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High *VISTA*, *SNAI1* and *SNAI2* mRNA expression in resected non-small cell lung cancer is associated with shorter patients' overall survival

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Introduction: Non-small cell lung cancer (NSCLC) is often diagnosed at an advanced stage and can be surgically removed in only 30% of patients. The resected tumour tissue is valuable for investigation of tumour metastatic potential, epithelial-mesenchymal transition (EMT) and tumour immune microenvironment (TIM), as relevant factors for follow-up of these patients. Oncoprotein IMP3 is associated with the metastatic potential of tumour cells, *SNAI1* and *SNAI2* genes are expressed in EMT, while *VISTA* gene is associated with tumour immune evasion. Our aim was to analyse this interaction between EMT and tumour immune evasion on human specimens.

Methods: 55 patients who underwent lobectomy/lymphadenectomy for NSCLC at University Hospital of Split, Croatia from 2013 to 2017 were included. Their clinical data were collected from hospital records and paraffin blocks from the Department of Pathology. *SNAI1*, *SNAI2* and *VISTA* mRNA were analysed by q-RT PCR, and IMP3 by immunohistochemistry. The results were compared with clinical-pathological parameters and overall survival (OS). $p<0.05$ was set as statistically significant.

Results: There were 37 (67%) men and 18 (33%) women, median age 64 (range 49-83) years. Histological types were 34 (62%) adenocarcinoma and 21 (38%) squamous carcinoma. 35 (63%) tumours were in stage T1, and 50 (90%) in N1. Lympho-vascular invasion was found in 55% of cases. The resection margin was positive in 6 cases. High *SNAI1 and SNAI2* mRNA were associated with lympho-vascular invasion, high *SNAI2* with higher T stage, and high *VISTA* with positive resection margin. IMP3 expression, as well as higher mRNA expression of all analysed genes were connected with lower OS.

Conclusions: High expression of *VISTA, SNAI1* and *SNAI2* mRNA in surgically resected NSCLC is correlated with shorter patient's overall survival.

Key words: NSCLC, surgery, overall survival, *VISTA, SNAI1, SNAI2,* IMP3 oncoprotein

The Japanese Approach; Bathing and Dietary Habits as COVID-19 Deterrents

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Introduction: The COVID-19 pandemic has affected countries worldwide. Further investigation was conducted to explore the correlation between two Japan's unique cultural habits, traditional Japanese cuisine and bathing, and the incidence of COVID-19. However, more research is still needed to determine if these habits have the potential to reduce the infection rate.

Methods: We conducted a Google Forms survey targeting Japanese medical students from March 20th to April 12th. We collected data on bathing and dietary habits and multiple factors such as water temperature, sleep time, risk factors, and vaccination status. After gathering the data, we executed descriptive analytics and logistic regression on Microsoft Excel.

Results: 224 medical students, 81 males and 143 females aged 19 to 29, responded to our questionnaire. The estimated coefficients for three predictor variables were -1.18, -0.30, and 0.32 for constant term, frequency of bathing, and dietary habits, respectively. The coefficient for the frequency of bathing (-0.30) indicates that an increase in the frequency of bathing is associated with a decrease in the log odds of having COVID-19 when all other variables are held constant. The coefficient for dietary habits (0.32) suggests that individuals with a higher level of interest in their diet are associated with increased log odds of having COVID-19. The COVID-19 infection rate was 44% for individuals bathing at a temperature of 39°C or lower, and 33% for those bathing at 40°C or higher. Among individuals who used bath additives almost daily, the infection rate was 48%, whereas those who used them occasionally or did not use them had a 34% infection rate.

Conclusions: Dietary habits affect the infection rate of COVID-19 negatively. Bathing has a positive effect and can lower the risk of COVID-19 infection if done under appropriate conditions. A more effective diet for immunity is focusing on Japanese food and actively incorporating Western-style food as supported by data published by Japanese government

agencies such as the Ministry of Health, Labor and Welfare in Japan. However, caution has to be paid in interpreting this study as this is an ecological study.

Keywords: Bathing habit; Dietary habit; COVID-19; Japan

Allergy detection to a panel of selected nutritional allergens: the presence of common epitopes

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Introduction: Allergy is the body's reaction to substances (allergens) that the body perceives as foreign and harmful. When the body detects a substance (pollen, animal hair, drug, or food ingredient) as a foreign body, it triggers a whole cascade of defensive reactions in the immune system, which is called an allergic reaction. It can appear immediately or after a few hours after contact with the allergen. Food allergy is mediated by IgE, T-cells or both. IgE-mediated allergy (eg. urticaria, asthma, anaphylaxis) is initially acute, usually develops in early childhood and is more common in those with a positive family history of atopy. After testing, if the prick skin test is positive, that food is excluded from the diet, and if the symptoms improve, the patient takes that type of food again (a double-blind trial) to determine if the symptoms reappear. The main aim of this research was to determine the potential presence of food allergy in individuals from 20 to 22 years and prone to allergies.

Material and methods: Nine allergens (peanut, almond, walnut, strawberry, egg white, kiwi, apple, garlic, honey) and a positive control - histamine were tested at the same time. After applying the allergen, these places are lightly pricked with a lancet so that the allergen reaches the surface layer of the skin. After 20 minutes, skin reactions were read at the places of applied allergens. The appearance of redness, swelling and itching in a certain marked place is a reliable sign of an allergic reaction of the body to the corresponding allergen. Current study included 23 respondents in Laboratory for Biochemistry and Physiology, aged 20-22 that were part of a preliminary assessment based on possible allergy symptoms.

Results: Results showed that most (of examinees had no allergy reactions to all potential allergens, while 13.05% (n=3) of them had a moderate allergy to almonds, peanuts, and walnuts. Two of them (8.7%) had a strong allergic reaction to peanuts (urticaria diameter greater than 15 mm). One examinee showed a strong allergic reaction to egg white (redness diameter 28 mm); while one examinee showed extremely strong allergic reactions to egg white, peanut, walnut, and kiwi (urticaria diameters: 29 mm, 21 mm, 16 mm and 15 mm respectively).

Conclusion: These results suggest the existence of common antigenic epitopes in different types of food (nutritional allergens). Purified proteins that are specific to a certain food may be useful to include in patient routine prick tests. Further investigations should be obtained in terms of IgE analysis in allergic individuals to different types of food.

Keywords: food allergy, IgE, urticaria, epitopes

A phylogenetic method for estimating COVID-19 transmission rate using coronavirus genomes

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Introduction: The COVID-19 pandemic brought a wealth of genomic data, revealing shortcomings in current methods for interpreting phylogenetic data. Traditional techniques for estimating the transmission rate of viral infections rely on transmission equilibrium between populations and are not suitable for large datasets. To address this, a phylogenetic software package called transRate was developed to estimate transmission rates using pathogen genomes. This method is better suited for transmission rate estimation on a large dataset in a non-endemic state.

Methods: The software package, transRate, estimates a phylogenetic tree from pathogen genomes and identifies significant clades based on sequence labels and bootstrap support. Our simulation study showed that transRate can accurately estimate transmission rates even in the presence of phylogenetic tree uncertainty and transmission events that occur through various stochastic processes. In real data analysis, clades are identified by geographical origin and bootstrap support, and geographical outliers are inferred to define possible transmission events. Using transRate, we analyzed 40,028 high-quality sequences of SARS-CoV-2 in human hosts during the early pandemic.

Results: In February 2020, notable transmission occurred between the Americas, Asia, Europe, and Oceania. The average transmission rate for this time point is approximately 6.8%. An estimated average transmission rate of around 2.3% was found in the time-point of March 2020. There was an increased prevalence of transmission events. The World Health Organization had recorded a total of 750,890 laboratory-confirmed cases as of March 31, 2020.

Conclusion: Our analysis revealed that travel restriction mandates did affect transmission between populations in March 2020, but there was likely an increase in transmission events within populations at this time. The development of transRate enables scientists and public health officials to better understand the progression of the COVID-19 pandemic and any future viral outbreaks. During the early stages of the pandemic, public health officials had to take decisive action with limited information about the method of infection and transmission rates of the emerging threat. With the growing abundance of public databases for genomic

sequences and the ease of use of transRate, this methodology could become more prevalent in tracking and preventing the spread of such diseases.

Keywords: Phylogenetic tree, cladistics, gene flow, SARS-CoV-2, COVID-19, species tree, gene tree, transmission rate

Vaping cessation resources for young adults: an observational audit of north american quitline consortium websites

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Introduction: Over 55% of young adults (YAs, ages 18-24) express a desire to quit vaping, but only about 37% report trying. The National Cancer Institute (NCI) prioritizes YAs as a vulnerable population needing more interventions. Some support exists for youth (under 17), but fewer resources are targeted to YAs. The present study assessed the availability of vaping information and cessation resources targeted for YAs on North American Quitline Consortium (NAQC) Quitsites.

Methods: NAQC Quitsites were observationally audited to locate vaping cessation resources for each of the 50 US states, the US Capital, and 13 Canadian provinces. An audit checklist was used to assess websites on the presence of cessation information and resources targeted to YAs. Inclusion criteria included "ENDS, vaping, e-cigarettes, nicotine, cessation, quitting," etc.

