VILNIUS UNIVERSITY HOSPITAL SANTAROS KLINIKOS

RESEARCH AND INNOVATION

2021

Dear readers and colleagues,

The year 2021 at Vilnius University Hospital Santaros Klinikos was marked by a never-ending, intense battle against COVID-19. Newly acquired data led to evidence-based decisions and significantly improved the diagnostic and therapeutic options for this disease. COVID-19 vaccines were developed in a record time and soon became one of the main tools for mitigating the pandemic. All this has served as a reminder that the rapid development of research and innovation is essential for effectively protecting the health of our patients and our country's society. This publication provides an opportunity to better understand the diversity of research conducted at Vilnius University Hospital Santaros Klinikos and to highlight scientific initiatives of both national and international significance. Most of the projects and research presented here are only possible because of the extensive experience and consistent work of our specialists that seek to unravel the causes and evolution of various disorders, and to discover new ways to help patients with unusual and dangerous health conditions. We are delighted that every year Vilnius University Hospital Santaros Klinikos is becoming an increasingly important international center of research and innovation.

Sincerely,
Director General Prof. Feliksas Jankevičius

Contents

Clinical and biomedical research department, Innovation and Technology Transfer Office

Biomedical research and Research and Experimental Development (R&D) projects at Vilnius University Hospital Santaros Klinikos

One of the many activities of specialists at Vilnius University Hospital Santaros Klinikos (VUH SK) is the independent conduct of biomedical research. VUH SK continuously promotes the development of biomedical research, contributes to the formation of Lithuanian and European Union (EU) health policy, and implements EU joint actions and biomedical projects. In 2021, the following biomedical research activities were carried out in the hospital: epidemiological, retrospective, prospective studies, drug and medical device clinical trials, research and experimental development (R&D) projects in cooperation with other research institutions, research done by PhD as well as undergraduate students. Biomedical research activities are focused on clinical trials (both drug and medical device), studies on chronic non-infectious and infectious diseases. VUH SK has a modern infrastructure that allows patients to participate in phase I-IV clinical trials and other biomedical research.

In 2021, a total of 443 biomedical trials in various therapeutic areas were conducted at VUH SK – 219 biomedical studies (71 of which were initiated in 2021), 224 drug or medical device clinical trials (44 of which were initiated in 2021), Figure 1. Further, 55 new preliminary and 44 final clinical trial contracts as well as 37 research collaboration agreements with other Lithuanian and foreign institutions were signed. In 2021, 40 R&D and innovation projects were carried out at VUH SK and another 13 were submitted for evaluation.

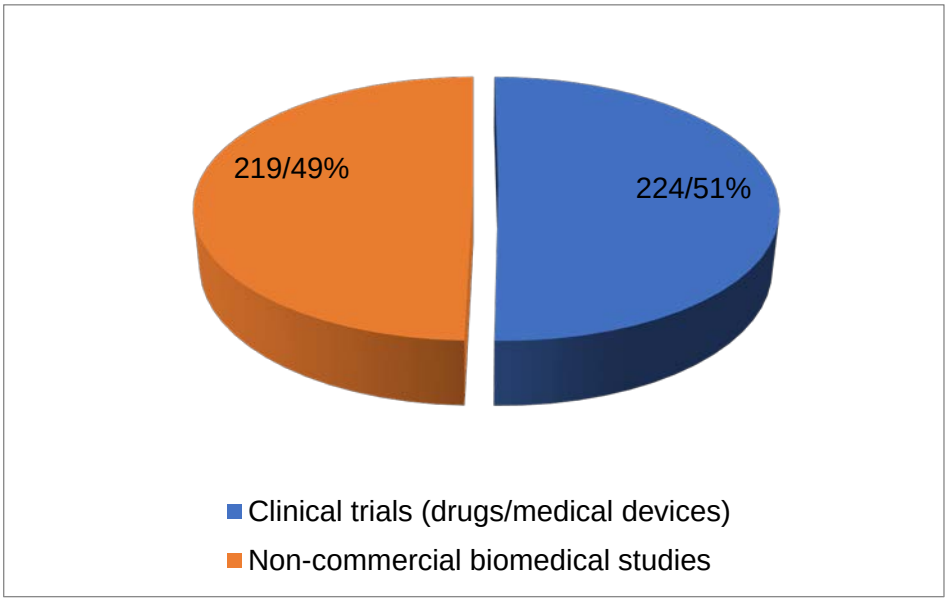

Figure 1. Types of research at VUH SK in 2021.

The main research areas are cardiology and angiology, transplantology and regenerative medicine, oncohematology, oncology and surgery, radiology and nuclear medicine, advanced therapeutics, pediatric diseases and rehabilitation, hepatology and gastroenterology, fertility treatment, neurology, rheumatology, orthopedics-traumatology, endocrinology, obstetrics and gynecology, and others (Figures 2 and 3).

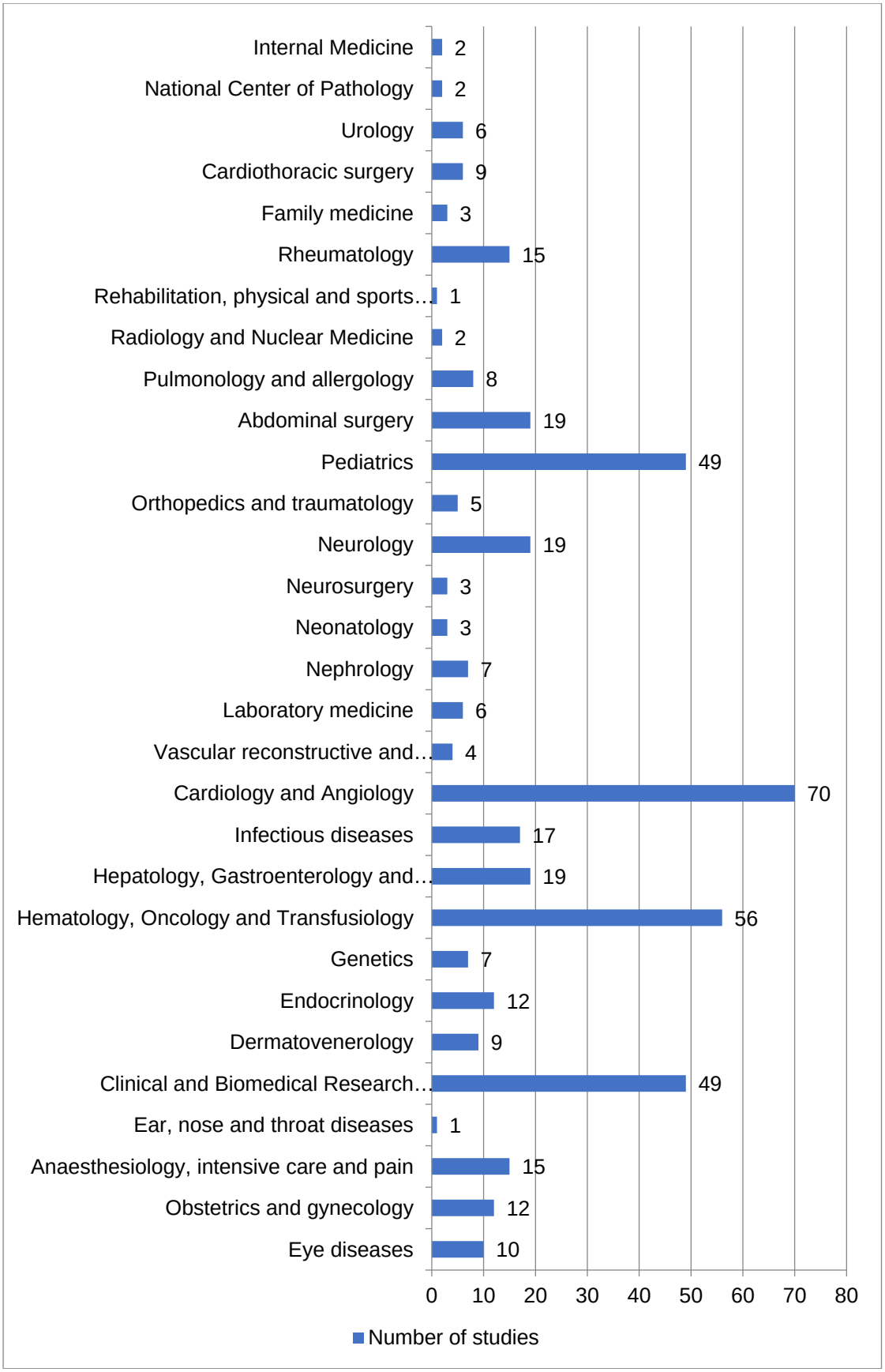

Figure 2. The main therapeutic areas of biomedical research at VUH SK in 2021.

