transplantation remains the optimal treatment for end-stage renal disease, offering improved quality of life and increased survival rates compared to long-term dialysis. However, despite advances in surgical techniques, immunosuppression regimens, and post-operative care, there are still significant challenges in predicting the long-term outcomes of transplantation. Among the many factors that influence graft survival, the quality of the donated organ plays a fundamental role. Syndecan-1 is found in the endothelial glycocalyx and shed in a higher rate into the blood during systemic pathological conditions. In this study, we aimed to investigate the potential role of syndecan-1 as a biomarker of kidney donor organ quality. We hypothesized that elevated syndecan-1 levels in donors correlate with short-term outcome in kidney recipients. To test this hypothesis, we analyzed a cohort of kidney transplant donors and recipients and evaluated the relationship between syndecan-1 levels in the donor organ and various clinical and laboratory parameters in recipients, including graft function.

Methods: In this single-center, retrospective, observational cohort study we investigated 80 donors' serum syndecan-1 levels and correlated them with donor characteristics and short-term outcome in corresponding 104 kidney recipients.

Results: Higher syndecan-1 levels correlated with last creatinine before organ harvest and were marginally higher in donors with acute kidney injury. However, syndecan-1 levels were not associated with short-term outcome in kidney recipients (delayed graft function and renal function at 3 months post kidney transplantation).

Discussion: Numerous pathophysiological phenomena are evidently connected to structural and functional aberrations of the endothelial glycocalyx. The most substantial proof for this connection is found in the context of organ damage following ischaemia, sepsis and inflammation, diabetic vascular disease, atherosclerosis, and kidney diseases. Our understanding of the role of syndecan-1 in kidney transplantation is just beginning to be elucidated. There is an apparent need for more robust, prospective studies that investigate the relationship between donor syndecan-1 levels and post-transplant kidney function at different times. Gaining a better understanding of this correlation could open the door for improvements in donor organ selection, pre-transplant risk assessment, and post-transplant patient management, potentially leading to better patient outcomes.

Conclusion: Our findings suggest that syndecan-1 could serve as a novel indicator of graft quality, ultimately contributing to a more personalized and effective approach in kidney transplantation. However, this finding needs validation in larger cohorts.

DIAGNOSTIC ACCURACY OF CAROTID PLAQUE INSTABILITY BY NONINVASIVE IMAGING: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: There is increasing evidence that plaque instability in the extracranial carotid artery may lead to an increased stroke risk independently of the degree of stenosis. We aimed to determine diagnostic accuracy of vulnerable and stable plaque using noninvasive imaging modalities when compared to histology in patients with symptomatic and asymptomatic carotid atherosclerosis.

Methods: Medline Ovid, Embase, Cochrane Library, and Web of Science were searched for diagnostic accuracy of noninvasive imaging modalities (CT, MRI, US) in the detection of 1) vulnerable/stable plaque, and 2) vulnerable/stable plaque characteristics, compared to histology. The quality of included studies was assessed by QUADAS-2 and univariate and bivariate random-effect meta-analyses were performed.

Results: We included 36 vulnerable and 5 stable plaque studies in the meta-analysis, and out of 211 plaque characteristics from remaining studies, we classified 169 as vulnerable and 42 as stable characteristics (28 CT, 120 MRI, 104 US characteristics). We found that MRI had high accuracy (90% [95% CI: 82–95%]) in the detection of vulnerable plaque, similar to CT (86% [95% CI: 76–92%]; $p > 0.05$), whereas US showed less accuracy (80% [95% CI: 75–84%]; $p = 0.013$). CT showed a high diagnostic accuracy to visualize characteristics of vulnerable or stable plaques (89% and 90%) similar to MRI (86% and 89%; $p > 0.05$); however, US had lower accuracy (77%, $p < 0.001$ and 82%, $p > 0.05$).

Discussion: High accurate CT and MRI may be used as the first-choice modality to select high-risk patients for clinical trials aiming to reduce the rate of stroke recurrences and the burden of new ischemic lesions on follow-up MRI. All characteristics of a vulnerable plaque were detected by MRI with high accuracy, suitable for assessing the effect of specific treatments targeting the vulnerable plaque characteristics, such as lipid-lowering and anti-inflammatory strategies.

Conclusions: CT and MRI have a similar, high performance to detect vulnerable carotid plaques, whereas US showed significantly less diagnostic accuracy.

Registration: PROSPERO ID CRD42022329690

ROTATIONAL THROMBOELASTOMETRY AND PSORIASIS

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Background: It has been repeatedly described that patients with severe psoriasis are at higher risk of thrombotic complications. Rotational thromboelastometry (ROTEM) is a viscoelastic hemostatic test that enables sophisticated evaluation of hemostasis in whole blood sample. The aim of this study was to evaluate changes in hemostasis in psoriatic patients using ROTEM.

Methods: We performed a pilot, observational, prospective study. Totally 60 patients with severe psoriasis (PASI > 15) were enrolled in this study. The control group consisted of 11 healthy blood donors. Blood samples were tested with ROTEM using INTEM, EXTEM and FIBTEM reagents.

Results: We detected significant changes in INTEM clot forming time, INTEM maximum clot firmness, EXTEM clotting time, FIBTEM clotting time and FIBTEM maximum clot firmness compared to healthy controls.

Conclusion: We observed abnormal changes in hemostasis using rotational thromboelastometry in patients with severe psoriasis.