Results: Five broken Quitsites were excluded, and 32/59 (54.2%) of Quitsites had no observable information or support for YA-targeted vaping cessation. 48/59 (81.4%) Quitsites had no YA-targeted vaping cessation resources linked from the homepage or dedicated pages for the topic, while 18/47 (38.2%) of Quitsites had no mention of vaping or ENDS anywhere, whether concerning cessation or general information – all Canadian Quitsites contained vaping information. 47/59 (79.7%) Quitsites had no YA representation in images or videos anywhere.

Conclusion: NAQC Quitsites currently offer little support for YAs interested in quitting vaping, as more than half lack YA-targeted vaping cessation resources. About one-third of Quitsites lacked any mention of vaping. As we learn more about why YAs are unable to quit as much as they desire, Quitsites are encouraged to add resources targeted to this demographic to facilitate their journey toward quitting vaping. Closing this information and support gap will improve our perception of the risk associated with vaping, leading to healthier decisions by Young Adults and other vulnerable populations. Supported by the NCI and FDA (TCORS Grant U54CA228110).

Keywords: Young Adults, E-cigarettes/Electronic Nicotine Delivery Systems (ENDS), Flavored tobacco, Cessation

Characterization of immune signaling in African spiny mouse in response to skin wounding

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Introduction: Prior studies have detailed the unique mammalian regenerative capability of the African spiny mouse *Acomys Cahirinus (Acomys)* that lacks distinct fibrotic scarring observed among other mammals across numerous tissues including skin, muscle, spinal cord, and heart. Identifying molecular mechanisms which confer this regenerative capability offer an opportunity to further understand and extrapolate the regenerative process to non-regenerating mammals such as humans. Analysis between *Acomys* and closely related, non-regenerative mouse *Mus Musculus (Mus)* provides a comparative basis for the enabling of successful wound-healing. This study involves characterizing the involvement of cytokines, immune response signaling proteins, to theregeneration process.

Methods: Characterizing immune response is complicated by individual cytokine signaling factors often acting as mutual regulators. As such, this project consisted of profiling cytokines present in bothregenerative and non-regenerative wounds. This provided a comparative basis of immune signaling factors that might alter the course of the wound-healing process. Following experiments determined whether differences in cytokine presence influenced the migration of skin cells across a simulated wound. Cells from both models were exposed to each respective signaling factor solution to determine how rates of simulated wound closure were affected.

Results: Profound differences in cytokine profiles exist between regenerative and non-regenerative models. Relative to *Acomys*, *Mus* expressed over thirty unique pro-inflammatory cytokines. Moreover, *Mus* similarly exhibited greater concentrations of all common immune signaling factors. *Acomys* exhibited only a single unique cytokine. The regenerative model additionally exhibited 1/3 total protein concentration relative to its fibrotic counterpart. Results were corroborated by both models' cells demonstrating faster rates of simulated wound closure when exposed to regenerative signaling factor solution. Over an observed 25hr period, dermal fibroblast cells of both species covered a simulated wound *in vitro* faster upon exposure to *Acomys* cytokines as opposed to *Mus*. *Acomys* cells exhibited an exponential rate of closure overthe first 6hrs following exposure while *Mus* migration occurred at a linear rate. Both species' cells migrated at a constant, linear rate following the initial 6hr period.

Conclusions: Rather than unique signaling factors, the regenerative model exhibits suppression of numerous proteins implicated in immune response. This suggests that a lesser activation of immune response contributes to regeneration. Following the identification of dysregulated cytokine expression implicated in immune response following injury, future work will determine specificmolecular pathways that direct regeneration rather than a fibrotic, scarring response. Identifyinga cytokine responsible for immune system repression in the regenerative model might provide evidence for potential development of therapeutic wound-healing treatments.

Keywords: Regeneration, Fibrosis, Inflammation, Cytokine

The Incidence of opportunistic viral infections in hematopoietic stem cells pediatric recipients

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Introduction: Opportunistic viral infections represent major causes of morbidity and mortality among children with hematopoietic stem cell transplantation (HSCT). Modern molecular diagnostics has enabled continuous patient monitoring after HSCT, which might prevent the development of disease, rapid graft failure, and graft-versus-host disease (GVHD) and decrease the health burden.

Methods: This study aimed to determine the incidence of Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Herpes simplex virus (HSV), Human herpesvirus 6 (HHV6), and BK virus among pediatric patients after HSCT. This is a prospective study involving 21 children with HSCT. The frequency of viruses in blood and urine was examined using PCR and Real-time PCR methods.

Results: During the early post-engraftment period, the determined incidences of CMV and EBV were 16.7% and 31.3%. The highest viral load of CMV was 27300 copies/ml and the lowest was 63 copies/ml. A significant increase was observed for positive tests of EBV in boys (p=0.022) and a significant decrease for the urine-sample BKV in girls (p=0.031). The presence of HSV was identified only during the 6th,7th, 8th, and 12th testing (4.8%; 4.8%; 4.8%; 5.9%) and for HHV6 only during the 7th testing (4.7%). The observed incidences of BK virus in blood and urine were between 56.3-78.6% and 50-70%, respectively.

Conclusion: The results showed that the frequency of the observed viruses after HSCT was maintained at high levels, especially for the BK virus in blood and urine samples, which were continued even after three months of transplantation, unlike other viruses. In addition, during the early post-engraftment period, when the risk of infections is the highest, the highest frequencies of CMV and EBV were observed. Studies on larger cohorts of pediatric HSC recipients and of longer duration would provide more reliable data on the frequency of opportunistic viral infections, the development of disease, and the impact on GVHD.

Keywords: hematopoietic stem cells transplantation (HSCT); opportunistic viral infection; Herpesviruses (CMV, EBV, HHV6, HSV); BK Polyomavirus

Newly diagnosed DM1 in children and adolescent during the COVID-19 pandemic in the Republic of Serbia: experience from the University Children's Hospital

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Introduction: Public health measures, parental fear of infection and redeployment of medical resources in response to the COVID-19 pandemic might have led to a decrease in pediatric healthcare access and utilization. As a result, a delay in type 1 diabetes diagnosis might have occurred, leading to the worsening of its clinical presentation in the pediatric population.

Methods: The study population included children and adolescents less than 18 years old presenting to the University Children's Hospital with a new diagnosis of DM 1. The clinical and biochemical features of diabetes observed during the COVID-19 period from April 1, 2020, until December 31, 2022, were compared with the period from April 1, 2017, until December 31, 2019.In the COVID-19 pandemic group, the clinical and biochemical features were compared betweenchildren with and without SARS-CoV-2 infection at diagnosis or before the diagnosis of DM 1.

Results: During the COVID-19 pandemic, observed frequencies of DKA and severe DKA at diagnosis were both significantly higher than during the pre-pandemic period (47.6% vs 32.6%, p = 0.011, and 20.7 vs 9.4, p = 0.01, respectively). In the COVID-19 group, blood pH levels were significantly lower than in the pre-pandemic group (7.245 ± 0.175 vs 7.317 ± 0.139, $p < 0.001$) while HbA1c levels were higher (11.041 ± 2.052 vs 10.613 ± 1.389, $p = 0.045$). Clinical and biochemical features of diabetes in children with SARS-CoV-2 infection at or before the diagnosiswere not significantly different compared to children without an infection.

Conclusion: We report a significant worsening of the clinical presentation of newly diagnosed DM 1 and an increase in the frequency of DKA and severe DKA at diagnosis during the COVID-19 pandemic. Further studies are necessary to gain quantitative insight into pediatric healthcare availability and access in the Republic of Serbia.

Keywords: COVID-19; Type 1 diabetes mellitus; Diabetic ketoacidosis; children and adolescents

Amino acid supplementation partially rescues the growth defect of Taz1 deficient yeast cells

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Introduction: Barth syndrome (BTHS) is a recessive genetic disorder. It affects mitochondria-rich organs like the heart and skeletal muscles. BTHS is caused by a mutation in the tafazzin (TAZ) gene. TAZ participates in a mechanism of cardiolipin (CL) maturation. CL is a phospholipid located in the inner mitochondrial membrane. It provides membrane homeostasis and cell integrity. CL mutations make mitochondrial energy production less effective and shorter cell-life. The physiology behind CL synthesis is not completely understood patients with BTHS are treated symptomatically. One of new treatments is a high-protein diet. In some research amino acids (AA) improve cell growth. That is why we are interested in what effects supplementation of AA has on TAZ-deficient *S. cerevisiae* cells.

Methods: Study used *S. cerevisiae* model and harvesting included streak plate method, overnight liquid yeast cultures, spotting agar assay and cell washing.

