

International Conference

# “Autoimmune Diseases: Main Problems and Solutions”

November 9–10, 2023

Rīga Stradiņš University, Riga, Latvia

## ABSTRACT BOOK



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**“Autoimmune Diseases: Main Problems and Solutions”**

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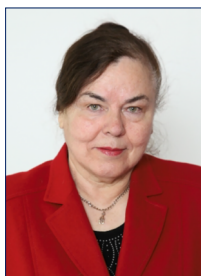
## WELCOME LETTER

It is our privilege and pleasure to welcome you to the International Conference “Autoimmune Diseases: Main Problems and Solutions” taking place in Riga. Conference is organized by the Rīga Stradiņš University within the frame of the EC Horizon 2020 Framework program project “Reducing networking gaps between Rīga Stradiņš University (RSU) and internationally-leading counterparts in viral infection-induced autoimmunity research (VirA)”.

The aim of the Conference is to bring together researchers exploring triggers and mechanisms of autoimmunity, leading to better understanding of chronic diseases and comorbidities in order to deliver precise and early diagnostic and to move towards the development of personalized medicine.

Exchange of opinions and communication are the most important components in the progress of scientific ideas. We are happy to present the scientific program of the Conference covering different aspects of autoimmunity in plenary lectures, oral presentations and poster sessions. The Conference has attracted about 100 participants from 7 countries.

We hope that you will enjoy the Conference and that your interactions with colleagues from different countries will stimulate a creative exchange of ideas and collaborative research projects in the future.



VirA project coordinator,  
Assoc. Prof. Modra Murovska

## Scientific committee

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## General information

### VirA Objectives and Activities

- Increase research excellence of the coordinating institution in the field of research as a result of the twinning exercise
- Enhance reputation, attractiveness and networking channels of the coordinating institution
- Enhance scientific and technological capacity of the linked institutions with a principal focus on the university or research organisation from the Widening Country

### Project Activities

- Raising experience of the staff with short term exchanges
- Bringing new knowledge to the institute from expert visits and short-term on-site or virtual training
- Conference participation and attendance to help with project dissemination and gathering of new information and global knowledge exchange
- Organisation of training workshops and joint summer schools to increase the networking ability of staff and foster knowledge transfer

## Table of Contents

WELCOME LETTER .....	3
Scientific committee .....	4
Organizing committee .....	4
General information	
VirA Objectives and Activities .....	5
Project Activities .....	5
<b>SESSION I – Understanding Autoimmunity .....</b>	<b>10</b>
Why Autoimmunity? Hyper-Stimulation of the Immune System CPI and Breast Silicone Implants Are Proofs of Concept <i>Yehuda Shoenfeld</i> .....	10
Gut Microbiome Analysis and Effect on Cytokine Levels in Individuals with Fibromyalgia <i>Zaiga Nora-Krūkle, Lauma Ieviņa, Santa Rasa-Dzelzkalēja, Anda Vilmane, Sabine Grāvelsiņa, Nikita Fomins, Viktorija Ķeniņa, Šimons Svirskis, Dita Gudrā, Dāvids Fridmanis</i> .....	12
Autoantibodies Directed Against Plasma Cytokines Mirror Autoimmune Characteristics in Patients with Inflammatory Diseases <i>Christian Scheiber, Alexander Dulovic, Tanja Schulz, Karl Bechter, Nicole Schneiderhan-Marra, E. Marion Schneider</i> .....	13
Phosphorylcholine (PC) – Mode of Activity and Therapy Development for Autoimmune Diseases <i>Miri Blank</i> .....	14
<b>SESSION II – Viruses and Autoimmunity .....</b>	<b>16</b>
Understanding the Infectious Origin of Autoimmunity from the SARS-CoV-2 Pandemic <i>Bhupesh K. Prusty</i> .....	16
Novel Compounds to Control Human Herpesvirus-6 Infection <i>Claudio Trapella, Daria Bortolotti, Sabrina Rizzo, Giovanna Schiuma, Paolo Marchetti, Andrea Alogna, Roberta Rizzo</i> .....	17
Neurodegenerative Role of West Nile Virus Non-Structural Protein 1: Effect on TLR3 and Amyloid Beta Expression <i>Silvia Beltrami, Sabrina Rizzo, Valentina Gentili, Giovanna Schiuma, Roberta Rizzo, Daria Bortolotti</i> .....	18

The Highest EBV DNA Copy Numbers Are Detected in Untreated Patients with the High-Risk Chronic Lymphocytic Leukemia <i>Irina Kholodnyuk, Laura Zvejniece, Svetlana Kozireva, Olga Kornilova, Mariia Nazarenko, Zanna Rudevica, Ainars Leonciks, Alla Rivkina, Sandra Lejniece, on behalf of the VirA project No 952376</i> . . . . .	19
Possible Role of EBV in the Pathogenesis of Oral <i>Lichen Planus</i> <i>Ingrīda Čēma, Jagriti Kakar, Modra Murovska</i> . . . . .	21
<b>SESSION III – Myalgic Encephalomyelitis / Chronic Fatigue Syndrome . . .</b>	<b>22</b>
The Role of the Gut Microbiome in the Pathogenesis of Autoimmune Disorders and Non-Pharmacological Interventions for Its Modification. An Example Chronic Fatigue Syndrome <i>Paweł Zalewski, Sławomir Kujawski, Joanna Słomko, Hanna Tabisz, Monika Prylińska</i> . . . . .	22
Selection of Biomarkers in ME/CFS for Patient Stratification and Treatment Surveillance / Optimisation <i>Modra Murovska, Angelika Krūmiņa, Sabīne Grāvelsiņa, Anda Vilmane, Santa Rasa-Dzelzkalēja, Zaiga Nora-Krūkle, Diāna Arāja, Šimons Svirskis, Uldis Berķis</i> . . . . .	23
Complementary Disease Management Approaches in Myalgic Encephalomyelitis / Chronic Fatigue Syndrome Patients <i>Diāna Arāja, Edgars Vasiļevskis, Zaiga Nora-Krūkle, Angelika Krūmiņa, Uldis Berķis, Modra Murovska</i> . . . . .	24
Kynurenine and Human Herpesvirus-6B in Myalgic Encephalomyelitis / Chronic Fatigue Syndrome <i>Santa Rasa-Dzelzkalēja, Daniel Alexander Bizjak, Šimons Svirskis, Sabīne Grāvelsiņa, Jasmine-Leonike Buhl, Angelika Krūmiņa, Zaiga Nora-Krūkle, Anda Vilmane, Marion Schneider, Modra Murovska, on behalf of the VirA project</i> . . . . .	26
<b>SESSION IV – Inflammation and Autoimmunity . . . . .</b>	<b>28</b>
Mitochondrial Myopathy as a Mimic of Inflammatory Myopathy <i>Anda Kadiša, Mihails Tarasovs, Sofija Semenistaja</i> . . . . .	28
Low-Grade Inflammation Contribution to Neuropsychiatric State in Osteoarthritis Patients <i>Mihails Tarasovs, Sofija Semenistaja, Andris Vikmanis, Dmitrijs Omeļčenko, Valērija Groma, Aivars Lejnīeks</i> . . . . .	29

<b>SESSION V – COVID-19 Autoimmune Aspects</b> .....	31
Functional Single Nucleotide Polymorphisms (SNPs) Linked to Autoimmune Manifestations in Long COVID	
<i>Marion Schneider, Julian M. Schneider, Jürgen M. Steinacker, Christian Scheiber, Alex Dulovic, Nicole Schneiderhan-Marra</i> .....	31
Gestational COVID-19: Maternal and Foetal Morphological Alterations Caused by SARS-CoV-2 Infection	
<i>Sabrina Rizzo, Silvia Beltrami, Giovanna Schiuma, Roberta Rizzo, Angelina Passaro, Pantaleo Greco, Roberta Gafà, Sandra Skuja, Daria Bortolotti, Valērija Groma</i> .....	32
Association of Liver-Related Autoantibodies with Possible Post-COVID Condition	
<i>Ieva Vanaga, Oksana Kojesova, Jeļena Storoženko, Santa Rasa-Dzelzkalēja, Elvira Hagina, Aleksandrs Kojesovs, Ludmila Viksna</i> .....	33
New Onset of Autoimmune Diseases Following COVID-19 Diagnosis and Vaccination	
<i>Hele Everaus</i> .....	34
Determination of Population Health Biomarkers in Wastewater Samples	
<i>Vadims Bartkevičs, Iveta Pugajeva, Ingus Pērkons, Arvis Prikulis, Deniss Fedorenko, Juris Ķibilds</i> .....	35
<b>SESSION VI – Challenges and Solutions</b> .....	37
Infra-Red Spectroscopy in Diagnosis of Autoimmune Diseases	
<i>Boris Gilburd</i> .....	37
Integrative Medicine Approach in the Treatment of Autoimmune Diseases	
<i>Edgars Vasiļevskis, Diāna Arāja, Irina Evansa, Sandra Vasiļevska</i> .....	38
Personalized Physical Activity as a Therapeutic Strategy for Autoimmune Diseases	
<i>Yuri Ostrinski</i> .....	39
Circulating miRNAs Expression in Myalgic Encephalomyelitis / Chronic Fatigue Syndrome	
<i>Irene Soffritti, Sabine Grāvelsiņa, Maria D'Accolti, Francesca Bini, Eleonora Mazziga, Anda Vilmane, Santa Rasa-Dzelzkalēja, Zaiga Nora-Krūkle, Angelika Krūmiņa, Modra Murovska, Elisabetta Caselli</i> .....	41
Coinfection of Dermal Fibroblasts by Human Cytomegalovirus and Human Herpesvirus-6 Can Boost the Expression of Fibrosis-Associated Micrnas	
<i>Maria D'Accolti, Irene Soffritti, Clara Maccari, Francesca Bini, Eleonora Mazziga, Maria-Cristina Arcangeletti, Elisabetta Caselli</i> .....	43