Keywords: severe psoriasis; rotational thromboelastometry; ROTEM

APPLIANCE OF CONTINUOUS GLUCOSE MONITORING SYSTEM AS AN EDUCATIONAL TOOL IN PATIENTS WITH TYPE 2 DIABETES IN THE EARLY STAGE OF DISEASE

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Introduction (aim of the study): Type 2 diabetes mellitus is an economical, medical and social problem. Patients with type 2 diabetes live shorter compared to peers without diabetes, mainly because of cardiovascular diseases. The basis of the treatment of these illness is multifactorial therapy focused on maintaining healthy levels of blood glucose and prevention of cardiovascular complications. Continuous glucose monitoring (CGM) is a small device, which measures glucose level in tissue fluid every few minutes and allows to observe glucose trends on the graph. One of the most popular systems is Freestyle Libre, it is based on scanning. A sensor is placed on the posterolateral part of the arm and then a mobile phone or reader with the NFC function is brought close to the sensor to obtain a reading. Compared to people using glucometers, patients using CGM systems, including Freestyle Libre, achieve better diabetes control, have fewer hypoglycaemic episodes, and their quality of life improves. The hypothesis that the temporary, educational use of the CGM system may have clinical significance in patients in the early stage of type 2 diabetes requires verification. The aim of the study is to evaluate the effectiveness of an educational intervention involving the periodic usage of the Freestyle Libre 2 CGM system in patients in the early stage of type 2 diabetes.

Methods: Both qualitative and quantitative methods are used. In-Depth Interview is conducted among men to

access the impact of the sensor on motivation to long-term lifestyle change. The quantitative methods belong laboratory blood tests, anthropometric measurements, and psychological assessment. Psychological tools such as QoL-Q Diabetes, Diabetes Distress Scale (DDS), Athenian Scale of Insomnia are used.

Results: The study is still in progress. In-Depth Interviews are analysed. Freestyle Libre is perceived as small and useful tool. A few patients consider using continuous glucose monitoring system on their own temporarily, without refund. Patients report a desire to change lifestyle, compare food products and introduce physical activity.

Discussion: Recent years have provided convincing evidence that CGMS use results in clinical benefits, namely improvement in glycemic control and quality of life, in both T1DM and T2DM treated intensively with insulin. Sparse data exists concerning patients with T2DM on less intensive therapeutic models, such as oral medications.

Conclusions: The results of the study may influence the guidelines and clinical practice in diabetes by providing the evidence that short-term use of CGM soon after T2DM diagnosis improves long term glycemic control.

INVESTIGATING HOST-BACTERIA INTERACTIONS IN AGING SKIN USING LONG-READ SEQUENCING

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Our skin serves as a habitat for millions of microorganisms that play vital roles in both health maintenance and disease. This bacterial community can be influenced by diverse factors, including antibiotics, diet, exercise, and age. The latter is known to have a significant impact on microbiota composition, yet the intricate cause-effect relationship remains poorly understood. Our current study aims to unravel the link between human skin ageing and skin microbes. To achieve this, we recruited healthy volunteers from two age groups: young individuals aged 18 to 30 and older adults aged 60 and above. For our investigation, we collected swabs from the forehead, arm, and foot aiming to isolate bacteria and perform metagenomic profiling via long-read Nanopore sequencing. Concurrently, we accessed a range of age-associated biophysical parameters at each body site using the multi-probe DermaLab Combo device. Our analysis revealed *Staphylococcus* species as the prominent constituents of the foot microbiome in both age groups. Interestingly, *Corynebacterium* exhibited a threefold increase in abundance on the aged forehead skin. Moreover, older individuals displayed a lower abundance of *Cutibacterium* and *Malassesia*, in line with prior findings. The forearm site exhibited highly diverse bacterial profiles, where the prevalence of *Staphylococcus* was reduced in the older cohort alongside a higher prevalence of *Acinetobacter* and *Moraxella*. While alpha and beta diversity, as well as biophysical parameters, did not show statistically significant differences among the groups, a strong negative correlation was notably observed between thickness and collagen intensity in the forehead and arm. Further analysis of the species and strain level is still required to solidify our conclusions. Ongoing studies are currently exploring functional differences in bacterial isolates from old versus young individuals through the analysis of biofilm formation, UV resistance, and influence on host/bacteria. This project is significantly advancing our understanding of the aged skin microbiome. Our findings will provide a foundation for future perspectives and applications, from managing strategies to address skin age to understanding the potential contribution of bacteria to impaired wound healing and skin disorders in the elderly.

TOWARDS NEW BIOMARKERS OF PSORIATIC ARTHRITIS – THE ROLE OF MICROTRAUMA, ALARMINS AND LIPID MEDIATORS IN DEVELOPMENT AND RESOLUTION OF INFLAMMATION

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Introduction: Psoriatic arthritis (PsA) is the most prevalent chronic systemic inflammatory arthritis. Because of significant heterogeneity and lack of autoantibodies that can help pose the diagnosis, it remains a clinical challenge for practitioners. Recent studies suggest that that balance between biomarkers of microtrauma at entheseal sites leads to inflammation, which then spreads to secondarily involved structures. Among those mediators are Lipid mediators, which have both pro and anti-inflammatory properties like Leukotrienes, lipoxins, and Alarmins. The general goal of the study has been divided into several specific objectives with 3 research models. The aim of this presented study is to compare the influence of lipoxin A4 on the synthesis of pro-inflammatory cytokines by PBMCs from patients with PsA and healthy controls.

Methods: The peripheral blood will be collected from patients with psoriatic arthritis (n = 12) and healthy controls (n = 12). PBMC isolated from peripheral blood will be further stimulated with LPS and the modulatory effect of analogue of lipoxin A4 on generation of cytokines will be assessed. The level of cytokines in PBMC culture supernatants will be quantified with Cytometric Bead Array kit. The samples will be measured on BD LSR Fortessa flow cytometer and the results were analyzed with BD FCAP Array software.

