8TH SLOVENIAN
PNEUMOLOGY, ALLERGOLOGY AND IMMUNOLOGY CONGRESS

APRIL 18-19 2024
Four Points by Sheraton Ljubljana Mons

Združenje pnevmologov Slovenije
Slovenian Respiratory Society

SLOVENIAN
ASSOCIATION
OF ALLERGY AND
CLINICAL IMMUNOLOGY

SEKCIJA MEDICINSKIH SESTER IN ZDRAVSTVENIH TEHNIKOV V PULMOLOGIJI

8TH SLOVENIAN PNEUMOLOGY, ALLERGOLOGY AND IMMUNOLOGY CONGRESS

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PNEUMOLOGY

PLENARY SYMPOSIUM

SLEEP-DISORDERED BREATHING

SLEEP APNOEA IN THE FUTURE – FROM DIAGNOSIS TO TREATMENT

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Sleep-disordered breathing, with obstructive sleep apnoea (OSA) being the most prevalent form, is estimated to affect at least 425 million people globally. OSA, characterized by intermittent upper airway obstruction during sleep at the soft palate, tongue, and epiglottis levels, carries significant cardiometabolic and neurocognitive implications. Consequently, addressing this disease burden will necessitate novel diagnostic and therapeutic strategies in the foreseeable future.

Key pathophysiological mechanisms driving cardiovascular and neurocognitive sequelae in OSA include intermittent hypoxemia and sleep fragmentation. While the severity of OSA is conventionally gauged using the apnoea-hypopnea index (AHI), it proves oversimplified, failing to capture the nuances of nocturnal hypoxemia. Future assessments may pivot towards incorporating additional parameters, such as hypoxic and ventilatory burdens, which have shown stronger correlations with cardiovascular and overall mortality.

It's imperative to recognize that OSA transcends mere AHI or hypoxic burden; rather, it manifests as a multifaceted disorder with distinct genotypes, endotypes, and phenotypes. Understanding these diverse profiles will pave the way for tailored treatment modalities.

The evolving landscape of OSA diagnostics demands alternatives to current methodologies like polysomnography and respiratory polygraphy, which are resource-intensive and require specialized expertise. Future diagnostics should accommodate the variability observed across consecutive nights, necessitating accessible and efficient solutions. While consumer devices hold promise, their integration warrants caution due to unknown algorithms, inadequate validation, and privacy concerns. Nevertheless, artificial intelligence, particularly deep learning, stands poised to streamline data processing, facilitating broader patient access to timely interventions.

The paradigm of OSA management is shifting towards personalized medicine, wherein treatment decisions hinge on patient-specific endotypes and phenotypes. Upcoming therapeutic innovations include hypoglossal nerve stimulator therapy and novel pharmacotherapeutic agents. Notably, selective norepinephrine reuptake inhibitors, when combined with antimuscarinics, show therapeutic potential. Additionally, while acetazolamide demonstrates efficacy in both OSA and central sleep apnoea (CSA), its utilization remains underexplored in this context. Given obesity's pivotal role in OSA pathogenesis, medications from the GLP-1 and SGLT-2i classes offer promising avenues. Addressing residual excessive daytime sleepiness, unresponsive to continuous positive airway pressure (CPAP), may entail selective histamine receptor antagonists, and dopamine and norepinephrine reuptake inhibitors in the future.

HOW WE DO IT: OSA PATIENTS IN OUR OUTPATIENT CLINIC

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BACKGROUND: The diagnosis and effective treatment of obstructive sleep apnea (OSA) in adults is a health priority. A home sleep apnea testing (HSAT) is an alternative to polysomnography for the diagnosis of OSA in uncomplicated adults.

METHOD: We have been performing HSAT at our institution for 11 years, since year 2016 we have permission to prescribe CPAP. With a retrospective analysis, we evaluated our data from 2016 to 2023.

RESULTS: The number of HSAT in our clinic increases every year, with the highest number in 2022 when we performed 615 investigations. Last year, as many as 31% of referred patients, had a diagnosis of sleep disordered breathing (SDB). We are one of the largest institutes in terms of completed HSATs reimbursed by our main health insurance company. We perform sleep recording with a classic 4-channel respiratory polygraph, with a 5-channel one, where 1-channel EEG is added, or with a peripheral arterial tonometry (PAT). In patients where there is a clinical suspicion of allergic rhinitis, we do a skin test for allergy. In 2021, as many as 18% of patients had a positive house mite test. In the case of a family history of asthma and shortness of breath on exertion, methacholine challenging testing is performed. In 2021, 12% of patients had a positive methacholine challenging test. The percentage of SBD eligible for PAP therapy (likelihood ratio) is increasing; the highest was 76% in the year 2020. After that time, it is slowly decreasing due to more and more patients opting for PAT technology, to which we do not triage because they are self-paying. Auto-titrating intolerance or so-called primary CPAP failure varies between 10-12%. Every year we prescribe more and more CPAP devices, last year, for example, 260. To rule out obesity hypoventilation syndrome and also in patients who experience prolonged and deep desaturation after CPAP treatment, we also perform a gas blood analysis. In patients who have primary CPAP failure, we do an endoscopy of the upper respiratory tract to rule out “floppy” epiglottis.

CONCLUSIONS: Despite the fact that there are poor road connections to our clinic, the number of HSATs are constantly increasing. Considering that from year 2023 on, the HSAT service can only be reimbursed to pulmonologists, it would be necessary to impress the majority of outpatient pulmonologists in order to acquire knowledge in the field of SDB and to obtain permission to prescribe CPAP at the expense of the health insurance company. From a financial point of view, it would be necessary to encourage the performance of cheaper HSATs. As part of the comprehensive treatment, we believe that certain patients should undergo allergy and methacholine testing, arterial blood gas analysis, and endoscopy of the upper respiratory tract. Last but not least, we must define comorbidities with lung X-ray, ergometry and heart ultrasound.

ORAL APPLIANCE THERAPY IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

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INTRODUCTION: Oral appliance (OA) therapy is recommended for patients with mild to moderate obstructive sleep apnoea (OSA) and is second line therapy for severe OSA patients when CPAP therapy is not tolerated. However, the efficacy of OA therapy can vary depending on the type of device used. This study aims to assess the effectiveness and tolerance of custom-made OAs produced by Maxillofacial Surgery Department at the University Medical Centre Ljubljana.

METHODS: This retrospective study included the last 100 patients diagnosed with OSA at University Clinic Golnik who received custom-made OAs from the Maxillofacial Surgery Department and were subsequently evaluated for treatment effectiveness. Patient data were obtained from medical records in the University Clinic Golnik database.

RESULTS: Out if 97 participants (3 were excluded due to lack of data) included in the analysis, 68% were male, age 49,9±9,0 years, body mass index (BMI) 28.2±4.2, AHI 20.3±0.9/h. The initial distribution of OSA severity was mild 32%, moderate 50% and severe 18%. 26% of patients had enlarged neck circumference, 25%were obese, 40% had ESS >11, and 41% did not tolerate CPAP therapy. Following OA treatment, the distribution of OSA severity improved, with 17% having no OSA, 42% mild OSA, 35% moderate OSA, and 6% severe OSA. Significant improvement in AHI (>50% reduction or change in OSA severity) was observed in 47% of patients. Overall, 81% of patients tolerated OA therapy well. Among the 25 patient's intolerant to CPAP therapy, 68% responded positively (significant improvement in AHI) to OA therapy. Patients who were intolerant to CPAP therapy (68% vs. 37%, p=0.018) and those with higher initial severity of OSA showed better responses to OA therapy (response rates: 19% mild, 56% moderate, 70% severe OSA, p=0.001).In multivariant regression analysis adjusted to age, sex, neck circumference, BMI, CPAP tolerance, initial OSA severity, only OSA severity predicted good response to OA therapy (OR 3.4, CI 1.0-11.8). In univariate regression analysis, CPAP tolerance also predicted response to OA (OR 0.28, CI 0.09-0.83).

CONCLUSIONS: Custom-made OA therapy demonstrates efficacy in approximately half of the patients referred for treatment and is generally well tolerated. Patients' intolerant to CPAP therapy and those with higher initial severity of OSA tend to exhibit better responses to OA therapy.

OBSTRUCTIVE SLEEP APNEA SYNDROME IN A PATIENT WITH PRIMARY HYPOGONADISM UNDERGOING TESTOSTERONE REPLACEMENT THERAPY: A CASE REPORT

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INTRODUCTION: Primary risk factors for obstructive sleep apnea syndrome (OSAS) include male gender, age, and obesity. In men, hypogonadism is either a cause or result of obesity, creating a link between OSAS, obesity, and hypogonadism. While testosterone replacement therapy (TRT) may improve metabolic parameters in hypogonadal men, it can also increase hematocrit levels, causing polycythemia *(1).*We present a case of a hypogonadal man, who after years of TRT and weight gain, developed polycythemia, requiring further investigations.

CASE REPORT: A 40-year-old man with primary hypogonadism (following hemicastration of the right testis and atrophy of the left testis), treated with TRT since puberty, has been under the care at the Endocrinology Department of UKC Ljubljana since 2006. Initially, he weighed 86 kg, but his weight gradually increased, reaching 108 kg by 2014 when elevated hematocrit was first detected. Despite transitioning from testosterone undecanoate injections to dermal gel and reducing the dosage, polycythemia persisted even with low total testosterone levels. Further investigations included a sleep study, which confirmed moderate OSAS with an apnea-hypopnea index (AHI) of 29. The patient received a CPAP mask, which gradually decreased his hematocrit levels, allowing for safe continuation of TRT.

DISCUSSION: OSAS and male hypogonadism (MH) share common risk factors, but the link between OSAS and MH is more complex. OSAS can worsen MH through hypoxia and disrupted sleep, while MH may exacerbate OSAS due to sleep disturbances associated with low testosterone levels *(2).*
Polycythemia is a recognized side effect of TRT and a complication of OSAS, both independently elevating cardiovascular morbidity risk *(3).* Limited data exist regarding TRT use in patients with OSAS and MH. However, TRT is generally contraindicated in untreated and/or severe OSAS due to the potential symptom exacerbation and secondary polycythemia risk. The Endocrine Society recommends close monitoring of hematocrit levels during TRT, with discontinuation if levels exceed 54%, and assessment of patients for OSAS *(2, 4, 5*). Upon normalization of hematocrit, reintroduction of TRT can proceed using a lower dose or alternative formulation *(4).*

CONCLUSION: Personalized management of TRT in hypogonadal men with OSAS is essential, with careful dosage consideration to mitigate exacerbating OSAS symptoms and improve long-term outcomes.

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SLEEP DISORDERS IN PATIENTS WITH DIFFICULT-TO-TREAT ASTHMA

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INTRODUCTION: Sleep disturbances are prevalent among individuals with asthma, and some symptoms of disrupted sleep coincide with those of asthma. These sleep disorders may impact asthma control or serve as indicators of insufficient asthma management. This study aimed to assess the prevalence of sleep disorders in patients with difficult-to-treat asthma.

METHODS: This prospective monocentric study was performed at the University Clinic Golnik. Patients were recruited from the outpatient clinic with a multidisciplinary team approach for patients with difficult-to-treat asthma. All consecutive patients were approached to participate in the study from August 2022 to October 2023. Polysomnography was performed at a time of clinical stability in all. Their medical records were reviewed.

RESULTS: A total of 108 patients were enrolled in the study, of whom 55 were male (51%), with a mean age of 57.9 ± 12.6 years. Among them, 42 (39%) were classified as obese. Patients reported a range of sleep problems, including snoring (34; 32%), breathing pauses during sleep (30; 28%), night awakenings (63; 59%), night cough (45; 42%), nocturia (76; 70%), symptoms of restless leg syndrome (46; 43%), restless sleep (39; 36%), unrefreshed sleep (40; 37%), and morning headaches (20; 18%). Obstructive sleep apnoea (OSA) was diagnosed in 53 (49%) patients (3 [3%] with mild OSA and Epworth Sleepiness Scale [ESS] >10, 25 [23%] with moderate OSA, and 25 [23%] with severe OSA). Polysomnography results revealed that 76 (70%) had prolonged sleep latency (SL), 87 (81%) experienced prolonged wake after sleep onset (WASO), 82 (76%) had suboptimal sleep efficiency (SE), 62 (58%) exhibited an arousal index >15/h, and 49 (46%) had a periodic leg movement index >15/h. Asthma control (Asthma Control Test [ACT] score <20) was significantly worse in patients experiencing breathing pauses (39% vs. 16%, p=0.007), night awakenings (71% vs. 45%, p=0.007), night cough (60% vs. 23%, p<0.001), restless sleep (51% vs. 21%, p=0.002), and unrefreshed sleep (50% vs. 23%, p=0.005).

CONCLUSIONS: Patients with difficult-to-treat asthma frequently exhibit symptoms of disrupted sleep and demonstrate poor sleep quality. Clinically significant OSA is present in half of these patients, and nearly half experience symptoms indicative of restless leg syndrome. Patients presenting with breathing pauses, night awakenings, night cough, restless sleep, and unrefreshed sleep demonstrate poorer asthma control.

SURVIVAL OF PATIENTS WITH CENTRAL SLEEP APNOEA ON ASV THERAPY

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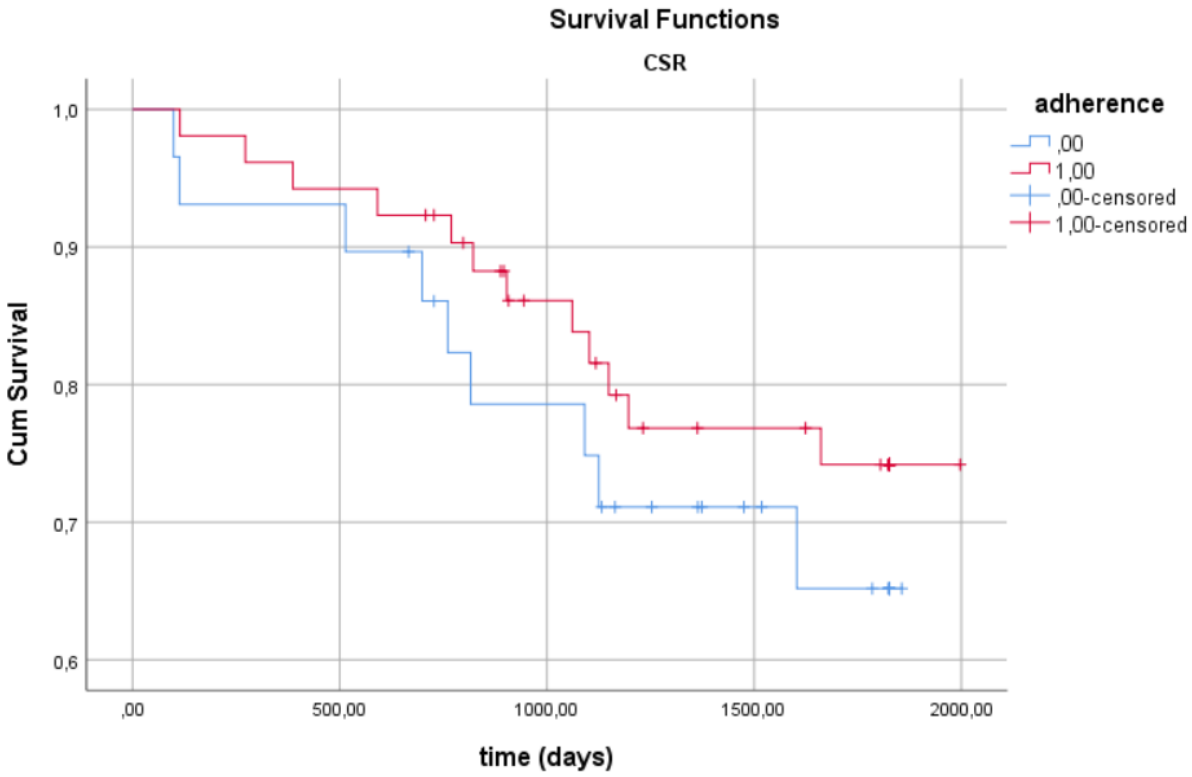
INTRODUCTION: Patients diagnosed with central sleep apnoea (CSA) often receive adaptive servo ventilation (ASV) therapy to stabilize their breathing during sleep. However, the survival benefit of ASV therapy remains uncertain. This study aims to assess the survival benefit of ASV therapy among different CSA subgroups.

METHODS: Medical data of all patients diagnosed with CSA and prescribed ASV therapy at University Clinic Golnik between May 2015 and January 2023 were collected. Patients were categorized into three groups based on their initial diagnosis: CSA with Cheyne-Stokes Respiration (CSR), treatment emergent CSA (TeCSA), and other types of CSA. Adherence to therapy data was obtained from ASV machine software. Comparison between CSR and TeCSA was made.

RESULTS: The study included 182 patients with CSA, of whom 144 (79%) were male, with a mean age of 68.9 ± 9.0 years, BMI of 36.7 ± 7.2 kg/m2, and AHI of 55.3/h ± 20.8/h. During the follow-up period of 4.5 ± 2.4 years, 49 (27%) patients deceased. 81 (45%) patients had CSR, 76 (42%) had TeCSA, and 23 (13%) had other types of CSA. A total of 114 (63%) patients were adherent to ASV therapy, with a mean usage of 6h6 min ± 2 h24 min. Patients with CSR were more likely to be male (88% vs. 73%, p=0.026), had lower BMI (34 ± 7.3 kg/m2 vs. 40.6 ± 6.9 kg/m2, p=0.045), higher morning pO2 (9.8 ± 1.7 kPa vs. 9.0 ± 1.3 kPa, p=0.003), less likely to have diabetes mellitus (28% vs. 45%, p=0.033), and more likely to have elevated NTproBNP (74% vs. 51%, p=0.045) compared to patients with TeCSA. There was no significant difference in adherence to therapy between CSR and TeCSA groups. In the entire cohort, there was no difference in 5-year survival regarding adherence to therapy. However, a trend towards better 5-year survival was observed in adherent CSR patients compared to non-adherent CSR patients (71% vs. 61%, graph 1), although the difference was not statistically significant.



Graph1: 5-year survival of patients with CSR on ASV therapy – adherent vs. non-adherent

CONSLUSION: Patients with CSA receiving ASV therapy demonstrate good adherence to treatment regardless of their initial diagnosis. While there was no significant difference in 5-year survival based on adherence to therapy in the entire cohort, there was a trend towards better survival in adherent CSR patients compared to non-adherent ones.

PLENARY SYMPOSIUM

CONGENITAL LUNG DISEASES

MULTIDISCIPLINARY MANAGEMENT IN COMPLEX CONGENITAL ANOMALIES OF THE RESPIRATORY SYSTEM: A CASE REPORT

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AIM: Congenital anomalies of the respiratory system are rare. Proper management is vital as it significantly affects the child's breathing and overall health. We aim to stress that optimal outcomes can only be achieved through a multidisciplinary approach, as presented in this case report.

METHODS: We analysed the clinical records of a 16-year-old boy with congenital laryngeal cleft, tracheomalacia, right-sided bronchomalacia, left lung agenesis, and right lung hypoplasia, resulting in severe obstructive-restrictive ventilatory impairment and chronic respiratory failure.

RESULTS: In early childhood, the boy underwent treatment with invasive ventilation via tracheostomy. After laryngeal cleft repair at the age of 7, he was treated with supplemental oxygen at night. In later years, severe obstructive-restrictive ventilatory impairment (FEV1 26%, Z -5,89; TLC 55%, Z -3,5; RV/TLC 248%, Z 3,83) and low oxygen saturation during exercise were detected. Chest CT showed just developed middle and lower right lobes, signs of large and small airway disease and trapped air. His attending multidisciplinary team created a comprehensive treatment plan to escalate his treatment. The respiratory physiotherapist educated him on coughing techniques, the use of an incentive spirometer, and a PEP device. The physiotherapist devised a physical rehabilitation plan including brisk walking, interval training, and anaerobic exercises. The clinical dietitian modified the nutrition plan considering his body mass index (16,52 kg/m^2), diet, nutritional requirements, and the results of a bioimpedance analysis. Besides an inhaler combining a long-acting β2 agonist, anticholinergic and steroid (Trimbow), his physicians and nurses started treatment with non-invasive ventilation (NIV) with supplemental oxygen during night-time and physical activity. The NIV use was supported due to its potential to improve respiratory function and address the energy and weight losses associated with chronically elevated respiratory effort.

CONCLUSION: This case report underscores the complexity and challenges of managing congenital anomalies of the respiratory system. Managing such diseases requires a multidisciplinary approach, emphasising the role of allied health professionals. Additionally, with advances in medicine, more children with congenital anomalies of the respiratory system have the potential to reach adulthood, suggesting an increased need for collaboration between paediatric and adult pulmonologists.

CASE REPORT: RECIDIVANT PNEUMOTHORAX IN BIRT HOGG DUBE SYNDROME

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A 59 year old female nonsmoker (administration worker) with arterial hypertension is sent to an outpatient pulmonary check up after being hospitalized 2 months ago for left spontaneous pneumothorax. At that time a CT scan showed small cystic formation up to 11 mm in basal areas on both sides with differential diagnosis of lymphangioleiomyomatosis, alpha 1 antitrypsin deficiency or Birt Hogg Dube syndrome. VATS excision of left upper lobe apex was done and histology analysis confirmed panacinar emphysema.

Thorough anamnesis revealed she already suffered 2 spontaneous right pneumothorax both 9 years ago a few months apart. CT scan of lungs has not been done at that time. She always instantly felt sharp pain in the upper side of the lung not related to strenuous effort or possible infection. She never presented haemoptysis or any other sign or symptom, she never had any surgery and was otherwise healthy. Her brother died years ago of cystic fibrosis, her sister also suffered pneumothorax.
Lung function at first visit was not made for safety precautions. Laboratory results showed normal levels in complete blood count and biochemical values, normal level and phenotype MM of alpha 1 antitrypsin, immunological markers were negative.

Patient was sent to genetic testing which confirmed heterozygous pathogen form in the FLCN gene. Birt Hogg Dube syndrome (BHD) is a rare autosomal-dominant multiorgan systemic disorder manifesting as benign cutaneous fibroma or fibrofolliculomas, lung cysts with or without spontaneous pneumothorax and increased incidence of renal tumors, thyroid, parathyroid and intestinal tumors. Currently there are no guidelines for BHD, recommendation is: CT or MRI scan of abdomen every 1 to 3 years, annually dermatology and endocrinology checkup, colonoscopy after 40 years of age, cessation of smoking.
Follow up: in the last 3 years the pneumothorax did not repeat, all above mentioned investigations are normal. The patient's son and sister were invited to genetic testing but have not responded yet.

CYSTIC FIBROSIS

Invited lecture

UNRAVELING COMPLEXITY: CFTR-RELATED DISORDER PRESENTING WITH DISSEMINATED BRONCHIECTASIS

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BACKROUND: The connection between genotype and phenotype in CF (cystic fibrosis) is complex. This diversity stems from numerous mutations affecting the CFTR (cystic fibrosis transmembrane conductance regulator) gene. Clinical presentation and test results are essential for understanding a patient's condition fully. The term "CFTR-related disorders" (CFTR-RDs) encompasses diverse conditions, including mono-symptomatic disorders in adults.

CASE SUMMARY: A 36-year-old man, former smoker (15 pack years), presented with a chronic cough he noticed for 20 years, producing brownish, mucoid sputum, exacerbated by irritants, cold and carbonated drinks. He experienced a squeezing sensation in his lungs during coughing fits but had no exertional dyspnea or nocturnal cough. His medical history was negative for bronchitis, bronchiolitis or previous asthma. He has no other complaints, has not yet had children.
On a CT scan, performed after a traumatic shoulder fracture, extensive bronchiectasis were found in the upper and middle thirds of the lungs, along with small airway impaction. Further investigations ruled out allergic bronchopulmonary aspergillosis (ABPA), non-tuberculous mycobacterial (NTM) infection or systemic inflammatory diseases. Spirometry was normal, without response to bronchodilator, diffusion capacity for CO was reduced to 68.1%. Due to productive cough and culture isolates (S. aureus +++), he was treated with flucloxacillin 1g/12h for 10 days. 6% NaCl inhalations and flutter device usage were prescribed, which he tolerated well.
He was referred for iontophoresis, which showed elevated sweat chloride concentration of 74.7 mmol/L. Subsequently, he was referred for genetic testing. Heterozygous mutation for F508del on the CFTR gene was confirmed.
The multidisciplinary council concluded it was CFTR-RD. Given the discrepancy between the discovery of the genetic mutation (typically present in homozygous form in CF patients), clinical presentation affecting only one organ system and high iontophoresis values, additional genetic testing (Next Generation Sequencing) is planned. He was also referred for additional diagnostics with other specialties (ENT for sinus evaluation, gastroenterology, infertility clinic).

CONCLUSION: For now, the patient is categorized as CFTR-RD, but reclassification to CF is possible pending high iontophoresis results, further genetic testing, and assessments by other specialties.

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Invited lecture

EFFECT OF COMBINED DRUG ELEXACAFTOR/TEZACAFTOR/IVACAFTOR ON A CLINICAL COURSE OF DISEASE IN A PATIENT WITH CYSTIC FIBROSIS – SINGLE CASE STUDY

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BACKGROUND: Cystic fibrosis (CF) is a rare monogenic disease characterized by dysfunctional/absent chloride channel (CFTR), expressed on many cells, leading to multi- organ involvement. Premature death occurs primarily due to respiratory failure. Novel, "break-through" treatment with CFTR modulators elexacaftor/tezacaftor/ivacaftor (ETI), has been shown to improve symptoms and lung function. However there is only limited data of long-term outcomes, especially in patients with advanced lung disease.

OBJECTIVES: To analyze the long term clinical course after the introduction of ETI in a case study of advanced lung disease.

METHODS: We analyzed clinical data for the three years preceding and three years following the introduction of ETI in F508del homozygous female, aged 27, who was considered being listed on the active-lung transplant list. The treatment was started in agreement with the patient, although there was a serious consideration whether to introduce the drug at all, as the patient's condition could worsen due to the advanced form of the disease.

RESULTS: Notable improvements were observed in the reduction of acute pulmonary exacerbation (APE) frequency and severity – the patient had 13 (10 required hospitalization) APE before, and only three mild APE (no hospitalizations) after ETI. Structural changes in the lungs were partially reversible. Respiratory insufficiency has resolved. Unstable values of FEV1 before(18-29%) stabilized after ETI(39-45%) and remind stable during all three APE. Body mass index status fluctuated(16,2-19) before and remained stable after ETI(19,3). Major improvements in laboratory parameters, such as iron, hemoglobin and erythrocytes; vitamin A; liver enzymes and albumin; leukocytes, neutrophils and C-reactive protein, have been observed. Patient also estimated importantly improved quality of life, enabling her to finish her studies.

CONCLUSION: The introduction of ETI can significantly stabilize the disease – in the short and long term including improvement in quality of life even in cases of advanced and unstable CF already approaching terminal respiratory failure.

Invited lecture

FAMILY FUNCTIONING AND QUALITY OF LIFE IN FAMILIES WITH CYSTIC FIBROSIS – A SINGLE CENTRE STUDY

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BACKGROUND: In cystic fibrosis(CF) diagnosis is mostly made in childhood and is a challenge to cope for the whole family. Having CF or a child with CF affects family functioning, especially communication, proper control and the distribution of family roles. How patients/family respond to the new circumstances is crucial for prognosis and quality of life (QOL). It is therefore important to know how families cope with CF, what are protective and risk factors.

OBJECTIVES: To evaluate what are the patterns of association between family functioning and the patient's/family's experience of QOL on a sample of Slovenian families with CF patient (FM-CF).

METHODS: We included 32 FM-CF (44persons) in the quantitative section, which we then supplemented with a qualitative section (semi-structured-interviews). Data was gathered with a combination of a content analysis and elements of the grounded theory. We used two questionnaires, measuring family functioning (FAM-III-family-assessment-measure), and the QOL (WHOQOL–BREF: The WHO-QOL-Questionnaire).

RESULTS: FM-CF report that patterns of fulfilling family cycle tasks are also effective under stress. Scale values of family functioning are in the subclinical range (T=52), with no areas of strength. Questionnaires reveals that the most important predictors of quality of life in patients/families with CF are: adequate family supervision, absence of depressive symptoms, adequate family cohesion and adequate expression of negative emotions. Avoidance strategies are strongly and significantly associated with worse QOL.

CONCLUSION: According to our results, psychological support is very important to help patients/families develop and maintain appropriate family control, cohesion and expression of negative emotions, and positive coping with the challenges of the CF, all of which have an impact on higher QOL and prognosis. Modular treatment of CF also increases psychosocial challenges, so monitoring effects is important for a holistic treatment. We need new knowledge and measures; our results are already a fraction of that.

PLENARY SYMPOSIUM

INTERSTITIAL LUNG DISEASES

Invited lecture

GENETIC BACKGROUND OF PROGRESSIVE PULMONARY FIBROUS DISEASES

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SUMMARY: Idiopathic pulmonary fibrosis (IPF) is one of the most common interstitial lung diseases. IPF is characterized by a progressive fibrosing phenotype, with decline in lung function and early mortality. In addition to environmental risk factors, the most important of which is smoking, the development of IPF is also influenced by genetics. A common genetic risk factor for IPF is the rs35705950 polymorphism in the MUC5B gene promoter; likewise, among individuals with IPF - especially those with the familial form of IPF - pathogenic variants in the telomerase complex genes (TCG) may also be present. The presence of pathogenic variants in TCG should be considered in patients who, in addition to IPF, have other symptoms/signs that are characteristic of short telomere syndrome (STS), or in individuals where such a clinical picture is found in the patient's family. The clinical spectrum of STS includes: i.) early greying (significant greying before the age of 30), ii.) interstitial lung disease in the family, iii.) cirrhosis of the liver of unknown origin, iv.) aplastic anemia and v.) myelodysplasia/leukemia.
The rs35705950 polymorphism and pathogenic variants in the telomerase complex genes are a risk factor/cause of the disease also in other fibrosing interstitial lung diseases (e.g. in hypersensitivity pneumonitis, in autoimmune interstitial lung diseases and in unclassified interstitial lung diseases), but the genetic contribution is less apparent, as in IPF.

Invited lecture

LONG TERM FOLLOW UP OF PATIENTS WITH COVID 19 FOR OCCURRENCE OF SECONDARY FIBROSIS USING MEASUREMENT OF TRANSFER FACTOR FOR CARBON MONOXIDE (DLCO)

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BACKGROUND: The Pandemic COVID 19 shook the whole world, not only groups dealing with the global aspect of health but also all fields of human activity. Although it is primarily a respiratory infection, many other organs are affected by pathological processes. These processes are present in all parts of the lungs, blood vessels, central nervous system, and peripheral nerves. Inflammation was taken as the dominant process, but proteolysis and thrombosis are no less important. How many cases with secondary lung fibrosis will remain in the inadequate reparation process is the goal of this paper.

METHOD: Patients treated for COVID 19 infection were followed one year after discharge. Control examinations were performed after one month, three months, six months and after one year. In addition to routine clinical and laboratory analyses, the transfer factor for carbon monoxide (DLco) was also analyzed. The single breath real time method, approved by the ERS (European Respiratory Society), was applied. Results of DLco < 80% of predicted were considered as inappropriate recovery.

RESULTS: In a group of middle and moderate form 32 (15 Female, 17 male) cases were analyzed. The group with severe and very severe form includes 36 (16 female, 20 male) patients. In the middle-moderate group six months after discharge 10 (31.25 %) patients had DLco below lower limit of normal, and in sever-very sever group 13 out of 36 pts (35.11 %). Using Mann-Witney test statistical significance was at level $p<0.05$. In measurement one year after discharge 6 pts out of 32 (18 %), in mild-moderate group had DLco <80%, and in severe-very severe group 9 out of 36%. (25 %). There was no statistical significance. It is to be mentioned that no other parameters of patient's status were included in analysis, except the DLco, what can be the limitation of the work.

CONCLUSION: A reduced DLco was found in all groups of patients, with statistically significant difference on six months after discharge, in the comparison of subjects with mild and moderate form with those with severe and very severe disease. But there is no statistically significant difference after one year from discharge. So, recovery of DLco was present in all groups, but it was slower in the severe and very severe group.

PULMONARY MANIFESTATIONS OF INFLAMMATORY BOWEL DISEASE: A CASE REPORT

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INTRODUCTION: While Inflammatory bowel disease (IBD) is primarily characterized by gastrointestinal manifestations, its extraintestinal involvement can extend beyond the skin. This case report explores a rare presentation in an 18-year-old female, emphasizing the complex interplay between IBD and pulmonary manifestations.

CASE PRESENTATION: An 18-year-old female presented with acute ankle pain and swelling, and a two-month history of occasional mild gastrointestinal symptoms with diarrhea. Past medical history included anemia and a family history of thrombophilia. Examination revealed tender erythema nodosum on her legs. Laboratory tests showed slightly elevated inflammatory parameters, but her chest X-ray was normal. Rheumatologists did not identify any systemic connective tissue disease but observed elevated PR3-ANCA antibodies.

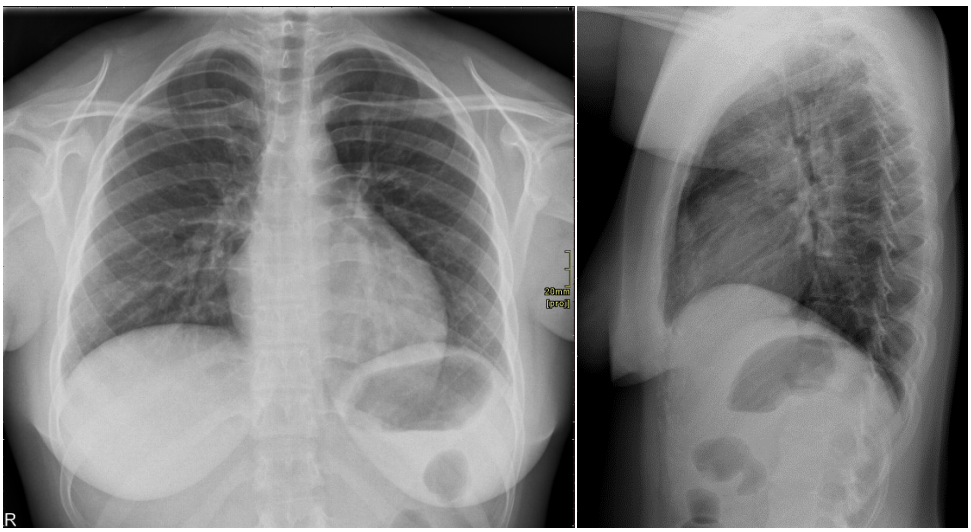

Figure 1: Chest X-ray in AP and lateral projection of the patient with suspected sarcoidosis, revealing no abnormalities.

A subsequent low-dose chest CT scan showed multiple diffuse small ground-glass opacities in the lungs without lymphadenopathy. The pattern and distribution were nonspecific, not typical for sarcoidosis or hypersensitivity pneumonitis, which were the main differential diagnoses. Consultation with a pulmonologist advised diagnostic bronchoscopy, revealing lung capillaritis in transbronchial biopsy. Upon reevaluation of her clinical history, the focus shifted to gastrointestinal symptoms, which raised suspicion of IBD with erythema nodosum and capillaritis as extraintestinal symptoms. This prompted a PET-CT scan, which revealed intense segmental metabolic activity in the colon, suggestive of an active IBD, later confirmed on colonoscopy. Initiation of treatment with corticosteroids and infliximab resulted in complete resolution of ground-glass opacities on follow-up low-dose chest CT.

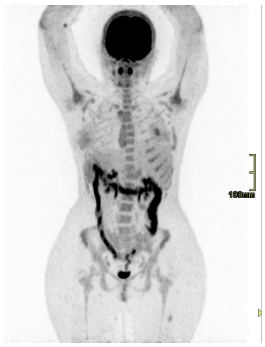

Figure 2: Full-body PET-CT scan revealed segmentally increased metabolic activity in the colon along with enlarged lymph nodes, characteristic of inflammatory bowel disease.

DISCUSSION: Pulmonary involvement in IBD is a rare condition, that reflects the intricate gut-lung axis, influenced by dysbiosis and immune dysregulation *(1)*.

Differential diagnosis between IBD-associated pulmonary manifestations and other granulomatous lung diseases is crucial, and making a definitive diagnosis requires careful clinical assessment, imaging, and sometimes histopathological examination *(2-4).* Clinical manifestations range from asymptomatic disease to symptoms of dyspnea, cough, fever, and pleuritic chest pain, on top of an underlying IBD *(5).* Chest CT often reveals non-specific findings, such as ground-glass opacities seen in our patient with pulmonary capillaritis *(6).* Treatment typically involves corticosteroids, with infliximab as a potential alternative option *(2-5).*

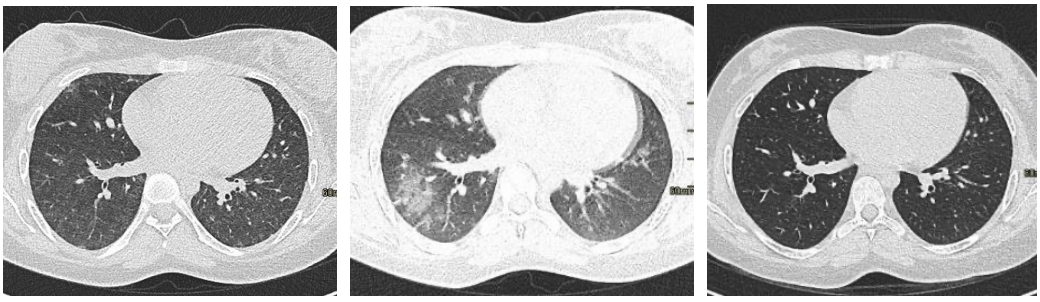

Figure 3: Low-dose HRCT of the lungs in the axial plane. 3a (left): multiple diffuse small ground-glass opacities in the lung. 3b (middle): progression of changes in the right lower lobe at PET-CT one month later. 3c (right): complete resolution of ground-glass opacities in the lungs 3 months after initiating therapy.

CONCLUSION: This case highlights the diagnostic challenges in rare extraintestinal presentations of IBD, necessitating multidisciplinary collaboration for accurate diagnosis and management.

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INTERSTITIAL COUNCIL OF UKC MARIBOR – ANALYSIS OF DATA FROM 2019 TO 2024

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INTRODUCTION

When a patient is suspected of having interstitial lung disease (IPD), he or she is referred to our department for further diagnostics via a personal physician, regional pulmonologist or other medical institutions.

All necessary diagnostic tests are performed in patients with suspected IPB. Each treatment begins with a detailed and concurrently directed history, clinical examination, laboratory tests and X-ray of the lungs. Patients also undergo pulmonary function measurements, a 6-minute walk test, and, if necessary, ergospirometry. We continue with CT or HRCT of the chest and invasive examinations, if necessary. In our department, we perform bronchoscopy with the possibility of transbronchial biopsy (TBB), bronchoalveolar lavage (BAL) and endobronchial ultrasound (EUZ).

After all the necessary examinations have been carried out, the patient is then presented at an interstitial council, which takes place virtually once per month. In addition to pulmonologists, radiologists and rheumatologists from our institution, a pulmonologist, pathologist and radiologist from the Golnik Clinic are also present at the council. At the council, after a detailed analysis of the patient's case, we reach an agreement on the most optimal form of treatment and further handling of the patient. We can also decide on additional more invasive tests, such as open lung biopsy (VATS) or cryobiopsy.

At the council, we also present patients whose condition is worsening or we believe that they need intensified or maybe even a change of therapy.

It is necessary to be aware that not all patients in whom we suspect and find IPB are presented at the council. In total, many more patients with IPB were treated, diagnosed and managed in our department. These patients did not require treatment at the interstitial council for various reasons.

The following presents an analysis of the data of patients who were presented at the interstitial council at the Department of Pulmonary Diseases of the University Hospital Maribor in the period from January 2019 to January 2024 (a 5-year period).

INTERSTICIAN COUNCIL FROM 2019 TO THE END OF 2023

From the beginning of January 2019 to the end of December 203, we presented a total of 367 cases at the Interstitial Council at the Department of Pulmonary Diseases of the University Medical Center Maribor. 276 patients, some of whom were presented several times. 191 men and 176 women were presented. Of these, 79 were active smokers, 85 ex-smokers and 202 non-smokers.

In 144 cases, after the presentation at the council, we made the first diagnosis of changes in the lungs. Of these, the most cases were sarcoidosis in various stages, a total of 24 cases. This was followed by hypersensitivity pneumonitis with 23 new diagnoses, in 15 patients we diagnosed non-specific changes in the interstitium. In 13 cases, lung damage was caused by smoking. We diagnosed 12 new cases of idiopathic pulmonary fibrosis (IPF) and 7 new cases of progressive pulmonary fibrosis (PPF). We had 7 cases of lung changes caused by SARS-CoV-2 and 4 cases of organizing pneumonia. A total of 8 patients were diagnosed with toxic lung damage (4 cases of cordarone, 2 cases each of welding lungs and methotrexate lungs).

The interstitium of the lungs is also affected by various rheumatological and systemic connective tissue diseases. Changes in the lungs in 17 cases were the result of rheumatoid arthritis, 5 in Sjörgren's

syndrome, the same number in various vasculitis. In 2 patients, the changes were associated with systemic sclerosis, and in 1 case each with dermatomyositis, antiphospholipid and antisynthetase syndrome.

In a total of 96 cases, we decided on further or more invasive diagnostic measures. 49 of them were referred for additional imaging tests (CT, HRCT, PET CT, US), 24 for cryobiopsy and 23 for VATS. 1 patient was a candidate for lung transplantation and was therefore referred to the transplant council.

In the case of 64 patients, given the already known diagnosis, we decided for follow-ups. In the same number of cases, we also decided to introduce, adjust the dose, or discontinue therapy with methylprednisolone. According to the council's decision, antifibrotics, biological drugs, immunosuppressants, etc. were introduced when a new diagnosis was made or when an already known disease worsened. Among them, nintedamib (29), rituximab (18), methotrexate (11) and mycophenolic acid (10) were most often introduced. Other drugs were introduced in less than five cases (pirfenidone, azathioprine, mepolizumab). Therapy had to be discontinued in one case due to hepatopathy.

CONCLUSION

The interstitial pleural effusion is an important and crucial part of the diagnosis of many patients with suspected or confirmed interstitial lung disease. With a multidisciplinary approach, different aspects and views of interstitial diseases are discussed. With the presence of specialists from various disciplines from the Maribor University Hospital and the Golnik Clinic, the interstitial council became and remains an indispensable part of the quality and good treatment of patients with interstitial diseases.

Invited lecture

ALPHA-1 ANTITRYPSIN DEFICIENCY – SINGLE CENTRE EXPERIENCE

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BACKGROUND: Alpha-1-antitrypsin (AAT) deficiency is an underdiagnosed autosomal recessive disease. The burden of under-diagnosis is high since risk factor modification (primarily smoking cessation), because of diagnosis awareness, can profoundly alter the natural course of the disease. The current study aimed to illuminate clinical aspects of the disease by characterising the cohort of patients with AAT deficiency managed at Golnik University Clinic.

METHODS: Genetic testing for the two most common pathogenic variants, PI*Z (c.1096G>A, p.Glu366Lys) and PI*S (c.863A>T, p.Glu288Val), in *SERPINA1* gene followed by sequencing was performed, and clinical information of patients was obtained from the hospital's information system: i.) age, ii.) the serum concentration of AAT, iii.) pulmonary function parameters (FEV1 (%), TI, DLCO (%)), iv.) liver function parameters (AST, ALT, yGT), v.) presence of emphysema, and vi.) smoking status.

RESULTS: Out of 700 patients with a genetic test, 210 had one (187 PI*MZ, 23 PI*MS), 39 had two pathogenic variants (1 PI*SS, 6 PI*ZZ, 32 PI*ZZ genotype). Serum AAT concentration differed significantly between the groups (single-allele impairment/bi-allele non PI*ZZ/bi-allele PI*ZZ) with the lowest concentration present in PI*ZZ group. Pulmonary function tests, namely FEV1(%), and DLCO(%) were likewise the lowest in the PI*ZZ group. A similar trend was apparent with emphysema.
CONCLUSIONS: Among adult patients with AAT deficiency, lung disease – manifested as a decrease in pulmonary function and emphysema – was substantial. As expected, lung disease was more pronounced among smokers, even in patients with only single-allele impairment.

PRIKAZ DRUŽINE S POMANJKANJEM ALFA-1 ANTITRIPSINA – KLINIČNI PRIMER

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prim. Dušanka Vidovič, ., specialistka interne medicine in pnevmologije, UKC Maribor
prim. Katarina Osolnik, ., specialistka interne medicine in pnevmologije, Klinika Golnik
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UVOD: Pomanjkanje alfa-1 antitripsina je dedna bolezen, ki se deduje avtosomno ko-dominantno. Najpogosteje se pojavi med 30. in 40.letom. Posledica je predvsem prizadetost pljuč, tudi jeter. Vodi lahko v razvoj pljučnega emfizema, KOPB, jetrne okvare, lahko pa se kaže tudi kot panikulitis, vaskulitis. Zlati standard za potrditev diagnoze je fenotipizacija.

PRIKAZ PRIMERA: 37-letna pacientka je bila v pulmološki obravnavi zaradi bolečine na levi strani prsnega koša, opažala je tudi, da se hitreje zadiha. Sicer je bila zdrava, nekadilka, v otroštvu izpostavljena pasivnemu kajenju. Opravljen RTG prsnega koša je pokazal nespecifičen infiltrat v linguli, zato je opravila še CT prsnih organov: omenjen infiltrat bi lahko predstavljal manjšo nekrotično pljučnico. Prisotni so bili še noduli v preostalih segmentih pljuč - lahko majhna vnetna žarišča ter areali destruiranih pljuč - lahko po pljučnici v preteklosti. Pljučna funkcija je bila v mejah normale. V laboratorijskih preiskavah je bilo ugotovljeno hudo pomanjkanje AAT, blago zvišana sedimentacija, vnetni pokazatelji nizki, negativni tumorski in imunološki markerji, KKS z biokemijskimi preiskavami, vključno z jetrnimi testi so bili normalni.

REZULTATI: Genetska analiza je potrdila prisotnost dveh mutacij - genotip ZZ, v genu za AAT (homozigot). Vrednosti AAT smo preverili tudi pri bratih. Pri 51- letnem bratu, ki je bil brez težav s strani dihal, z normalno pljučno funkcijo, je bilo ugotovljeno blago pomanjkanje AAT, genetsko testiranje je potrdilo prisotnost 1 mutacije - genotip MZ (heterozigot), ki se ne povezuje s klinično pomembnim pomanjkanjem AAT. Pri drugem, 53-letnem bratu, ki je bil prav tako asimptomatski, je bila vrednosti AAT normalna, genetsko testiranje je pokazalo, da gre za genotip MM.

ZAKLJUČEK: Pri treh družinskih članih smo potrdili tri različne genotipe in sicer pri sestri ZZ (homozigot), za katerega je značilno hudo pomanjkanje AAT in visoko tveganje za razvoj pljučne bolezni pri kadilcih kot nekadilcih ter tudi visoko tveganje za jetrno bolezen. Prvi brat je prenašalec, z blagim pomanjkanjem AAT, s fenotipom MZ (heterozigot), za katerega je značilno visoko tveganje za razvoj pljučne bolezni predvsem pri kadilcih, drugi brat je brez okvare gena za AAT, genotip MM.
Ključne besede: pomanjkanje alfa-1 antitripsina, KOPB, emfizem, jetrna bolezen, genetsko testiranje

PLENARY SYMPOSIUM

COPD

COPD IN THE 21ST CENTURY

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In the management and understanding of COPD as a disease, therapeutic nihilism is characteristic, accompanied by a stigma attached to the condition. There is a large gap between the burden of COPD in terms of morbidity and mortality and the attention is given to research funding and focus on COPD. Consequently, comparatively fewer new drug classes have been developed in recent decades to improve clinical management and prognosis. However, recent years have witnessed a shift in our understanding of disease development, emphasizing the importance of early detection and personalized approaches, alongside the emergence of new targets and drug classes, thus promising to reshape the practice of treating COPD patients in the coming years.

Currently, COPD is typically diagnosed at a stage where pathological changes are irreversible, attributed to factors such as a prolonged period of asymptomatic disease activity and reliance on spirometry, an insensitive diagnostic tool. Revising the diagnostic criteria for COPD could facilitate earlier diagnosis, allowing intervention before irreversible pathological changes occur and the development of disease-modifying drugs. GOLD guidelines have proposed two new disease entities: PRISM and preCOPD, recognizing the limitations of defining COPD solely based on decreased Tiffeneau index and advocating for the inclusion of HRCT as a helpful tool for early disease recognition. Studies have demonstrated that small airway disease is an early pathological feature of mild and moderate COPD, with early loss of half of the terminal and transitional bronchioles. Consequently, new diagnostic tools must be developed to detect these disease processes early.

In COPD management, new concepts have gained importance, including precision medicine, the treatable trait approach, and predictive biomarker development. Improved predictive biomarkers enable more certain prediction of therapeutic responses at the individual patient level, facilitating targeted therapeutics to increase benefits and decrease harm. In eosinophilic airway inflammation phenotype, present in 10 to 40% of COPD patients, blood eosinophils serve as a predictive marker for identifying patients who will benefit from treatment with inhaled corticosteroids in terms of decreased acute exacerbation (AE) risk. Several studies in recent years have investigated the use of anti-IL5 monoclonal antibodies in COPD patients with higher blood eosinophils and high AE risk, with mixed results reflecting the complexity of airway pathology in COPD. Notably, a monoclonal drug targeting the IL-4/IL-13 receptor, dupilumab, demonstrated effective reduction in AE risk, improvement of lung function, and quality of life in a recent study. Ensifentrine, a novel inhalation drug with dual inhibition of phosphodiesterase (PDE) 3 and 4 enzymes, has shown anti-inflammatory and bronchodilatory effects, improving lung function and lowering AE risk in clinical studies and is awaiting regulatory approval.

For patients with the chronic bronchitis phenotype, associated with worse symptoms, quality of life, faster lung function decline, higher AE risk, and higher mortality, new modes of clinical diagnosis and treatments are being developed. HRCT appears useful as a radiological alternative to the clinical diagnosis of chronic bronchitis, providing additional information about mucus plugging and its association with

clinical outcomes. Some interventional procedures, such as metered cryospray and bronchial rheoplasty, show promise in improving outcomes by targeting pathological bronchial epithelium and inflammation.

In conclusion, the future of COPD will combine advancements in early detection facilitated by novel methods, early intervention strategies, precision medicine with targeted therapies tailored to specific treatable traits and endo- and phenotypes, development of predictive markers, and the introduction of innovative therapies.

HRCT – THE NEW LUNG FUNCTION IN COPD?

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BACKGROUND: The standardized characterization of COPD and other smoking-related lung changes via CT scans is increasingly recognized as essential due to the emerging role of reduced-dose CT and the widespread availability of CT technology. While quantitative CT offers valuable insights into emphysema, airways, and air trapping, visual assessment of CT scans remains crucial for describing patterns of altered lung structure in COPD. Moreover, visual assessment provides distinct phenotypes currently unidentified by quantitative CT. In addition to evaluating lung parenchyma and airways, chest CT scans often reveal comorbidities that may not be diagnosed through clinical management but are independently associated with all-cause mortality. This study aimed to assess chest CT results in COPD patients to sub-phenotype obstructive lung disease and evaluate associated comorbidities.

METHODS: We analyzed patients primarily referred for chest CT to examine lung structural changes. The prevalence and severity of emphysema, bronchial thickening, and other airway abnormalities (such as mucus plugs, bronchiectasis, and bronchomalacia) were visually assessed, along with parenchymal lung abnormalities (interstitial lung abnormalities - ILA) and other comorbidities detected on CT scans (including ascending aorta dilatation, pulmonary artery enlargement, coronary artery calcification, liver steatosis, and osteoporosis). Correlations with pulmonary function tests were made for emphysema, bronchial wall thickening, CT index of air trapping (E/IMLD), and pectoralis muscle area (PMA).

RESULTS: The final sample consisted of 61 patients, 62% men, with mild to severe COPD, with an average age 68±8,9 years, FEV1 1880±914 ml (67±27% predicted), and DLCO 70±28%. Emphysema was found in 49 patients (80%, mild in 18, moderate in 13, severe in 18), bronchial wall thickening in 56 patients (92%, mild in 38, moderate in 17, severe in 1), bronchiectasis in 17 patients (28%), bronchomalacia in 19 patients (31%), mucus plugs in 24 patients (39%), ILA in 6 patients (10%), ascending aortic enlargement in 14 patients (23%), pulmonary artery dilatation in 17 patients (28%), coronary artery calcification in 52 patients (85%, mild in 24, moderate in 13, severe in 15), liver steatosis in 10 patients (16%), and osteoporosis in 53 patients (87%). Additionally, clinically important changes in the thorax or upper abdomen, not visible on chest X-rays, were found in 9 patients. Statistically significant correlations were observed between visually assessed emphysema severity, bronchial wall thickening, CT air trapping index, PMA, and lung function parameters (FEV1, Ti, DLCO).

CONCLUSIONS: The visual and quantitative analysis of chest CT in COPD patients offers detailed insights into altered lung structure, correlating well with lung function and enabling the categorization of COPD into distinct subtypes defined by structure and function. Additionally, many clinically important comorbidities are often detected through chest CT.