<b>SESSION VII – Challenges and Solution II</b> .....	45
Immune-Related Adverse Events During the Treatment with Anti PD-1 Antibodies in Melanoma Patients: Literature Review and Two Year Experience at REUH <i>Simona Doniņa, Ieva Vaivode</i> .....	45
Suboptimal Selenium Serum Concentrations in Latvian Patients with Autoimmune Gastritis and Autoimmune Thyroid Disorders <i>Ize Konrāde, Vita Rovīte, Andrejs Šķesters, Indra Zeltiņa, Justine Kaupe</i> .....	46
Resistance of <i>Enterococcus</i> Isolates in Hospitalised Patients from Two Multidisciplinary Hospitals: From Science to Practice <i>Inga Mauliņa, Linda Labecka, Juris Ķībils, Renārs Erts, Dace Bandere, Angelika Krūmiņa</i> .....	48
<b>SESSION VIII – WIDESPREAD Project’s Achievements</b> .....	50
Implementation of VirA Project “Reducing Networking Gaps Between Rīga Stradiņš University (RSU) and Internationally-Leading Counterparts in Viral Infection-Induced Autoimmunity Research” <i>Modra Murovska, Asja Lunga, Simona Doniņa, Zaiga Nora-Krūkle, Valērija Groma, Angelika Krūmiņa, Santa Rasa-Dzelzkalēja</i> .....	50
Strategies for Advanced Development of Antibacterials – Springboard Project’s Achievements <i>Raivis Žalubovskis</i> .....	51
Autoimmunity Research in European Agendas <i>Uldis Berķis</i> .....	52
<b>Poster session</b> .....	53
HHV-7 Protein Expression in the Synovial Tissue of Osteoarthritis Patients and Its Possible Contribution to the Degree of Inflammation <i>Sofija Semenistaja, Mihails Tarasovs, Sandra Skuja, Anda Kadiša, Valērija Groma, Pēteris Studers</i> .....	53
Vascular Presence of HHV-6 in <i>Substantia Nigra pars compacta</i> of Chronic Alcohol Users and Controls: A Histopathological Evaluation <i>Nityanand Jain, Sandra Skuja</i> .....	54
Infections During the Avacopan Early Access Program (EAP) for Anca-Associated Vasculitis (AAV) <i>Tamara Popov, Achim Obergfell, Javier Villacorta</i> .....	55
Development of Benzoxaphosphepine 2-Oxides as Carbonic Anhydrase Inhibitors <i>Anastasija Balašova, Aleksandrs Pustenko, Raivis Žalubovskis</i> .....	56
<b>Authors Index</b> .....	58

## Session I – Understanding Autoimmunity

### Why Autoimmunity? Hyper-Stimulation of the Immune System CPI and Breast Silicone Implants Are Proofs of Concept

**Yehuda Shoenfeld**

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Autoimmune diseases are induced by hyper-stimulation of the immune system ("adjuvant" ASIA Syndrome) [1, 2], in a genetically prone individuals, i.e. HLA-DRB1 [3]. We will describe several proofs of concepts to this hypothesis:

1. Check point inhibitor (CPI) [4], which unleash a hyper-stimulation of the immune system [5] leads to an emergence of avalanche of autoimmune diseases. This occurs specifically in HLA-DRB<sub>1</sub> individuals [3].
2. The silicone breast implants (SBI) [6] are also associated with autoimmune autonomic nervous system in prone individuals with HLA-DRB<sub>1</sub> bearer [7].
3. Moreover, induction of the B large cell lymphoma is specifically more frequent in HLA-DRB<sub>1</sub> individuals [8].

These proofs of concept support the "ASIA" (Shoenfeld's) syndrome [9–11] and the mosaic of factors involved in autoimmunity (combination of hyper-stimulation of the immune system in a genetically prone individuals).

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## Gut Microbiome Analysis and Effect on Cytokine Levels in Individuals with Fibromyalgia

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### Background and Aim

Fibromyalgia (FM) is one of the most common forms of chronic widespread pain, with an estimated prevalence of 0.2–6.6% (2.4–6.8% in women, 0.7–11.4% in urban areas and 0.1–5.2% in rural areas). Pharmacological treatments for FM patients are mainly directed to palliate some symptoms, with relevant clinical benefits experienced only by a minority of individuals from any intervention. Indirect evidence (including conducted pilot study) hints that alterations can be detected in FM patient gut microbiome. Growing data indicate microbial control of neurological functions by the immune system.

To investigate the influence of specific bacterial species on cytokine profiles and symptomatology in individuals with fibromyalgia.

### Methods

The pilot-study involved a cohort of 17 patients with FM and samples from 24 healthy blood donors.

Cytokines levels were measured – using bead-based multiplex assay. The analysis of the intestinal microbiome – performed by constructing a genomic library and conducting next generation sequencing.

Statistical analysis and data visualization were performed with GraphPad Prism and JMP software.

### Results

Significant differences in the  $\beta$ -diversity of gut microbiome have been observed in people with FM compared to apparently healthy subjects. In the FM group, there is a significant positive correlation between the cytokine IL-6, IL-9 and IL-8 and *Anaerobutyricum halii*, along with several other bacterial species, indicating that an increase in these bacteria is associated with higher cytokine levels. Additionally, the “Widespread Pain Index” shows a strong positive correlation with *Bacteroides stercoris*, suggesting a potential link between this bacterium and increased pain in individuals with FM.

## Conclusions

The findings suggest that specific bacterial species may play a significant role in the cytokine profiles and symptomatology observed in individuals with FM, potentially opening avenues for targeted therapeutic interventions and further research into the microbiome's impact on this condition.

## Autoantibodies Directed Against Plasma Cytokines Mirror Autoimmune Characteristics in Patients with Inflammatory Diseases

**Christian Scheiber**<sup>1</sup>, Alex Dulovic<sup>2</sup>, Tanja Schulz<sup>1</sup>, Karl Bechter<sup>3</sup>,  
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## Background

Autoantibodies (aAbs) directed against inflammatory cytokines such as IL-6, TNF- $\alpha$ , or interferon- $\gamma$  constitute important fine-tuners of cytokine-driven inflammatory responses. However, under pathological conditions like chronic inflammation, elevated aAb levels may enhance the susceptibility to various infections.

## Study Subjects and Methods

In this study, plasma/serum samples of patients suffering from inflammatory diseases were screened for aAbs against IL-6, IL-8, TNF- $\alpha$ , GM-CSF, as well as interferons - $\alpha$ , - $\beta$ , - $\gamma$ , - $\lambda$ , and - $\omega$ . The patient cohort consists of patients with COVID-19, long-COVID, chronic fatigue syndrome (CFS), virus-treated malignancies, affective spectrum disorder (AF, ICD-10 F30-F33), schizophrenic spectrum disorder (SZ, ICD-10 F20-F25), hemophagocytic lymphohistiocytosis (HLH), macrophage activation syndrome (MAS), and was completed by healthy donors (HDs). Plasma aAb titers were determined by LuminexR technology using recombinant cytokines and interferons (peptrotech.com). Some patient's samples were tested on several occasions of the observation time. In addition, patient's plasma samples were tested for IL-1 $\beta$ , -6, -8, -10, TNF- $\alpha$ , sCD25, and ferritin using the Immulite 1000R system (siemens.com).

## Results

Autoantibody titers were found to be highly elevated in individual patients throughout all cohorts. Interferon-directed aAbs were also found in a number of HDs. For AF and SZ patients, IL-6-directed aAbs correlated to aAbs against IL-8.

## Conclusion

Infections and inflammation, leading to higher levels of inflammatory cytokines and interferons, may lead to aAb formation in patients and also in HDs. Although the functionality of such aAbs is not known, the results confirm a current hypothesis that infections and chronic as well as acute hypercytokinemia would trigger aAb formation in a given host. Longitudinal observation provides evidence that aAb profiles may be attenuated by physical training.

## Phosphorylcholine (PC) – Mode of Activity and Therapy Development for Autoimmune Diseases

**Miri Blank**

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Where there are helminthes, autoimmunity is rare. The aim of the helminthes is to protect themselves *via* immunomodulation of the host immune network. The immunoregulatory functions of helminthes were attributed to the phosphorylcholine (PC) moiety on the helminthes' secretory molecules. We have constructed a bi-functional molecule PC-tuftsins (TPC) and showed its immunomodulatory activity in 4 mouse models (lupus, rheumatoid arthritis, colitis, experimental autoimmune encephalomyelitis) [1–3]. Treatment *ex vivo* with TPC decreased the production of IL-1 $\beta$ , IL-2, IL-5, IL-6, IL-9, IL-12(p70), IL-13, IL-17A, IL-18, IL-21, IL-22, IL-23, IFN $\gamma$ , TNF $\alpha$ , GM-CSF by CD3/CD28 activated PBMCs whereas it negligibly affected cell viability of inflamed TABs [4]. Phosphorylcholine (PC) is a small 82 zwitterionic molecule present in those helminthes secretory molecules which permit helminthes to survive in the host, inducing a situation of immune tolerance as well as on the surface of apoptotic cells and of several bacteria regulating adhesion to epithelial cells and immune recognition.

Additional interesting molecule is Sphingosylphosphorylcholine (SPC). In an experimental model that imitates multiple sclerosis, it was found SPC strongly blocked plasma cell differentiation from B cells and antibody production *in vitro* [5]. SPC downregulated LPS-stimulated IRF4 and Blimp 1, which are required for the generation of plasma cells. Administration of SPC against an experimental autoimmune encephalomyelitis (EAE), significantly attenuated the symptoms of disease,

showing decreased demyelinated areas of the spinal cord and decreased numbers of cells infiltrated into the spinal cord. SPC also elicits therapeutic outcomes against EAE, an experimental model of MS, suggesting SPC as a new material to control MS.

Collectively, PC in various forms demonstrate has anti-inflammatory inhibitory activities *in-vitro*, in animal models and *ex-vivo* in human.

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## Session II – Viruses and Autoimmunity

### Understanding the Infectious Origin of Autoimmunity from the SARS-CoV-2 Pandemic

**Bhupesh K. Prusty**

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Germany*

Autoimmunity is a characteristic overlapping feature associated with many post-viral chronic illnesses like ME/CFS and long COVID. However, the exact antecedent to the development of autoimmunity and its potential contribution to chronic disease development is largely unknown. Recently, our group has shown potential effects of immunoglobulins as a serum-transferrable factor in ME/CFS patients that induced alterations in mitochondrial architecture in healthy cells. Viral infection is known to induce molecular mimicry, a condition that can induce autoimmunity. For the first time, we show that virus infection can also alter natural antibody levels in humans, potentially contributing to the development of autoimmunity. Using longitudinal serum samples from individuals infected with SARS-CoV-2, we explain the potential mechanism of the development of autoimmunity.