Results: A set of preliminary experiments funded by the Medical University of Lodz was performed in our group. The basal and LPS-induced pro-inflammatory cytokine production by PBMCs was lower in PsA patients as compared to healthy subjects. Strikingly, co-stimulation with LXA4 resulted in a significant increase in pro-inflammatory cytokine secretion in PsA, in contrary to the reduction observed in healthy subjects.

Discussion: In one study lipoxin A4 was postulated to be important factor in the resolution of tendon inflammation in patients experiencing pain before and after surgical subacromial decompression treatment of supraspinatus

tendon. However, it is important to stress that role of lipoxins has not been studied up till now in pathogenesis of psoriatic arthritis. The following results would possibly help in establishing the role of lipoxin A4 in resolution of inflammation in Psoriatic arthritis.

Conclusion: Psoriatic arthritis is one of the most common inflammatory arthritis, leading to deterioration in the quality of life and disability. Understanding the process which initiates inflammation in PsA will potentially enable rapid therapeutic intervention and contribute to the prognosis improvement.

LOW FIBRINOGEN LEVELS IN THE TREATMENT OF ISCHAEMIC STROKE BY INTRAVENOUS THROMBOLYSIS: A POSSIBLE PREDICTOR OF HAEMORRHAGIC INTRACRANIAL COMPLICATIONS?

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Introduction: Intravenous thrombolysis (IVT) is the recommended recanalization therapy for ischaemic stroke. One complication of IVT is intracranial haemorrhage. Its incidence as a consequence of IVT is 5–12% in stroke centres in the Czech Republic.

The aim of this study is to evaluate whether a decrease in fibrinogen levels below the reference range of 1.8 g/l is associated with a higher likelihood of intracranial haemorrhage in patients with ischaemic stroke treated with IVT.

Methods: This multicentre retrospective study was conducted in 7 centres of the National Stroke Research Network STROCZECH. The study population consists of 280 patients enrolled in the observational group (divided into subgroups according to the type of haemorrhagic complication after IVT) and the control group (without haemorrhagic complication) according to the model 1:1 principle (same NIHSS, age, sex). Parameters monitored include personal and pharmacological history, laboratory and neurological parameters and results of selected imaging examinations. For statistical analysis, a non-parametric one-way ANOVA test with the Friedman and Dunns tests and a non-paired non-parametric Mann-Whitney test were selected.

Results: In all treatment groups (all observational subgroups as well as the control group), a decrease in fibrinogen levels was noted after IVT administration, with varying degrees of signification. A statistically significant difference ($p = 0.0394$) was found at 6 h after IVT administration in patients with the most clinically serious form of bleeding (parenchymal haematoma type 2), fibrinogen value 1.84 g/l vs 2.53 g/l.

An interesting finding, where signification was not reflected, concerns the baseline fibrinogen (i.e. before IVT administration), which is higher in all subgroups of patients with haemorrhagic complications compared to the control group.

Discussion: Current guidelines for the intravenous thrombolytic therapy include the examination of coagulation parameters, including fibrinogen levels, before IVT, 6 hours and 24 hours after IVT, but there is no official recommendation on when to substitute fibrinogen, and whether to do so at all. Substitution is available in hospitals (Haemocomplettan i.v.). Fibrinogen substitution may be a way to prevent the development of bleeding complications during IVT treatment.

Conclusions: Patients with the clinically most severe form of haemorrhagic complication (parenchymal haematoma type 2) had significantly lower fibrinogen levels than those in the control group 6 hours after IVT administration.

The study also leads us to a new hypothesis: a haemorrhagic complication of IVT could be associated with a relative decrease in fibrinogen levels from baseline, despite the fact that the values are still within the physiological range.

IN VITRO ACTIVITY OF NEW SMALL-MOLECULE PERK INHIBITOR IN PARKINSON'S DISEASE CELLULAR MODEL

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Introduction: Abnormal aggregation of α-synuclein is the major molecular event underlying Parkinson's disease (PD) pathogenesis. Accumulated α-synuclein induces Endoplasmic Reticulum (ER) stress and activates the Unfolded Protein Response signaling pathway, that ultimately leads to PERK-dependent apoptosis and neurodegeneration. Therefore, the aim of the present study was to determine an in vitro effect of new small-molecule PERK inhibitor LDN-27339 (LDN) in cellular models of PD.

Methods: The study was carried out using SH-SY5Y and DI TNC1 cell lines with dopaminergic and astrocytic phenotype, respectively. The cytotoxic effect of LDN was evaluated by the colorimetric XTT assay. Cells were treated with LDN in wide concentration range (0.75 μM – 0.5 mM) or with vehicle (0.01% DMSO) and incubated for 16, 24 and 48 h. Untreated cells constituted a positive control, and cells treated with 99.9% DMSO served as a negative control. After incubation, the XTT/PMS mixture was added to each well and the absorbance was measured after 3 h. The inhibitory activity of the tested compound was evaluated by Western blotting. Cells were pretreated with LDN at the concentration range of 3–50 μM for 1h and then exposed to an ER stress inducer, thapsigargin (Th), at 500 nM for 2h. Untreated cells constituted a negative control, and cells treated with Th only served as a positive control. The detection of immune complexes was performed by means of chemiluminescence.

Results: LDN significantly inhibited PERK-dependent phosphorylation of eIF2α in SH-SY5Y cells at 25 μM and in DI TNC1 cells at 50 μM. The inhibitor had negligible cytotoxic effect at any concentration and incubation time in both tested cell lines.

Discussion: The findings indicate high activity of LDN against PERK in both SH-SY5Y and DI TNC1 cell lines, and the inhibitory effect was stronger in dopaminergic cells. At the same time, the compound did not significantly impair cell viability at the effective concentrations.