Invited lecture

ALPHA -1 ANTITRIPSIN DEFICENCY: THE BEGININGS OF AUGMENTATION THERAPY IN SERBIA

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Alpha-1 antitrypsin (AAT) deficiency has been known as the genetic cause of lung disease for decades, nevertheless it remains clinically under-recognized. Delayed diagnosis may have substantial consequences. The first step in the management of individuals with AAT deficiency is making a diagnosis in a timely manner and raising awareness about the disease. Treating patients with AATD includes the modification of risk factors, non-pharmacological and pharmacological measures and, as the last step, augmentation therapy. Unfortunately, access to augmentation therapy is limited in many countries and insurance policies often do not cover its cost. From the end of 2023, replacement therapy has become available in Serbia, and it's covered by national health insurance. The decision on therapy is made by consulting body consisted of four members. So far, 10 patients, mean age 49.50±9.46, have been started on the human alpha -1-proteinase inhibitor on a weekly regimen (60mg/kg) in a hospital setting. Out of 10, 7 patients were male, 9 out of 10 patients were index cases. One patient never smoked tobacco, while others are former smokers. Median time from onset of symptoms to diagnosis was 5 (1-34) years, and all patients had confirmed Pi*ZZ genotype. Percent-predicted FEV1 values ranged from 14-62, consistent with very severe to moderate obstruction. Most of the patients were non-exacerbators (7 patients). After completing the total of 145 weekly doses, there were no adverse reactions to therapy, and there were no differences in exacerbation rate. Therapy is still early in the course to assess lung function and survival. Besides optimizing medical management in select patients with AAT deficiency, introducing AAT augmentation therapy in Serbia should help raise awareness about this condition and increase the diagnostic rates.

Invited lecture

WHAT HAVE WE LEARNT FROM THE CZECH COPD REGISTRY?

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BACKGROUND For the purpose of this study, we used the data from the Czech Multicenter Research Database of COPD (COPD Registry), a prospective multicenter study aiming to assess patterns of mortality and morbidity in patients with moderate to severe COPD. Patient recruitment took place between years 2013 and 2016 in fourteen centers providing respiratory care; 5-year follow up was completed in December 2021. During the study and since its completion, several importnant observation have been made, improving current knowledge in the field.

METHODS Individual data of 784 patients from the COPD Registry with forced expiratory volume in 1 second (FEV_1) <60% of predicted value were analyzed in multiple studies. In two studies, we analyzed the prognostic value of the 2017 and the updated 2023 GOLD classification systems. In a next study, we analyzed the risk of mortality in relation to clinical phenotypes of COPD. We also aimed to develop a new prognostic instrument with improved ability to predict mortality. We also aimed to develop a easy-to-use instrument for assessment of patients´ application (inhalation) technique. In a recent study, we assessed the specific mortality in relation to clinical phenotypes. In most studies, Kaplan-Meier survival analyses and Cox model of proportional risks were used. Estimates of survival probability, survival median and hazard ratios (HR) were supplemented with 95% confidence intervals (CI).

RESULTS We found that: a) the GOLD 2017 and 2023 classification systems have poor prognostic properties, b) emphysematous, cachectic and frequent exacerbator phenotypes are associated with worse prognosis. We developed the CADOT index, outperforming the BODE and ADO indices. The „five steps assessment“ was developed as a simple and universal instrument for assessment of correct inhalation technique. In the last study (yet unpublished data), we observed that respiratory causes of death were associated with presence of emphysematous, frequent excacerbator or cachectic phenotype (all $p<0.05$), while cardiovascular causes of death were associated with overlap of emphysematous and frequent excacerbator phenotypes ($p=0.038$) and frequent exacerbators were less likely to die of a malignancy (HR 0.59; $p=0.04$). ACO appeared to be a protective factor of cardiovascular mortality ($p<0.001$).

CONCLUSIONS The COPD Registry has been an important source of morbidity- and mortality- related scientific knowledge.

Invited lecture

PRELIMINARY RESULTS OF MEDICATION COVERAGE ANALYSIS IN SLOVENIAN COPD PATIENTS

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INTRODUCTION: Adherence to guideline-directed treatments for Chronic Obstructive Pulmonary Disease (COPD) is associated with improved quality of life, reduced acute exacerbation rates, and fewer hospital admissions. Single-inhaler combinations have shown superior adherence compared to multiple-inhaler combinations. However, there is a lack of national data from Slovenia on this subject. Therefore, our study aimed to evaluate the utilization of various classes of chronic COPD medications within the Slovenian COPD patient population. Data for this nationwide retrospective observational study were obtained from medication claim records of the Health Insurance Institute of Slovenia, covering the entire population of 2.1 million inhabitants.

METHODS: Subjects were identified as COPD patients upon their first acquisition of any COPD chronic therapy (LAMA, LABA, and ICS combinations) and were at least 45 years old, starting from 2015 to 2022. Follow-up began with the first acquisition of a long-acting therapy (of any kind) or the second acquisition of short-acting medications within two years. We then assessed coverage for the entire follow-up period and on a yearly basis. Patients ceased to be followed up one day before the last medication acquisition in our database.
The Proportion of Days Covered (PDC) was computed for chronic bronchodilator therapies, including at least one anticholinergic agent in any inhaler, single-inhaler dual bronchodilator treatments (LAMA+LABA), and single-inhaler triple therapy regimens (LAMA+LABA+ICS) both for the entire follow-up period and for each year of follow-up since the first acquisition of the medications in question. We determined the proportion of patients with PDC > 80% and PDC < 50% for the entire follow-up period, indicating good and poor coverage, respectively. Annual coverage rates were also calculated for all three groups of therapy users (LAMA, single-inhaler LAMA+LABA and single-inhaler LAMA+LABA+ICS). Additionally, we calculated the proportion of patients with treatment gaps.

RESULTS: Between 2015 and 2022, we identified 21,121 new COPD patients (60% men, 40% women). The mean follow-up duration was 3.84 years (range: 30 – 2827 days). There were 20,457 any-time users of a LAMA agent in any inhaler, with a mean PDC of 0.52. The proportion of patients with PDC for LAMA of at least 80% and less than 50% was 24% and 47%, respectively. Additionally, 81% of patients experienced at least one 60-day gap in coverage during follow-up.
We identified 10,879 (52% of COPD patients) any-time users of a single-inhaler LAMA+LABA. Since the initiation of LAMA+LABA use, the mean PDC increased from 0.64 in the first year to 0.88 in the eighth year of follow-up for those who received the medication at least once a year. However, the overall PDC for all patients during the entire follow-up period remained low (0.46) due to many patients experiencing treatment gaps. 8.9% of these patients experienced treatment gaps totalling more than one year and less than two years between the start and stop of LAMA+LABA therapy.
We also identified 2,901 any-time users of a single-inhaler LAMA+LABA+ICS. From the first acquisition of LAMA+LABA+ICS, the mean PDC increased from 0.49 in the first year to 0.90 in the eighth year of follow-

up for those who received the medication at least once a year. Again, the overall PDC for the entire follow-up period was low (0.30) due to many patients experiencing treatment gaps.

CONCLUSION: The proportion of COPD patients in Slovenia consistently using at least one guideline-directed basic therapy (LAMA) is satisfactory, whereas the proportion of patients consistently using single-inhaler combinations throughout the entire follow-up period (either LAMA+LABA or LAMA+LABA+ICS) is relatively low. The coverage for the combined inhalers increases with follow-up time, presumably because of the disease's progressive nature.

SKELETAL MUSCLE PARAMETERS AS PREDICTORS OF SURVIVAL IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AFTER PULMONARY REHABILITATION

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INTRODUCTION: Patients with chronic obstructive pulmonary disease (COPD) exhibit alterations in body composition characterized by low strength, diminished muscle mass, sarcopenia, and reduced exercise capacity, all of which can influence the survival of patients. This study aimed to identify clinical indicators of skeletal muscle condition that influence the risk of mortality after pulmonary rehabilitation (PR) program.

METHODS: We included consecutive patients with COPD who completed our 4-week inpatient PR in the University clinic Golnik from 2017 to 2021. All were clinically stable at the start and completed a predefined protocol of tests and investigations at the beginning and the end of PR. We used ESPEN criteria to determine low fat free mass index (FFMI) (<17 kg/m^2 for males and < 15 kg/m^2 for females) and BODE criteria for low body mass index (BMI) (<21 kg/m^2). Multiple logistic regression was used to determine predictors of low FFMI. We used Cox proportional hazards regression models to investigate predictors of all-cause mortality. Survival data was censored on 20th February 2024.

RESULTS: The final sample included 141 patients (age 65±7.2y, women 34%, FEV_1 40±18% predicted, BMI 26.5±5.9 kg/m^2). 14% of patients experienced acute exacerbation during PR. Over a median follow-up of 1619 days (IQR 1152-2018), 46 patients (33%) died. Survival rates at 1 and 2 years post-PR were 97% and 90%, respectively. Table 1 outlines the characteristics of deceased versus surviving patients. In multivariate logistic regression, current smoking was significantly associated with a 4.5-fold increased risk of low FFMI (95% CI: 1.6-12.5, p=0.004); additionally, FEV_1% was a significant predictor with an OR 0.97 (95% CI: 0.942-0.995, p=0.019). In the two multivariate Cox models, we adjusted for pre-PR FEV_1%, age, gender, and either pre-PR low FFMI status or pre-PR low BMI status, along with pre-PR 6-minute walk test distance (6MWTD), handgrip strength, and pre-PR MIP. We found that pre-PR 6MWTD (HR 0.996, 95% CI 0.992-0.999, p=0.022), low pre-PR FFMI (HR 2.2, 95% CI 1.1-4.3, p=0.030), and low pre-PR BMI status (HR 2.7, 95% CI 1.3-5.8, p=0.008) remained significant predictors of all-cause mortality. MIP showed a trend towards significance (HR 0.983, 95% CI 0.964-1.002, p=0.072), whereas handgrip strength did not predict mortality (p=0.575). Change in 6MWTD did not predict survival (p>0.05). Within a subgroup of BMI above 21 kg/m^2, a low FFMI was associated with an increase in mortality risk (log-rank, p=0.033).

CONCLUSION: In our study, we observed that 38% of patients had a decrease in FFMI according to ESPEN criteria at the beginning of PR. Current smoking emerged as a robust independent predictor of low FFMI, while low FFMI itself was identified as an independent predictor of increased mortality. Although the 6MWTD was a significant predictor of mortality, the improvement from pre- to post-PR did not predict survival. Regarding muscle strength parameters, lower MIP exhibited a trend toward significant prediction of mortality, while handgrip strength showed no association with mortality.

Overall, our survival models did not distinctly favor low FFMI over BMI; however, low FFMI provided additional prognostic information in patients with a BMI exceeding 21 kg/m². Our findings highlight the importance of focusing on body composition, particularly muscle mass, across varying degrees of COPD severity and body weight.

TABLE 1: Characteristics of patients according to survival

Characteristic	***All***	***Dead***	***Alive***	***P value***
No. of subjects	141 (100%)	46 (33%)	95 (67%)	
Age, yr	65.5(7.2)	66.7 (6.6)	64.9(7.5)	0.578
Sex, F, n (%)	48 (34%)	14 (30%)	34 (36%)	0.574
BMI, kg/m²	26.5(5.9)	25.0 (6.5)	27.2 (5.6)	**0.037**
BMI <21kg/m²	24 (17%)	13 (28%)	11 (12%)	**0.018**
FFMI, kg/m²	17.3 (3.6)	16.5 (4.1)	17.6 (3.4)	0.097
Lower FFMI, n (%)	62 (38%)	26 (59%)	36 (38%)	**0.027**
Handgrip [kg]	319 (132)	319 (121)	320 (137)	0.963
Smoker/ex-smoker, n (%)	29 (21%)/112 (79%)	10 (22%)/36 (78%)	19 (20%) /76 (80%)	0.827
MRC scale, points	3.0 (0.9)	3.3 (0.9)	3,0 (0.9)	0.496
FEV_1, mL	1151 (546)	947 (459)	1251 (559)	**<0.001**
FEV_1, % predicted	40 (18)	33 (14)	44 (18)	**0.019**
6MWTD, post, m	393 (110)	338 (98)	420 (105)	**<0.001**
6MWTD, pre, m	341 (108)	287 (100)	367 (103)	**<0.001**
Change in 6MWTD, ≥30 m	99 (70%)	31 (67%)	68 (72%)	0.61
MIP, cm H2O	75 (26.6)	63.4 (22.5)	80.5 (26.7)	**<0.001**
LTOT, n (%)	28 (20%)	12 (26%)	16 (17%)	0.260
Comorbidities n (%)	136 (97%)	46 (100%)	91 (96%)	0.303
Arterial hypertension, n (%)	61 (43%)	22 (48%)	39 (41%)	0.473
Diabetes, n (%)	18 (13%)	8 (17%)	10 (11%)	0.286
Osteoporosis, n (%)	114 (89%)	41 (98%)	73 (85%)	**0.035**
Depression, n (%)	51 (36%)	20 (44%)	31 (33%)	0.192

THE ROLE OF IMPULSE OSCILLOMETRY IN CLINICAL ASSESMENT OF COPD

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BACKGROUND: Spirometry is routinely used to assess COPD patients. On the other hand, impulse oscillometry (IOS) has been proposed as an alternative and may provide additional information for COPD management. This study aims to evaluate the associations between spirometry and IOS parameters in the clinical assessment of COPD.

METHODS: The study included 73 COPD patients (age 67.8 ± 14.4 years, 27 [37%] females). Flow-volume, IOS and clinical assessment were done at the same visit. Patients were also evaluated with the COPD Assessment Test (CAT), SARC-F questionnaire and the 1-minute sit-to-stand test (STS).

RESULTS: The results are summarised in Table 1. In general, similar associations were found between FEV1 and IOS parameters. In patients with ACO the increase in R5-20 was most pronounced. There was a positive correlation of IOS parameters with CAT (FEV1% r = 0,460, p < 0,001; R5% r = 0,445, p < 0,001; X5% r = 0,195, p = 0,100; R5-20% r = 0,486, p < 0,001)
and negative correlation with STS (FEV1% r = 0,479, p < 0,001; R5% r = 0,380, p < 0,003; X5% r = 0,247, p = 0,061; R5-20% r = 0,434, p<0,001).

Table 1:

	FEV1 %	R5 %	X5 %	R5-20 %
GOLD groups				
COPD A	72 (60-75)	103 (79-127)	203 (65-517)	100 (15-160)
COPD B	47 (37-60)	171 (135-206)	425 (271-708)	522 (187-766)
COPD E	39 (30-57)	183 (146-236)	853 (431-1585)	525 (310-797)
	p=0,002	p<0,001	p<0,001	p<0,001
COPD phenotypes				
Non-exacerbator	57 (39-72)	142 (104-198)	421 (224-708)	329 (138-679)
AE-CB	55 (40-64)	173 (147-205)	649 (439-1025)	543 (350-708)
AE-non-CB	34 (25-46)	182 (146-236)	1222 (425-2665)	420 (281-787)
ACO	50 (38-65)	203 (152-248)	421 (323-871)	669 (436-916)
	p=0,014	p=0,072	p=0,008	p=0,115
COPD and sarcopenia				
Negative SARC-F	57 (41-71)	150 (122-195)	432 (235-769)	331 (160-612)
Positive SARC-F	35 (25-50)	192 (147-248)	650 (414-1644)	693 (404-928)
	p<0,001	p=0,096	p=0,139	p=0,018

*values are median (IQR 25 – 75)

CONCLUSIONS: IOS measurements show similar performance to standard flow-volume spirometry in assessment of COPD and shows good correlation with exercise capacity (assessed with STS) and COPD symptoms (assessed with CAT). It may offer additional insight in some COPD subtypes (such as ACO).

OVERVIEW OF ECG ANALYSIS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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We aimed to investigate the association between COPD and electrocardiography (ECG) abnormalities and their relation to the disease severity.

Cross-sectional study, including 220 patients with stable COPD as investigated group (IG), aged 40-75 years and 58 non-COPD subjects, matched by gender, age, BMI, smoking-status, as control group (CG). All study subjects underwent pulmonary evaluation, resting-ECG, 24-hour-ECG-Holter monitoring.

Results presented statistically significant difference between presence of sinus tachycardia with decrease of FEV1(GOLD1→GOLD4), the frequency increased significantly, 29(59.18%) in GOLD4 vs. 8(14.04%) in GOLD1, p=0,00001. Higher prevalence of atrial fibrillation (AF) was detected in GOLD4, 15(30.61%) vs. 6(10.53%) in GOLD1, with no statistically significant difference, p=0,0668. AF was present in IG in 49(22.3%) patients vs. CG 2(3.45%) with clinically significant difference ($p<0.05$). With decrease of FEV1(GOLD1→GOLD4), the frequency of P - pulmonale increased significantly, p=0,00001. There was no significant association between subgroups of IG and the presence of Right Bundle Branch Block (RBBB), but there was clinically significant difference between IG 26(11.82%) vs. CG 3(5.17%), $p<0.05$. Right axis deviation presence increased significantly with decrease of FEV1(GOLD1→GOLD4), p=0,0221.

As a conclusion, ECG abnormalities are present even in the early stages of the disease. (GOLD 1, 2). Cardiovascular assessment in COPD patients is an urgent need to develop strategies for detection and early treatment.

PLENARY SYMPOSIUM

TUBERCULOSIS

Invited plenary lecture

EPIDEMIOLOGY AND TREATMENT OF MULTIDRUG-RESISTANT TB IN SLOVENIA

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Tuberculosis is still the most common and most lethal infectious disease. One of the major reasons for that is TB caused by multi-drug resistant *M. tuberculosis* which has worse treatment outcome. In recent years we have seen an increase of multidrug resitent TB cases (RR-TB, MDR-TB, preXDR-TB and XDR-TB). In Slovenia we have treated only sporadic cases of MDR-TB in the past few years. Our analysis will reveal epidemiological and treatment data of multidrug-resistant TB cases in the past 10 years.

METHOD: We performed retrospective analysis of TB cases registered in Registry of TB of Republic of Slovenia from 2014 to 2023. In all microbiologically confirmed cases a susceptibility test for the first line antituberculous drugs (DST 1) was performed. When resistance to 1st line DST was detected, additional testing for 2nd and 3rd line DST was performed.

RESULTS: 1024 TB cases were registered between 2014 till 2023 and diagnoses was microbiologicaly determined in 946 patients (92,4%). In all microbiologicallly confirmed cases a susceptibility test for DST 1 was performed. Resistance to antituberculous drugs was determined in 46 patients (4,5 % all TB patients or 4,9% all microbiologically confirmed cases). Among resistant TB cases only a small fraction had MDR-TB (8.7%) (1 patient RR-TB, 2 pts. MDR-TB, 1 preXDR-TB). Share of MDR-TB among all TB patients was 0,39%. Treatment success was good. Three patients successfully completed the treatment, one patient moved back to home country before completing the treatment in Slovenia.

CONCLUSIONS: Slovenia has a low share of resistant TB cases and MDR-TB cases are detected only sporadically. The most common risk factor for MDR-TB is relocation from countries with higher shares of resistant TB and MDR-TB.
Treatment success in patients that completed their treatment in Slovenia is good. Treatment and follow up of these patients in one centre with appropriate multidisciplinary team and expertise for the whole duration of treatment is crucial. Due to several socio-political crisis globally there's an increase in migration from those areas and subsequently an increase of resistant TB cases is expected in Slovenia. Hence we follow the WHO recommendation, including the introduction of novel treatment regimens for resistant TB treatment.

IS WHOLE GENOME SEQUENCING FUTURE OF DRUG SUSCEPTIBILITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS*?

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BACKGROUND: National reference laboratory for Mycobacteria on University Clinic Golnik is performing drug susceptibility testing (DST) on all *M. tuberculosis* (MT) isolates. DST is performed phenotypically and since 2021 all isolates are sequenced with whole genome sequencing method (WGS). High sensitivity and specificity of molecular methods, drop of cost and ease to perform are main reasons why WGS already replaced phenotypic testing for first line drugs in some western countries in Europe. Phenotypic DST for pyrazinamide (PZA) is inaccurate due to large inoculum which can reduce activity of PZA. Therefore WGS is recommended to be used to rule in PZA resistance. Moreover, specific mutation in *oxyR-ahpC* gene is conferring isoniazid resistance while phenotypic DST reports it as sensitive. In 2024 we stared testing diagnostic kit Deeplex Myc-TB which enables sequencing also from positive sputum sediment.

METHODS: WGS was used for retrospective analysis of selectively chosen isolates obtained from our National Mycobacterial collection in years between 1995 and 2023. Since 2021 WGS is used on all MT isolates.

RESULTS: Retrospective analysis of 57 isolates from National mycobacterial collection revealed six isolates sensitive to isoniazid (INH) and mutation in region *oxyR-ahpC* was detected with WGS . Same mutation was found in relapse TB case in 2023, in 2016 isolate was reported phenotypically as INH sensitive. Six isolates were found to be sensitive to PZA but mutation in genes conferring resistance to PZA were found only in three isolates.

CONCLUSION: WGS is useful tool to combine with phenotypic DST to obtain most accurate results of drug resistance profile. As the catalogue of mutations in MT and their association with drug resistance will be more refined, we are expecting that phenotypic DST for first line drugs will eventually be replaced with WGS. In the future easier to use diagnostic kits will be developed based on sequencing which will shorten turn-around time from specimen collection to report generation. One of major challenges for the future is use of sequencing methods for identification and DST of non-tuberculous mycobacteria (NTM).

Invited lecture

TUBERCULOSIS AND RISK GROUPS – CURRENT PROBLEMS AND OVERVIEW OF SITUATION IN SERBIA

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BACKGROUND: With the rate of 7/100.000 in 2023, Serbia belongs to the low tuberculosis (TB) burden countries. This has been the first notification rate increase since 2004, and is due to several reasons.

METHODS: The data of the Annual reports on infectious diseases in the Republic of Serbia of the Institute of Public Health 'Batut' from 2010 were analyzed.

RESULTS: From 2010-2023 in Serbia we registered 30 migrants with TB, mostly coming from Afghanistan (11), 6 from Somalia, 3 from Pakistan and China each, 2 from Morocco and Ivory Coast each, and 1 each from Indonesia, Iraq and Uganda. Pulmonary TB had 26 migrants and 4 non-pulmonary TB. All were new TB cases. Twenty two migrants (73,3%) stopped treatment, dropping out of follow-up system, while 5 were successfully cured (16,6%). One patient died of TB, one returned to the country of origin, and for one there were no data on outcome. In all migrants, tests confirmed sensitivity to first-line anti-TB agents. None had TB and associated HIV infection. General percentage of TB patients tested for HIV in Serbia in period 2010-2022 was rather small (0,6-12,6%). In 2021, 2022 and 2023 there were 2, 3 and 3, respectively, registered cases of multidrug-resistant TB (MDR-TB), which is significantly less than in 2010-2019 period. In the last two years there were no repeated treatments for MDR-TB. Presence of extensively drug-resistant tuberculosis (XDR-TB) in Serbia cannot be verified, since testing on second-line anti-TB drugs is impossible. In 2022, only 52% of samples were tested for resistance to first-line anti-TB drugs. TB notification rate in prisons shows the trend of significant decline from 39/100.000 (2010) to 7/100.000 (2019). What raises concern is the fact that in 2022 in Serbia were registered twice as many cases of TB among children than in previous period with scope of investigated contacts of TB patients at only 75%. Potential risk groups are labor migrants, particularly ones from high TB burden countries, who currently aren't universally tested neither for active nor latent TB.

CONCLUSION: Epidemiological surveillance, contact control, increased scope of testing for resistance to anti-TB drugs and HIV, and chemoprophylaxis, are the most important measures for prevention and curbing spread of TB, especially in risk groups.

Invited lecture

STATUS ABOUT TUBERCULOSIS IN TUZLA REGION

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The annual report on the state of tuberculosis (TB) in the Federation of Bosnia and Herzegovina is a regular activity of the Tuberculosis Control Unit in the FBiH (NTP unit of the FbiH).
Tuberculosis control at the cantonal/county level is carried out in close cooperation with cantonal coordinators, i.e. contact persons, who are required to collect tuberculosis reports, and TB registration after completion of treatment, through cantonal tuberculosis control units (NTP units), which submit individual data to the FBiH Tuberculosis Control Unit, located in the Epidemiological Service of the Clinic for Pulmonary Diseases and TB. Podhrastovi UCC Sarajevo, where active tuberculosis is registered on the prescribed forms, in the FBiH tuberculosis register, and electronically.
In the analysis of the annual report on the state of TB in FBiH, 287 reports of TB patients, registered during 2020, were included. Registered TB cases of all localizations (pulmonary, extrapulmonary, newly discovered cases or relapses, sputum BK positive or negative, direct or cultural, sensitive or resistant to antituberculosis drugs) from all ten cantons/counties of FBiH, whose data were obtained from cantonal coordinators (specialists pneumophthisiologists), epidemiologists and microbiological laboratories, (NTP units of cantons/counties), and refer to the calendar year 2020. Treatment outcomes, according to WHO recommendations, refer to the previous year, 2019.
Patients were treated according to the principles of the modern DOTS strategy in a hospital according to a 6-month treatment regimen for newly discovered cases (initial phase of treatment 2 months) and after that 4 months through the pulmonary care service of health centers, and according to an 8-month treatment regimen for relapses, and with the tuberculosis treatment record card (WHO Treatment card) returned for a final opinion to the Epidemiological-Statistical Service of the NTP unit FBIH, the supervisory institution for tuberculosis control in the FBiH.
Compared to previous years (870 TB cases registered in 2012; 800 TB cases in 2013, 795 patients in 2014, 728 in 2015, 573 in 2016, 515 in 2017, 461 in 2018, 436 2019, 278 2020). there is a slight decrease in the total number of patients, which is the result of the good implementation of the NTP of the FBiH and continues the trend of reducing tuberculosis in the FBiH, and the average annual rate of reduction of tuberculosis in the FBiH from 2012 to 2016 is 9.6% per year, and in 2017 Mr. compared to 2016. a decrease of 10.1% was recorded, while in 2018 compared to 2017. rate lower by 9.02% (23.2:20.8/100,000); in 2020, that drop is 34.17% in the pandemic era, very large; we will have to analyze the same in the coming period, because it is very large. (WHO follows the global trend of tuberculosis decline in the world by 2.5 per year). In 2019 the rate of tuberculosis in FBiH recorded a further decrease to 19.6/100,000 inhabitants, which is 8.4% less compared to the previous year, and in 2020. 12.93/100,000.
The migrant crisis in Bosnia and Herzegovina has seen migrants falling ill, and the number has increased compared to 2019. (5 in 2020), which is a big challenge due to the possibility of the emergence of drug-resistant, especially multi-resistant tuberculosis, for which there is no drug available in Bosnia and Herzegovina. The treatment of migrants takes place in the hospital in the initiation phase, but the

phase of continuation of treatment is without adequate control of the pulmonologists of the health centers, because the IOM took over the treatment of migrants in the camps. During 2020, the trend of a gradual decline in reported cases of active tuberculosis in all locations in the FBiH continued. Pulmonary localization of TB disease was more prevalent (250-87.1%) compared to extrapulmonary localization. Men were somewhat more often represented (176 - 61.3%) compared to women (111 – 38.7%). The largest number of tuberculosis patients was in the age group above 64 years, age, and the least number of patients are up to 14 years of age. A positive sputum smear recorded an increase in the percentage in 2020 compared to 2019. In 2020, a slight increase in the percentage of average positivity of M. Tuberculosis culture findings (176 – 61.3%) in FBiH was recorded compared to 2019. It can be seen that the success rate of treatment was 163 (81.9%) for newly diagnosed, 12 (80%) for relapses, and 175 (81.81%) in total, while there were 221 (50.8%) unevaluated patients, slightly less compared to the previous year. Tuzla Canton (TC) has a significant decrease in registered tuberculosis patients compared to 2020. Morbidity rate in TC (14.6) in 2020. is slightly above the rate in FBiH (12.93). The largest number of patients is in Tuzla municipality (16), Živinice municipality (11), Lukavac municipality (10), but the rate of reported active tuberculosis is again highest in Banovići municipality (30.7), Teočak municipality (27), Klladanj and Lukavac. No cases of TB were registered in the municipalities of Sapna, Kalesija and Čelić in 2020. Resistant TB in FBiH, 2020 recorded a total of 1 patient, from monoresistant (MR-TB), and no cases of polyresistant (PR-TB) and multidrug-resistant tuberculosis (MDR-TB) were recorded.

During the previous two years, 66 patients were treated at the hospital in Tuzla Canton in 2022 yr and 55 patients in 2023 yr. In the first four months of this year, 18 patients were treated at the Clinic for the Lung disease in TC.

PATIENTS WITH RISK FACTORS FOR DEVELOPING TUBERCULOSIS (TB) IN SLOVENIA BY COUNTRY OF BIRTH FROM 2011 UNTIL 2023

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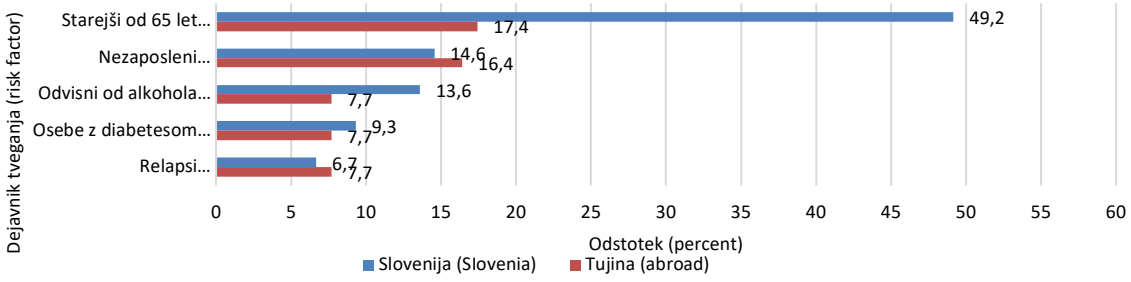
Some people with certain risk factors have an increased risk of infection with *M. tuberculosis* and also higher risk of TB reactivation. Due to covid-19 pandemic and several socio-political crisis around the globe in the past couple of years which impacted the TB occurance, we wanted to check if the analysis of risk factors would show any change as well as tendency of risk factors to change through certain time periods.

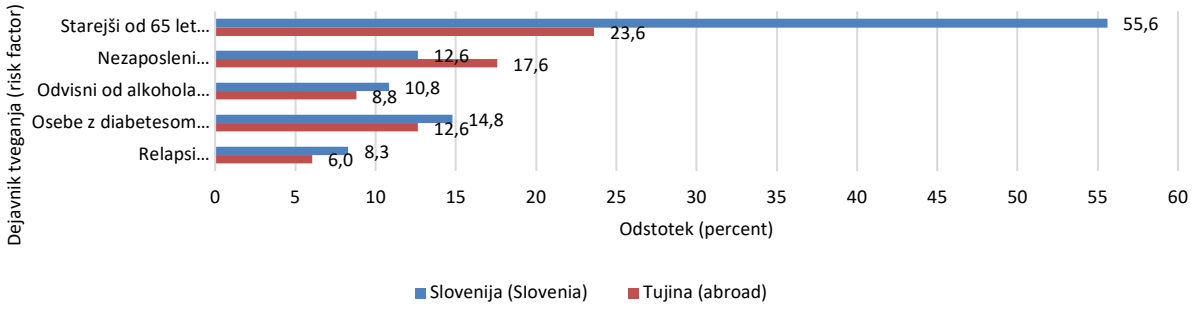
METHOD: we analysed retrospectively the documentation of patients entered into Registry of TB between 2011 and 2023. The whole time period was devided into three groups: 2011-2014 – first period; 2015-2018 – second period; 2019-2023 – third period. We determined 5 most common risk factors in patients born in Slovenia and abroad. We also evaluated the tendency of risk factors to change during the aformentioned time periods.

Results:

Graf 1: Delež dejavnikov tveganja pri TB bolnikih glede na državo rojstva, 2011 - 2014
Graph 1: Proportion of risk factors in TB patients by country of birth, 2011 - 2014

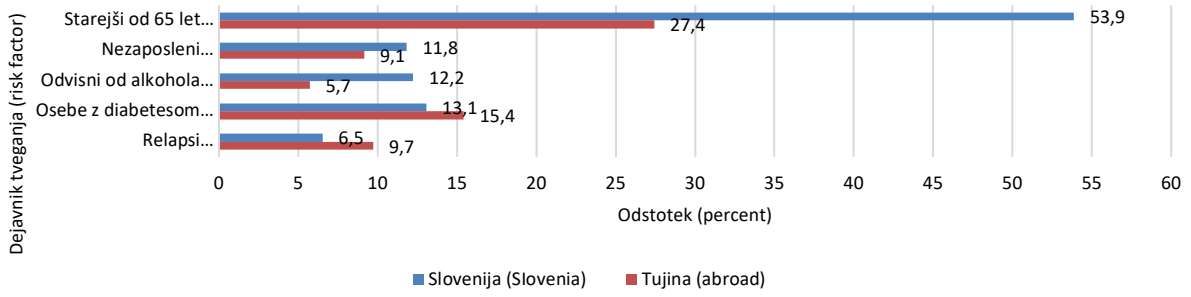


Graf 2: Delež dejavnikov tveganja pri TB bolnikih glede na državo rojstva, 2015 - 2018
Graph 2: Proportion of risk factors in TB patients by country of birth, 2015 - 2018



Graf 3: Delež dejavnikov tveganja pri TB bolnikih glede na državo rojstva, 2019 - 2023

Graph 3: Proportion of risk factors in TB patients by country of birth, 2019 - 2023



In TB patients born in Slovenia age is the most common risk factor. Unemployment as a risk factor was in decline in this group of TB patients while risk of TB in diabetics was increasing. In the past few years share of TB patients with alcohol abuse increased again while the share of relapses dicreased
In TB patients born abroad age is the most common risk factor as well, although the share is smaller than in patients born in Slovenia. In patients born abroad the share of unemployed patients and share of relapses dicreased, share of TB patients with diabetes is increasing.

CONCLUSION: In Slovenia age is the most common risk factor for TB. The share of patients born abroad among all detected TB cases is also increasing. Among TB patients there are still low numbers of patients from vulnerable groups, e.g. homeless people, drug addicts, prisoners, HIV infected persons, refugees, which is a consequence of good control and prevention activities carried out by Registry of TB and multidisciplinary teams.

ANALYSIS OF DEMOGRAPHIC CHANGES IN PATIENTS WITH TUBERCULOSIS (TB) IN SLOVENIA BETWEEN 2014 AND 2023, WITH A FOCUS ON FOREIGNERS

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Slovenia is categorized among nations with a low tuberculosis (TB) incidence rate (i.e. fewer than 10 cases per 100,000 population). In such countries, a considerable portion of TB cases is attributable to individuals born outside the country, who migrate from regions with higher TB incidence rates. The socio-political unrest of recent years has significantly influenced migration patterns into Europe. As such, we wanted to investigate the shifts in the demographic composition of patients receiving TB treatment over the past decade. This analysis places particular emphasis on TB patients who were born abroad.

METHODS: We conducted a retrospective analysis of patient data entered into the TB Register of the Republic of Slovenia between 2014 and 2023. We divided the 10-year period into two intervals: the first interval from 2014-2018 and the second interval from 2019-2023. We examined the gender and average age of the patients, as well as the country of birth (foreigners = born outside Slovenia). The birth countries of the foreign-born patients were categorized into "Near" (Croatia, Bosnia and Herzegovina, Serbia, Montenegro, Macedonia, Kosovo) and "Distant" (Afghanistan, Eritrea, Thailand, China, Pakistan, and other countries).

RESULTS:

	Combined	**Foreign**	**Slovenia**	**Proportion of foreign born**
2014-2023	1022	393	629	38,5%
2014-2018	602	218	384	**36,2%**
2019-2023	420	175	245	**41,7%**

Table 1: Number of TB patients from 2014 to 2023 by country of birth

		Male	Female	Average age (combined)	Average age (men)	Average age (women)	Proportion of women
2014-2023	**ALL**	618	404	59,6	56,5	64,4	39,5%
	FOREIGN	276	117	51	50	53,2	29,8%
	SLOVENIA	342	287	65,1	61,8	69	45,6%
2014-2018	**ALL**	362	240	59,8	56,1	65,4	39,9%
	FOREIGN	157	61	51,2	49,7	55,2	**28,0%**
	SLOVENIA	205	179	64,7	61,1	68,9	46,6%
2019-2023	**ALL**	256	164	59,4	57,1	63	39,0%
	FOREIGN	119	56	50,6	50,5	51	**32,0%**
	SLOVENIA	137	108	65,6	62,8	69,2	44,1%

Table 2: Demographic characteristics of TB patients from 2014-2023 by country of birth

	Foreign combined	Near countries	Distant countries	Proportion of patients from distant countries
2014-2023	393	352	41	10,4%
2014-2018	218	198	20	**10,1%**
2019-2023	175	154	21	**12,0%**

Table 3: Foreign-born TB patients categorized into "Near" and "Distant" country groups

Over half of TB patients were born in Slovenia – 61.5%, but this proportion is declining. Conversely, the share of patients born abroad has increased – from 36.2% to 41.7%.
The gender ratio and average age in the Slovenian population have not significantly changed in recent years. Among patients born abroad, males predominated – 70.2%. In recent years however, the proportion of women among foreign-born patients has increased – from 28.0% to 32.0%.
Foreign-born patients are generally younger than Slovenian ones by 8.6 years. In recent years, the average age of foreign-born female patients has decreased - by 4.2 years.
Among foreign-born patients, a majority were born in countries near Slovenia – 89.6%. The proportion of patients who came to Slovenia from distant countries is slowly increasing – from 10.1% to 12.0%.

CONCLUSIONS: In Slovenia, from 2019-2023, the share of foreign-born TB patients rose, aligning with low TB incidence country trends. Among these foreign-born patients, there has been an increase in the proportion of women. These women are on average much younger than foreign-born female TB patients in the period 2014-2018. There is also a mild increase in the number of TB patients born abroad who move to Slovenia from distant countries. These immigrants usually settle here permanently and establish families. The demographic structure of TB patients born in Slovenia has remained largely unchanged in recent years.

REVIEW OF PATIENTS WITH TUBERCULOSIS FROM 2014 TO 2023 IN THE DEPARTMENT OF PULMONARY DISEASES AT THE UNIVERSITY MEDICAL CENTRE MARIBOR

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In the last ten years (2014–2023), a total of 145 patients (89 males and 56 females) were treated at the Department of Pulmonary Diseases of the University Medical Centre Maribor.
In 4 patients, the diagnosis of tuberculosis was made during an autopsy; 10 patients died before the start of treatment or the diagnosis based on positive sample cultures was confirmed only after death; 15 patients died during treatment, of whom only 3 died due to tuberculosis; 4 patients emigrated from Slovenia during treatment; and the treatment was successfully completed by 101 patients (70%).
The average age of the patients was 62 years: the youngest patient was 23 years old, and the oldest was 92 years old.
117 patients had pulmonary tuberculosis, with 20 patients having an additional site of disease: 6 had tuberculous pleurisy, 3 had tuberculosis of the throat, 1 had lymph node involvement, 8 had genitourinary involvement, and 2 had spinal involvement.
Extrapulmonary tuberculosis was demonstrated in 26 patients: most commonly lymph node tuberculosis (13), pleurisy (10), genitourinary tuberculosis (1), and subcutaneous abscess (2).
76 patients, or 52%, had the infectious form of pulmonary tuberculosis. Of these, 49 were male (55%) and 27 were female (48%).
In reviewing the data, we focused on risk factors for tuberculosis reactivation. Factors that increase the likelihood of infection progressing to disease are typically host-dependent.
The most important are all conditions that alter the immune response, with HIV infection being the most prominent. Other significant factors include immunosuppressive medications, diabetes, alcohol, smoking, and malnutrition. We were also interested in how many of our patients come from countries with a higher incidence of tuberculosis.
We also checked how many patients were residing in a nursing home at the time of diagnosis.

PLENARY SYMPOSIUM

ASTHMA

Invited lecture

BASOPHILS AND MAST CELLS IN OMALIZUMAB TREATMENT OF SEVERE ALLERGIC ASTHMA

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ABSTRACT

BACKGROUND: Patients with severe allergic asthma are suitable candidates for omalizumab therapy, however some patients do not respond to treatment. Currently, reliable biomarkers to predict the response of severe asthma patients to omalizumab are not available. Treatment of patients with omalizumab results in several consistent changes in circulating basophils and mast cells, which are associated with beneficial response in patients with chronic urticaria. Additionaly, higher basal serum tryptase levels and higher active tryptase allele number were associated with poor clinical responses to omalizumab. Most common casue of elevated basal tryptase is hereditary α-tryptasemia (HαT), caused by increased alpha-tryptase–encoding *TPSAB1* copy number.

OBJECTIVE: Our aim was to assess if the changes in circulating basophils or higher active tryptase allele numbers are associated with efficacy of omalizumab treatment in patients with severe allergic asthma.

METHODS: In a cohort (N=99) with severe allergic asthma from a referral centre in Slovenia treated with omalizumab we evaluated the dynamics of basophil count, FcεRI count on the surface of basophils, and IgE count at three time points: before starting treatment, at 2–4 weeks, and at 3 months. Additionally, we conducted tryptase genotyping to determine the prevalence of HαT.

RESULTS: Poorer response to omalizumab therapy was demonstrated in 31% of patients. Patients with severe asthma treated with omalizumab did not exhibit a change in the quantity of circulating basophils, but there was a significant reduction in the expression of FcεRI and the number of IgE on the cell surface, after 1 month and 3 months of therapy . However, the dynamics of basophils, FcεRI, and IgE on basophil surfaces did not correlate with the long-term clinical response to omalizumab treatment in asthma patients. Overall, HαT was common in severe allergic asthmatics (10%) and detected in 23% of individuals with poor clinical responses to omalizumab versus 4% of those with positive responses (P = .010).

CONCLUSION: Changes on basophils in peripheral blood during omalizumab treatment are not associated with clinical response, while patients with HαT had and poorer clinical response to anti-IgE treatment in severe allergic asthmatics.

INTRODUCTION: Severe asthma is a subtype of asthma where patients remain inadequately controlled despite high doses of inhaled corticosteroids and β-adrenergic agonists. This leads to a greater burden of symptoms and exacerbations, necessitating additional oral corticosteroid therapy, frequent hospitalizations, diminished quality of life, and high healthcare costs. Although its prevalence is estimated at 5-10% of all patients with asthma, it has significant impacts on health and socio-economic status (1). Patients with severe asthma may benefit from biologic treatments, which target various immunological pathways such as IL5, IL4/IL13, TSLP, or IgE antibodies. Severe allergic asthma, a specific subset of severe asthma cases, is characterized by heightened immune reactions to specific allergens like pollen, dust mites, fungi, or pet dander. Patients with severe allergic asthma are suitable candidates for omalizumab therapy (2).

Omalizumab is a biologic monoclonal anti-IgE antibody used not only for the treatment of severe allergic asthma but also for the treatment of chronic spontaneous urticaria and in some cases, allergic rhinitis. Chronic spontaneous urticaria (CSU) is a skin disease where spontaneous urticaria (hives) occurs most days of the week, lasting six weeks or more. CSU is not a T2 inflammation-related disease nor an atopic disease. Severe cases can be successfully treated with omalizumab, but not all CSU patients respond well (3,4). Treatment of patients with CSU with omalizumab results in several consistent changes in circulating basophils, reducing the circulating basophil numbers in peripheral blood, and also number of IgE and FcεRI receptors on basophil surface. All these immunological changes are associated with clinical efficacy and good response to omalizumab treatment (5) .

In patients with asthma omalizumab reduces the concentration of total IgE in plasma, indirectly reducing the expression of the FcεRI receptor on the surface of inflammatory cells such as basophils, mast cells, dendritic cells, eosinophils, and also on structural cells such as epithelial cells and smooth muscle cells in the airways. Omalizumab thus interrupts the inflammatory cascade of asthma mediated by IgE in both the early and late asthmatic responses (6). However, despite the potential effectiveness of omalizumab, some patients do not respond to treatment. Currently, reliable biomarkers to predict the response of severe asthma patients to omalizumab are not available.

Mast cells are crucial immune cells found in various external environments, including the skin, gut, and lungs. They play a pivotal role in the body's defense against pathogens and allergic reactions, serving as frontline defenders against bacteria, viruses, and other foreign pathogens. Mast cell precursors from the blood migrate to the airway wall, including within airway smooth muscle bundles, where they are exposed to a host of local factors that cause them to mature. In the asthmatic lung, mast cells can directly promote smooth muscles contraction and bronchoconstriction by releasing agents (e.g. CysLTs, histamine) that serve as contractile agonists (7–12). The best-characterised mechanism by which mast cells secrete pro-contractile agonists is via activation of the high affinity immunoglobulin E (IgE) receptor, FcεRI. Airway IgE levels are elevated during allergic lung inflammation associated with asthma, causing mast cell FcεRI activation and release of contractile agonists (primarily CysLTs and histamine in human mast cells) that cause smooth muscle contraction and thus bronchoconstriction. In the bronchoalveolar lavage (BAL) of asthma patients, an association has been found between tryptase concentration and disease severity. This connection is also present with peripheral blood (basal tryptase), but it is not as strong. In the group of patients with severe asthma, tryptase is increased in BAL and peripheral blood regardless of the degree of eosinophilic inflammation (13).

Hereditary alpha-tryptasemia (HαT) is a genetic condition marked by increased levels of alpha-tryptase, a specific type of tryptase enzyme, in the bloodstream. HαT is common and has been found to be present in approximately 5% of people in Western Europe and the United States. In individuals with HαT, there is an amplification of the gene encoding alpha-tryptase, leading to higher-than-usual

levels of this enzyme within their mast cells. In these patients, the heightened production of tryptase by mast cells translates to increased levels of alpha-tryptase in tissues. While certain tryptases stored inside mast cells are believed to contribute to allergy symptoms, the version of tryptase (pro-tryptases) that can be detected in people regularly at baseline – and that is elevated in HαT – has currently no known function in human health or disease. The majority of individuals (estimates are that up to 2/3) with HαT appear to be asymptomatic or have few symptoms. Among individuals presenting to medical attention, HαT is associated as a modifier of the disease, such as the severity of anaphylaxis and irritable bowel disease (14,15)
In this study, our objective was to investigate whether alterations in basophils or mast cells are linked to the response of patients with severe asthma to omalizumab, and if they could serve as predictors of a clinical response to omalizumab.

METHODS: All adult patients with severe allergic asthma treated with omalizumab (N=99) between 2007-2019 at the University Clinic Golnik were included in this retrospective analysis. Severe asthma was diagnosed according to ERS/ATS guideline criteria and the GINA guidelines. All included patients were on high doses of inhaled corticosteroids (ICS) combined with long-acting beta-agonist (LABA); despite the treatment, all had at least 2 exacerbations requiring oral corticosteroids (OCS) per year or were on maintenance OCS treatment. Patients with severe allergic asthma were eligible for omalizumab treatment if sensitization on skin prick test or specific IgE to inhalant allergens was confirmed and total IgE ranged from ≥30 to ≤1500 IU/mL. All eligible patients were treated with omalizumab as an add-on therapy to concomitant asthma treatment; the dose (mg) of omalizumab and dose frequency was based on the serum total IgE level (IU/mL) and the patient's body weight (kg). A positive response to omalizumab was defined as at least 50% reduction of exacerbations and/or 50% reduction of maintenance OCS after 12 months of treatment.
For all patients, the following baseline clinical/laboratory parameters were collected: demographic data (age, sex), body mass index (BMI), forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), FEV1/FVC, diffusing capacity for carbon monoxide (DLCO), nitric oxide in exhaled air (FeNO), asthma control test (ACT), asthma exacerbations, asthma therapy, smoking status, total serum IgE levels, absolute number of peripheral blood eosinophils and baseline serum tryptase.
We evaluated whether the dynamics of basophil count, FcεRI count on the surface of basophils, and IgE count on the surface of basophils could predict the clinical response to omalizumab treatment in severe asthma patients. We assessed these parameters at three time points: before starting treatment, at 2–4 weeks, and at 3 months. Additionally we performed tryptase genotyping in all patients with severe allegic asthma treated with omalizumab.

RESULTS: We analyzed the treatment course of 99 patients with severe asthma who were treated with omalizumab. After one year of treatement with omalizumab a good response (higher ACT scores, fewer exacerbations per year and a decrease in maintenance OCS) was observed in 68 patients, and treatment was continued in this group. However, 31 patients had poorer clinical responses to omalizumab, and treatment with omalizumab was discontinued (Table 1).

	Good responders			Poor responders		
	Before	After 12 months	*P* value[a]	Before	After 12 months	*P* value[a]
No.	68			31		
Exacerbations in 12 months, median (IQR)	3 (2-4)	0 (0-1)	**<.0001**	4 (3-5)	3 (2-4)	**0.048**
ACT, median (IQR)	18 (12-22)	22 (18-24)	**<.0001**	18 (15-20)	17 (15-20)	0.576
OCS maintenance, No. (%)	22 (32)	8 (12)	**0.007**	22 (71)	18 (58)	0.426

Table 1: Clinical characteristics of patients before and after starting omalizumab treatment.

The most significant clinical differences between good and poor responders were that patients with a poor response did not have allergic rhinitis. They also exhibited lower FEV1, lower TI, lower DLCO, and were more frequently on continuous systemic steroid therapy.

Patients with severe asthma treated with omalizumab did not exhibit a change in the quantity of circulating basophils. However, there was a significant reduction in the expression of FcεRI and the number of IgE on the cell surface, after 1 month and 3 months of therapy . However, the dynamics of basophils, FcεRI, and IgE on basophil surfaces did not correlate with the long-term clinical response to omalizumab treatment in asthma patients (Figure 1)

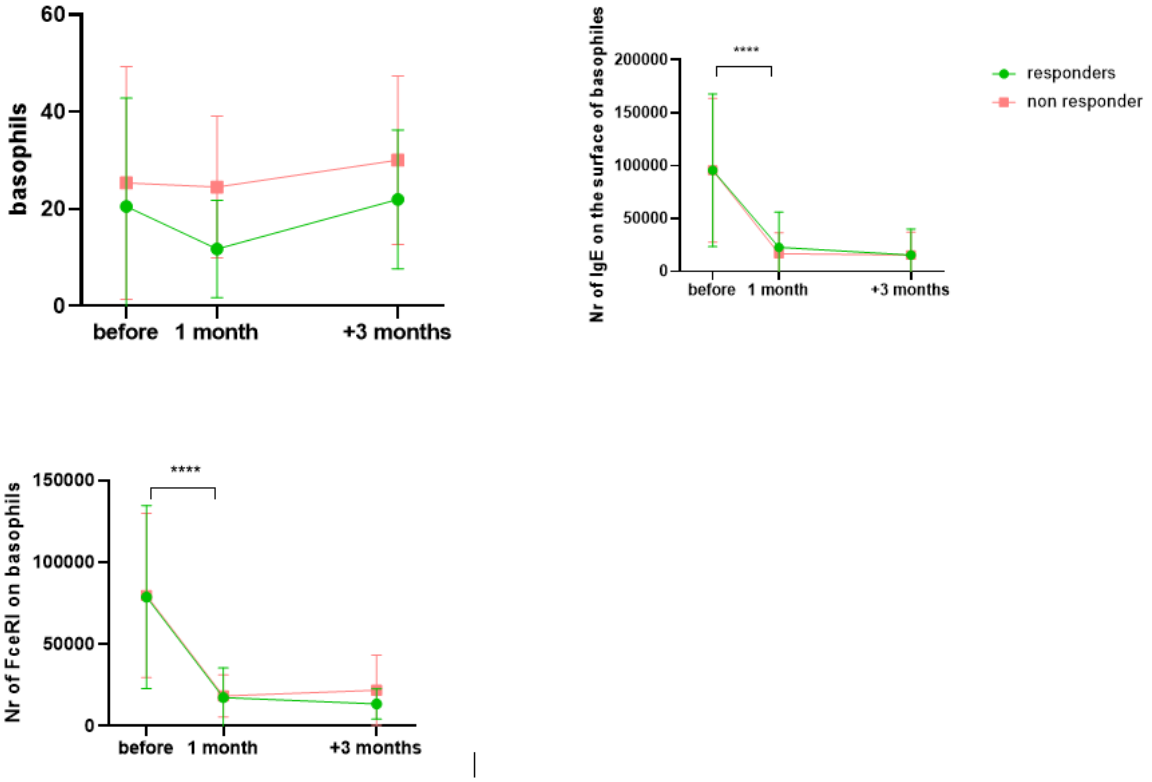

Figure 1: Dynamics of basophil count, IgE count on the surface of basophils, and FcεRI count on the surface of basophils during treatment with omalizumab in patients with severe asthma.

Next, we analysed the prevalence of HαT in our cohort stratified according to response to omalizumab treatment. A total of 23% (7 of 31) of individuals with poor clinical responses were found to have HαT versus 4% (3 of 68) of those responding well to the add-on omalizumab treatment. The prevalence of HαT among individuals with poor responses to omalizumab was also significantly greater than the reported 5.7% prevalence in the general Caucasian population.

CONCLUSIONS: Omalizumab treatment affects cells that express IgE and FcεRI receptors, such as basophils and mast cells. In this study, we investigated the correlation between the clinical response to treatment and the basophils and mast cells in patients with severe allergic asthma.

Treatment of patients with omalizumab results in several consistent changes in circulating basophils, reducing the circulating basophil numbers in peripheral blood, and number of IgE and FcεRI receptors on basophil surface. In patients with CSU, omalizumab promptly reverses the decrease in peripheral blood basophil count, while also diminishing the levels of FcεRI and IgE on the cell surface and these immunological shifts are closely associated to clinical effectiveness (5). In contrast, individuals with severe asthma treated with omalizumab do not exhibit a change in the quantity of circulating basophils. There is a reduction in the expression of FcεRI and the number of IgE on the cell surface, but the dynamics of basophils, FcεRI, and IgE on basophil surfaces do not indicate the long-term clinical response to omalizumab treatment in asthma patients. Therefore, studying basophils in peripheral blood may not reflect the situation in the lungs accurately. A more informative assessment of the effect of omalizumab on structural cells, such as mast cells, epithelial cells and smooth muscle cells of the respiratory tract, would likely be challenging due to their difficult accessibility.