## Novel Compounds to Control Human Herpesvirus-6 Infection

**Claudio Trapella**, Daria Bortolotti, Sabrina Rizzo, Giovanna Schiuma, Paolo Marchetti, Andrea Alogna, Roberta Rizzo

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### Introduction

An increased awareness of diseases associated with Human herpesvirus 6 (HHV6) infection and reactivation in both immunocompetent and immunocompromised patients has resulted in a growing interest in the evaluation of the best treatment options available for the clinical management of HHV6 disease. However, no compound has yet been approved exclusively for the treatment of HHV6. Thus, clinicians most often utilize the anti-cytomegalovirus (CMV) agents (ganciclovir, cidofovir and foscarnet) for the clinical treatment of HHV6, as in cases of HHV6 encephalitis. For this reason, the identification of anti-HHV6 compounds provides a valuable opportunity for developing efficient antiviral therapies.

### Materials and Methods

We synthesized two different classes of molecules that present in one case the substituted rhodanine nucleus and the second one the thiobarbituric moiety. The substituted furan was obtained via Suzuki coupling using the corresponding 5-formyl-furanyl-2-boronate and the 5-iodo-salicylic acid as a starting material for the synthesis of both compounds. The aldehyde obtained by this strategy has been used for a Knoevenagel condensation with rhodanine derivative to produce compound 1 and thiobarbituric acid to obtain compound 2. Compounds 1 and 2 has been characterized by mono-dimensional and bi-dimensional NMR and by HPLC to confirm the structures and the purity grade. The two compounds were tested on HHV-6A and HHV-6B infected T cells. The levels of viral DNA, RNA and proteins were determined by Real Time PCR, RT-PCR and immunofluorescence.

### Results

We report two compounds displaying an anti-HHV-6A and HHV-6B activity. The compounds inhibited both viral entry ( $p = 0.02$ ) and cell-to-cell transmission ( $p = 0.015$ ) in in vitro models of infection. These compounds are not cytotoxic and do not alter cell functions (protein expression, cell viability, mitochondrial activity). Due to their lipid oxidation ability, we hypothesize a mechanism on the viral envelope that affects the fluidity of the lipid bilayer, thus compromising the efficiency of virus-cell fusion and preventing viral entry.

## Conclusions

These compounds present a selectivity for HHV6 envelop, without any effect on cell membrane. These results might be associated with the staticity of viral envelopes that are without any repair mechanism, in contrast with the biogenic membranes of the cells that are endowed with plenty of tools to repair membrane damage or alteration. We suggest a possible use of these new compounds to inhibit HHV6 life cycle and prevent disease progression.

## Neurodegenerative Role of West Nile Virus Non-Structural Protein 1: Effect on TLR3 and Amyloid Beta Expression

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## Introduction

In the last years, the North-Est region of Italy, in particular Veneto and Emilia-Romagna (Riccò M. et al., 2022), has been characterized by a significant increase of West Nile Virus (WNV) infection rate. Neuroinvasive WNV viral infection may be linked epidemiologically and mechanistically to neurodegeneration, which have been associated with a significant prevalence of sequelae such as memory loss, confusion, and fatigue years later.

Non-structural protein 1 (NS1) is a highly conserved protein among Flaviviruses, which is actively secreted by infected cells and detected in the serum between days 3 and 8 post-infection, peaking on day 5, the day prior to the onset of clinical disease. Extracellular forms of NS1 are implicated in immune modulation and in promoting endothelial dysfunction at blood-tissue barriers, facilitating WNV dissemination to the brain and affecting disease outcomes.

## Aim

Focusing on the recently discovered antimicrobial roles of amyloid beta (Bortolotti et al., 2019), we connected WNV late pathology to overlapping features encountered in neurodegenerative diseases such as Alzheimer's disease. We aimed to investigate the possible effect of soluble NS1 on neurodegenerative and dysfunctional biomarkers (e.g. amyloid beta (A $\beta$ ), GFAP,  $\beta$ III-tubulin) expression in neuronal cells (neurons and glial cells), to clarify the mechanism underlying the CNS sequelae associated to WNV infection.

### Methods

2D cultures and 3D neuronal model were obtained with iPS (Induced Pluripotent Stem) cells and treated with purified WNV NS1. The mRNA and proteomic profiles were evaluated.

### Results

We observed the ability of soluble NS1 to affect the expression of neurodegenerative and dysfunctional biomarkers. In particular, NS1 induced A $\beta$  altered expression via TLR3, an endosomal Pathogen Pattern Receptors (PPRs) involved in RNA viruses sensing (Wang T. et al., 2004).

### Conclusion

Our preliminary results suggest a possible role of soluble NS1 on CNS damage associated to WNV infection. Interestingly, TLR3 increased expression has been found associated to A $\beta$  plaque in AD brains (Walker et al., 2018) and A $\beta$  itself stimulates TLRs expression, prompting the neurodegeneration (Caldeira et al., 2017). NS1 released by WNV infected cells might participate in CNS neurodegenerative process by altering TLR3 signaling and A $\beta$  expression, suggesting a novel pathogenetic role.

## The Highest EBV DNA Copy Numbers Are Detected in Untreated Patients with the High-Risk Chronic Lymphocytic Leukemia

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### Objectives

Chronic lymphocytic leukemia (CLL), the most common adult leukemia, remains incurable despite of introduced targeted therapies. CLL is frequently complicated due to immune system dysregulations. Autoimmune complications account for up to 25% of CLL patients. Among them, the most frequent are hemolytic anemia and thrombocytopenia, – both are the clinical symptoms of the high-risk CLL at the late stages. Epstein-Barr virus (EBV) is implicated in a number of autoimmune

diseases. Previously, three reports associated the high EBV DNA loads in peripheral blood with poor overall survival of CLL patients.

The form of CLL with the high-risk for progression is characterized by the CLL cells expressing the unmutated immunoglobulin heavy chain variable region genes (IGHV).

The aim of this work was to determine the quantity of EBV DNA in peripheral blood mononuclear cells (PBMC) of 71 untreated CLL patients and to analyze associations with the indicators of the high-risk disease.

### **Methods**

IGHV mutation statuses were assessed applying multiplex-PCR and sequencing. To detect the EBV DNA copy numbers, the commercial quantitative real-time PCR kit was used. Expression of the cell-surface markers, including CD38, CCR1 and CCR2, was analyzed by multiparameter flow cytometry.

### **Results**

The EBV DNA ( $> 5$  copies per  $10^5$  of PBMCs) was detected in 46.5% ( $n = 33$ ) of patients. The largest number of the EBV-positive patients was observed in the group with the late clinical stages III–IV (50.0% were EBV-positive). Unmutated IGHV (umIGHV) prevailed in the EBV-positive group, – in 64.0% ( $n = 21$ ). Among EBV-positive patients with  $> 100$  copies per  $10^5$  of PBMCs ( $n = 9$ ), 7 patients (77.8%) had umIGHV.

### **Conclusion**

The impact of EBV in CLL is not defined. According to our results, EBV can be one of the factors influencing progression of CLL. The personalized treatment selection can be improved by considering the all factors in the CLL disease progression.

## Possible Role of EBV in the Pathogenesis of Oral *Lichen Planus*

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### Objective

Oral *lichen planus* (OLP) is considered a T cell-mediated chronic inflammatory process activated by an unknown antigen, making basal keratinocytes vulnerable to a cytotoxic cell mediated immune response. The aim is to summarize information on the role and pathways of Epstein–Barr virus (EBV) and immune cells in inducing OLP as an autoimmune lesion.

### Methods

A search was conducted across databases of PubMed, Scopus, Research Gate, Web of Science, Science Direct, and Google Scholar. A total of 127 studies were identified that examined the potential correlation between EBV and oral epithelial cells with OLP.

### Results

We can assume that EBV can act both as an exogenous and an endogenous antigen in the pathogenesis of OLP. An important role in detecting and capturing antigens and modulating the adaptive immune response plays antigen-presenting cells (APC), such as dendritic cells (Langerhans cells, LC). Although EBV shows tropism for B cells and epithelial cells, under certain conditions it can infect monocytes, LCs, NK, and T lymphocytes. It means that under some circumstances of the chronic inflammatory process, EBV particles can react as endogenous agents. During the development of the autoimmune process, a decisive role is played by the loss of immune tolerance.

### Conclusions

Factors like the activity of cytokines, chemokines, and autoantibodies secreted by EBV-positive plasma cells, autoantigens formed due to virus protein mimicry of human proteins, new self-peptides released from damaged tissues, self-reactive B and T cells, dysregulation of LC function, the anti-apoptotic effect of EBV early lytic antigens, and an imbalance between inflammatory and anti-inflammatory immune cells facilitate the development of an autoimmune process.

## Session III – Myalgic Encephalomyelitis / Chronic Fatigue Syndrome

### The Role of the Gut Microbiome in the Pathogenesis of Autoimmune Disorders and Non-Pharmacological Interventions for Its Modification. An Example Chronic Fatigue Syndrome

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#### Introduction

The first part of the study aimed to assess the composition of the intestinal (gut) microbiome in Chronic Fatigue Syndrome (CFS) patients as compared to healthy controls (HC). A decrease in symptoms severity has been noted in response to whole-body cryotherapy (WBC) in multiple autoimmune disorders. Therefore, the effects of WBC and static stretching (SS) on symptoms severity and gut microbiome in CFS and HC have been examined.

#### Methods

Twenty-five CFS and sixteen HC fecal bacterial composition was examined using Illumina sequencing of 16S rRNA gene amplicons. Gut microbiota from 22 patients with CFS (18 females) and 10 HC (8 females) were examined before and after WBC+SS.