Conclusions: The inhibitor may rescue neural and glial cells from ER stress-mediated apoptosis in the course of PD, in which the PERK-dependent pathway is overactivated. Further studies are needed to assess the full potential of LDN and its possible application in novel therapeutic strategies against PD. This work was supported by the Medical University of Lodz, Poland (grant no. 503/1-156-07 /503-11-001) and by the National Science Centre, Poland (grant no. 2021/43/O/NZ5/02068).

BINOCULAR VIDEO HEAD IMPULSE TEST: NORMATIVE DATA STUDY

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Introduction: The video head impulse test (vHIT) evaluates the vestibulo-ocular reflex (VOR). It's usually recorded from only one eye. Newer vHIT devices allow a binocular quantification of the VOR.

Purpose (Aim): To investigate the advantages of simultaneously recorded binocular vHIT (bvHIT) to detect the differences between the VOR gains of the adducting and the abducting eye, to define the most precise VOR measure, and to assess gaze dys/conjugacy. We aimed to establish normative values for bvHIT adducting/abducting eye VOR gains and to introduce the VOR dysconjugacy ratio (vorDR) between adducting and abducting eyes for bvHIT.

Methods: We enrolled 44 healthy adult participants in a cross-sectional, prospective study using a repeated-measures design to assess test–retest reliability. A binocular EyeSeeCam Sci 2 device was used to simultaneously record bvHIT from both eyes during impulsive head stimulation in the horizontal plane.

Results: Pooled bvHIT retest gains of the adducting eye significantly exceeded those of the abducting eye (mean (SD): 1.08 (SD = 0.06), 0.95 (SD = 0.06), respectively). Both adduction and abduction gains showed similar variability, suggesting comparable precision and therefore equal suitability for VOR asymmetry assessment. The pooled vorDR here introduced to bvHIT was 1.13 (SD = 0.05). The test–retest repeatability coefficient was 0.06.

Conclusion: Our study provides normative values reflecting the conjugacy of eye movement responses to horizontal bvHIT in healthy participants. The results were similar to a previous study using the gold-standard scleral search coil, which also reported greater VOR gains in the adducting than in the abducting eye. In analogy to the analysis of saccade conjugacy, we propose the use of a novel bvHIT dysconjugacy ratio to assess dys/conjugacy of VOR-induced eye movements. In addition, to accurately assess VOR asymmetry, and to avoid directional gain preponderance between adduction and abduction VOR-induced eye movements leading to monocular vHIT bias, we recommend using a binocular ductional VOR asymmetry index that compares the VOR gains of only the abduction or only the adduction movements of both eyes.

DIAGNOSTIC FEATURES OF POLYNEUROPATHY IN PATIENT WITH 2 TYPE DIABETES MEIIITUS

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Introduction: Polyneuropathy is the most common and prognostically unfavorable complication of diabetes mellitus (DM). Distal symmetrical polyneuropathy (DSPN) is the most common type of peripheral neuropathy in patients with type 2 diabetes. Electroneurophysiological and clinical manifestations DSPN precede the manifestations of the main disease and appear 2–8 years before the appearance of the first clinical symptoms.

Most polyneuropathies are of metabolic origin, including DSPN, which is accompanied by an axonal type

of nerve damage. However, patients with DM also have a demyelinating type of nerve damage, which makes it necessary to differentiate DSPN from chronic inflammatory demyelinating polyneuropathy (CIDP).

Methods: We examined 32 patients with type 2 diabetes and a concomitant diagnosis of polyneuropathy of the lower extremities of varying degrees of severity. All patients underwent a neurological examination, evaluation of NISS-LL, TSS scales and stimulation ENMG examination of the nerves of the upper and lower extremities.

Results: All patients were divided into three groups according to ENMG data. The first group included patients with a predominantly demyelinating form of nerve damage – 16 patients. The second group with predominant axonal degeneration – 12 patients. The third group includes 4 patients with a mixed type (axonal-demyelinating). The sums of points in patients according to diagnostic scales (NISS-LL, TSS) depending on the stage of polyneuropathy increased with increasing severity of DPNP. During the analysis of disease histories, it was found that the experience of diabetes among patients of the 1st group is 1–5 years, they have a controlled blood glucose level and regularly do sports. In the II group, the majority are patients with diabetes for more than 5 years, whose blood glucose level is insufficiently controlled, episodes of hypo- or hyperglycemia are noted. Patients of this group are also characterized by moderate or low physical activity. In the III observation group, most patients have been sick for more than 10–13 years, they have an elevated blood glucose level, with frequent hypo- or hyperglycemic states, excess body weight and very low physical activity.

Conclusion: Based on the results of our study, we can conclude that the development of severe axonal-demyelinating polyneuropathy is directly correlated with diabetes experience and blood glucose control. Adequate physical activity of patients also plays an important role. At the same time, identification of the morphological type of polyneuropathy is important for prescribing specific therapy.

SEEING BEYOND THE SURFACE: ADVANCED ENDOSCOPY IN LARYNGEAL MALIGNANCY CLASSIFICATION

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Introduction: Enhanced contact endoscopy (ECE) – combination of contact endoscopy and NBI (Narrow band imaging) or IMAGE1S, is a non-invasive optical technique used for assessment of superficial vascular changes of mucosal lesions in high magnification. The aim of our study was to evaluate the diagnostic value of ECE in an intraoperative settlement.

Methods: Patients with laryngeal lesions underwent microlaryngoscopy with a structured assessment of the lesion using white light, NBI and ECE. Lesions were classified according to the European Laryngological Society Classification that divides the vascular pattern changes into longitudinal (unsuspicious) and perpendicular (suspicious). Evaluation was correlated with histopathology.