Mast cells serve as the primary source of tryptase in the body, with tryptases playing roles in severity of asthma, in airway balance, vascular regulation, and being found at elevated levels in the bronchoalveolar lavage of patients with severe asthma. In HαT extra copies of the *TPSAB1* gene result in increased production of alpha-tryptase and elevated tryptase levels. In certain circumstances HαT is associated with more severe allergic reactions and more severe irritable bowel syndrome symptoms (14)(15). Our study demonstrated that HαT is linked to reduced response to omalizumab in patients with severe allergic asthma. However, how alpha-tryptase contribute to symptoms associated with HαT remains unclear. Elevated alpha tryptase levels may lead to increased autocrine activation of mast cells. This heightened mast cell activity can result in the release of various inflammatory mediators and cytokines, contributing to asthma symptoms. Further research aims to determine whether the presence of HαT is associated with a specific phenotype of severe asthma and the response to omalizumab treatment. If proven, genotyping of tryptase could help identify patients likely to have a better response to omalizumab, guiding treatment choices.

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Invited lecture

CHALLENGES IN PHENOTYPING OF SEVERE ASTHMA AND LESSONS FROM BIOMARKERS

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The successful management of severe asthma requires consideration of clinical heterogeneity and characteristics that are driven by varying clinical and inflammatory phenotypes (1). Clinical asthma phenotypes are the products of underlying inflammatory processes. These might be categorized into two basic inflammatory phenotypes which are defined by the predominant immunological pathways driving the disease pathology: eosinophilic and noneosinophilic (1). The major downstream T2 cytokines in the airways are IL-5, IL-4, and IL-13 which play distinct roles in the airway: Increased production of IL-5 induces hypereosinophilia, IL-4 mediates a switch in B cells leading to the synthesis of IgE and subsequent elevations in levels of total and specific IgE. IL- 13 regulates the production of nitric oxide (FeNO), muscle contraction and bronchial hyperreactivity (3). Biomarkers are important not only for phenotyping asthma but also for diagnosis, evaluation of disease risk and severity, prognosis, treatment selection and monitoring the response to treatment (4). Elevated blood eosinophil count (BEC) and FeNO levels are associated with an increase in airflow limitation, mucus plugging, and an increased risk of severe asthma exacerbations (5). Total IgE levels may be useful in the diagnostic procedure of alternative or overlapping diagnoses (asthma with fungal sensitisation), but they have not demonstrated prognostic or theragnostic value in identifying a suitable biologic therapy. The concept of phenotypisation includes as well clinical characteristics including age of asthma onset and comorbidities. A recent study found a median number of 3 comorbidities per patient attending a specialist-referral difficult asthma clinic (6). Patients should be assessed using a systematic, multidisciplinary evaluation to determine the contribution of all comorbid conditions, including extrapulmonary factors such as obesity, anxiety or depression, and chronic rhinosinusitis with nasal polyps. This is particularly necessary for symptomatic patients with aT2-low phenotype. In fact, a patient with T2-low asthma who is very symptomatic and receiving high-dose asthma treatment is often indicative of comorbid disease (1). Common inflammatory ground for example links obesity, insulin resistance, and asthma. As recognition of their interplay, one worsening the natural course of the other, is recognised, questions remain about how to adequately address them altogether to improve clinical outcomes (6).

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PLENARY SYMPOSIUM

PULMONARY VASCULAR DISEASES

Invited plenary lecture

PULMONARY THROMBOENDARTERECTOMY PROGRAM IN ROYAL PAPWORTH HOSPITAL CAMBRIDGE

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Royal Papworth Hospital (RPH) is one of the four reference centers in the world for pulmonary endarterectomy for the treatment of chronic thromboembolic pulmonary hypertension (CTEPH). To date, over 2800 pulmonary endarterectomies have been performed for the entire population in the United Kingdom since 1997 as well as international referrals.

The most important component of the CTEPH program at RPH is the expert multidisciplinary team (MDT) that review all the cases of CTEPH referred by the 7 national pulmonary hypertension centers in England, Wales, Scotland, and Northern Ireland. The team consist of a dedicated team of cardiothoracic surgeons, respiratory physicians, cardiologists, and radiologists. They are supported by a dedicated team of anaesthetists, intensivists and clinical perfusionists.

The team review all referred cases of CTEPH every week which amounts to 450—500 cases per year. From this weekly MDT meeting, patients are triaged to pulmonary endarterectomy (PEA), balloon pulmonary angioplasty (BPA) or targeted medical therapy. This has resulted in 180-200 cases of PEA yearly at RPH for the last 10 years with in-hospital mortality of less than 3%.

We have the world's largest continuous database of over 2500 patient with chronic thromboembolic disease (CTED) with up to 5 years of post-procedural follow up. This has allowed us to publish the world's largest long term outcome work on patient with CTED as well as continual clinical research in collaboration with University of Cambridge at the Cambridge Heart and Lung Research Institute.

We share our expertise in helping centers around the world to establish PEA program by training healthcare professionals in all aspect of treating patients with CTED including surgeons, anaesthetist, intensivists and clinical perfusionist. Royal Papworth has the only dedicated PEA fellowship program in the world, having trained surgeons from India, Singapore, Australia, and the United States.

Invited lecture

RIGHT HEART CATHETERISATION IN UNIVERSITY MEDICAL CENTRE LJUBLJANA, SLOVENIA

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PURPOSE: Pulmonary hypertension (PH) is a pathophysiological state defined as an increase in mean pulmonary artery pressure (mPAP) ≥ 20 mmHg at rest as measured by right heart catheterization (RHC). PH can be categorized into five classes based on similar pathophysiology, hemodynamics, and management: pulmonary arterial hypertension (group 1), PH as a cause of left heart disease (group 2), PH due to lung disease / chronic hypoxemia (group 3), chronic thromboembolic pulmonary hypertension (group 4) and PH with unclear and multifactorial mechanisms (group 5). RHC is minimally invasive procedure that allows direct hemodynamic measurement of pulmonary pressures. While echocardiography is the best screening method, RHC is the gold standard for establishing the diagnosis of PH. It is necessary to differentiate between pre- and post-capillary PH. RHC is also a tool to establish prognosis and assess response to treatment. Hemodynamic diagnosis of PH and its subtypes requires measurement of PAP, pulmonary artery wedge pressure (PAWP), cardiac output (CO), and calculation of pulmonary vascular resistance (PVR). Although RHC is an invasive procedure, it is safe when performed in experienced centers, and the risk of complications is low (the overall rate of serious adverse events is 1.1%, and fatal complications are less than 0.1%).

METHODS: We analyzed the data of all the patients in whom the RHC was performed in the years 2018 to 2023. The procedure was performed via right internal jugular vein using a standard 7F fluid-filled thermodilution pulmonary artery flotation catheter (Swan Ganz type). We measured pressures in the right atrium, right ventricle, pulmonary artery, and PAWP. CO was measured via the thermodilution method, and PVR was calculated from these variables. We also analyzed the saturation of arterial and mixed venous blood. Diascopy was used when we had problems with catheter guiding and/or wedging. Besides basic measurements, we performed a fluid stress test with 500 mL 0.9% saline solution in all the patients and a vasodilation test with nitric oxide when necessary. Patients stayed in the observation and were dismissed the same or the next day.

RESULTS: In the years 2018 - 2023, we performed 249 RHCs. Usually, we perform more than 50 procedures per year (except in the pandemic era). 72 (41.4%) patients were male and 102 (58.6%) were females. The average age was 61.4 years; the youngest was 18, and the oldest was 86. Follow-up was performed in 75 cases. Group 1 PH was diagnosed in 24%, group 2 in 7%, group 3 in 5%, group 4 in 37% and group 5 in 4% of the cases. In 12% of cases, patients had combined pre- and postcapillary pulmonary hypertension, and in 11%, we excluded pulmonary hypertension. Diascopy was used in 39% of the cases, mainly in group 4 PH, because of wedging problems. Complications were primarily hematomas on the puncture site (6.4% of the cases).

CONCLUSIONS: Right heart catheterization is an indispensable tool for diagnosing different types of pulmonary hypertension, assessing response to treatment, and assesing the prognosis. The procedure must be performed in experienced centers where results are standardized, concise and where all complications can be managed, although the rate is meager.

Invited lecture

ONSET OF PULMONARY THROMBOENDARTERECTOMY PROGRAM FOR TREATMENT OF CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION IN UNIVERSITY MEDICAL CENTRE LJUBLJANA, SLOVENIA

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PURPOSE: Chronic thromboembolic pulmonary hypertension (CTEPH) is an unique form of pulmonary hypertension, that can be completely cured with pulmonary thromboendarterectomy (PTE), which is a demanding surgical procedure. In the past we had been sending Slovenian patients to AKH Vienna for PTE, but since the accessibility of treatment abroad had decreased during pandemy and the proportion of newly diagnosed patients is rising, we decided to start our own PTE program with the valuable help of experts from Royal Papworth hospital Cambridge, one of the world's most established centres for this intervention.

METHODS: We have analyzed the data from all patients, to whom PTE was performed in our center from November 2022 to end of December 2023 All the patients were carefuly discussed at the Cambridge PTE multidisciplinary meeting, and later on operated by our cardiovascular surgenos, supervised by expert PTE surgeon from Cambridge. The follow up of the patients was performed 6 months after the surgery (including invasive mesurement of pulmonary pressures).

RESULTS: PTE was performed on 10 patients, 6 male, 4 women, age 22-76, most had at least one comorbidity. All of the patients clinical state improved after the surgery. After the surgery the mean pulmonary artery pressure (43 to 22 mmHg), pulmonary vascular resistance (5,8 to 2,3 wood units) and NT pro BNP (374 to 262 pg/L) all almost normalized. WHO function class improved for most of the patients (3,5 vs 1,1). In only 2 patients we observed residual pulmonary hypertension that needed further medical treatment. We observed no severe complications during or after the procedure.

CONCLUSIONS: Commencement of PTE program is a great novelty for patients with CTEPH in Slovenia. The results of our first 10 patients indeed confirm that with the solid support of collegues from Cambridge and maintainance of decision making at their multidisciplinary PTE meetings, we feel confident that we can offer our patients treatment of the same quality as it is in high volume expert centers.

Invited lecture

SURGICAL ASPECTS OF ESTABLISHING A NATIONAL PTE PROGRAM AT UMC LJUBLJANA

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OBJECTIVE: Pulmonary thromboendarterectomy (PTE) is the mainstay of therapy for patients with chronic thromboembolic pulmonary hypertension (CTEPH) since the procedure leads to major clinical improvement and represents a potential cure for a large majority for patients suffering from CTEPH. However, PTE is a highly complex surgical intervention with a multitude of potential morbidity and surgery-related risks. Slovenian CTEPH patients identified as surgical PTE candidates were traditionally referred to a medium-volume program at Vienna Medical Centre with the disadvantage of international travel, pre- and post-operative care, and funding. Since the acceptance of our patients to Vienna program became inadequate or even temporarily impossible during Covid pandemics, the establishment of a national PTE program at UMC Ljubljana was proposed by leading national experts in the field.

METHODS AND RESULTS: Organization of national PTE program is a complex process involving diagnosis of patients, identification of surgical candidates, pre-, intra- and immediate post-operative management, and appropriate follow up. Training a team of medical professionals with adequate expertise is challenging as the number of patients is relatively small and the incidence of surgery-related risks can be extremely high, especially in unexperienced medical teams. Thus, a multidisciplinary team of cardiovascular surgeon, pulmonologists, interventional and imaging radiologists, anesthetists, and perfusionist were trained in a high-volume center at Royal Papworth Hospital in Cambridge, UK. Currently, all Slovenian patients who present with symptoms of CTEPH are comprehensively discussed at the Papworth Multidisciplinary meeting. The first 10 PTE surgeries at UMC Ljubljana were successfully performed by Slovenian medical team under close on-site supervision of the lead Papworth PTE surgeon. Later, surgeries were performed by the same medical team under off-site supervision using strict adherence to Papworth surgical protocol.

CONCLUSIONS: The launch of the PTE program in Slovenia, with a clear framework, coupled with good surgical outcomes is very encouraging and offers a curative solution for our CTEPH patients and potentially for patients from a broader Balkan region. A clear referral process and an increase in disease and treatment awareness in medical community are crucial for future success of the program, offering a definitive solution and avoiding treatment delays.

CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION IN A PATIENT WITH ADVANCED CYSTIC FIBROSIS - A CASE PRESENTATION

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INTRODUCTION: Cystic fibrosis is the most common monogenic disease of Caucasians. It is a multisystem disease predominantly affecting the lungs. In the advanced stage, it may be associated with significant pulmonary arterial hypertension (PAH) and cor pulmonale. When pulmonary hypertension is diagnosed, it is necessary to exclude other possible causes, firstly chronic thromboembolic pulmonary hypertension, especially in patients in whom anticoagulant therapy has not yet been initiated.

CASE PRESENTATION: A 40-year-old man was diagnosed with cystic fibrosis in adulthood at UMC Maribor, where he was admitted for acute respiratory insufficiency due to the exacerbation of inflammation in bronchiectasis (FEV1 29%). He was referred to UMC Ljubljana for further treatment. Due to advanced pulmonary disease and signs of right-sided heart failure, we performed a cardiac ultrasound, which showed a severely enlarged right ventricle with impaired systolic function (TAPSE 1.3 cm, FAC 28%) with normal right ventricular filling pressures and signs of severe pre-capillary pulmonary hypertension (estimated sPAP 73 mmHg) with normal cardiac output. Investigations were also performed to exclude other possible causes of pulmonary hypertension, where CT-A of the lung showed pulmonary embolisms of chronic appearance in most of the arteries of the left lung wing. Anticoagulant therapy was initiated, initially with heparin, then switched to warfarin, after which the patient's condition stabilised. The specific treatment of chronic thromboembolic pulmonary hypertension (pulmonary endarterectomy, specific drugs, balloon pulmonary angioplasty) and lung transplantation should be considered if the disease progresses in the future.

CONCLUSION: In any advanced pulmonary disease with severe PAH and cor pulmonale, other possible causes of PAH should be excluded, firstly chronic thromboembolic hypertension, as the introduction of anticoagulant treatment can prevent further deterioration due to recurrent pulmonary embolisms and specific treatment modalities can significantly improve the course of the disease.

PULMONARY CEMENT EMBOLISM AFTER VERTEBRAL KYPHOPLASTY - CLINICAL CASE

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INTRODUCTION: We present an interesting and rare case of a severe pulmonary embolism with bone cement after vertebral kyphoplasty. This condition is often asymptomatic but can also result in an obstructive shock. D-dimer, a serum marker usually present in pulmonary embolism, can be normal and management guidelines are not clearly established.

CASE REPORT: An 81-year-old male was admitted to the hospital following a fall and a fracture of the 11th thoracic vertebrae. Kyphoplasty of the fractured vertebrae was performed to reduce pain and vertebral body collapse. Two days later he became dyspnoic, hypoxic, hypotensive, tachycardic, oliguric, and distended jugular veins indicating increased central venous pressure were noted on physical examination.
Urgent cardiac ultrasound revealed a distended right ventricle and a broad, non-collapsible inferior vena cava. Computed tomography angiography of pulmonary arteries revealed hyperdense cement material occluding the anterior segment artery for the right upper lobe, and the same material in the subsegmental arteries for the right upper, middle, and lower lobes. The same material was observed in the paravertebral veins at the level of the fracture. Signs of right ventricular load, including right ventricular enlargement and interventricular septal bulging towards the left, were also noted. The patient was immediately started on noradrenaline, supplemental oxygen, and continuous heparin infusion. After three days of hospital treatment, the patient recovered.

PLENARY SYMPOSIUM

LUNG CANCER DIAGNOSTIC METHODS, DO WE NEED TO RECONSIDER OUR APPROACH

MALIGNANT SOLITARY Ground-Glass nodules – DO THEY NEED PET-CT?

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OBJECTIVE: According to recommendations, ground-glass nodules (GGN) are usually only monitored through imaging. If a solid component appears, invasive diagnostics are recommended. Sometimes, GGN persist or even indicate growth, prompting diagnostic procedures even without a present solid component. Our aim was to study diagnostic and treatment strategy of solitary GGNs: feasibility of performing PET-CT in preoperative disease staging, assessing the correlation between clinical and pathological stages, and determining the percentage of disease progression and survival rates.

METHODS: We retrospectively analyzed all patients diagnosed with newly detected non-small cell lung cancer (NSCLC) with a clinical stage T1 (tumors smaller than 3 cm) at the Golnik Clinic between 2014 and 2020, regardless of the clinical stage N and M. The exclusion criterion was the presence of simultaneously detected two or more lung carcinomas.
We reviewed the chest CT scans performed during the diagnostic process, classifying lesions as subsolid without solid component - GGN, subsolid with solid component, or solid. We focused on GGN. We were interested in whether a PET-CT was performed before surgery and if it influenced the clinical stage N/M or altered the course of treatment. We compared the clinical and pathological stages. We examined survival rates and whether there was disease recurrence after treatment completion.

RESULTS: Between 2014 and 2020, 889 solitary NSCLC of clinical stage T1 were diagnosed at the Golnik Clinic, of which 44 carcinomas had a radiological ground-glass opacity pattern. 5 lesions were smaller than 1 cm, 22 lesions were 1-2 cm, and 17 lesions were 2-3 cm. Lymph nodes or distant metastases were not detected in any case with CT scans performed during the diagnostic process.
Pathological diagnosis was confirmed before the planned treatment in 41 cases (either adenocarcinoma or atypical lepidic proliferation). In 3 cases, surgical treatment was indicated on MDT without a confirmed diagnosis based on lesion growth on consecutive CT scans.
PET-CT was performed in 27 cases (61.3%), confirming the clinical stage in 26 cases, and falsely raising suspicion of mediastinal lymph node metastases in 1 case (subsequently, EBUS staging of possible lymph node metastases did not confirm it). Course of the treatment was therefore not altered in single case. In most cases, the PET-CT report indicated a low SUV of the primary tumor and unreliable assessment of potential metastases.
41 patients were treated surgically, 2 patients received radical radiation therapy due to comorbidities and poor lung function, and 1 patient was managed conservatively. Anatomical resection with lymphadenectomy (mostly lobectomies and some segmentectomies) were performed in 30 cases, and

wedge resection without lymphadenectomy in 11 cases. All resections were radical - R0. pN stage was 0 in all 30 cases where lymphadenectomy was performed, thus matching the clinical N stage 100%.
Postoperative histologic results revealed, that 35 were invasive adenocarcinomas (mostly lepidic, some acinary predominant), 4 were minimally invasive adenocarcinoma and 2 adenocarcinoma in situ. Micropapillary and solid component were present rarely, only once each.
Disease progression occurred in one case during the 3-year follow-up, but it was merely radiological suspicion, as invasive diagnostics were not possible due to the patient's poor condition and pathological confirmation and correlation were not done, so there is a strong possibility that new primary tumor was present. The three-year survival and/or progression free rate was 97.7%

CONCLUSIONS: With our research we showed, that PET-CT adds very limited value in preoperative disease staging of GGN. This might be because of two reasons:
- GGN in our study didn't metastasize to local lymph nodes or distant organs,
- the assessment of potential metastases is unreliable due to the low SUV of the primary tumor.

PET-CT can pe potentially harmful due to unnecessary ionizing radiation exposure and false positive findings, which may necessitate unnecessary invasive investigations. The findings from our study also question the guidelines for staging with EBUS-TBNA in cases where the tumor is smaller than 3 cm and is not avid on PET-CT, as in no instance did we find metastases in lymph nodes or record lymph node recurrence.
Because the survival of patients with GGN carcinoma in stage T1 is good and disease recurrence is rare, it is worth considering the necessity of PET-CT and EBUS in this group of patients before definitive treatment. Furthermore, in the choice of treatment, especially in surgery, it is reasonable to opt for the least invasive option.

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CORRELATION OF CLINICAL AND PATHOLOGICAL N STAGE IN OPERATED PATIENTS WITH NON-SMALL CELL LUNG CARCINOMA

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BACKGROUND: The clinical nodal (N) stage of non-small cell lung cancer (NSCLC) plays an important role in deciding on the most appropriate approach to cancer treatment. This stage is determined through imaging examinations such as CT or PET scans, or through pathological tests conducted on lymph node (LN) samples obtained via techniques like EBUS-TBNA or mediastinoscopy. Previous studies have highlighted a notable disparity between clinical (cN) and post-operative, pathological (pN) nodal stages, resulting in suboptimal treatment decisions for a considerable number of patients. Our objective was to assess the agreement between cN and pN stages among patients who underwent surgery after the diagnosis of NSCLC at the Clinic Golnik.

METHODS: We have retrospectively reviewed 84 patients with NSCLC from the clinical registry of the Clinic Golnik who underwent surgery in 2018. Data about cN and pN stages, clinical staging techniques, as well as other important factors associated with patient or cancer characteristics were obtained from the local information system. To assess agreement between cN and pN a simple percentage agreement was calculated. Sensitivity, specificity, NPV and PPV of each diagnostic technique were calculated.

RESULTS. Out of 84 patients, 51 patients were in pathologic stage I, 19 in stage II and 14 in stage IIIa disease. Final diagnosis was adenocarcinoma in 65, SCC in 16 and NSCLC in 3 patients. CT was performed in all patients, PET CT in 83, and EBUS-TBNA in 22 patients. According to clinical stage had 69 patients cN0, 10 cN1, and 5 cN2 stage. 67 stage pN0, 9 patients stage pN1 and 8 patients had stage pN2, The overall agreement between cN and pN was 78.6%; in stage I 96.3%, stage II 63.2% and stage IIIa 36%. Sensitivity, specificity, NPV and PPV for N2 stage were 38%/ 97.4%/ 54.5%/ 100% for CT, 38%/ 98,7%/ 54.5%/ 50% for PET, and 60%/ 100%/ 88.9%/ 100% for EBUS-TBNA. Sensitivity and NPV for metastasis in the sampled LN by EBUS-TBNA were 100%.

CONCLUSION. The agreement between clinical and pathological nodal stage was suboptimal, poor in stage III. Additional analysis is needed to investigate the possible underlying reasons for the discrepancies in cases of inconsistency, and reveal potential improvements in clinical staging.

Invited lecture

EBUS CRYOBIOPSY FOLLOWING UNSUCCESSFUL EBUS NEEDLE PUNCTURE OF MEDIASTINAL LYMPH NODES.

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INTRODUCTION: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is the preferred investigation for diagnosing mediastinal diseases and assessing the extent of lung cancer. EBUS-TBNA demonstrates excellent diagnostic yield and very high negative predictive value for excluding metastases of non-small cell lung cancer in lymph nodes, but it is less effective for benign lesions, lymphomas, and other rare lung tumors. Attempts to improve the diagnostic yield of EBUS-TBNA with different needles and methods have not been successful. In cases of inadequate samples and the need for further diagnosis, mediastinoscopy was performed. The recent invention of a new cryoprobe with a diameter of 1.1 mm allows it to be introduced through the EBUS bronchoscope under ultrasound guidance for performing cryobiopsy of mediastinal lesions. The advantage of this procedure is the collection of larger histological samples, the ability to examine the architecture of the tissue sample, and perform molecular diagnostics. Compared to mediastinoscopy, the procedure is safer and less invasive. In our study, we aimed to determine the contribution of cryobiopsy with histological samples in selected patients where definitive diagnosis was not possible with transbronchial needle aspiration.

METHODS: We retrospectively analyzed cryobiopsies of pathological lesions in the mediastinum performed at the Golnik Clinic in 2023 / 2024 after the introduction of EBUS-guided cryobiopsy of mediastinal lesions. Cryobiopsy was performed in selected patients: as a synchronous examination with EBUS-TBNA in cases of suspected lymphoma or as a repeat examination with inconclusive cytological findings from EBUS-TBNA. We conducted a comparative analysis of the data obtained from cytology/histology with EBUS-TBNA using a "histological" 19G needle and then with histological findings from cryobiopsy.

RESULTS: Until the time of analysis we performed EBUS-guided cryobiopsy of the mediastinum in 16 patients. Final diagnosis was accieved in all cases (100%). EBUS- cryobiopsy was successful in 13 cases (81%) and EBUS-TBNA in 7 cases (44%). In 9 cases, a definitive diagnosis was established with cryobiopsy after a non-representative/nonspecific finding on needle biopsy. In 4 cases where a diagnosis was initially made from the needle biopsy, additional immunohistochemical examinations were performed on the histological sample for a more precise diagnosis. In 3 cases EBUS-TBNA was diagnostic and EBUS – cryobiopsy unsuccesful. In 2 cases needle biopsy provided a diagnosis of small cell carcinoma and sarcoidosis whereas histology revealed necrosis. In the third case EBUS – TBNA yielded a diagnosis of adenocarcinoma, whereas EBUS – cryobiopsy was just suspicious for carcinoma. We did not record any complications from the performed cryobiopsies.

CONCLUSIONS: EBUS-guided cryobiopsy represents a valuable adjunct to the diagnostic armamentarium for mediastinal lesions. Its ability to yield larger tissue samples with acceptable safety makes it a promising tool for clinicians managing patients with challenging mediastinal pathology.

Furthermore, it enhances diagnostic accuracy and may obviate the need for additional more invasive procedures in certain cases. Based on existing literature and accumulated experience, we believe it is not sensible for this method to be routinely used in all patients with suspected mediastinal diseases, as EBUS-TBNA proves to be an appropriate examination in the group of patients with lung cancer. The contribution of cryobiopsy is observed in selected patients where cytology was inconclusive, or more material is needed for diagnosis (granulomatous diseases, rare cancers, lymphomas). For these patients, mediastinoscopy would be indicated as a third-line examination, which is a more invasive procedure with more potential complications. Further research and prospective studies are warranted to establish standardized protocols, optimize procedural outcomes, and refine its role in clinical decision-making.

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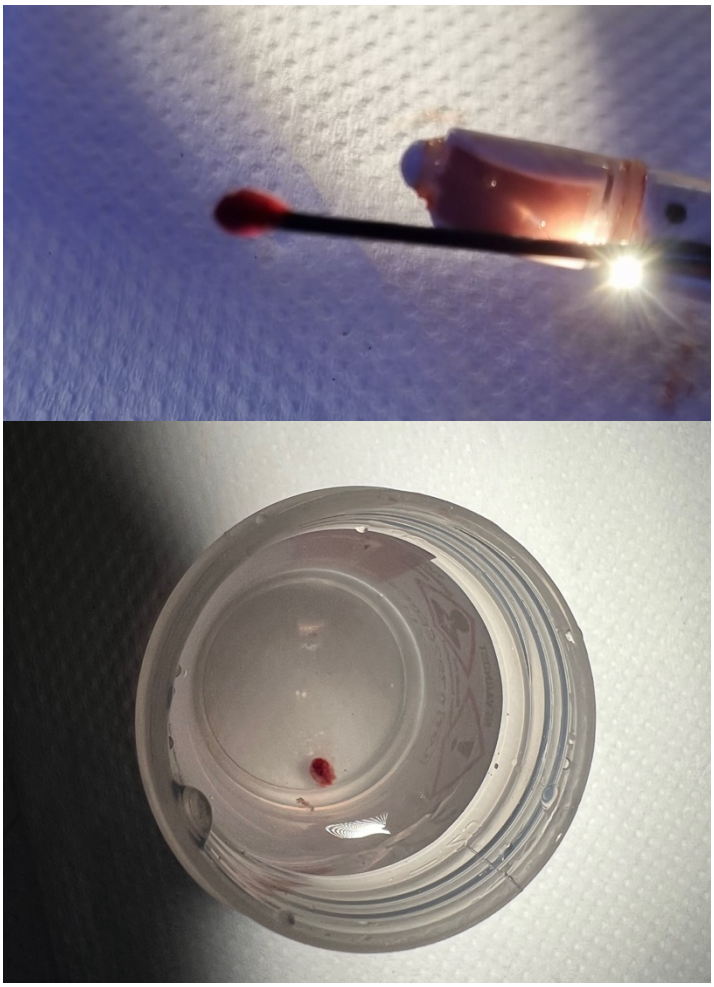

Figure 1,2: EBUS – guided cryobiopsy – the sample size.

Invited lecture

NEW MODELS FOR PREDICTION OF POSTOPERATIVE PULMONARY COMPLICATIONS IN LUNG RESECTION CANDIDATES.

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BACKGROUND: Following the yet outdated ERS/ESTS guidelines (pubslished in 2009), spirometry, diffusion capacity for carbon monoxide (DLCO) and/or spiroergometry (CPET) have been used for preoperative evaluation of lung resection candidates. In the recent decade, ventilatory efficiency for carbon dioxide (V_E/VCO_2 slope) and partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) emerged as independent predictors of post-operative complications (PPC) in lung resection surgery. However, single parameters give only partial information regarding peri-procedural hazards. Accordingly, our aim was to create new prediction models with improved ability to stratify the risk of post-operative pulmonary complications (PPC) in patients scheduled for elective lung resection surgery.

METHODS: This *post-hoc* analysis was comprised of consecutive lung resection candidates from two prior prospective trials. All the included individuals completed lung function tests and cardiopulmonary exercise testing (CPET) and were PPC were recorded from the first 30 postoperative days. We compared demographic, functional, CPET and in-hospital data of subgroups with and without PPC. Logistic regression analyses were used for identification of risk factors for PPC that were entered into the final risk prediction models. Two risk models were developed; the first used $P_{ET}CO_2$ at rest (for patients with no available CPET data), the second used V_E/VCO_2 slope (for patients with available CPET data). ROC analysis with the De-Long test and area under the curve (AUC) were used for comparison of both models.

RESULTS: The dataset from 423 patients was randomly split into the derivation (n=310) and validation (n=113) cohorts. Two final models were developed, both including FEV_1/FVC ratio, sex, thoracotomy and „atypical“ resection as risk factors. Above that, the first model also included rest $P_{ET}CO_2$, while the second model V_E/VCO_2 slope from CPET. AUCs of the new models were 0.777 (95% CI: 0.722–0.832) and 0.782 (95% CI: 0.727–0.837); both $p<0.001$. We have found no differences in AUCs between the derivation and validation cohorts.

CONCLUSIONS We created two new multicomponental models for PPC risk prediction, both having outstanding predictive properties.

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Invited lecture

UTILITY OF NGS FOR MOLECULAR PROFILING OF TUMORS FROM CELL-FREE DNA IN THE DIAGNOSIS OF NON-SMALL CELL LUNG CANCER (NSCLC)

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BACKGROUND: The next-generation sequencing method (NGS) allows for the simultaneous testing of multiple predictive biomarkers, and is essential for patients with metastatic NSCLC before determining the optimal systemic treatment approach. The tumor tissue is considered as optimal sample for molecular testing. Studies of free DNA analysis of in the blood (cfDNA) with NGS have shown a high success rate of DNA sequencing. The mutation concordance detected from the tumor and cfDNA has been reported in the range of 50-100% with the best concordance observed in more advanced disease. Our aim was to ascertain the concordance between detected mutations in the tumor tissue and blood of our patients with metastatic NSCLC.

METHODS: We collected 8ml of blood in cfDNA collection tubes from consecutive patients diagnosed with metastatic NSCLC at the interventional pulmonology ward at Clinic Golnik. The blood was frozen at -75°C within 6 days. Among the collected samples, we selected 10 for NGS analysis, all of which exhibited specific mutations in the tumor tissue. Nucleic acid extraction was done using Genexus purification system (Genexus cfTNA Purification Combo, ThermoFischer Scientific), and NGS was performed by Genexus Integrated Sequencer (Oncomine Precision Assay GX Combo, ThermoFischer Scientific).The concordance between mutations detected in tumor and cfDNA was calculated using a simple percentage agreement calculation.

RESULTS: Among the 10 included patients, KRAS mutation was detected in tumor tissue in 4 (G12C in 3 and Q61H in one) patients, while EGFR DEL, ALK-EML4 fusion, RET-KIF5B fusion, ROS-C74 fusion, NRAS-Q61K and BRAF V600E mutations were each detected in one patient. The overall concordance between detected mutations in the tumor and cfDNA was 80% (100% - 7 of 7 for SNPs and deletions detected from DNA, and 33% 1 of 3 for fusions detected from RNA.

CONCLUSIONS: The results of this pilot study demonstrating a good concordance between tumor and cfDNA are promising. Liquid biopsy could potentially replace the more invasive sampling in patients only suitable for targeted treatment and at higher risk for complications in the future.

FOLLOW UP OF NON-DETERMINED EXUDATIVE PLEURAL EFFUSIONS

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BACKGROUND: The study aimed to examine the long-term outcome of patients with non-determined exudative pleural effusions and assessed the frequency of false-negative diagnosis after non-diagnostic thoracoscopy.

METHODS: Among 409 patients who underwent thoracoscopy from 1.1.2000 until 31.12.2013 in University Clinic Golnik we retrospectively reviewed the data of 138 patients (33%) with non-diagnostic thoracoscopy - histological diagnosis of nonspecific pleuritis. Patients were evaluated in two analyses, conducted in 2014 and 2024.

RESULTS. Initial data analysis in 2014: Follow up of 138 patients revealed that the majority (90.5%) of non-diagnostic pleural effusions had a benign course. The most common causes were parapneumonic pleuritis (25% of patients). 23% had pleuritis with known exposure to asbestos, 22% of patients had idiopathic pleuritis - without any known cause. Other known causes were pleuritis due to systemic connective tissue and autoimmune diseases (11% of patients), paramalignant pleuritis (7% of patients), pleuritis as a consequence of chest trauma (7% of patients), pleuritis due to congestive heart failure (3% of patients) and pleuritis in connection with pulmonary embolism (2% of patients). 9.5% of non-determinated pleuritis were false-negative. The malignant disease was found after mean interval of 16 months, most often the mesothelioma. All of these patients were previously exposed to asbestos. **Subsequent analysis in 2024:** Among the 138 patients with undiagnosed pleural effusions, 42 (30.4%) were still alive at the follow-up in 2024. We sent them invitations for examination, and 14 individuals responded. In all cases, we confirmed regression of the pleural effusion; however, some exhibited residual fibrothorax. Two patients were referred for further diagnostic procedures due to chest pain and weight loss; the results are pending. Among the causes of death, pleural mesothelioma was confirmed in 20 patients (14.5%) during the entire follow-up period. All but one of these cases had a documented history of asbestos exposure. Additionally, 36 patients (26%) had a malignancy as the cause of death. However, information on whether pleural carcinosis accompanied the underlying malignancy remains undisclosed.

CONCLUSION. The majority of non-diagnostic pleural effusions had a benign course. In long-term follow up 15% of non-determinated pleuritis were false-negative. Patients with exposure to asbestos require attentive monitoring and according to the clinical course and dynamics of pleural effusion additional diagnostic procedures.

PLENARY SYMPOSIUM

NEOANTIGENS

Invited lecture

NEOANTIGENS – AN ACHILLES HEEL OF CANCER

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The immune system is inherently connected to the biology of cancer through the process of mutagenesis (the process of mutations formation). Mutagenesis is essential for the cancerous transformation of tissues (through its actions on tumour driver genes) and, at the same time, mutagenesis is also responsible for the formation of neoantigens.
Neoantigens are modified self-antigens that have been modified to a point for the immune system to recognize and subsequently remove them. Since neoantigens are created after birth (as part of the mutagenesis process), they bypass central thymic tolerance and, unlike the body's own antigens, are potentially highly immunogenic. Consequently, they represent a good target for antitumor immunity.
Recognition and removal of neoantigens takes place through their presentation to the cells of the immune system (killer T-cells and helper T-cells) via MHC (Major Histocompatibility Complex) molecules.
The clinical success of treatment with immune checkpoint inhibitors in certain cancer subtypes (i.e. malignant melanoma, non-small cell lung cancer, etc.) is most probably attributable to the high neoantigen burden of these cancer subtypes (usually, these are cancer subtypes that are highly influenced by mutagenic factors like smoking or UV-radiation).
Furthermore, an additional potential therapeutic venue connected to neoantigens, namely treatment of cancer with personalized vaccines directed against the individual's own neoantigens, which cancer cells present to the immune system via MHC molecules is showing promising preclinical as well as clinical results.

Invited lecture

PRECLINICAL AND CLINICAL EVIDENCE OF EFFICACY OF NEOANTIGEN BASED THERAPEUTIC STRIDES

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Neoantigens are modified self-antigens that have been modified sufficiently for the immune system to recognize and subsequently remove them.

Recently, a new, potential, therapeutic approach connected to neoantigens and cancer, namely treatment with personalized vaccines directed against the individual's own neoantigens, which cancer cells present to the immune system via MHC (Major Histocompatibility Complex) molecules, has shown promising results both in preclinical and clinical studies.

In several preclinical models in different organisms, the treatment of many histological types of cancer by vaccination with immunogenic neoantigens in combination with immune checkpoint inhibitors (ICIs) has been shown to be more effective than treatment with only ICIs.

Furthermore, two recent clinical studies have demonstrated the efficacy of vaccination therapy with personalized mRNA vaccines directed against the individual's own neoantigens.

The first was a small study of 16 patients with ductal pancreatic carcinoma. Patients were vaccinated with neoantigen based vaccines composed of neoantigens with the highest predicted immunogenicity. Patients who developed an immunological response to the vaccine (n=8) had a statistically significant longer recurrence free survival than patients who did not develop an immunological response to the vaccine.

In another study, 157 patients with malignant melanoma were either treated with personalized mRNA vaccine directed against neoantigens with the highest predicted immunogenicity in combination with ICIs or with just an ICI therapy. Patients on a combination therapy (vaccine + ICIs) had recurrence free survival, which was significantly higher than the recurrence free survival of patients treated with only ICIs.

Invited lecture

GENETIC PLATFORM FOR THE CHARACTERIZATION OF NEOANTIGEN-SPECIFIC T CELL RECEPTORS

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ABSTRACT: T cells equipped with transgenic immune receptors such as T cell receptors (TCR) targeting neoantigens have great potential as therapeutic agents. However, their identification and comprehensive characterization is necessary to ensure safety and efficacy and to support clinical translation[1]. Such characterization is challenging. Here we summarize our recent paper[2] on an innovative genetic platform called Uni-Vect that enables the development of cell-based assays to study signaling of neoantigen-specific TCRs. Implementing Uni-Vect as a platform to characterize the antigen specificity and avidity of neoantigen-targeting TCRs provided a rapid screening assay to qualify receptors for further study.

KEY WORDS: TCR; T-cell receptor, T-Cells; Adoptive cell therapy; Neoantigens; Activation-dependent expression; Single-lentiviral expression system; CRISPR/Cas9; Reporter cell lines

INTRODUCTION: The success of T-cell based cellular immunotherapy is offset by challenges in efficacy in solid tumours[1]. The causes of limited efficacy are diverse and include an immunosuppressive tumor microenvironment and intrinsic properties of the T cells and tumor cells[3]. However, one of the major challenges is targeting the tumor, which should allow anti-tumor activity without damaging healthy tissue. Since cancer cells arise from normal cells, they are difficult for the immune system to distinguish. Even if tumor-specific targets are identified, these targets are usually not uniformly expressed on the cancer cells, which results in the tumor cells being invisible to the tumor-specific T cells, leading to evasion.

It is worth noting that CD19, a target for the clinically approved CAR-T cells for certain B-cell leukemias and lymphomas, is not tumor-specific. It is also expressed in normal B cells, and what makes it a viable target is that we can live without B cells providing an appropriate supportive treatment to overcome antibody deficiencies. Therefore, a major challenge in the development of T cell-based immunotherapies is to identify homogeneously expressed tumor-specific targets.

Recently, neoantigens have proven to be an attractive tumor-specific target[4,5]. This is because cancer cells carry unique somatic mutations that distinguish them from healthy cells and make them a clean target. Tumor neoantigens are recognized by T cells, which recognize specific mutations presented as mutated peptides in the context of major histocompatibility complexes (MHC). Neoantigens are also at the center of mechanisms by which immune checkpoint inhibitors work. In addition, neoantigens are being explored in the context of tumor vaccines, such as those based on mRNA lipid nanoparticles, which recently showed an unprecedented response in pancreatic cancer[4].

Neoantigens are also an attractive target for cellular immunotherapies. The ability of TCRs to target neoantigens[5–8] is the basis of cellular immunotherapy using tumor-infiltrating lymphocytes (TILs)[9,10]. The next steps are genetically modified T cells into which TCRs specific for the desired targets have been introduced, which was also the focus of one of our studies[5]. In this study, activating RAS missense mutations, which are among the most common mutations in human cancers were targeted[5].

Recently, clinical success has also been achieved in solid tumors and established approaches targeting intracellular antigens presented in the context of MHC molecules, including neoantigens[6–8]. Overall, tumor targeting via neoantigens represents one of the most attractive opportunities but also a challenge for innovative approaches, and this contribution summarizes our recent study[2] aimed at establishing a pipeline for the evaluation of neoantigen-specific TCRs, particularly in solid tumors.

MATERIALS AND METHODS: In our recently published work, we have developed a genetic platform that combines autonomous antigen-induced production of an accessory molecule, along with constitutive CAR expression in a single lentiviral vector called Uni-Vect[2]. This platform can be used for therapeutic and research purposes and in this contribution, we summarize its use for the characterization of neoantigen-specific TCRs.

First, we generated an engineered Jurkat T cell line in which we knocked out TCR alpha and beta chains using CRISPR/Cas9. This TCR negative Jurkat cell line was then transduced with human CD8 alpha and beta heterodimers and Uni-Vect constructs to generate reporter lines. Cells were flow cytometry sorted on TCR negative, CD8 positive, mCherry positive populations and low basal expression of the inducible reporter cassette. TCRs specific for neoantigens from melanoma were introduced into the reporter cell line via lentiviral delivery. All these steps together enabled us to generate engineered reporter cell lines for cell-based assays to study T-cell activation signaling of neoantigen-specific TCRs.

To validate TCRs function we pulsed monoallelic antigen presenting cell lines with various concentrations of control wild-type and mutated peptides. TCR signaling was monitored by reporter gene (eGFP) expression driven by NFAT T-cell activation signaling. To validate built-in NFAT sensor we monitored T-cell activation also via conventional staining for activation markers and also by secretion of cytokines. We also performed experiments to detect processed and presented neoantigens via engineered target cell lines.

RESULTS: Uni-Vect was designed to enable the simultaneous constitutive and antigen-inducible transgene expression in one lentiviral vector called Uni-Vect[2]. This platform can be used as a built-in NFAT-inducible reporter of T cell activation signaling and thus provides a platform to functionally evaluate immune receptors that signal via NFAT, such as TCRs or CARs.

To enable the reporter platform, we developed a genetically engineered Jurkat cell line that lacks endogenous TCR expression and is equipped with CD8 alpha beta and the Uni-Vect system featuring NFAT-inducible eGFP and constitutive mCherry reporter genes. We further engineered this cell line with TCR specific for neoantigen derived from melanoma[11,12]. Expression of this TCR resulted in reactivity to a corresponding peptide/HLA complex.

Next, we stimulated reporter cell lines with the TCR specific for the selected neoantigen using titrated peptide concentrations in the presence of antigen presenting cells. Increasing peptide concentrations led to a gradual increase of eGFP expression demonstrating the utility of the Uni-Vect system as a platform for monitoring TCR activation. The upregulation of surface activation marker and production of cytokines correlated with eGFP expression as an indicator of T cell activation.

To evaluate our platform with additional immunoreceptors, 6 different TCRs targeting HLA-A*02:01-restricted neoantigen-peptide complexes were introduced into reporter cell lines developed with the Uni-Vect. Titrations of the neoantigen peptides revealed a range of avidities among the TCR-engineered reporter cells. Interestingly, two TCRs recognized the wild-type peptide at high peptide concentrations. For the discovery and development of neoantigen specific TCRs, our system would need to enable response to endogenously processed and presented neoantigens. Therefore, target cells were engineered to express the tandem mini-gene constructs encoding a mutated (MUT) or wild-type (WT) peptides. Expressing the various TCRs upregulated eGFP expression when co-cultured with target cells

expressing MUT but not WT TMC and as shown before, we identified a TCR that was activated by WT TMC.

DISCUSSION

Engineered T cells expressing transgenic receptors have immense potential as therapeutics, but comprehensive characterization of neoantigen-specific TCRs is required to increase therapeutic efficacy and reduce the risk of adverse events[1]. Although testing receptors in primary T cells is essential, a cell-based Uni-Vect system that can assess NFAT activation as an indicator of TCR signaling provides a rapid screening test to qualify receptors for further study. We have used Uni-Vect to develop reporter cell lines and have shown how TCRs introduced into these reporter cell lines can be characterized for antigen specificity and avidity with a recognition pattern identical to that of T cells from which the receptors were isolated[11,12]. Overall, our results show that the Uni-Vect as a sensor based on the T cell activation signaling can be used as a robust and user-friendly platform to characterize the expression, specificity, and functional avidity of novel TCRs.

DECLARATION OF INTERESTS

A.S. is co-inventor on PCT International Patent Applications by The Trustees of the University of Pennsylvania, which incorporate discoveries and inventions described here.

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Invited lecture

MANUFACTURING OF MRNA VACCINES - A NEOANTIGEN-BASED PERSPECTIVE

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The COVID-19 pandemic triggered an unprecedented surge in development of mRNA-based vaccines and other therapeutics, such as protein replacement therapy and cancer. mRNA is produced by a cell-free process based on in vitro transcription (IVT) reaction, a RNA-polymerase-catalyzed polycondensation of NTPs into a nascent mRNA chain guided by DNA template. A particularly interesting new application for mRNA are neo-antigen mRNA vaccines, which harness the uniqueness of each patient's cancer neo-antigens combined with the ability of mRNA production platform to be rapidly adapted to produce a desired genetic material which can be delivered to antigen-presenting cells both ex vivo and in vivo, to provide a truly personalized cancer treatment.

We developed an mRNA production workflow adaptable to production from mg to multi-g scale, based on rapid at-line high pressure liquid chromatography (HPLC) monitoring of consumption of nucleoside triphosphates (NTPs) with concomitant production of mRNA, with a sub-3 min read-out, allowing for adjustment of IVT reaction parameters with minimal time lag. IVT was converted to fed-batch resulting in doubling the reaction yield compared to batch IVT protocol, reaching 10 mg/ml for multiple constructs, thus decreasing the per-gram cost by up to 50%. A purification train including affinity chromatography selective for polyadenylated mRNA (Oligo dT) coupled with reverse-phase chromatography was developed to remove IVT components (NTPs, DNA, T7), and IVT by-products, in particular dsRNA, a major immunogenic impurity which activates dsRNA-dependent enzymes and leads to inhibition of protein synthesis. Elimination of dsRNA improves translation and minimizes the activation of innate immune response. In context of clinical applications of neoantigen mRNA vaccines, which require as many as nine administrations, minimization of innate immune response may be critical to clinical success.

TREATMENT ADJUSTMENT AND IMPACT OF NOVEL ORAL ANTICOAGULANTS ON COMPLICATIONS IN PATIENTS REQUIRING DIAGNOSTIC BRONCHOSCOPY

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BACKGROUND: Therapy with novel oral anticoagulants (NOACs) carries a heightened risk of bleeding following invasive sampling during bronchoscopy. To minimize the potential for complications, it is important to hold NOACs prior to bronchoscopy. The published recommendations for discontinuing NOACs prior to bronchoscopy, rely on drug half-life and insights from other invasive procedures. Our aim was to assess the safety of adhering to our local recommendations for discontinuing NOAC therapy before bronchoscopy.

METHODS: We conducted a retrospective review of all patients who received treatment with NOACs and underwent bronchoscopy in the year 2023. We followed the recommendations for NOACs hold before the procedures with high risk of bleeding. We recorded the incidence of bleeding during bronchoscopy and potential peri-procedural thromboembolic events, comparing these outcomes to those of a group of 150 consecutive patients receiving acetylsalicylic acid (ASA) and of a group of 150 patients without anticoagulant (ACT) or antiplateled (APT) therapy.

RESULTS: Out of 1300 patients who underwent bronchoscopy in 2023, 100 patients were receiving treatment with NOACs (48 rivarixaban, 36 apixaban, 12 dabigatran, 4 edoxaban). We further analysed 61 patients in whom transbronchial biopsy (TBB, 49 patients), or bronchial biopsy (BB, 12 patients) were performed. Mild to moderate bleeding after TBB occurred in 8 out of 49 patients (16.3%) receiving NOACs, compared to 12 out of 81 patients (14.8%) on ASA therapy and 6 out of 60 patients (10%) without ACT or APT. Patients who experienced bleeding were diagnosed with either lung cancer (13) or interstitial lung disease (12) with one exception. Age, thrombocytes, INR or OGF levels were not associated with bleeding incidence. No severe bleeding or thromboembolic events were reported in any of the groups studied.

CONCLUSION: Adhering to local recommendations for NOACs discontinuation prior to bronchoscopy ensures safe invasive procedure for patients treated with NOACs.

Ca + IPB (TBB)	Ca - 97	IPB - 72	all
NOAK all	29	18	47
bleeding	4	4	8
ASA all	40	33	73
Bleeding	6	5	11
Controls all	28	21	49
bleeding	3	3	6

Invited plenary lecture

FOCUSED ECHOCARDIGRAPHY IN CARDIO-ONCOLOGY (FECO)

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ABSTRACT
Transthoracic echocardiography (TTE) is the cornerstone of imaging in patients with a malignancy in all stages of their treatment—before, during, and after the completion of it—to identify most of the cardiotoxic complications. However, the restricted time and resources of cardio-oncology services and the high volume of oncological patients and survivors on the other hand limit the access of this population to this modality.
Focused Echo in Cardio-Oncology (FECO) in proportion to other focused cardiac protocols is proposed as a valuable tool after the initial standard complete TTE to: (a) identify the potential toxicity expected by the specific cancer therapy applied; (b) assess sequentially the pre-existing abnormality, if any, in relation to therapy; (c) assess the effect of any cardio-protective intervention; (d) identify any cardiac origin of patient complaints during or after therapy; (e) assess cardiac function in asymptomatic patients who develop significant changes in cardiac biomarkers during cancer therapy.
The application of FECO protocols is aimed to ensure accuracy, reliability, and effectiveness in the early identification of cardiovascular complications, improving quality of life, and being at the same time cost-effective.

KEY WORDS
cancer, cardio-oncology, cardiotoxicity focused echo, echocardiography

INTRODUCTION
New systemic therapies in the treatment of non-small cell lung cancer (NSCLC) enable longer survival for patients with advanced NSCLC. Preservation of quality of life is becoming more and more important for these patients, which is why we must pay attention to the acute and chronic side effects of systemic cancer therapy during and after cancer treatment. Many anticancer drugs can disrupt the cardiovascular balance, triggering cardiovascular events such as arterial hypertension, myocardial dysfunction with or without heart failure, QT prolongation, arrhythmias, and thromboembolic complications during treatment or many years after treatment has ended, to name just a few . [1] Knowledge of the potential cardiovascular toxicity of oncological drugs is very important for patients with curable as well as curable cancer, as it enables the prevention and early detection of cardiovascular diseases with the possibility of appropriate action, which is reflected in the quality and length of life of these patients.
In the past, due to their cardiotoxicity, they were the first to be exposed to anthracyclines, which cause the collapse of myocytes upon receiving a certain cumulative dose of the drug and thereby cause irreversible damage to the myocardium. [2] It is the so-called type I cardiotoxicity, which most often manifests itself as a late complication and causes progressive damage to the heart muscle. [1, 3]
With the advent of biological drugs, in addition to better treatment results, we also hoped for fewer side effects. Despite our wishes, we learned from the example of the monoclonal antibody trastuzumab that even biological drugs cause unwanted side effects. In the case of trastuzumab, acute cardiotoxicity was shown, which is characterized by the fact that it usually does not cause myocyte collapse, but rather a reversible defect in the contractility of the heart muscle due to the action on the HER2 signaling molecule in the heart muscle cells - type II cardiotoxicity. In studies, trastuzumab in combination with anthracyclines has been shown to cause symptomatic heart failure in as many as 27%, and out of this combination, symptomatic heart failure has occurred in up to about 4%. [4] Type II cardiotoxicity is also associated with small molecule tyrosine kinase inhibitors that act on various single or multiple signaling molecules in cardiac cells (e.g. lapatinib, sunitinib, sorafenib and others). Some of these small tyrosine kinase inhibitors—for example, crizotinib, lapatinib, vemurafenib,

sorafenib, sunitinib, ceritinib, and others—are also associated with QT prolongation. Treatment with antiangiogenic drugs is associated with increased blood pressure in 15 - 45%, dasatinib can cause pulmonary arterial hypertension, and nilotinib is associated with generalized atherosclerosis and ischemic events such as acute myocardial infarction, cerebrovascular insult and peripheral occlusive arterial disease. Among biological drugs, antiangiogenic drugs can also be associated with ischemic events. [1, 3, 5, 6]

So far, little is known about the impact of the new method of systemic cancer treatment, immunotherapy, on the cardiovascular system. Considering the side effects known so far, which occur during treatment with immunotherapy, we could expect mainly cardiovascular complications in connection with the formation of autoimmune antibodies, such as e.g. vasculitis, myocarditis, pericarditis. As far as we know, the literature mainly describes cases of myocarditis, which, according to the data known so far, occurs rarely - in the case of treatment with a combination of two checkpoint inhibitors in 0.27%, and in the case of therapy with a single such drug in an even lower proportion. [7] Myocarditis caused by immunotherapy has often been shown to progress quickly, it can be accompanied by many complications, conduction disturbances or arrhythmias often occur, which can subsequently lead to cardiac arrest and even death of the patient. [7, 8]

The gold standard for the assessment of potential cardiotoxicity is the assessment of left ventricular ejection fraction, which can be assessed with the help of cardiac ultrasound, scintigraphy or MRI. [1, 9] Unfortunately, this method is not the best for detecting initial defects of the myocardium, when it is still possible to preserve or restore the function of the left ventricle with appropriate measures. For this reason, new methods are being sought to predict the potential cardiotoxic effect of an anticancer drug at an early stage. There is more and more evidence that the so-called myocardial strain could be used for these purposes, the decline of which in the course of cancer treatment indicates subclinical heart cell damage. Various cardiac biomarkers are also being investigated in this role, including a biomarker that indicates cardiac cell damage (troponin) and a biomarker that is released when the heart muscle is strained (BNP/NT-proBNP). [1, 9] When interpreting cardiac biomarkers, it should be noted that many cancer patients have elevated values, despite the absence of signs of heart disease, which can significantly complicate the use of these indicators in practice, since higher values do not necessarily mean damage to the heart muscle, but they can be indicators of the activity or progression of the cancer. [10] Despite the fact that the above-mentioned methods show great promise, their use in the standard treatment of cancer patients is not yet clearly defined at a given moment.