Results: Yeast took three days to grow on glucose (SMD, YPD, and SCD), four days to grow on YPPyruvate and SCPyruvate, and seven days to grow on SMPyruvate media. SMPyruvate has the same composition as SCPyruvate except for a minimal AA mix. Based on these findings, we decided to test what AA in SCPyruvate cause better growth than in SMPyruvate. AA from SCPyruvate were added to SMPyruvate, separately. The yeast took nine days to grow on SMPyruvate, SMPyruvate + Typ, SMPyruvate + Thr, SMPyruvate + Arg, and SMPyruvate + Lys, and three days on SMD media. Results showed that WT and taz1Δ strains, on SMPyruvate + Lys and SMPyruvate + Arg, grow similarly. However, *S. cerevisiae* grows best on SMD media.

Conclusion: Results establish that AA supplementation can potentially improve altered taz1Δ mitochondria causing BTHS. AA are used in anaplerotic pathways to compensate for the mitochondrial inability to use oxidative phosphorylation and act as antioxidant to diminish the accumulation of reactive oxygen species and thicken the mitochondria membrane to slow down proton leak.

Keywords: Barth syndrom, amino acids, anaplerotic pathways, pyruvate media.

Significant improvement in BCVA after EDOF lens implantation in patients with post-phacovitrectomy IERM

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Introduction: Vitreoretinal diseases and cataracts commonly coexist, and combined phacovitrectomy is shown to be a safe and effective therapy for these conditions. In this research, we aimed to evaluate the best-corrected visual acuity (BCVA) of extended depth-of-focus intraocular lens (EDOF IOL) implantation and to compare them with monofocal IOL implantation in patients with idiopathic epiretinal membrane (IERM) after phacovitrectomy.

Methods: Our study included 20 participants, who were randomized into two groups: 10 patients with monofocal IOLs and 10 patients with EDOF IOLs. All patients underwent standard 25-GA three-port sutureless pars plana vitrectomy (PPV), followed by IERM and inner limiting membrane (ILM) peeling. In all patients, phacoemulsification was performed, and depending on the group, monofocal or EDOF IOLs were implanted. All patients were examined one day and one week after the surgery in order to detect possible post-surgery complications. Furthermore, BCVA was determined one month and sixth months after the procedure.

Results: A significant improvement in BCVA was found at one and six months after EDOF IOL implantation (when comparing baseline vs. one month, one month vs. six months, and baseline vs. six months, the p-values were <0.001 for all comparisons). On the other hand, patients with monofocal IOLs showed a significant improvement in BCVA only for the comparison between the baseline BCVA and BCVA 6 months post-surgery ($p<0.001$). However, there was no significant difference when comparing the BCVA of patients with monofocal IOLs and the BCVA of patients with EDOF IOLs at three months and six months postoperatively ($p=0.739$ and $p=0.606$, respectively).

Conclusion: Both monofocal and EDOF IOLs yielded similar outcomes in terms of BCVA. PPV with EDOF IOL implantation is an effective procedure for improving BCVA in patients with IERM.

Keywords: monofocal intraocular lens, extended depth-of-focus intraocular lens, phacovitrectomy, idiopathic epiretinal membrane

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Schnitzler syndrome: a case report

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Introduction: Schnitzler syndrome (SchS) is a rare late onset autoinflammatory disease, characterized by the association of monoclonal immunoglobulin (Ig) M gammapathy and urticarial rash, and at least two additional minor criteria, such as fever, arthralgia, lymphadenopathy, hepatosplenomegaly, increased markers of systemic inflammation, or abnormal findings on bone imaging. Although not yet fully understood, interleukin-1 (IL-1) plays a critical role in the pathogenesis of SchS, which is supported by the excellent responses of patients to IL-1 blocking therapies.

Case description: A 46-year-old female patient presented to our department with recurrent fever, a disseminated burning urticarial rash, as well as bone pain for several years. Laboratory investigations revealed leukocytosis with neutrophilia, elevated C-reactive protein, and high levels of bone alkaline phosphatase. Immunofixation detected IgM kappa monoclonal gammopathy. Neither high-dose antihistaminics, colchicine (1 mg/day) nor dapsone effectively prevented the relapse of inflammatory episodes. Histopathology of lesional skin showed a dermal neutrophilic infiltrate. Radiological imaging of the bones revealed multiple osteolytic lesions, particularly in the lower limbs. SchS was definitively diagnosed based on the Strasbourg criteria. Therefore, anakinra (100 mg/day), a recombinant human IL-1 receptor antagonist, was initiated and led to a rapid improvement of the skin symptoms within 24 hours. However, bone pain persisted, and temporary treatment discontinuation for two days resulted in a relapse with re-emergence of disseminated urticaria accompanied by fever, bone pain, and elevated inflammatory markers. Treatment with canakinumab (150 mg administered subcutaneously once every 4 weeks), a human IgGκ IL-1β monoclonal antibody, was secondarily started which completely resolved her symptoms few days after the first injection.

Discussion: More than 350 cases have been reported to date, but it is likely to be highly underdiagnosed. The effectiveness of anakinra implies a crucial pathophysiological role of IL-1, but does not discriminate between IL-1α and IL-1β. The burden of painful daily injections increased the need for longer-acting agents, and in vitro findings hinted at IL-1β as the key proinflammatory cytokine involved in the pathophysiology which might explain the benefit of long-acting IL-1β antagonist in our patient. Follow-up of a large cohort of patients with SchS is necessary to better assess the response and safety of canakinumab.

Keywords: Schnitzler syndrome, autoinflammatory disease, urticaria, monoclonal gammopathy, Interleukin-1 blocking therapy

Lactose as a common excipient vs. lactose intolerant patients- a literature review

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Introduction: Lactose intolerance, characterized by the inadequate enzymatic hydrolysis of lactose, poses a significant challenge to individuals consuming medications containing lactose as an excipient. The prevalence of lactose intolerance necessitates a comprehensive examination of the implications of such consumption on patient outcomes and safety.

Methods: A literature review was conducted to identify studies assessing the effects of consuming drugs containing lactose among individuals with lactose intolerance. Databases including PubMed and Web of Science were searched using relevant keywords such as "lactose intolerance," "drugs," and "adverse effects." Studies written in English were included. Case reports, observational studies, and clinical trials were considered to provide a comprehensive overview.

Results: A total of 10 studies met the inclusion criteria and were analyzed. The literature highlights a lack of standardized reporting on lactose content in medications, hindering accurate assessment of exposure risk. Adverse reactions ranged from mild gastrointestinal symptoms, such as bloating and flatulence, to more severe manifestations like abdominal pain and diarrhea. In some instances, patients with lactose intolerance demonstrated increased frequency and severity of adverse effects compared to those without the condition. However, variations in study design, lactose content measurement, and patient populations limit the ability to establish a definitive correlation. Notably, a subset of studies indicated that adverse effects can be mitigated by communicating with pharmacists to identify lactose-free alternatives, prescribing lactase formulations and reassuring patients of minimal lactose doses to induce nocebo effect.

Conclusion: The consumption of drugs containing lactose by individuals with lactose intolerance presents a complex clinical scenario. While existing literature suggests a potential for adverse reactions, the heterogeneity of studies, variability in lactose content reporting, and the lack of standardized testing methods impede definitive conclusions. Healthcare professionals should exercise caution when prescribing medications to lactose-intolerant patients, particularly in cases of self-administration. Consideration of patient history, severity of intolerance, and the formulation of the medication should guide clinical decision-making. Further research employing rigorous study designs, precise lactose content quantification, and larger patient cohorts is warranted to provide a more conclusive understanding of the interplay between lactose-containing drugs and lactose intolerance.

Keywords: Lactose Intolerance; Pharmaceutical Preparations; Adverse Events

Severe celiac disease following immune checkpoint inhibitor treatment – a case report

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Introduction: Immune checkpoint inhibitors (ICIs) have developed into a potent clinical approach for the treatment of malignancies, although they can cause inflammatory side effects, known as immune-related adverse events (irAEs) by boosting immune system. Celiac disease (CD) induced by ICIs is a rare entity, only a few case reports being documented in the literature. CD is an autoimmune condition, impacting the small intestine, caused by gluten-intake in genetically predisposed individuals. Here, we present a case of a patient with melanoma experiencing CD alongside a lymphoproliferative lesion after initiation of nivolumab (anti-PD-1)/ipilimumab (anti-CTLA4) therapy.

Methods: This report describes a clinical case of a 53-year-old Caucasian male patient who had received a diagnosis of cutaneous melanoma three years prior. Due to the detection and progression of cerebral metastases Yervoy/Opdivo therapy was initiated. After 2 doses, he manifested symptoms of reduced appetite, abdominal discomfort, and a notable weight reduction of 12 kg over a span of six weeks. Esophago-gastroduodenoscopy (EGD) was performed, accompanied by subsequent biopsy procedures.

Results: Histological examination revealed subtotal villous atrophy in the duodenum along with an elevated number of intraepithelial lymphocytes, suggesting CD. Laboratory findings showed positivity for tissue transglutaminase IgA (tIgA), IgG, endomysial IgA (EMA), and deamidated gliadin peptide (DGP) antibodies, confirming the diagnosis.
Immunohistochemistry showed atypical T lymphocytes in the lamina propria with pathological immunophenotype (CD3+, CD4-, CD5-, CD8-, CD56-). Analysis of T-Cell Receptor γ Gene rearrangement showed a monoclonal T cell population in a polyclonal background, suggesting an inflammation associated with a lymphoproliferative process. Gluten-free diet was initiated while the immunotherapy was maintained, resulting in the amelioration of abdominal symptoms within four months. Histological and molecular analyses provided evidence of regression, reinforcing the diagnosis of CD.