Results: 60 patients with 76 lesions were enrolled. Sensitivity, specificity, and accuracy for NBI assessment reached 71.4%, 100% and 78.6%, resp., Kappa index of 0.556. Sensitivity, specificity, and accuracy for ECE 86.4%, 89.5% and 87.3%, resp., Kappa index of 0.716. Additional 12% (9/76) of the lesions could be assessed with ECE compared to NBI.

Conclusions: Our data support the assumption that ECE is a useful tool for pre-histological examination of mucosal lesions, however it cannot fully replace biopsy sampling. ECE shows higher accuracy in detecting malignant lesions compared to NBI and can be especially helpful in the assessment of vocal fold leukoplakia.

Keywords: enhanced contact endoscopy; narrow band imaging; squamous cell carcinoma; laryngeal lesion; leukoplakia

INCREASED CARBOHYDRATE INTAKE DOES NOT WORSEN DIABETES COMPENSATION IN OLDER TYPE 2 DIABETICS

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Introduction: Especially in seniors the carbohydrate (CHO) intake is usually reduced due to high prevalence of type 2 diabetes (T2DM). However, low CHO intake can lead the lack of building blocks for regeneration and other anabolic processes that are accompanied by insulin resistance. The aim of insulin resistance can be inhibition of glucose oxidation and redistribution of glucose for growing ans regenerating tissues. The aim of our study was to assess the effect of increased CHO intake on blood levels of glucose, insulin and C-peptide in older patients with and without type 2 diabetes.

Methods: Twenty-eight patients were enrolled to prospective study (20 T2DM patients, and 8 non-diabetic). They received standard hospital diet during the first week of study. Throughout the second week they received 150 g of maltodextrin (Fantomalt Nutricia) divided to 3 daily doses immediately after the normal meal. Blood glucose level was monitored continuously by a subcutaneous sensor Medtronic. Mean glycaemia, time in range (TIR) and calculated HgA1 were evaluated. Additionally, plasmatic levels of C-peptide, insulin were measured on the first, seventh and fourteenth day in 7 subjects.

Results: Increased CHO intake led to transient increase of blood glucose levels in T2DM patients during the day

1–3. After 3 days the postprandial peak hyperglycaemias were blunted. CHO did not influence the percentual range of high glucose level and decreased a risk of hypoglycaemia. The change of plasmatic levels of C-peptide and insulin was minimal after addition CHO to diet.

Discussion: We found that increased CHO intake gradually decreased insulin resistance. This supports the idea that insulin resistance cause by preferred non-oxidative glucose metabolism can be improved by increased CHO intake. Therefore, CHO intake should not be decreased in patients who require nutritional support due to muscle loss and sarcopenia.

Conclusion: Liberal intake of CHO in diet can be recommended in malnourished patients with type 2 diabetes.

CLINICAL PATTERNS OF PSYCHICAL DISORDERS CREATED IN THE COURSE OF A FULL-SCALE INVASION OF UKRAINE IN MILITARY PERSONNEL

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Relevance: In recent times due to the situation in Ukraine related to the defense of its territorial integrity, starting with the situation in eastern Ukraine in 2014 with the operation of the Joint Forces (JF), in which tens of thousands of people are involved. Fighting in the country is characterized by a high level of stress. More than 85 thousand servicemen have been in the active combat zones of the country. It is important to determine what influence on the psyche has their military experience.

Objective: to study the peculiarities of the clinical picture of mental disorders acquired during the period of environmental protection.

Materials and methods: Taking into account the rules of bioethics and deontology and in the presence of informed consent for participation in the study, 40 patients were examined in 2023 on the basis of the Military Medical Clinical Center of the Northern Region of the Ministry of Defense of Ukraine. The age of all male patients ranged from 18 to 53 years. The following psychodiagnostic techniques were used: Mississippi scale of posttraumatic stress disorder, CES-D scale and traumatic events assessment scale.

Results: according to the results of psychodiagnostic study of Mississippi scale of post-traumatic stress disorder 30% of respondents (12 people) had a good level of adaptation with scores 35–96, and 70% (28 people) had an adaptation disorder with scores 100 and more when taking this test. According to the results of psychodiagnostic survey on CES – D scale 20% (8 people) of respondents had low level of fighting (10–13 points), 16% (7 people) average level (14–17 points), 28% (11 people) elevated level (18–21 points), 28% (11 people) with high level (22–25 points) and 8% (3 people) with very high level (26–28 points). Based on the results of the traumatic event impact assessment scale, 17% of respondents (7 people) had avoidance symptoms, 33% (13 people) had obsessive compulsive symptoms, and 50% (20 people) had agitation symptoms.

Conclusion: The combat experience of the majority of patients in the active combat zone ranged from medium to high. Only 8% (3 people) had a very high level of combat experience and 28% (11 people) had a high level of combat experience. The respondents' length of stay in the active combat zone ranged from 60 to 340 days. 17% of the respondents (7 people) had avoidance symptoms, 33% (13 people) had obsessive compulsive symptoms, and 50% (20 people) had arousal symptoms. Therefore, further in this direction the targeting of psychotherapeutic and psychoeducational programs in this category of patients will be considered.

LONG-STAY ICU PREDICTORS AFTER BENTALL PROCEDURE: RETROSPECTIVE STUDY

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Aim: To analyze the frequency of early postoperative complications and predictors of long-stay patients in the intensive care unit (ICU) after the Bentall procedure.