Rare cardiovascular adverse events have been reported for tyrosine kinase inhibitors (TKIs) in clinical studies. Among EGFR-directed TKIs, afatinib is particularly mentioned in connection with cardiotoxicity, as it also exhibits activity on HER2 in addition to EGFR inhibition. Nevertheless, in a retrospective analysis of data from clinical studies with afatinib, no significant cardiotoxicity was identified. [14] The anti-ALK TKI crizotinib causes QT prolongation in approximately 4%, and ceritinib is also associated with mild to moderate QT prolongation. [1, 6]

Despite the relatively low perceived cardiotoxicity of the described drugs, it should be noted that today the development of new oncological drugs is very fast. During their development, sufficient attention is often not paid to cardiovascular side effects, since most of these drugs are approved through the fast-track process and there is no data on late toxicity. They were additionally tested on a relatively small sample of ideal patients without significant associated cardiovascular comorbidities. In everyday practice, however, patients with lung cancer are anything but free of accompanying diseases. These patients are usually elderly people, often also smokers/ex-smokers with a high proportion (20-36%) of associated various cardiovascular comorbidities. [11] Because of the above, it will be important in the future to systematically monitor possible side effects in patients on standard treatment, because only in this way will we properly assess the relationship between the risk and benefit of these drugs in everyday care and enable a better quality and longer life. Only the prospective observation of cardiovascular side effects of new biological drugs in the standard treatment of cancer patients will give us a complete picture. In the follow-up of our patients, we performed two clinical studies.

STUDY I

METHODS

This was a prospective, observational study conducted at a single academic center from June 2012 to September 2013. Patients with lung cancer scheduled for first-line platinum-based chemotherapy were included in the study. Cardiac biomarkers including ultra-sensitive troponin T (usTnT), N-terminal pro-BNP (NT-proBNP) and echocardiography examination with tissue Doppler were assessed at baseline, at the end of chemotherapy (visit two) and at the follow-up (visit three). Results were reported as means ± standard deviation and number (percentage) for numeric and categorical variable, respectively. Elevated usTnt and NT-proBNP was defined as more than 30% elevation from the baseline value. Lowered left ventricular ejection fraction (LVEF) was defined as reduction of LVEF ≥ 10% to value ≤ 55% whereas diastolic dysfunction was defined using European Society of Cardiology guidelines.

RESULTS

Overall, 41 patients (54% men, 61 ± 9 years, 68% with advanced lung cancer) were included at baseline. Patients received 4.6 ± 1.1 cycles of chemotherapy. 1 patient died before visit two, additional 8 patients were not assessed at visit three (2 died, 6 were lost to follow up). Values at baseline, visit two and visit three for usTnt, NT-proBNP and LVEF were 0.011 ± 0.005 pg/ml, 0.011 ± 0.005 pg/ml, 0.008 ± 0.003 pg/ml, 266.4 ± 250.1 pg/ml, 257.7 ± 378.1 pg/ml, 225.9 ± 430.0 pg/ml and 68% ± 8%, 67% ± 8%, 68% ± 9%, respectively. Diastolic dysfunction was found in 9 (27%), 6 (27%) and 4 (24%) patients at baseline, visit two and visit three, respectively. Elevated usTnT and NT-proBNP at visit two and three was found in 3 (16%), 2 (25%) and 6 (35%), 2 (25%) patients, respectively. Lowered LVEF was observed in one patient at visit three.

CONCLUSION

Patients with lung cancer had similar values of bio and echocardiographic markers of cardiotoxicity immediately and 6 months after platinum-based chemotherapy. However several patients were shown to have elevated levels of usTnT and NT-proBNP after chemotherapy, which indicates the importance of careful evaluation of cardiotoxicity and further research . [12]

STUDY II

METHODS

This was a prospective, observational study conducted at a single academic center from January 2017 to June 2021. We followed NSCLC pts treated with either immune checkpoint inhibitor (CPI) or endothelial growth factor receptor tyrosine kinase inhibitor (EGFR TKI) who consented to additional monitoring. During their routine treatment, they had a directed clinical examination, echocardiogram recording, proBNP and troponin T sampling at treatment initiation, months 2 and 4, and then every four months until the end of treatment. Only pts with normal baseline cardiac function were included in the study.

RESULTS

We included 61 pts with advanced NSCLC and a mean age of 63,7±9 years, 34 of them female (56%). 48% of pts (29 pts) were treated with monotherapy with CPI (atezolizumab, nivolumab, or pembrolizumab), while 52% of pts (32 pts) received EGFR TKI (afatinib, gefitinib, erlotinib, or osimertinib). We analyzed recorded data for up to 24 months of treatment; at 4, 12, and 24 months, there were 59, 29, and 15 pts still included, respectively. No pts on either CPI or EGFR TKI developed signs of heart failure during their treatment. In a joint study cohort, left ventricular ejection fraction stayed normal at all time points (p=0.71), the values of troponin T and NTproBNP stayed stable throughout treatment (p=0,45 and p=0,85, respectively).

CONCLUSION

Our observational study has shown no cardiac toxicity in pts treated for advanced NSCLC with either immunotherapy or targeted therapy in routine clinical practice. Analysis of more sensitive parameters and longer observation times are needed.

DISCUSION

TTE is a fundamental diagnostic method for monitoring cancer patients who are treated with known cardiotoxic drugs or new drugs whose side effects are not yet known. A complete echocardiographic examination adds important information to the clinical assessment of the patient, improving risk prediction before treatment, defining the appropriate control intervals according to the initial cardiotoxicity and predicting the need for the introduction of protective drugs.

During cancer treatment, TTE can identify potential cardiotoxic effects of the regimen, allowing for treatment adjustments. TTE can further identify late cardiotoxic effects, months or even years after therapy has already been completed.

Echocardiography has advantages over other imaging methods: no exposure to radiation, no complications, easy to use, reasonable cost, widely available and satisfactorily reproducible. The examination is non-invasive and provides information on most cardiotoxic complications of cancer treatment: left ventricular (LV) dysfunction, heart valve disease, pericardial disease and pulmonary hypertension.

However, cardio-oncology is a relatively new field that has been developing slowly in recent years and has limited staff, knowledge and techniques. In addition, the number of oncology patients who require TTE is large, as are the number of patients with cancer and associated cardiovascular disease who require even more careful monitoring because they have a high risk of cardiotoxicity. This, however, raises issues of cost, feasibility, lack of staff and resources, and in some cases allows investigation only for high-risk patients or patients with already known cardiovascular complications.

Cancer patients require echocardiographic examination at various stages. A complete baseline TTE examination is necessary in most patients diagnosed with cancer before any therapy (surgery, chemotherapy, radiotherapy or interventional radiology). Later, examination is required at different intervals depending on the cardiovascular risk, diseases and cardiotoxicity of the therapy. The number of patients who need TTE is increasing, a special problem is patients who received anthracyclines in childhood and need monitoring throughout their life.

Point of care ultrasound (POCUS) or bedside ultrasound is a term used for the use of focused ultrasound examination in different clinical settings: FICE (Focused Intensive Care Echo), FEEL (Focused echocardiography in emergency life support), BEAT (Bedside Echocardiographic Assessment in Trauma). FoCUS (Focused Cardiac Ultrasound) is a focused ultrasound of the heart that helps answer clinical questions in various clinical situations (International Liaison Committee on Focused Cardiac Ultrasound). This paper proposes FECO (Focused Ultrasound in Cardio-Oncology) as a variation of FoCUS adapted to cardio-oncology needs. There are three important differences between FoCUS and FECO [13]

First, FECO is performed by cardio-oncology specialists who look at the cardio-oncology patient holistically.

Second, FECO should not be performed with emergency ultrasound machines that do not offer the possibility of recording ECG, storing and processing data.

Third, FECOs incorporate advanced imaging techniques such as spackle tracking and automated three-dimensional (3D) TTE software. These techniques increase the ability to detect minor changes in myocardial function and greater reproducibility than conventional 2D echocardiography.

Instead of a full TTE, FECO is given to a wider range of specialists and trainees

in cardiology and oncology who have little or limited experience with echocardiography, the possibility to perform a focused echocardiographic examination that can then be analyzed

by an expert. Undoubtedly, basic echocardiography training and adherence to appropriate protocols are prerequisites to ensure proficiency, reliability, and reproducibility. This will ultimately increase the

number of staff who can effectively perform ultrasound examinations of oncology patients who need the examination for various reasons.
There is no doubt that the initial assessment of the oncology patient requires a cardio-oncology specialist and extended echocardiography to assess the risk of cardiotoxicity by identifying pre-existing cardiovascular disease. However, it is on subsequent visits
FECO could serve as a valuable tool in clinically stable patients.
FECO has similar starting points to FoCUS, but is more flexible.
Oncology patients develop four main types of toxicities identified by echocardiography: LV dysfunction, valvular heart disease, pericardial disease, and pulmonary hypertension. Consequently, four different protocols have been proposed according to the type of cardiotoxicity:
FECOm (targeted echocardiography in cardio-oncology in patients on chemotherapeutic agents causing myocardial dysfunction),
FECOv (FECO in patients at risk of heart valve disease),
FECOpd FECO in patients at risk of pericardial disease) and
FECOph (FECO in patients at risk of pulmonary hypertension)
FECO may not be suitable for patients with multiple cardiovascular abnormalities
in the baseline TTE study, such as heart failure, valvular heart disease, or multivalve disease. The same applies to patients receiving multiple concurrent or sequential therapies with different cardiotoxicity profiles. FECO is also not suitable for monitoring patients on new therapeutic regimens with unknown cardiotoxicity and for hemodynamically unstable patients. Patients with a poor ultrasound window are also not suitable for FECO.

CONCLUSION

FECO can have a central role in the treatment of cancer patients, ensuring their wide access to cardio-oncology services in a costeffective manner. Oncological patients of any risk can be serially monitored with FECO during and after cancer therapy, including lifelong follow-up in the presence of specific indications. Standard and specialized FECO protocols maximize accuracy, reliability, and effectiveness in early identification of CV complications, thus saving
time, limiting costs, and improving quality of care.

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ENDOBRONCHIAL HAMARTOMA - A RARE CAUSE OF OBSTRUCTIVE PNEUMONIA

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INTRODUCTION: Endobronchial hamartomas (EH) are the most common benign lung tumors. Usually, they are found at the periphery of the lungs, while the endobronchial location is less common. They are comprised of an abnormal mixture of tissues native to the bronchial tree. We present a case of EH presenting with acute respiratory failure and pneumonia, successfully removed with bronchoscopy.

CASE PRESENTATION: A 67-year-old former smoker presented with acute respiratory failure due to pneumonia. He complained of exertional dyspnea, headache, persistent productive cough, and wheezing, with symptoms that had worsened over the past few months. His medical history included diabetes, obstructive sleep apnea, arterial hypertension, and adrenal incidentaloma. On examination, he was afebrile, with an oxygen saturation of 87%. Lung auscultation showed decreased breath sounds at the lung bases, with inspiratory and expiratory wheezes. Chest X-ray showed alveolar consolidation in the right lung. A chest CT scan showed a tumor 20x14 mm in size in the right intermediate bronchus, occluding its lumen, and causing pneumonitis. Bronchoscopy showed a polypoid tumor on a peduncle within the right main bronchus, without malignant cells in the biopsy. Consequently, endoscopic resection with a combination of electrocautery and cryotherapy was done. Histopathological examination confirmed the diagnosis of EH. Follow-up showed improvement in respiratory symptoms and lung function.

DISCUSSION: Although benign, EH may cause severe symptoms, depending on their location. While chest X-rays are often unremarkable, CT scans can reliably detect low-attenuation endobronchial masses. Bronchoscopy is considered the preferred diagnostic and therapeutic tool, allowing for both visualization and potential removal of the lesion in cases where malignancy is not suspected. Bronchoscopic excision with argon plasma coagulation, electrocautery, and tumor debulking with a flexible cryoprobe is the treatment of choice. For larger lesions with chronic parenchymal changes distal to the affected area, surgical resection may be considered. Once removed, the chances of recurrence are estimated to be about 10%. Despite bronchoscopic resection being the preferred treatment, the lack of cases hinders the establishment of standardized guidelines.

CONCLUSION: Due to their rarity, EH poses a diagnostic and therapeutic challenge. The lack of standardized protocols highlights the ongoing need for research aimed at developing optimal diagnostic and therapeutic approaches.

MIDDLE LOBE SYNDROME – CASE DESCRIPTION

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This case report details the presentation, diagnosis, and management of a 48-year-old male patient with a significant smoking history and a reported allergy to Lidaprim. The patient presented with a persistent cough and right-sided chest pain over a period of five months, accompanied by systemic symptoms such as weight loss and fatigue. Despite multiple courses of antibiotic therapy prescribed by his primary care physician, the symptoms failed to improve, prompting further investigation.

Imaging studies, including chest X-ray and subsequent CT scan, revealed consolidation with positive air bronchogram in the right middle lobe, suggestive of middle lobe syndrome. Additionally, bilateral basal chronic deformative bronchitic changes were noted, likely secondary to chronic smoking. Further evaluation revealed slightly enlarged mediastinal lymph nodes and an osteolytic lesion at the thoracic vertebra (Th 12), raising suspicion for possible metastatic involvement.

Histopathological examination of biopsied tissue obtained during bronchoscopy confirmed the presence of non-small cell lung carcinoma, predominantly adenocarcinoma. Notably, bronchoscopic findings revealed significant obstruction of the right segmental bronchi due to mucosal edema and infiltration, indicative of advanced disease.

Laboratory analyses revealed leukocytosis and elevated inflammatory markers, consistent with an active inflammatory process. Imaging studies, including PET/CT scan, demonstrated evidence of metastatic disease involving the left iliac bone and subcarinal lymph node, further confirming the advanced stage of the malignancy.

Management involved a multidisciplinary approach, including palliative radiotherapy targeting the Th12 lesion and subsequent chemotherapy. Despite initial challenges, the patient exhibited a favorable response to treatment, prompting consideration for ongoing immunotherapy in a specialized facility abroad.

This case highlights the complex presentation and management of advanced non-small cell lung carcinoma in a heavy smoker, underscoring the importance of timely diagnosis, multidisciplinary care, and access to advanced treatment modalities in optimizing patient outcomes in advanced lung cancer.

A COMPLICATED CASE OF PANCREATICOPLEURAL FISTULA WITH A LEFT-SIDED PLEURAL EFFUSION

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INTRODUCTION: We present a patient with a suspected malignant left-sided pleural effusion with a complicated course and a final diagnosis of chronic pancreatitis of unknown etiology and a pancreaticopleural fistula as a cause of the effusion.

CASE SUMMARY: A 69-year old male patient, an active smoker with COPD and type 2 diabetes, presented to the emergency department of a regional hospital with a history of dyspnea lasting several weeks and unintentional weight loss of 10 kg in 2 months, without other complaints. Laboratory findings, including hepatic and pancreatic enzymes, were unremarkable. A left-sided pleural effusion was discovered. A diagnostic tap was performed, the fluid was found to be a hemorrhagic neutrophillic exudate. No bacteria were found and repeated cytological analyses were negative for malignant cells. Level of amylase in the effusion was high and found to be rising on repeated pleural taps. A computed tommography (CT) of abdomen and chest did not show any malignancy, only signs of chronic pancreatitis, a pancreatolith in the pancreatic duct and two fluid collections around the pancreas were found. He was presented to the gastroenterological-surgical multidisciplinary team and an endoscopic stent placement was indicated. The procedure was successful, but fluid drainage was inadequate and pleural effusion enlarged again. An infection with sepsis and delirium prolonged the diagnostic course. Based on repeated abdominal CT and magnetic resonance cholangiopancreatography (MRCP) the MDT suggested an endoscopic retrograde cholangiopancreatography (ERCP) procedure to remove the pancreatolyth as it was deemed a possible cause of chronic pancreatitis and fluid accumulaton. The procedure was unsuccessful, so we transferred him to the Clinical department for abdominal surgery, University clinical centre Ljubljana. A distal pancreaticotomy with splenectomy, suturing of the pancreaticopleural fistula and thoracic drainage was performed. A pleural empyema complicated the recovery course, so video-assisted thoracic surgery (VATS) was needed. After a protracted antibiotic and antimycotic treatment his condition stabilised and he was released home. Follow-ups did not show a repeat of pleural effusion.

CYTOKINE RELEASE SYNDROME IN LUNG CANCER PATIENT RECEIVING IMMUNE CHECKPOINT INHIBITORS

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INTRODUCTION Immune checkpoint inhibitor (ICI) therapy has revolutionized the treatment of non-small cell lung cancer (NSCLC), resulting in improved overall survival in both the first-line and second-line settings in advanced stage disease. While efficacious, such treatments are associated with a variety of immune-related adverse events (IRAEs).
Cytokine release syndrome (CRS) is well-described IRAE following CAR-T cell therapy, but has rarely been reported following ICI.

CASE PRESENTATION We present a clinical case of a 62-year-old female patient treated for lung adenocarcinoma stage cT2bN0M1a with a combination of chemotherapy and immunotherapy using ipilimumab, nivolumab, pemetrexed, and carboplatin. After the third cycle, the patient experienced deterioration with extreme weakness, fever, hypotension and somnolence. Despite antibiotic and supportive therapy, her clinical condition continued to worsen, requiring intensive care unit admission. Excluding infectious causes raised suspicion of CRS. The patient received methylprednisolone and tocilizumab therapy, leading to rapid clinical improvement.
Subsequently, we found 17 clinical cases, published in medical journals, involving advanced NSCLC patients experiencing CRS as an adverse effect of treatment with ICI. We describe differences and similarities of those cases, from types of ICI used, number of treatment cycles prior to adverse event, CRS severity, management of IRAE and ultimately the outcome of the underlying disease.

CONCLUSION CRS is a serious, life-threatening complication, rare but increasingly occurring as an adverse effect of ICI treatment. When presented with the described clinical symptoms, considering CRS is crucial, as early recognition is key to timely intervention.

Short oral presentations

OBSTRUCTIVE LUNG DISEASES

RESLIZUMAB IN THE MANAGEMENT OF SEVERE EOSINOPHILIC ASTHMA (SEA): INSIGHTS FROM NORTH MACEDONIA

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Reslizumab as a humanized anti-IL-5 antibody, which interrupts eosinophil maturation, stimulates programmed cell death and plays an important role in the treatment of SEA. The aim of this retrospective analysis is to evaluate the need for additional biologic (anti-IL-5) therapy in severe eosinophilic asthma, to showcase the possible positive changes in the clinical and laboratory tests and to present our first practical experience with this therapy in the Republic of North Macedonia.

We have evaluated the effect of anti-IL5 therapy in SEA in 6 patients regarding Absolute Eosinophil Count (AEC), Asthma Control Test (ACT), Forced Expiratory Flow in 1 second (FEV1), Asthma Exacerbations (AE), eventual adverse events (SAE) and side effects in six patients with SEA. We determined AEC, ACT, FEV1 and AE at the beginning of the treatment and consecutively at every next treatment visit thereafter. Duration of the anti-IL-5 treatment ranges from 4 months up to 3 years.
We found that Reslizumab improves asthma control (AC), significantly reduces the use of concomitant asthma therapy and drastically reduces the AEC. In two patients with continuous maintenance oral corticosteroid therapy (mOCS), the dose is reduced by 50% (5 mg/day and 2.5 mg/day respectively). Although we have found improvement of FEV1, the effect was more evident in the patients with lower baseline values of FEV1. During treatment with Reslizumab, 6 episodes of AE requiring the use short course of OCS (or higher dose for a short time) were noted. Most common residual disease manifestations in our patients were impaired lung function (33.3%), uncontrolled sinonasal disease (16.6%) and uncontrolled asthma symptoms (33.3%). The common side effect was increased CK blood level and was detected in both of our male patients, which is inconclusive as they both had increased CK at baseline visit. One female patient, had increased CK level, on 2 from 36 separate blood samples. No SAEs occurred during the course of the study.
We can conclude that Reslizumab improves AC and FEV1, reduces the number of AE, AEC and the usage of concomitant asthma therapy, with a generally well-tolerated safety profile.

THE USE OF EXPIRATORY VARIABILITY INDEX FOR MEASURING CLINICAL OUTCOMES IN CHILDREN WITH MODERATE/SEVERE BRONCHIAL OBSTRUCTION

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BACKGROUND: Moderate or severe bronchial obstruction in preschool children are an important cause of morbidity and often lead to hospital admissions. After the initial treatment at the ER, establishing a good clinical and diagnostic assessment of the patient`s respiratory tract status is crucial for further management. Children under the age of 5 years are rarely capable of performing lung function tests, which can guide treatment regimes in these cases. A novel method based on measuring the expiratory variability index (EVI) using expiratory flow-volume curves during night-time tidal breathing has emerged as a beneficial tool in management of bronchial obstruction in small children.

AIM: to determine differences in EVI in preschool children with bronchial obstruction in association with treatment outcomes.

METHODS: 19 children aged 2-5 years, hospitalized due to a lower respiratory tract infection were recruited to a prospective, observational study at the Srebrnjak Children`s Hospital, Zagreb, Croatia. EVI was calculated using the Ventica® device. A subgroup of participants underwent available standardized lung function tests (high frequency oscillation technique). Groups of participants were defined by diagnosis (bronchitis/asthma with or without pneumonia). Mean EVI values for each group were calculated and additional clinical assessment data was collected.

RESULTS: Median EVI was 15,2±3,75. EVI was significantly correlated with initial diagnosis- participants with pneumonia had a higher EVI (p= 0,000, Spearman`s Rho= 0,75763), and age of participants (p=0,000, Spearman`s Rho= 0,71608). There was a positive clinical correlation between EVI and moderate or severe disease manifestation (according to treatment type, need for oxygen supplementation and length of hospital stay).

CONCLUSION: EVI correlated well with referral diagnosis and age of participants, meaning older participants and those with pneumonia had a higher EVI and better disease outcomes. EVI seems to be a good assessment tool for young pediatric patients with bronchial obstruction, as it calculates an exact value of breathing patterns.

CLINICAL REMISSION OF ASTHMA IN SEVERE ASTHMA PATIENTS ON TREATMENT WITH BIOLOGICALS IN OUR OUTPATIENT CLINIC FOR SEVERE ASTHMA

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Asthma remission is a proposed new asthma treatment goal. We preformed retrospective analysis of our severe asthma patients that are receiving biologicals for severe asthma in our outpatient clinic. Currently we have 49 patients with severe asthma on biological treatment. 40 of them, which have been receiving current biological treatment for at least 12 months on March 15th 2024, were included in an evaluation for asthma clinical remission. We checked their symptoms and ACT score, evaluated their lung function, frequency off exacerbations and evaluated how the patient is feeling about their asthma, as well as whether we agree that they are in remission.
RESULTS: 22 (55%) off our patients achieved proposed criteria for clinical asthma remission, 9 on omalizumab, 6 on mepolizumab, 5 on benralizumab and 2 on dupilumab.
Conclusion: Our results show that clinical remission is achievable in severe asthma patients on biological treatment.

CLINICAL OUTCOMES AFTER BIOLOGICAL THERAPY IN THE SHARP SINGLE CENTRE SEVERE ASTHMA PATIENT COHORT

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INTRODUCTION: Severe asthma patients have a decline in lung function and frequent exacerbations that adversely affect quality of life. Oral glucocorticoids (OCS) are often required to keep the disease under control. It has been consistently shown that the initiation of biological therapies is effective in improving clinical outcomes and reducing oral glucocorticoid burden in such patients.

AIMS: We aimed to determine the OCS burden in our severe asthma centre cohort, the effectiveness of biological therapy in reducing OCS and its impact on exacerbation rate, lung function, and quality of life.

METHODS: We included severe asthma patients enrolled in SHARP registry of the University Clinical Centre Ljubljana from June 2016 with at least one entry before biological therapy initiation and one entry after 12-18 months of follow-up. We analysed OCS burden, number of exacerbations, lung function and ACT questionnaire before and after biologics introduction.

RESULTS: 80 patients, 59% females, were included in the study. Their mean age was 57 years (SD 12 years). Most patients (86,3%) had adult-onset asthma. Forty (50%) patients were taking OCS before the biological therapy introduction, with a median dose of 5 mg (IQR 25-75, 5 - 10 mg) prednisolone. After initiation of biological therapy 15 (37.5%) patients were able to discontinue OCS use, while the OCS dose did not change significantly in the remaining patients. The number of exacerbations per year decreased significantly after biological therapy (patients with >2 exacerbations 34% vs. 3.2%, $p < 0.001$). After biological therapy there was a significant improvement in FEV1 (mean [SD]: 66% [20] vs. 75% [22], $p = 0.009$), FeNO (median [IQR 25-75]: 48 [26-85] vs. 36[19-56], p 0.07) and ACT (mean [SD]: 14.3 [4.9] vs. 19.1 [5.2], $p < 0.001$).

CONCLUSIONS: We have shown that in our cohort the introduction of biological therapy reduced the number of patients receiving OCS and improved several clinical outcomes, which is consistent with other studies. Nevertheless, a significant part of patients remained dependent on OCS even after biological therapy which shows that there are still unmet needs in the management of severe asthma patients.

NOT ALL SMOKERS HAVE COPD: A CASE REPORT

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INTRODUCTION: Heterogeneity of chronic obstructive lung diseases has been widely recognized. In practice, many patients with obstructive airway disease do not fully fit the diagnosis of either classical asthma with eosinophilic airway disease, bronchial hyperresponsiveness and reversibility of airflow limitation, or chronic obstructive pulmonary disease (COPD) with irreversible airflow limitation and neutrophilic airway disease (1). A degree of overlap between the two is often found. It should be actively looked for, especially in patients with a hard-to-manage disease course, to provide an additional treatment approach or - treatable trait (1,2).

CASE REPORT: Herein we present a case of a 54-year-old long-time smoker with frequent exacerbations of a previously diagnosed obstructive airway disease (COPD) with non-reversible airway obstruction noted on spirometry and two previous episodes of lower left lobe atelectasis. On chest CT imaging, only mucoid impactions of the airways were seen along with known centrilobular and paraseptal emphysema, and bronchoscopy never showed clear underlying pathology for the cause of lobar collapse. On follow-up, marked peripheral eosinophilia with high total serum IgE and positive IgE and IgG antibodies for Aspergillus fumigatus were noted. He was diagnosed with concomitant asthma and fulfilled the criteria for serological allergic bronchopulmonary aspergillosis (ABPA). Because of a frequent need for oral corticosteroids (OCS) he was considered for OCS sparing treatment and started on anti-IL-receptor alpha (IL5/R) biologic therapy. On anti- IL5/R he had a complete normalization of spirometry and remarkable symptomatic improvement.

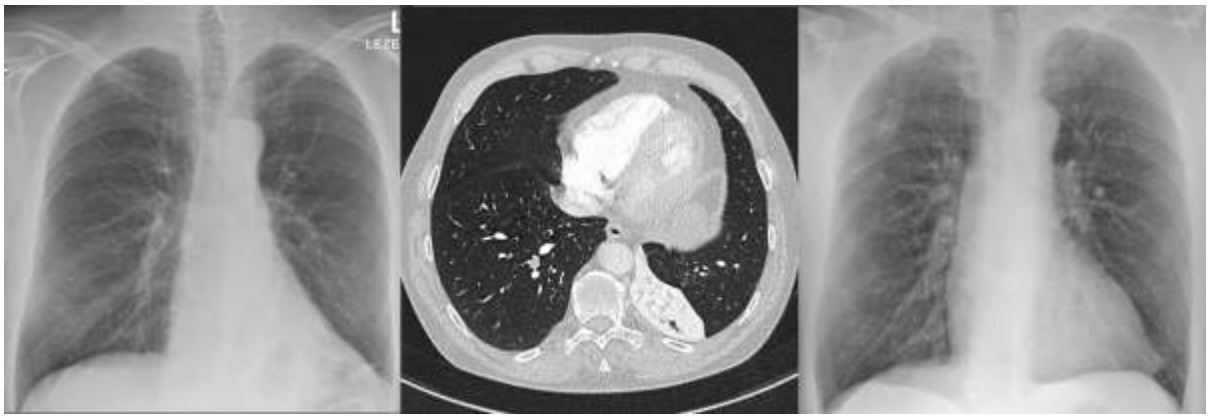

Figure 1. Radiologic imaging, July 2023; from left to right: atelectasis on chest X-ray and CT and the spontaneous resolution.

CONCLUSION: Eosinophilic inflammation of the airways has recently become one of the more approachable treatable traits with the development of several biologics that target this pathway (2, 3). While biologic therapy has proven efficacy in difficult to treat asthma, its uses in COPD and ABPA, are still a matter of ongoing clinical trials (4, 5, 6, 7). Patients with frequent exacerbations requiring OCS or visits to the ER despite maximal inhaled therapy should be evaluated for OCS sparing approaches.

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ARTERIAL HYPERTENSION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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We aimed to investigate the association between COPD and Arterial Hypertension (AH) and the relation to the severity of airflow limitation.

Cross-sectional study including 220 patients with initially diagnosed COPD, aged 40 to 75 years and 50 non-COPD subjects matched by age, smoking status, body mass index, as controls. All study participants underwent anthropometric measurements, blood pressure measurement (three times in each patient, after 10 minutes rest, the median value was taken for analysis), medical history analysis, routine laboratory, pulmonary evaluation (dyspnea severity assessment, baseline and post-bronchodilator spirometry, gas analyses, chest X-ray).

Results presented that there was no statistically significant difference in presence of AH in COPD patients compared to controls 81(36.82%) vs. 16(27.59%); p=0.189. According to the systolic blood pressure there was clinically significant difference between IG vs. CG, 133.61±15.23mmHg vs.121.03 ± 12.31mmHg, p=0.0317. According to the diastolic blood pressure there was also clinically significant difference between IG vs. CG, 81.04±11.19mmHg vs. 72.37±13.17 mmHg, p=0.0411, higher values were measured in the IG. AH was detected in different COPD stages according to GOLD classification I, II, III, IV, with frequency 29.82%, 35.48%, 42.31%, 40.82%, respectively in each stage of the disease.

We found higher values of mean systolic and diastolic blood pressure in patients with COPD even in early COPD stages, compared to non-COPD controls. Despite the frequent coincidence, current guidelines are still mostly restricted to the management of the individual disease. Future diagnostic and therapeutic strategies should therefore be guided by an integrative perspective for prevention, screening and start of combined treatment in early stage.

MODIFIABLE RISK FACTORS IN COMPLEX COPD: A FOCUS ON PHARMACOLOGICAL THERAPY

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INTRODUCTION Chronic Obstructive Pulmonary Disease (COPD) causes high morbidity of patients. Effective management involves subphenotyping and both pharmacological and non-pharmacological approaches, with adherence to medication playing a crucial role, especially in complex cases. In this study, we explored modifiable risk factors related to pharmacological therapy in complex COPD, including adherence, inhalation technique, and medication usage. Understanding these factors is essential for optimizing treatment outcomes in this vulnerable patient group.

METHODS We enrolled patients receiving care for complex COPD within a multidisciplinary team at the University Clinic for Pulmonary Diseases and Allergy Golnik between December 2022 and December 2023. Clinical data were systematically reviewed, incorporating reports from a specialist nurse and a clinical pharmacist regarding adherence to inhaled medications, proper inhalation technique, and appropriate medication usage. Low adherence was defined as <80% of days covered. Excessive use of SABA) was characterized by >1 SABA canister per month, and high use of oral corticosteroids (OCS) was defined as an average coverage of >3 mg/day of metilprednisolon.

RESULTS The study included 45 patients: 49% females, a mean age of 68.4y (±10.2), and a mean FEV1 of 50% (±25). Low adherence was identified in 21% of patients, while incorrect inhaler administration technique was observed in 61% of patients. Following instructions, 23% of patients improved their inhalation technique, while 48% required a spacer device to improve. Excessive SABA usage was noted in 13.5% of patients, with 54% using SABA daily, and 8% not utilizing SABA at all. 80 % of patients had CAT scores> 10, and 50% > 20. Twenty percent of patients demonstrated high use of OCS. 42.% of patients did not experience an acute exacerbation (AE) of COPD in the past 12 months, and 6.7% of patients had more than three AE COPD.

CONCLUSIONS Approximately one-fifth of patients with complex COPD exhibited nonadherence to inhalation therapy, while a substantial majority (61%) demonstrated incorrect inhalation technique. Notably, 13.5% of patients exhibited excessive SABA usage, and 20% demonstrated high use of OCS. These findings underscore the importance of targeted interventions to improve adherence and inhalation technique in patients with complex COPD, aiming to optimize treatment outcomes and reduce exacerbation rates.

SEROLOGICAL DETECTION OF ATYPICAL RESPIRATORY INFECTIONS - A DECADE-LONG EXPERIENCE AT THE UNIVERSITY CLINIC GOLNIK

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BACKGROUND: Serology is a valuable tool for diagnosing acute infections caused by various pathogens. In this study, we aimed to assess the prevalence of positive serological results for *Mycoplasma pneumoniae* (Mp), *Chlamydia pneumoniae* (Cp), *Bordetella pertussis* (Bp), and *Legionella pneumophila* (Lp) in patients presenting with respiratory infection symptoms at the University Clinic for Respiratory and Allergic Diseases, Golnik.

METHOD: Serological testing for Mp, Cp, Bp, and Lp was conducted at the Laboratory for Clinical Immunology and Molecular Genetics. We retrospectively analyzed results from patients treated between January 2013 and December 2023. ELISA assays were utilized for Mp (IgM, IgG, and IgA), Bp (IgM, and IgA), and Lp (IgM and IgG) antibody detection. Immunofluorescence staining was employed for Cp (IgM and IgG) antibody detection.

RESULTS: Overall, in the last ten years, Cp was tested in 1087 serum samples from 1006 patients, showing 24 (2.21%) positive results for IgM antibodies and 552 (50.78%) for IgG antibodies. Notably, 8.28% of positive IgG results had a titer ≥ 1:512, indicating acute infection. Mp was tested in 1199 serum samples from 1094 patients, and 94 (7.84%) samples were positive for IgM, 328 (27.36%) were positive for IgG, and 128 (10.68%) were positive for IgA antibodies. Furthermore, Lp was positive in 25 (2.33%) samples for IgM and 68 (6.35%) samples for IgG antibodies out of 1071 serum samples from 988 patients. As for Bp, 565 serum samples from 528 patients were tested, and 82 (14.51%) were positive for IgM and 126 (22.3%) for IgA antibodies. Before the SARS-CoV-2 pandemic (in 2019), there were very few Bp IgM positive results and 48 (48.0%) IgG positive results, whereas, after the pandemic (in 2023), there were 9 (5.6%) IgM positive results and 121 (75.2%) IgG positive results.

CONCLUSION: In the past decade, *Bordetella pertussis* has emerged as the most commonly identified pathogen indicating acute infection, with the highest proportion of positive IgM results. However, when examining the trend over the last 10 years, *Chlamydia pneumoniae* stands out as the pathogen with the most significant annual increase in incidence. Serological testing for atypical pathogens causing acute pulmonary infections is highly important for selecting the correct antibiotic therapy, epidemiological surveillance, outbreak investigation, and research efforts to control these respiratory infections.

THE IMPACT OF COVID-19 PANDEMIC ON THE EPIDEMIOLOGY AND ETIOLOGY OF LOWER RESPIRATORY TRACT INFECTIONS IN CHILDREN

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PURPOSE: Changes in the epidemiology of communicable diseases in children are one of the direct and measurable consequences of the COVID-19 pandemic. Therefore, we aimed to evaluate the impact of the recent pandemic on the epidemiology of lower respiratory tract infections and the relevant causative pathogens other than SARS-CoV-2.

METHODS: We performed a retrospective study and included all children who were hospitalized due to lower respiratory tract infections in 2019 and 2021. Nasopharyngeal swabs were used to identify the most common respiratory viruses and atypical bacteria using polymerase chain reaction.

RESULTS: In 2019, 356 children were hospitalized due to lower respiratory tract infections, and 250 children in 2021 (a reduction of 29.8%). The proportion of children who were hospitalized due to atypical pneumonia and bacterial pneumonia decreased from 10.1% and 21.9% in 2019 to 2.8% and 12.0% in 2021, respectively ($p < 0.01$ for both). We observed a complete disappearance of influenza viral infections in 2021 and a profound decrease in the proportion of *Mycoplasma pneumoniae*-positive specimens from 9.0% in 2019 to 2.7% in 2021 ($p < 0.01$). However, the proportion of rhinovirus-positive specimens increased from 8.2% in 2019 to 41.2% in 2021 ($p < 0.01$).

CONCLUSION: The COVID-19 pandemic affected the incidence and etiology of lower respiratory tract infections in children, resulting in a decrease in atypical and bacterial pneumonia, a complete disappearance of influenza, and a seasonal shift in the epidemiology of the respiratory syncytial virus. These observations are important for planning healthcare resources and preventive measures for future pandemics, which will undoubtedly occur.

KEYWORDS: COVID-19, lower respiratory tract infections, children, epidemiology, etiology, SARS-CoV-2

ANALYSIS OF BACTERIAL COLONIZATION IN PATIENTS WITH EXACERBATION OF BRONCHIECTASIS INFLAMMATION

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BACKGROUND: Bronchiectasis are present in many patients with COPD. Cough is more or less present in nearly all patients with COPD. But more expectorance than usual, aggravation of dyspoea are suggestible for COPD exacerbation. It is provoked by increase level of inflammation in the lung, with infection or without. Microbiological analysis of sputum specimen is the main goal of this paper.

METHOD: We analyzed the specimen of sputum in COPD patients with bronchiectasis any time if exacerbation is suspected. The analysis was performed according to routine guidelines in microbiological laboratory of General hospital Tešanj. Diagnosis of bronchiectasis was performed using High Resolution CT scan. Microbiological specimen was collected according to adopted protocol of hospital in first three days of hospitalization to avoid nosocomial hospital infection.

RESULTS: We analyzed 128 patients with COPD hospitalized during 2022 and 2023 years treated in Department of Pneumonology of General hospital Tešanj. Patients were divided in two groups those with clinical signs and symptom of exacerbation, and those without. Signs of exacerbation were found in 35 patients (27.34%). Among these patients with exacerbation sputum was positive in 24 cases (68.37 %). Next bacterias were found: Klebsiella Pneumoniae in 11 cases, Escherichia coli in 7cases Serratia marcescens 5 cases, Acinetobacter species in 2 cases, Streptococcus pneumoniae in 3 cases, Enterobacter species and staphylococcus koagulaza negative in one case both. Some patients had two or three bacterias. Positive results was found even in those patients without signs of exacerbation, as follow: Klebsiella Pneumoniae 3 cases, Acinetobacter species 2 cases, Escherichia coli 2 cases, Serratia marcescens one case, Streptococcus pneumonia 2 cases, In three patients two bacterias were found. Statistical analysis was performed by Chi-square test. No statistical difference between two groups was founded.

CONCLUSION: Positive results of microbiological analysis was found in the patients with and without signs of exacerbation, and use of antibiotics should be reserved only for those with positive results of microbiological results and for those with wors overall clinical status. This approach is necessaire for avoidance of resistance to antibiotics. Viral infection as a cause of exacerbation should be considered any time.

EOSINOPHILIC BRONCHIECTASIS AND MICROBIOLOGICAL PROFILES IN A SLOVENIAN COHORT

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INTRODUCTION Bronchiectasis has traditionally been considered a neutrophilic disorder. However, recent evidence suggests that eosinophils, typically associated with allergic and type 2 inflammatory responses, may also play a significant role in bronchiectasis. Blood eosinophil counts (BEC) exceeding 300 cells/µl are found in a subgroup of patients comprising of approximately 20% - eosinophilic bronchiectasis. Some studies have linked BEC exceeding 300 cells/µl with Pseudomonas-dominated microbiome profiles. Therefore, we aimed to investigate the association between elevated BEC and colonization with Pseudomonas aeruginosa (PA), as well as the prevalence of nontuberculous mycobacteria (NTM) infection.

METHODS We conducted a retrospective study at the University Clinic Golnik, including consecutive adult patients diagnosed with non–cystic fibrosis bronchiectasis (NCFBE) based on ICD coding and treated between July and December 2023. Our cohort comprised 175 patients, with 7 excluded due to incomplete data. Medical records were systematically reviewed to collect demographic, clinical, and microbiological data. Patients were stratified into two groups based on their BEC: < 300 Cells/µL and ≥ 300 Cells/µL. Multivariate analysis was employed to identify variables potentially associated with the risk of PA and NTM infection.

RESULTS In the analysis of 168 patients, 41.7% had BEC of ≥ 300 cells/µL. Table 1 presents the characteristics of the patients. In both groups, a predominance of females was noted (73.5% vs. 64.3%). There were no statistically significant differences in comorbidities between the groups, except for asthma and ICS treatment. In our multivariate analysis, adjusting for COPD status, FEV1%, number of lobes affected, inhaled corticosteroid use, age, and eosinophil count, only higher FEV1% was significantly associated with decreased risk of PA infection (OR = 0.97, 95% CI: 0.95-0.99, $p < 0.001$), while an increased eosinophil count showed a trend towards higher risk of PA infection (OR = 1.00, 95% CI: 1.001-1.003, $p = 0.067$). In the same model, the risk of NTM infection was not associated with any of the included variables.

CONCLUSION In our cohort, the eosinophilic group was larger than previously reported (42%), potentially due to the high prevalence of comorbid asthma among patients. We found no significant differences in the frequencies of PA and NTM between the two BEC groups. Notably, while lower lung function, as measured by FEV1, was independently associated with an increased risk of PA, the number of involved lung lobes showed no association. Elevated eosinophil levels showed a trend toward increased risk of PA. The use of ICS was not associated with either PA or NTM risk.

Table 1: Patient characteristics according to blood eosinophil count

Characteristics	< 300 Cells/µl	≥ 300 Cells/µl	P value
No. of subjects	98	70	0.202
Age, yr	58.9 (12.0)	59.2 (12.8)	0.942
Sex, F, n (%)	72 (73.5%)	45 (64.3%)	0.202
BMI, kg/m2	23.8 (5.6)	26.8 (6.9)	0.074
Ever smokers, n (%)	39 (39.8%)	36 (51.4%)	0.135
FEV1, mL	2164 (806)	2065 (885)	0.346
FEV1 % predicted	78.3 (24.4)	71.7 (26.7)	0.575
Lobes involved, n	2.53 (1.24)	2.41 (1.26)	0.931
ICS therapy, n (%)	32 (32.7%)	36 (51.4%)	**0.015**
Comorbidities n (%)			
Asthma	26 (26.5%)	31 (44.3%)	**0.017**
COPD	12 (12.2%)	14 (20.0%)	0.171
GORD	19 (19.4%)	15 (21.4%)	0.745
postTB	5 (5.1%)	5 (7.1%)	0.228
Sputum microbiology			
Pseudomonas aeruginosa, n (%)	11 (14.5%)	12 (19.4%)	0.444
Haemophilus influenzae, n (%)	11 (14.5%)	12 (19.4%)	0.444
Multiple bacteria isolation, n (%)	18 (23.7%)	10 (16.1%)	0.272
NTM infection, n (%)	13 (16.7%)	6 (9.8%)	0.245
T2 markers			
F_{eNO}, ppb	18,0 (18.6)	33.7 (36.4)	**0.047**
Elevated total IgE (>100), n (%)	4 (10.3%)	11 (29.7%)	**0.033**
Eosinophil count, cells/µl	138.3 (72.1)	576.7 (319.9)	**< 0.001**

Legend: BMI = body mass index; COPD = chronic obstructive pulmonary disease; GORD= Gastro-oesophageal reflux disease; NTM = nontuberculous mycobacteria; TB = tuberculosis. All values are mean (SD) unless otherwise stated.

PNEUMOCOCCAL PNEUMONIA IN AN ELDERLY EX-SMOKER: A FATAL ENCOUNTER EMPHASIZING THE CRUCIAL ROLE OF VACCINATION

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INTRODUCTION: Community-acquired pneumonia (CAP) is one of the leading causes of hospital admission and also the most common cause of septic shock necessitating treatment in the intensive care unit (ICU) (*1*). Despite rapid recognition and advances in supportive therapy, it remains a significant cause of morbidity and mortality, especially among vulnerable populations. *Streptococcus pneumoniae* remains the most common bacterial cause of CAP. Besides pneumonia, it also causes sinusitis, otitis media, meningitis, sepsis, and joint infections (*2,3*).

CASE PRESENTATION: A 78-year-old patient, a former heavy smoker, presented to the Emergency Department in January with a 5-day history of dyspnea, productive cough, and sharp pain on the right side of the chest, aggravated by coughing. A chest X-ray revealed extensive right-sided pneumonia. Urine pneumococcal antigen was positive and empirical antibiotic treatment with amoxicillin-clavulanate and azithromycin was initiated. Due to worsening clinical status, the patient required intubation and mechanical ventilation the following day and slowly developed symptoms of septic shock. From blood cultures grew *S. pneumoniae*, leading to therapy de-escalation to penicillin G. Due to acute renal failure the patient required hemodialysis. Pleural ultrasound raised suspicion of empyema, confirmed by pleural punction, leading to thoracic drainage insertion. Further course was complicated with ventilator-associated pneumonia and penicillin G was replaced with piperacillin-tazobactam. Despite comprehensive directed and supportive therapy, the patient's condition progressively deteriorated. The patient died on the day 12 after admission. Postmortem serotype 3 was proven.

DISCUSSION: CAP represents a significant cause of morbidity and mortality, partly preventable through vaccination (*3*). Slovenian recommendations include three vaccines against pneumococcal infections: 23-valent polysaccharide vaccine (PPV23), 13-valent (PCV13), and as of 2023, a 20-valent conjugate vaccine (PCV20). The main advantage of the polysaccharide vaccine is its coverage of a broader range of serotypes, while the conjugate vaccine elicits a superior immune response compared to the polysaccharide vaccine, with higher titers, more sustained antibody response, and greater antigenic strenght (*4*). In presented patient vaccine-type strain caused the infection, implying that prior vaccination could have prevented the adverse outcome of the disease, as randomized controlled trials showed up to 75% efficacy of pneumococcal vaccines in preventing invasive pneumococcal infections in persons aged 65 years or older (5).

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CASE REPORT: COMPLICATED PARAPNEUMONIC EFFUSION AND ESOPHAGO-PLEURAL FISTULA

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CASE PRESENTATION: A 72 year old male with arterial hypertension and dyslipidemia presented to hospital was dyspnea at minimal physical effort lasting 10 days. He was a smoker (8 pack-years) and no alcohol abuse. There was no history of fever, cough, haemoptysis or thoracic pain. Laboratory tests revealed evidently elevated CRP 319 mg/l and leukocytosis, hyponatremia and acute renal failure. Chest radiograph showed moderate right sided pleural effusion estimated at around 1000 ml and no pneumonic infiltrates, on ultrasound exam signs of organization with septa and fibrin debris were seen. Diagnosis of right sided pneumonia with pleural effusion and respiratory failure was made and furthermore there was suspicion of empyema. Doctor in charge performed drainage presented with no pus secretion.

Cytological examination showed no malignant cells, but was positive for acute pleuritis with 98 % of neutrophilic granulocytes, biochemically it was exudate with 42 g/l protein (corresponding serum proteins were 43 g/l), lactate dehydrogenase (LDH) concentration was 16.5 microkat/l (corresponding serum LDH was 3.4 microkat/l), acidic pH 7.1, elevated alpha amylase 35 microkat/l (corresponding serum value 0.6 microkat/l), glucose was 3,6 mmol/l.

He was treated with amoxicillin clavulanic acid and azithromycin. Fourth day patient reported abdominal pain in the left upper quadrant, but biochemically in serum there was normal value of alpha amylase and lipase so pancreatitis was not suspected. The Gram stain results of pleural fluid were known and, surprisingly, Candida glabrata was isolated. Repeated pleural effusion analysis showed a rise in alpha amylase value to 45 microkat/l and LDH value to 34 microkat/l.

CT scan of lung and abdomen was performed for clarification. Rupture of distal esophagus on right side with a communication to pleural space was confirmed with air-fluid collection and no mediastinitis. Urgent EGDS was performed and a penetrating ulcer was seen, consequently stent was inserted. Patient was transferred to thoracic surgery department, active pleural drainage was continued, antibiotic therapy was changed to imipenem cilastatin, antifungal anidulafungin was added. Patient clinically stabilized and repeated lung CT after a few days excluded mediastinitis, there was only a slight residual pleural effusion. Histopathology examination of esophagus revealed nonmalignant ulcer. Patient improved completely.

CONCLUSION: Pleural effusion due to esophageal perforation is a rare condition. Delayed diagnosis can be fatal, because this condition is associated with high morbidity and mortality rates. The cause of perforation may be traumatic (iatrogenic or spontaneous), inflammatory or neoplastic.
Spontaneous esophageal rupture generally occurs at the level of the lower esophagus.

CONVENTIONAL VERSUS MOLECULAR METHODS FOR DETECTION OF ANTIBIOTIC-RESISTANT BACTERIA FROM RECTAL SWABS IN A TERTIARY CARE HOSPITAL

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Antibiotic-resistant bacteria, especially carbapenemase-producing *Enterobacteriaceae* (CPE), vancomycin-resistant *Enterococci* (VRE) and extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL), continue to be a major challenge in clinical setups worldwide. Rapid diagnostic is cruical for implementation of appropriate treatment strategies and prevention of spread of resistant strains.

The aim of our study was to evaluate molecular assay Allplex™ Entero-DR (Seegene) from rectal swabs and bacterial cultures compared to routine microbiology METHODS: Allplex™ Entero-DR Assay is a quantitative PCR-based method, for the detection of 5 Carbapenemase genes, Extended Spectrum Beta-Lactamase gene (CTX-M), and Vancomycin resistance genes (VanA, VanB).

In the Laboratory for Respiratory Microbiology at University Clinic of Respiratory and Allergic Diseases Golnik we received 291 rectal swabs as screening samples between October 2023 and January 2024 for detection of CPE, VRE and ESBL. All samples were analysed by conventional methods.
Out of 291 samples 50 rectal swabs were collected *concurrently for molecular diagnostic and included in the study. These samples were analysed with* Allplex™ Entero-DR.
Also a retrospective analysis of 45 previously characterized antibiotic-resistant bacteria from our collection of culture from year 2018 and 2023 (15 VRE, 15 ESBL and 15 CPE) was done.

Out of 50 rectal swabs Allplex™ Entero-DR detected 7 VRE, 2 CPE and 4 ESBL-producers. Conventional methods identified 4 VRE, 3 CPE and 6 ESBL.
Out of 45 bacterial cultures 15 VRE (100%), 15 ESBL (100%) and 15 CPE (100%) were also detected with Allplex™ Entero-DR. Additionally, in 5 out of 15 CPE molecular assay also detected ESBL.

Allplex™ Entero-DR assay did not detect 2 ESBL-producers and one CPE which could be due to poor collection of rectal swabs for molecular diagnostic or presence of other ESBL or CPE gene, which is not detectable by Allplex™ Entero-DR. On the other hand it showed better detection of VRE and also additional detection of ESBL in carbapenemase-producing *Enterobacteriaceae.*
In conclusion, our results show that the Allplex™ Entero-DR assay is a useful and fast method which could be benefitial for our laboratory together with conventional methods.

UROPATHOGENICITY OF HUMAN *ESCHERICHIA COLI* STRAINS EMPLOYING AN *IN VITRO* BIOMIMETIC MODEL OF PORCINE UROTHELIUM

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INTRODUCTION: Urinary tract infections can be severe, sometimes fatal diseases, whose etiological pathogens are predominantly uropathogenic strains of *Escherichia coli* (UPEC). To investigate different aspects of UPEC pathogenesis, many models have already been established with minor or major disadvantages.

AIM: The aim of this research was to develop a simple, fast, ethically acceptable and affordable biomimetic *in vitro* model based on normal porcine urothelial (NPU) cells, which are genetically and physiologically similar to human bladder urothelial cells and to perform basic studies on the pathogenicity of *E. coli*.

METHODS: Initially, the model was established using a set of control *E. coli* strains – two well-described human UPEC strains (J96 and 536), non-pathogenic human commensal strain SE15 and laboratory strain MG1655. Testing of the model was subsequently performed using human *E. coli* strains isolated either from urine of patients with urinary infections or from faeces of healthy individuals. Pathogenicity of the individual tested strains was determined by assessing the viability of NPU cells following infection. Transmission and scanning electron microscopy were performed to visualize the presence of bacteria on the surface and inside NPU cells, as well as ultrastructural changes resulting from bacterial pathogenicity. We also assessed the cytokine response of NPU cells to different *E. coli* strains as one aspect of the immunological response to infection. Bacterial strains were further tested for the presence of different virulence-associated genes. In addition, the phylogroup, the core lipid type, the O-serotype, the type of lipopolysaccharide, and the ability to form biofilms were determined. Statistical analysis of possible correlations between strains' characteristics and the effect on survival of the NPU cells was performed.

RESULTS: Results showed that our model has the discriminatory power to distinguish pathogenic from non-pathogenic *E. coli* strains. The NPU cells of our model showed unique cytokine responses to infection with each *E. coli* strain, and ultrastructural changes clearly demonstrated the pathogenic effect of the strains on the morphology of the model compared to an uninfected model. Statistically significant correlations were found between the highly pathogenic group of strains and *cnf1*, *hlyA*, *clbAQ*, *papGIII*, *sfaDE* and *tcpC* genes, as well as between the *cnf1* gene and the group of strains that induced a high fold-change in total cytokine synthesis.

CONCLUSIONS: Given the close resemblance between human and porcine urothelium, the results are highly relevant to human medicine.

The study was supported by the Slovenian Research Agency (Grant No. P1-0198, P3-0108, J7-2594 and MRIC UL IP-0510 Infrastructure program).