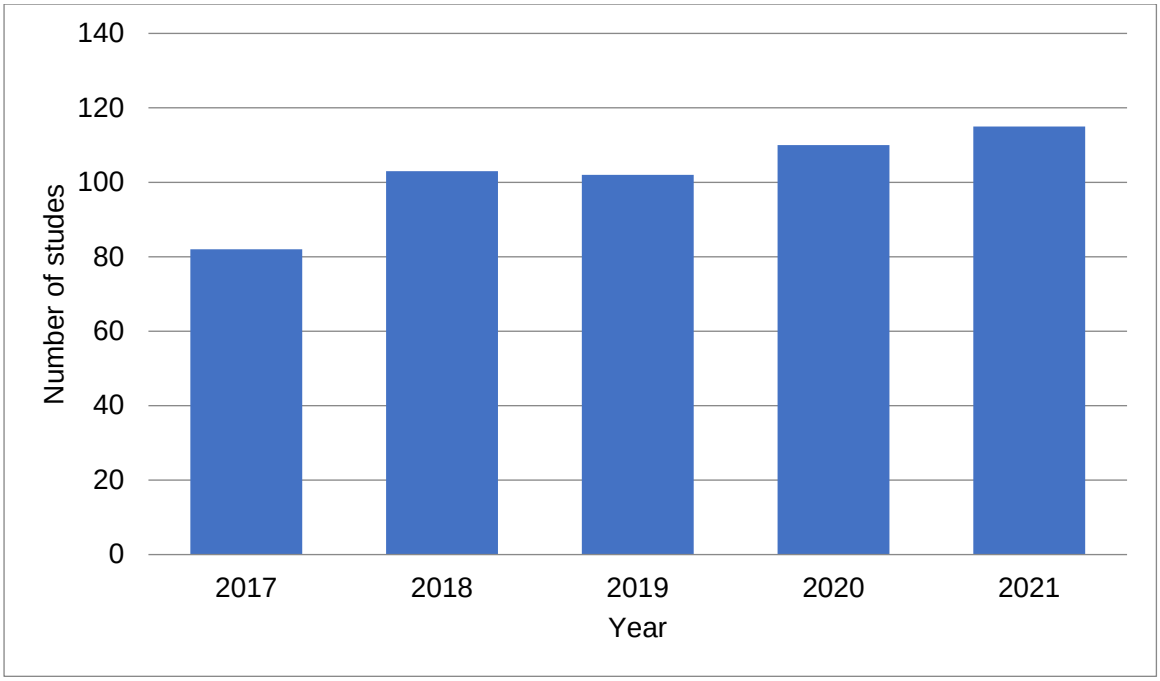

Figure 3. The number of newly initiated biomedical studies from 2017 to 2021.

The following medical device trials have been (or are being) carried out at VUH SK:

- APAMA 1 – a balloon for pulmonary vein isolation (treatment of atrial fibrillation),
- AFERA 2 – a catheter and navigation system (for atrial fibrillation),
- Beat to beat – arterial blood pressure-lowering pacemakers,
- CCM – pacemakers that improve myocardial contractility,
- Double-Check AF – a non-commercial study with Kaunas University of Technology – a wearable watch to detect atrial fibrillation and extrasystoles
- HYDRA – a transcatheter aortic valve implantation study (monitoring),
- Accu-CINCH – a left ventricular annulus to restore left ventricular geometry and reduce mitral valve leakage,
- FAME III – percutaneous intervention drug-eluting stents,
- TRISTAR – a tricuspid valve ring for valve correction,
- PLA – Pulsed Field Ablation System trial for the treatment of paroxysmal atrial fibrillation,
- E-SAFE – a study to measure esophageal temperature and retraction probe temperature during ablation (for atrial fibrillation),
- KALPA™ – a study to assess the safety and efficacy of a medical device and the mapping, imaging and management capabilities in patients undergoing left atrial appendage closure,
- Leaflex Performer – a clinical study to demonstrate the safety and efficacy of the medical device,
- NuVera ICE – a study of a catheter insertion during percutaneous procedures that use septal puncture to access the left atrium,
- CCM-HFpEF – the assessment of safety and performance of an implantable device, the Sphere-9 catheter and the Affera tagging and radiofrequency pulsed field ablation system for the treatment of atrial fibrillation.

The partners of clinical research of VUH SK are Abbvie, Amgen, Merck, Servier, Sanofi, Novartis, Bayer, Biotex, Boehringer-Ingelheim, Takeda, Hoffmann-La Roche, Shire, Celgene, Odonate Therapeutics, Gilead, Biogen, Pfizer, Dr. Falk Pharma and other representatives of innovative solutions in the pharmaceutical industry. VUH SK's medical device research partners are Micro Interventional Devices Inc., St. Jude Medical Coordination Centre, Medtronic, Millipede, PiCardia, NuVera Medical, K2 Medical Ltd. and other representatives.

VUH SK's research partners include Vilnius University (Lithuania), the Research Council of Lithuania (Lithuania), the National Cancer Institute (Lithuania), the Center for Innovative Medicine (Lithuania), Vilnius Gediminas Technical University (Lithuania), Kaunas University of Technology (Lithuania), the Institute of Biotechnology (Lithuania), the New York University School of Medicine (USA), the University of Cologne (Germany), the University of Rostock (Germany), Stanford University (UK), the University of Aalborg (Denmark), the University Hospital Erlangen (Germany), Heidelberg University Hospital (Germany), the Menzies Research Institute (Tasmania), the INSERM Research Institute (France), the Dutch-Belgian Cooperative Trial Group for Hematology Oncology HOVON, the Medical University of Vienna, the Hamilton Health Sciences Corporation, the EuroSurg Collaboration, the Karolinska University Hospital, the University of Leipzig, the University of Heidelberg, University College Dublin, the National University of Ireland Dublin and other research institutions.

Based on the order of the Ministry of Education, Science and Sport of the Republic of Lithuania, the Research Council of Lithuania (RCL) performs an annual expert evaluation of (R&D) activities in Lithuanian universities and research institutes. From October 2021, the results of the evaluation of R&D in 2020 are available to the public. The annual evaluation of Lithuanian institutions' R&D activities helps to quantify research dissemination (units of published works) and the funds received from R&D projects and contracts in each institution.

When evaluating articles in the fields of natural, technological, medical and health, agricultural and social sciences, the experts decide on a list of journals in which the published articles are counted. These are updated during each assessment and can serve as a guideline to help researchers choose the right journal for disseminating their research.