Conclusion: In this case we observed the clinical manifestations of CD with lymphoma prone clonal lymphoproliferative lesion. While the preexistence of CD cannot be definitively ruled out, it is evident that immune checkpoint inhibitor (ICI) treatment exacerbated the condition and possibly triggered a lymphoproliferative process. This is a rare but severe complication of

ICI therapy. Clinicians should be attentive for such complications in ICI-treated patients, optimizing diagnosis and management.

Keywords: Immune Checkpoint Inhibitors, Immune-related adverse events, Melanoma, Celiac Disease, Lymphoproliferative process

The impact of chronic systemic inflammation on the outcome of oropharyngeal carcinoma treatment

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Introduction: The incidence of oropharyngeal cancer in Croatia and worldwide is increasing, which represents a significant public health problem. Despite the identification of various risk factors such as smoking, alcohol consumption, and HPV infection, the complete pathogenesis of these cancers is still not fully understood. In recent years, research on the impact of chronic inflammation on head and neck cancers, including oropharyngeal cancer, has been receiving increasing attention, with efforts to find highly specific and sensitive, cost-effective, and widely available biomarkers that could be used in routine laboratory tests as reliable indicators of disease outcomes in patients.

Methods: The aim of this study was to investigate the impact of inflammatory parameters from routine laboratory findings taken before the start of treatment on the treatment outcomes of oropharyngeal cancer. This retrospective cohort study included 92 patients who were treated for histopathologically confirmed oropharyngeal cancer at the Clinic for Ear, Nose, and Throat Diseases of the Clinical Hospital Center Zagreb during a ten-year period.
Cox univariate and multivariate regression analysis was performed for progression-free survival and overall survival, taking into account NLR, PLR, SIRI, SII, CRP, and fibrinogen, along with demographic and clinicopathological characteristics of the patients. ROC analysis and the application of the Youden index yielded cutoff values of 2.94 for NLR, 162.3 for PLR, 1.18 for SIRI, 728.4 for SII, 4.2 and 4.3 for fibrinogen and CRP, respectively. Kaplan-Meier survival curves were stratified according to the cutoff values of NLR, PLR, SIRI, SII, CRP, and fibrinogen.

Results: In the univariate analysis for progression-free survival, elevated SIRI and CRP were identified as indicators of poorer outcomes, while elevated NLR and PLR were found significant in the multivariate analysis. For overall survival, NLR, PLR, SIRI, SII, fibrinogen, and CRP were identified as significant indicators of poorer survival in the univariate analysis, while PLR, SIRI, fibrinogen, and CRP remained significant in the multivariate analysis. The most significant indicator of poorer outcomes in this study was the platelet-to-lymphocyte ratio (PLR).

Conclusion: Further prospective studies on larger patient populations are needed to determine the clinical applicability of these biomarkers in the prognosis of patients with oropharyngeal cancer.

Keywords: inflammation, inflammatory parameters, oropharyngeal carcinoma, overall surival, prognostic factor

Metachronous breast and ovarian cancer in BRCA1 mutation carrier - an unusual course of disease

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Introduction: Breast cancer genes (*BRCA*) 1 and 2 encode proteins involved in the DNA damage repair essential for genome protection. *BRCA1/2* mutations are associated with considerably higher risk of developing cancer most often breasts and ovarian. Cancer risk management of mutation carriers includes intensive screening to increase the chance of early detection. However, surgical procedures such as prophylactic bilateral mastectomy and adnexectomy are the most effective options for preventing breast and ovarian cancer in women with *BRCA1/2* mutation.

Case description: A fifty-year-old woman was referred to a CT-scan due to sharp chest pain and dry cough during follow-up for T2N0M0 breast cancer she developed eight years earlier when underwent left breast segmentectomy and axillary dissection, followed by chemo-, radio- and tamoxifen therapy. The CT-scan detected mediastinal lymphadenopathy suspicious of metastasis. Video-assisted mediastinoscopy with biopsy was performed. Histopathology revealed noncaseating granulomas consistent with sarcoidosis. QuantiFERON test excluded tuberculosis. Spontaneous resolution of symptoms occurred soon after. Six years later, during regular follow-up, elevated tumor marker CA 15-3 443.6 U/mL was observed. PET/CT scan revealed increased metabolic activity in bilateral mediastinal, lumbar, iliac, inguinal lymph nodes and an enlarged right axillary lymph node. Fiberoptic bronchoscopy and bronchoalveolar lavage confirmed reactivation of sarcoidosis requiring no treatment. Nevertheless, regular CT scan discovered stranding and distortion of mesentery, scarce perihepatic ascites and fluid in the pouch of Douglas. Cytology of ascites indicated possible ovarian cancer. Laparoscopic exploration subsequently confirmed serous high-grade ovarian cancer. Initially, patient was treated with chemotherapy followed by extensive debulking surgery. Somatic *BRCA1* mutation was confirmed on tumor tissue with consecutive confirmation of germline mutation. After good therapeutic response to platinum-based chemotherapy, maintenance therapy with PARP (poly-ADP ribose polymerase) inhibitor olaparib began and complete response to the therapy was achieved.

Discussion: This case report emphasizes the significance of distinguishing metastatic breast cancer from another non-malignant condition like sarcoidosis or another primary neoplasm. Such conditions could mimic a pattern of metastatic breast cancer. Metachronous breast and ovarian malignancies are indicative for hereditary cancer and those patients should undergo

genetic counseling and testing important to discover inherited pathogenic germline mutations like *BRCA1/2* mutations.

Keywords: Breast cancer, hereditary, ovarian cancer, sarcoidosis

Metastatic mediastinal synovial sarcoma's prognosis for pediatric patient and side effects oftreatment influence the therapeutic decisions

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Background: Mediastinal synovial sarcoma (SS) is an oncological condition, very rare in children, challenging to diagnose. It imposes a multimodal treatment: surgery, chemotherapy, radiotherapy. Although in the localized stages and locally advanced SS has a better prognosis in children than in adults, in the metastatic stage the prognosis is very poor, affecting the quality of life of the patient andconsequently the therapeutic decisions.

Most metastatic SS become resistant to chemotherapy. Due to the toxicity and the limited benefit, terminal patients may choose to forgo the treatment and proceed with palliative care only.

Case Report: 14-year-old patient was admitted to the emergency service with pain in the right shoulder and acute respiratory failure. CT scanning indicates right mediastinal tumor with multiple metastases associated with hemothorax and pneumothorax. After the surgical resection of the tumor, the histopathological examination reveals the synovial sarcoma. The patient is treated according to the CWS-2009 protocol (chemotherapy, surgery, external radiotherapy). The radiation therapy had to be abandoned due to SARS-CoV-2 infection and the radiological progression.

Other 3 lines of chemotherapy were used: the first one involved Ifosfamide, Doxorubicin, Vincristine, the second one - Carboplatin, Epirubicine, Vincristine, the last one - Gemcitabine, Taxotere.It leaves its mark on the patient's quality of life by determining haematological, infectious, and gastrointestinal toxicity.

Due to the toxicity and the inefficiency of treatment, we opted together with the family for home therapy and the use of morphine derivatives. The psychosocial distress interfered with the abilityto cope effectively with cancer, its physical symptoms and treatment. The palliative care had a great significance for the child who had been suffering for a long time.

Conclusions: SS treatment has a limited effect on metastatic patients and leads to physical and psychologicaldamage. The adverse effects and the limited benefit may determine the patients to choose palliative careas a last resort to promote a sense of well-being for the remainder of their life.

Keywords: mediastinal synovial sarcoma, chemotherapy, inefficiency of treatment, palliative care.

Prevalence of Irritable Bowel Syndrome in Medical Students: A Literature Review

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Introduction: Stress plays an important role in gut function due to the bidirectional communication between the brain and the gut, (the brain-gut axis). Irritable bowel syndrome (IBS) is described as one of the most common functional gastrointestinal disorders, caused by alterations in the gut-brain axis. Emotional stress increases gastrointestinal motility, visceral sensitivity and intestinal permeability. It changes the amount of secretions and promotes inflammation. All this can cause problems with digestion, absorption, and intestinal microbiota, leading to characteristic symptoms of IBS such as abdominal pain, bloating and altered bowel habits. Due to the overwhelming amount of material to learn and busy schedules, stress is common among medical students. In fact, medical education is considered one of the most academically and emotionally demanding programs. The focus of this review was to summarize a complex literature describing the association between stress and the prevalence of IBS among medical students.

Materials: Data were obtained through searches in PubMed and Google Scholar database. The MeSH Terms used included “irritable bowel syndrome”, “irritable colon”, “IBS” and “medical students”. A total of 23 articles were reviewed after application of exclusion criteria. Additionally, other review articles and primary studies were used to obtain further relevant information.