ALLERGY/CLINICAL IMMUNOLOGY

EARLY HEMPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN CHÉDIAK HIGASHI SYNDROME

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OBJECTIVE: Chédiak Higashi syndrome (CHS) is a rare autosomal recessive lysosomal disorder caused by LYST pathogenic variants leading to defects in the lysosomal trafficking regulator protein characterized by oculocutaneous albinism, immunodeficiency, easy bruising, and progressive neurological deterioration. CHS is a genetic condition that can lead to hemophagocytic lymphohistiocytosis (HLH). Severe clinical manifestations are linked to certain genetic variants. We present a Slovenian case series of CHS patients, who developed accelerated phase - HLH early in life.

DESIGN AND METHOD: We present three patients from the same Roma population who were treated at the University Children's Hospital Ljubljana, Slovenia in the last 25 years. We have analysed their clinical documentation and have compared their clinical picture, time to clinical, genetic diagnosis and development of HLH.

RESULTS: Patient 1, a brother was first evaluated because of albinism at 2 years. CHS was confirmed with LYST NM_000081.3 c.8127_8131delinsTTCTGATATGTA homozygous genetic variant causing frameshift mutation leading to earlier stop termination codon (p.Val2710Serfs*4). He developed clinical and laboratory signs of HLH at the age of 3. HSV-1 infection causing gingivitis was confirmed as possible trigger. In addition, low CMV viraemia was detected. After stabilisation of his activated immune system, he was treated with allogeneic stem cell transplantation at 3,5 years.
Patient 2, a sister presented with respiratory symptoms and pancytopenia at 3 months. The same homozygous genetic variant of CHS as in her brother was confirmed. As a possible trigger of an accelerated phase, only rhinoviral infection was identified. She was treated with allogeneic stem cell transplantation at 8 months of age.
Patient 3, a boy with CHS from the same Roma community was treated at our hospital 20 years ago. In the first year he presented with recurrent bilateral pneumonias. When he was already in the accelerated phase at 1,5 years CHS was confirmed. He died after various relapses of HLH at 3,5 years.

CONCLUSIONS: We present three patients with CHS who all developed accelerated phase early in life and all have confirmed c.8127_8131delinsTTCTGATATGTA homozygous genetic variant. We found only one additional CHS patient with the same homozygous genetic variant in the literature, also of Roma origin who died at 3 years. This homozygous genetic variant seems to be associated with early development of HLH.

INBORN ERRORS OF METABOLISM LINKED WITH INBORN ERRORS OF IMMUNITY: DATA FROM THE SLOVENIAN NATIONAL REGISTRY

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OBJECTIVE: Inborn errors of metabolism (IEM) are a heterogeneous group of disorders, some of which can affect the immune system through multiple mechanisms. Immunological problems may be overlooked due to the systemic features of metabolic disease. Patients with IEM without genetic diagnosis can have life threatening
infections. The diagnosis of metabolic diseases that lead to immunological disorders is likely under-recognized.

DESIGN AND METHOD: A national Slovenian register of inborn errors of immunity was established in 2007. All patients with clinical and laboratory findings consistent with inborn errors of immunity (excluding simple IgA deficiency) are included. 64% of patients in the registry have genetically confirmed disease. In this abstract we present data of patients from our registry with confirmed metabolic errors that are linked with inborn errors of immunity (IEI).

RESULTS: In chart 1 we present by type all 335 patients diagnosed with IEI in Slovenia. 19 patients with IEM causing IEI in our cohort are presented in table 1. Most of the patients with IEM have a congenital defect in phagocyte function. Neutropenia dysfunction led to the deaths of two patients with glycogen storage disease Ib.
In addition, currently three patients with organic aciduria and immune dysfunction are followed at our hospital.

Chart 1. A pie chart showing the proportion of inborn errors of immunity in Slovenian register.

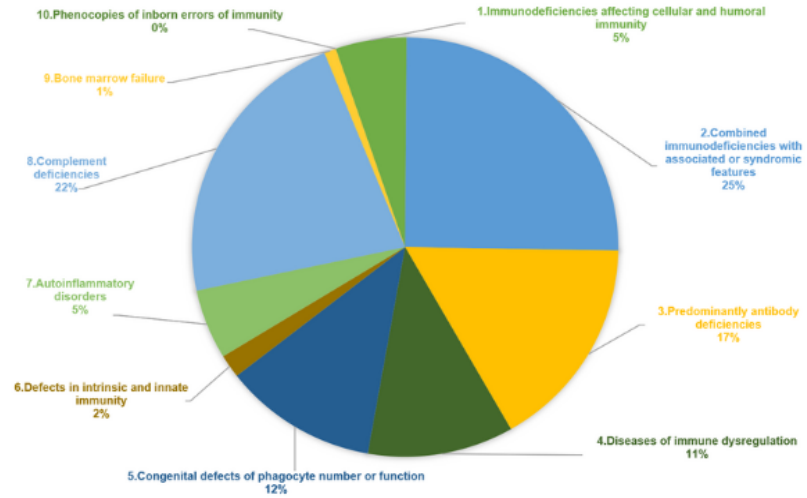

Table 1. Inborn errors of metabolism linked with inborn errors of immunity in Slovenian register.

	Number of patients		Deceased	Adult	Males	Type of inborn errors of metabolism	Treatment
Immunodeficiencies affecting cellular and humoral immunity	1			0	0	Adenosine deaminase (ADA) deficiency	prophylaxis, enzyme replacement therapy
Combined immunodeficiencies with associated or syndromic features	2			100%	50%	Cartilage-hair hypoplasia	prophylaxis
Predominantly antibody deficiencies	1			100%	100%	Catalytic phosphatidylinositol-kinase δ subunit hyperactivity	stem-cell transplantation
Congenital defects of phagocyte number or function	13			53,8%	69 %		
		6	2			Glycogen storage disease type Ib	empagliflozin
		6				Papillon-Lefevre syndrome	stomatological
		1				Barth Syndrome	cardiological
Autoinflammatory disorders	3			33,3%	55,6%	Aicardi-Goutières syndrome	baricitinib (JAK-inhibitor), syptomatic
Total	20		2 (10%)	10 (50%)	11 (52,6%)		

CONCLUSIONS: We present Slovenian data on patients with inborn errors of metabolism that cause inborn immune disorders. Presence of recurrent infections or autoimmune findings in a patient with a suspected metabolic disease should suggest that immune deficiency may also accompany the picture. Immunologic diagnostic examinations should be performed. In addition, in patients with an undefined immunodeficiency, a comprehensive assessment should be performed to evaluate possible IEM.

For optimal treatment and prognosis, it is crucial to have good cooperation between metabolic medicine specialists and immunologists.

Metabolic diseases leading to immunological disorders are probably underdiagnosed. Newborn screening tests for metabolic disease can detect some patients with IEI.

SODIUM-GLUCOSE COTRANSPORTER-2 INHIBITOR CAN IMPROVE NEUTROPHIL FUNCTION IN PATIENTS WITH GLYCOGEN STORAGE DISEASE TYPE 1B

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BACKGROUND: Neutropenia and neutrophil dysfunction with complications significantly influence the quality of life and life expectancy in Glycogen storage disease type 1b (GSD-1b). It appears to be caused by 1,5-anhydroglucitol-6-phosphate accumulation in the neutrophils. Sodium-glucose cotransporter-2 (SGLT2) inhibitors, such as empagliflozin, can remove this toxic metabolite from the body. Several case reports show that this treatment could ameliorate neutropenia-related symptoms, such as severe infections and inflammatory bowel disease (IBD).

METHODS: We present a Slovenian cohort of GSD-1b patients and evaluate the course of empagliflozin treatment in three pediatric patients treated for more than two years. Clinical and laboratory data were assessed using symmetrical periods before and after empagliflozin administration.

RESULTS: The Slovenian cohort of GSD-1b patients (3 male, 2 female) includes two deceased patients not treated with empagliflozin. They passed away at the ages of seven and eleven, both because of septic complications of IBD. In all three patients treated with empagliflozin, we observed significant overall clinical improvement characterized by resolution of aphthous stomatitis and anaemia, termination of abdominal pain, improved stool consistency, increased appetite, independence from tube feeding during the day, reduced frequency and severity of infections and improved postoperative wound healing. In a patient with IBD, we achieved long-term remission confirmed endoscopically and histologically. Therefore, infliximab could be tapered down. Increased and stabilized neutrophil count and improved neutrophil function enabled the discontinuation of G-CSF treatment in all patients. During empagliflozin treatment, we observed a trend of decreasing inflammatory parameters, immunoglobulins and thrombocytes.

CONCLUSION: SGLT2 inhibitors are an innovative treatment option for treating the cause of neutropenia in GSD-1b. Our report exhibits the most extended follow-up of empagliflozin treatment in GSD-1b patients to date: 30 months. Based on clinical observations and laboratory results, targeted metabolic treatment could improve immune function in GSD-1b patients.

PLENARY SYMPOSIUM

VENOM ALLERGY-OUR WAY OF WORKING

COMPUTATIONAL APPROACHES FOR EPITOPE IDENTIFICATION IN ALLERGIC DISEASES

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AIM: Utilise the recently developed bioinformatic tools for epitope identification, following phage display, in order to reduce the amount of experimental ('wet-lab') work through computational analysis.

METHODS: Phage display libraries containing phages expressing linear or cyclic, 7 or 12 amino acid long peptides on their surface underwent three rounds of selection (binding of peptides to polyclonal anti-Ves v 5 antibody, washing unbound phages, elution of bound phages, amplification of eluted phages), to obtain peptides with strong binding affinity for the antibodies. DNA was isolated from eluted phages and subjected to next-generation sequencing (NGS). The bioinformatic analysis included computational tools, such as BBDuk for high-quality sequence retrieval, PuLSE to obtain peptide sequences, MEME and Hammock for motif identification, SAROTUP for non-target binding sequence elimination and Pepitope for epitope localisation on the allergen. Visualisation was performed in Pymol. The pipeline was validated on the major wasp allergen Ves v 5.

RESULTS: Replacing the preparation and Sanger sequencing of individual phage colonies with a predominantly computationally oriented approach, we obtained sequences of the most abundant peptides recovered after three panning rounds. The non-target binders were identified and removed from the set of sequences, resulting in only potential true binders. We detected between 8 and 15% non-target binding sequences in linear and less than 1% in cyclic library. Clear enrichment of specific amino acids could be demonstrated, and three distinct motifs, TKQE, GKI and KPN, were identified, indicating the main epitope regions on the allergen Ves v 5. Finally, we localised the most prominent epitope regions on the allergen.

CONCLUSION: We introduced a pipeline that significantly reduced the time and cost of B-cell epitope identification, making it, therefore, more available for broader use. Using this approach, we identified three major epitope regions on the allergen Ves v 5.
This research was supported by the Slovenian Research Agency; grant numbers P1-0420, P3-0360 and 51978.

CHARACTERISTICS OF IGE BINDING TO WASP ALLERGEN VES V 5 COMPARED BETWEEN SYMPTOMATIC AND ASYMPTOMATIC SENSITIZATION

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AIM: The study aimed to evaluate if IgE antibodies of wasp venom symptomatic and asymptomatic individuals differ in their recognition of epitopes on the major wasp allergen Ves v 5.

METHODS: We tested the sera of 36 symptomatic (Mueller grade III or IV) and 36 asymptomatic (no reaction or large local reaction) individuals referred to University Clinic Golnik. Criteria for inclusion in the study were values for specific IgE (sIgE) against whole wasp venom extract and against allergen Ves v 5 > 0.35 kU/L. The samples were incubated on a peptide microarray slide, with immobilised 7 or 12 amino acid long peptides representing the three dominant epitope regions of a major wasp allergen Ves v 5. The prominent amino acid sequences (major motifs) of the three epitope regions were GKI, TKQE or KPN. Peptides were immobilised on the glass slide in linear and cyclic conformation. The binding of IgE was detected with fluorescently labelled anti-human-IgE antibodies, and fluorescence intensity was detected on a high-resolution laser scanner at 635 nm (JPT Peptide Technologies, Berlin, Germany).

RESULTS: The peptide microarray analysis revealed that the IgE antibodies bound better to the linear conformation of the peptides versus cyclised peptides. Antibodies also bound significantly better to peptides containing motifs TKQE and KPN compared to motif GKI. Even though symptomatic individuals had significantly higher values of sIgE for allergen Ves v 5, antibodies of asymptomatic individuals showed significantly higher binding affinity to the epitope-like peptides, representing the major epitope regions. Finally, both groups of individuals bound the epitope-like peptides with all three identified motifs; however, they recognised different amino acids surrounding the major epitope amino acid motif.

CONCLUSION: Antibodies of symptomatic and asymptomatic individuals recognise the same epitope regions on the allergen; however, they differ in recognising the surrounding amino acids (those that contribute less of the binding affinity). Additionally, higher affinity does not appear to be a key factor for triggering the effector cell degranulation.
This research was supported by the Slovenian Research Agency; grant numbers P1-0420, P3-0360 and 51978.

Invited lecture

HYMENOPTERA VENOM ALLERGY - THE CURRENT STATE OF DIAGNOSTICS AND THERAPY IN THE CZECH REPUBLIC

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The main insects responsible for the vast majority of sting allergic reactions in our region are wasps and bees. Unfortunately, we don´t have our own epidemiological data reflecting the prevalence of large local and systemic sting reactions in our region. Our national recommendations for diagnostics and treatment of hymenoptera venom allergy are based on the EAACI guidelines. For diagnostics, we routinely use skin prick tests and we measure specific IgE to venom extracts. In case of unclear results, we can routinely perform molecular diagnostics (with the use of commercially available panel of 5 bee and 2 wasp venom allergens). There is also basophil activation test with venom extracts available in our country. Intradermal tests are not widely used, as the preparation suitable for intradermal testing isn´t registered in our country. Venom immunotherapy is indicated in accordance with the EAACI position paper from 2018. For venom immunotherapy, we can use only depot preparation. For build-up phase, we preferentially use conventional schedule, faster protocols are not widely used. The sting challenges with living insects aren´t performed in our country. Epinephrine auto-injectors are commonly available, there used to be available two preparations, nowadays we have only one on the market. In 2021, the Working Group for Insect Venom Allergy within the Czech Society of Allergy and Clinical Immunology was established. This group focuses on organizing lectures and meetings with HVA topics to share recent knowledge in our Czech allergist community.

This study was supported by the grant of Ministry of Health of the Czech Republic Conceptual Development of Research Organization Faculty Hospital in Pilsen FNPl, 00669806).

Invited lecture

APPROACH TO PATIENTS WITH STINGING INSECT ALLERGY. RECOMMENDATIONS FOR PATIENTS AND MEDICAL SPECIALISTS IN BOSNIA AND HERCEGOVINA

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The estimated number of annual mortalities due to insect sting induced anaphylaxis ranges from 0.03 to 0.45 per one million worldwide inhabitants. However, this number could be underestimated as many fatal reactions following insect stings may remain undetected. Large local reactions at the site of the sting that is characterized by a swelling with a diameter exceeding 10 cm and lasting for more than 24 h, occur in 2.4% to 26.4% of the general population.

Bosnia and Hercegovina doesn't have a register of patients with allergic diseases. Accidental reports of sudden death after insect sting is still present annually and are mostly reported by social media. Local reaction at the site of the sting is not precisely defined which is misleading for patients. Therefore is important to provide adequate information for patients and health care workers as well. The Society of Pulmologyst BiH is starting a campaign to provide recommendations regarding suspected insect sting allergy.

The campaign aims to provide adequate information to the general population, and recommendations for an adequate approach to suspected insect sting allergy reactions in medical care facilities. It is important to raise awareness of recognition and urgency in the treatment of anaphylactic reaction to Hymenoptera sting.

A secondary aim is to register patients with suspected insect sting allergy, provide adequate diagnostic evaluation, and based on that plan future treatment.

VENOM ALLERGY IN KOSOVO

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INTRODUCTION: Insect venom is one of the most frequent elicitor of anaphylaxis.The majority of cases of anaphylactic reaction occur after a sting by honeybee, due to the higher frequency of attacks.

THE AIM OF STUDY: The registration of prevalence of allergic reactions to insect sting, in certain group of patients presented in Private policlinic in Prishtina, Kosovo.

MATERIAL AND METHOD: 459 patients were examined, from the period August 1, 2023-December 31, 2023, presented for diagnosis at the "Ylli" Clinic, Pristina.

DIAGNOSIS: For diagnosis we have used Polycheck Allergy Diagnostic method, Scanner Based Assay for Screening and Quantification of Allergen-Specific IgE.

RESULTS: We examined 459 patients from the time period August 1, 2023-December 31, 2023).Most of our patients have demonstrated: Rhioconjunctivitis 86(18.73%),Bronchial asthma 83 (18.08%),44 Urticaria (9.58%),42 Dermatitis (9.15),Rash 33(7.18),Angioedema (3.7%),17 Anaphylaxis (3.7%),11 Urticaria with Angioedema (2.3%),11Conjunctivitis (2.3%),12 Prurit (2.6%),8 Dermografismus (1.74%),Rhinosinusitis 6(1.3%),4 Sinusitis (0.87%),2 Contact dermatitis (0.4%),Vasculitis 2(0.4%),1 Reactio medicamentosa (0.2%),1 Headache(0.2%),1 Rash(0.2%),1 AERD(0.2%),1 Epistaxis (0.2%),and 125(27.23%) without initial dgn. 450 of them were positive for pneumoallergens (92.02%), 296 (60.54%) were allergic to certain foods, and only 15 of them (3.07%) were allergic to insects. Patients allergic to insects 15 (m/f=8/7), most of them clinically manifested Anaphylactic Reaction (7), 3 of them Generalized Urticaria, Rhinoconjunctivitis (2), Bronchial Asthma (1), Generalized Erythema (1) as well as Dermographism (1) patients. Of those 15 allergic patients, 6 (40%) of them were allergic to bee venom, 6 (40%) to wasp venom, 6 (40%) to vVespv 5, hornet venom 4 (26.67%), mosquito 7 (46.67%). The highest level of sensitization of our patients was level 4, with crusts registered. 2 patients with rVesv5 and 1 patient with wasp venom. While patients sensitized to Bee venom, Wasp venom and Hornet venom, most of them belong to level 2 and 3. Most of our patients have been sensitized to two or more insect poisons.

CONCLUSION: Even the systemic reaction to the insect venom ranges from 3.3-5% of general adult population, it could be life threatening reaction.

IMPORTANCE OF COMPONENT RESOLVED DIAGNOSTICS AND CROSS-REACTIVE CARBOHYDRATE DETERMINANTS-INHIBITION TEST IN HYMENOPTERA VENOM ALLERGY: SINGLE CENTRE EXPERIENCE

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BACKGROUND: Adults with systemic allergic reactions (SAR) to insect sting show often multiple-positivity of serum specific IgE (sIgE) to Hymenoptera venoms and cross-reactive carbohydrate determinants (CCD). Specific IgE-reactivity to CCD (CCD-sIgE) complicates the interpretation of sIgE to insect venoms, especially in patients with multiple/CCD-positivity. Unnecessary long-term venom-specific immunotherapy (VIT) in false-positive patients increase the risk of recurrent SAR. We analyzed the clinical importance of the molecular component-resolved diagnostics (CRD) and CCD-inhibition for selection of allergens for VIT.

METHODS: In 71 patients we measured sIgE-reactivity to venom extracts (honeybee venom, wasp venom and hornet venom), CCD and recombinant allergens: phospholipase A2 (rApi m1), hyaluronidase (rApi m 2), icarapin (rApi m 10), antigen 5 (rVes v 5), and phospholipase A1 (rVes v 1) using Immunoblot (Euroimmun, Germany, Euroline DPA-Dx insect venoms). In 40 multiple/CCD-positive patients CCD- inhibition test (anti-CCD Absorbent, Euroimmun, Germany) was performed. According to CRD and CCD-inhibition we identified true sensitization and defined groups of multiple/CCD-positive patients who needed CCD-inhibition before starting VIT. We also analyzed the correlation of ImmunoCAP (Thermo Fisher Scientific/Phadia, Upsala, Swedish) and Immunoblot for venom extracts, rApi m 1 and rVes v 5 in 39 patients.

RESULTS: In honeybee venom allergic patients using rApi m1, rApi m 2 and rApi m10, CRD sensitivity was 86.8% while in wasp venom allergic patients, sensitivity of sIgE-rVes v5 was 94% by Immunoblot. In multiple/CCD-positive honeybee venom allergic patients, CRD is not necessary in all patients. CCD- inhibition was helpful in 33% multiple/CCD-positive patients who were initially negative to all tested recombinant honeybee allergens. This patients require CRD to other honeybee recombinant allergens. There was a significant correlation between levels of sIgE to venom extracts ($p<0.0001$) and recombinant allergens rApi m 1 ($p=0.029$) and rVes v 5 ($p<0.0001$) measured by ImmunoCAP and Imunoblot.

CONCLUSION: Our study demonstrates the clinically importance of molecular CRD using profile of the five important recombinant allergens and CCD-inhibition of multiple/CCD-positive sera in the daily clinical practice. Molecular CRD and CCD-inhibition help synergistically in determining true sensitization and this diagnostic approach should be extremely important for the selection of adequate venom for long- lasting VIT.

STINGING REALITY: EXAMINING THE QUALITY OF LIFE IN HYMENOPTERA VENOM-INDUCED ANAPHYLAXIS AND IMPLEMENTING INTERVENTIONS FOR ENHANCED WELL-BEING

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This study aimed to assess the impact of Hymenoptera venom-induced anaphylaxis as graded by the World Allergy Organization (WAO) from I to V on the quality of life (QoL) of affected individuals,
Seventeen patients with a history of anaphylaxis to hymenoptera venom participated in the study and completed the validated Anaphylaxis QoL (A-QoL) questionnaire and its subscales ; Social QoL, Emotional Qol, Limitiation of life and Venome-QoL. The data were analyzed using GraphPad Prism statistical software, employing T-student and ANOVA tests to compare normal quantitative data and Pearson correlation for normal quantitative data.
The mean A-QoL was 2.20 (SD 0.8550), and the Venom QoL mean was 2.794 (SD 2.251), with no significant difference (p=0.1182). Subscale analysis revealed that Emotional QoL (mean 2.484, SD 0.9985) was significantly (p=0.0204) more affected than Social QoL (1.682, SD 0.5823) and Limitation of life (2.106, SD 0.7771). No significant differences were observed in A-QoL (p=0.0826) and Venome-QoL (p=0.1630) between patients who experienced recent anaphylaxis (within the last year) and those with a longer interval since the reaction. Additionally, there was no correlation between WAO severity grade of anaphylaxis and A- QoL (p=0.9236), Venome-QoL (p=0.9696), Social QoL (p=90.8268), Emotional QoL (0.2119) and Limititation on life (p=0.8809)
The type of Hymenoptera (wasp, yellow jacket, or bee) that triggered anaphylaxis did not influence A-QoL (p=0.5117). In response to the observed impact on QoL, a School of Anaphylaxis has been initiated to educate patients on recognizing signs of anaphylaxis, self-administering adrenaline autoinjectors, and post-anaphylaxis care, aiming to enhance overall QoL for these individuals and their families and partners.

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EFFECTS OF VENOM IMMUNOTHERAPY ON QUALITY OF LIFE IN PATIENTS WITH BEE AND WASP STING HYPERSENSITIVITY

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INTRODUCTION: The research evaluates the effects of venom immunotherapy (VIT) on the Quality of Life (QoL) in individuals with bee and wasp venom hypersensitivity. Results encompass both cross-sectional and longitudinal validations of the Slovene version Vespid Allergy Quality of Life Questionnaire – VQLQ (developed by Oude Elberink et al.) in patients with allergic reactions to wasp and honey bee venom.

METHOD: The study utilized the Slovene version of the Health-Related Quality of Life Questionnaire for Hymenoptera venom allergy (HRQLH-S) to conduct cross-sectional validation among 288 patients with confirmed Hymenoptera venom hypersensitivity. Comparisons were made between the results of the HRQLH-S and Expectation of Outcome questions (EoO), using a Likert scale to assess the impact of insect sting allergy on the quality of life (QoL). Longitudinal validity was established by administering the questionnaire to 49 adult patients undergoing VIT.

RESULTS: Highly statistically significant correlations were confirmed between HRQLH-S results and EoO for patients with both wasp venom hypersensitivity (Q16r=0.67; Q17r=0.63; $p<0.001$) and bee venom hypersensitivity (Q16r=0.62; Q17r=0.64; $p<0.001$). Additionally, a significant difference ($p<0.001$) in QoL was observed between with VIT treated patients (Me=3.18) and untreated ones (Me=4.2), evident as early as 6-12 months into Venom Immunotherapy (VIT). After 3-5 years of VIT, the results revealed even greater improvement in QoL for treated patients (Me=2.47) compared to untreated individuals. The Cronbach's alpha for the cross-sectional validation of HRQLH-S was 0.96. In the longitudinal validation, we demonstrated a significant correlation between the EoO questions and HRQLH-S (Q16 r=0.77; Q17 r=0.72; $p<0.001$), indicating good internal consistency (Cronbach $\alpha=0.97$). Additionally, a noteworthy difference ($p<0.001$) in the QoL was identified for pretreatment patients, with an average median value (Me=3.91), in comparison to the value after 5 years of VIT (Me=2.06).

CONCLUSION: VIT for wasp and honey bee venom has shown significant improvements in the quality of life for individuals with venom hypersensitivity. The study confirms that HRQL-S is a reliable and effective tool for assessing the quality of life in patients with allergy to insect venoms.

PLENARY SYMPOSIUM

VENOM ALLERGY – DIAGNOSTICS

CLINICAL PROFILE AND MANAGEMENT IN BONE MARROW MASTOCYTOSIS.

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ABSTRACT

INTRODUCTION: Mastocytosis manifests diversely due to mast cell mediator release, including osteoporosis, gastrointestinal disturbances, and severe allergic reactions. Insights into severe anaphylaxis highlight hereditary alfa-tryptasemia (HAT) and systemic mastocytosis (SM) triggers, with bone marrow mastocytosis (BMM) emerging as a distinct entity featuring heightened osteoporosis and allergic events alongside low progression risk.

METHODS: This retrospective study analyzed 147 mastocytosis patients, focusing on demographics, clinical characteristics, and outcomes. Longitudinal data on serum tryptase levels, liver enzymes, bone densitometry and severe allergic reactions were collected and analyzed.

RESULTS: The study revealed a heightened incidence of BMM, with 105 patients diagnosed. Anaphylaxis was prevalent in BMM, with 98% receiving venom immunotherapy (VIT) and 3% requiring additional epinephrine for breakthrough anaphylaxis. BMM patients exhibited reduced bone density (38%) and osteoporosis (13%), with none showing disease progression during follow-up. ISM patients had higher mean serum tryptase levels (31.9 µg/L) compared to BMM (15.9 µg/L) and a higher risk of tryptase elevation during follow-up.

CONCLUSION: BMM presents a distinct clinical profile characterized by heightened anaphylaxis risk and osteoporosis burden, yet low progression risk. Lifelong VIT and osteoporosis screening are crucial in BMM management. Treatment advances, including omalizumab, midostaurin and avapritinib offer promising outcomes in reducing symptom burden and progression. Osteoporosis management involves bisphosphonates or denosumab. Further research is warranted for refining management strategies in mastocytosis.

KEYWORDS: Mastocytosis, Bone Marrow Mastocytosis, Systemic Mastocytosis, Indolent Systemic Mastocytosis, Anaphylaxis, Osteoporosis, Venom Immunotherapy, Tryptase, Midostaurin, Avapritinib.

INTRODUCTION: Mastocytosis is a disorder characterized by an aberrant proliferation of clonal mast cells and presents with diverse manifestations attributable to the release of mast cell mediators. These symptoms encompass osteoporosis, gastrointestinal disturbances, and severe allergic reactions. Moreover, the infiltration of mast cells in the bone marrow and other organs can culminate in bone marrow failure, hepatosplenomegaly accompanied by liver failure and hypersplenism, and organ dysfunction.[1] Recently heightened interest in the genetic basis of severe allergic reactions has led to recognition of hereditary alfa-tryptasemia (HAT) and systemic mastocytosis (SM) as triggers.[2] This increased attention has led to a shift in the classification of SM, with the acknowledgment of bone

marrow mastocytosis (BMM) as a distinct entity.[1,3,4] BMM is characterized by a low risk of disease progression yet a heightened burden of osteoporosis and allergic adverse events.[3] Despite these advancements, clinical management of mastocytosis remains challenging, compounded by the limited guidance available for the clinical follow-up care of affected patients. In this study, we retrospectively analyzed patients diagnosed with mastocytosis to delineate the disease's evolution and inform strategies for improved patient care.

METHODS: This single center retrospective study involved the analysis of electronic patient records to gather data on various parameters including liver enzymes, tryptase levels, complete blood counts, bone marrow densitometry, occurrences of severe allergic reactions, and concurrent venom immunotherapy (VIT). The collected data were subjected to descriptive analysis, and statistical comparisons were performed using the Kruskal-Wallis test with pairwise comparison with Dwass-Steel-Crichtlow-Fligner test and the Wilcoxon test, which were executed using Jamovi software built on top of the R statistical language.

RESULTS: The study encompassed a cohort of 147 patients diagnosed with mastocytosis, among whom 53% were male, with a median age of 50 years. Table 1 provides an overview of the demographic and clinical characteristics of the patients. The median duration of follow-up for the entire cohort was 22 months, with the longest follow-up period extending to 220 months.

N = 147	
Male / Female N (%)	78 (53 %) / 69 (47 %)
Age; Median (Range)	50 (18 – 82)
Diagnosis N (%)	
Urticaria pigmentosa	1 (1 %)
Bone marrow mastocytosis	105 (71 %)
Indolent systemic mastocytosis	32 (22 %)
Smoldering mastocytosis	1 (1 %)
SM-AHN	4 (3%)
Aggressive systemic mastocytosis	4 (3 %)
Bone densitometry in BMM N = 60	
Osteopenia in BMM cohort	15 (25 %)
Osteoporosis in BMM cohort	8 (13 %)

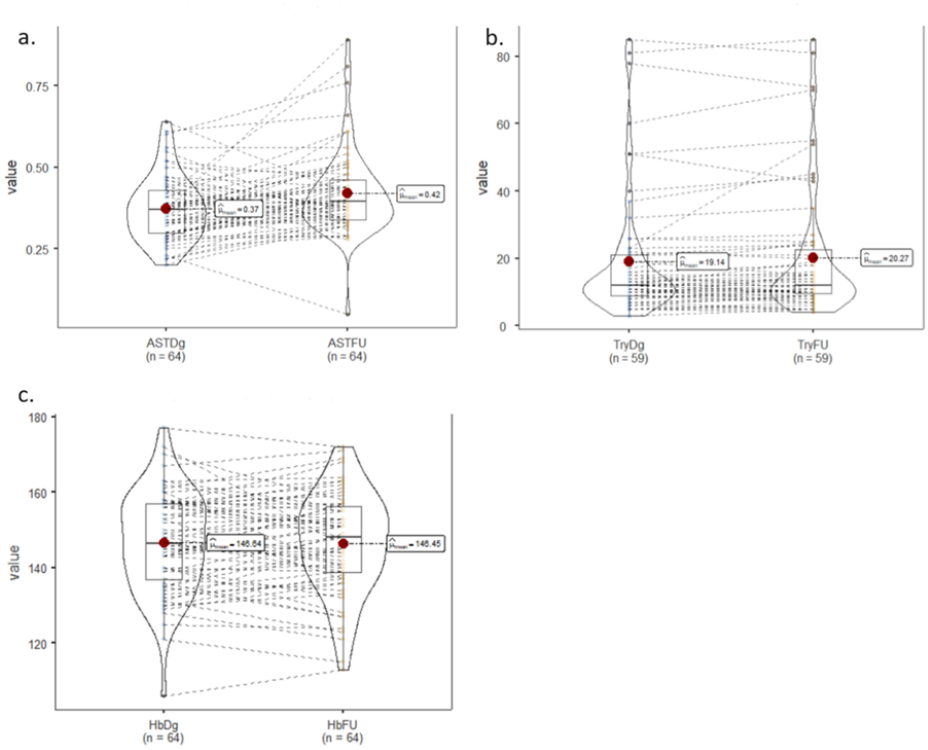
Table 1: Patients characteristics. SM-AHN- systemic mastocytosis with associated hematologic neoplasm; BMM- bone marrow mastocytosis.

INCIDENCE AND PREVALENCE: The incidence and prevalence of mastocytosis remain contentious due to underdiagnosis, contributing to varied data across studies. A recent nationwide register study conducted in Denmark revealed the highest recorded incidence and prevalence rates, standing at 2.77 per 100,000 and 28.44 per 100,000, respectively.[5]
In Slovenia, our study calculated an estimated incidence rate for systemic mastocytosis of 1.2 per 100,000 in 2023, with a corresponding prevalence rate of 7.4 per 100,000. These figures likely underestimate the true rates due to the utilization of single-center data. However, it's worth noting that the hematology department at the University Medical Centre Ljubljana (UMC) serves as the largest referral center for mastocytosis diagnoses in Slovenia, diagnosing most cases in the country.

BONE MARROW MASTOCYTOSIS Bone marrow mastocytosis is characterized by involvement of the bone marrow and absence of skin involvement making the diagnosis without clinical suspicion difficult. Most patients with BMM (94 %) were referred due to severe allergic reactions to Hymenoptera and a

positive KIT p.D816V mutation in peripheral blood. The high prevalence of patients with BMM (74 %) in our cohort of patients can be attributed to the heightened awareness of allergology specialists in Slovenia.
Anaphylaxis emerged as the predominant presenting symptom among patients with BMM, with 98% of these individuals receiving venom immunotherapy (VIT). Despite VIT, a small subset of patients (3%) required additional epinephrine due to severe allergic reactions during follow-up.
Patients with BMM are at increased risk of osteoporosis and fractures necessitating screening with bone densitometry (DXA). Due to limitations in our electronic patient records results were only available for 60 (57 %) patients with 38 % having a reduced bone density and 13 % meeting criteria for osteoporosis.

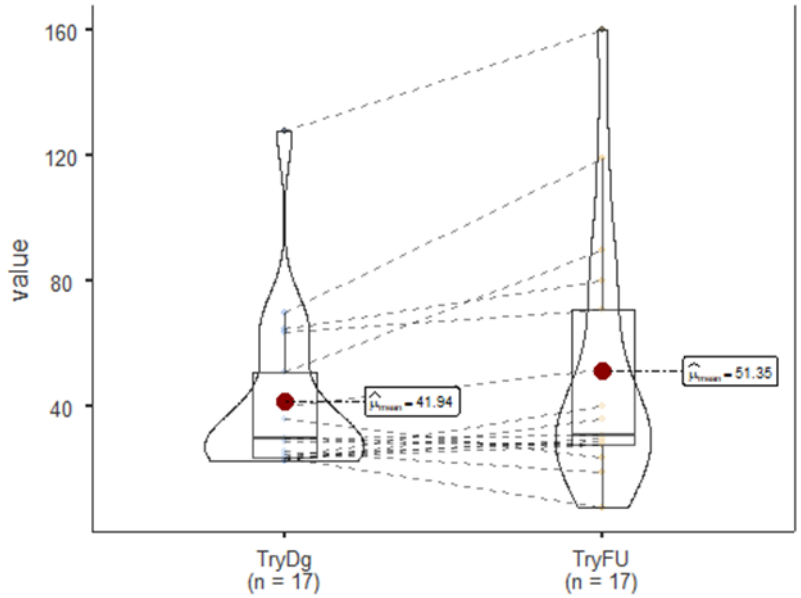
FOLLOW-UP DATA ON PROGRESSION FOR BMM: During the follow-up period, none of the 105 patients showed progression of the disease. Picture 1 illustrates the data on aspartate aminotransferase (AST), tryptase levels, and hemoglobin counts at the time of diagnosis and at the last follow-up. Although a statistically significant difference was observed for AST (Wilcoxon p = 0.006), the magnitude of this difference is small and likely not clinically significant. Conversely, no statistically significant differences were found for tryptase and hemoglobin values during follow-up.
It's important to note that due to limited data on follow-up for patients recently diagnosed, not all 105 patients were included in the analysis.



Picture 1: Box-violin plots for a. aspartate aminotransferase at diagnosis (ASTDg) and follow-up (ASTFU); Wilcoxon p= 0.006, b. tryptase levels at diagnosis (TryDg) and follow-up (TryFU); Wilcoxon p=0.1 and c. hemoglobin at diagnosis (HbDg) and follow-up (HbFU); Wilcoxon p=0.94.

COMPARING BMM WITH ISM
Patients with indolent systemic mastocytosis (ISM) exhibited higher mean serum tryptase levels compared to those with bone marrow mastocytosis (BMM), with respective values of 15.9 µg/L (ref < 10 µg/L) for BMM and 31.9 µg/L (ref < 10 µg/L) for ISM (Kruskal-Wallis p<0.001). We found no statistically significant differences for AST, prevalence of osteoporosis, hemoglobin and leucocyte count between the groups. Patients with ISM also demonstrated a higher risk of experiencing

additional increases in serum tryptase levels during follow-up, particularly among patients presenting with levels above 20 µg/L (p=0.04) as presented in picture 2.



Picture 2: Box-violin plots for serum tryptase at diagnosis (TryDG) and follow-up (TryFU) for patients with indolent systemic mastocytosis and a basal serum tryptase level at diagnosis above 20 µg/L (p=0.04)

DISCUSSION

In recent years, significant progress has been achieved in the management of systemic mastocytosis (SM). The utilization of midostaurin and avapritinib has demonstrated improved outcomes in patients with advanced SM and enhanced symptom control in those with indolent systemic mastocytosis (ISM).[6,7] The heightened awareness among physicians has substantially increased the incidence of bone marrow mastocytosis (BMM), presenting hematologists with unique challenges and necessitating a tailored approach. The progression rate in patients with BMM at 1.7 % is lower than in patients with ISM.[8] This is in line with our data where we had no progression in our patient cohort albeit the patient numbers are still small and follow-up short.

Patients with BMM exhibit a heightened risk of severe anaphylaxis, surpassing 90 %, compared to patients with ISM, underscoring the critical importance of accurate diagnosis in mitigating mortality associated with Hymenoptera stings.[3] In our BMM cohort, breakthrough anaphylaxis occurred in 3% of patients despite VIT, emphasizing the need for additional interventions in these cases. Patients with repeated anaphylaxis have a significant risk of mortality and additional intervention is warranted.[9] Treatment with omalizumab might improve symptoms, including skin symptoms and prevention of anaphylactic shock, but is probably less effective in mitigating gastrointestinal, musculoskeletal, and neuropsychiatric symptoms. Data regarding the efficacy comes from 2 randomized trial and from small retrospective cohorts and case reports.[10-12]

Reducing mast cell burden holds promise in symptom management and anaphylaxis risk reduction. In the past only cytoreductive therapy was available with a safety profile prohibiting its use in patients with nonadvanced SM. Avapritinib is registered for the treatment of advanced mastocytosis after at least one treatment and in patients with ISM with severe symptoms not controlled by other means. In the registration study anaphylaxis requiring epinephrine was observed in 7 patients before inclusion in the study. During study treatment with avapritinib 5/7 patients did not experience anaphylaxis with

only grade 1-2 treatment-related adverse events.[13] Although the patient numbers are small, this data is promising, showing favorable results with a good safety profile.

Another important aspect is osteoporosis and increased risk of bone fractures in BMM. The prevalence of osteoporosis in BMM is 8 – 40 %, preferentially involving the spine and less the hip.[3] Our data shows a reduced bone density in 38 % of patients and osteoporosis in 13 % which is in line with published data. Current biomarkers such as serum tryptase failed to clearly correlate with the risk of reduced bone density warranting DXA in all patients with SM. In a Dutch study there was a tendency to higher prevalence of osteoporosis in patients without skin involvement.[14] Currently these patients would be classified as BMM. Whether this tendency is due to different biology or due to diagnostic delay in patients without skin involvement is unknown. Patients with osteoporosis should receive antiresorptive treatment with bisphosphonates.[15] In patients with contraindications for bisphosphonates denosumab should be used.[16] A small study showed a concurrent reduction in tryptase levels in denosumab treated patients raising the possibility of additional benefit.[16]

Our data reinforces the distinct nature of BMM within the SM spectrum, characterized by a high burden of anaphylaxis and osteoporosis, coupled with a low progression risk. Lifelong VIT and osteoporosis screening are imperative for all Hymenoptera venom-sensitive patients. Follow-up protocols, including physical examination, complete blood count, liver enzyme assessment, and serum tryptase levels, should be tailored to individual patient needs, with intervals of once every two years likely sufficient given the low progression risk, although further long-term data is essential for refinement.

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LONG-TERM STABILITY OF KIT P.D816V ALLELIC BURDEN IN PERIPHERAL BLOOD LEUKOCYTES IN HVA PATIENTS

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BACKGROUND: Recent studies have highlighted the importance of somatic missense *KIT* p.D816V variant in patients with *Hymenoptera* venom-triggered anaphylaxis (HVA). However, the dynamics in *KIT* p.D816V allelic burden in peripheral blood in HVA patients over time remains unknown.

METHODS: We evaluated *KIT* p.D816V allelic burden in peripheral blood leukocytes using a highly sensitive quantitative PCR test in 24 patients with HVA (initial grade III or IV according to the Mueller severity score) followed at the University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia for 12.5 years (median; range 4 – 14 years). Basal serum tryptase (BST) was measured from blood samples at the same sampling points.

RESULTS: Our results show an overall stable *KIT* p.D816V allelic burden over a long period of time, as only a slight and non-significant increase was observed (median from 0.019% to 0.026; P = 0.23). The same results were observed when HVA patients were stratified according to gender (female vs male), culprit (honey bee vs wasp), or venom immunotherapy (receiving VIT vs not). Similarly, no significant increase over time was observed for basal serum tryptase levels (median from 14.4 ng/mL to 14.1 ng/mL; P = 0.16). Nevertheless, in 13 of 24 (54%) patients, we observed a more than two-fold increase in *KIT* p.D816V allelic burden (median from 0.010% to 0.043%) and in 6 of 24 (25%), a more than two-fold decrease in *KIT* p.D816V allelic burden (median from 0.056% to 0.006%), while in 5 of 24 (21%) only minor or no changes in *KIT* p.D816V allelic burden, showing high variability in the dynamics between different HVA patients.

CONCLUSIONS: These results suggest the high variability in the dynamics of *KIT* p.D816V allelic burden detected in peripheral blood leukocytes in HVA patients over a long period of time. Future studies should determine the clinical significance of changes of *KIT* p.D816V allelic burden.

FUNDING: *The research was supported by the Slovenian Research and Innovation Agency (grant no. P3-0360).*

SENSITIZATION TO HYMENOPTERA VENOMS AMONG HEALTHY ADULTS IN SLOVENIAN POPULATION

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BACKGROUND: This retrospective study evaluates the presence of sIgE antibodies against *Hymenoptera* venoms and corresponding sensitization in a cohort of 111 healthy adults in Slovenian population.

METHODS: We included 111 healthy adults (82.9% female, 39.73 ±11.54 years) with no history of systemic reaction to insect stings. Data on birth year, gender, number of times stung by a bee or wasp in their lifetime, last sting occurrence, large local reaction (LLR) to the sting and the number of times stung in their lifetime were collected. We measured sIgE antibodies against native bee and wasp venom as well as recombinant epitopes and correlated the results with the data from the questionnaire. Data are presented as means and standard deviation. The cut-off value for detection of sIgE antibodies was >0.1 kIU/L, and a positive result was regarded as >0.35 kIU/L.

RESULTS: 9 subjects were positive (>0.1 kIU/L) only for bee, 21 only for wasp and 11 for both venoms. In 1 bee venom positive subjects and in no wasp venom positive subjects the IgE positivity was explained with crossreactivity at the level carbohydrate epitopes. No bee venom positive subject was positive with Api m 2 only, leaving 40 genuinely sensitized subjects.
Individuals stung more than once by bees had positive IgE results for bee allergen in 25.4%, while those stung once had a 6.7% positivity rate ($p>0.05$). Those stung more than 5 years ago had lower positive IgE rates (19.4%) than those stung within the last 5 years (30.3%) ($p>0.05$). A significant difference was revealed in individuals with no to little reaction vs. individuals with a large local reaction (LLR) at the bee sting site, who were more likely to be IgE positive (14.6% vs. 35.7%, $p<0.05$). Individuals who reported that they were never stung by a bee or wasp still showed positive IgE values in 28.6%. For wasp allergens, 28.3% of individuals stung more than once had positive IgE results, and those stung once had a 13.3% positivity rate ($p>0.05$). Those with LLR to wasp stings also had a higher rate of positive IgE (40% vs. 17.4%, $p<0.05$). Interestingly, 33.3% of individuals with no history of wasp sting still had positive IgE antibodies. Our results also indicated that the incidence of *Hymenoptera* venom IgE-positive patients increases with age, especially for the bee >0.35 kIU/L, wasp >0.35 kIU/L, bee 0.1-0.35 kIU/L and wasp 0.1-0.35 kIU/L categories ($p>0.05$).

CONCLUSIONS: We found that there is a relatively high frequency of accidentally discovered sensibilization to *Hymenoptera* venom in the general population. Our data indicated, that people who were stung more than once by the insect and those that had a LLR had greater sensitization. Our results also implied that the sensitization of *Hymenoptera* venom IgE positive patients increases with age. Prospective studies are needed to confirm the clinical importance of sensitization in up-to-now healthy subjects and the development of tailored therapeutic plans to prevent possible severe allergic reactions in the future.

FUNDING: *The research was supported by the Slovenian Research and Innovation Agency (grant no. P3-0360).*

INCREASED DIAMINE OXIDASE DURING HYMENOPTERA VENOM-TRIGGERED ANAPHYLAXIS IS SUGGESTIVE OF UNDERLYING CLONAL MAST CELL DISORDER

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BACKGROUND: Diamine oxidase (DAO) is the main enzyme associated with histamine degradation. Even though histamine is one of the primary mediators released from mast cells during anaphylaxis, the relationship between DAO, mast cells and anaphylaxis is not well understood. An increase in DAO concentrations in severe anaphylaxis was shown in animal models, while data in human samples is scarce. One small study showed an increase in DAO during anaphylaxis in patients with mastocytosis. We aimed to determine how DAO concentration changes during severe *Hymenoptera* venom-triggered anaphylaxis, particularly in patients with clonal mast cell disorder (cMCD).

METHODS We retrospectively evaluated 26 patients presenting with an acute episode of *Hymenoptera* venom-triggered anaphylaxis to the emergency department at the University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia. All patients experienced severe anaphylaxis (grade III or IV according to the Mueller severity score). Tryptase and DAO levels were measured from blood samples collected during anaphylaxis and convalescent samples using commercially available fluorescence enzyme immunoassay and ELISA tests, respectively. *KIT* p.D816V missense variant in peripheral blood leukocytes was assayed using a highly sensitive quantitative PCR test.

RESULTS The *KIT* p.D816V variant, demonstrating cMCD, was detected in 6 (23%) of evaluated patients. A marked increase (median 433%) in DAO during anaphylaxis was observed in patients with the *KIT* p.D816V variant (cMCD); on the other hand, the increase was only modest (median 18%) in the subgroup of *KIT* p.D816V-negative patients ($P < 0.001$). Specifically, using the ROC-determined DAO cut-off, during anaphylaxis of 50 ng/ml, DAO levels were raised in 5 (83%) of *KIT* p.D816V-positive, and 1 (5%) of *KIT* p.D816V-negative patients. Similarly, with the cut-off of 220% increase of DAO during anaphylaxis, 5 (83%) of *KIT* p.D816V-positive and none of *KIT* p.D816V-negative patients could be identified. The increase in acute tryptase was similar in patients with and without detected *KIT* p.D816V variant (197% vs 177%; $P = 0.84$).

CONCLUSION Our data highlight the importance of DAO determination during anaphylaxis. The determination of DAO in serum during anaphylaxis represents a valuable and easily accessible screening test for patients suspected of clonal mast cell disorder.

REASSESSING THE CLINICAL UTILITY OF BEE VENOM ALLERGEN MOLECULES

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BACKGROUND: Molecular allergy diagnostic of bee venom allergy is currently of limited use. The sensitivity of 5 available bee venom allergens is not optimal, and some relevant allergens are still unavailable for routine use. It has been confirmed that primary sensitization to bee venom is indicated by sensitization to Api m 1, Api m 2, Api m 3, Api m 4, or Api m 10. Therefore, we decided to reassess the usefulness of the available panel extended by sApi m 4, which is available in the „research use" regimen.

METHODS: 57 bee venom allergic patients were included in our analysis. 28 wasp venom allergic served as a control group. Specific IgE to all commercially available bee venom allergen molecules rApi m 1, rApi m 2, rApi m 3, rApi m 5, rApi m 10, and moreover to sApi m 4 were assessed in all patients. The sensitivities and specificities achieved by all molecules and their combinations were calculated.

RESULTS: We detected sensitivity and specificity of rApi m1 85.96% and 82.14% resp., rApi m 2 47.36% and 85.71% resp., rApi m 3 43.85% and 78.57% resp., rApi m 4 28.00% and 100% resp., rApi m 5 31.57% and 57.85% resp., and rApi m 10 56.14% and 82.14% resp. The sensitivity and specificity of the rApi m 1 + 2 combination was 91.22% and 75.00% resp. Adding other molecules in different combinations revealed a maximal sensitivity 92.98% and a specificity 71.43%.

CONCLUSION: We confirmed our previously published findings that by using a complete panel of 5 bee venom molecules, almost 10% of bee-allergic patients can be missed. The addition of Api m 4 did not improve the sensitivity. On the other hand, the excellent specificity achieved using Api m 4 confirms its high benefit for detecting primary sensitization to bee venom.

This study was supported by the grant of Ministry of Health of the Czech Republic Conceptual Development of Research Organization Faculty Hospital in Pilsen FNPl, 00669806).

HIGH PREVALENCE OF SEVERE SYSTEMIC ALLERGIC REACTION TO BEE VENOM AMONG SLOVENIAN BEEKEEPERS: A PILOT STUDY

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BACKGROUND: Epidemiological data regarding systemic allergic reaction (SAR) to bee venom among Slovenian population of beekeepers are currently lacking. A pilot study was conducted to assess the feasibility of a national epidemiological cross-sectional study, aiming to estimate the lifetime prevalence of self-reported SAR to bee venom, and to identify associated risk factors among Slovenian beekeepers.

METHODS: The first 50 beekeepers of any age, registered with the Slovenian Beekeepers' Association, were recruted through an online convenience sampling survey approach. Data were collected via telephone interviews using the validated questionnaire APISS-Q. Self-reported symptoms were subsequently verified by general practitioners and/or allergologists, with the observed health outcome documented in medical records. For the analysis, only the first SAR to bee venom occurring at any point during a beekeeper's lifetime was included.

RESULTS: Out of 50 beekeepers, 86.0% were male, and 14.0% female, with an average age 51.14 years. The majority (84.0%) had been engaged in beekeeping for over a decade, pursuing it as a hobby (96.0%). 48.0% reported an estimated exposure to 100 or more bee stings annually, yet only 20.0% utilized complete protective equipment. The estimated overall prevalence of the first self-reported SAR to bee venom, graded according to the Müller classification, was 24.0%, while the overall prevalence for severe SAR (grades III and IV) to bee venom was 10.0%. Most allergic beekeepers (66.6%) accessed medical services at the primary healthcare level, with referrals to allergologists for further diagnostic evaluation and treatment. We found a high agreement between the prevalence of mild/moderate SAR (grade I and II) assessed by APISS-Q and verified by allergologists. A tendency among allergic beekeepers to apply herbal remedies/alcohol or local heating at the sting site over the prescribed medications was noted.

CONCLUSION: Pilot study highlighted a high prevalence of severe SAR to bee venom among Slovenian beekeepers, as confirmed by allergologists, along with deviations from recommended treatment guidelines. Our survey demonstrated the feasibility of national epidemiological cross-sectional study within this high-risk population.

FUNDING: The work was supported by the Slovenian Research and Innovation Agency [grant No. P3-0429].

PLENARY SYMPOSIUM

VENOM ALLERGY – THERAPY

Invited lecture

BASOPHIL ACTIVATION AND CLONAL MAST CELL DISORDER ARE ASSOCIATED WITH SEVERE SIDE EFFECTS DURING HONEYBEE VIT.

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BACKGROUND: Venom immunotherapy (VIT) is the only causative treatment for severe *Hymenoptera* venom anaphylaxis. However, severe side adverse events (SAEs), such as anaphylaxis, can also occur during VIT. Due to these repeated severe SAEs, sometimes VIT could not be continued and has to be stopped. Our objective was to identify factors associated with severe SAEs during VIT.

METHODS: We recruited 332 patients undergoing honeybee VIT. We ascertained predictorsof the severity (Cox grade) and threshold of SAEs during VIT. We assessed baseline serum tryptase (BST) levels; sIgEs to HB venom, rApi m 1 and rApi m 10; and basophil activation test (BAT) response. Additionally, in patients with the most severe SAEs, we conducted examinations for *KIT* p.D816V missense variant and tryptase genotyping to determine the presence of increased α-tryptase encoding germline copy number at *TPSAB1*(α-tryptasemia – HαT).

RESULTS: During VIT in the whole group 20% of patients had grade 1 SAEs, while 8% had severe grade 2-4 SAEs. Predictors of severe SAEs during honeybee VIT were age (P=0.025), BST (P=0.006), and BAT response (P=0.001). BAT response was also an individual and significant predictor of any SAEs and SAEs at a low cumulative allergen dose (median 55 mcg) during VIT build-up (P<0.001). Prevalence of cKIT mutation in group of patients with most severe SAEs was 26%, and prevalence of HαT was 8.7 %

CONCLUSIONS: Basophil activation and clonal mast cell disorder are associated with severe side effects during honeybee VIT. These risk factors can help guide recommendations for VIT and overcome systemic reactions to honeybee VIT.

KIT P.D816V VARIANT IS ASSOCIATED WITH HYMENOPTERA VENOM IMMUNOTHERAPY COMPLICATIONS AND FAILURE

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BACKGROUND: Lately, recent studies have highlighted the importance of routine screening for somatic missense *KIT* p.D816V variant and its relation to *Hymenoptera* venom induced anaphylaxis. Our study aimed to evaluate clinical relevance of missense variant *KIT* p.D816V on the course and efficiency of *Hymenoptera* venom immunotherapy (VIT).