Materials and methods: This retrospective study is based on the obtained medical records of adult patients (aged 18–75), who underwent Bentall procedure for an ascending aortic aneurysm between 2012 and 2021. Depending on the length of ICU stay, all patients were divided into two groups: length of ICU stay up to 3 days (group 1) and length of ICU stay longer than 3 days (group 2). Univariate and multivariate analysis (logistic regression) was used to determine prognostic risk factors.

Result: Patients of the second group were characterized by reliably older age (p = 0.005), more frequent presence of arterial hypertension (p = 0.044) among concomitant diseases (p = 0.044) and significantly lower baseline glomerular filtration rate (GFR) (p = 0.045). In case of the second group patients in comparison with the first one, cell saver was used 3.6 times (p = 0.0005) more often and almost 6 times (p = 0.0037) more often, as well as the need for rethoracotomy due to bleeding, 4.3 times (p = 0.0002) more often acute renal failure renal failure and 3.3 times (p = 0.0004) more often acute respiratory failure. Multivariate logistic analysis revealed two independent predictors of long-stay ICU: duration of mechanical ventilation (OR 1.204 (CI 1.053–1.377), p = 0.007) and development of acute renal failure renal failure (OR 4.069 (CI 1.040–15.923), p = 0.044).

Discussion: Aortic reconstruction can pose a significant risk of postoperative complications and, accordingly, a long-stay ICU. Among postoperative complications after surgery for AAA, the prevalence of arrhythmias was 33.7%, acute renal failure – 14.6%, bleeding/tamponade – 14.6%, while hospital mortality was recorded at 15.6% [3]. In our opinion, such a high mortality rate, in contrast to our work, is due to the high frequency of emergency operations (43%) and the presence of concomitant

cardiac surgery. At the same time, the prevalence of acute renal failure and arrhythmias in the postoperative period was relatively similar to our study – 52.4% and 18.4%, respectively.

Conclusions: All patients must be treated in the same way to avoid per- and postoperative complications, but patients with risk factors for long-stay ICU should be under special attention to avoid additional complications and, subsequently, to reduce length of ICU stay.

Keywords: Bentall's procedure, length of ICU stay, ascending aortic aneurysm, risk factors.

EIF4F CONTROLS AMPK ACTIVITY IN MALIGNANT MELANOMA

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Introduction: Malignant melanoma is an aggressive form of cancer with poor prognosis, often caused by mutations in the MAPK ERK signaling cascade. Clinically relevant small-molecule drugs targeting BRAF and MEK kinases can prolong survival, but resistance rapidly emerges. Recently, the eukaryotic translation initiation complex (eIF4F) has been reported as the nexus of resistance to BRAF/MEK inhibitors. Furthermore, simultaneous inhibition of BRAF and eIF4F synergized in killing cancer cells. Therefore, we aimed to precisely characterize the crosstalk between the ERK and eIF4F pathways in melanoma.

Methods: We used MS-based proteomic approach and small-molecule MEK/eIF4F inhibitors to identify common targets of the ERK and eIF4F pathways in NRAS- and BRAF[V600E]-mutant melanoma cells. Then we validated the targets using RNA interference and western blotting.

Results: The proteomic analysis revealed a significant overlap of ERK and eIF4F targets in both melanoma subtypes. Interestingly, apart from the cell cycle and DNA repair regulators, we found regulators of the primary cellular energy sensor, AMP-dependent protein kinase (AMPK).

MO25, part of an AMPK-activating complex (LKB1-STRAD-MO25), and PP2Aα, an AMPK-inhibiting phosphatase, were found to be downregulated. Upon eIF4F inhibition, we observed ERK pathway activation and surprisingly, AMPK activation despite the downregulation of LKB1, the canonical activator of AMPK. This was also confirmed in LKB1-deficient BRAF[V600E]-mutant melanoma cells. These results suggest the existence of a novel, LKB1-independent mechanism of AMPK activation in melanoma cells.

Furthermore, PP2Aα downregulation seems to play an essential role, as RNAi-mediated knockdown of PP2Aα

and a small-molecule PP2A inhibitor both potently promoted AMPK activity.

Conclusion: Our findings reveal new insights into the molecular mechanisms of melanoma resistance and indicate the cooperation of the ERK and eIF4F pathways in controlling essential cellular processes. eIF4F inhibition promoted non-canonical, LKB1-independent AMPK activation with simultaneous ERK activation. We describe a novel mechanism of AMPK activity control in BRAF[V600E]-mutant melanoma cells.

LACTATE: THE ENEMY OF THE NEURONS IN THE PRESENCE OF GLUTAMATE TOXICITY – A STUDY IN CORTICAL PRIMARY CELLS

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Introduction: Limited substrate availability because of the blood brain barrier has made the brain develop specific molecular mechanisms to survive, using lactate synthesized by astrocytes, as an energy source in neurons. In primary cortical cells, lactate showed a beneficial and deleterious effect against glutamate toxicity depending on the conditions.

Methods: To understand if lactate was used as a preference energy source to rescue neurons after inducing glutamate shock, we treated primary cortical cells with oxamate (inhibitor of lactate dehydrogenase (LDH)), 50 mM glutamate, and lactate and/or glucose for 24 hours. LDH activity was detected in single neurons linked or not linked to glia cells by a redox reaction. The total cell number was counted in three random fields. Nuclei were stained with Dapi and the viable neurons were detected by MAP2.

Results: We showed that neurons linked to glial cells presented a higher LDH activity compared to not-linked neurons, supporting the hypothesis that lactate shuttle exists and interacts between astrocytes and neurons. Next, we examined the effect of glucose, lactate, and oxamate on neuron viability. In the presence of glucose, inhibition of LDH by oxamate decreased viability of neurons. However, addition of oxamate to cells incubated in media containing lactate elevated survival of neurons.