#### Results

Before WBC+SS, 143 (46%) microbiome genera were characteristic just for the CFS group. In addition, the gut microbiome in the CFS patient's group is characterized by a higher abundance of the 10 most common types of bacteria compared to the HC group. A significantly higher richness, indicated by an observed number of OTUs was observed in CFS compared to HC ( $p = 0.045$ ). Significant between-group differences in beta diversity of gut microbiome in CFS compared to HC were

noted. The three most discriminating *amplicon* sequence variants (ASVs) were: ASV 135, ASV 101, and ASV 5. In addition, we have obtained an excellent performance of machine learning methods in discriminating gut microbiome from CFS vs. HC. When analysing subjects who completed WBC+SS, no significant interaction between group and effect of intervention was noted ( $p = 0.39$ ). Supervised and non-supervised methods revealed no clear subgroups could be distinguished based on the gut microbiome.

### Conclusions

The gut microbiome is different in CFS patients in comparison to healthy people. After WBC+SS gut microbiome became more similar in CFS patients in comparison to HC. Further studies should assess the pathophysiological consequences of differences as well as the effectiveness of therapies aimed at modifying gut microbiome in CFS patients to a more comparable to healthy counterparts.

## Selection of Biomarkers in ME/CFS for Patient Stratification and Treatment Surveillance / Optimisation

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### Objective

We hypothesized the cohort of ME/CFS patients is divided into several groups/subsets of which one is having primary autoimmune aetiology, another one is triggered by viral infection. The group/subset triggered by viral infection also could have markers of autoimmunity. This study aimed to examine diagnostic potential of serum biomarkers depending on the presence/absence of viral infection biomarkers by direct comparing ME/CFS cases to healthy controls and applying the results from laboratory testing and pathology to the pattern recognition algorithm random forest to widen marker patterns usable in ME/CFS patient stratification.

### Methods

Various PCRs were used to detect presence of viral infection, activity phase and viral load; ELISA to detect antibodies against beta-2 adrenergic and M3/M4

acetylcholine receptors, and activin B level; Luminex multiplex technology to detect anti-human immunoglobulin class antibodies and cytokines' level. Selected statistical/machine learning methods were undertaken to explore the joint potential of biomarkers for classification of ME/CFS patients into severity groups, and this way also monitor the course and treatment effects.

### **Results**

A dataset was used originating from 188 persons, including 54 healthy controls, 30 patients classified as "mild", 73 as "moderate", and 31 as "severe", clinically assessed by ICC 2011 criteria. Activin B concentration was detected higher in subgroups of moderate and severe ME/CFS. The ROC analysis showed that anti- $\beta$ 2AdR has the best potential to be a good marker of ME/CFS in general, but, as with anti-M4, cannot be a discriminating factor for the severity of ME/CFS. Differences between ME/CFS and control group were observed IgG4 and also IgA levels. The high scores of IgG4 and IL-2 may indicate the presence of an autoimmune process underlying the ME/CFS onset.

### **Conclusion**

The highest classification power from data analysis is attributed to anti- $\beta$ 2AdR, anti-M4, IgG4, but also IL-2 (autoimmune processes marker) and IL-6 (marker for innate immunity).

## **Complementary Disease Management Approaches in Myalgic Encephalomyelitis / Chronic Fatigue Syndrome Patients**

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### **Objective**

There are over 100 autoimmune-related diseases affecting roughly 20% of the population, most undiagnosed. Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a chronic post-viral illness, for which immune dysregulation, autoimmunity, and virus reactivation are some of the key hypothesized mechanisms. Respecting the challenges of diagnosing such complex conditions and the limited specific therapies, this study aimed to provide insight into the complementary disease management approaches in ME/CFS.



## **Methods**

To achieve this aim, the guidelines of the National Institute for Health and Care Excellence (NICE, UK) on “Myalgic encephalomyelitis (or encephalopathy)/ chronic fatigue syndrome: diagnosis and management” (NICE guideline [NG206]) were reviewed and the Scopus, Web of Science (WoS), and PubMed databases were used to identify relevant scientific articles.

## **Results**

NICE guideline [NG206] notes that ME/CFS symptoms can be managed but there is currently no cure, and recommends energy management (EM) as the option for managing ME/CFS. EM is a self-management strategy that includes all types of activity (cognitive, physical, emotional, and social), led by the person themselves with support from an ME/CFS specialist team. The following keyword search for “energy management” AND “myalgic encephalomyelitis / chronic fatigue syndrome” in the databases found a limited number of items: in Scopus (5), WoS (3), PubMed (5), and ‘Pacing’ was identified as a type of EM provided for ME/CFS patients. Although ‘Pacing’ has been used and studied for at least 10 years, a scoping review published in October 2023 highlighted the challenges of unambiguously evaluating this approach.

## **Conclusions**

EM has been defined as one of the recommended disease management modalities for ME/CFS patients, but it is currently mainly expressed as a single ‘Pacing’. In the authors’ view, there are other EM methods (such as Qigong, Yoga, and Coaching) whose potential should be explored for improving the quality of life of ME/CFS patients.

## Kynurenine and Human Herpesvirus-6B in Myalgic Encephalomyelitis / Chronic Fatigue Syndrome

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### Objective

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex and poorly understood illness potentially triggered by human herpesvirus (HHV) infection. Kynurenine is an amino acid involved in the metabolism of tryptophan, producing metabolites that regulate immune response, inflammation, and brain function. Abnormalities in kynurenine metabolism may play a role in the development and progression of ME/CFS. Aim of this study was to determine contribution of HHV-6B infection and kynurenine level in the course of the ME/CFS.

### Methods

29 patients with ME/CFS and 23 controls were enrolled in the study. DNA was isolated with phenol-chloroform method, concentration measured spectrophotometrically and quality assessed by analysis of  $\beta$ -globin gene. HHV-6 Real-TM Quant and RealStar HHV-6 kits were used for quantitative analysis of HHV-6 load.

Kynurenine concentration was determined spectrometrically in blood plasma. Fluorescence was measured with a plate reader and the concentration was calculated by the linear regression of the optical density from 492 nm minus 620 nm. Statistical analysis was performed using GraphPad Prism v.9.0.2.

### Results

Based on adapted semi-structured interview questions by Minnock et al., 2015, 22 patients had a moderate course of ME/CFS and 7 patients exhibited mild ME/CFS symptoms.

Mean  $\pm$  SD (standard deviation) kynurenine concentration in blood plasma from patients with ME/CFS was  $2.418 \pm 0.572 \mu\text{M}$  and in control group individuals  $2.692 \pm 0.52 \mu\text{M}$ .

Analysing levels of kynurenine and patients' age, a significant difference between groups of control individuals, patients with mild and moderate ME/CFS was found ( $p = 0.0413$ ).

Mean HHV-6B load in PBMC from patients and controls was 10.21 and 0.18 copies in  $10^6$  cells, respectively.

### **Conclusions**

The current results support the involvement of HHV-6B infection in patients with ME/CFS. Kynurenine was selectively upregulated in patients with ME/CFS and controls.

More research is needed to fully clarify the possible link between kynurenine, HHV-6B infection and course of ME/CFS.

## Session IV – Inflammation and Autoimmunity

### Mitochondrial Myopathy as a Mimic of Inflammatory Myopathy

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#### Objectives

Mitochondrial myopathies (MM) are a genetically and phenotypically diverse group of mitochondrial diseases, with broad involvement severity of the muscle tissue. Resulting in a muscle weakness, pain, exercise intolerance, even life-threatening manifestations, it can mimic inflammatory myopathies.

#### Case Description

We present a case of 22-year-old Latvian male who initially presented with a 6-month history of fatigue, generalized weakness, and dyspnea on exertion, furthermore, a right lower leg oedema for a week.

Physical examination revealed weakness in distal and proximal muscle groups of upper and lower extremities. Laboratory analysis depicted elevated serum creatinine kinase, creatinine kinase-MB, high sensitivity troponin-T, aspartate aminotransferase and alanine aminotransferase. Autoantibody tests for autoimmune rheumatic disorders, including myositis, were negative. Paraneoplastic syndromes, endocrine pathologies, storage diseases, chronic infections were ruled out. Electromyography revealed myogenic injury with higher prevalence in leg muscles, and no associated polyneuropathy. Histological finding revealed signs of inflammatory myopathy. Based on the obtained data clinical diagnosis of unspecified myositis and myopericarditis was established.

The patient received methylprednisolone therapy with no long-standing clinical improvement. He returned to the hospital repeatedly due to progressive heart failure, oedema of the extremities, myalgias, muscle atrophy with contractures in both elbows. Following implementations of corticosteroids, L-carnitine, and thiamine resulted in gradual depletion of efficacy and clinical deterioration. Patient's mother recalled her son avoiding continuous physical activities during youth,

moreover patient's grandmother's 2 out of 7 sisters suddenly dying at the age of 45. Subsequently, genetic testing revealed MT-TL1 gene mutation (m.3260A>G transition), confirming the diagnosis of mitochondrial myopathy with skeletal and cardiac muscle involvement. The treatment with mitochondrial cocktails was emerged. Now the patient is physically active with no impairment of daily routine.

### Conclusions

This case report raises the awareness of genetic disorders like mitochondrial myopathy as a mimic of inflammatory myopathy, to prevent the delay in diagnosis.

## Low-Grade Inflammation Contribution to Neuropsychiatric State in Osteoarthritis Patients

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### Objectives

Osteoarthritis (OA) is no longer viewed as only a degenerative disease but rather a complex, multifactorial disease involving the joint as a whole organ. The pathophysiological process includes articular cartilage degeneration, decreased repair, subchondral bone sclerosis, chondrocyte apoptosis, and low-grade inflammation, as inflammation plays an integral role in the pathogenesis of osteoarthritis, leading to joint destruction. Depressed mood and cognitive decline are frequently found in OA patients. Both depression and OA are disabling conditions with a significant impact on a patient's life. This study evaluates the possible connection between chronic low-grade inflammation and cognitive changes in OA patients.

### Materials and Methods

Fifty patients with no underlying chronic diseases affecting cognition (such as diabetes mellitus, strokes, myocardial infarction, and others) were enrolled in the study. PHQ-9, GAD-7, and WOMAC tests were performed for all of the patients; thirty-three patients also had the MoCA test. Tests were made before the surgical operation and without the use of narcotic analgesia or premedication. Synovial

biopsies were obtained during the surgical procedure. The histopathology of synovitis was analysed according to the Krenn histopathological grading system under a light microscope.