According to the results published by the RCL in 2021, 154 published papers were submitted by VUH SK for the year 2020. **Compared to the previous five years, the number of papers published in 2020 is about 2-3 times higher: there were 57 papers submitted for evaluation in 2015 and 74 in 2016.** The assessment of research dissemination resulted in a weighted sum of 334.37 points for VUH SK (Figure 4). Based on these results, VUH SK ranks third in a list 18 Lithuanian institutions.

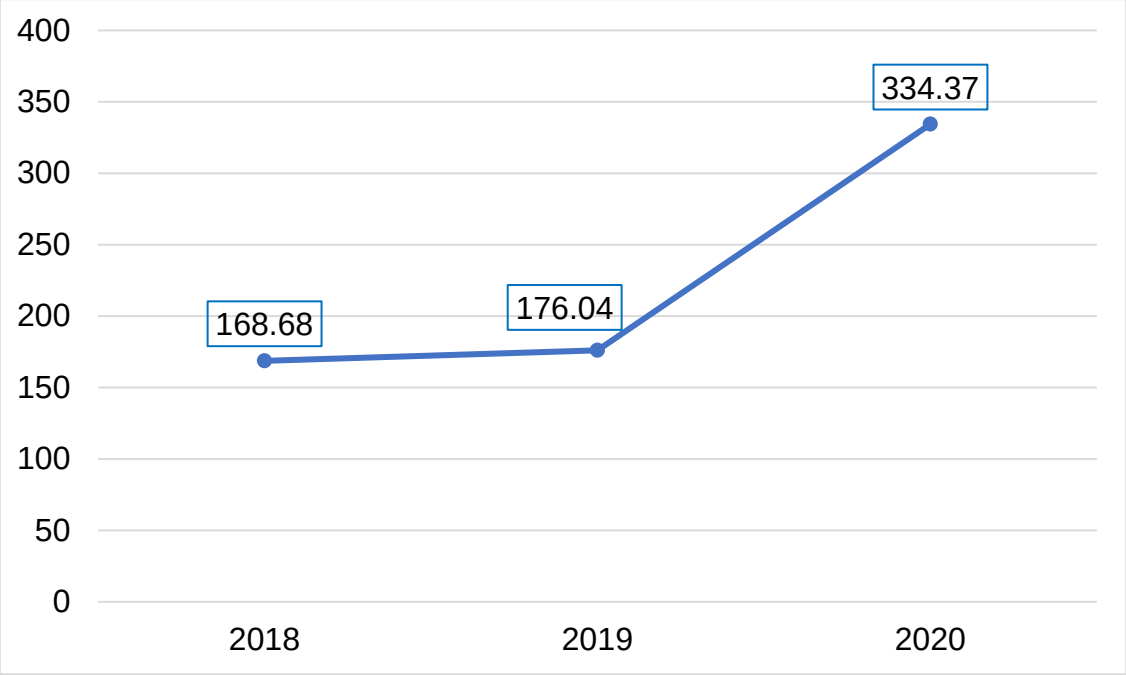

Figure 4. Points received during the expert evaluation of research and experimental development (R&D) of universities and research institutes in Lithuania from 2018 to 2020.

According to the results of the evaluation of R&D projects and contracts published by the RCL, VUH SK is the second-ranked institution in the field of medical and health sciences when considering funding received through projects of international research programs (221.94 thousand euros), Figure 5. VUH SK has also received 585.32 thousand euros through R&D contracts with business entities and is the leader among other medical and health sciences institutions in Lithuania.

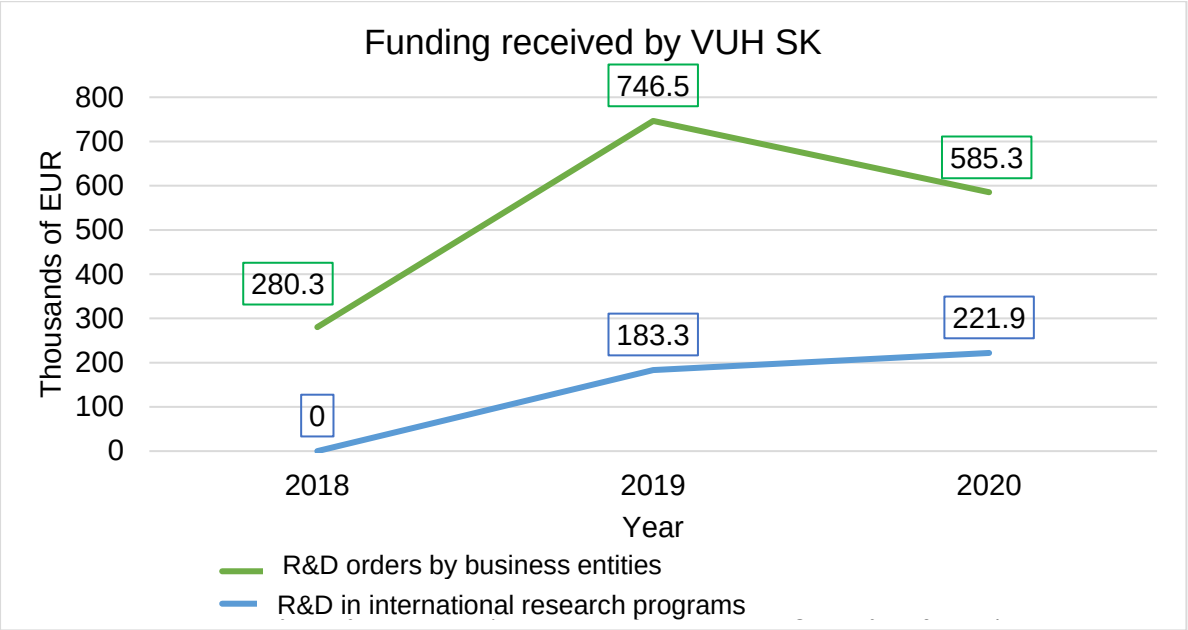

Figure 5. Funding received for R&D at VUHSK from 2018 to 2020.

In 2021, 14 PhD and dozens of bachelor and master theses were prepared at VUH SK (with supervision by the hospital's own specialists), which is a teaching base for the Faculty of Medicine of Vilnius University (Figure 6). VUH SK is one of the main training facilities for students in medicine, nursing and public health in Lithuania.

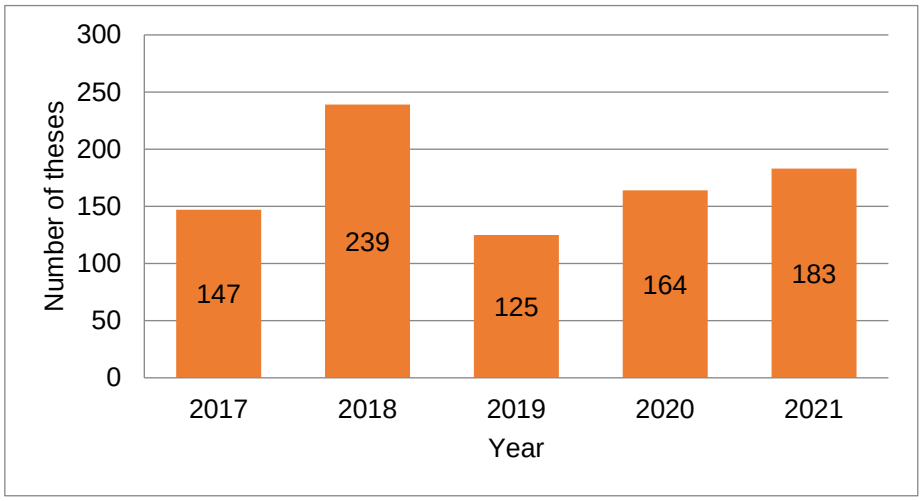

Figure 6. Undergraduate research (number of theses) at VUH SK from 2017 to 2021..