Results: All reviewed studies were cross-sectional, employing various questionnaires for IBS-related factors like anxiety, socioeconomic status, and diet. Diagnostic criteria used were Rome criteria: Rome III (15 studies), Rome II (5 studies), Manning, Rome I, and Rome IV (one each). Sample size was 181 to 6105 participants, with IBS prevalence being 7.8% to 61.7%. Factors that were linked to higher likelihood of IBS development were anxiety, poor sleep quality and sleep disturbances, female sex, living in a school dormitory and low physical activity.

Conclusion: IBS was found to have a relatively high prevalence among medical students. While many factors play a role in its etiopathogenesis, the review of relevant literature showed that one of the critical factors is the emotional stress medical students are exposed to as part of their education.

Keywords: irritable bowel syndrome, medical students, stress

Creating a model system for studying DNS-protein crosslink repair in human cells using Crispr/dCAS9

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Introduction: DNA-protein crosslinks (DPCs) are highly toxic DNA lesions that originate from endogenous (aldehydes, ROS) and exogenous (chemotherapeutics, UV radiation) agents. They interfere with replication and transcription and lead to genomic instability, consequently causing carcinogenesis and neurodegeneration. DPC induction is used as a mechanism of chemotherapeutic agents like camptothecins and etoposide which crosslink topoisomerases to DNA, thus stopping the division of cancer cells. CRISPR/Cas9 is a gene-editing tool that uses single-guided RNA (sgRNA) to target and cleave specific DNA sequences, while dCas9 is a modified version used for transcriptional regulation without cutting DNA. A model system for investigating DPC repair (DPCR) in human cells has not yet been developed. Therefore, we aim to create a DPC consisting of dCas9 crosslinked to the specific site in the genome and measure the rate of DPCR using RT-qPCR (reverse transcription quantitative PCR). We anticipate that developing a model system to quantify DPCR will enhance our understanding of DPCR pathways and will provide an impetus for future drug development.

Methods: We selected seven non-essential target genes and designed 2-3 sgRNAs for each of them. These sgRNAs were then synthesized and combined with dCas9 in a complex, which was subsequently transfected into HEK293T cells. Experimental conditions were optimized for each target, including complex concentration, transfection, and silencing duration. Target gene silencing was quantified using RT-qPCR.

Results & Conclusions: Out of seven tested genes, we detected successful silencing of two genes: TDP2 (tyrosyl-DNA phosphodiesterase 2) and MATE1 (multidrug and toxin extrusion protein 1) with different silencing efficiencies. The silencing efficiencies were 18,07% and 26,64% for TDP2 with sgRNA1 and sgRNA2, respectively, and 38,43% and 62,70% for MATE1 with sgRNA1 and sgRNA2, respectively. Observed silencing indicates dCas9 presence at the target DNA site. Due to superior silencing efficiency with sgRNA2 for MATE1, we proceeded with transfecting the sgRNA2/dCas9 complex to assess dCas9-DNA crosslink formation. We are currently optimizing complex concentration and isolation of dCas9-DNA crosslinks from HEK293T cells before and after exposure to DPC inducers. These experiments will answer our question: whether dCas9 forms DPCs in human cells and if it can be used for measuring DPCR by tracking target gene expression.

Keywords: DNA repair, DNA-protein crosslinks, CRISPR/dCas9, human cells, model system

Gastropancreatic neuroendocrine neoplasms in Slovenia

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Introduction: Gastropancreatic neuroendocrine neoplasms (GEP-NENs) are heterogeneous group of neoplasms with certain common characteristics. They arise from regulatory cells of the neuroendocrine system in gastrointestinal tract and their prognosis closely correlates with the grade of the disease. GEP-NEN patients are usually treated in selected tertiary centers due to multidisciplinary and narrowly specialised treatment approach. GEP-NEN incidence in Slovenia is unknown due to limitations of international classification of the diseases and Slovene cancer registry data. Our aim was to review yearly GEP-NEN patient data in the only national tertiary referral center and report on demographic and estimated epidemiologic data.

Methods: In this retrospective study, data of all referred GEP-NEN patients who were diagnosed in 2021 was analyzed. Diagnosis of included patients was confirmed by patohistology or functional imaging in different hospitals across Slovenia. Demographic data, method of diagnosis confirmation, grade, stage, primary tumor site, sites of metastasis and first line treatment were collected. Descriptive statistics was applied and GEP-NEN incidence was estimated.

Results: 91 new patients with GEP-NENs were referred to our center in 2021, with a median age of 64,1 years (range: 21,1 – 88,2) 50% were female. Patients were predominantly diagnosed by patohistology (77,8%) followed by imaging (22,2%). The most common primary tumor sites were pancreas 42,2%, ileum 16,6%, stomach (13,3%), rectum and appendix (both 8,9%). Neoplasm grading was G1/2/3 in 53,3/22,2/10% patients respectively. Out of total 20% of patients with metastasis, majority, 72,2% had liver-only metastatic disease. Most common firstline treatment was surgery (78,9%), followed by combination of somatostatin analogue and everolimus (2,2%) and systemic chemotherapy (2,2%). Estimated incidence of gastropancreatic neuroendocrine neoplasm in Slovenia in year 2021 was at least 4,5/100.000.

Conclusion: This retrospective study aimed to address the knowledge gap concerning the incidence and characteristics of GEP-NENs in the Slovenian population. Estimated incidence and other findings are similar to other reported European data.

Key words: neuroendocrine neoplasms, retrospective analysis, epidemiology

Systemic juvenile idiopathic arthritis: rollercoaster diagnosis and therapy

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Introduction: Systemic juvenile idiopathic arthritis (sJIA) is a subtype of JIA dominated by general symptoms such as recurrent fever, rash, lymphadenopathy, serositis, hepatosplenomegaly and arthritis. Unspecific and variable symptoms make the diagnosis of sJIA especially challenging.

Case description: A 2-year-old boy with unremarkable family history was hospitalised in a lower-rank hospital due to high, spiky pattern fever accompanied with fluctuating patchy rash. As broad-spectrum antibiotics had no treatment effect, incomplete Kawasaki was suspected. Despite the intravenous immunoglobulins, initial symptoms were accompanied by newly developed generalised lymphadenopathy and hepatosplenomegaly. From there on, the boy was transferred to the referral centre for paediatric rheumatology for further evaluation and therapy. Haematologic malignancies were excluded by bone marrow biopsy and imaging procedures and sJIA was deemed the most probable diagnosis. Since there was no clinical and laboratory response to the glucocorticoids as the first-line therapy and IL-1 antagonist as a second-line therapy, prompt clinical and laboratory improvement was finally achieved after the introduction of the IL-6 inhibitor.

Discussion: Systemic juvenile idiopathic arthritis represents a unique form of JIA with complex, unspecific clinical manifestations. It should always be considered as a possible cause of a prolonged fever in a child under 16 years of age. The diagnostic algorithm is formed to primarily exclude other, especially infectious, and haematological diseases. Finally, all patients with suspicion of sJIA should always be transferred to a referral centre since it requires highly specific diagnostic and treatment procedures.

Keywords: recurrent fever, systemic juvenile idiopathic arthritis (sJIA), IL-1 antagonist, IL-6 inhibitor

Twin Pregnancy with Hydatidiform Mole and Coexisting Fetus Following IVF–ET

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Introduction: An unusual twin pregnancy consisting of hydatidiform mole, and a fetus is a rare condition. In vitro fertilization and embriotransfer are thought not to increase the risk for this obstetric condition. These cases are always difficult to manage, and one should always weigh between a possible fetal survival and a risk of molar pregnancy's complications.

Methods: After 3 years of trying to conceive naturally and after one ectopic pregnancy followed by salpingectomy a couple of 39 years has undergone 2 unsuccessful in vitro fertilizations and embriotransfers. The third attempt has resulted in a twin pregnancy with hydatidiform mole and coexisting fetus and has been successfully terminated by cesarean section in 39th week of pregnancy.

Results: 12 days after embriotransfer of 2 blastocysts βhcg level was 2020 and 48 hours after βhcg level was 6222. After 3 weeks of pregnancy βhcg level was 158154 and after 4 weeks βhcg level was >225000 IU/L. During the 6th week of bichorial-biamniotic twin pregnancy a hydatidiform mole and coexisting fetus have been confirmed. On ultrasound two separated gestational bladders were seen: one with and the other one without a heartbeat. During the whole pregnancy a patient had persistent vaginal bleeding. During 39th week of the pregnancy, a patient had fever 37,9 °C and an induction of delivery with prostaglandine was performed. Due to CTG 175/min with decelerations 130/min and a risk for perinatal asphyxia, a cesarean section has been performed and a healthy child was born. During the postpartum CRP was 126,4. After giving amoxiciline clavulanate the next day CRP was 203,0 and the antibiotic therapy was replaced by gentamicine resulting in reaching a normal range CRP level.

Conclusion: Twin pregnancies with hydatidiform mole and coexisting fetus are hard to manage due to the rarity of this condition and it is even harder to decide whether to terminate the pregnancy among women who have undergone treatments for infertility and whose pregnancies have long been waited for.