### **Results**

Mean synovial inflammation, Krenn score was measured as 3.3 (0-7), consistent with low-grade synovitis. VAS mean pain score was 6.3, ranging from 2 to 10. The mean BMI score was 30.5, ranging from 19.5 up to 45.2. The mean PHQ-9 was 6.8, ranging from 0 up to 21, and the mean GAD-7 was 5.08 (0-19). WOMAC score means value was determined as 46.2, ranging from 7 up to 91. MoCA mean value was 26, ranging from 20 u to 30. Statistical analysis using the Spearman method found a correlation between PHQ-9 and Krenn score ( $r = 0.305$ ;  $p = 0.037$ ).

### **Conclusions**

The findings state that inflammation affects depressive states in OA patients.

## Session V – COVID-19 Autoimmune Aspects

### Functional Single Nucleotide Polymorphisms (SNPs) Linked to Autoimmune Manifestations in Long COVID

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In hyperinflammatory states of acute virus infections, homologous sequence regions in viral genes can lead to crossreactive autoantibodies and complement mediated tissue destruction, danger signaling and chronicity of inflammation.

To identify the most relevant single nucleotide polymorphisms (SNPs) causing autoimmunity by virus infections, long COVID patients were genotyped for 90 functional SNPs related to methylation, endogenous antioxidants, hormonal stress competence, cytokine driven inflammation, phase I/II detoxification, and immune capacity genes.

The identification of functional mutations in the above group of genes might contribute to a lower threshold of activation for low-affinity T cell synapse formation. High cytokine conditions would allow low-affinity clones to be selected and lead to an autoimmune phenotype. Specifically, methylation deficiencies may explain chronicity of clinical signs of virus infections, especially in states of low natural killer cell (NK-cell) activity.

SNPs were determined by The Infinium Global Screening Array-24 v3.0 BeadChip (Illumina.com). Immune phenotypes of T-, B-, NK-cells and monocytes and dendritic cells were identified by flow cytometry. Plasma cytokines were quantified by Immulite 1000® (Siemens) and ELLA™ multiplex assays (Biotechné). Autoantibodies against interferons (IFN- $\alpha$ , - $\beta$ , - $\gamma$ , - $\omega$ , - $\lambda$ ), IL-6, IL-8, and TNF- $\alpha$  were detected using Luminex.

Following visual programming work, long COVID patients were found to be unique by multiple SNPs in methylation genes (MTR, MTRR, MTHFR), and PEMT, further related to anti-oxidant gene mutations (GST-M, GST-P, SOD-2). Neurological disease phenotypes in long COVID had multiple SNPs in phase 2 detoxification genes

such as NAT2, genes involved in stress compensation, and P2X7 linked to inflammatory activation and calcium signalling. These SNPs were combined with dopamine metabolism and adenosine signalling pathways. Increased cytokine driven chronic inflammation could be explained by gain-of function SNPs in cytokine genes, TLRs, MyD88 and elements of the NFκB pathway.

## **Gestational COVID-19: Maternal and Foetal Morphological Alterations Caused by SARS-CoV-2 Infection**

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### **Background**

The evaluation of the effect of SARS-CoV-2 infection during pregnancy has raised interest. Even if virus vertical transmission is still controversial and the rate of mother-to-newborn transmission is low (Flannery et al., 2022) several researches have focused on the possible distinctive markers associated with different susceptibility to SARS-CoV-2 infection during pregnancy.

### **Aim**

Evaluate the effect of SARS-CoV-2 infection at tissue level in gestational COVID-19.

### **Methods**

Morphological alterations were assessed in the placental / chorionic villi, chorionic plate, basal plate, and umbilical cord tissues obtained from 7 subjects with symptomatic respiratory SARS-CoV-2 infection and compared with 7 non-COVID control subjects, and also in tissues obtained from 27 weeks old COVID-19 positive foetus dead after 37 days by pulmonary embolism and thrombosis of the superior vena cava. The expression of SARS-CoV-2 Nucleoprotein (NP) and Human Leukocyte Antigen-G (HLA-G) was estimated by the use of immunohistochemistry.

### **Results**

The 57%, 42,8%, and 28,6% of placental / chorionic villi, chorionic plate, and basal plate, respectively, were found positive for NP antigen ( $p < 0.01$ ), while none of the umbilical cords stained for NP. The presence of NP positivity correlated with



high levels of the fibrinoid component in placental / chorionic villi samples and leukocyte infiltration in basal plate. All the NP positive chorionic plate and half of the NP positive basal plate samples expressed HLA-G, but with lower H-score compared to non-COVID subjects ( $p < 0.05$ ).

SARS-CoV-2 NP protein were detected in several foetal tissues, particularly in the oesophagus, stomach, spleen, and heart, with a significantly higher H-Score than the placenta ( $p < 0.05$ ) (Greco et al., 2023).

### **Conclusions**

The presence of SARS-CoV-2 infection in gestational tissues correlates with morphological alterations and a decreased HLA-G expression compared to the control group and intrauterine SARS-CoV-2 transmission might be considered as a possible cause of complications in the newborn, suggesting prompt diagnosis and therapy as beneficial for both maternal and newborn health.

These data suggest a possible implication of SARS-CoV-2 infection in morphological and protein expression modification during pregnancy, which might impact on infection susceptibility, pregnancy complications and vertical transmission.

## **Association of Liver-Related Autoantibodies with Possible Post-COVID Condition**

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### **Objective**

The post-COVID condition involves symptoms lasting weeks, months, or years after COVID-19 recovery. From a theoretical perspective, immune-mediated reactions with cross-reactive antibodies triggered by the SARS-CoV-2 virus and SARS-CoV-2 vaccines may impact liver cells. Our study aimed to assess the possible development of an autoimmune process in the liver by detection of circulating antinuclear antibodies (ANA), anti-liver kidney microsomal type 1 antibodies (anti-KLM1), antibodies against soluble liver antigen (anti-SLA), and the total immunoglobulin G (IgG) and investigate the association between blood parameters and post-COVID condition.

### **Methods**

The study included 27 patients 2.5–3 years after the first acute COVID-19 in 2020. All patients were clinically examined and interviewed regarding their subjective recovery, long-lasting symptoms, comorbidities, reinfections with SARS-CoV-2, and SARS-CoV-2 vaccination. Autoantibodies were detected by ELISA (Alegria ORGENTEC Diagnostika GmbH, Germany). Blood tests included IL-6, CRP, ALT, AST, and other parameters.

### **Results**

Forty-four percent of patients had reinfection with the SARS-CoV-2 without hospitalization, 88% were vaccinated, and 63% reported new long-lasting symptoms after the first COVID-19. The subjective perception of health was not associated with reinfections, SARS-CoV-2 vaccination, and blood parameters except for total IgG and autoantibodies. Despite clinically insignificant levels of total IgG (median 11.2 g/l), ANA IgG (median 0.3), anti-LKM1 (median 2.0 U/l), and anti-SLA (median 1.1 U/l), we found higher levels of ANA and anti-LKM1 ( $p = 0.013$ ) in patients who reported no recovery at 2.5–3 years than in patients reported recovery. In parallel, long-lasting symptoms were associated with higher levels of anti-SLA ( $p = 0.042$ ) and IgG ( $p = 0.031$ ).

### **Conclusions**

Despite the exclusion of autoimmune hepatitis after COVID-19 or SARS-CoV-2 vaccination, levels of anti-LKM1, ANA, anti-SLA, and total IgG were positively associated with reported post-COVID symptoms, supporting the opinion on the autoimmune genesis of the post-COVID condition.

## **New Onset of Autoimmune Diseases Following COVID-19 Diagnosis and Vaccination**

**Hele Everaus**

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The diagnosis of COVID-19 increases the risk of new-onset autoimmune diseases that affect the nervous system, musculoskeletal system and endocrine system.

Right now, we are seeing Tsunami of autoimmune issues after SARS-CoV-2 infection (COVID-19) and after COVID vaccination. Recent findings suggest that the incidence of autoimmune diseases triggered by COVID-19 vaccines is on rise, highlighting the pressing need to identify high-risk and vulnerable populations. It is important not to ignore the potential side effects of vaccination. The true incidence

of these diseases after vaccination remains difficult to determine, as not all cases are or will not be reported.

What can we do?

We should not never ignore any symptom of patient. Chest pain, any cardiac symptoms, especially in our youth who have been vaccinated.

We need to check antiphospholipid antibody.

Patients autoimmune laboratory tests should be researched yearly if not every six months.

The long-term implications for emerging autoimmune issues after COVID vaccination and after contracting COVID are unknown. Whether we see more instances of pericarditis, as well as new onset multiple sclerosis, vasculitis, autoimmune neuropathy, thyroid disease, hepatitis, and potentially other illnesses is something that remains to be seen and about which we must stay vigilant going forward.

And we must take time to really listen to patient input and any complaint in order truly identify the causes behind their symptoms.

It is our responsibility to remain vigilant and actively understand the serious adverse events associated with COVID-19 vaccines, critically evaluate vaccine safety and increase public and healthcare worker awareness regarding vaccination.

This will enable prompt identification, diagnosis, and treatment of these autoimmune diseases following vaccination through the recognition of their clinical and laboratory features. We need this approach in order to develop an efficient COVID-19 vaccination long term strategy with a low risk side effects.

## **Determination of Population Health Biomarkers in Wastewater Samples**

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### **Objective**

Wastewater-based epidemiology (WBE) aims to monitor the population's health and the prevalence and spread of infectious diseases such as SARS-CoV-2 by analysing biomarkers in wastewater and providing valuable early detection and surveillance data to public health authorities. This report overviews results obtained by Institute "BIOR" scientists related to applying the WBE approach to assess community-wide trends and identify potential outbreaks, helping inform targeted public health interventions.

### **Methods**

Real-time polymerase chain reaction methods and high-resolution mass spectrometry were used to obtain results from wastewater samples. Sampling was conducted from December 2020 to July 2023 in wastewater treatment plants in Latvia's most populous cities.

### **Results**

This study used the WBE to investigate temporal trends of 40 biomarkers in wastewater over 32 months in several cities of Latvia. During the pandemic, there was a rise in the intake of specific pharmaceuticals, including antihypertensives, antidepressants, and ibuprofen, mainly while strict restrictions were in place. Distinct seasonal trends were discovered in the consumption patterns of antibiotics, the anti-asthmatic drug salbutamol and the decongestant xylometazoline, where higher consumption occurred during colder seasons, as expected.