VUH SK has also been authorized by the State Medicines Control Agency of Lithuania to organize Good Clinical Practice (GCP) training. The training can be executed remotely: investigators may attend the course and complete the final test at any convenient time and place. A certificate of Good Clinical Practice are provided upon successful completion of the test.

COVID-19: challenges, experience and future outlook

In September 2021 representatives of Vilnius University Hospital Santaros Klinikos and the Faculty of Medicine of Vilnius University organized the first hybrid conference titled “COVID-19: challenges, experience and future outlook” dedicated for professionals of all fields of medicine and psychology. The program was composed of various topics: this allowed to better grasp the different perspectives of the approach towards healthcare during the COVID-19 pandemic (Figure 1). With the simultaneous need to mitigate the spread of SARS-CoV-2 and ensure the availability of medical services, the pandemic provided an opportunity for specialists to gain experience and resilience while providing essential services during a public health emergency. The speakers’ presentations were preceded by a remote press conference.

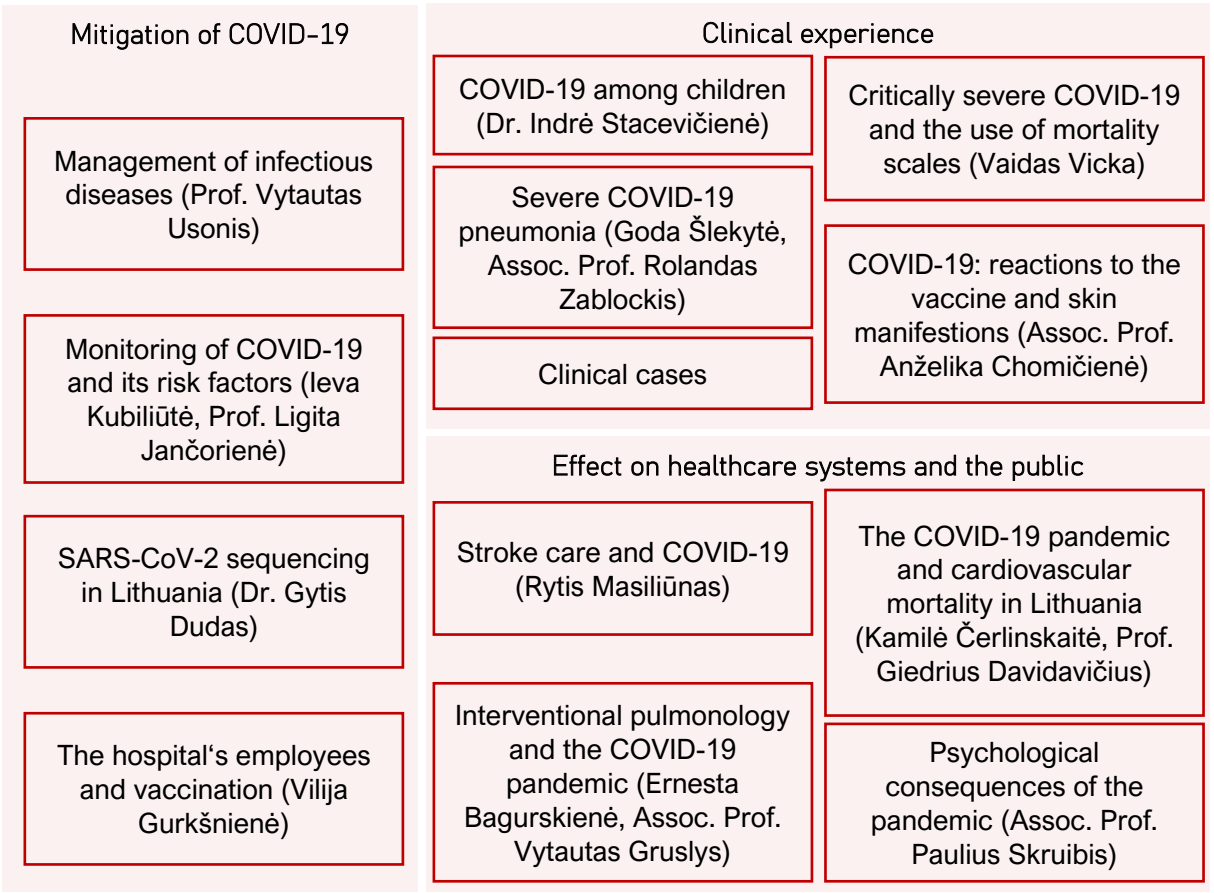

Figure 1. The program of the conference.

Center for Infectious Diseases

Vilnius University Hospital Santaros Klinikos has joined the novel vaccine research network VACCELERATE

The European Union (EU) has allocated 12 million euros from the HORIZON 2020 fund to create a novel network for vaccine research VACCELERATE (**project coordinator in Lithuania – Prof. Ligita Jančorienė**), which seeks to connect all European countries to develop COVID-19 vaccines and form a consortium for second and third phase vaccine clinical trials in Europe. The latter will be coordinated by VACCELERATE and are necessary to ensure immunity against SARS-CoV-2 among European residents. VACCELERATE is led by representatives of the University of Cologne, Germany, and currently includes 26 partners from 16 countries in the EU. VACCELERATE is going to increase preparedness for future pandemics by creating a network of specialists and institutions that can effectively test new vaccines even after the end of the COVID-19 pandemic. VACCELERATE will also include a task force of stakeholders, which will ease strategic discussions and state major public health needs together with healthcare institutions, vaccine developers and international vaccine initiatives. VACCELERATE is also going to unite different sites for clinical trials and aggregate expert opinion to ensure the exchange of experience while creating new vaccines. In summary, the initiative will help to coordinate vaccine trials across all EU member and associated countries under one strategic and research umbrella.

An essential step in creating the VACCELERATE network is the identification of sites and laboratories in Europe capable of conducting clinical trials. More than 200 such sites have already been registered (www.euvap.eu) and their practices are expected to undergo harmonization. VACCELERATE will also ease access to clinical trials for volunteers by creating a respective registry (https://www.vaccelerate.eu/volunteer-registry/index.html), Figure 1. Joint data gathering among VACCELERATE centers will help to exchange information and help to create a consolidated database. Data analysis will then reveal and help resolve the main issues associated with vaccine development during a pandemic. Therefore, VACCELERATE is one of the pathways for the EU to have sufficient resources to ensure sufficient resources to develop and use vaccines as a powerful tool against new infectious agents.