Keywords: Coexisting fetus; hydatidiform mole; twin pregnancy

Navigating Delirium and Agitation in Geriatric Patients

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Introduction: Delirium frequently arises in hospitalized older adults, particularly after surgical interventions. Although less prevalent than the hypoactive subtype, hyperactive delirium poses a significant care dilemma, notably in critical scenarios. This variant, marked by heightened agitation and aggression, challenges optimal care provision. This case report examines the potential downsides to employing sedation as a strategy in hyperactive delirium.

Case description: A 66-year-old male with a past medical history of chronic obstructive pulmonary disease, hypertension, diabetes mellitus, cirrhosis due to alcohol abuse, chronic kidney disease, frailty, dementia, and dysphagia, presented to the hospital with altered mental status and suspected seizure activity. A recent fall and escalating abdominal and back pain were noted. Imaging disclosed a ruptured suprarenal abdominal aortic aneurysm, needing urgent vascular surgery intervention. Upon arrival, he was promptly taken to the operating room for open repair. The surgery proceeded uneventfully. However, post-operative extubation challenges emerged. He experienced one self-extubation and two mucus-plugging incidents within ten days, leading to multiple re-intubations. Subsequent examination revealed Lewy body dementia, a vascular stroke history and alcohol abuse contributing to his condition. To manage the agitation, haloperidol 5 mg/mL was administered, exacerbating his delirium, and causing an acute change in mental status. A geriatrician consultation team prompted the discontinuation of haloperidol.

Discussion: Employing haloperidol in a patient requiring possible intubation poses concerns. The medication's potential for excessive sedation, muscle rigidity, anticholinergic effects (including decreased mucous production), and interference with intubation procedures warrants careful consideration. Alcohol withdrawal can contribute to difficulties in extubation and is likely a significant factor in delirium development. Patients with Lewy Body Dementia are especially sensitive to antipsychotics, heightening the risk of severe confusion, sedation, and exacerbation of Parkinsonian features. Given the multitude of comorbidities in delirious patients, cautious antipsychotic use is advisable. Effective management of delirium requires a focus on identifying and addressing underlying causes, with nonpharmacological interventions preferred due to their minimal risk profile. Should antipsychotics remain necessary and oral or nasogastric administration is feasible, quetiapine is a favoured option.

Keywords: Antipsychotic Agents; Delirium; Psychomotor Agitation

Monitored Anesthesia Care in the Management of a Patient with Subcutaneous Thrombotic Vasculopathy: A Case Report

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Introduction: Chronic lower extremity ulcers are linked to significant morbidity and complications, requiring regular wound care accompanied by treatment-resistant pain. Monitored anesthesia care (MAC) is a special approach to anesthesia that enhances patient comfort and safety during a variety of medical procedures by creating a controlled state of sedation and pain relief.

Case description: A 32-year-old female with a history of psoriasis and Ehlers-Danlos syndrome presented to the emergency department with bilateral leg pain caused by extensive necrotic areas and ulcerations. Initial biopsy and presentation indicated cutaneous polyarteritis nodosa, but response to treatment with prednisone and colchicine was inadequate. Extensive negative laboratory workup followed, and magnetic resonance angiography did not indicate vasculitis. A repeat biopsy prompted the reclassification of her condition to subcutaneous thrombotic vasculopathy. Despite aggressive treatment including therapeutic anticoagulation, sodium thiosulphate, intravenous immunoglobulins, plasmapheresis, and surgical debridements, her lesions worsened, renal function declined, and she passed away five months post-admission.

The patient underwent thirty dressing changes under MAC during hospitalization. Medical history and health status assessment, alongside midazolam premedication, were regularly conducted. Propofol was employed as both an induction and maintenance agent, delivered through intravenous infusion and occasional bolus. Lidocaine was added to help mitigate the propofol-induced injection pain. Dexmedetomidine, ketamine and fentanyl were administered due to their sedative and analgesic properties. Vital signs were closely monitored. The patient breathed spontaneously with mask-delivered oxygen, avoiding any instances of hypoxia. During five of the procedures, a bolus of phenylephrine was needed due to short-lasting episodes of hypotension, but apart from that the patient was hemodynamically stable. No complications were noted, and she reported no pain or anxiety during any procedure.

Discussion: The successful application of MAC in this complex case highlights its adaptability and effectiveness. It played a crucial role in facilitating pain-free dressing changes, improving the patient's experience, and aiding the technicians and nurses performing the change. Importantly, MAC proved safe for the patient even as her primary disease progressed. The

safety and efficacy of MAC make it a valuable approach for managing similar clinical scenarios and further research of MAC's applications is warranted.

Keywords: chronic ulcer, monitored anesthesia care, pain management, thrombotic vasculopathy

Reconstruction of a COVID-19 vaccine adenovirus from negative stain electron microscopy data

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Introduction: Vaccines against SARS-CoV-2 proved essential in protecting against hospitalization and death from COVID-19 and are still widely used to control the global pandemic. Two most employed vaccine types were ARNm vaccines and modified adenovirus vaccines. The aim of this work was to obtain a three-dimensional (3D) model of the icosahedral nucleocapsid of the adenovirus found in one of two commercially available vaccines, and this reconstruction then be used for demonstration purposes for first-year medical students.

Methods: Vaccine was deposited on carbon-coated electron microscopy grids, then samples were stained using 2% uranyl-acetate. Imaging was done using the 4x4k Ceta camera of a Talos 200keV transmission electron microscope. Single-particle analysis (SPA) was done using the software RELION and involved particle picking, 2D class averaging and 3D processing of data.

Results: A 3D model was obtained and refined, then compared with existing reconstructions of wild-type adenoviruses, showing typical symmetry.

Conclusions: Negative stain electron microscopy (NS-EM) remains a quick method to visualize the geometry and structure of viruses, molecular complexes, and various synthetic particles, and can be successfully used to generate 3D models with relatively high accuracy.

Keywords: 3D model; COVID-19 vaccines; NS-EM; adenovirus; SPA.

Novel likely pathogenic variant in *ABCD1* gene identified by Joint Genome Analysis in a child at-risk for childhood cerebral adrenoleukodystrophy (cCALD) – approach to management

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Introduction: Childhood cerebral adrenoleukodystrophy (cCALD), alternatively X-linked adrenoleukodystrophy (X-ALD), is caused by mutations in the *ABCD1* gene and is characterized by accumulation of saturated very long-chain fatty acids (VLCFA), especially hexacosanoic acid (C26:0), in the nervous system and adrenal cortex. Despite the vast spectrum of clinical phenotypes, neurologic manifestations present in nearly all males by adulthood. Regardless of age, the biochemical phenotype of elevated plasma concentration of VLCFA has 100% penetrance in males. The phenotype cannot be predicted by VLCFA plasma concentration or by the nature of the *ABCD1* pathogenic variant. Furthermore, the same pathogenic variant can be associated with all the known phenotypes. Early neurologic, cognitive, and/or behavioral signs and symptoms are often nonspecific.

Methods: Under the CROseq-GenomeBank project, whole genome sequencing and joint analysis of the child's and his parents' genome was performed.

Results: We present a five-year-old boy with delayed speech and language development, cognitive impairment, and clumsiness. His MRI and EEG are normal. We detected a novel variant of the *ABCD1* gene classified as likely pathogenic (ACMG criteria: PM2, PM5, PP2, PP3), of which the mother is a carrier. This variant is not described in ClinVar nor has it been reported in The Genome Aggregation Database (gnomAD). Family history is negative for X-ALD. On

biochemical testing, although elevated, acylcarnitine and LPC levels are not suggestive for X-ALD. Synacthen test was borderline.

Conclusion: Screening for early detection of cCALD, including neurologic examinations and contrast-enhanced brain MRI every 6 months, and surveillance for primary adrenocortical insufficiency every 4-6 months should be scheduled. Unpublished data suggest that a variant in the same c. position with a different p. change was identified in a single X-ALD case at the Moser Center for Leukodystrophies, Kennedy Krieger Institute. Many *ABCD1* variants are private missense variants, found only in a single family or a small population, and are likely novel. Consequently, many are of uncertain clinical significance and without clear management strategies in terms of who will benefit from targeted therapy (hematopoietic stem cell transplantation or an ex vivo gene therapy). Clinical outliers highlight the importance of a structured approach to management involving a multidisciplinary team.

Keywords: Childhood cerebral adrenoleukodystrophy; novel ABCD1 variant, protein, human; Whole Genome Sequencing, Joint analysis

Von Hippel-Lindau Syndrome: A Case Study of Diagnosis, Treatment, and Genetic Implications in a Young Adult

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Introduction: Von Hippel-Lindau (VHL) syndrome is an autosomal dominant disorder characterized by the development of cysts and tumors affecting various parts of the body. These tumors can be benign or malignant and typically emerge during early adulthood. A hallmark of VHL syndrome is the presence of hemangioblastomas, tumors composed of newly formed blood vessels. The condition arises from VHL gene mutations, which function as a tumor suppressor. Approximately 20% of cases involve de novo mutations in the gene. Treatment strategies for VHL syndrome depend on tumor location and size.