The study confirmed that WBE-based SARS-CoV-2 RNA monitoring is extremely useful and acquired viral RNA data correlated with a 35-day cumulative incidence of SARS-CoV-2 infection cases. Data from mobile calls and the content of population size biomarkers in wastewater samples could be used effectively to better understand and improve the accuracy of WBE.

### **Conclusions**

This study demonstrated the effectiveness of WBE in monitoring temporal trends and detecting changes in pharmaceutical consumption, emphasising the utility of advanced methodologies in tracking public health-related information.

## Session VI – Challenges and Solutions

### Infra-Red Spectroscopy in Diagnosis of Autoimmune Diseases

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Infrared spectroscopy a technique that can be used to analyse the chemical composition of a sample by measuring the absorption or transmission of infrared radiation. This technique has shown potential in the diagnosis and management of autoimmune diseases (AIDS). Infrared spectroscopy (IR) can provide a molecular fingerprint that can be used to compare samples and identify functional groups. IR spectrometry has been used to differentiate between the sera of rheumatoid arthritis (RA) patients and blood donors and to identify unique IR spectral patterns that correlate with typical RA autoantibody. IR has also been used to discriminate fibromyalgia from other rheumatologic disorders based on blood plasma samples. [1–3].

The results showed that FT-IR spectroscopy combined with chemometrics could be useful for fibromyalgia diagnosis [4, 5]. Extracellular vesicles (EVs) have been studied in various contexts related to fibromyalgia: EVs from mesenchymal stromal cells (MSCs) have therapeutic potential in musculoskeletal regeneration, MSCs were shown to inhibit and revert fibrosis progression in mouse models of diabetic nephropathy [6, 7]. Ore preliminary data on using FTIR spectroscopy of plasma and plasma-derived EVs from patients with fibromyalgia and different control subjects pointed to possibility of using this tool for diagnosis and follow-up of patients with autoimmune disorders. While research on the role of EVs in fibromyalgia is still limited, the potential therapeutic applications of EVs warrant further investigation.

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## Integrative Medicine Approach in the Treatment of Autoimmune Diseases

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### Objective

Autoimmune diseases pose significant health challenges, and conventional treatments often have limitations. The objective of this study is to assess the frequency of use of integrative medicine in the treatment of autoimmune diseases through evidence-based data analysis. Integrative medicine combines the principles of conventional Western medicine with complementary therapies to create a holistic and patient-centered approach to healthcare.

### Methods

A systematic literature review was conducted, including studies published until October 2023. PubMed database was searched systematically for relevant articles. Inclusion criteria considered randomized controlled trials (RCTs), cohort studies, and case-control studies focusing on integrative medicine interventions for autoimmune diseases. Data from selected studies were extracted and synthesized to assess the impact of integrative medicine approaches.

### Results

A total of 3145 studies met the inclusion criteria and were included in the analysis. Integrative medicine interventions encompassed acupuncture (470), herbal remedies (2434), yoga (128), taichi (13) and meditation (100). Studies covered a range

of autoimmune diseases, including rheumatoid arthritis, lupus, multiple sclerosis, and inflammatory bowel disease. The results demonstrated a variable reduction in disease activity scores for autoimmune patients undergoing integrative medicine treatments. Improvements in quality of life, pain management, and psychological well-being were observed across multiple autoimmune diseases.

### **Conclusions**

This evidence-based data analysis supports the potential of integrative medicine approaches as valuable adjunct therapies for autoimmune diseases. Integrative medicine interventions show promise in improving disease activity, enhancing the quality of life and addressing psychological well-being among patients. A healthier workforce is more productive, leading to economic growth and reduces the strain on public health resources. However, the varying degrees of effectiveness across different autoimmune diseases emphasize the importance of personalized treatment strategies. Further research should explore the long-term effects, safety profiles, and optimal combinations of integrative medicine approaches in managing autoimmune diseases.

## **Personalized Physical Activity as a Therapeutic Strategy for Autoimmune Diseases**

**Yuri Ostrinski**

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The clinical management of autoimmune diseases, in which the immune system aberrantly targets self-tissues and organs, has historically centered around pharmacological interventions.

Recent insights, however, show the potential of personalized physical activity regimens to complement traditional therapeutic approaches. Given the heterogeneity of autoimmune diseases presentations and courses, a one-size-fits-all approach to physical activity is not optimal.

This presentation reveals the basic principles of personalized targeted physical activity, clinical applications, and patient-centered considerations for integrating personalized physical activity regimens into autoimmune diseases maintenance and treatment strategies.

By tailoring a physical exercise regimen to a patient's disease specificity, severity of symptoms, physical capacity, age and other clinical markers, healthcare professionals can harness the immunomodulatory and symptom-mitigating effects of tailored physical activity, thereby facilitating holistic, patient-centered care.

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## Circulating miRNAs Expression in Myalgic Encephalomyelitis / Chronic Fatigue Syndrome

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### Objective

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex multifactorial disease that causes increasing morbidity worldwide, and many individuals with ME/CFS symptoms remain undiagnosed due to the lack of diagnostic biomarkers. Its aetiology is still unknown, but increasing evidence support a role of Herpesviruses (including Human Herpesvirus 6 species, HHV-6A and HHV-6B) as potential causative agents [1]. Interestingly, the infection by these viruses has been reported to impact the expression of microRNAs (miRNAs) [2, 3], short non-coding RNA sequences, which have been suggested to be epigenetic factors modulating ME/CFS pathogenic mechanisms. Notably, the presence of circulating miRNAs in plasma has raised the possibility to use them as valuable biomarkers for autoimmune diseases [4]. Thus, this study aimed at determining the role of eight miRNAs, which were selected for their previous association with ME/CFS, as potential circulating biomarkers of the disease.

### Methods

Forty ME/CFS patients and 20 healthy controls were recruited at the Rīga Stradiņš University Ambulance outpatient clinic and miRNA presence was quantitatively evaluated in plasma from by specific Taqman assays.

### Results and Conclusions

Six out of the eight of the selected miRNAs were differently expressed in patients compared to controls; more specifically, five miRNAs were significantly upregulated (miR-127-3p, miR-142-5p, miR-143-3p, miR-150-5p, and miR-448), and one was downmodulated (miR-140-5p). miRNA levels directly correlated with disease severity, whereas no significant correlations were observed with the plasma levels of seven pro-inflammatory cytokines or with the presence/load of HHV-6A/6B genome, as judged by specific PCR amplification. Target genes of altered miRNAs were involved in pathways related to transcriptional control of herpesvirus reactivation, immune response to viral infection, extracellular matrix remodeling,

inflammation, cell viability and immune cell death. The results may open the way for further validation of miRNA as new potential biomarkers in ME/CFS and increase the knowledge of the complex pathways involved in the ME/CFS.

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## **Coinfection of Dermal Fibroblasts by Human Cytomegalovirus and Human Herpesvirus-6 Can Boost the Expression of Fibrosis-Associated Micrnas**

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### **Objective**

Human cytomegalovirus (HCMV) and Human herpesvirus type-6A (HHV-6A) have been reportedly suggested as triggers of the onset and/or progression of many autoimmune diseases, including Systemic sclerosis (SSc), a severe autoimmune disease with still unclarified etiology, causing progressive fibrosis of skin and internal organs [1]. Reactivation of such viruses and specific antiviral immune responses have been detected in SSc patients, and infection by HCMV or HHV-6A was shown to induce the expression of fibrosis-associated transcriptional factors and miRNAs in human dermal fibroblasts [2, 3]. However, it is unlikely that such viruses have separated effects on infected cells since both viruses are ubiquitously present in the human population and can mutually boost each other. Consistently, we recently reported that the simultaneous presence of HCMV and HHV-6 induced a higher expression of fibrosis-associated factors associated, compared to what observed in single infected cells [4]. Based on these observations, this study aimed to investigate the effect of HCMV/HHV-6A coinfection on primary human dermal fibroblasts, focusing on the expression of miRNAs associated with pro-fibrotic pathway.

### **Methods**

Human primary dermal fibroblasts were infected *in vitro* with cell-free inocula of HCMV (T40E) and HHV-6A (U1102), and samples were collected at different times post infection (0, 1, 2, 4, 7 and 10 d.p.i.). Total nucleic acids were extracted from collected cells and analysed by virus-specific real time quantitative PCR (qPCR), and by qPCR microarrays simultaneously detecting and quantifying 84 human microRNAs associated with cell fibrosis.

### **Results and Conclusions**

The results evidenced increased HCMV and HHV-6A replication in coinfecting cells, accompanied by increased induction of fibrosis-miRNAs in coinfecting

compared to single-infected cells, thus supporting the hypothesis that HCMV and HHV-6 can enhance each other and may cooperate at inducing enhanced miRNA-driven fibrosis. These data also suggest the use of virus-induced miRNAs as novel diagnostic or prognostic biomarkers for SSc and its clinical treatment.

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## Session VII – Challenges and Solution II

### Immune-Related Adverse Events During the Treatment with Anti PD-1 Antibodies in Melanoma Patients: Literature Review and Two Year Experience at REUH

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#### Objective

To describe the association between use of anti PD-1 antibodies and immune-related adverse events (IrAEs) among patients with cutaneous melanoma based on literature data and single centre experience.

#### Method

Literature review and analysis of medical records from 72 locally advanced or metastatic cutaneous melanoma patients who received > 1 cycle of anti PD-1 antibodies at REUH Outpatient clinic from November 2021 to October 2023. IrAEs were graded using the CTCAEv5.

#### Results

IrAEs typically arise between 3 and 14 weeks from starting anti-PD-1 based therapy but may occur at any point during treatment, and even after discontinuation of

the drug. IrAEs are common and have been reported to occur in 70% of patients treated with this type of monoclonal antibodies.

Among the cohort (387 patients) observed in literature, during the treatment of melanoma IrAEs was reported in 69% of patients, including 44.2% with grade 2 or higher and 13.4% with grades 3 through 5.

IrAEs are organ specific, with skin-related adverse events being the most common – 26–42% followed by endocrine IrAEs (thyroiditis/ hypothyroidism 16–34%), arthralgia 11–16% and colitis/diarrhea (10%). Studies have reported that melanoma patients have a higher risk of vitiligo than other people, and the incidence is much higher than that of other tumour types.