Discussion and preliminary conclusions: Considering mechanisms related to the tricarboxylic acid cycle, we assume that these effects of lactate are the result of switching between pyruvate and glutamate consumption in mitochondria. For example, the addition of oxamate in media with lactate decreases the formation of pyruvate and as a compensation, increases consumption of glutamate, reducing glutamate toxicity. In the brain, when neurons do not have access to glucose, lactate is transported to neurons from astrocytes. However, this energy source is deleterious in the presence of toxic glutamate concentrations because it facilitates uptake of pyruvate by mitochondria.

PLATELET FUNCTION TESTING – A SINGLE CENTRE EXPERIENCE

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Introduction: Aim of the study is to evaluate the laboratory effectiveness of the most used antiplatelet drugs in our centre (clopidogrel, ticagrelor, aspirin) and to assess the effectiveness of the changed treatment in patients in whom we found in vitro resistance, or high on-treatment platelet reactivity (HTPR) to clopidogrel.

Methods: In our set of patients, the effectiveness of antiplatelet drugs was tested using the light transmission aggregometry (LTA) method from 16. 9. 2020 to 31. 12. 2022. The effectiveness of treatment in patients switched to an alternative medicine was monitored and the success of the change in therapy was evaluated.

Results: In the monitored period, a total of 529 blood samples were tested for clopidogrel (n = 216), ticagrelor (n = 59) and aspirin (n = 254). The efficacy of clopidogrel treatment was 45.4%. On the other hand, the efficacy of ticagrelor was 93,2%, statistically significantly higher (p < 0.000). In case of conversion from clopidogrel to ticagrelor, treatment was successful in 49 of 54 patients (90.7%).

Discussion: To effectively prevent thromboembolic events during endovascular procedures, it is necessary to correctly identify patients in whom ineffective antiplatelet treatment could cause thrombosis, occlusion of an implanted stent or an ischemic stroke. Currently, there is no standardization among neurointerventional centers in testing the effectiveness of antiplatelet treatment, nor antiplatelet therapy guidelines. Identifying the incidence of HTPR in our population is the first step on the way to standardization.

Conclusion: For the antiplatelet drugs tested, statistically significant differences in the effectiveness were demonstrated. In the case of clopidogrel, ticagrelor is a suitable alternative, significantly reducing incidence of HTPR. The number of patients with HTPR undergoing stent/flow diverter implantation can be significantly reduced by including platelet function testing in the algorithm of endovascular procedures.

CIRCADIAN LOCOMOTOR DYSRHYTHMIA AND THERAPEUTIC POTENTIAL OF METHYLPHENIDATE IN A RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Introduction: Early diagnosis of sporadic Alzheimer's disease (sAD) presents a difficult challenge, with only recently emerging disease-modifying therapy. Epidemiological and animal studies have highlighted circadian dysrhythmia and an Attention Deficit Hyperactivity Disorder-like phenotype as risk factors and plausible etiopathogenetic factors, scarcely investigated in AD patients and inadequately modelled in animal AD models. We aimed to investigate these parameters in a rat model of AD induced by intracerebroventricular streptozotocin (STZ) and how they are affected by oral methylphenidate (MPH).

Methods: Three-month old male Wistar rats (n = 40) were injected intracerebroventricularly with citrate buffer (control/CTR) or STZ (3 mg/kg) split in two doses 48 hours apart after which MPH therapy was initiated daily in a dual-bottle dosage regimen (4 and 10 mg/kg) for 6 weeks to half of CTR and STZ groups. Cognitive ability and locomotor activity were assessed by Novel Object Recognition (NOR) and Open Field (OF) at 2, 4, and 6 weeks after STZ. Baseline and 6-week continuous measurements of cognition, locomotion and impulsivity were done by custom home cage apparatus in an operant conditioning task (VlaDiSlav test), and the Multicage InfraRed Open Source Locomotor Activity eValuator (MIROSLAV).

Results: At 2 weeks, STZ demonstrated worse performance than CTR (NOR) but this deficit was ameliorated by MPH. No change in NOR were observed at 4 and 6 weeks between the groups. Learning curves in continuous testing using VlaDiSlav show a severely diminished learning rate in STZ group, however, STZ+MPH learning rate was similar to CTR values. STZ+MPH group also showed reduced impulsivity compared to all other groups. OF thigmotaxis was slightly increased in CTR relative to other groups, with no differences in total distance traversed. Preliminary data (MIROSLAV) demonstrated reduced nocturnal and increased diurnal activity in the STZ group.

Discussion: VlaDiSlav continuous measurement gives insight into the learning process suggesting that STZ induced a severe learning deficit that might be normalized by MPH treatment. These novel findings also highlight the benefits of supplementing conventional behavioural testing with continuously operating, automatised, programmable home cage-based devices. Preliminary MIROSLAV data indicate the presence of a circadian dysrhythmia with similar parameters to those observed in AD patients, unlike those observed in other AD animal models.

Conclusion: Novel non-invasive methodology points to preliminary robust beneficial effects of MPH on cognitive performance in the STZ model of sAD,but further

analysis is necessary to better describe the complex STZ phenotype and the effects of MPH.

DISTANCE FROM MAIN ARTERIES IS ASSOCIATED WITH MICROSTRUCTURAL AND FUNCTIONAL BRAIN TISSUE CHARACTERISTICS

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Introduction: Given the substantial dependence of neurons on continuous supply of energy, the distribution of major cerebral arteries opens a question whether the distance from the main supply arteries constitutes a modulating factor for the microstructural and functional properties of brain tissue.