Case presentation: This report describes a 20-year-old male patient, previously healthy and lacking any notable risk factors in his medical history. He presented with symptoms including headaches, vertigo, and recent-onset weakness in his right arm. Imaging using a 3T MRI revealed a cystic formation in the right cerebellum and a pituitary gland tumor. Subsequent transcranial surgery targeting the cerebellum was successfully performed with no complications. Pathohistological analysis identified a hemangioblastoma. Endocrinological assessments revealed a non-functional pituitary macroadenoma. A subsequent transsphenoidal surgery effectively removed the pituitary tumor, which was found to be an aggressive variant upon pathological examination. Genetic testing identified a mutated VHL gene, with the patient being heterozygous, thus confirming the diagnosis of von Hippel-Lindau syndrome. Given residual hemangioblastoma lesions, gamma knife radiosurgery was employed to manage further growth. Ongoing monitoring is in place to track the residual macroadenoma lesion. A comprehensive evaluation of the patient's kidney and adrenal glands is also scheduled.

Conclusion: Von Hippel-Lindau syndrome constitutes a severe genetic condition associated with potential adverse outcomes. In this instance, the syndrome was newly diagnosed in a young patient, necessitating a vigorous approach to address the associated tumors.

Keywords: genetic testing, hemangioblastoma, pituitary gland, von Hippel-Lindau

The impact of Talin 2 knockdown on actin cytoskeleton in the melanoma cell line MDA-MB-435S

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Introduction: Chemotherapy does not work as well against melanoma as it does for some other types of cancer. Therefore, there is an urge to characterise novel targets within melanoma cell to improve chemotherapy effectiveness. Integrins are proteins that connect the extracellular matrix to the cytoskeleton via multi-protein structures called focal adhesions (FAs) and regulate cell proliferation, survival, and motility. Although integrins have proven to be promising targets, clinical trials have not been successful, so more fundamental research is required. One of the main adapter molecules within FAs are talins, which are essential for connection with F-actin and recruitment of microtubules (MTs) to adhesion sites via interaction with KANK proteins. We have previously shown that melanoma cell line MDA-MB-435S adheres preferentially via integrin αVβ5 FAs containing both talin1 and 2, but that KANK2 functionally interacts with talin2 to regulate MT dynamics, sensitivity to paclitaxel (PTX) and cell migration (Lončarić et al., Cellular & Molecular Biology Letters, 2023, 28:56). Here, we investigate how talin2 knockdown affects the actin cytoskeleton and actin binding proteins.

Methods: Experiments were performed using the human melanoma cell line MDA-MB-435S. Gene-specific siRNA transfection was used for transient knockdown. Cell lysates or integrin adhesion complexes (IACs) isolated using previously standardized protocol were analysed by SDS-PAGE and Western blot. IACs were also analysed by mass spectrometry (MS)-based proteomics. The immunofluorescence was done using confocal microscope.

Results: Talin2 knockdown, previously shown to slightly enlarge FA size, alter MT dynamic, and increase actin stress fiber levels, increased the levels of the actin-binding proteins, filamin B and α-actinin-1 in IAC isolates. This result was supported by immunofluorescent analysis. MS data after talin2 knockdown showed increased vinculin and zyxin which is in line with increased actin stress fibers.

Conclusions: Here we show that the increased level of actin stress fibers after transient talin2 knockdown is accompanied by an increased amount of two actin-binding proteins, filamin B

and α-actinin-1. It remains to be determined whether and how these proteins interact with other cellular proteins, such as signalling molecules and/or transcription factors that may directly or indirectly regulate cellular response and cell motility.

Key words: melanoma, focal adhesion, talin, stress fibers, filamin B, α-actinin-1

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Testing ChatGPT's capacity to write essays on ethical dilemmas: A cross-sectional study

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Introduction: Advances in the field of artificial intelligence (AI) have brought about concerns about possible breaches of integrity regarding its use in education. We aimed to examine whether ChatGPT, an AI chatbot that uses natural language processing to create human-like conversation, has the potential to produce unstructured essays on medical students' personal experiences of challenges and ethical dilemmas they faced during studies that are indistinguishable from human-written texts.

Methods: We collected 48 essays written by 5th and 6th year medical students at the University of Split School of Medicine (USSM) in 2023, enrolled in the Medicine in English program. Following anonymization by a course professor (MFŽ), two researchers (MK, MFŽ) selected keywords (range = 12-14) that would be used to generate the prompts for ChatGPT. Two other researchers (LU, JK) then used the keywords to generate two levels of prompts: full keyword prompts and partial keyword prompts (which contained half of the total extracted keywords). These prompts were then fed to the ChatGPT via its online user interface. We analyzed the essays using the Language Inquiry and Word Count (LIWC) 22 software. We used the Kruskal-Wallis test to compare the overall LIWC scores and conducted the Dwass-Steel-Critchlow-Fligner test for pairwise comparisons between each essay group. We considered $P < 0.05$ as statistically significant. All analyses were conducted in jamovi version 2.3.16 (jamovi project, Sydney, Australia).

Results: We found that student-produced essays had significantly lower scores on the “Analytical thinking”, “Authentic”, and “Emotional tone” LIWC summary variables. While having significantly more “Words per Sentence” and “Dictionary words”, the student-produced essays contained fewer “Big words” than the ChatGPT-produced essays. Regarding variables related to psychological processes for the student-produced essays, the student essays had significantly lower results on the “Drive” and “Affect” variables, but higher on the “Cognition” variables in comparison to the ChatGPT-produced essays.

Conclusion: This study sheds light on the linguistic difference between human-generated and AI-generated text based on a multitude of parameters and shows the latter can be differentiated.

Keywords: ChatGPT, AI, LIWC analysis, prompts, keywords

MDMA- From recreational to therapeutic use: A review

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Introduction: Posttraumatic stress disorder (PTSD) is a chronic, debilitating psychiatric disorder. Anxiety, depersonalization, derealization, insomnia, recurrent nightmares, depression, and suicidal thoughts are common symptoms. Moreover, it can result in stress-related physical health issues such as cardiovascular disease, type 2 diabetes, and vicious habits. Currently, the treatment includes trauma-focused psychotherapies and antidepressants. Commonly, treatment resistance occurs when PTSD sufferers are overwhelmed by the negative memories of their trauma that they cannot engage in therapy.

Methods: This review aims to present recent findings regarding the effectiveness and safety of 3,4 methylenedioxymethamphetamine (MDMA)-assisted therapy for treating chronic PTSD in both civilians and military personnel. Studies support the idea that the use of MDMA improves patients' compliance during psychotherapy. This analysis comprehends the latest studies on the topic published by the Multidisciplinary Association for Psychedelic Studies (MAPS) and by the most relevant databases such as PubMed, Google Scholar, Microsoft Academics, and EMBASE, from the last 4 years. Data was gathered regarding finished clinical trials.

Results: In comparison to anxiolytics, antidepressants, and antipsychotics, MDMA does not require daily dosing. The treatment strategy may lessen subsequent medication adherence issues seen in PTSD patients as well as the frequency of adverse events. Anxiety, headaches, exhaustion, muscle tension, and insomnia were the most frequently reported adverse reactions during experimental sessions. As a releaser of serotonin, noradrenaline, dopamine, and oxytocin, MDMA increased levels of self-confidence, awareness, and closeness among participants and motivated them to engage in therapy by reducing reactivity to trauma reminders.

Conclusion: Compared to current treatments, MDMA-assisted therapy has the potential to help patients suffering from treatment-resistant PTSD. There remains a long way to convince critics that a compound experienced recreationally by people may have benefits in its clinical form, only under medical supervision and in strictly regulated doses.

Keywords: MDMA; PTSD; MAPS; Assisted Therapy; Antidepressants

The impact of prenatal alcohol exposure on cardiovascular system in a rat model

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Introduction: Fetal alcohol spectrum disorders belong to serious problems, which can cause a range of negative physical and cognitive consequences. Alcohol exposure can damage the developing brain, resulting in learning difficulties and behavioral deficits. Unfortunately, the today's knowledge remains incomplete. The aim of our study was to fill in the gaps in what we know and deal with the many consequences of these disorders.

Methods: Four pregnant Wistar rats were divided into two cohorts based on the substance administered during pregnancy: 0.9% NaCl (as a control) and 28.5% ethanol. A total number of 16 born rats from both groups were investigated in our study. The electrocardiograms of these rats were analyzed regarding parameters of heart rate variability (HRV), including heart rate (HR), high-frequency band (HF), low-frequency band (LF), very-low-frequency band (VLF) and detrended fluctuation analysis (DFA). We examined correlations of parameters within the experimental and control groups.

Results: Mean HR was found to be higher in the control group (388.57 vs 343.55, $p=0.08$), while the mean HR correlation between both groups was positive (0.05). We observed a significant positive HF power correlation between the experimental and control groups (0.52, $p<0.05$). Moreover, the negative correlation of VLF power was also statistically significant (-0.59, $p<0.05$), while LF power correlation was at the level of 0.04 with no significance. There was also a significant negative correlation of DFA alfa between females in both groups (-0.67, $p<0.05$), while there was no statistical difference between males.