15 out of 72 melanoma patients treated with anti PD-1 antibodies at REUH Outpatient clinic developed IrAEs during the treatment: hypothyroidism was diagnosed in 8 patients, lichenoid dermatosis – in 3 patients, vitiligo – in 2 patients and colitis – in 2 patients.

### **Conclusion**

According to the literature data cutaneous IrAEs are the most common, followed by endocrine IrAEs in melanoma patients treated with antiPD-1 antibodies. Hypothyroidism was the leading adverse event in our observation.

## **Suboptimal Selenium Serum Concentrations in Latvian Patients with Autoimmune Gastritis and Autoimmune Thyroid Disorders**

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### **Introduction**

The prevalence of different autoimmune disorders (AID) constantly increases. Autoimmune gastritis (AIG) is a non-self-limiting, chronic inflammatory disorder affecting the oxyntic mucosa leading to progressive mucosal atrophy. The inflammatory process in AIG seems to be mediated by autoreactive T cells, although the exact causative agent is unknown. Selenium is an essential trace element with fundamental effects on human biology because seleno-proteins are essential for protecting against oxidative stress and play a vital role in the immune system regulation and production of active thyroid hormone. Increasing serum selenium concentration up to about 135 µg/L is associated with decreased mortality.

### **Objective**

We aimed to assess selenium serum concentrations in Latvian patients diagnosed with autoimmune gastritis or autoimmune thyroid gland diseases and compare it with selenium concentrations in healthy controls.

## Methods

One hundred and twenty-eight AIG patients, 47 Hashimoto's thyroiditis (HT) patients, 13 Graves' disease (GD) patients, and 49 healthy subjects were included in this study. Plasma selenium concentration was determined fluorometrically by using a fluorescence spectrophotometer.

## Results

AIG group consisted of 87 women and 41 men with mean age 66.41, SD  $\pm$ 11.43. In HT group were 44 women and 3 men with mean age 41, SD  $\pm$ 11.53. In GD group were 11 women and 2 men with mean age 41, SD  $\pm$  13.03 and controls consisted of 38 women and 11 men with mean age 30, SD  $\pm$  10.25. Median plasma selenium levels were 83.29 (19.03–151.31)  $\mu$ g/L for AIG patients, 92.19 (80.89–103.49)  $\mu$ g/L for HT patients, 76.42 (55.76–97.08)  $\mu$ g/L for GD patients and 94.76 (17.75–173.60)  $\mu$ g/L for the controls. We found a significantly lower selenium concentrations in AIG patients compared to controls ( $p < 0.05$ ). No differences were observed in selenium levels between HT, GD and controls and no differences were observed in selenium levels between AIG and HT or GD patients.

## Conclusions

Selenium status in Latvian patients with diagnosed AIG, HT, and GD disease is at a suboptimal level, although there is significant difference between AIG disease patients and controls. It might be suspected that AIG causes low plasma selenium levels and low plasma selenium levels further facilitate AIG.

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## Resistance of *Enterococcus* Isolates in Hospitalised Patients from Two Multidisciplinary Hospitals: From Science to Practice

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### Objectives

The aim of the study was to investigate the enterococci containing isolates, their antimicrobial resistance in hospitalized patients of two multidisciplinary hospitals.

### Methods

*Enterococci* containing isolates were collected from two multidisciplinary hospitals. Genomic DNA sequences of these isolates were consequently obtained through Illumina short-read sequencing and *de novo* assembly. To identify the relevant antimicrobial resistance genes and point mutations in *Enterococcus* genomes, ResFinder tool and database was utilised. Furthermore, we determined multi-locus sequence types (MLST) to facilitate epidemiological comparisons.

### Results

We discovered five various *Enterococcus* genera – *E. faecalis* (n = 85), *E. faecium* (n = 55), one of each *E. gallinarum*, *E. avium*, *E. durans*. The most frequent were *E. faecalis* and *E. faecium* species. Our analysis revealed a diverse array of AMR determinant genes and mutations within *Enterococcus* isolates obtained from hospitalised patients. Predominantly, genes encoding resistance to aminoglycoside and tetracycline antibiotics were observed in the dataset. 60.00% *E. faecalis*, 100% *E. faecium*, and one *E. avium* were multiresistant isolates. Obtaining results from analysed samples, 21 genes were present causing resistance against antibacterial agents, including aminoglycosides, macrolides, lincosamides, glycopeptides, tetracyclines, trimethoprim, and amphenicols. *ClpL* gene, which is responsible for bacteria to be stress tolerant (high temperatures and better biofilm producers), was also detected.



### **Conclusions**

Enterococcus isolates were multiresistant in most of the isolates, especially *E. faecium* containing samples. The results show that lincosamides are not efficient against *E. faecalis* infections. Aminoglycosides, macrolides, and penicillins are not efficient against *E. faecium*. *ClpL* gene in obtained isolates are responsible for stress and high temperature tolerance. Hospitalised patients are often immunocompromised; therefore, prevention and eradication of such multi-resistant bacteria is crucial. Furthermore, the misuse of antibiotics can drive the emergence of resistant bacterial strains, intensifying the burden of healthcare. Addressing these issues requires the expertise in infectious diseases, following antimicrobial stewardship programs, and in immunology.

## Session VIII – WIDESPREAD Project’s Achievements

### Implementation of VirA Project “Reducing Networking Gaps Between Rīga Stradiņš University (RSU) and Internationally-Leading Counterparts in Viral Infection-Induced Autoimmunity Research”

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The aim of VirA project is to promote autoimmune disease research capacity and fill networking gaps in the institution (RSU) of the low-performing Member State – Latvia, by establishing a consortium with leading research institutions from Italy – University of Ferrara, Germany – Ulm University, and Israel – Tel-Aviv University.

The planned project activities are:

- Raising experience of the staff with short term exchanges;
- Bringing new knowledge to the institute from expert visits and short-term on-site or virtual training;
- Organization of training workshops, joint summer schools and seminars to increase the networking ability of staff and foster knowledge transfer;
- Conference participation and attendance to help with project dissemination and gathering of new information.

Within the frame of the project 8/8 outgoing and 8/9 incoming ECR visits are organized, 10/10 expert visits with short-term on-site training have been implemented bringing new theoretical and practical knowledge to the RSU.

Six workshops on “Importance of differential diagnostic in rheumatic and other autoimmune diseases”, “New trends in autoimmune diseases immunological profiling”, “Viral infections as aetiological or trigger factors of autoimmune

diseases", "Morphological studies in autoimmune disease research", "Basic aspects of biostatistics, clinical and laboratory data management" and "A practical workshop on proposal preparation and project management"; one seminar "Enhancement of scientific manuscripts preparation quality" and two Summer schools "New trends in molecular and immunological detection methodology of persistent viral infections", and "Clinical and laboratory data management and modelling" have been organized. Two LATVIA-ISRAEL Symposiums of Autoimmunity (one on Zoom, another in Israel) have been organized, as well as regular participation in the Friday Mosaic of Autoimmunity International e-Meetings organized by Prof. Yehuda Shoenfeld. The number of publications in RSU (in the field of autoimmunity) has increased by 24.4% per year on average, researchers have participated in 18 international conferences/congresses with 59 presentations.

## **Strategies for Advanced Development of Antibacterials – Springboard Project's Achievements**

**Raivis Žalubovskis**

*Latvian Institute of Organic Synthesis*

Springboard – European Union's Horizon 2020 Twinning project for building an excellence platform in the area of advanced discovery of novel antibacterial drugs to strengthen the research potential of the Latvian Institute of Organic Synthesis.

The project deals with four main scientific topics for the development of novel antibacterials:

- 1) the identification of new enzymatic targets;
- 2) application of natural products and their synthetic analogues;
- 3) biopharmaceuticals, antisense, and peptide antibiotics as antibacterials;
- 4) biofilm formation and quorum sensing molecules as targets for new antibacterial therapies.

The discussions on four scientific topics as well as achievements of Springboard project will be presented.

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## Autoimmunity Research in European Agendas

**Uldis Berķis**

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Research and Innovation, Ministry of Education and Science, Latvia*

VirA project has covered a very important niche – evolving autoimmunity research under the emerging long-COVID nosology. European Union Framework programmes have several funding modes – ranking from traditional focused work programme topic-based calls to member state and Commission partnerships on main health challenges. Autoimmunity is presented in all instruments, however, a focused instrument only on autoimmunity is absent yet. Member state initiatives have traditionally focused towards main blocks in disease classification – ERA-NET NEURON and the emerging European Brain partnership, translational cancer research (including immunotherapies) in ERA-NET TRANSCAN and Cancer mission, European partnerships on health research ERA4HEALTH and THCS. Forthcoming calls on nanomedicine and health system transformation best coincide with the autoimmune diseases research agenda.

Building on VirA focus, also digital agenda is of importance, as autoimmunity is characterised by complex interlay of various processes, reflected in multitude of biomarkers. Artificial intelligence affects both medical profession and the patient awareness. Digital assistance is covering needs not addressed before.

In 2023 after prolonged gap period, a national research programme in public health will be reinstated. Autoimmune diseases in the health system is a topic of importance. VirA project best combines modern views on care and prevention.

It is not an easy task to navigate in research funding network where narrow topic-based approach combines with priority ranking. Autoimmunity due to better understanding is a domain which importance has shown permanent increase and advanced therapies have contributed to patient perspectives.

## Poster session

### HHV-7 Protein Expression in the Synovial Tissue of Osteoarthritis Patients and Its Possible Contribution to the Degree of Inflammation

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#### Objectives

Osteoarthritis (OA) is an acknowledged degenerative joint disease with a variable degree of inflammation. HHV-7 viral proteins are found in the blood specimens and synovial tissues of patients diagnosed with chronic arthritides, but their impact on the course of the disease must be elucidated. The aim of the study was to analyse the presence of HHV-7 proteins and assess the extent of tissue inflammation based on the Krenn et al. score and TNF $\alpha$  expression within different synovial membrane compartments of patients with OA.

#### Materials and Methods

Thirty-one OA synovial tissue specimens were stained routinely as well as immunohistochemically with antibodies both against the HHV-7 protein and the pro-inflammatory cytokine TNF $\alpha$ . The histopathology of synovitis was analysed according to the Krenn histopathological grading system under a light microscope. Both HHV-7 and TNF $\alpha$  expression were assessed quantitatively.