Methodology: To tackle this question, multimodal MRI acquisitions of 102 healthy volunteers over the full adult age span were utilised to quantify microstructural (cellularity, myelin density, iron concentration) and functional (connectivity and neural activity) tissue characteristics in various brain regions and ascertain their dependence on the distance from the closest major artery.

Results: Our result point to higher iron concentration and higher cellularity in areas more distant to main arterial trunks. In the cortex, these findings were combined with lower intracellular volume fraction and higher myelin density, suggesting relatively higher abundance of smaller cells and/or myelinated small axons farther away from main arteries. In the white matter, bigger distance from main arteries was associated with relatively higher intracellular volume fraction and increased myelin density. Furthermore, age emerged as a substantial factor influencing this pattern.

Conclusion: With higher age, cortical areas more distant from arteries show lower intracellular volume fraction, pointing to their potentially higher vulnerability. All in all, this pilot study provides a novel insight on brain regionalisation based on artery distance, which merits further investigation to validate its biological underpinnings.

THE EFFECTS OF PRENATAL PRAVASTATIN TREATMENT IN THE RABBIT FETAL GROWTH RESTRICTION MODEL

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Introduction: Fetal growth restriction (FGR) remains one of the main contributors to perinatal mortality and morbidity. Although there is some clinical evidence supporting the benefit of early postnatal strategies, no prenatal strategies have accomplished positive clinical results. Research on murine FGR models suggests the potential benefits of prenatal pravastatin. Given that the rabbit hemodichorial placenta more closely resembles the human condition, we investigated the effects of prenatal maternal pravastatin administration in the rabbit FGR model.

Methods: On gestational age 25 (term 31 d) pregnant dams (n = 14) underwent partial uteroplacental vessel ligation (UPVL) in one uterine horn to induce FGR, leaving the other horn as control. Dams were randomized to either receive pravastatin 5 mg/kg/d dissolved in their drinking water or normal drinking water until delivery. On GA 30d rabbits were delivered and grouped into four categories: controls without pravastatin (C/NoPrav) (n = 33), FGR without pravastatin (FGR/NoPrav) (n = 36), FGR with pravastatin (FGR/Prav) (n = 30), and controls with pravastatin (C/Prav) (n = 32). Newborn rabbits underwent pulmonary function assessment using the FlexiVent system, neurobehavioral assessment, and were harvested for alveolar morphometry or neuropathology. Placentas were analyzed for histological and RNA expression.

Results: Birthweight was lower in the FGR groups (FGR/Prav, FGR/NoPrav), but there was no difference between FGR/Prav and C/NoPrav. No differences were observed in placental zone proportions; however, eNOS in FGR/Prav placentas and VEGFR-2 in FGR/Prav and C/Prav were upregulated. There were no differences in pulmonary function assessment and alveolar morphometry. FGR/Prav kittens had increased neurosensory scores on postnatal day 1, but there were no differences in neuromotor tests, neuron density, apoptosis, and astrogliosis.

Discussion: Hydrophilic pravastatin primarily affects maternal and placental tissue. Thus, improvement of fetal or neonatal outcomes would likely depend on enhanced placental function. However, in our study, this was not the case, as our primary outcome, birthweight,

was not improved. Additionally, no differences were observed in pulmonary function, alveolar morphometry, or brain neuron density. On the other hand, pravastatin was associated with a small but significant increase in neurosensory scores. Although this study was not powered to detect small effects on secondary outcomes, these findings warrant further investigation.

Conclusions: In the rabbit FGR model, pravastatin upregulated expression of VEGFR-2 and eNOS in FGR placentas and was associated with higher neurosensory scores, without a measurable effect on birthweight, pulmonary function and morphology, or neuron density.

LYMPHOCYTE SUBPOPULATION CHANGES IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder caused by increased platelet destruction and altered production. Although some patients achieve long-term remission after 1st line therapy, almost 80% ITP patients require further treatment. Current guidelines consist of monotherapy with different drugs, with no tool to predict individual response.

Aim: In our study, we evaluated laboratory parameters in patients with ITP with the aim of identifying a prognostic marker for a poor response leading to chronicity and/or refractoriness to treatment.

Methodology: To our project, we collected data from 35 patients with primary ITP (newly diagnosed or relapsed disease). We assessed blood count, antiplatelet autoantibodies, and T lymphocyte subsets in peripheral blood at 0, 3, 6 and 12 months during follow-up. Using multicolour flow cytometry, we determined relative and absolute numbers of lymphocytes, T cells, and their subpopulations: CD4+ helper T cells (including IFN-γ+/CD4+ T cells, IL-4+/CD4+ T cells, IFN-γ+/IL-4+/CD4+ T cells and IL-17A+/CD4+ T cells), CD8+ cytotoxic T cells (including IFN-γ+/CD8+ T cells, IL-4+/CD8+ T cells and IFN-γ+/IL-4+/CD8+ T cells) and ratio of CD4 + to CD8+ T cells.

Results: Our data show that the value of antiplatelet autoantibodies, the percentage of cytotoxic T lymphocytes, and the immunoregulatory index (IRI, CD4+/CD8+ T lymphocyte ratio) differ significantly by treatment response. Responders have a higher IRI (P = 0.04), higher antiplatelet autoantibodies (P = 0.01) and lower CD8+ T cells relative count (P = 0.2) before treatment.

Discussion: The results suggest that antiplatelet autoantibodies and subsets of T lymphocytes in peripheral blood (especially CD8+ CD3+ lymphocyte relative count of CD8+ CD3+ lymphocytes and ratio CD4+ / CD8+) could be used as a prognostic tool for a worse clinical outcome in patients with ITP.

Conclusion: These biomarkers could be utilised for stratification and eventually selection of treatment preferring combination therapy in patients with unfavourable immunological setting.