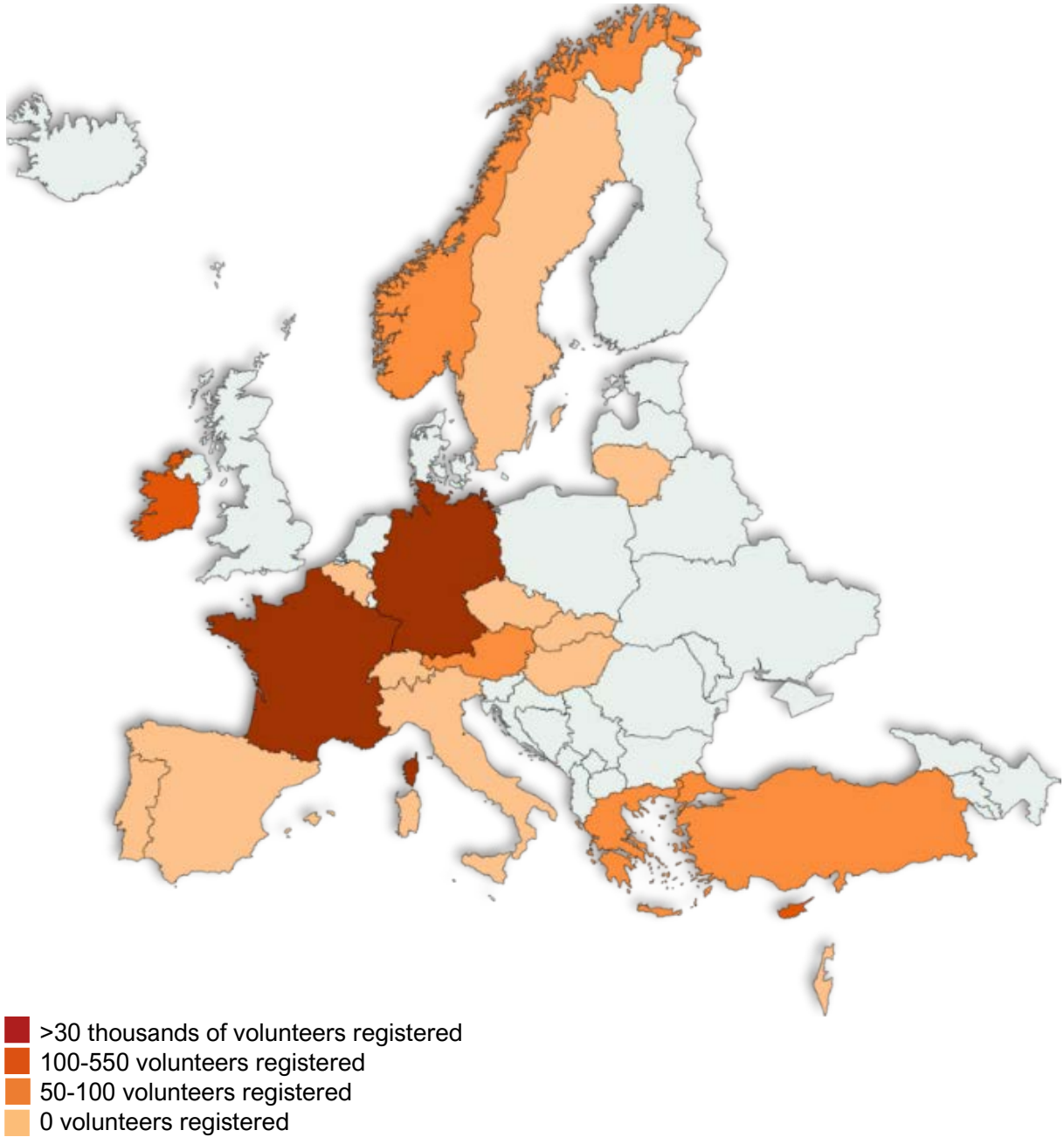

Figure 1. Countries with an active VACCELERATE volunteer registry (www.vaccelerate.eu/volunteer-registry/index.html)

Follow-up of patients after COVID-19

Since the beginning of COVID-19 pandemics, Vilnius University Hospital Santaros Klinikos (VUH SK) has become one of the main medical centers for COVID-19 patients in Lithuania. Most patients infected with SARS-CoV-2 and hospitalized at VUH SK survived even after critical COVID-19 disease and were successfully discharged from the hospital. Because of a high number of COVID-19 survivors, it became necessary to understand the possible long-term outcomes of this disease, especially the impact on the respiratory system, and to define the need for further outpatient management. The outpatient monitoring of COVID-19 survivors' respiratory system was provided by the outpatient service of VUH SK.

Data of the first 51 patients were analyzed by a multidisciplinary team of specialists (pulmonologists, radiologists, specialists in infectious diseases; **coordinator – Prof. Ligita Jančorienė**) in the beginning of 2021. The aim of this analysis was to evaluate pulmonary function, exercise capacity, residual radiological changes, and health-related quality of life (HRQoL) at follow-up in a cohort of SARS-CoV-2 pneumonia survivors.

The mean age of the study group subjects was 56 years (35.3% of patients were followed after moderate, 41.2% after severe, and 23.5% after critical COVID-19 disease)[1]. The results revealed that pulmonary function at follow-up was impaired in 47.2% of individuals, and almost one third of all patients showed reduced physical capacity during the six-minute walking test. Different levels of abnormalities were found in most patients (96%) on follow-up chest computed tomography scans, with the ground-glass opacity being the most common radiological feature.

The Health-Related Quality of Life Short Form (SF-36) Questionnaire scores demonstrated a reduction in health status across all domains, with the lowest scores for limitations in social activities because of physical problems, vitality, and general health (Figure 1).

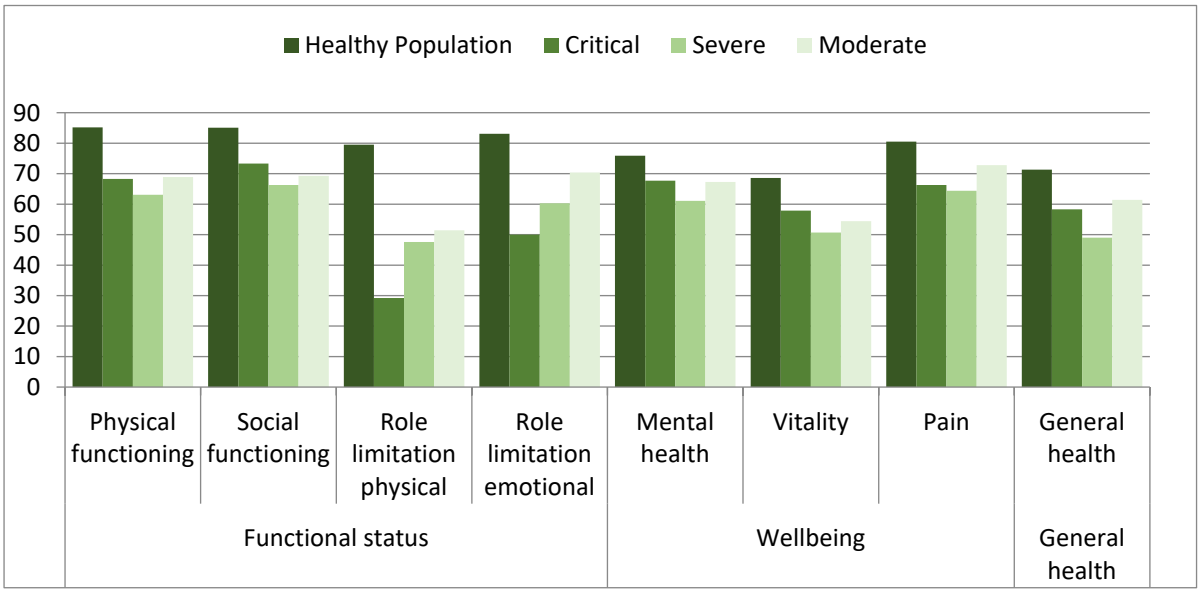

Figure 1. HRQoL results in comparison between disease severity groups and with general (healthy) population.[1]

Our data suggested that, in the group of COVID-19 pneumonia survivors, a period of 2-3 months after hospital discharge might not be sufficient for full recovery, thus, additional outpatient medical services (e.g., rehabilitation, psychological support, etc.) should be recommended.

It is also important to evaluate long-term sequelae of COVID-19 disease at longer follow-up periods. Therefore, monitoring of COVID-19 survivors is continued and follow-up data from a larger group of patients after a longer follow-up period are being collected.

As the clinical manifestation of SARS-CoV-2 infection varies widely – from asymptomatic infection to severe pneumonia or even multiple organ damage – and the determinants of the severity of the disease are not known, it is thought that the risk of developing severe COVID-19 pneumonia and residual lung damage may be determined by individual genetic factors leading to a "stormy" and prolonged immune response.

The follow-up data is expected to be supplemented by specific immune markers and genetic analysis to reveal the potential risk factors for severe COVID-19 pneumonia and assess its sequelae, taking into account not only general clinical symptoms but the possible person-specific immune and genetic factors.