METHODS: A retrospective study including 839 patients treated with *Hymenoptera* venom immunotherapy from our in-house registry was performed. The activating *KIT* p.D816V missense variant was assayed from whole blood with allele-specific quantitative PCR.

RESULTS: *KIT* p.D816V variant was detected in blood of 125 (15%) evaluated patients. The majority, 88 (70%), had normal basal serum tryptase levels. Significant difference in complications during the build-up phase of VIT can be observed between honeybee and wasp venom immunotherapy. Roughly half of the *KIT*-positive patients receiving honeybee venom immunotherapy had adverse systemic reactions during the treatment. Furthermore, presence of *KIT* p.D816V variant strongly predicts wasp VIT failure. Higher allele burden of *KIT*-positive patients with VIT failure compared to successful VIT was identified.

CONCLUSION: Our study presents an important novelty regarding clinical relevance of *KIT* p.D816V variant in *Hymenoptera* venom immunotherapy. *KIT* p.D816V variant assayed from whole blood, indicative of clonal mast cell disease, seems to be a positive predictor for systemic adverse reactions during the honeybee venom treatment, and a strong predictor of VIT failure in wasp venom.

FUNDING: The research was supported by the Slovenian Research and Innovation Agency (grant no. P3-0360 and 53537).

STING CHALLENGES - OLDIES BUT GOLDIES

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BACKGROUND: Effectiveness of Hymenoptera venom immunotherapy (VIT) can be determined unequivocally only by exposing the patient to the venom of the culprit insect. Various biomarkers have just a limited role serving this purpose.
The aim of this presentation was to show that Hymenoptera sting challenges are the best means to confirm VIT effectiveness.

METHODS: 76 Hymenoptera venom-allergic patients (33 bee venom- and 43 wasp venom-allergic) were challenged by the culprit insect sting, 1-2 years after initiating VIT. Local and systemic reactions were recorded. Dynamics of venom-specific IgG_4 antibodies (analyzed in a subgroup of patients at various time points - before, after initial phase, and after 1 year of VIT, at the time of a sting challenge), sensitization profiles (analyzed in patients who developed a systemic reaction after a sting challenge - "reactors"), and markers of clonal mast cell disorders and hereditary α-tryptasemia (KIT p.D816V, tryptase genotyping, basal serum tryptase levels) were all considered as possible predictors of VIT effectiveness.

RESULTS: 32 of 33 bee venom-allergic patients (97%) were protected against future bee sting anaphylaxis by VIT. Only one patient (3%) developed a severe systemic reaction (Müller grade IV) after a bee sting challenge, and consequently, is now treated with a double maintenance dose (200 µg of bee venom extract). Among wasp venom-allergic patients, 39 of 43 (91%) were initially protected against further wasp sting anaphylaxis by VIT. Four patients (9%) developed systemic reactions - 3 patients severe (Müller grade IV), and 1 patient mild (Müller grade II). All of them were treated with a double maintenance dose (200 µg of wasp venom extract) from then on, and re-challenged after one year. On re-challenge, 3 patients were protected, and 1 developed a severe systemic reaction (Müller grade IV) again. She was then switched to another manufacturer's venom extract.
Dynamics of venom-specific IgG_4 antibodies, sensitization profiles, and markers of clonal mast cell disorders and hereditary α-tryptasemia failed to predict VIT effectiveness in this study group.

CONCLUSIONS: Hymenoptera sting challenges remain the golden standard to confirm VIT effectiveness, and as such, are extremely useful in therapeutic decision-making. They should be performed under maximum precaution measures, only in centers equipped to deal with anaphylaxis.

LONG-TERM EFFICACY AND RE-STING OUTCOMES FOLLOWING VENOM IMMUNOTHERAPY DISCONTINUATION: A 978-PATIENT ANALYSIS

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BACKGROUND: This retrospective study evaluates the long-term efficacy of VIT after cessation, observing reactions to re-stings in 687 patients 3-23 years post a 5-year VIT regimen.

METHODS: Analysing data from 2217 identified patients who underwent VIT between 1995 and 2020, 1049 responded, leaving 978 after exclusions. We assessed re-sting reactions in 383 BVIT and 671 WVIT recipients, some treated for both. Reactions were graded by severity; emergency kit use post-VIT was tracked. Reaction rates post-re-sting were compared using the two-proportion Z-test. Relatives of 164 deceased patients were contacted to identify insect sting-related deaths, 64 responded with 1 confirmed sting-related death.

RESULTS: 687 (71.9%) reported re-stings during a follow-up period of 3-23 years post-treatment. Systemic allergic reactions were observed in 25.5% of re-stung BVIT patients and 15.5% of WVIT patients, with severe reactions in 11.3% and 5.3%, respectively. Hornet stings post-WVIT led to SARs in 29.5% of cases, with severe reactions in 15.9%. Statistical analysis revealed significant differences in the rates of systemic and severe reactions between BVIT and WVIT groups. Additionally, 13.3% of bee and 10.4% of wasp VIT recipients continued to carry auto-injectors post-VIT.

CONCLUSIONS: This study provides insights into the long-term efficacy of VIT up to 23 years post-treatment, with a significant portion of patients experiencing re-stings. There was a slight expected increase in systemic allergic reactions compared to the frequency observed at the end of VIT treatment, with WVIT proving more effective than BVIT. Additionally, WVIT offers somewhat effective protection against hornet stings.

The research was supported by the Slovenian Research Agency (grant no. P3-0360).

ALLERGY

Short oral presentations

FOOD ALLERGY

PREDICTING REACTION SEVERITY IN CHILDREN ALLERGIC TO PEANUTS: A PROSPECTIVE, MULTI-CENTER, CROSS-SECTIONAL STUDY

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BACKGROUND: Peanut allergy (PA) affects approximately one percent of children and presents a high burden for families of children at risk for unexpected allergic events. We aimed to predict reaction severity in children allergic to peanuts and to identify risk factors for anaphylaxis in a Slovene pediatric patient cohort.

METHODS: As part of a study, we performed a prospective, cross-sectional study of all peanut allergic patients (n = 106, 35 females) aged 3 months to 18 years (median 85 months) attending three pediatric allergy clinics between 1st January 2020 and 31st December 2022. PA diagnosis was based on a history of an allergic reaction within two hours of peanut consumption and a positive allergy test. Patients with negative allergy tests and negative oral food challenge (OFC) were excluded. Medical data was provided by patients or their caregivers and missing data retrieved from the hospital information system. Skin prick testing was performed and serum samples were sent for determination of total and peanut-specific IgE antibodies, peanut component-resolved-diagnostics (CRD), and basophil activation testing (BAT). Based on the results, 52 OFCs were performed.

RESULTS: In 72 children initially evaluated due to an allergic reaction to peanuts, atopic dermatitis was present in 57 children (79%). 45 children (63%) had several food allergies. 1st allergic reactions occurred mostly between 1.5 to 3 years of age (n = 35, 49%) and within 30 minutes after contact (n = 64, 89%). Adrenaline was administered in 15 (21%) of patents after 1st reaction. The severity of the allergic reaction was only weakly correlated (p = 0.04) with the amount of peanuts consumed. 21 OFCs (40%) resulted in an allergic reaction, 11 of which were anaphylactic events. The optimal cutoff for distinguishing between anaphylaxis and a less severe allergic reaction to peanuts was a specific IgE Ara h2 level of 0.92 IU/mL with 90% sensitivity and 47.5% specificity for predicting anaphylaxis (p = 0.04). BAT at the highest peanut concentration (33.3 ng/ml) showed important differences in basophil reactivity between groups (p = 0.017).

CONCLUSIONS: Based on our data, epidemiological and clinical characteristics could not predict the severity of allergic reactions to peanuts in children. Even standard allergy testing, including CRD, was only a moderate predictor of the severity of an allergic reaction. More accurate diagnostic tools that can also predict dose–response relationships (e.g., BAT) may be useful in clinical practice in selected patients.

ANALYSIS OF PRICK TEST RESULTS FOR ALIMENTARY ALLERGENS, SIX MONTHS EXPERIENCE IN ONE ALLERGOLOGY CENTER

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BACKGROUND: Appropriate treatment of allergic diseases should include "in vivo" (PRICK test) and "in vitro" (laboratory analyses of IgE) analyzes. Both results should be take in mention in process of decision making for treatment of these diseases. Because of long term treatment with specific immunotherapy (SIT) appropriate diagnosis should be as much precise as possible.

METHOD: Results of PRICK tests in period of six months were analyzed in this paper. Skin test were performed as PRICK method approved of EAACI. Standard skin PRICK testing for sensitization to inhalant and nutritional allergens in Europe in the according to GALEN network were used. Tests for alimentary allergens were performed using original foods. A positive PRICK result was considered if the swelling was 3 mm or more, regardless of the diameter of the redness around the puncture point. Total IgE and specific IgE were performed for all subjects (inhalation or alimentary specific IgE, as appropriate. Measurement of IgE was performed by ELISA method using ALLEGRIA devices.

RESULTS: In six months period 168 patients were tested. Among them 114 female (67.86 %) and 54 male (32.14 %). Out of all, 19 patients (11.31 %) were positive to potatoes, 17 patients to banana (10.12 %), 14 patients (8.33 %) to tomato, 12 to strawberry (7.43 %), 10 to peanut (5.952 %), 8 to cabbage (4.76 %), and so on. The in vitro measurement of specific IgE antibodies is an important complementary test mostly for those subjects who cannot undergo PRICK test. That's the situation if the skin of forearm share permanent skin manifestation of allergy, or something else (a lot of time pityriasis versicolor with redness, skin eczema, inflammation of skin, and so on. If the subcutaneous specific immunotherapy is considered the precision of diagnosis should be as correct as possible. The results with the most common potato allergy were surprising to us.

CONCLUSION: In the PRICK tests for alimentary allergens, we found a high percentage of potato allergy, which was a surprise to us, although it partly similar with the data from the literature.

KEY WORDS: PRICK test, Total IgE, Specific InE, Allergy, Alimentary allergen.

REGIONAL DIFFERENCES IN SENSITIZATION RATES TO FOOD ALLERGENS IN CHILDREN IN CROATIA

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BACKGROUND: The H2020 Imptox project focuses on the possible human health effects of micro- and nanoplastics (MNP), predominantly on the development and clinical manifestations of allergic diseases in children. The prevalence of allergic conditions has been on a global rise in the past few decades. While there is data on the prevalence rates of asthma, allergic rhinitis and eczema, such data is largely lacking for food allergy. The development and clinical presentation of allergic diseases, including food allergy, are affected by a number of intrinsic and environmental factors, including pollution, dietary and other lifestyle habits etc.

AIM: to determine the prevalence rates of sensitization to food allergens in pediatric population in Croatia from different exposure sites regarding lifestyle habits, air pollution, exposure to MNPs etc.

METHODS: 933 children aged 6-18 yeras were recruited to the cross-sectional Imptox study in 3 distinct geographical regions in Croatia, differening in levels of exposure to air pollution, presumably to MNPs, dietary and lifestyle habits: the capital city of Zagreb and its surroundings, Slavonia and Dalmatia. Participants underwent skin prick test (SPT) to a standard set of aeroallergens and additionally, to food allergens (prick to prick method), blood sampling and stool sampling at 2 timepoints 6 months apart. Additional clinical, demographic and other data was collected, including the ISAAC II, socioeconomic questionnaire, food diaries, food frequency questionnaire (FFQ) and data on exposure to MNPs.

RESULTS: A total of 123 participants (13,18%) were sensitized to at least 1 food allergen (48,78% boys). 5,36% (N=50) participants were sensitized to at 2 or more food allergens (58% boys). The highest sensitization rate to food allergens was in the Slavonia region (N=71, 13,84%), while the lowest was in the region of Dalmatia (N=24, 11,94%), as was the polysensitization rate (to inhaled and food allergens, N=19, 9,45%).

CONCLUSIONS: The sensitization rate to food allergens and the polysensitization rate was the highest in the continental region of Slavonia and the lowest in theMediterranean region of Dalmatia, suggesting that environmental factors (such as environmental pollution and diet) may influence allergic sensitization and the development of food allergy.

DIAMINE OXIDASE DEFICIENCY – A CASE REPORT

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AIM: With this report, we aim to raise awareness about diamine oxidase (DAO) deficiency. DAO is an enzyme that breaks down histamine and, if deficient, can lead to histamine intolerance. We present the case of a 5-year-old girl with recurring acute urticaria after ingesting certain foods, such as specific bonbons, cookies, multivitamin tablets, and pâtés. Urticaria also appeared following a few days of treatment with certain antibiotics and painkillers, including miocamycin and phenoxymethylpenicillin syrup, amoxicillin tablets, and paracetamol syrup, while azithromycin syrup and paracetamol tablets were interestingly tolerated. Usually, the urticarial rash first appeared around larger joints like ankles, knees, and elbows, and then affected the trunk or gluteal region. Urticaria was alleviated by antihistamines and never accompanied by angioedema. The girl also reported eye dryness, redness, and discharge, nasal congestion, dry skin, and persistent epigastric pain. She followed a balanced diet. Her family history was negative for histamine intolerance or allergies.

METHODS: Abdominal ultrasound and extensive allergy testing were performed, including skin testing; provocation with penicillin V; serum immunoglobulins (Ig) against tissue-transglutaminase; Ig specific for certain fruits, antibiotics, jelly, and latex; an IgE inhalation panel; serum tryptase level; an enteric pathogen panel; and serum levels of DAO. Additionally, foods and medications that caused urticaria were closely screened for common ingredients.

RESULTS: Ultrasound did not show any abnormalities. Provocation with penicillin V excluded acute urticaria. There was no eosinophilia. All specific IgE levels were within the normal range. Only the serum level of DAO was reduced (4 U/ml; normal ≥10 IU/ml). The shared ingredients among the medications and foods tested were fruit aromas and beeswax.

CONCLUSION: The girl’s symptoms could be explained by DAO deficiency. She was advised to follow the “urticaria” diet, which means avoiding foods that have a high histamine content or promote its endogenous synthesis. Additionally, she was prescribed antihistamines and recommended to take preventive measures against mold, dust mites, and animal dander. In the future, only tolerated medications, apart from antibiotics in essential situations, are indicated. DAO deficiency should always be considered when recurrent urticaria presents without evidence of an allergic reaction.

OTHER

HEREDITARY ALPHA TRYPTASEMIA AND PRESENCE OF KIT P.D816V IN SLOVENIAN CHILDREN WITH ANAPHYLAXIS

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BACKGROUND: Hereditary alpha tryptasemia (HαT) is associated with an increased risk for severe Hymenoptera venom-triggered anaphylaxis, idiopathic anaphylaxis and an increased prevalence of anaphylaxis in adult patients with clonal mast cell disorders.

OBJECTIVE: This study aimed to assess the presence of HαT and KIT p.D816V missense variant in children with anaphylaxis.

METHODS: Children with anaphylaxis underwent tryptase genotyping by droplet digital PCR and KIT p.D816V in peripheral blood using a highly sensitive PCR. Children's clinical data were retrospectively examined.

RESULTS: We enrolled 180 children with anaphylaxis (mean age 10.5 years, 72.0% male). Number of anaphylaxis were one/two/three/four/five/six episodes in 115/36/15/7/5/2 children. Anaphylactic reaction was treated with adrenalin in 69.7% of episodes.

HαT and KIT p.D816V missense variant were identified in 7.8% and 3.3% of children with anaphylaxis, respectively. Only one KIT p.D816V missense variant and no HαT was present among nine children with idiopathic anaphylaxis. HαT was associated with elevated basal serum tryptase (BST) level above 10 ng/mL. Mean BST in children with KIT p.D816V missense variant was 5.1 ng/mL.

In cases where worst anaphylaxis was graded as 3-4 by Muller or 3-5 by Dribin, the presence of the HαT and KIT p.D816V missense variant was observed in 6.1% or 6.1% and in 3.0% or 3.7% children, respectively. When worst anaphylaxis was graded as 1-2 by Muller or 1-2 by Dribin HαT and KIT p.D816V missense variant were present in 12.5% or 21.0% and in 4.2% or none, respectively. Among the 20 children with worst anaphylaxis graded as 4 by Muller and 32 with anaphylaxis graded as 4-5 by Dribin, only one child had the KIT p.D816V missense variant, and none had HαT.

HαT and KIT p.D816V missense variant were present in 4.8% and 3.8% of children treated with adrenaline at least at one episode of anaphylaxis and in 7.7% and 1.5% of children with two or more episodes of anaphylaxis, respectively. At first anaphylaxis, 36.0% of children with HαT and 17.0% with KIT p.D816V missense variant were under six years old.
CONCLUSIONS: Elevated baseline serum tryptase above 10 ng/mL was consistent marker forHαT. At the current time of our research, neither KIT p.D816V nor HαT indicated a higher risk for more severe/repeated/early/idiopathic anaphylaxis.

REAL-WORLD CLINICAL EXPERIENCE WITH DUPILUMAB USE IN 33 CHILDREN WITH SEVERE ATOPIC DERMATITIS

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AIM: Dupilumab is a relative newcomer among treatment options for patients with moderate or severe atopic dermatitis (AD) and has been in use in children and adolescents in Slovenia since 2021. We have collected and analysed the data on the efficacy and safety of dupilumab use in a cohort of children and adolescents with severe AD followed in a single centre.

METHODS: The retrospective study included children undergoing dupilumab therapy for AD at the Department of Allergology, Rheumatology and Clinical Immunology at the University Children's Hospital, University Medical Centre Ljubljana, from January 2021 to February 2024. The medical records pertaining to the outpatient clinic visits were analysed to assess the clinical data regarding their disease symptoms and treatment.

RESULTS: We analysed a cohort of 33 patients (18 male, 15 female, mean age 8.7 years). The median age of onset of their AD symptoms was 3 months (IQR: 2-3.75). 26 patients had food allergies, 29 inhalant allergies, 13 asthma and 1 eosinophilic esophagitis. Only 6 patients had no prior systemic treatment, while 27 had received cyclosporine before, with 5 still receiving both cyclosporine and dupilumab. One patient was transitioned to MMF after disease relapse on dupilumab. Another underwent various immunomodulatory therapies (cyclosporine, MMF, methylprednisolone, methotrexate, omalizumab) before successfully discontinuing cyclosporine 3 months post-initiation of dupilumab.
Median age at the start of dupilumab treatment was 7.6 years (IQR: 4.4-13.4). The patients have been receiving dupilumab for an average of 1.1 years, the longest duration of treatment being 3 years. The decrease in AD severity is shown in Figure 1. The extent of AD improvement (in %) was assessed by the patients and their parents. The median time for treatment effect onset was 3 months (IQR: 2-3). Two patients experienced conjunctivitis as a side effect, prompting one to discontinue treatment.

CONCLUSION: Prior to the availability of dupilumab, cyclosporine was the primary systemic treatment for severe AD in our centre. Dupilumab has emerged as a successful alternative, demonstrating very good effectiveness and safety profile.

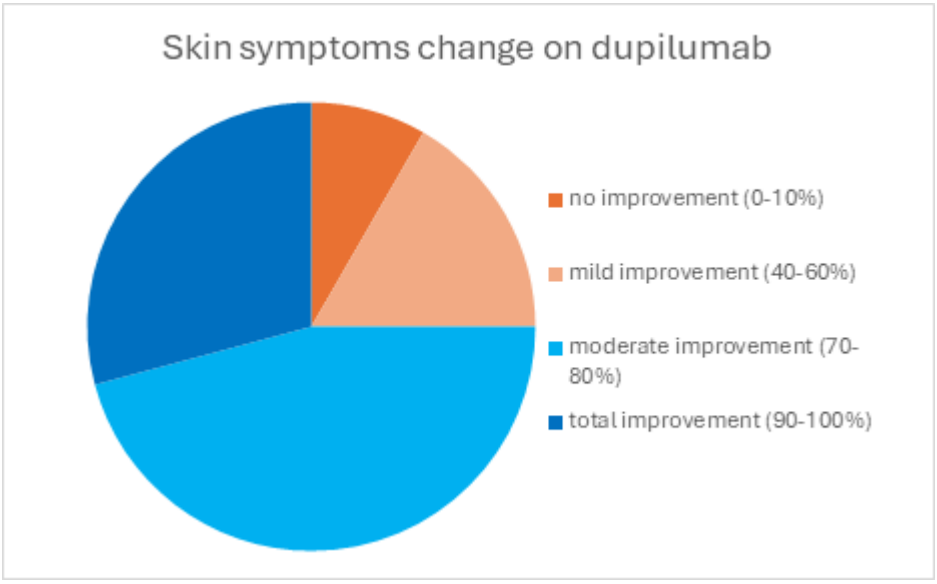
Skin symptoms change on dupilumab
no improvement (0-10%)
mild improvement (40-60%)
moderate improvement (70-80%)
total improvement (90-100%)

Figure 1

CHRONIC URTICARIA SUBTYPES IN SLOVENIAN CHILDREN: INTERTWINING THE CAUSES AND CONTINUING DESPITE TRIGGER IDENTIFICATION

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BACKGROUND AND AIMS: Chronic urticaria (CU) is divided into inducible (CindU) and spontaneous forms. In CU, mast cell activation, including autoimmunity, plays a role. After defining possible triggers, our study aimed to evaluate the causes and natural course of CU subtypes in children.

METHODS: 37 children's data (1-18 years old, an average age of 9.8 years, with female predominance in 67%) diagnosed with CU between August 2019 -2023, were analysed.

RESULTS: The clinical manifestations of hives occurred in 27 children, and hives and angioedema combined in 10 children. One girl had anaphylaxis after taking nonsteroidal antirheumatics. Gastrointestinal complaints were present in six children (three diarrhoea and three epigastric pain). Eight children had eosinophilia and two eosinopenia.
Different potential triggers were simultaneously present in 84% of children with CU (in 18/6/3/2 children 2/3/4/5 different triggers, respectively).
Specific IgE to inhalant allergens were present in 62%, but only 28% had some clinical meaning. CU worsened after contact with pets in only two children, but avoiding them did not diminish CU. Three children were allergic to food.
The infectious trigger was suspected in 47% (in five children with *Mycoplasma pneumonia*e, in four SARS-CoV-2, in three streptococcal, in three other viral infections, two parasitosis and two *Helicobacter pylori*). Children with parasitosis had less symptoms after the mebendazole treatment. In one child treated with macrolides, CU ended afterwards, but CU continued after antibiotics in others.
Low serum diamine oxidase (DAO) level was found in 31% of children with CU. All children benefited from a low-histamine diet. In one girl with celiac disease, a gluten-free diet did not influence the CU course.
Half of CU cases had a physical trigger (53% cold urticaria, 33 % dermographism). All children with CindU also had other triggers of spontaneous CU.
Detectable autoimmune antibodies were present in 10 % of children with CU. One girl with CIndU had previously systemic juvenile arthritis.
Two children were successfully treated with omalizumab.

CONCLUSIONS: Different potential triggers were found in 84% of children with CU. A low-histamine diet, mebendazole, and sometimes macrolide treatment were associated with fewer CU symptoms.

KAWASAKI DISEASE COMPLICATED BY MACROPHAGE ACTIVATION SYNDROME (MAS) IN A 5 – MONTH OLD GIRL WITH TURNER'S SYNDOME AND CONGENITAL AORTIC STENOSIS CORRECTED BY BALLOON DILATATION AND THE ROSS PROCEDURE

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The patient was admitted for fever and enteritis. She had been febrile up to 38,5°C for a day and except for changes in stool consistency and frequency no other signs of illness were seen.
A stool PCR found *Clostridium difficile* toxin A and B, the infection was confirmed by stool culture. She had an elevated ESR and CRP value and elevated LFTs. Due to risk factors present, she was treated with metronidazole. We noticed a petechial rash on her torso on day 4. The rash later became macular and confluent on the head, chest and abdomen. A cervical, axilar and inguinal lymphadenopathy was found.
On day six peripheral oeodema of the feet, leucopenia (L 2,92 $x10^9$/L) and thrombocytopenia (tr 78 $x10^9$/L) appeared. Her pro-BNP level was high. Fever persisted up to 39°C twice per day for 7 days. We excluded viral infections by nasopharyngeal swab and blood PCR for EBV, CMV, HHV-6 and HSV. We performed a heart ultrasound which found no changes in cardiac function or changes to the right coronary artery. The left coronary artery could not be shown adequately, due to postoperative anatomic changes to the heart.
Due to clinical criteria compatible with incomplete Kawasaki disease we treated her with IVIG and acetylsalicylic acid (50mg/kg:4) on day 8. Her general condition improved. She remained afebrile for 48 hours, her rash and oedema of the feet completely disappeared.
She became febrile again on day 11. Hepatosplenomegaly appeared. WBC and thrombocytes were low and there was a further increase in her LFTs. An extremely high ferritin level was measured (>33511,0 μg/L), D-dimer, LDH, fibrinogen and triglicerides were normal and CRP remained low. She received treatment with high dose methylprednisolone and cyclosporine for suspect macrophage activation syndrome (MAS).
Her clinical condition and laboratory values improved rapidly. On follow-up a slight elevation of thrombocytes and peeling of the skin was noticed on her fingers. No changes to the coronary arteries were shown up to six weeks after treatment.

Table 1: Laboratory values.

Laboratory parameter (unit)	**Day1**	**Day 5**	**Day 6**	**Day 8 (4.12.)**	**Day 11**	**Day 12 (8.12.)**	**Day 22**
L (x10^9/L)	10,05	/	**2,92**	5,14	10,84	12,3	15,0
Hb (g/L)	**103**	**/**	**91**	**85**		**65**	130
Tr (x10^9/L)	253	/	**78**	**95**		**91**	409
CRP (mg/L)	**21**	**33,6**	**23**	**23**		**62**	<8
ESR (mm/h)	/	/	/	37	/	/	/
ALT (μkat/L)	**5,14**	**2,55**		**12,01**		**19,5**	**2,36**
AST (μkat/L)	/	**2,67**		**14,66**		**25,87**	0,97
GGT (μkat/L)	0,81	**1,01**		**2,18**		**3,99**	**3,41**
LDH (μkat/L)	/	/	/	/	/	**58,12**	**8,10**
Alb (g/L)	/	/	/	/	/	32	
Pro-BNP (ng/L)	**4917**	**2847**				**5811,0**	**1972**
Ferritin (μg/L)	/	/	**> 1676**			**>33511,0**	230
D-dimer (μg/L)	/	/	/	/	/	**12,376**	/
Fibrinogen (g/L)	/	/	/	/	/	1,8	/
Triglicerides (mmol/L)	/	/	/	/	/	1,9	/

*L – Blood leukocyte value, Tr – thrombocytes, Hb – haemoglobin value, CRP – C-reactive protein, ESR – erythrocyte sedimentation rate, ALT – alanine aminotransferase, AST – aspartate aminotransferase, GGT – gamma glutamyl transferase; LDH – lactate dehydrogenase, Alb – albumin, NT-pro BNP – N-terminal proB-type natriuretic peptide;

Table 2: Treatment.

Days of disease	**Day 1-5**	**Day 8**	**Day 9-11**	**Day 12 -14**	**Day 15 - 18**	**Day 19 – 4 wks**
Treatment	metronidazole	IVIG 2gr/kg ASA 50mg/kg :4	ASA 50mg/kg :4	ASA 50mg/kg:4 Metyhlprednisolone 100mg iv Transfusion of concentrated erythrocytes	ASA 50mg once daily Cyclosporine 15mg BID iv Metyhlprednisolone 4 mg BID iv	ASA 50mg once daily Cyclosporine 15mg BID oral Metyhlprednisolone 4 mg BID oral

*IVIG – intravenous immunoglobulin; ASA – acetylsalicylic acid; BID – twice per day; TID – three times daily;

ANGIOEDEMA

ABDOMINAL MANIFESTATION OF HEREDITARY ANGIOEDEMA: "PAIN FOR THE PATIENT, ENIGMA FOR THE CLINICIAN."

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INTRODUCTION Hereditary angioedema (HAE) is a rare genetic disorder characterized by recurrent episodes of tissue swelling, primarily affecting the skin, respiratory tract, and gastrointestinal system. It is caused by a deficiency in the C1 esterase inhibitor, leading to uncontrolled activation of the complement system. Abdominal manifestations of HAE are often challenging to diagnose due to their nonspecific symptoms and resemblance to other conditions. Early recognition and management are crucial to prevent life-threatening complications*(1)*.

CASE REPORT A 38-year-old patient came to the allergology outpatient clinic due to suspicion of HAE. The suspicion was raised by the pediatrician, who diagnosed HAE in the patient's daughter. Over the past 6-7 years, he experienced frequent episodes of swelling in his hands, feet, and occasionally his face. Additionally, he reported severe abdominal pain since childhood, which would spontaneously resolve after several hours or days. Laboratory investigations at the clinic revealed low C4 levels, a deficiency in the C1 esterase inhibitor, with reduced C1 inhibitor activity to 29%. These findings confirmed the diagnosis of hereditary angioedema. The patient was initiated on appropriate treatment and provided with education regarding trigger factors and management strategies. One month after the initial examination, the patient experienced acute swelling in the abdomen and self-administered icatibant. The pain, which used to last for several hours or days before the diagnosis, completely disappeared within 20 minutes.

DISCUSSION: Abdominal manifestations of HAE are often underrecognized, resulting in delays in diagnosis and inappropriate management. Patients with HAE may initially present with abdominal pain, which can mimic more common conditions like appendicitis or gastrointestinal disorders. Nevertheless, a meticulous clinical history and evaluation of family history can help raise suspicion for HAE. Confirmatory diagnosis relies on laboratory testing, including the measurement of C4 levels and C1 esterase inhibitor concentration/activity *(1)*. A survey reported an 8.6-year delay in recognizing HAE *(1, 2)*. Early diagnosis of HAE is critical to prevent unnecessary interventions and complications. The main focus of treatment strategies is to prevent and manage acute attacks using medications. Furthermore, patient education plays a vital role in enhancing treatment adherence and reducing the disease's impact on the quality of life*(3)*.

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BEYOND THE SMILE: HAE EXPERIENCES IN DENTAL CARE

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PURPOSE: This study aims to investigate the experiences of patients with HAE in the context of dental care. The primary objective is to gain insights into patient interactions with dental professionals, evaluate the effectiveness of preventive measures, and identify potential areas for improvement in dental care for patients with HAE.

METHODS: A comprehensive survey of seventeen HAE patients yielded responses from nine participants, covering demographics, HAE diagnosis, treatment regimens, dental practices, communication with dentists, and the impact of dental procedures on HAE episodes.

RESULTS: The surveyed cohort comprised six females and three males with diverse ages at HAE diagnosis (10 to 45 years). Medication regimens varied, including plasma-derived C1-esterase inhibitor, icatibant, reported for as-needed use, and danazol or lanadelumab as long-term preventive therapy. Dental care visits ranged from regular bi-annual to sporadic, as-needed, with each patient having an assigned dentist. Discrepancies in dentist awareness of HAE were noted, with seven confirming awareness and two reporting otherwise. Notably, eight out of nine participants did not receive preventive medication before dental procedures, underscoring the need for tailored care. One of the nine patients experienced swelling during a dental procedure, that was his first-ever episode. Only one participant consulted with a physician before a dental visit, indicating potential areas for improved coordination. Additionally, four patients stressed the urgency of enhancing dentists' awareness about HAE, emphasizing the pivotal role of education in dental care settings.

RECOMMENDATIONS: Based on the findings, it is recommended to raise awareness among dental professionals about HAE. Patients with HAE should receive plasma-derived C1-esterase inhibitor before any invasive dental procedures. Another option is danazol several days in advance of the planned procedure. In cases of episodes during or after dental interventions, prompt treatment with appropriate medications is crucial, with the added note that these medications should be readily available in the dental office.

CONCLUSIONS: Based on responses from nine surveyed HAE patients, this study emphasizes intricate interactions with dental care providers. While satisfaction exists, findings highlight the need for heightened awareness and improved communication by dental professionals. Future initiatives should close the awareness gap, tailoring recommendations for better access and affordability in preventive measures and treatment during dental interventions.

LIMITED ABILITY OF STIMULATED KALLIKREIN ACTIVITY TO DIFFERENTIATE BETWEEN DIFFERENT TYPES OF ANGIOEDEMA

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BACKGROUND: Discriminating between different angioedema (AE) types, specifically histamine-mediated (histaminergic) AE (H-AE) and bradykinin-mediated AE (Bk-AE), remains challenging, translating into misdiagnosis and delays in diagnosis. The absence of specific biomarkers for bk-AE makes the diagnosis difficult and currently relies on exclusion. We aimed to test the kallikrein activity as a reliable biomarker for differential AE diagnosis.

METHODS: To develop and optimise the procedure of stimulated kallikrein activity, we tested blood serum and plasma (heparin and EDTA) of healthy controls and patients with hereditary angioedema due to C1 inhibitor (HAE-C1-INH) and different possible stimulators of kallikrein activity. Next, we included additional controls and patients with varying types of angioedema, specifically H-AE and bk-AE (ACEi-AE, HAE with normal C1-INH).

RESULTS: Measurements of spontaneous kallikrein activity were consistently high in blood serums of patients with HAE-C1-INH, compared to other samples that showed higher variability. However, the level of activity achieved was not significantly higher than in control samples. Measurements of dextran sulfate (DXS) stimulated activity did not provide a distinguishable difference between any of the tested control and patient groups. Similar results were obtained with heparin and EDTA plasma samples and using ellagic acid or kaolin to stimulate the kallikrein activity. Furthermore, results were inconsistent between different directions of fluorescence measurement (top or bottom). In some cases, stimulated activity was also lower than spontaneous activity, which was unexpected.

CONCLUSIONS: The stimulated kallikrein activity assay results were highly variable, and the procedure was difficult to standardise. Therefore, the assay demonstrated limited ability as a diagnostic tool for the diagnosis of bk-AE.

RESPIRATORY ALLERGY

THE INFLUENCE OF BIRCH AND BIRCH RELATED SPECIES ON THE ALLERGENIC POLLEN SEASON

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BACKGROUND: Understanding the course of the pollen season is an important tool in preventive behaviour of pollen allergy sufferers, diagnosis, and medical treatment. Pollen loads noticeably vary from year to year due to weather conditions and plant characteristics. This study aims to analyse two consecutive years with different pollen season dynamics compared to the 10-year average.

METHODS: The sampling and analyses were carried out by the recommended methodology, summarized in the standard EN 16868:2019. Hirst type samplers were used in Ljubljana and Maribor. The results were given as daily average pollen concentrations (number of pollen grains/cubic meter of air/day). Annual (APIn) and monthly (MPIn) pollen integrals were analysed, which are defined as the sum of average daily pollen concentrations over a year or month. Linear regression was used to study the relationship between APIn and MPIn for the reference 10-year data set (2012-2021).

RESULTS: Based on the observed period of 10 years, MPIn in April has the greatest influence on the APIn (correlation for Ljubljana R^2= 0,90; for Maribor R^2= 0,71), indicating one seasonal peak on average. Year 2022 was outstanding due to a large amount of pollen, APIn values exceeded the average, in Ljubljana for 48,6 % and in Maribor for 79,4 %. An additional peak was observed in both cities, the first already in February and the second in April. The differences between pollen peak spectrums in February and April were large, alder (Alnus) and hazel (Corylus) dominated in February, whereby in April the most abundant pollen types were birch (Betula), hornbeam (Carpinus), hop-hornbeam (Ostrya) and beech (Fagus). Unlike 2022, the dynamics of the 2023 season were completely different. Due to lower pollen production and unfavourable conditions during the flowering of birch and related species, APIn values were lower than average, in Ljubljana for 26,0 % and in Maribor for 17,2 %. In the months following April (flowering period of grasses and weeds), no significant deviations from average were noticed.
CONCLUSIONS: Noticeable differences in pollen loads appeared in the first quarter of the year, during the season of birch and species closely related to it. Besides April, it is worth noting February, when an alder and hazel pollen peak can occur, containing allergens belonging to the pathogenesis-related protein class 10 (PR-10).

SENSITIZATION TO CAT: WHEN IS NASAL CHALLENGE NEEDED?

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INTRODUCTION: Although the skin prick test (SPT) is a reliable diagnostic tool in perennial allergic rhinitis (PER) for patients allergic to cats, the minimum necessary SPT wheal size required to distinguish cat sensitization from true allergy remains controversial. The cat nasal challenge test (cNCT) could be considered the gold standard for detecting true cat allergy.

AIMS*:* To assess the difference in the frequency of cNCT positivity between cat owners and non-owners and to determine an appropriate cut-off level for SPT wheal size in detecting positive cNCT in PER patients who are candidates for allergen immunotherapy (AIT) with cat allergen extracts.

SUBJECTS AND METHODS: cNCT in the form of a nasal spray was administered to 60 adult patients with PER, i.e., cat owners (*n* = 19) and cat non-owners (*n* = 41) with positive SPT to cat fur allergen (Diater, Spain). Subjective (total nasal symptom score [TNSS]) and objective measurements (peak nasal inspiratory flow [PNIF]) for assessment of nasal patency and nasal eosinophil count [NEo]) were used to assess the nasal response. Peak expiratory flow (PEF) was used as a safety parameter during cNCT.

RESULTS: No differences were obtained in SPT wheal size and cNCT positivity between cat owners and non-owners. Positive cNCT detecting true cat allergy could be predicted by a cat SPT wheal size >6.5 mm with 71.11% sensitivity and 100% specificity.

CONCLUSIONS: In adult patients with PER, the frequency of cat allergy was similar among cat owners and non-owners. A cat SPT wheal size ≥6.5 mm could be helpful in detecting true cat allergy by avoiding the demanding, time-consuming, and often unavailable cNCT when cat AIT is needed.

INDOOR AIR POLLUTION AFFECTS ALLERGIC DISEASE MANIFESTATION IN CHILDREN

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BACKGROUND: Allergic diseases are the most common chronic conditions in children and represent a significant global healthcare issue. Environmental factors, especially air pollution, have a crucial role in the development and clinical manifestations of allergic diseases in children.

AIM: In order to gain better insight into the complex relationships between indoor air pollution and allergic diseases, we analyzed large-scale cohort and air quality data from the FP7 Atopica (Grant agreement number: 282687) cohort.

METHODS: 4015 children aged 4 to 10 years were recruited the cross-sectional Atopica study in 3 distinct geographical regions in Croatia differing in air quality data (and other lifestyle factors: Slavonia, the capital city of Zagreb and its surroundings and the Mediterranean region of Dalmatia. All participnats underwent a skin prick test to a standardized palette of inhaled allergens, blood samples were taken and additionally, clinically relevant data (ISAAC II questionnaire) along with other data-demographic, socioeconomic, lifestyle data, pollen symptom and medication diaries during ragweed pollination seasons, and outdoor and indoor air quality data were collected.
Within the Horizon EDIAQI project (Grant agreement number: 101057497) we analyzed this retrospective cohort data in relation to indoor air quality data using state-of-the-art machine learning algorythms (random forrest) and logistic regression.

RESULTS: The vairaible „using central heating" was significant in predicting sensitization to house dust mite (p= 0.03, coef.= -0.50). Similar effects were observed for "using central heating" and wheezing or whisting in the chest, a common symptom in asthma (p= 0.00, coef.= -0.50). Additionally, the model revealed an inverse trend for "heating using fossil fuels" (p= 0.02, coef.= 0.52) in predicting allergy symptoms to ragweed pollen.

CONCLUSIONS: Indoor air pollution, more specifically source of heating in household, affects allergic diseases and allergy symptoms in children. Central heating seems to have a protective role in the development of allergic phenotypes, whereas using fossil fuels (coal or wood) seems to increase the risk for manifesting allergy symptoms (primarily rhinitis and rhinoconjuctivitis) to ragweed pollen, probably due to the increased production of different pollutants (CO_2, SO_2, particulate matter) when burning fossil fuel in households.

EVALUATION AND COMPARISON OF THE EFFICACY OF SCIT AND SLIT FOR THE TREATMENT OF ALLERGIC ASTHMA IN CHILDREN

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BACKGROUND: Allergen immunotherapy, using subcutaneous (SCIT) or sublingual (SLIT) administration, is the only disease-modifying treatment for allergic diseases, primarily for allergic rhinitis and increasingly for allergic asthma. Currently, few studies assess the impact of immunotherapy on children's allergic asthma. Thus, our study aims to evaluate and compare the efficacy of SCIT and SLIT in treating this condition in children.

METHODS*:* Our retrospective study, conducted at Department of Pediatrics, University Medical Centre Maribor, Allergology outpatient clinic, involved 69 children with allergic asthma treated with immunotherapy over the last five years. Treatment duration was three years for dust mite allergies or at least 6 months per year for 3 consecutive years for pollen allergies. Following the European Academy of Allergy and Clinical Immunology (EAACI) recommendations, we assessed and compared the effects of SCIT and SLIT on lung function, eosinophilic airway inflammation, asthma symptoms, and the need for long-term controller medication.

RESULTS: Significant improvements were noted with both SCIT and SLIT. SCIT showed a notable increase in the median forced expiratory volume in the first second (FEV1) from 99% to 105.5% predicted ($p < 0.01$), and median Asthma Control Test (ACT) scores improved from 23 to 25 ($p < 0.01$). Asthma exacerbations reduced from the median of two to zero over three months ($p < 0.01$). SLIT also showed effectiveness, with median ACT scores increasing from 24 to 25 ($p < 0.01$) and exacerbations decreasing from the median of one to zero ($p < 0.01$). Comparatively, SCIT was significantly superior only in improving lung function, with an 8% increase in FEV1 median, against a 1% decrease with SLIT ($p < 0.01$). Both treatments significantly reduced the need for long-term controller medication. Post-treatment, 87.5% of children on long-term controller medication could discontinue or reduce their use after SCIT ($p < 0.01$), and 65.6% could do the same after SLIT ($p < 0.01$), with no significant difference between the treatments ($p = 0.50$).

CONCLUSION: SCIT and SLIT are effective in treating allergic asthma in children, significantly reducing asthma symptoms, exacerbations, and the need for long-term controller medication.

OUTCOME OF DRUG PROVOCATION TESTING IN CHILDREN WITH SKIN SIGNS ONLY ASSOCIATED ANTIBIOTIC HYPERSENSITIVITY

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BACKGROUND AND AIMS: In drug hypersensitivity risk stratification has become an important tool for adjusting the diagnostic strategy. Skin tests (ST) are recommended before drug provocation test (DPT) but may be omitted in low-risk patients. Aim of the study was to describe the outcomes of DPT in children having previously skin signs only when suspected antibiotic hypersensitivity.

METHODS: A retrospective study of children with suspected antibiotic hypersensitivity because of only skin signs who completed DPT from October 2023 to January 2024 at a paediatric allergy centre in Ljubljana is presented. Suspected hypersensitivity reactions were classified 1. as immediate or delayed, 2. according to skin morphology and 3. according to risk stratification from history and clinical features from recent EAACI/ENDA position paper on DPT.

RESULTS: We identified 94 children (55 boys, 39 girls) who reported 99 suspected hypersensitivity reactions. Commonly implicated antibiotics were amoxicillin in 52%, penicillin V in 23% and amoxicillin-clavulanate in 18% of suspected hypersensitivity reactions. The timing of suspected hypersensitivity reaction was delayed in 90% and possibly immediate in 10%. Reported symptoms were urticaria in 44%, maculopapular rash in 37%, unclear rash lasting less than a week and requiring no hospitalisation in 12%, urticaria with angioedema in 3%, urticaria and maculopapular rash in 3% and angioedema in 1%. Risk stratification classified 52% as intermediate, 36% as low and 12% as intermediate or low risk reactions.
All STs (n = 11) were negative. There were 95 DPTs to index drugs. A negative challenge result was obtained in 92% (87 of 95) of DPTs. During eight positive DPT both, different timing of reaction and different type rash as previously described, occurred in 50% (4 of 8). Vomiting occurred twice during DPT with amoxicillin-clavulanate and with amoxicillin in one child with previously unclear rash. Signs during DPT occurred at home in 62% (5 of 8).

CONCLUSIONS: Our study supports the opinion that prior skin tests may not be necessary for children with skin signs only suspected antibiotic hypersensitivity, not just in low risk but also in intermediate risk skin reactions.

EPIDEMIOLOGY AND DIAGNOSIS OF PERIOPERATIVE ANAPHYLAXIS IN SLOVENIA

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AIM: Drug-induced anaphylaxis is a significant cause of anaphylactic deaths, particularly in the perioperative setting, where it can result from the administration of anesthetics and other drugs. This study aimed to determine the frequency of allergic reactions during general anesthesia, identify the frequency of reactions for each drug used, estimate the frequency of IgE-mediated reactions, and determine the incidence of perioperative anaphylaxis in Slovenia. The study also sought to assess potential differences in reactions based on the type of surgery or gender.

METHODS: A retrospective analysis was conducted on examinations performed at the Golnik Clinic's allergy unit between 2017 and 2022 for suspected allergic reactions during surgery. Patient data were analyzed based on various factors such as age, sex, type of surgery, severity of reaction, reaction time, and symptoms. Additionally, the study involved the analysis of drugs used, tryptase blood levels, IgE antibodies, skin test results, provocation tests, and basophil activation tests.

RESULTS: Among the 173 patients analyzed, 56.6% were diagnosed with perioperative anaphylaxis. The majority of affected patients were women, with the most frequent reactions occurring during the induction of anesthesia, particularly in orthopedic surgery. The most common causative agents were neuromuscular blocking agents (37.8%), chlorhexidine (18%), antibiotics (10.8%), and alpha-gal (5.4%). In 17.1% of cases, no specific causative agent could be identified.

CONCLUSION: The study highlighted the prevalence and causative agents of perioperative anaphylaxis in Slovenia, emphasizing the need to raise awareness among medical staff about the importance of tryptase collection and the necessity for collaboration between anesthesiologists, allergologists, and immunologists.

EVALUATING THE USE OF BASOPHIL ACTIVATION TEST IN PERIOPERATIVE ANAPHYLAXIS TO NEUROMUSCULAR BLOCKING AGENTS

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BACKGROUND Immediate hypersensitivity reaction during surgical procedure or perioperative hypersensitivity (POH) has an estimated incidence between 1/1250 to 1/20000 cases. Mechanism of most POH is IgE-mediated (60%), although there are other purposed mechanisms. The main culprit in IgE-mediated POH are neuromuscular blocking agents (NMBA). Skin testing (ST) usually plays the main role in confirming the diagnosis of POH, although the use of other *in vitro* tests can be beneficial. The Basophil activation test (BAT) can help resolve the diagnosis of POH but lacks standardisation.

METHODS The BAT was performed from whole blood samples that were stimulated with allergens and controls. Degranulation was stopped by chilling on ice, after which CD123-PE/HLA-DR-PerCP/CD63-FITC-labeled antibodies were added. Between the years 2017 and 2021 we conducted 83 BAT-NMBA tests. Out of that initial group we were able to identify a group of 14 POH NMBA patients (culprit = NMBA) and a group of 24 non-NMBA POH patients (control group).

RESULTS A full clinical workup was done in both patient and control group as well as ST and BAT to rocuronium. The results of BAT were expressed as the percent of activated or CD63-positive cells. Stimulation index (SI) between basal response and response to the highest concentration of allergen was also calculated. Optimal cut-off of SI > 2 was established with ROC curve (AUC = 0,75; p = 0,01). Aforementioned cut-off criteria presented a specificity of 91,67% and a sensitivity of 57,14% with positive predictive value of 80,0 % and a negative predictive value of 78,6 % respectively. A second criteria for cut-off value of SI > 2 and % CD63+ > 5 was also studied but returned lower diagnostic values (specificity of 87,50% and a sensitivity of 53,85%).

CONCLUSION In combination with ST and clinical workup BAT-NMBA showed useful diagnostic parameters. According to our data a cut-off value of SI>2 for the positive test is optimal for the specificity and sensitivity of the BAT-NMBA. Low sensitivity remains a challenge that could also be explained with non-IgE-mediated POH to NMBA.

IMMUNOLOGY

Invited plenary lecture

ON THE MODULATION OF ALLERGEN-SPECIFIC T CELL RESPONSES

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Today, more than 30% of the population is affected by allergies, which is due to the fact that their frequency has increased considerably in recent decades. Allergic symptoms range from hay fever, allergic conjunctivitis and atopic dermatitis to asthma and life-threatening anaphylaxis. However, not all individuals who are exposed to a certain allergen source are sensitized. Thus, in many individuals, tolerance to exogenous allergens develops, in which case blocking IgG antibodies neutralizing allergen and regulatory T cells counteracting ensuing Th2 responses and antibody producing B cell and plasma cell responses play a major role. Why does tolerance induction against usually innocuous allergens fail in a large proportion of individuals? Among different important factors (epithelial barrier integrity, age at first exposure, frequency and dose of exposure) it is worth taking a closer look at the critical requirements for the development of T regulatory cells (Treg). Notably, in the absence of interleukin-2 (IL-2) or its specific high-affinity receptor CD25, severe forms of autoimmunity develop. In contrast, stabilization of IL-2 by specific monoclonal antibodies not only prolongs the biological half-live of IL-2, but can also be used to preferentially direct its action towards the expansion of Tregs. In a preclinical allergy model, we have shown that IL-2 antibody complexes (IL-2C) significantly reduce subsequent sensitization and also have a therapeutic effect when sensitization has already occurred. So far, however, no long-lasting increase of Treg levels could be achieved with IL-2C alone or when combined with allergen. To solve this shortcoming, we have performed a search for small molecule inhibitors (SMI), which would induce IL-2 overproduction during T cell receptor (TCR) triggering while only moderately stimulating T cell proliferation. We hypothesized that such SMI would preferentially differentiate Treg-like cell types. The aminopyrimidine compound BX-795 was identified as a SMI with such a capability which additionally inhibited Th2 responses. RNA-Seq analyses showed that the BX-795-induced T cells, referred to as Th-IL-2 cells, closely resemble induced (i) Tregs, but that their lineage-specific transcription factor is Helios rather than Foxp3. IL-2 neutralization experiments confirmed that IL-2 was required for distinct aspects of Th-IL-2 differentiation, such as for instance the high-level expression of the adenosine-generating molecules CD39 and CD73. Administration of BX-795 *in vivo* reduced allergic lung inflammation and pathology upon allergen exposure and was associated with a reduced influx of inflammatory cells such as eosinophils, downregulation of the Th2 lineage transcription factor GATA-3 and an increase of lung-resident T cells exclusively expressing IL-2. In summary, SMI that influence TCR-signaling and lead to an overproduction of IL-2 but not to excessive T cell proliferation, such as BX-795, may become useful new agents for the prevention and therapy of allergies.

Invited lecture

BIOMARKERS IN ANAPHYLAXIS

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BACKGROUND

Anaphylaxis is a potentially life-threatening, rapidly progressing systemic hypersensitivity reaction, often following exposure to a small amount of allergen, including insect venom, food, and medications. Measurement of acute serum tryptase, released by mast cells during activation/anaphylactic reaction, is currently one of the main diagnostic tests to confirm an anaphylactic reaction. However, in up to 30% of cases, tryptase levels may not increase during the reaction, especially for food-related systemic reactions and certain medications. In the pursuit of a more reliable biomarker for predicting anaphylaxis, the chemokine CCL2 has emerged as a promising biomarker. Our study compared the results of tryptase and chemokine CCL2 to determine their diagnostic value in Slovenian individuals with anaphylactic reactions of different grades caused by different triggers.

METHODS AND RESULTS

Two hundred four individuals with allergic reactions and 203 healthy controls underwent examination for two anaphylactic biomarkers, tryptase and chemokine CCL2. Among the patients, 96 had reactions due to insect stings, 54 due to medication, 18 due to food, and 36 due to other triggers. One hundred twenty-six experienced Grade 3 anaphylactic reactions by Brown grading, 58 experienced Grade 2, and 20 experienced Grade 1 reactions.

Tryptase exhibited higher sensitivity and specificity compared to CCL2: 70.1% vs 63.2% and 97.5% vs 93.6%, respectively. When both markers were used sequentially, their combined specificity increased to 82.4%.

A similar trend was observed at the grade and trigger level, where tryptase showed higher specificity compared to CCL2 at all levels. However, as seen before, specificity increased even further when biomarkers were used sequentially, one after another. At Grade 3, the sensitivity and specificity of tryptase, CCL2, and combined tryptase/CCL2 were 80.0%, 73.6%, 90.4% and 97.5%, 93.6%, 92.6%, respectively. At grade level 2; 62.1%, 51.7%, 79.3% and 97.5%, 93.6%, 92.6%, respectively. Furthermore, at grade level 1 the figures were 40.0%, 35.0%, 50.0% and 97.5%, 93.6%, 92.6%, respectively. Reactions due to insect sting showed the higest sensitivity (89.6%) with combined biomerkers, followed by 88.9% for food and 75.0% by unknown trigger. The lowest sensitivity appeared at medication level, with 72.2% (Figure 1).

CONCLUSIONS

Serum tryptase, demonstrated superior sensitivity compared to CCL2. This trend persisted across different grades and triggers of anaphylactic reactions. However, the highest sensitivity score was accomplished when using both biomarkers together. Our study underscores the importance of sequential biomarker assessment in enhancing the accuracy of anaphylactic reaction diagnosis, offering valuable insights for clinical practice and future research endeavours.