#### Results

Synovial inflammation severity was evaluated as 2 (IQR 0–5), consistent with low-grade synovitis. TNF $\alpha$  expression demonstrated a positive correlation with the severity of synovitis ( $p = 0.005$ ). HHV-7 protein expression was found in synovial lining cells ( $p < 0.001$ ), lymphocytes ( $p < 0.001$ ), macrophages ( $p < 0.001$ ), and vasculature cells ( $p < 0.001$ ). The statistically significant difference in HHV-7 protein expression between low-grade ( $8.47 \pm 6.06$ ) and moderate-grade ( $18.3 \pm 13.3$ )

synovitis groups was established consisting with 9.82, CI [1.63–18.0],  $p < 0.02$ ,  $d = 0.948$ . The positive correlation between HHV-7 protein expression in synovial cells and macrophages ( $r = 0.638$ ;  $p < 0.026$ ), as well as lymphocytes ( $r = 0.608$ ;  $p < 0.001$ ) was established. The positive correlation between HHV-7 protein expression and Krenn score was observed ( $r = 0.540$ ;  $p < 0.003$ ). However, there was no statistically significant role of HHV-7 protein expression in the relationship between synovial inflammatory infiltrates and the Krenn score.

### Conclusions

The findings state that OA is characterized by mostly low-grade inflammation. HHV-7 proteins are found in different synovial membrane compartments. HHV-7 immunoreexpression correlates with the degree of synovial inflammation.

## Vascular Presence of HHV-6 in *Substantia Nigra pars compacta* of Chronic Alcohol Users and Controls: A Histopathological Evaluation

Nityanand Jain, Sandra Skuja

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### Objectives

Human herpesvirus-6 (HHV-6) is a double-stranded herpesvirus that has a tropism for endothelial cells, particularly in organs such as the brain and liver. Despite uncertainties about its exact pathogenesis, recent research suggests a connection between the virus and a number of central nervous system disorders. We hypothesized that the virus and chronic alcohol abuse may act synergistically to alter the blood-brain barrier, given the virus's known propensity for hematogenous spread. Herein, we investigated the presence of HHV-6 proteins in endothelial cells of gray (GM) and white matter (WM) within the dopaminergic-neuron-rich region of the *Substantia Nigra pars compacta* (SNpc).

### Methods

18 brain autopsy specimens with a history of chronic alcohol use were compared with 13 young non-alcohol users (controls). 10 visual fields per region in each slide were evaluated by light microscopy (40×) and quantitative estimation of HHV-6 expression in vascular bed was performed. Results were analysed using SPSS version 29.

## Results

The median number of HHV-6+ vessels per vision field in GM and WM were three and five vessels, respectively. This difference in the distribution of HHV-6+ vessels between GM and WM was statistically significant (Wilcoxon signed rank test;  $p < 0.001$ ). In WM, there were significant differences in distribution of HHV-6+ vessels between controls and chronic alcohol users (Mann-Whitney U;  $p < 0.001$ ). Similar results were obtained in GM of SNpc.

## Conclusions

Our results indicate the potential involvement of endothelium (especially in the WM) in the transmission of the virus from the periphery to the subcortical and basal ganglia regions through a modified blood-brain barrier in the background of chronic alcohol abuse.

## Infections During the Avacopan Early Access Program (EAP) for Anca-Associated Vasculitis (AAV)

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## Background

Infections remain a concern with many immune targeted therapies. Infections are one of the common causes of early mortality in AAV. Avacopan, a selective C5aR inhibitor is approved for the treatment of adults with severe and active AAV in combination with rituximab or cyclophosphamide. Avacopan does not block C5b-9 production, leaving the membrane attack complex (MAC) intact. Integrated safety data from two Phase 2 and one Phase 3 studies in 439 AAV patients have shown fewer infections in patients on avacopan versus comparator groups. Our objective was to assess infections in patients under real world conditions by analysing avacopan EAP data.

## Methods

Pharmacovigilance data for EAP participants was obtained for the period February 2019 and September 2023. Criteria for EAP participation included newly diagnosed or relapsing AAV and high unmet need.

## Results

Data from 216 patients were analyzed with a median treatment duration of 6 months (range 1–45 months). 14 episodes of infections were reported in 10 patients (5%). Eight were classified as serious, with four resulting in hospitalization

and one with fatal outcome. The patient who died had a relapsing MPA with pulmonary involvement and died due to complicated pneumothorax and multiple infections in an intensive care unit setting. COVID-19 was the most frequent type of infection, occurring in 4 patients with full resolution in 3 and in one outcome unknown. One patient required hospitalization. Eight events in four patients were assessed as related to avacopan by the reporter, leading to permanent avacopan withdrawal, temporary avacopan withdrawal, no change and unknown action in 1 patient each.

### Conclusions

Infection in these patients appears similar to what would be expected in this immunosuppressed population. Limitations of this program include potential under-reporting and incomplete data.

## Development of Benzoxaphosphepine 2-Oxides as Carbonic Anhydrase Inhibitors

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Carbonic anhydrases (CA, EC 4.2.1.1) are essential metalloenzymes found across all kingdoms of life. These enzymes are involved in many important physiological processes, as they catalyse the reversible hydration of carbon dioxide [1]. Abnormal levels or activities of CA have been associated with multiple diseases such as cancer, glaucoma, epilepsy, inflammatory illnesses, and many more [2]. Hence, inhibition of CA has become a major target of drug development.

Herein we report our results on the development of a new class of CA inhibitors – benzoxaphosphepine 2-oxides. These compounds show remarkable selectivity and good inhibitory activity against the cancer-associated CA isoforms IX and XII [3, 4]. Furthermore, aforementioned compounds can be used as starting points for the design of more potent CA IX/XII inhibitors.

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## Authors Index

### A

Alogna, Andrea 17  
Arāja, Diāna 23, 24, 38  
Arcangeletti, Maria-Cristina 43

### B

Balašova, Anastasija 56  
Bandere, Dace 48  
Bartkevičs, Vadims 35  
Bechter, Karl 13  
Beltrami, Silvia 18, 32  
Berķis, Uldis 23, 24, 52  
Bini, Francesca 41, 43  
Bizjak, Daniel Alexander 26  
Blank, Miri 14  
Bortolotti, Daria 17, 18, 32  
Buhl, Jasmine-Leonike 26

### C

Caselli, Elisabetta 41, 43  
Čēma, Ingrīda 21

### D

D'Accolti, Maria 41, 43  
Doniņa, Simona 45, 50  
Dulovic, Alex 13, 31

### E

Erts, Renārs 48  
Evansa, Irina 38  
Everaus, Hele 34

### F

Fedorenko, Deniss 35  
Fomins, Ņikita 12  
Fridmanis, Dāvids 12

### G

Gafā, Roberta 32  
Gentili, Valentina 18  
Gilburd, Boris 37  
Grāvelsiņa, Sabīne 12, 23, 26, 41  
Greco, Pantaleo 32  
Groma, Valērija 29, 32, 50, 53  
Gudrā, Dita 12

### H

Hagina, Elvīra 33

### I

Ieviņa, Lauma 12

### J

Jain, Nityanand 54

### K

Kadiša, Anda 28, 53  
Kakar, Jagriti 21  
Kaupe, Justīne 46  
Ķēniņa, Viktorija 12  
Kholodnyuk, Irina 19  
Ķibilds, Juris 35, 48  
Koļesova, Oksana 33  
Koļesovs, Aleksandrs 33  
Konrāde, Ilze 46  
Kornilova, Olga 19  
Kozireva, Svetlana 19  
Krūmiņa, Angelika 23, 24, 26,  
41, 48, 50  
Kujawski, Sławomir 22

### L

Labecka, Linda 48  
Lejniece, Sandra 19

Lejnieks, Aivars 29  
Leonciks, Ainars 19  
Lunga, Asja 50

## M

Maccari, Clara 43  
Marchetti, Paolo 17  
Mauliņa, Inga 48  
Mazziga, Eleonora 41, 43  
Murovska, Modra 21, 23, 24, 26,  
41, 50

## N

Nazarenko, Mariia 19  
Nora-Krūkle, Zaiga 12, 23, 24, 26,  
41, 50

## O

Obergfell, Achim 55  
Omeļčenko, Dmitrijs 29  
Ostrinski, Yuri 39

## P

Passaro, Angelina 32  
Pērkons, Ingus 35  
Popov, Tamara 55  
Prikulis, Arvis 35  
Prusty, Bhupesh K. 16  
Prylińska, Monika 22  
Pugajeva, Iveta 35  
Pustenko, Aleksandrs 56

## R

Rasa-Dzelzkalēja, Santa 12, 23,  
26, 33, 41, 50  
Rivkina, Alla 19  
Rizzo, Roberta 17, 18, 32  
Rizzo, Sabrina 17, 18, 32  
Rovīte, Vita 46  
Rudevica, Zanna 19

## S

Scheiber, Christian 13, 31  
Schiuma, Giovanna 17, 18, 32  
Schneider, Marion 13, 26, 31  
Schneider, Julian M. 31  
Schneiderhan-Marra, Nicole 13, 31  
Schulz, Tanja 13  
Semenistaja, Sofija 28, 29, 53  
Shoenfeld, Yehuda 10  
Šķesters, Andrejs 46  
Skuja, Sandra 32, 53, 54  
Słomko, Joanna 22  
Soffritti, Irene 41, 43  
Steinacker, Jürgen M. 31  
Storoženko, Jeļena 33  
Studers, Pēteris 53  
Svirskis, Šimons 12, 23, 26

## T

Tabisz, Hanna 22  
Tarasovs, Mihails 28, 29, 53  
Trapella, Claudio 17

## V

Vaivode, Ieva 45  
Vanaga, Ieva 33  
Vasiļevska, Sandra 38  
Vasiļevskis, Edgars 24, 38  
Vikmanis, Andris 29  
Viksna, Ludmila 33  
Villacorta, Javier 55  
Vilmane, Anda 12, 23, 26, 41

## Z

Zalewski, Paweł 22  
Žalubovskis, Raivis 51, 56  
Zeltiņa, Indra 46  
Zvejniece, Laura 19

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