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Towards a simple hepatitis C virus infection screening tool in Lithuania

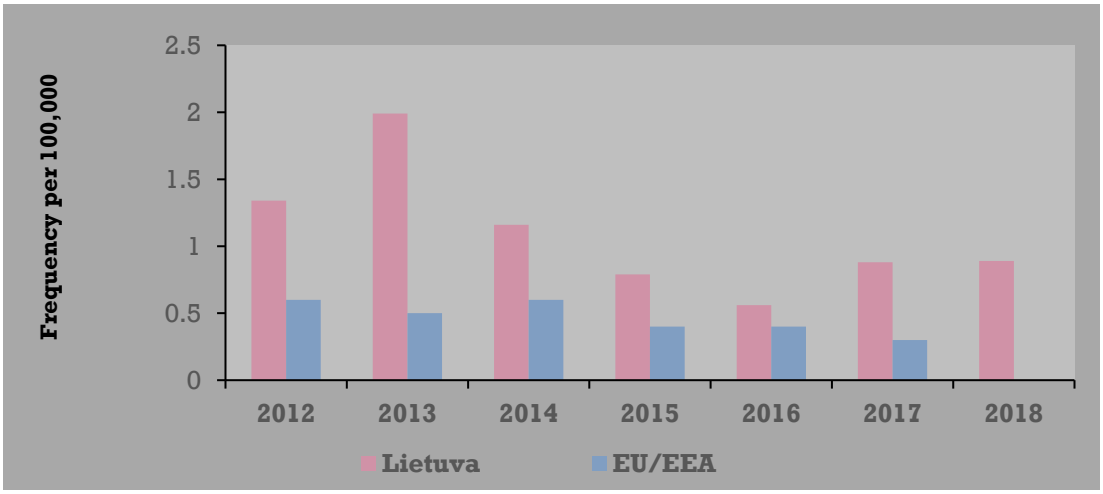
Hepatitis C virus (HCV) infection is one of the major global causes of death and morbidity, so it remains an important public health concern. Previously, HCV infection was difficult to eradicate, but with the discovery of very effective direct-acting antivirals, almost all HCV infection cases can now be cured. **The World Health Organization released the "2030 Agenda for Sustainable Development" and called for international action to drastically reduce the HCV burden by 2030.** This can be achieved by the identification of people who have the disease and making sure that they are appropriately linked to care. However, routine screening is presently limited to just blood donations in Lithuania.

Specialists of the Center for Infectious Diseases of Vilnius University Hospital Santaros Klinikos (VUH SK) (**head – Prof. Ligita Jančorienė**) in collaboration with the Faculty of Medicine of Vilnius University (**PhD student Samanta Grubytė and others**) performed two sets of analyses to identify the main epidemiological patterns and risk factors of HCV infection:

— A descriptive analysis of all the acute HCV cases officially registered via the Lithuanian mandatory notification system from 2005 to 2018.

— Evaluation of the prevalence, incidence rates of HCV infection and its transmission residual risk at the National Blood Center of Lithuania from 2004 to 2018.

During the studies above, the main risk factors that may be crucial to develop efficient policies and evidence-based national HCV screening strategy in Lithuania have been evaluated. Both studies showed that even as the number of HCV cases diagnosed in Lithuania is decreasing, our country has one of the highest incidence rates when compared to other European countries. This indicates that HCV infection control in Lithuania requires improvement



1 pav. Rates of acute HCV infection reported in Lithuania and Europe, 2012-2018.

In the future, specialists are planning to design a simplified questionnaire for HCV risk assessment that might be an appealing instrument for clinicians. Specialists have already performed a case-control study (119 patients and 119 controls were included) to identify risk factors that should be included in the HCV risk assessment questionnaire.

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Participation in the Joint Action on integrating prevention, testing and linkage to care strategies across HIV, viral hepatitis, TB and STIs in Europe (INTEGRATE)

In its third Health Programme (2014–2020) the European Commission endorsed and prioritized a cross-disease integrated approach to combine efforts and promote cost-effective, affordable and effective interventions. Infectious diseases are generally managed in parallel structures with disease specific policies and actions at both European and national levels. In this framework, a three-year Joint Action on integrating prevention, testing and linkage to care strategies across human immunodeficiency virus (HIV), viral hepatitis, tuberculosis (TB) and sexually transmitted infections (STIs) in Europe (INTEGRATE) was launched in Europe in 2017 (**representative at Vilnius University Hospital Santaros Klinikos, VUHSK – Prof. Raimonda Matulionytė**).

The approach in INTEGRATE has been to explore how effective tools for diagnosis and linkage to prevention and care for one disease can be used for other diseases; the applied methodology was to review existing tools and then adapt and pilot these tools in other disease areas

An important focus area of INTEGRATE has been to investigate missed opportunities for combined testing for HIV, viral hepatitis, STIs and TB. Studies and testing services designed for HIV testing have demonstrated the benefits of introducing a combination of tests for these infections depending on target group and service set-up. Advances in HIV testing approaches have been explored to examine how they could potentially be expanded to include testing for other diseases and to evaluate their effectiveness.

The pilot study was conducted in the Center for Dermatovenerology of VUH SK, where individuals presenting with HIV indicator conditions (IC) were offered testing for both HIV and hepatitis C virus (HCV), in addition, individuals presenting with an STI were also offered testing for hepatitis B virus (HBV). It was started out by offering HIV tests to all individuals presenting with one of the following ICs: seborrheic dermatitis, candidiasis, psoriasis, *herpes zoster* and *herpes simplex* and STIs, after 10 months of implementation, HCV testing was added to the offer to all individuals presenting with one of the ICs; HBV testing was also added the last 6 months of the pilot period to individuals presenting with an STI. To facilitate pilot implementation of IC testing, training of staff and frequent staff meetings were conducted in the clinics before the pilots. At VUH SK, plan-do-study-act cycles (PDSA) were performed. PDSA provides a framework to implement and test changes on a small scale, in a structured way and build on the learnings and act immediately.

3 664 consecutive individuals aged 18–65 years participated: 1 592 individuals with an STI, and 2 072 individuals with other dermatological ICs. During the 18-month pilot, the overall HIV testing rate for all IC increased from a baseline of 10.6% (range 0—37% for *herpes simplex* and STI respectively) to 71% (range 43—97% for *herpes simplex* and STI respectively). The overall pilot HCV testing rate for all IC was 83% (range 57–98% for *herpes simplex* and STIs respectively) over a period of 11 months. For STI individuals with HCV testing increased from 0,8 to 98% and HBV testing increased from 0.6 to 92% over a period of 6 months. Three new cases of HIV (0.08% positivity) were identified during the pilot; one with *herpes zoster* (3.7% positivity), one with seborrheic dermatitis (0.5% positivity) and one with an STI (0.06% positivity) – all cases were linked to care. A total of eight new diagnosis of active HCV (0.47% positivity) were identified: five with an STI (0.54% positivity) and three with severe psoriasis (0.53% positivity). Two new cases of HBV were identified in individuals with an STI (0.44% positivity). All HIV, HCV and HBV tests performed were antibody tests and all individuals with a positive test were referred for further investigation and linked to treatment and care in an infectious disease hospital.

The pilot study demonstrated that the ICT strategy can successfully be applied to increase testing rates for HIV/HCV/HBV/STI in a health care setting. This approach was adapted and implemented thereafter into the daily practice of Center for Dermatovenerology.

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Participation in the EuroSIDA study: Clinical and Virological Outcome of European Patients Infected with HIV

EuroSIDA study is a prospective observational cohort study following adult people living with HIV (PLHIV). The study aims to follow the long-term clinical prognosis for the general population of HIV-infected patients living in Europe and to assess the impact of antiretroviral drugs on these patients.