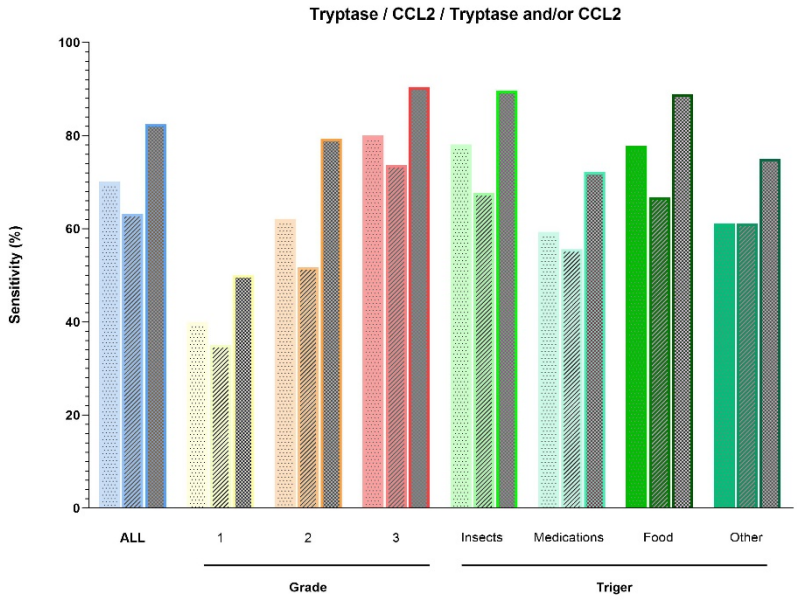

Figure 1: The serum biomarkers tryptase and CCL2. Their sensitivity overall, by grades and by different triggers (insect stins, Medication, Food, Other). The highest sensitivity was accomplished when using both biomarkers (tryptase and CCL2) together.

Invited lecture

WHY AND HOW: REGULATORY T CELLS FOR IMMUNOTHERAPY OF AUTOIMMUNE DISEASES

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ABSTRACT: Autoimmune diseases affect a growing proportion of the population and cause considerable individual and social costs. Patients therefore need more effective, more targeted and safer treatment strategies to reduce the use of general immunosuppressants[1]. In search for safe and efficient therapies that would resolve dysregulated and detrimental immune responses in autoimmune disorders, eliminate or reduce dependence on immunosuppressive drugs, and possibly stimulate tissue repair [2,3], there is a great motivation to develop cell-based immunotherapies[4]. In the context of adoptive cell therapies for autoimmune disorders, one of the promising approaches is to exploit regulatory T cells (Treg) which are one of the most important immune cells responsible for homeostasis and prevention of excessive immune responses at sites of inflammation and peripheral tolerance.

Regulatory T cells account for approximately 1% of human peripheral blood mononuclear cells (PBMCs) and 5-10% of $CD4^+$ T cells. They are distinguished from other cells of the $CD4^+$ compartment by the high expression of CD25 and the transcription factor Forkhead Box P3 (FOXP3), which is a master regulator of Treg identity and is required for their development, maintenance, and suppressive function[5–7]. Functional defects in Foxp3 gene in humans cause severe immune system dysregulation like - X-linked syndrome (IPEX), characterized by high levels of circulating cytokines, type 1 diabetes (T1D), atopic dermatitis, hypothyroidism, enteropathy, arthritis, and sepsis, leading to early death of patients[8]. Treg cells have ability to suppress various immune cells including effector T cells, B cells, dendritic cells, natural killer cells, macrophages, and neutrophils by direct and indirect mechanisms (Fig. 1). In animal models of multiple sclerosis and influenza lung infection, they even promote remyelination and tissue repair, respectively [2,3,9].

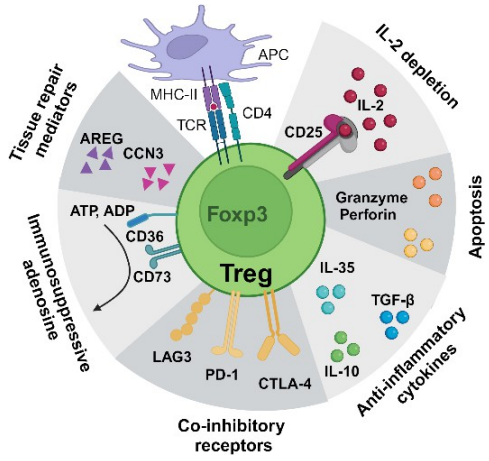

Figure 1: Mechanism employed by Treg cells to control immune responses. Treg cells recognize peptides presented by MHC-II on antigen-presenting cells (APC). Upon activation, they use direct and indirect mechanisms to suppress immune responses. The α-subunit of the IL-2R receptor, CD25, significantly increases the affinity of the receptor for IL-2. Depletion of IL-2 from the inflammatory environment is one of the major immunosuppressive mechanisms of Treg cells. In addition, they can induce apoptosis[10] by releasing granzyme or perforin and release several anti-inflammatory cytokines[11,12], express several co-inhibitory receptors such as CTLA-4[11,13], convert ATP and ADP into immunosuppressive adenosine[14], and promote tissue repair [2,3,9]. Adapted from Romano *et al.,* 2019; Plitas *et* Rudensky, 2016 [12,15]. Created with BioRender.com.

In general, we distinguish two types of Treg cells. In the thymus, where T cell differentiation takes place, cells with strong T cell receptor (TCR) signals induce the differentiation of CD4 cells into thymic Tregs (tTreg), which are responsible for tolerance to tissue self-antigens [16,17]. They are stable due to demethylation of Foxp3 locus and as such ideal to use for therapies. Peripheral Treg (pTreg) cells on the other hand arise from conventional $CD4^+$ T cells (Tconv) in the presence of tolerizing antigens [18],

TGF-β, IL-2[18–20] in periphery and contribute to tolerance to food antigens, gut microbiota[19], and resolution of inflammation[21,22].

REGULATORY T CELLS IN AUTOIMMUNE DISEASES

Even in the absence of a debilitating mutations in Foxp3, Tregs can fail to regulate excessive autoimmune responses. Usually, the frequency of Tregs in patients with autoimmune diseases is not reduced, but they are suppressed by an inflammatory environment [23,24]. No significant differences were found in the number of Treg cells in SLE[23] and MS[25,26], but they had an abnormal immunosuppressive function[23,26,27], lower cloning frequency[25], and decrease in FOXP3 mRNA[27]. In the EAE animal model, antigen-driven activation and inflammation in the CNS promoted FOXP instability and reprogramming selectively in autoreactive bona fide thymic Treg cells[28]. Certain effector cytokines can either release T cells from Treg suppression (IL-21)[29], inhibit Treg cells (TNF, IL-6, Type I IFN)[27,30,31], convert them into ex-FOXP3 Th2 cells with proinflammatory features (IL-4)[32] or reduce levels of FOXP3 (TNF)[27]. The stability of phenotype and function of Treg cell therapeutics thus poses a major challenge for the development of safe and efficient cellular therapies[33–35]. Unstable expression of FOXP3 can lead to conversion from the therapeutic state of a Treg to an activated memory or effector T cell phenotype and subsequent expression of inflammatory cytokines, particularly in the autoimmune environment[36] and under conditions of constant TCR engagement and signalling[37]. Such cells may then promote rather than regulate inflammatory responses[36,38]. We showed that MOG reactive genetically engineered Treg cells effectively suppress early autoreactive T cell responses in the early stages of disease, but not after 30 days in animals, although they still exhibit an activated suppressive phenotype (CTLA-4$^+$, PD-1$^+$, LAG-3$^+$, CD44$^+$CD62L$^-$) and control EAE[39].

REGULATORY T CELLS IN CELL-BASED IMMUNOTHERAPIES

The success of CAR-T cell therapies accelerates the development of other cell-based immunotherapies- including those for autoimmune diseases. Therapies with autologous or allogeneic *ex vivo* expanded polyclonal Treg cells showed a good safety profile, and reduced the need for immunosuppressants after solid organ transplantation[1,40–42] and in chronic graft-versus-host disease (GvHD)[43]. They transiently improved metabolic function in patients with T1D[44,45], and reduced proinflammatory cell counts [46] and proinflammatory cytokine expression[43,46] in patients with GvHD and systemic lupus erythematosus (SLE) without risks associated with opportunistic infections[1,40,41,44]. In addition, numerous animal studies have shown that Tregs can be used to suppress common autoimmune diseases (e.g., T1D[47], MS[48], RA[49]) and prevent organ rejection[50] in an antigen-dependent manner [39,48,51] emphasizing the importance of TCR signalling in Treg biology[11]. However, antigen-specific Tregs are rare and difficult to isolate [34,52]. A more robust approach utilizes genetically engineered antigen-specific Treg cells (eTreg cells) obtained via the introduction of a TCR or CAR into polyclonal Treg cells to make them specific for target antigens and tissues, thereby increasing efficacy and limiting off-target effects [33,53–55].

POLYCLONAL TREG CELLS – CLINICAL STUDIES

In kidney transplant recipients, treatment with polyclonal Treg therapy resulted in decreased dependence on immunosuppressive drugs and thus fewer adverse effects related to infections [1,40]. Adoptively transferred *ex vivo* expanded donor Treg cells in patients with chronic GvHD resulted in symptom relief, a reduction in steroidal immunosuppressants, and a decrease in peripheral blood cytokine levels [43]. In T1D, the phase I clinical trial demonstrated an excellent safety profile for *ex vivo* expanded autologous polyclonal Treg cells. The cells persisted in patients even 1 year after transfer. The well-tolerated treatment paved the way for new phase II clinical trials [56]. A phase I clinical trial showed that adoptive transfer of autologous Treg cells after liver transplantation transiently

increased the pool of circulating Tregs, reduced T cell responses directed against the donor, was well tolerated, and safe [42].

ANTIGEN-SPECIFIC TREG CELLS – PRECLINICAL STUDIES

Antigen-specific Tregs have better therapeutic potential compared with polyclonal Tregs, but are rare and difficult to isolate [57,58]. The results of numerous animal studies show that Tregs could be used to suppress autoimmune diseases (e.g., T1D, MS, RA, colitis), prevent organ rejection, and attenuate GvHD [51].

Antigen-specific Treg cells expanded from NOD mice (non-obese diabetic mice - a preclinical model for T1D) transgenic for TCR recognizing islet antigen significantly suppressed diabetes and helped keep blood glucose levels low. Treg cells retained characteristic expression of signature genes as well as immunosuppressive cytokines [47]. After adoptive transfer, T cells co-transduced with Foxp3 and TCRαβ genes accumulated in the inflamed paw and significantly suppressed collagen-induced arthritis, bone destruction, and reduced the expression of inflammatory cytokines [49]. Ellis et al. showed that CAR-Treg cells specific for HLA-B7 alloantigen migrate and persist in transplanted allogeneic pancreatic islets and exhibit a suppressive phenotype in a nonhuman primate transplantation model[50]. An important step toward introducing antigen-specific Treg cell treatments into the clinic is the STEADFAST study. A first-in-human, phase I/IIa clinical trial will evaluate the safety and tolerability of HLA-A*02-specific CAR Treg therapy to prevent rejection after kidney transplantation[59].

During my postdoctoral project I led at the Institut Necker Enfantes Malades (INEM) in Paris in the group of Prof. Dr. Simon Fillatreau, we investigated the molecular mechanisms by which genetically engineered autoreactive Treg cells expressing high-avidity TCR[39] exert their therapeutic effects. To understand the molecular mechanisms involved in the protective role of genetically engineered antigen- specific Treg cells and to evaluate the strengths and limitations of this approach, we used a preclinical mouse model of central nervous system – experimental autoimmune encephalomyelitis (EAE) which induced by immunization with a peptide derived from myelin oligodendrocyte glycoprotein (MOG). Treg cells modified to express TCRs that recognize the MOG_{35-55} peptide protected mice from developing EAE. After disease onset, they initially accumulated in draining lymph nodes and increased levels of LAG-3, CTLA-4, and PD-1. The genetically engineered Treg cells also significantly reduced the acute autoreactive $CD4^+$ T cell response in treated mice. Moreover, they persisted in the target organs for more than 30 days after immunization and exhibited an effector/memory $CD44^{high}CD62L^{low}$ phenotype. To define the molecular characteristics of autoreactive Treg cells in the CNS of diseased mice, we performed single cell RNA sequencing and bulk transcriptome analyzes of autoreactive Tregs and conventional T cells (Tconv) at the peak of EAE. Among other genes, *Il10*, *Ctla4*, and *Areg* were highly expressed in Treg cells in the CNS compared with their Tconv counterparts. Using genetic approaches, we generated antigen-specific Treg cells in which the genes for Il10, Ctla4, or Areg were silenced and showed that Il10 and Ctla4 are non-redundantly required for protection against EAE, whereas Areg has no effect. Our research highlights the importance of several suppressive factors for the functionality and therapeutic potential of antigen receptor-engineered Treg cells[39].

DISCUSSION: The increase in incidence and prevalence, especially in Westernized societies, urges policy makers and researcher to focus on autoimmune disease surveillance, prevention, health services, and research[60]. Several challenges remain before current Treg-based therapies reach the clinic and become the therapy of choice[4,61]. *In vitro* expansion of Treg cells relies on incubation with TCR activation beads, cytokines (IL-2), and mTOR inhibitors (rapamycin) to promote proliferation and a stable phenotype. However, when injected into patients or experimental animals, Treg cells encounter an inflammatory environment (e.g. IL-6 and TNF expression effector T cells - Teff) that negatively affect the stability of their phenotype and suppressive capacity similarly as endogenous Treg cells when encountering inflammatory stress environments[30,62]. Therefore, it is important to understand what drives the

instability of Treg cells and how their function is influenced by the immune environment to rationally develop cellular products for immunotherapies that remain in the patient and successfully regulate the immune response.

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Invited lecture

THE IMPACT OF THE HLA TYPE ON HUMORAL IMMUNITY AFTER VACCINATION

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It is well established that there is marked biological variation in how individuals respond and maintain immunity after vaccination. Low antibody responder group after vaccination was identified and found to be more commonly male, elderly and with long-term health conditions. On the other hand the response could be attributable to genetic factors. Human leukocyte antigens (HLA) molecules have been recognized as significant influencers in adaptive immune responses to various vaccines, including hepatitis B, measles, and influenza. Recent studies revealed the influence of HLA, coded by particular alleles, on humoral and cellular immune response to vaccination against COVID-19.

In the present study we sought to investigate the contribution of vaccinated individuals' HLA type to the variation in strength of humoral response to the vaccination with mRNA SARS-Cov-2 (Comirnaty, Pfizer/BioNTech) vaccine.

A group of 106 health care workers were vaccinated for the first time at the beginning of 2021. After 6 to 8 weeks concentration of antibodies was determined in the sera of vaccinated individuals using Architect SARS-CoV-2 IgG II Quant reagents. Control group (CG) was composed of 147 randomly chosen subjects. Both groups were typed for HLA-A, B, C, DRB1 and DQB1 by next generation sequencing (NGSgo-GenDx). Vaccinated individuals were divided in the groups of very strong responders (>20000 AU/ml) (VSRG), responders (50-20000 AU/ml) (RG) and non-responders (<50AU/ml).

Statistical analysis was performed using two tailed Fischer's exact test. Extremely significant increase of the A*03:01 frequency was observed in the VSRG compared to CG ($p = 9 \times 10\text{-}4$) as well as when compared to RG ($1 \times 10\text{-}4$). Moreover, while DQB1*06:02 was also found significantly associated with very strong response to vaccination, A*24:02 seem to have an influence on non-responsiveness.

Our results confirm the influence of vaccinated individual's HLA-type on humoral immunity, especially on the strength of the response to vaccination, where A*03:01 plays a crucial role.

Invited lecture

BIOBANKS: AN IMPORTANT AND CRITICAL INFRASTRUCTURE IN BASIC RESEARCH AND CLINICAL STUDIES - AN EXAMPLE OF A BIOBANK AT THE NATIONAL TRANSFUSION CENTRE

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Biobanks are repositories of human biological material that responsibly receive, store and distribute biological samples and relevant clinical data for research, education purposes and for clinical use. They are a complex system of processes through which unique and valuable samples are managed. Biobanks are an important part of development and progress in the medical field and are crucial for the implementation of high-quality clinical studies and research, including the field of personalized medicine. A pilot biobank was established at the Blood Transfusion Centre of Slovenia (BTCS) in the scope of Interreg project C3B. The goals of the project were also to check the interest in biological samples of healthy donors for research, education and clinical studies, to arrange and organize the collection, storage and use of samples of blood from healthy donors, to develop a pilot model and to prepare the prerequisites for the establishment and operation of a national biobank of the blood samples of healthy donors (approval of Medical Ethics Committee obtained in August 2023), based on our experience and good practices in blood banking and according to the Strategy of the Blood Transfusion Centre of Slovenia 2023 - 2030. The project C3B was chosen twice as one of the best projects in the program - in the field of management and in the field of health.

Our model is based on ethical, moral and medical principles that BTCS takes into account when managing the blood bank. We implemented good practices from the field of biobanking, used our own good practices and quality standards, and established a pilot model of a biobank of blood samples from healthy donors. The samples are intended to be used for education, research and clinical studies, approved by the Medical Ethics Committee. The establishment of a pilot model of a biobank of blood samples from healthy donors will represent the integration of the national biobank into already existing protocols at BTCS with an emphasis on the processes by which we monitor the blood sample from the reception of the donor to the release of the blood sample or its components from the biobank to the end users. A quality system is included in the biobanking and the activities are supported by information system which ensures the traceability of the handling of samples and enables the protection of personal data.

Invited plenary lecture

AN IFNg-DEPENDENT IMMUNE-ENDOCRINE CIRCUIT LOWERS BLOOD GLUCOSE TO POTENTIATE THE INNATE ANTI-VIRAL IMMUNE RESPONSE

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Viral infection causes changes to our metabolism that are relative to the severity of disease. To what extent blood glucose levels are subject to infection-induced changes is mostly unknown. Here we show that strong, non-lethal infection causes restriction of systemic glucose availability which promotes the antiviral IFN-I response. Following systemic viral infection of mice, we find that IFNγ produced by γδ T cells directly stimulates pancreatic β-cells to increase glucose-induced insulin release. Subsequently, hyperinsulinemia lessens endogenous glucose output by the liver. Glucose restriction enhances type-I interferon production by curtailing lactate-mediated inhibition of IRF3 and NF-κB signaling. Induced hyperglycemia constrained IFN-I production and increased mortality upon infection. These findings identify glucose restriction as a physiological mechanism to bring the body into a heightened state of responsiveness to viral pathogens. This immune-endocrine circuit is disrupted in hyperglycemia, which explains why people with metabolic disease are more susceptible to viral infection.

Invited lecture

DECONSTRUCTION OF THE INFLAMMASOME IN CANCER CELLS FOR INDUCTION OF IMMUNOGENIC CELL DEATH THAT BOOSTS ANTITUMOR IMMUNITY

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Inflammasomes serve as crucial defense mechanisms, employing cytokines and immunogenic cell death components to activate the immune system against pathogens. However, in the context of cancer, inflammasomes play ambiguous and context- dependent roles, often downregulating effectors of immunogenic cell death. In this study, we drew inspiration from the natural inflammasomes to design diverse deconstructed versions, employing one or both inflammasome signaling arms, and explored whether their local activation in tumors can induce antitumor immunity. To induce immunogenic cell death, we designed tightly regulated GSDMD variants comprising different pore-forming capabilities and diverse modes of activation. We show that electroporation of plasmids encoding the pyroptotic component into the B16 melanoma tumor model leads to tumor regression and complete remission in a quarter of treated C57BL/6J mice. The inflammatory cytokines IL-1β and IL-18, along with the T-cell activator IL-12, did not provide protection when produced solely within tumors, however, they significantly boosted anti-tumor immunity when combined with pyroptosis. Hence, these molecules act as adjuvant therapy, igniting inflammatory responses that together with pyroptotic cancer cells facilitate the immune-recruitment and maturation in the tumor microenvironment and drive potent and persistent antitumor effect. The transcriptome analysis showed that careful choice of immunostimulatory molecules is imperative, as combining IL-1β and IL-18 or introducing IFNγ can antagonize the protective effects of pyroptosis by increasing the expression of multiple immune checkpoints. Moreover, our findings demonstrate that local deconstruction of the inflammasomes, without inducing systemic inflammation, provides long-lasting protective immunity against distant tumors and proves effective across various tumor types. Deconstructed inflammasomes emerge as a potent, tunable and tumor-agnostic strategy to enhance antitumor responses, even against the most resilient tumor types.

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CLUSTERING: A UNIFYING MECHANISM FOR NLRP3 INFLAMMASOME ASSEMBLY

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NLRP3 is an essential inflammasome-forming sensor that contributes to the first line of defense against pathogens. The ability of NLRP3 to respond to diverse endogenous triggers and its dysregulated activity contribute to the progression of common noncommunicable diseases, such as neurodegenerative and cardiovascular diseases. Despite significant progress in understanding the structural mechanism of NLRP3 inflammasome assembly, it remains enigmatic how so many distinct triggers and cellular locations facilitate its activation.

NLRP3 driven to different subcellular locations, including endoplasmic reticulum, Golgi apparatus, plasma membrane, centrosome, lysosomes, mitochondria, peroxisomes, was introduced into murine NLRP3-deficient macrophages. We determined the subcellular localization of prepared variants using confocal microscopy. Various canonical triggers were employed to activate NLRP3 variants, while small molecule inhibitors were used to dissect the activation process. Activation of NLRP3 inflammasome was assessed by cytokine release measurements using enzyme-linked immunosorbent assay and by cell death assays (propidium iodide uptake, lactate dehydrogenase release).

NLRP3 variants, when targeted to organelles, responded to canonical activators similarly to wild-type NLRP3. While wild-type NLRP3 relies on a basic segment (K127-K130) for membrane binding and inflammasome activation, the membrane-enriched variants retained the ability to promote inflammasome assembly when this segment was mutated. This demonstrates the need for NLRP3 binding to membrane scaffolds to successfully nucleate the inflammasome. Engineered NLRP3 fusions demonstrated that a protein-based scaffold can replace the membrane and revealed NLRP3 clustering as a crucial step preceding inflammasome assembly. Using well-characterized small molecule NLRP3 inhibitors, we demonstrated that inhibitors binding to the NACHT domain can suppress constitutively clustered NLRP3, while MCC950, which tethers several NLRP3 subdomains into inactive conformation, fails to prevent inflammasome activation.

Our results demonstrate that NLRP3 can be activated from diverse subcellular locations and emphasize clustering of NLRP3 at membrane or protein scaffolds as an important step in its activation distinct from the final active NLRP3 oligomer. Diverse NLRP3 triggers can engage different activation pathways, inducing ultrastructural changes in cell architecture, ultimately leading to the scaffold-mediated clustering of NLRP3 and subsequent formation of the NLRP3 inflammasome. In this way NLRP3 senses cellular anomalies exhibited via lipid or protein assemblies, establishing NLRP3 as the master sensor of cellular well-being.

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OVERCOMING T CELL EXHAUSTION BY TARGETING CELL METABOLISM

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CAR (chimeric antigen receptor) T cell therapy (CAR-T) and immune checkpoint inhibitors are now at the forefront of anticancer immunotherapies. Despite their success in several cancer types, there is a need for improved overall response to these treatments. One of the major factors that contribute to the limited success of immunotherapies is the immunosuppressive tumor microenvironment that leads to T cell exhaustion marked by increased inhibitory receptors and decreased effector cytokines. Metabolic reprogramming is now considered to be one of the central features of immune cells that provides unique opportunities for the development of drugs and combinational strategies for improved anticancer immunotherapies. Very recent studies demonstrated that by targeting T cell metabolism it is possible to improve anticancer immune response in immune checkpoint therapies and in CAR-T therapy. We explore mechanisms and strategies that would enable modulation of T cell metabolism for enhanced metabolic fitness and functionality of T lymphocytes.

Invited lecture

IMMUNOSTAINING PROTOCOLS OF THE TEN MOST COMMONLY USED DIAGNOSTIC MARKERS IN CYTOPATHOLOGY LABORATORY ON BENCHMARK ULTRA AND DAKO OMNIS STAINING PLATFORMS: VALIDATION AND IMPLEMENTATION STUDY

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INTRODUCTION: The withdrawal of the iView detection kit used for the BenchMark Ultra immunostainer (Ventana, Roche Diagnostics) forced many cytopathology laboratories, including ours, to substitute immunocytochemical staining (ICC) protocols for routine practice with other detection kits. Our objective was to optimize, validate, and implement ICC staining protocols with OptiView and EnVision FLEX detection kits by comparing the staining results with those of the iView detection kit.
METHODS: Residual cytology samples with known diagnoses were used. Ten most commonly used antibodies in routine cytopathology diagnostics were tested (anti-: calretinin, Ber-EP4, MOC-31, CKAE1/AE3, CK5/6, CD68, LCA, desmin, HBME-1, and WT1). Different staining parameters were tested with OptiView on the BenchMark Ultra (Ventana, Roche Diagnostics) and EnVision on the Dako Omnis immunostainers (Agilent). An optimal staining protocol was selected for each antibody and validated on 10 positive and 10 negative cases. The staining results were compared with the existing iView protocol by evaluating UK-NEQAS and our internal scores.

RESULTS: The optimal OptiView and EnVision protocols differed among the antibodies tested but showed similar or even better results than the existing iView protocol, followed by stronger intensity. Conclusion: We have established and validated optimal ICC staining protocols with the most commonly used antibodies in a cytopathology routine practice that may be useful for other cytopathology laboratories that have the same immunohistochemical staining platforms. However, the challenge of standardizing ICC protocols across different cytopathology laboratories remains unresolved.

Invited lecture

ASSESSMENT OF TUMOR-INFILTRATED LYMPHOCYTES IN TUMOR TISSUE SECTIONS OF PRIMARY HIGH-GRADE SEROUS CARCINOMA WITH EYEBALLING AND AI-DRIVEN IMAGE ANALYSIS: A COMPARISON STUDY

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INTRODUCTION: The assessment of tumor-infiltrating lymphocytes (TILs) as a prognostic variable in high-grade serous ovarian carcinoma (HGSC) has not seen broad adoption due to a lack of standardization. The International TIL Working Group has standardized semiquantitative TIL scoring, but only for breast cancer. In the era of artificial intelligence, digital imaging image platforms have also been widely used to overcome the challenges of TIL assessment. We aimed to compare TIL scores in HGSC achieved with semiquantitative analysis (eyeballing) based on breast cancer guidelines and digital image analysis (DIA).

METHODS: Formalin-fixed paraffin-embedded tumor sections of patients diagnosed with primary HGSC, immunohistochemically stained with CD3, CD4, and CD8 antibodies, were used. Intra-tumoral and stromal TILs were scored separately. For the eyeballing assessment, breast cancer guidelines were adopted, and for DIA, an algorithm using AI-driven precision pathology software VisioPharm was created. In both assessment methods, the result was calculated as a percentage of positively stained intra-tumoral TIL area per intratumoral area and stromal TIL area per stromal area. Assessments from both methods were compared using Bland Altman, Cronbach's α, and Spearman's rank tests.

RESULTS: The Bland-Altman analysis showed a percentage difference in TILs assessed with eyeballing and DIA of 8.4% (HR (95% CI): 1.999, range 0.485-3.505, p=0.010). Moreover, internal consistency between eyeballing and DIA assessed with Cronbach's α was very good (α=0.848). A significantly moderate positive monotonic relationship was also observed with Spearman's rank correlation test ($p<0.001$; rho=0.581).

CONCLUSION: We observed strong agreement between eyeballing and DIA in analyzing TILs in HGSC, suggesting that the DIA could be a reliable alternative for TIL scoring in comparison to eyeballing.

Invited lecture

CAR-T CELL THERAPY

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CAR T cells are genetically modified T lymphocytes that can be used to target and destroy pathological, usually tumour cells. CAR T cells are derived from the patient's own T lymphocytes, which are genetically modified to express CAR receptors (chimeric antigen receptors) on their surface. The synthetic CAR receptors are designed on the basis of a single-chain immunoglobulin molecule to allow the T lymphocytes to recognise specific target molecules on the surface of tumour cells. Unlike TCR receptors, CAR receptors are able to recognise surface antigens independently of major histocompatibility complex (MHC) molecules. As reduced MHC expression is one of the mechanisms of tumour cells to evade the immune system, CAR-T cells are an additional option for cytotoxic immunotherapy of cancer as well as other diseases (infections, autoimmune diseases, posttransplant rejection reactions).

Invited lecture

THE ITOCI STORY: AUTONOMOUS AMELIORATION OF CYTOKINE RELEASE SYNDROME BY CAR-T CELLS

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ABSTRACT: Remarkable clinical results in the treatment of hematologic malignancies[1,2] have recently led to the first FDA-approved CAR-T therapy[3] and opened up unprecedented possibilities for the treatment of complex diseases. Unfortunately, adoptive cancer immunotherapy poses safety risks such as cytokine release syndrome (CRS) and neurological toxicities that can cause life-threatening adverse events[4,5]. Systemic administration of tocilizumab, which blocks the IL-6 signaling axis, is currently used to treat such adverse events. Early detection and administration is necessary to manage CRS, while control of neurological toxicities is difficult as the blood-brain barrier impedes entry of the drug into the central nervous system[5,6]. To address these gaps, we developed and tested an innovative approach in preclinical humanized mouse model in which CAR-T cells were equipped with autonomous antigen-induced release of IL-6 receptor alpha (IL-6Rα) blocking antibody to ameliorate CRS[7]. We will summarize and present this study here.

KEY WORDS: CAR; T-Cells; Adoptive cell therapy; Inducible systems; Activation-dependent expression; Transient expression; Single-Lentiviral expression system; tocilizumab; IL-6 receptor alpha blocking antibody; cytokine release syndrome; CRS

INTRODUCTION: Immunotherapy has shown extraordinary clinical responses in the treatment of various blood cancers[1,2], leading to the first FDA-approved chimeric antigen receptor T cell (CAR-T) therapy, initially for patients with relapsed or refractory acute lymphoblastic leukemia (ALL)[3]. Unfortunately, adoptive cancer immunotherapy carries safety risks such as cytokine release syndrome (CRS) and neurologic toxicities[4], which have led to life-threatening complications[5]. Current management strategies, which include the systemic use of the anti-IL-6 receptor-blocking antibody tocilizumab, need to be administered early to control the adverse effects associated with immunotherapy[8]. The control of neurological toxicities is challenging because the blood-brain barrier (BBB) impedes the entry of tocilizumab into the central nervous system (CNS) and may require invasive intra-thecal administration[5,6]. In this contribution, we present our recently published data on the development of an innovative approach to ameliorate CRS via CAR-T cells themselves[7].

MATERIALS AND METHODS: To address the safety issues associated with cellular immunotherapy for cancer, we engineered CAR-T cells with autonomous tumor-restricted release of tocilizumab-based biological drug. We first developed an innovative, genetically integrated system that combines autonomous antigen-induced production of biological drug along with constitutive CAR expression in a single vector system. To implement our proposed system for limiting toxicities associated with cancer immunotherapy, we have combined clinically utilized CD19-targeting CAR with *in situ* secretion of an IL-6 receptor alpha (IL-6Rα) blocking antibody based on tocilizumab (referred to as iToci) as a means to treat CRS and neurological toxicities by the therapeutic cells themselves. The materials and methods are described in detail in our research paper[7] and briefly summarized here.

The molecular cloning of the designed lentiviral transfer plasmids based on our newly developed Uni-Vect genetic platform was performed using standard molecular biology techniques. For this study, we used the reverse architecture of the Uni-Vect[7]. The lentiviral particles were produced using standard procedures. CAR-T cells were generated from healthy donor T cells that were activated, transduced with lentivirus, and expanded. The biological activity of designed iToci was demonstrated by biochemical and cell-based assays. iToci-CAR-T cells were tested in an *in vivo* study in humanized mice. 6- to 8-week-old female and male SGM3 mice were sublethally irradiated and infused with human CD34+ cells from umbilical cord blood. After hematopoietic reconstitution, the mice were injected intravenously (i.v.) with acute lymphoblastic leukemia cells NALM6 expressing a secreted luciferase and the LNGFR marker gene. Seven days later, the mice received control CD19-targeting CAR-T cells, iToci CD19-targeting CAR-T cells or untransduced T cells by i.v. infusion. Tumor growth was monitored using a bioluminescence assay. Circulating human T cells were analyzed by flow cytometry and counted with flow-count fluorospheres. To assess CRS toxicity, mouse weight loss was monitored daily, and human cytokine levels were analyzed in the peripheral blood. To assess signs of neurotoxicity, the brains of experimental mice were removed at necropsy, fixed, embedded in paraffin, sectioned, and stained. Hematoxylin and eosin-stained paraffin sections were examined blindly and independently by two pathologists for histopathologic analysis.

RESULTS: Systemic administration of the IL-6Rα blocking antibody tocilizumab is the predominant clinical strategy for the control of CRS associated with CAR-T cells targeting CD19[8]. However, control of neurological toxicities is difficult because the BBB impedes entry of tocilizumab into the CNS, necessitating invasive intra-thecal administration in some cases[5,6].

In our recently published paper[7], we have developed and utilized the novel genetic platform called Uni-Vect to upgrade CAR-T cells with the ability to autonomously deliver a tocilizumab-based antibody. Uni-Vect enables antigen-dependent expression of an inducible accessory molecule together with the constitutive expression of an immune receptor such as CAR. We first investigated the functionality of Uni-Vect in the context of hematologic malignancies and found robust performance where coculture with antigen positive cell line led to the activation of inducible module while system remained non-responsive to antigen-negative cell line.

We then designed and developed IL-6Rα blocking antibody based on the heavy and light chains of tocilizumab[9], termed iToci. When iToci was integrated into the Uni-Vect platform and transduced into healthy donor T cells to generate iToci-CAR-T cells targeting CD19, antigen-inducible expression of iToci was observed along with lysis of target cells. To demonstrate the functionality of the developed antibody, binding ability to human IL-6Rα was demonstrated. Next, we repurposed the Uni-Vect system for developing cell-based assays to monitor the IL-6 signaling and utilized this system to evaluate the biological activity of iToci.

Finally, we tested whether iToci-CAR-T cells can ameliorate CRS *in vivo* in a humanized mouse model[10,11]. We first demonstrated that iToci does not prevent control of tumor growth. Further, we show that, unlike control conventional CAR-T cells, iToci-CAR-T cells enable improvement of CRS as evidenced by only transient weight loss and improved survival. Levels of inflammatory cytokines associated with CRS were decreased in the iToci group on day 4 post infusion. We also investigated

whether iToci has the potential to control neurological toxicities and observed evidence of activity, although this needs to be further investigated.
In a repeated experiment the improvement of CRS was tested in a more stringent model where rapid weight loss and higher levels of secreted cytokines were observed. The explanation for such challenging conditions is not clear and may have to do with donor-intrinsic factors of the cord-blood HSCs or CAR-T cells themselves. Even under these difficult conditions, iToci was still effective at providing advantages in terms of survival and decreased release of cytokines, although to a lesser extent than in the first experiment, described above.
Overall, these data show that Uni-Vect can be used for autonomous antigen-triggered secretion of biologically active IL-6Rα blocking antibody by CAR-T cells *in situ* to improve CRS[7].

DISCUSSION: In our recently published study[7], which we summarize in this contribution, we have shown that antigen-inducible secretion of tocilizumab-based antibody from CAR-T cells enables amelioration of CRS *in vivo* in a humanized mouse model. In this study[7], we made the interesting observation that the expansion of iToci-CAR-T cells was lower compared to control CAR-T cells. This could indicate that several factors contribute to the improvement of CRS. There could be several explanations, e.g. that blockade of IL-6 signaling has an impact on overall inflammatory milieu that affects CAR-T cell expansion in a complex fashion or that iToci CAR-T cells have different intrinsic functionalities in addition to the secretion of iToci, although *in vitro* expansion, phenotype and cytotoxic activity *in vitro* and *in vivo* showed no differences compared to control CAR-T cells. We conclude that further studies are needed to better understand the underlying mechanisms of iToci effects. However, experimental evidence indicates that iToci-CAR-T cells have achieved the balance between *in vivo* expansion and effector functions including cytokine secretion to be effective in tumor eradication without deleterious toxicity. We envision that iToci-CAR-T cells could enable autonomous prevention of CRS before it can cause damage and without the need to introduce recombinant tocilizumab.

DECLARATION OF INTERESTS: A.S. is co-inventor on PCT International Patent Applications by The Trustees of the University of Pennsylvania, which incorporate discoveries and inventions described here.

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Invited lecture

AUTOIMMUNE AND AUTOINFLAMMATORY MANIFESTATIONS IN INBORN ERRORS OF IMMUNITY

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The knowledge of human inborn errors of immunity (IEI) is rapidly expanding and currently we are able to classify > 500 monogenic IEI. Diseases across the entire spectrum of IEI can be associated with immune dysregulation leading to various allergic, autoimmune and inflammatory complications. These complications can arise across all age groups and all stages of disease, ranging from presenting complaints to late complications.

Based on the data from the Slovenian Registry of IEI, non-infectious and non-malignant manifestations occur in 29% patients with IEI, including autoimmune manifestations in 22%, lymphoproliferative/granulomatous in 12%, autoinflammatory in 5%, and allergic manifestations in 4% of patients with IEI. Autoimmune and autoinflammatory manifestations preceded the diagnosis of IEI in 80% of patients included in our registry, therefore, physicians treating these conditions should have a low threshold for performing the evaluation for possible underlying IEI. The clinical clues to the identification of patients with IEIs include severe or unusual infections, early or atypical autoimmune disease, positive family history or consanguinity, syndromic features, lymphoproliferation or granulomatous inflammation. On the other hand, patients with known IEIs should be regularly monitored not only for infections but also for other manifestations as they share common genetic factors for autoimmunity, lymphoproliferation, granulomatous inflammation, autoinflammation and even allergy.

In the last decade, a new group of monogenic diseases with combined features of immunodeficiency, autoimmunity, autoinflammation and/or allergy was recognized. The pathogenic mechanisms in these diseases include defects of both innate and adaptive immunity as well as increased risk of infection. Characteristic examples in this group of IEI are cytoskeletal disorders, interferonopathies and relopathies. Phenotypic presentations in these diseases frequently includes combined immune- and nonimmune-mediated organ and tissue damage, such as CNS involvement in interferonopathies.

With the improved diagnostics including upfront application of next-generation sequencing and additional omics technologies, we could expect earlier genetic, molecular and immune characterization of IEI and underlying autoimmune/inflammatory complications. Better knowledge and the multilayer concept of autoimmune mechanisms and manifestations in IEIs could provide also clinical guidance on the use of novel targeted therapeutic approaches.

Invited lecture

ASSESSING B LYMPHOCYTE ACTIVATION AFTER CPG STIMULATION: A DIAGNOSTIC APPROACH FOR PRIMARY IMMUNODEFICIENCIES

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ABSTRACT: Flow cytometric immunophenotyping plays a central role in the diagnostic work-up of patients with suspected primary immunodeficiencies (PID), particularly those involving lymphoid cells. Depending on the genetic defect, a part of the immune system or a cell type may be missing, reduced or not functioning properly. The majority of PID patients (60–65%) have a defect in the lymphoid system that affects B and/or T cells alone or in combination with other cells. An accurate immunophenotypic diagnosis is essential for further functional testing as well as for genetic testing. In the presentation, functional activation test of B cells will be presented and the associated pitfalls addressed. For new and rare PIDs, "diagnosis by research" is essential for conclusive diagnosis. Functional activation test of B cells are crucial for the assessment of the humoral immune system in PID. CpG is a synthetic oligonucleotide that mimics bacterial DNA and activates Toll-like receptor 9 (TLR9) on B cells, leading to activation and proliferation. In vitro tests to assess proliferation potential, differentiation to plasmablasts and immunoglobulin secretion after CpG stimulation reliably show B cell activation. Abnormal activation may indicate potential PID. In summary, measuring the activation of B lymphocytes after stimulation with CpG can be a valuable tool for the diagnosis of PIDs, providing insights into the functionality of the immune system, especially the B cell compartment. In combination with other diagnostic methods, this approach contributes to a more comprehensive understanding of the patient's immune status.

INTRODUCTION: B lymphocytes serve as carriers of humoral immunity, and a defect in any of the molecules that control their function or intercellular interactions can lead to primary immunodeficiency (PID). More than half of PID cases are associated with a defect in antibody production. B lymphocyte deficiencies exhibit significant variability and stem from various genetic deficiencies. These deficiencies can manifest as a partial or complete loss of B lymphocytes, decreased or absent serum immunoglobulin levels, or impaired antibody function[1]. The diagnosis of PID relies on clinical signs and laboratory tests. Clinical immunological laboratory tests play a crucial role in confirming suspected primary immunodeficiency disorders (PID)[2]. Initial assessments typically involve a complete blood cell count, followed by specific immune parameter testing, such as serum immunoglobulin levels and the presence of specific antibodies. Flow cytometry offers the most comprehensive evaluation of the cellular immune system, allowing for differentiation between normal activation and severe deficiencies of lymphocyte subpopulations. General cellular examination with immunophenotyping of B lymphocytes, natural killer (NK) cells, and T lymphocytes, along with activated T lymphocyte analysis, serves as the primary step. However, further differentiation of B lymphocyte, T lymphocyte, and NK lymphocyte subpopulations is often necessary in most cases. Besides immunophenotyping, functional tests also play a vital role in indicating many immunodeficiencies[3]. The proliferative potential of B lymphocytes, differentiation into plasmablasts and immunoglobulin secretion can be reliably assessed by CpG stimulation of B lymphocytes via the T cell-independent (TI) pathway [4]. Early diagnosis is important to initiate appropriate therapy.

Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by defective T lymphocyte development and/or function, along with impaired antibody responses. The latter may result from inadequate activity of T helper cells or inherent defects in B lymphocytes. Accurate assessment of lymphocyte count and differentiation is crucial for diagnosing patients with suspected cellular immunodeficiency. Subsequent laboratory investigations involve immunophenotyping of T lymphocytes and in vitro functional tests to assess T and B lymphocyte proliferation and cytokine production. It is important to compare the values with reference values of the appropriate age group[5].
The aim of our study was to introduce a new functional test into routine. The functional assay of B lymphocyte activation after stimulation with CpG provides information on B lymphocyte proliferation and differentiation into plasmablasts, as well as on immunoglobulin production during B lymphocyte activation via TI pathway, allowing us to assess more precisely the functions of the humoral immune system, whose main promoters are plasmablasts and their precursors.

METHODS: The study included two distinct groups: 25 healthy volunteers and 6 adult patients diagnosed with common variable immunodeficiency (CVID). The healthy cohort included 20 adults aged between 20 and 58 years and 5 children aged 6 to 9 years. The adult participants included 12 women with an average age of 32 years and 7 men with an average age of 31.8 years. The group of children included 2 girls with an average age of 9 years and 3 boys with an average age of 7.3 years. The patient cohort included 4 women and 2 men with an average age of 37 years. The youngest patient was 22 years old, while the oldest was 52 years old.
To establish the optimal experimental conditions for B lymphocyte proliferation, we conducted a series of investigations comparing various parameters. Peripheral blood mononuclear cells (PBMCs) were subjected to different stimulations, with variations in CpG concentrations, stimulation durations, and cell suspension volumes.
For subsequent experiments, including both negative controls and variations of CpG stimulation with or without EdU, cells were incubated for 7 days at 37°C in a CO_2 incubator. EdU (Invitrogen) was added 20 hours before the end of the isolation period, as per the Click-iT EdU Alexa FluorTM 488 Flow Cytometry Assay Kit protocol.
Cell labeling included CD19 APC, CD27 PerCP Cy5.5, and CD38 BV450, along with intracellular staining with EdU AlexaFluor 488. Flow cytometry analysis was conducted using a BD FACSCantoTM II Flow Cytometer equipped with BD FACSDivaTM Software, measuring 100,000 events per sample.
Statistical analysis of the data was performed using GraphPrism, calculating mean, median, standard deviations, and 10th and 90th percentiles. A paired T-test was employed to determine statistically significant differences, with a significance level (α) set at 0.05.

RESULTS: In various studies, researchers have employed diverse stimulation protocols, including variations in stimulation duration, CpG concentrations, and PBMC concentrations. This variability necessitated a comprehensive examination to establish optimal experimental conditions.
To address this, B lymphocyte proliferation was evaluated following stimulation periods of 2, 5, 7, and 10 days with 5 µM CpG. Initial observations after 2 days of stimulation revealed a minimal difference in proliferation between unstimulated and stimulated PBMCs. However, proliferation increased significantly with extended incubation periods. Notably, after 5 days of stimulation, a substantial increase to proliferation was observed, reaching its peak at 7 days. Prolonged stimulation for 10 days resulted in a slight decline proliferation.

Different concentrations of CpG were tested to stimulate B lymphocytes, and proliferation was measured after 7 days of stimulation with 2.5 µM, 5 µM, and 10 µM CpG. The lowest proliferation was measured when stimulated with 2.5 µM CpG. A proliferation plateau was reached when stimulated with 5 µM CpG, and a similar proliferation rate was measured when stimulated with 10 µM CpG.
In our investigation, the impact of different cell suspension volumes was examined by testing three distinct volumes while maintaining consistent cell concentration (1×10^6 cells/ml) and CpG concentration (5 µM A correlation between cell suspension volume and proliferation was observed in our observations, with the highest proliferation being observed for B lymphocytes incubated in 1 ml of cell suspension. Conversely, the lowest proliferation was measured in a 0.25 ml cell suspension.
Normal proliferation values after CpG stimulation were determined on whole B lymphocytes (CD19+), as well as the proliferation of subpopulations of naïve B lymphocytes (CD19+CD27), memory B lymphocytes (CD19+CD27+) and plasmablasts (CD19+CD27+CD38+). The obtained values of proliferations of stimulated and unstimulated cells were statistically evaluated by the paired T-test. Statistically significant proliferation of stimulated B lymphocytes was measured in all B lymphocyte phenotypes, in both children and healthy adults.
We determined normal B lymphocyte proliferation values for children and adults by calculating the median of the measured proliferations and identifying the range of values between the 10th and 90th percentile values.). After stimulation with CpG, the proportion of plasmablasts was also statistically significantly increased.
We aimed to test the hypothesis that B lymphocyte proliferation and immunoglobulin production are decreased in patients with PID. To do this, we enrolled 6 patients diagnosed with CVID. All CVID patients exhibited a reduced percentage of plasmablasts. In four patients, we observed significantly reduced proliferation of B lymphocytes. Additionally, a decreased concentration of IgM was measured in the supernatant following stimulation with CpG.

DISCUSSION: In the study, we analyzed a culture system using CpG DNA with sequential steps for T-cell–independent activation of different subpopulation of human peripheral blood B cells that induces plasmablast differentiation. Differences in the phenotype of B lymphocytes and their function help us to narrow down further research to identify the genetic cause of the disease. Insight into the functional status of B lymphocytes is obtained by measuring proliferation and differentiation into plasmablasts and the amount of immunoglobulins secreted. The proportion of plasmablasts and their proliferation give us information on whether the lymphocytes are capable of final differentiation into plasmablasts. Low proliferation after stimulation with CpG and a low rate of differentiation into plasmablasts, as well as the absence of secreted immunoglobulins, indicate a BCR-related defect[6].
CVID belongs to the family of primary immunodeficiencies in which patients are more susceptible to infections due to hypogammaglobulinemia and impaired antibody response[7]. Most of the underlying genetic causes have been described in receptors and ligands that are required for B cell signaling or interaction between B and T cell interactions, thus interfere with B cell activation and differentiation[8]. The association between mutations in certain genes and the development of CVID is well established in only 2-10% of cases[7]. Whole genome or exome sequencing has revealed new mutations in genes associated with CVID. However, how most of these mutations affect B-cell differentiation and function is still poorly understood. Immunophenotypic characterization of immunodeficient patients is useful to define subcategories, as patients may have different B-cell numbers and lower frequencies of memory B cells and plasma cells with altered isotypes [9].

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Invited lecture

MAST CELL ACTIVATION TEST IN ALLERGY DIAGNOSIS

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BACKGROUND: The mast cell activation test (MAT) is a new cellular test for allergy diagnosis. Testing for serum IgE to recombinant venom component and the basophil activation test (BAT) can improve the diagnosis and monitoring of Hymenoptera venom allergy (HVA). The main limitation of BAT is that it requires fresh blood for testing and that approximately 10% of the patients have basophils that do not respond to IgE/FcεRI-mediated positive control or the allergen. In this group of patients, the result of BAT is uninterpretable. The usefulness of the MAT in diagnosing patients with nonresponding basophils in BAT has not yet been addressed. It should be further assessed if the results of MAT are associated with the severity of the allergic reaction.

METHODS: We recruited 39 *Hymenoptera* venom allergic (HVA) patients, 22 non-sensitized controls, and 37 BAT nonresponding HVA patients. Specific IgE levels for honey bee venom (HBV), yellow jacket venom (YJV) and total IgEs were measured, and BATs were performed. MAT was done using LAD2 mast cells after passive sensitization with participants` plasma, afterwards LAD2 CD63 response to HBV and YJV was measured with flow cytometry.

RESULTS: We first optimized the susceptibility of LAD2 cells to IgE-mediated degranulation in HVA and showed that prestimulation with IL-33 and IL-6 significantly increased the LAD2 cells' responsiveness to allergen stimulation ($P<0.01$). MAT results correlated with BAT results, and patients with severe sting reactions (Mueller grades IV or III) had a median 2-fold higher MAT than the patients with nonsevere sting reactions (Mueller grades II, I or LLR) ($P<0.05$). Further, MAT provided conclusive results in 54.1% (20 of 37) of HVA patients with nonresponding basophils in the BAT.

CONCLUSIONS: The MAT represents a new diagnostic tool for HVA patients with nonresponding basophils. Further, MAT can identify patients at risk of severe sting reactions and thus can help guide recommendations for venom immunotherapy and improve the management of patients with HVA.

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Invited lecture

THE RESULTS OF CEMIPLIMAB TREATMENT IN PATIENTS WITH SKIN CANCER (SCC) IN SLOVENIA

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BACKGROUND: Cemiplimab is a first PD1 inhibitor for treatment of locally advanced cutaneous squamous cell carcinoma (laSCC) and metastatic SCC (mSCC).
Objectives: The aim of this retrospective cohort study is to provide valuable information on the efficacy and safety of cemiplimab in routine clinical practice in Slovenia.

METHODS: In this retrospective cohort study we analyzed medical records of patients with laSCC and mSCC inappropriate for surgery or radiotherapy treated with cemiplimab in first line between May 2020 and November 2022. Baseline characteristics, treatment outcomes and immune-related adverse events (irAEs) were systematically evaluated to determine the actual efficacy of cemiplimab in this group of patients.

RESULT: 28 patients were included in the analysis, 24 patients with laSCC and 4 with mSCC, with a mean age of 76.1 years. 18% of patients had concomitant autoimmune diseases. Median progression-free survival was 4.4 months (95% CI: 1.5-7.3) Median overall survival was 7.3 months (95% CI: 4.6-9.9 months), indicating a modest improvement in survival in this challenging patient population. Of the 27 patients with evaluable responses, 3 (11%) achieved a complete response and 5 (19%) achieved a partial response. In addition, 6 patients (22%) remained stable and 13 patients (48%) experienced disease progression, indicating the heterogeneity of observed treatment responses. The overall response rate (ORR) of 30% and the disease control rate (DCR) of 52% reflect the moderate efficacy of cemiplimab in this real-world setting. It is important to note that a quarter of patients experienced an irAEs, although most were mild to moderate in severity. Importantly, the response rate was significantly higher in patients who experienced irAEs with an effective response rate of 43% and a distributed immune response rate of 71%, highlighting the complex interplay between treatment efficacy and immune modulation.

CONCLUSION: Cemiplimab has shown meaningful efficacy with DCR of 52 % in patients with laSCC and mSCC in Slovenia. Patients developing irAE had higher response rate.

Invited lecture

COMBINATION OF ELECTROCHEMOTHERAPY AND IL-12 GENE ELECTROTRANSFER IN VETERINARY MEDICINE

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The combination of electrochemotherapy (ECT) with gene electrotransfer of interleukin-12 (IL-12) presents a promising approach in veterinary oncology for the treatment of tumors. ECT uses electrical impulses to facilitate the uptake of chemotherapeutic drugs, enhancing their effectiveness. Meanwhile, IL-12 is a potent immunostimulatory cytokine that promotes the anti-tumor immune response. This protein stimulates immune cells such as T cells and natural killer cells to attack and destroy tumor cells more effectively. In addition, the release of IL-12 in the tumor microenvironment enhances the anti-tumor immune response. Several clinical studies have been published using the combined treatment of electrochemotherapy (ECT) and interleukin-12 (IL-12) gene electrotransfer (GET) to treat various histological types of spontaneous tumors. Although the findings of these studies show that the treatment is safe and effective, different treatment conditions were used for IL-12 GET (intratumoral and peritumoral application). Therefore, the aim of this study was to compare the antitumor efficacy of two IL-12 GET administration routes in combination with ECT and their contribution to the enhanced ECT response. We enrolled 77 dogs with spontaneous mast cell tumors (MCTs) treated with three different modalities: ECT + GET peri. t. (29 dogs), ECT + GET i.t. (30 dogs) and ECT alone (18 dogs). In addition, immunological aspects of the treatment were observed with respect to the immune status of tumors sampled prior to treatment and immune cell subpopulations of peripheral blood mononuclear cells (PBMCs) before and after treatment. The results of this study showed that ECT + GET i.t. was significantly better than the other groups in terms of local tumor control, disease-free interval and progression-free survival. This was confirmed by immunological tests, in which an increased percentage of antitumor immune cells in the blood was observed after treatment in the ECT + GET i.t. group, but without any unwanted severe or long-lasting side effects. We concluded that considering the treatment modality of IL-12 GET, we opt for the intratumoral application of IL-12 GET as a treatment method.