Conclusion: The prenatal alcohol exposure was associated with positive correlation of HRV parameters. Our study reveals that fetus alcohol exposure during pregnancy, may affect the cardiovascular system, what is visible in mentioned parameters. We hypothesized that ethanol influences the autonomic nervous system of fetus, including the sinoatrial node, a component of heart rhythm regulation. This influence can lead to disruptions in HRV parameters and other unfavorable effects. Nevertheless, the prolonged outcomes on diverse systems are still unknown.

Keywords: Fetal alcohol spectrum disorders, prenatal alcohol exposure, heart rate variability, autonomic nervous system

A preliminary overview of the CROseq-GenomeBank Research Project

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Introduction: CROseq-GenomeBank Research Project is an international multi-institutional collaboration between "MILA za SVE" Foundation, University Hospital Centre Zagreb, and MGB/Harvard Medical School. The primary aim of this pilot project is the introduction and application of genomic medicine in Croatian paediatrics healthcare to aid early diagnosis, personalised therapy, and potential prevention of rare disorders. The secondary purpose is the development of an infrastructure to facilitate the 1000 sample library/database of population-based variants observed in the Croatian genome. With these goals in mind, CROseq-GenomeBank aspires to improve wellbeing of affected children and their families.

Methods: DNA was extracted from whole blood of patients with undiagnosed rare disorders (or disorders with uncertain genetic cause) and their parents (trio-based analysis). Whole Genome Sequencing (WGS) was done with Illumina library preparation, while the bioinformatic analysis on the germline and mitochondrial genome was performed with a custom in-house pipeline.

Results: Thus far, a total of 736 individual samples from 217 families were enrolled in this project. These comprised over 100TB of .vcf data stored in a GDPR-compliant cloud-format representing first genomic database established in Croatia (Croatian Genome Aggregated Database, CGAD and Croatian Genome-Phenotype Database, CGPD). In the on-going genome data analysis we identified; causal variants in 119 patients, more than 50 diagnoses of rare disorders, and more than 30 variants from the ACMG secondary findings reporting list. Furthermore, more than 10 patients received personalised therapy. Lastly, an educational program for medical professionals on genome variant interpretation has been established.

Conclusion: With the rise of new technologies, genomic and personalised medicine is steadily becoming more available in a day-to-day medicine. Steady implementation of clinical WGS and associated internal databases accelerates the development of genomics in medicine, which

ultimately allows for more clinical studies of novel therapeutics to be implemented. Therefore, future development of a Croatian genome database is crucial for a more accurate clinical interpretation of the genome variation, with the potential for implementation of new artificial intelligence technologies.

Keywords: Whole Genome Sequencing; Clinical Genome Joint Analysis; Rare Genetic Disorders; Population Genetics, Genomic Database.

Subcutaneous granuloma after HPV vaccine administration – case report

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Introduction: Subcutaneous nodules are a rare adverse event following immunization (AEFI). They have been frequently associated with aluminum containing vaccines, variations in injection technique and patient predisposition for allergy or delayed hypersensitivity. They are important as a reaction because they may influence completion of scheduled vaccinations.

Case Description: July 2021 a 16-year-old female patient with a positive test to inhalant allergens presented with a 20-day history of nontender subcutaneous proximal forearm nodule that developed after intramuscular administration of the amorphous aluminum hydroxy phosphate sulfate (AAHS)-containing HPV (Gardasil 9) vaccine. Patient was up to date on her immunizations, had no history of any adverse vaccine reactions, was not immunocompromised and had met developmental milestones of her age. The mass had developed approximately 3 weeks after receiving her third booster dose of HPV vaccine. At onset, ultrasound demonstrated a 24 x 15 x 6 mm cylindric hypoechoic area confined to the deep subcutaneous tissue and surrounded by region of isoechoic tissue, without the involvement of the underlying muscle. At the two-month follow-up visit, the initial swelling had subsided. Ultrasound demonstrated a 30 x 3 mm hypoechoic elongated mass surrounded by the region of increased echogenicity. The patient was not treated with topical and intralesional steroids. At the one-year follow-up visit, the mass showed no changes in size, vascularization or surrounding subcutaneous tissue. There were no symptoms present. She also reported tragus swelling after ear piercing. There was no clinical indication to proceed with histologic examination therefore the excision or biopsy was not performed.

Discussion: Subcutaneous nodules are not considered a contraindication for further vaccination. Benefits of vaccines containing aluminum far outweigh safety concerns regarding aluminum causing subcutaneous nodules. Clinical management is varied, but a priority should always be ensuring subsequent immunizations. To reduce risk of recurrence with subsequent immunizations, deep IM is recommended and seems to have prevented recurrence.

Keywords: Subcutaneous nodule, aluminum granuloma, pediatric radiology, vaccine site reactions, vaccines, human papilloma virus, HPV

Hidden pancreatic cancer in patient with type 2 diabetes mellitus – a case report

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Introduction: Pancreatic cancer is an aggressive and highly invasive malignant tumor. The diagnosis is very challenging as the cancer rarely shows symptoms early on. Additionally, there is a significant risk for developing pancreatic cancer in people with diabetes mellitus connected to hyperglycemia and hyperinsulinemia.

Case report: A 61-year-old Caucasian man, with medical history of arterial hypertension and type 2 diabetes mellitus for 9 years, started abruptly losing weight. He unintentionally lost 10% of his body mass in 6 months. Routine endocrine check-up revealed he had poorly regulated glycemic profile with HbA1c of 8,2% and random glucose level of 17,5 mmol/L despite his established diabetic drug therapy – vildagliptin 50mg, metformin 1000mg, dapagliflozin 10mg and gliclazide 60mg. Considering the weight loss and elevated levels of tumor markers in blood (CEA 7,1 µg/L; CA19-9 65,1 µg/L), the patient was referred to a gastroenterologist. Additionally, dapagliflozin and gliclazide were substituted with insulin glargine 100 U/ml. Abdominal ultrasound revealed inhomogeneous pancreatic tissue and dilated pancreatic duct (8mm) and MSCT confirmed changes in pancreatic parenchyma indicating chronic pancreatitis. In the following months the patient did not report to any of the physicians considering his diagnosis seemed benign. Eight months later, the patient urgently came to his endocrinologist with jaundice (bilirubin 156 µmol/L), dark urine, light colored stool, and stomach pain. MSCT revealed a neoplasm on the head of the pancreas. After 18 months of extensive surgical and oncological treatment, the patient died.

Conclusion: As an insidious disease, pancreatic cancer was not visible in the MSCT imaging the first time, which encouraged the patient not to seek physician's advice anymore. Sensitivity of any imaging technique is not a 100% and this time the outcome was fatal.

Keywords: MSCT, pancreatic cancer, type 2 diabetes mellitus

Impact of Sleep Deprivation on Reactive Oxygen Species and LipidAbsorption in the Gut

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Introduction: Sleep loss or sleep deprivation is a widespread public health problem that is associated withmany chronic health complications. In previous studies, the lab found that sleep deprivation in fruit flies led to increased levels of reactive oxygen species (ROS) in the gut. ROS are harmful oxygen derived molecules that can damage cells and DNA. The aim of this study was to investigate the effects of sleep deprivation on ROS levels and lipid absorption in mice and determine if the two are correlated. A secondary aim was finding reliable, minimally invasive biomarkers that correlate with ROS in the gut.

Methods: Mice were sleep deprived for 2 days and 5 days for the original experiments and fed melatonin. Control mice include non-sleep deprived mice and sleep deprived mice not fed melatonin. We also sleep deprived mice for 4 days, began feeding melatonin on day 5, andcontinued sleep deprivation and melatonin until day 10. Control mice again included non-sleepdeprived mice and mice sleep deprived for 10 days without melatonin.

Mice were then sacrificed, and we removed their small intestine and liver to stain for ROSand lipid levels. Blood was also collected to test for biomarkers either in lab with colorimetric/fluorometric kits or at a different company.

Results: We found that lipid accumulation in gut enterocytes appears alongside ROS accumulation at 2 days of sleep deprivation. Melatonin was able to both improve lipid absorption and reduce ROS in sleep deprived mice. We also found that there are less lipids in the liver in sleep deprivedmice. In the blood samples that we sent to another company, we determined that there were lower lipid levels (both triglycerides and cholesterol) in the blood of sleep deprived mice. We arestill in the process of imaging slides from the mice sleep-deprived for 10 days to evaluate if thereis a causal link between ROS and poor lipid absorption.

Conclusions: Our study provides evidence that sleep deprivation increases ROS levels and leads to poor lipid absorption in the gut of mice and that melatonin can improve these two symptoms. These findings are significant because they provide new insights into the mechanisms by which sleep deprivation can damage cells and affect health and suggest that antioxidants may be an effective treatment for sleep loss. Future studies are needed to understand the impact of intermittent sleeploss on lifespan and ROS in order to generalize our research to humans and clinical study.

Keywords: Sleep deprivation, lipid absorption, gut, oxidative stress, antioxidants