The study includes data from 118 collaborating clinics across 39 countries covering all European regions, Israel and Argentina. Any principal investigator is also eligible for the EuroSIDA Steering Committee, which is elected by the EuroSIDA study group for a 5-year period (**Prof. Raimonda Matulionytė elected for 2017-2022**). The EuroSIDA database holds data from more than 23 000 individuals contributing almost 200 000 person-years of follow-up, while EuroSIDA's unique plasma repository contains over 180 000 samples. Annual data collection includes essential demographic characteristics, information on clinical events, details about antiretroviral therapy (ART), hepatitis C treatment and other medications, together with a range of laboratory values and plasma samples. EuroSIDA was one of the cohorts to found The International Cohort Consortium of Infectious Disease (**RESPOND**) cohort consortium on infectious diseases in 2017.

The Center for Infectious Diseases of Vilnius University Hospital Santaros Klinikos (VUH SK) was involved in EuroSIDA in 2015. 120 patients were included in cohort 10 in 2015-2016 and 120 patients – in cohort 11 in 2019-2020.

In cohort 10, the included patients are consecutive patients in the outpatient clinic regardless of cluster of differentiation 4 (CD4) cell count and ART status, positive for antibodies against hepatitis C virus (regardless of HCV-RNA status, fibrosis stage and prior treatment against hepatitis C). For all HIV patients enrolled and under follow up, laboratory, therapeutic and clinical data are collected twice annually. Demographic data, date on pregnancy and serological evidence for infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) are collected once annually.

In cohort 11, the included patients are those, who have started integrase inhibitor (INSTI) based antiretroviral therapy (ART) after 1/1/2012 and have a CD4 cell count and HIV-RNA available in the 12 months prior to starting INSTI or within 3 months after starting INSTI; if participants have not started INSTI, they were included providing they have a CD4/HIV-RNA in the 12 months prior to baseline or within 3 months after baseline.

Following the introduction of direct-acting antiviral therapy in 2013, WHO launched the first Global Health Sector Strategy on Viral Hepatitis. In a recent publication[1], a hepatitis C virus (HCV) cascade of care in people with HIV (PWH) across Europe was described in terms of reaching the WHO elimination targets of diagnosing 90% and treating 80% of HCV-infected individuals. Care cascades were constructed across Europe, on a regional (n=5) and country (n=21) level, Lithuania included. In Eastern Europe, 78.1% of the estimated number of chronic infections have been diagnosed, whereas this proportion was above 95% in the other four regions. Overall, 3116 persons have ever started treatment (72.5% of the ever chronically infected, 95% CI 70.9-74.0) and 2404 individuals (55.9% of the ever chronically infected, 95% CI 53.9-57.9) were cured. Cure proportion ranged from 11.2% in Belarus to 87.2% in Austria.

In a pan-European cohort of PWH, all regions except Eastern Europe achieved the WHO target of diagnosing 90% of chronic HCV infections, while the target of treating 80% of eligible persons was not achieved in any of the regions.

The second study investigated the effectiveness, safety, and reasons for premature discontinuation of direct-acting antivirals (DAAs) in a diverse population of HIV / hepatitis C virus (HCV) coinfected individuals in Europe[2]. All HIV / HCV coinfected individuals in the EuroSIDA study that started interferon free DAA treatment between January 6, 2014, and January 3, 2018, with ≥12 weeks of follow-up after treatment stop were included in this analysis. 1042 individuals started interferon-free DAA treatment after 1/6/2014 and were included, 862 (82.2%) had a known response to treatment, and 789 (91.5%, 95% confidence interval (CI): 89.7 to 93.4) of which achieved sustained virologic response (SVR12). There were no differences in SVR12 across regions of Europe (p=0.84). After adjustment, the odds of achieving SVR12 was lower in individuals that received sofosbuvir / simeprevir ± ribavirin (RBV) (adjusted odds ratio 0.21 (95% CI: 0.08 to 0.53)) or ombitasvir / paritaprevir / dasabuvir ± RBV (adjusted odds ratio 0.46 (95% CI: 0.22 to 1.00)) compared with sofosbuvir / ledipasvir ± RBV. **Findings from real-world data on HIV/HCV coinfected individuals across Europe show that DAA treatment is well tolerated and that high rates of SVR12 can be achieved in all regions of Europe.**

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Center for Medical Genetics

The characterization of genetic diversity and association with COVID-19 disease indicators in the Lithuanian population (COVID-19_LT)

In May 2021, researchers from the Faculty of Medicine of Vilnius University (VU) and Vilnius University Hospital Santaros Klinikos (VUH SK) started conducting the biomedical study **COVID-19_LT**, which is led by the Dean of the VU Faculty of Medicine and the head of the VUH SK Center for Medical Genetics **Prof. (HP) Algirdas Utkus.**

Humanity is currently facing the global crisis caused by the COVID-19 pandemic and its consequences. SARS-CoV-2 induced COVID-19 infection is a multifactorial disease that most commonly presents with respiratory disorders but can be both asymptomatic and fatal. The mechanisms of the broad spectrum of COVID-19 symptoms are unknown, and it is hypothesized that the interaction between the virus and the host genomes plays an important role in the pathogenesis of the disease. Researchers are looking for evidence that the severity of the clinical signs of COVID-19 depends on the genome characteristics of the infected person. The interaction of this virus with the human body and the influence of genetic factors on the pathogenesis and indicators of the disease have been poorly investigated, so the data from COVID-19_LT are expected to provide new information for the development of SARS-CoV-2 infection.

Nearly 400 volunteers have already been involved in this biomedical study in 2021. By the end of the study, it is planned to include at least 1000 individuals (500 patients with COVID-19 infection and 500 healthy individuals who had high risk of exposure) and to collect their demographic, epidemiological, clinical data, venous blood samples, and to develop an informative COVID-19_LT biomedical research database. The analysis of the whole genome association will be performed for all subjects and the diversity of the genome will be determined and compared among of both infected and healthy groups as well as persons with either mild or severe forms of COVID-19. It is hoped that the data obtained during this study will provide new knowledge of fundamental biology on the genomic characteristics of the Lithuanian population with COVID-19 as well as healthy individuals who were at high-risk of falling ill with COVID-19, and will identify possible candidate genetic domains or variants to model the pathogenetic mechanisms of disease development and the body's response in relation to the severity of clinical signs of COVID-19 and other indicators of the disease. The identification of these genome variants / genetic domains / mechanisms is important in the search for effective COVID-19 therapies and predicting the clinical course of the disease in order to prevent and reduce severe complications and lethal outcomes.

This study, performed by geneticists and specialists of infectious diseases of VUH SK, is being conducted in collaboration with Finnish researchers and is part of the international *COVID-19 Host Genetics Initiative*, which aims to bring together researchers from around the world to study genetic factors relevant to the pathogenesis of COVID-19. More than 400 research institutions around the world have joined the initiative. The significant results obtained during the research *COVID-19_LT* are planned to be published in scientific publications, presented at Lithuanian and foreign scientific conferences, on the website of the Faculty of Medicine of Vilnius University.

Center for Anesthesiology, Intensive Care and Pain management

Comparison of mortality risk evaluation tools efficacy in critically ill COVID-19 patients

As the SARS-CoV-2 (COVID-19) pandemic has continued in the year 2020, the number of COVID-19 patients admitted to the intensive care unit (ICU) around the world has severely increased. Despite working at their maximum capacity and increasing the number of beds and personnel, these services are overstretched, leading to worse clinical outcomes. Having regard for the collapsing health care systems, this might raise the question of triage criteria amendments, since not all patients can or should be admitted to the ICU.