Invited lecture

CIRCULATING TUMOR CELLS IN BREAST CANCER PATIENTS

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Circulating tumor cells (CTCs) have become an important biomarker in breast cancer as they can provide critical information about disease progression and response to therapy. They represent an intermediate part of the metastatic cascade, therefore monitoring CTC levels in the blood has exceptional implications for the management of cancer patients. Due to CTC heterogeneity, different isolation techniques from whole blood samples were established. The aim of our study was to evaluate two isolation techniques, physical and biological method in metastatic breast cancer, on cell lines and in patients and to determine the prognostic significance of CTCs in our patient cohort. Both isolation techniques retained the viability and antigenic characteristics of MCF7 breast cancer cells. Some signs of degeneration were observed. In metastatic breast cancer patient cohort, morphological features of isolated CTCs were dependent on the separation technique. After physical isolation, CTCs with preserved cell morphology were detected. After biological isolation the majority of the isolated CTCs were severely degenerated and their identity was difficult to confirm by a cytopathological examination. Therefore, physical isolation technique was used for clinical evaluation in metastatic breast cancer patients. In total, 59 patients (median age 60.4 years) were included in the study. The numbers of CTCs and CTC clusters were evaluated as well as the presence of megakaryocytes and immune-inflammatory blood cells. The results were correlated with the overall survival (OS). The results showed that at least one CTC was present in 79.7% and more than 5 CTCs in 35.2% of the patients. CTC clusters were present in patients with more than 5 CTCs only (in 19.2% of them), and megakaryocytes were present in 52% of all patients. The presence of CTC clusters and megakaryocytes was positively associated with the CTC count. Patients with low pan-inflammatory value (PIV), low systemic immune-inflammatory index (SII), and low relative change from baseline (ΔPIV%, ΔSII%) were associated with significantly higher OS than their counterparts. In conclusion, the physical method of isolation is suitable for its use in clinical studies evaluating CTCs in breast cancer by cytopathological examination. In addition, CTC count in metastatic breast cancer tends to be prognostic in the case of low ΔSII% and low ΔPIV% values. These findings indicate a possibly important involvement of megakaryocytes in the metastatic process.

Invited lecture

ASSESSING IMMUNE CHECKPOINT INHIBITOR RESPONSE IN METASTATIC MELANOMA: A COMPREHENSIVE STUDY OF THE TUMOR MICROENVIRONMENT BIOMARKERS – A CLINICAL TRIAL PROTOCOL

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ABSTRACT: Immunotherapy has been successful in treating advanced melanoma, but a large proportion of patients do not respond to the treatment with immune checkpoint inhibitors (ICIs). The tumor microenvironment consists of cancer cells, stromal tissue, and extracellular matrix. The immune system is an important determinant of the tumor microenvironment, as the complex interplay between cancer cells and the host immune response is ongoing process. Tumor microenvironment (TME) characteristics are potential biomarkers of response to ICIs in metastatic melanoma. The methodology for profiling the TME has expanded with the use of multiplex tissue imaging technologies to profile the immune cell population more comprehensively in patients' biopsies. The use of multiplex immunofluorescence staining and quantitative pathology has enabled the assessment of multiple cell types and phenotypic markers (PD-L1) to generate a broader overview of the TME beyond T-cells alone.
The specific composition and biodiversity of the gastrointestinal microbiome, exosomal mRNA expression of PD-L1 and IFNγ, CTCs and ctDNA and dynamics predict the response to immunotherapy treatment with immune checkpoint inhibitors and could serve as prognostic and predictive markers in melanoma patients.
There is a need to implement biological markers into clinical practice to improve personalized treatment and predict treatment response, and the absence of clinically validated predictive biomarkers is one of the biggest causes of the unpredictable effect of ICIs.

KEYWORDS: immune checkpoint inhibitors (ICIs), metastatic melanoma, immune related adverse events (irAE), tumor microenvironment (TME), gastrointestinal microbiome, mRNA expression of PD-L1 and IFNγ

INTRODUCTION: Melanoma is a dangerous form of skin cancer, accounting for approximately 5% of all skin cancers and responsible for more than 90% of skin cancer deaths.[1-3] Although survival is improving, the prognosis depends on the stage at diagnosis.[4-7] In the last decade, immunotherapy has made the greatest progress in the treatment of advanced melanoma. Median overall survival has increased significantly, giving hope to many patients. However, only up to 50% of patients respond to immunotherapy treatment.[8] Immunotherapy with PD-1/PD-L1 and CTLA-4 immune checkpoint inhibitors (ICIs) in the treatment of patients with advanced unresectable and disseminated malignant melanoma has greatly improved prognosis and survival, with life expectancy increasing from less than 12 months to more than 40 months.[9] ICIs are monoclonal antibodies that, by binding to PD-L1/PD-1 (programmed death-ligand 1/programmed death 1) and CTLA-4 (cytotoxic T-lymphocyte-associate protein 4), enable immune cells to they act against tumors.[10] PD-1 is a transmembrane receptor on various cells of the immune system (T and B lymphocytes, macrophages, natural killer cells and dendritic cells). Transmembrane glycoprotein PD-L1 is found on immune cells such as macrophages, some activated T and B lymphocytes, dendritic and epithelial cells, and is also present on tumor cells, where it enables so-called "immune escape". Binding of PD-1 to PD-L1 initiates a negative regulatory loop of T lymphocytes and other immune cells, thereby enabling cancer cell proliferation. According to recent research, PD-L1 can also independently activate proliferative tumor pathways (PI3K/AKT, MAPK, JAK/STAT, Hedgehog and others).[11] CTLA-4 appears as a protein receptor in regulatory T

lymphocytes and, by binding to the B7 protein on antigen-presenting cells, initiates the inhibition of the immune response.[12] Immunotherapeutic agents from the group of PD-1 inhibitors (nivolumab, pembrolizumab), a combination of two-level immunotherapy of PD-1 and CTLA-4 inhibitors (nivolumab and ipilimumab) and BRAF (vemurafenib, dabrafenib) and MEK (cobimetinib, trametinib) signaling pathway inhibitors in case of BRAF V600 mutation are used as first-line treatment for metastatic melanoma according to ASCO and NCCN guidelines.[13] Unlike BRAF and MEK inhibitors, which inhibit signaling through the RAS – RAF – MEK signaling pathway, the action of ICIs immunotherapies is much more complex and therefore more unpredictable. About 40% to 60% of patients respond to immunotherapy and long-term responses are achieved. However, more than two-thirds of patients develop immune-related adverse events (irAEs) when treated with ICIs.[14] In approximately 10% of patients, the response to treatment with ICIs manifests as pseudoprogression with a discrepancy between the clinical and radiological response and the actual response due to a transient strong inflammatory response. Up to 30% of patients respond to treatment with ICIs with hyperprogression, where the cancer progresses rapidly for unknown reasons. More than a third of patients have irAEs, but regardless of interruption or discontinuation of treatment, patients show a better response and longer survival without disease progression. The irAEs themselves can be of varying degrees, sometimes irreversible, which indicates overtreatment especially in patients with a complete response.[14]

There is **A NEED TO IMPLEMENT BIOLOGICAL MARKERS INTO CLINICAL PRACTICE** to improve personalized treatment and predict treatment response, and the absence of clinically validated predictive biomarkers is one of the biggest causes of the unpredictable effect of ICIs.

The tumor microenvironment consists of cancer cells, stromal tissue, and extracellular matrix. The immune system is an important determinant of the tumor microenvironment, as the complex interplay between cancer cells and the host immune response is ongoing process. Tumor microenvironment (TME) characteristics are potential biomarkers of response to ICIs in metastatic melanoma. The methodology for profiling the TME has expanded with the use of multiplex tissue imaging technologies to profile the immune cell population more comprehensively in patients' biopsies. The use of multiplex immunofluorescence staining and quantitative pathology has enabled the assessment of multiple cell types and phenotypic markers (PD-L1) to generate a broader overview of the TME beyond T-cells alone.

The specific composition and biodiversity of the gastrointestinal microbiome, exosomal mRNA expression of PD-L1 and IFNγ, CTCs and ctDNA and dynamics predict the response to immunotherapy treatment with immune checkpoint inhibitors and could serve as prognostic and predictive markers.

The *human microbiome* is the genetic makeup of all microbes in the human organism, including bacteria, viruses, fungi, and protozoa. It consists of approximately 100 billion microorganisms that encode more than three million genes and thousands of metabolites.[15] The microbiome influences metabolic homeostasis, metabolism, neurological development, and also plays an important role in the immune response.[16,17] An imbalance between the microbiome and its host is believed to influence the occurrence of many diseases, such as cancer and autoimmune, metabolic and infectious diseases. The composition of the microbiome is influenced by environmental factors during and after birth, during childhood and adulthood, it also changes with diet and nutrition and drugs consumation.[18-23]

The *PD-1/PD-L1 signaling pathway* is an important mechanism for immune retreat and tumor progression. PD-L1 is a promising predictive biomarker for predicting response to immunotherapy treatment. Recent research has shown that patients with overexpressed PD-L1 in their tumor tissue benefit more from treatment with programmed cell death protein 1 (PD-1) and PD-L1 inhibitors.[24,25] Programmed cell death receptor 1 (PD-L1) on the surface of tumor cells inhibits the antitumor activity of T lymphocytes by binding to their PD-1 receptor and causes immunosuppression.[26,27] An alternative to membrane-bound PD-L1 is exosomal PD-L1 (ExoPD-L1), secreted from tumor cells, whose expression correlates with membrane-bound PD-L1 on the surface of the parental tumor cell.[26,27] The

prognostic role of PD-L1 is controversial, as approximately 10% of patients who do not express PD-L1 in tumor tissue also respond to immunotherapy. The reason is probably in the immune microenvironment of the tumor, where PD-L1 is also expressed on immune cells (on helper T cells - CD4+, killer T cells - CD8+, regulatory T lymphocytes - Treg, B lymphocytes, killer cells - CD56+ and monocytes - macrophage cells) and contributes to antitumor immunity. PD-L1 expression is dynamic and variable with patient and tumor heterogeneity and may depend on several factors, including prior therapies and the presence of tumor-infiltrating immune cells. Expression can vary from the tumor margin to the core, between the primary tumor and metastases, and changes dynamically during disease progression.[13] Consequently, a biopsy of tumor tissue at a given time is not representative enough to determine the PD-L1 status of a tumor. Given the lack of PD-L1 detection by conventional biopsy and IHC, research focuses on circulating PD-L1 expression in serum, plasma, circulating tumor cells, and exosomes, which are minimally invasive and allow real-time detection to more accurately represent heterogeneous PD-L1 expression.[25,28] The gut microbiome and its interrelationship with the tumor microenvironment, up- and down-regulation of the beneficial immune response could be a potential predictive biomarker for the efficacy of immunotherapy. Data showed that beneficial gut microbiota in responders increased CD cell activation and expression, promoting CD cell activation in contrast to non-responders with unfavorable gut microbiota and higher frequency of Treg cells.[29]

Interferon gamma (IFNγ) is a cytokine with antitumor and immunomodulatory activity.[30] It is formed by cells of the immune system - cytotoxic T lymphocytes (CD8+TLy), helper T cells (CD4+TLy) and natural killer cells (NK cells).[31] IFNγ acts by activating the JAK/STAT signaling pathway, which modulates the transcription of several genes.[32] Some of the end products of these genes inhibit the growth of tumor cells, while others contribute to it. The antitumor activity of IFNγ is manifested by the activation of the antigen-presenting cell, the arrest of the cell cycle in the G1 phase, the stimulation of cell ischemia and apoptosis. Antitumor activity is caused by suppression of T cells and inhibition of NK cell activity, stimulation of programmed cell death ligand 1 (PDL1) expression on tumor cells, and stimulation of angiogenesis and tumorigenesis.[30] More recent research has shown that IFNγ reflects the state of response to immunotherapy. Higher pre-treatment IFNγ concentrations are associated with a better response to treatment.[32-34] Reduced expression of genes encoding the synthesis of IFNγ itself or its receptor, as well as genes that are part of the JAK/STAT pathway, results in its reduced activity and, consequently, resistance to treatment with immune checkpoint inhibitors.[35-39] Some recent studies have also shown that the gut microbiome may modulate the IFNγ response and play a role in opportunistic infections and autoimmune diseases.[40,41]

Circulating tumor cells (CTCs) are cells that shed from a primary tumor and travel through the bloodstream, forming new metastasis. CTCs can be assessed at any point during the disease course and can serve as a 'liquid biopsy', particularly when a standard biopsy cannot be undertaken due to the inaccessibility of the tumor, or when multiple metastases are present in a patient.[42] Metastatic melanoma was the first malignancy in which CTCs were detected, when in 1991 Smith et al. reported the presence of melanoma cells in the peripheral blood of patients with metastatic cutaneous melanoma by identifying melanoma CTCs through mRNA transcript detection of specific markers.[43] Today CTCs are considered as a biomarker of a great potential in identifying, staging, and monitoring disease treatment, and play a crucial role in determining prognosis and predicting disease-free survival. As metastatic precursors, CTCs might provide valuable information about the tumor, facilitating metabolomic, proteomic, genomic, and transcriptomic studies in melanomas.[44-53]

Circulating tumor DNA (ctDNA) is a fraction of cell-free DNA shed into the bloodstream by cancer cells undergoing apoptosis or necrosis.[54] In the context of cancer, the presence of ctDNA fragments

containing specific mutations can be used for diagnosis, as a 'liquid biopsy' and for dynamic assessment of tumour burden and response to treatment by measuring the number of copies of ctDNA fragments. ctDNA carries genetic information specific to tumor cells, including somatic mutations, copy number variations, and other alterations found in the primary tumor.[55] The analysis of ctDNA can also be used to detect the molecular residual disease (MRD), identify known mechanisms of de novo and identify acquired resistance to therapy months in advance of imaging.[56]

RATIONALE FOR THE STUDY: Severe immune-related side effects can occur during treatment, and some patients do not benefit or receive minimal benefit from immunotherapy due to primary or acquired resistance.[8] There is a need to implement biological markers into clinical practice to improve personalized treatment and predict treatment response.[7] The absence of clinically validated predictive biomarkers is one of the biggest causes of the unpredictable effect of immunotherapy.[9]
The specific composition and biodiversity of the gastrointestinal microbiome, exosomal mRNA expression of PD-L1 and IFNγ, CTCs and ctDNA and dynamics predict the response to immunotherapy treatment with immune checkpoint inhibitors and could serve as prognostic and predictive markers.
The studies published so far have been carried out on animal models or a small number of subjects or samples. We attribute the conflicting results of the studies conducted so far to this. The importance of intestinal microbiome in health or pathology and the influence and connection of inflammation and immune cells is a developing medical topic. It is for this reason that many studies are based on small cohorts and a small number of samples, especially in the field of oncology. Our study is pioneering precisely in this respect, as it is composed thoroughly, with a clear hypothesis, on a large cohort and number of samples. Samples will be taken at three different treatment points and at any event such as hyperprogression, pseudoprogression or immune-related side events. We will study the dynamics of changes in the diversity of the GIT microbiome and the systemic immunological response during the treatment of metastatic melanoma with immunotherapy.
The study ***is first prospective study*** that simultaneously determines the expression of IFNγ and PD-L1 in tumor tissue and blood at different time points. Additionally, the number of CTCs and ctDNA will be evaluated at different time points. If we demonstrate that there is a correlation in the expression of IFNγ and PD-L1 in the tumor tissue and the blood, IFNγ could be obtained from the patient's peripheral blood by a minimally invasive method. Also, correlation between different biomarkers (CTCs, ctDNA and mRNA) from patient's blood and the treatment response could elucidate any of the chosen markers as a predictive marker for the successfulness of the therapy.

THE STUDY OBJECTIVES

Primary Objectives

- To determine the relationship between the predominant composition of the human gastrointestinal microbiome and the objective response to treatment with ICIs in patients with advanced malignant melanoma.
- To determine whether the dynamics of exosomal mRNA expression for PD-L1 and PD-L1 on the surface of immune cells is related to the response to immunotherapy treatment and has predictive value.
- To determine whether the exosomal mRNA expression for IFNγ is related to the response to immunotherapy treatment and has a predictive value.
- To determine the level of CTCs and ctDNA in patient's blood in different time points during treatment and the correlation with the treatment response and has predictive value

Secondary Objectives

- To determine whether a specific composition of the microbiome is associated with the occurrence of immune-related side effects in treatment with ICIS.

- To determine the effects of ICI on the gut microbiota over different time points

Exploratory Objectives

- To determine if there is a relationship between a certain composition of the microbiome and the cell population (CD3+, CD4+, CD8+, the ratio between CD4+ and CD8+, macrophages) in the peripheral blood.
- To determine whether patients who develop immune-related side effects when treated with immunotherapy have a better response to it and longer survival without disease progression compared to patients who do not develop immune-related side effects.
- To determine whether there are differences in the composition of the GIT microbiome in advanced melanoma patients treated in first line with ICIs in the region (Slovenia, Serbia, Croatia)

EXPECTED RESULTS: We expect to define the specific changes in GIT microbiome (bacteria and virome), expression of exosomal miRNA for PD-L1 and IFNγ, as potential biomarkers for clinical use in patients with advances and metastatic melanoma treated with immune checkpoint inhibitors. The expected results are based on the following hypotesis on which this project is based:

1. There is a difference in the diversity of the GIT microbiome (bacterial genera and source) between patients with and without response to ICIs treatment at baseline, 12 (+/- 2) and 28 (+/- 4) weeks.
2. Patients with response to treatment with ICIs have more frequent bacterial species from the genera Faecalibacterium, Akkermansia, Ruminococcus and Roseburia at baseline, 12 (+/- 2) and 28 (+/- 4) weeks than patients without response.
3. In patients with a response to ICIs treatment, the expression of CD4+ and CD8+ cells before the start of ICIs treatment, in the 12th (+/- 2) and 28th (+/- 4) weeks is higher than in patients without a response.
4. Expression of exosomal miRNA for PD-L1, PD-L1 from tumor tissue and PD-L1 on the surface of immune cells before treatment is associated with response to ICIs.
5. Expression dynamics of exosomal miRNA for PD-L1 and PD-L1 on the surface of immune cells is related to the response to ICIs and has predictive significance.
6. Expression of PD-L1 from tumor tissue, from immune cells and exosomal miRNA in metastatic patients treated with ICIs correlates with each other.
7. The concentration of IFNγ in the blood before the start of treatment is related to the response to ICIs.
8. The expression of IFNγ is related to the response to ICIs and has a predictive value.
9. Expression of IFNγ from tumor and exosomes correlates with each other in metastatic melanoma patients treated with ICIs.
10. In non-responders or patients developing resistance to ICI, the levels of CTC and ctDNA will increase.
11. Patients who develop immune-related side effects when treated with immunotherapy have a better response to it and longer survival without disease progression compared to patients who do not develop immune-related side effects.
12. There is no significant differences in the GIT microbiome composition between patients with advanced melanoma treated with ICIs in first line setting from different countries in the region

STATISTICS: The gastrointestinal microbiome, PD-L1 and IFNγ expression will be correlated with one year of PFS as the primary outcome of the study. An interim analysis will be made after the first 20 included patients.

We will analyze the 16S rRNA sequences with the appropriate software tools from the Mothur software package, and we will perform analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA). For the statistical analysis of the differences in the representation of the sequences of individual bacterial groups in the studied microbiomes, we will use the DeSeq 2 package from the R programming environment. The association between phylogenetic diversity, taxonomic

units, immune cells and the objective response to treatment will be determined using the Wilcoxon test. To compare values at different time points, we will use the paired *t*-test or corresponding non-parametric alternative. When comparing several groups at the same time, we will use the Kruskal–Wallis test. All differences will be considered statistically significant when $p < 0.05$.

The Kaplan–Meier method will be used to calculate survival (PFS), and the survival comparison of multiple groups will be calculated using the log-rank test. The association between the change in PD-L1 or IFNγ expression and response to treatment will be assessed using a logistic regression model and a multivariate model in which different variables will be included. Pearson's or Spearman's correlation test will be used to correlate changes in PD-L1 or IFNγ expression levels in tumor and blood.

CONCLUSION: Severe immune-related side effects can occur during metastatic melanoma patients ICIs treatment, and some patients do not benefit or receive minimal benefit from immunotherapy due to primary or acquired resistance. There is a need to implement biological markers into clinical practice to improve personalized treatment and predict treatment response. The absence of clinically validated predictive biomarkers is one of the biggest causes of the unpredictable effect of immunotherapy.

The molecular characteristics of the primary melanoma and metastases, the tumor microenviroment (TME) and the specific composition and biodiversity of the gastrointestinal microbiome, exosomal mRNA expression of PD-L1 and IFNγ, CTCs and ctDNA and dynamics predict the response to immunotherapy treatment with immune checkpoint inhibitors and could serve as prognostic and predictive markers.

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Invited lecture

NAVIGATING COMPLEMENT-LINKED KIDNEY DISORDERS: A CUSTOM GENETIC TESTING PERSPECTIVE

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BACKGROUND: Complement system is involved in the pathology of many diseases, including kidney disease. Evidence from genomics, animal models of kidney disease, and treatment with anti-complement drugs have confirmed a strong association of the complement cascade with kidney diseases such as immune complex-mediated glomerulonephritis, aHUS, C3G, ANCA vasculitis, renal ischemia-reperfusion injury, and rejection after kidney transplantation. Part of the factors influencing the occurrence and development of kidney diseases is also represented by genetic variants in complement system genes.

METHODS: Our study group consists of 21 patients (11 women and 10 men) with various kidney diseases associated with the complement system. We have designed a custom AmpliSeq Custom DNA Panel (Illumina) comprising 396 amplicons covering 16 different genes (FH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, CD46, FI, FB, C3, DGKE, THBD, PLG, VTN, MASP2, and CD36). Genomic DNA will be sequenced using the Illumina platform.

RESULTS: Variations were found in all tested patients; namely SNVs, insertions and deletions. A likely pathogenic variant in CFI (missense variant rs772044176; T/C) and a susceptibility variant in PLG (missense variant rs4252128; C/T) were detected in two separate patients. Multiple patients are carriers of age-related macular degeneration risk factor variation in C3 (missense variant rs2230199; G/C).
Conclusion: Our goal is to identify and evaluate the genetic basis of the complement system in kidney disease patients. A major challenge is the functional evaluation of observed genetic variants. Nevertheless, genetic testing has its place in modern medicine because it is used for diagnosis, presymptomatic assessment of the probability of disease development, pharmacogenetic treatment planning, and allows better information in family planning.

CAR-T IMMUNOTHERAPY FOR CANCER: OPPORTUNITIES FOR IMPROVEMENT OF MANUFACTURING AND FUNCTIONALITY

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Chimeric antigen receptors (CARs) are synthetic receptors comprised of the extracellular antigen recognition antibody-derived single chain variable fragment (scFv) and the intracellular T cell signaling domains. CARs endow T cells with the designed specificity and function. Since 2017, CAR-T cell immunotherapy is an approved cancer treatment approach for patients with certain types of leukemia and lymphoma. Here, we present the general principles of CAR-T cell therapy, the CAR structure, the production of the cellular product, and some of the major challenges in existing therapies, including the limited persistence of CAR-T cells and the lack of tumor-specific targets. We aim to produce safer and more effective mouse CAR-T cells by improving the individual design steps. One such improvement is developing CAR-T cells with the knock-out (KO) of the endogenous T-cell receptor (TCR). TCR KO prior to the introduction of the CAR molecule to the T cells reduces alloreactivity and contributes to the production of a more homogenous, and therefore safer, cellular product. We tested the knock-out of the TCR with the CRISPR/Cas9 system and designed sgRNAs targeting TCR α constant (TRAC) locus in the Jurkat cell line and primary mouse $CD4^+$ and $CD8^+$ T cells. Flow cytometry analysis showed that the KO efficiency was high in both Jurkat cell line (up to 90 %) and mouse primary T cells (up to 60 %). Therefore, in addition to upgrading CAR-T cell functions, manufacturing of the cellular product represents an opportunity to make CAR-T cells safer, more effective, and broadly available. The advantage and rationale for focusing on mouse CAR-T cells is the ability to comprehensively access mechanisms of action in the presence of an interacting immune system in future preclinical *in vivo* studies.

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TARGETING CELL MEMBRANE PROTEINS WITH ANTIBODIES

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BACKGROUND: Production of antibodies against membrane proteins still represents a challenge. Isolation and handling of membrane proteins are difficult and often result in alterations in the target proteins' three- dimensional structure compared to the native form expressed on live cells. Consequently, many antibodies that bind to membrane proteins *in vivo* can be missed during the hybridoma primary screening process with conventional immunological tests like ELISA. To test large numbers of antibodies on live cells, we developed a high-throughput screening method for testing hybridoma that produces antibodies targeting membrane proteins, called cell ELISA.

METHODS: Cell ELISA is conducted in 96-well round bottom plates and consists of two immunostaining and washing steps and a detection step. Firstly, seeded cells are incubated with hybridoma cell culture supernatants. After washing, cells are incubated with horseradish peroxidase-conjugated secondary antibodies and washed again. In the detection step, TMB substrate is added, and the colorimetric reaction is measured using a microplate reader. To validate the method, we performed a conventional ELISA test with peptide antigens that were used for immunization.

RESULTS: Primarily, we designed our assay on RPMI-8226 and Raji cell lines, of which RPMI-8226 (target cell line) expresses the antigen of interest, and Raji (control cell line) does not. Additionally, we tested the selected clones on Jurkat, Pfeiffer, and U-266 cell lines to confirm the specificity. We tested 336 different hybridoma supernatants using conventional and cell ELISA. Using conventional ELISA, we detected 31 clones against peptide antigens and using cell ELISA we detected 6 antibodies against the target membrane protein expressed on cells. Only three of the antibodies were detected in both test systems. Most clones producing antibodies binding to the native target protein can be identified using cell ELISA but are missed with conventional ELISA, which suggests the superiority of cell ELISA.

CONCLUSION: Using cell ELISA, we can easily recognize the antibodies that bind to membrane proteins *in vivo* in the primary screening process. It is a suitable, high-throughput, inexpensive, and easy-to-perform method for screening hybridoma that produces antibodies targeting membrane proteins.

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EXTRACELLULAR VESICLES AS KEY PLAYERS IN PROGRESSION OF HEMATOLOGIC MALIGNANCIES AND IMMUNOTHERAPY SUCCESS

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Extracellular vesicles (EVs) are small membrane-derived particles that are released by cells into the extracellular space and can be transported throughout the body. These vesicles play an important role in cell-to-cell communication and transport a variety of biological molecules from their cell of origin to target cells. EVs are involved in many physiological and pathological processes, for example, they can mediate immune responses, facilitate blood clotting, and contribute to the spread of cancer. In hematologic malignancies, understanding the role of EVs on cancer progression and immunotherapy success is particularly important due to elevated exposure of immune cells to EVs in the bloodstream. The aim of this study is to optimize and standardize procedures for isolation of lymphocyte EVs from patients with hematologic malignancies and to determine the potential inhibitory effect of these vesicles on immune cell effector function. EVs were isolated from blood of healthy donors and patients with hematologic malignancies with high-speed centrifugation and purified with tangential flow filtration. They were stained with various combinations of reagents and fluorescently labelled antibodies and preliminary measurements were obtained with a BD FACS Canto I cytometer. The origin and subsets of the vesicles were determined by measuring parent cell markers (CD19, CD3, CD4, CD8). Among reagents tested for staining EVs, carboxyfluorescein succinimidyl ester (CFSE) or CellMask Deep Red™ were shown to effectively stain the majority of the isolated lymphocyte vesicles. To determine the exhaustion and activation signaling of the EVs on immune cell effector function, potential markers of exhaustion and activation are currently being tested. The EVs hold great potential in predicting and monitoring the inhibitory effect and immune system anti-tumor response. However, standardization of handling procedures of EVs and identifying more precise vesicle biomarkers is needed. With further knowledge and optimized procedures in this field, a better understanding of cancer progression and the mechanisms of immunotherapy inhibition could be achieved.

FINDING REGULATORY T CELLS

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BACKGROUND AND PURPOSE OF THE STUDY: The immune system consists of a variety of immune cells, each of which plays a specific role in
mediating immune responses. Regulatory immune cells are critical for maintaining homeostasis and self-tolerance as they suppress the immune response after a threat has been eliminated. In autoimmune diseases, this process can be deregulated, and the immune system becomes overactivated. Patients with autoimmune diseases are usually treated with general immunosuppressants, a therapy with severe side effects that can make the patient susceptible to opportunistic infections and the development of malignancies. Cellular immunotherapy with modified regulatory T cells (Tregs) represents a step towards an alternative therapeutic option that restores immune homeostasis and induces regeneration of damaged tissue.
Tregs are a rare subset of CD4+ T cells characterized by a CD4+ CD25+ FoxP3+ phenotype. Optimization of the isolation and activation of Tregs for therapeutic purposes is required, especially in relevant animal models. The presented work will address the optimization of activation, expansion, and phenotypic purity of *ex-vivo* Treg.

METHODS: Mouse spleens and lymph nodes were harvested from female C57BL/6 mice. Treg population was isolated from the total cells by magnetic separation. The cells were activated, expanded in the presence of IL-2 for 14 days and monitored. Parameters such as the concentration of the initial cells, the concentration of IL-2 and the type of activation were altered, and their effect evaluated. The number of viable cells was determined after trypan staining and subsequent counting with an automated cell counter. Cell samples were stained for viability, relevant surface and intracellular markers and the immunophenotype was assessed by flow cytometry.

RESULTS: The aim of this work was to determine the conditions that result in a high number of viable cells, a high frequency of Treg cells and the most pronounced FoxP3+ CD25+ Treg phenotype.
Cell expansion peaked between day 4 and 8 and flow cytometry indicated successful Treg enrichment. The efficiency of Treg enrichment varied between the different conditions.

CONCLUSIONS We have successfully isolated an enriched Treg population and determined the conditions that yield the best result. We found that activity needs to be determined for individual lot numbers of mIL-2. Our results suggest using the concentration at the highest end of the manufacturer's recommendation.

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THE PROGNOSTIC SIGNIFICANCE OF PD-1 AND PD-L1 ON LYMPHOMA CELLS AND TUMOR-IMMUNE CELLS IN DIFFUSE LARGE B-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

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BACKGROUND: Diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) is the most common type non-Hodgkin's lymphoma, where the treatment of relapsed/refractory cases is the major challenge. Programmed cell death protein 1 (PD-1) and its ligand PD-L1 play a crucial role in the negative regulation of the immune response against the disease. Our aim was to analyze the expression of PD-1 and PD-L1 on lymphoma cells (LCs) and tumor-immune cells (TICs) and to investigate their correlation with outcome.

METHODS: Samples from 283 patients diagnosed with DLBCL, NOS (both germinal center B cell-like [GCB] and non-GCB subtypes) were included in the study. Expression of PD-1 and PD-L1 was determined using double immunohistochemical staining (D-IHC) for PD-1/PAX5 and PD-L1/PAX5 on tissue microarrays. LCs were highlighted by D-IHC to obtain more accurate results. Clinical data and histologic diagnoses were obtained from electronic data records. We correlated clinical characteristics, and PD-1 and PD-L1 expression on LCs and TICs with progression-free survival (PFS) and overall survival (OS).

METHODS: Expression of PD-1 on TICs was observed in 38.4% and on LCs in 8.8% of cases, while PD-L1 was expressed on TICs in 46.8% and on LCs in 6.5% of cases. PD-L1 expression on LCs was more frequent in non-GCB subtype ($p = 0.047$). In addition, patients with PD-L1 expression on LCs had significantly shorter PFS ($p = 0.015$), and the expression retained significant in the multivariate model ($p = 0.034$).

CONCLUSIONS: PD-L1 was more frequently expressed in LCs of the non-GCB subtype. Additionally, PD-L1 in LCs may predict shorter PFS time. D-IHC staining for PD-L1/PAX5 is a feasible method to assess PD-L1 expression on LCs of DLBCL, NOS patients and can be used to identify patients who may benefit from targeted immunotherapy with checkpoint inhibitors.

EX VIVO EXPANSION AND PHENOTYPIC CHARACTERIZATION OF MOUSE PRIMARY REGULATORY T CELLS

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BACKGROUND: $CD25^+$ $Foxp3^+$ regulatory T cells (Tregs) are a rare subset within the $CD4^+$ T cell compartment, playing a vital role in immune system regulation. Because of their ability to suppress autoimmune responses, they have been investigated as a potential new tool for treating autoimmune diseases and preventing graft rejection. Although the population of Treg cells is well-defined by the intracellular expression of the transcription factor Foxp3, there is no definitive surface marker that distinguishes Tregs from other T cells, making the fiducial phenotypic characterization and isolation challenging. Furthermore, most of the isolation and expansion protocols are designed for human Tregs, representing a significant drawback since mice are commonly used as model organisms in the preclinical phase of drug development.
The purpose of this study was to establish enrichment, activation and expansion protocols for primary mouse Tregs, and identify novel markers that distinguish Tregs from less stable subsets and contaminants, using flow cytometry and single-cell RNA sequencing.

METHODS: Immunomagnetic separation was used to isolate Tregs from mice spleens. The cells were activated and expanded under different conditions using different ratios of Treg cells to activation beads in the presence of IL-2. Samples were collected at different time points during 15 days, and stained for viability, and antibodies for eight surface markers and two intracellular markers. The phenotype of Tregs and other T cell subsets was analyzed on a flow cytometer. Single-cell RNA sequencing using the Chromium system (10x Genomics) was performed to determine the transcriptional profiles of subpopulations within the Tregs samples

RESULTS: The phenotype and transcriptome analysis revealed that the Treg population is already a heterogeneous population at the time of isolation. Over the course of 15 days, the percentage of $Foxp3^+$ Treg cells decreases from ≈85% at the time of isolation to ≈10% by day 15. Single-cell RNA sequencing confirmed our findings from flow cytometry.

CONCLUSION: We have successfully established isolation and expansion protocols for mouse $CD25^+$ $Foxp3^+$ regulatory T cells as well as comprehensive flow cytometry staining panels. During the expansion protocol, the proportion of Treg cells decreases, while the proportion of contaminants increases.
Single-cell RNA sequencing provided a deeper insight into the transcriptome of the Treg cells and the heterogeneity of *ex vivo* culture.

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ANTITUMOR EFFECT OF BLEOMYCIN ELECTROCHEMOTHERAPY COMBINED WITH ANTI-PD-1 IN MOUSE FIBROSARCOMA AND COLORECTAL TUMOR MODEL

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Electrochemotherapy (ECT) has emerged as a safe and effective treatment modality for various tumor types in both preclinical and clinical settings within human and veterinary oncology. Recent studies have revealed its ability to activate innate immunity by triggering a signaling pathway through damage-associated molecular patterns (DAMPs). This activation leads to specific antitumor immunity and subsequent immunogenic cell death. Since immunogenic effects have been reported with ECT with bleomycin (BLM), it could be a promising approach to combine it with immunotherapy, which is currently the most widely used treatment strategy. Especially PD-1 inhibitors achieve good treatment efficacy in various cancer types. Therefore, our study aimed to assess the antitumor efficacy of combined ECT BLM treatment with anti-PD-1 in mouse colorectal carcinoma and sarcoma.

In vitro sensitivity of colorectal MC38 carcinoma and WEHI 164 fibrosarcoma to bleomycin electrochemotherapy was determined using Presto Blue viability assay. Furthermore, the antitumor effect of combined ECT BLM and anti-PD-1 was evaluated in subcutaneous colorectal MC38 carcinoma grown in C57Bl/6 mice and WEHI 164 fibrosarcoma grown in Balb/c mice by tumor growth delay assay.

Our findings indicated that WEHI 164 cells exhibited greater sensitivity to ECT with BLM compared to MC38 cells, requiring a 250-times lower concentration of BLM to achieve a 50% reduction in cell viability post-electroporation. Consistent with these results, *in vivo* experiments showed a remarkable 100% cure rate of fibrosarcoma tumors, while colorectal carcinoma tumors exhibited a cure rate of 13%. Therefore, we combined ECT with BLM and immunotherapy specifically in the less sensitive colorectal carcinoma model, resulting in a 78% cure rate. Monotherapies, on the other hand, showed limited efficacy, with only up to an 8- day delay in tumor growth compared to controls in both tumor models.

Overall, our study underscores the enhanced antitumor effectiveness of combining ECT with immunotherapy, particularly in less responsive tumor types, i.e. colorectal carcinoma. These results highlight the potential of ECT to modify the tumor microenvironment in synergy with immunotherapy in the realm of cancer treatment. Nevertheless, further investigations are warranted to elucidate the underlying mechanisms of this combined therapeutic approach.

GENETICS

PLENARY SYMPOSIUM

GENETICS - CUTTING-EDGE ADVANCEMENTS IN DIAGNOSIS AND TREATMENT

Invited lecture

NEWBORN SCREENING PROGRAMME IN SLOVENIA

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Newborn Screening (NBS) is one of the most significant and successful public health initiatives globally. Implemented in various capacities across most developed countries, NBS programs vary in scope and focus, with the most comprehensive initiatives screening for over 50 different conditions. In Slovenia and its geographical surroundings of Southeastern Europe, the development of NBS programs reflects both regional trends and unique national efforts to improve neonatal healthcare. Slovenia initiated its NBS program in 1979, focusing initially on phenylketonuria (PKU), with congenital hypothyroidism (CH) added in 1981. 35 years later, the NBS was upgraded with mass-spectrometry (MS/MS) technology and expanded to cover multiple inborn errors of metabolism. This was a significant step forward, aligning with global movements towards early detection and treatment of congenital disorders to prevent severe disabilities and enhance the quality of life for affected children. Slovenia's approach to NBS, while comprehensive, has also faced challenges common to many countries in the region. The adoption of MS/MS, a technology that allows for the screening of multiple disorders simultaneously, has been slower compared to some Western countries. This technology significantly enhances the efficiency and scope of NBS programs by enabling the screening for a broader range of metabolic disorders from a single blood sample. Finally, in the year 2024, a second expansion of Slovenian NBS happened by introduction of dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) technology and quantitative RT-PCR to enable screening for cystic fibrosis, spinal muscular atrophy, congenital adrenal hyperplasia (CAH) and severe primary immunodeficiencies. Cumulatively, Slovenian NBS covers more than 40 disorders. The use of dried blood spots on filter paper facilitates easy transportation to the Paediatric Clinic in Ljubljana for analysis. Professional sample collection by maternity hospital staff ensures sample quality, critical for successful laboratory testing. Participation in the program is universal for all newborns, with an option for parental refusal. To date, over 150 newborns have been successfully treated, underscoring the program's importance. With the advent of new medicines and therapies, the scope of NBS is set to expand. The program's success strongly relies on the synergy between participating groups - maternity hospitals, the Paediatric clinic, and the laboratory.

Invited lecture

GENE THERAPY IN CHILDREN

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BACKGROUND Since 2018, when the first Slovenian patient with inborn error of metabolism (IEM) was referred abroad for experimental gene therapy, several other patients with various rare congenital diseases have been genetically treated at our institution. Aim was to report Slovenian experiences with gene therapy in children with focus on metabolic disorders, and to explore currently approved gene therapies in pediatrics.

METHODS Retrospective analysis of experiences with gene therapy in Slovenia and review of literature.

RESULTS After the year 2018, we referred four of our patients abroad for experimental gene therapy, one with MPS I, two with MPS IIIa, and one with metachromatic leukodystrophy (in collaboration with pediatric neurologists), who were later successfully treated and continue to be regularly monitored at our institution, in cooperation with both study centers. In December 2021, the first successful application of gene therapy in Slovenia was carried out at the University Children's Hospital, UMC Ljubljana, in a child with spinal muscular atrophy. It is also worth mentioning the very rapid progress that we are witnessing at the same time in the field of treating certain rare types of blood cancers with genetic therapy (i.e. treatment with CAR-T lymphocytes);

In 2012, the first gene therapy approved in Europe was the therapy for recurrent lipoprotein lipase deficiency pancreatitis (Glybera, uniQure), an extremely rare IEM that causes extremely elevated triglyceride levels. Twelve different gene therapies are currently approved by the European Medicines Agency (EMA). These include therapies for rare forms of blindness and leukaemia, spinal muscular atrophy, and a type of congenital immunodeficiency.

CONCLUSIONS The last decade has brought great progress in this field, including in Slovenia; gene therapy has been successfully used in several Slovenian patients in the last few years.

Invited lecture

GENETIC DIAGNOSIS OF PULMONARY DISEASES

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Possible genetic involvement should be considered in the following phenotypes presenting to the adult population pulmonary clinic, namely: i.) emphysema, ii.) idiopathic diffuse bronchiectasis, and iii.) idiopathic pulmonary fibrosis (IPF).

Alpha-1 antitrypsin (AAT) deficiency is the leading genetic cause of emphysema; in all individuals with a diagnosis of chronic obstructive pulmonary disease (COPD), it is necessary to consider and manage a patient in the light of possible AAT deficiency. Furthermore, the clinical picture of (serious) emphysema can also occur with pathogenic variants present in the telomerase complex genes (however, the more common presenting phenotype here is idiopathic pulmonary fibrosis).

In the case of diffuse bronchiectasis, the genetic causes can be the following: i.) primary ciliary dyskinesia - especially if the patient also has lateralization disorders, chronic sinus inflammation, recurrent otitis media with hearing disorders, impaired fertility, etc., ii. .) primary immunodeficiencies (especially common variable immune deficiency (CVID)), iii.) cystic fibrosis (CF); due to the presence of screening programs for the detection of CF in the neonatal period, presentation with CF in adulthood is increasingly rare, but in patients with diffuse bronchiectasis, it is also necessary to exclude CF as a possible genetic cause of the clinical picture; moreover, not only patients with CF but also carriers of pathogenic variants in the *CFTR* gene have an increased risk of developing (diffuse) bronchiectasis.

In patients with IPF, suspicion of the presence of pathogenic variants in the telomerase complex genes should be considered; suspicion is further strengthened if there is a personal/family history consistent with short telomere syndrome, namely: i.) early greying, ii.) interstitial lung disease in the family, iii.) liver cirrhosis of unknown origin, iv.) aplastic anaemia, and in v.) myelodysplasia/leukemia.

In addition to the conditions mentioned above, genetic causes can be sought for in recurrent spontaneous pneumothoraces (Birt-Hogg-Dubé syndrome (typically with kidney and skin involvement)), pulmonary fibrosis and oculocutaneous albinism (Hermansky Pudlak syndrome), pulmonary lymphangioleiomyomatosis (tuberous sclerosis), especially if other clinical signs characteristic of tuberous sclerosis are present.

Invited lecture

GENOMIC APPROACHES TO THE DIAGNOSIS OF PULMONARY AND IMMUNOLOGICAL DISEASES: THE EXPERIENCE OF A SINGLE INSTITUTION

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BACKGROUND: The majority of pulmonary diseases referred for genetic diagnosis in our laboratory are due to the determination of the genetic cause of cystic fibrosis. Most common pathogenic variants in the *CFTR* gene are primarily excluded with commercially available PCR test. Patients with *CFTR* negative results or patients with pulmonary diseases and immunodeficiency disorders are further referred to whole exome sequencing (WES). The aim of the work is to present the results of the comprehensive genetic diagnostic of our patients with pulmonary and immunological diseases over the last five years.

METHODS: For 69 patients analysis of the 29 most common mutations in the *CFTR* gene was performed with an Elucigene CF29v2 kit with in-house added allele-specific PCR for IVS8-5T variant. Patients with heterozygous F508del variant were further referred to WES.

Tree patients were referred to WES, due to the suspicion of a rare immunological disease, Netherton syndrome, Job syndrome, and Epidermodysplasia verruciformis. Additionally, in 6 patients, WES was performed due to the suspicion of pulmonary disease, mostly primary ciliary dyskinesia and primary spontaneous pneumothorax.

RESULTS: Three patients (3/69) referred for cystic fibrosis had homozygous F508del variant, of which one was a prenatal sample. One patient was compound heterozygous. In 6% (4/69) additional pathogenic variant in *CFTR* was detected by WES. These variants are often represented in the Slavic population but not included in commercial kit. In the immunological disease group of patients, for patient with Netherton syndrome differential diagnosis was performed, since the patient has Ichthyosis vulgaris due to two pathogenic variants in *FLG* gene in compound heterozygous state. Pathogenic variants in the *FLCN* gene, as a cause of primary spontaneous pneumothorax, were identified in two patients. Overall, in 17 patients tested by WES, we reached a genetic diagnosis in almost half the patients (8/17).

CONCLUSION: Our work represents a small but important contribution to genetic diagnostics of pulmonary and immunological Slovenian patients. Our results showed a need for broader *CFTR* mutation gene screening in our workflow. For diagnostic of other pulmonary and immunological diseases, WES has proven as essential diagnostic method, yet we still have not determined the genetic cause of the disease in all tested patients, which indicates the need for even more extensive genetic testing.

Invited lecture

GENOMIC LANDSCAPE OF SUSCEPTIBILITY TO SEVERE COVID-19 IN THE SLOVENIAN POPULATION

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INTRODUCTION: Several hundred genes have been implicated to contribute to the complex host genetic component of COVID-19 disease, with research indicating that the severe SARS-CoV-2 infection outcome may be population-dependent. Using whole genome sequencing, we aimed to identify rare genomic variants in genes associated with COVID-19 susceptibility by comparing a cohort of severe-disease patients requiring hospitalization with the Slovenian population background.

METHODS: Whole genome sequencing was performed on the DNA isolated from the whole blood of 60 patients with severe COVID-19 disease who were hospitalized during the second wave of the pandemic Sep-Dec 2020 and were a part of the severe COVID-19 hospitalized patient cohort from the University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia. Target genes associated with SARS-CoV-2 infection were obtained through an extensive review of the literature, and target genes identified by the COVID-19 Host Genetic Initiative were additionally included, as well as the curated Research COVID-19 associated genes from PanelApp, England Genomics. The background prevalence of identified variants in the general Slovenian population was determined by using de-identified sequencing data from 7901 individuals included in the Slovenian genomic database.

RESULTS: Rare pathogenic/likely pathogenic genomic variants in 517 target genes were identified and classified according to the ACMG criteria. At the level of the whole gene panel, we observed a burden of rare pathogenic/likely variants in our relatively small sample size of the severe COVID-19 cohort compared to the Slovenian population background, which might result in severe COVID-19 outcomes. At the individual gene level, several rare pathogenic/likely pathogenic variants were identified in genes associated with immunodeficiency, susceptibility to infections, autoimmunity, and inflammatory disorders. The results show the genomic landscape of susceptibility to severe COVID-19 in Slovenia.

CONCLUSIONS: Our results represent an important insight into the Slovenian genomic diversity associated with severe COVID-19 outcomes. Determining the genetic contribution of susceptibility to severe SARS-CoV-2 infection outcomes is important for public health measures and individualized treatment.

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TRANSCRIPTOMIC INSIGHTS IN CHRONIC RHINOSINUSITIS

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BACKGROUND: Chronic rhinosinusitis (CRS) is a condition characterized by inflammation of the nasal and sinus passages that persists for an extended period, typically lasting at least 12 weeks. Two significant phenotypes, chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP), do not account for substantial variability in the clinical course of the disease. Extended inflammation endotyping might bring better insight into the multifaced illness. Understanding the transcriptome of chronic rhinosinusitis involves studying the complete set of RNA transcripts in cells or tissues affected by the disease. This analysis helps identify the active genes and can provide insights into the underlying molecular mechanisms and potential therapeutic targets.

METHODS: The study was designed as a prospective cohort study of patients with CRS diagnosed and treated at a tertiary institution. The inclusion criteria were a clear-cut phenotype of primary CRS with or without polyps requiring surgical treatment. Using mRNA sequencing, differently expressed genes (DEGs) were characterized, and functional and pathway analysis was undertaken. To illustrate interactions, biological context, and bio-function among the top DEGs of different endotypes in CRS, a network and pathway analysis was performed using QIAGEN's Ingenuity® Pathway Analysis.

RESULTS: We identified 782 common CRS-associated nasal-tissue DEGs, while 375 and 328 DEGs were CRSwNP- and CRSsNP-specific, respectively. Common key DEGs were involved in dendritic cell maturation, the neuroinflammation pathway, and the inhibition of the matrix metalloproteinases. Distinct CRSwNP-specific DEGs were involved in NF-kβ canonical pathways, Toll-like receptor signalling, HIF1α regulation, and the Th2 pathway. CRSsNP involved the NFAT pathway and changes in the calcium pathway.

CONCLUSIONS: Research on gene expression patterns in CRS has provided valuable insights into the molecular mechanisms underlying the condition. The expression of various genes involved in inflammation, immune response, tissue remodelling, and other processes contributes to the pathogenesis of CRS.

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GENETIC MODIFIERS OF THE CLINICAL PHENOTYPE IN HEREDITARY ANGIOEDEMA DUE TO C1 INHIBITOR DEFICIENCY

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BACKGROUND: Hereditary angioedema due to C1 inhibitor deficiency (HAE-C1-INH) is a rare autosomal dominant disorder that causes recurrent episodes of oedema. It is caused by pathogenic variants in the *SERPING1* gene, which codes for C1-INH. The severity and frequency of symptoms can vary greatly between patients, with some suffering frequent, life-threatening attacks and others remaining asymptomatic throughout their lives. We aimed to identify novel modifying genetic factors that predispose to clinical symptoms.

METHODS: We performed whole exome sequencing (WES) and comprehensive bioinformatic analysis in symptomatic and asymptomatic (three duos) family members with HAE-C1-INH. Selected variants identified by WES, present in all asymptomatic patients and absent in symptomatic patients, were determined by Sanger sequencing. A total of 88 clinically well-characterised HAE-C1-INH patients from 42 unrelated families from southeastern Europe were included, among them nine asymptomatic adults.

RESULTS: By performing WES on symptomatic/asymptomatic family members with HAE-C1-INH we identified 39 variants in 23 genes that differ between asymptomatic and symptomatic patients. Based on the literature and possible involvement in mechanisms of angioedema, we selected variants in *CC2D2B* and *PLCL1* genes, which were analysed using Sanger sequencing in the entire group of HAE-C1-INH patients. We found significant differences in the frequencies of the *CC2D2B* c.190A>G (rs17383738) variant between symptomatic and asymptomatic patients, with heterozygotes being more common in asymptomatic HAE-C1-INH patients compared to symptomatic patients (55% vs 23%, Fisher's exact test: P = .049, OR = 4.24, 95% CI 1.07-14.69). On the other hand, no association was found between the *PLCL1* variant and the occurrence of clinical symptoms when analysing the entire cohort of HAE-C1-INH patients. Based on linkage disequilibrium analysis, *CCNJ* and *ZNF518A* genes may also be involved in the clinical expression of HAE-C1-INH.

CONCLUSIONS: Our study identified novel genetic factors that modify clinical variability in patients with HAE-C1-INH. Using the exome sequencing approach, we identified 39 possible genetic biomarkers in 23 genes that discriminate asymptomatic from symptomatic patients. We also demonstrated the importance of a variant in the *CC2D2B* gene as a disease-modifying factor in a large cohort of HAE-C1-INH patients from Southeastern Europe, suggesting the possible involvement of neprilysin in the development of angioedema attacks.

This research was supported by the Slovenian Research and Innovation Agency (grant nos. P3-0360 and J3-2532).

ADVANCES IN GENETIC TESTING: MULTIPLEX DDPCR ASSAY FOR TRYPTASE GENOTYPING

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BACKGROUND: Hereditary α tryptasemia (HαT) is an autosomal dominant trait characterized by increased *TPSAB1* copy number (CN) encoding α-tryptase. The determination of HαT is being discussed as an important biomarker to be included in risk assessment models and future diagnostic algorithms for patients with mastocytosis. Patients with HαT are also prone to more severe anaphylaxis. Therefore, tryptase genotyping should be considered in the clinical evaluation of individuals with a history of, or at risk for, severe anaphylaxis. Due to the complex genetic structure at the human tryptase locus, genetic testing for HαT is presently notably limited and infrequently pursued. The aim of this study was to develop, optimize and validate a multiplex droplet digital PCR (ddPCR) assay that can reliably quantify α- and β-tryptase encoding sequences in a single reaction well.

METHODS: Custom primers and probes targeting sequences encoding α- and β-tryptases were reported previously. For the detection of reference copy number invariant locus, additional primers, and probes targeting *AP3B1* and *AGO1* genes were designed. To optimize the ddPCR conditions and establish an amplitude-based multiplex ddPCR assay, a thermal gradient with varying annealing temperatures, different primers/probe concentrations, and various initial DNA quantities were tested. The multiplex ddPCR performance was compared with separate duplex ddPCRs in 114 DNA samples.

RESULTS: An annealing temperature of 60°C, DNA quantity of 55 ng and optimized primer and probe concentrations enabled a clear separation of positive droplets and clear, distinct differentiation of each target set within unique clusters. Results obtained from the multiplex ddPCR were concordant with those achieved with the duplex ddPCRs.

CONCLUSIONS: Utilizing this multiplex ddPCR assay, in contrast to conducting distinct duplex ddPCRs, presents noteworthy benefits for tryptase genotyping. These advantages encompass a substantial threefold decrease in material costs and considerable time savings. Consequently, this approach exhibits a high degree of suitability and particularly captures interest for routine clinical implementation.

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TRANSCRIPTOME DIFFERENCES IN HYMENOPTERA VENOM IMMUNOTHERAPY TREATED PATIENTS

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BACKGROUND: *Hymenoptera* venom immunotherapy (VIT) provokes venom tolerance in most treated patients, nevertheless the exact underlying mechanism remains unclear. Our study aimed to identify molecular mechanisms associated with VIT efficiency.

METHODS: We prospectively included 19 patients with *Hymenoptera* venom allergy undergoing VIT. Whole blood samples were collected before the beginning of VIT, after reaching the maintenance dose, after one year of VIT termination, and after the sting challenge. RNA-sequencing of whole blood was performed, and bioinformatic analysis was made using CLC Genomics Workbench and Ingenuity Pathway Analysis.

RESULTS: Longitudinal transcriptomic profiling revealed that the immune system became activated by reaching the maintenance dose and was suppressed after finishing VIT. Important is the inhibition of the NFκB pathway and downregulation of *DUX4* transcripts for early protection and induction of tolerance after finishing VIT. Furthermore, successful treatment was associated with inhibiting Th2, Th17 and macrophage alternative signalling pathways in synergy with the inhibition of the PPAR pathway. Finally, successful VIT restores the immune system's balance to a state similar to that of healthy individuals.

CONCLUSION: Transcriptome analysis of VIT reveals significant suppression of immune response after successfully finishing VIT. Our results outline the important role of inhibition of four pathways for the clinical effect of VIT: Th2, Th17, NFκB, and macrophage signalling. Two biomarkers specific for successful VIT, regardless of the time of sampling, were *C4BPA* and *RPS10-NUDT3.*

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TABLE OF CONTENTS

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