There are several scores used in the ICU to help clinicians estimate the mortality risk of patients. Three of the most common are the Simplified Acute Physiology Score (SAPS) II, Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) score. The 4C Mortality Score was developed in the year 2020 during the pandemic and is designed to be implemented at the moment of hospitalization rather than when admitting the patient to the ICU. However, it is highly specific to COVID-19, as it encompasses the parameters that are the most critical in this disease.

The aim of the study described was to estimate which of the conventional ICU mortality risk scores is the most accurate at predicting mortality in COVID-19 patients and to determine how these scores may be used in combination with the 4C Mortality Score.

This was a retrospective study of critically ill COVID-19 patients treated in tertiary reference COVID-19 hospitals during the year 2020. The 4C Mortality Score was calculated upon admission to the hospital. The SAPS II, APACHE II, and SOFA scores were calculated upon admission to the ICU. Patients were divided into two groups: ICU survivors and ICU non-survivors.

The main finding of the study is that the APACHE II score was the most accurate and had the best discrimination at predicting mortality risk in COVID-19 patients treated in the ICU. However, the best calibration was observed when the 4C Mortality Score was added to the model. In the final model, the APACHE II and 4C Mortality Score prevailed. For each point increase in the APACHE II, mortality risk increased by 1.155 (OR=1.155, 95% Confidence interval (CI): 1.085 to 1.229, $p<0.001$), and for each point increase in the 4C Mortality Score, mortality risk increased by 1.191 (OR=1.191, 95% CI: 1.086 to 1.306, $p<0.001$), demonstrating the best overall calibration of the model. **Therefore, the APACHE II Score and 4C Mortality Score independently predict mortality risk and can be used concomitantly.**

This study allowed to evaluate which of the scores currently used in clinical practice can best be used to estimate the severity of disease and mortality of COVID-19 patients and to pragmatically identify which patients should the limited resources be used on.

The results of this study were presented with a poster presentation at the 34th Annual Congress of the European Society of Intensive Care Medicine (ESICM) and published in “BMC Infectious Diseases “[1].

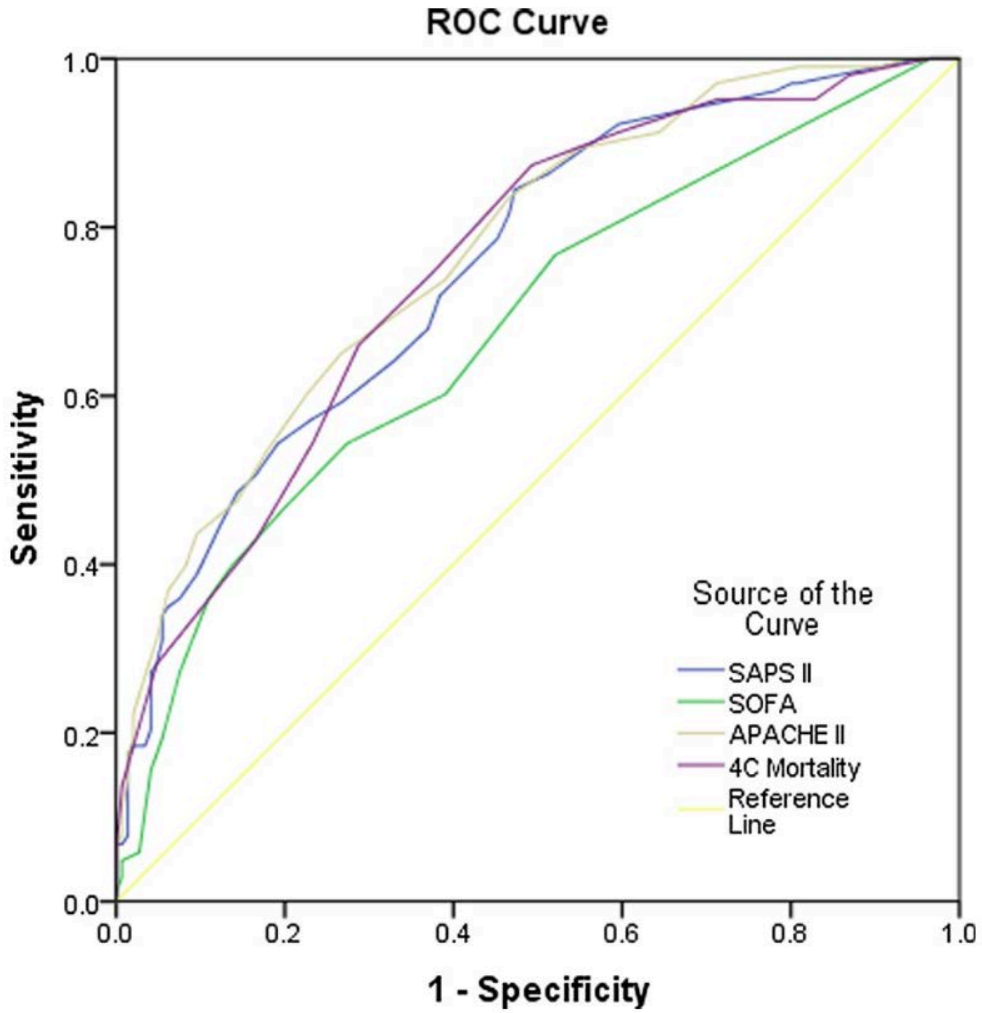

Figure 1. Accuracy of mortality risk scores. The ROC-AUCs for the mortality risk scores in the ICU and 4C Mortality Score reference lines denote the null hypothesis that the AUC really equals 0.50. ICU intensive care unit, ROC-AUC receiver operating characteristic area under the curve, SAPS II Simplified Acute Physiology Score II, SOFA Sequential Organ Failure Assessment, APACHE II Acute Physiology and Chronic Health II, 4C Mortality 4C Mortality Score.[1]

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Hematology, Oncology and Transfusion Medicine Center

COVID-19 research

A surveillance study of SARS-CoV-2 mutations

The SARS-CoV-2 virus that causes COVID-19, like all viruses, changes (mutates) over time. Most of the changes do not affect the properties of the virus, but some may be responsible for the virus spreading more rapidly in the community; a mutated virus may cause new symptoms, worsen the course of the disease, become less sensitive to drugs and vaccines, or change in such a way that diagnostic tests can no longer detect it. In the more than 20 months since the virus began to spread, more than a thousand new strains have been detected and named, but only a few of them have changed the course of the pandemic in a particularly noteworthy way.

Staff from the Biobank and the Molecular Medicine Unit at Vilnius University Hospital Santaros Klinikos (VUH SK), together with expert virologists from abroad, have initiated a national virus sequencing project. The project started with the sequencing of thousands of samples collected in Biobank during the second wave of the pandemic. It was found that between December 2020 and January 2021, various SARS-CoV-2 strains circulated in Lithuania, with B.1.177 being the most prevalent. This strain was one of the first SARS-CoV-2 strains to become widespread in Europe. The first cases of this strain were detected in Spain in June 2020, when many countries in the European Union and the Schengen area opened their borders following the quarantines and social restrictions imposed in response to the first wave of SARS-CoV-2 transmission. Spain was one of the most popular holiday destinations for Europeans in the summer of 2020, and as B.1.177 became the dominant strain in Spain, many of the holidaymakers who travelled from and were infected in Spain tended to bring B.1.177 with them when they returned home.

The SARS-CoV-2 1,000 genome-wide sequencing project has become a national-level sequencing project, currently involving VUH SK, the National Public Health Laboratory, Vilnius University, the Lithuanian University of Health Sciences, and the Kaunas Clinics of the Lithuanian University of Health Sciences Hospital. **The project revealed that the third wave of COVID-19 in Lithuania was caused by the alpha strain, first identified in the UK, while the fourth wave in Lithuania was caused by the delta strain (Figure 1).**

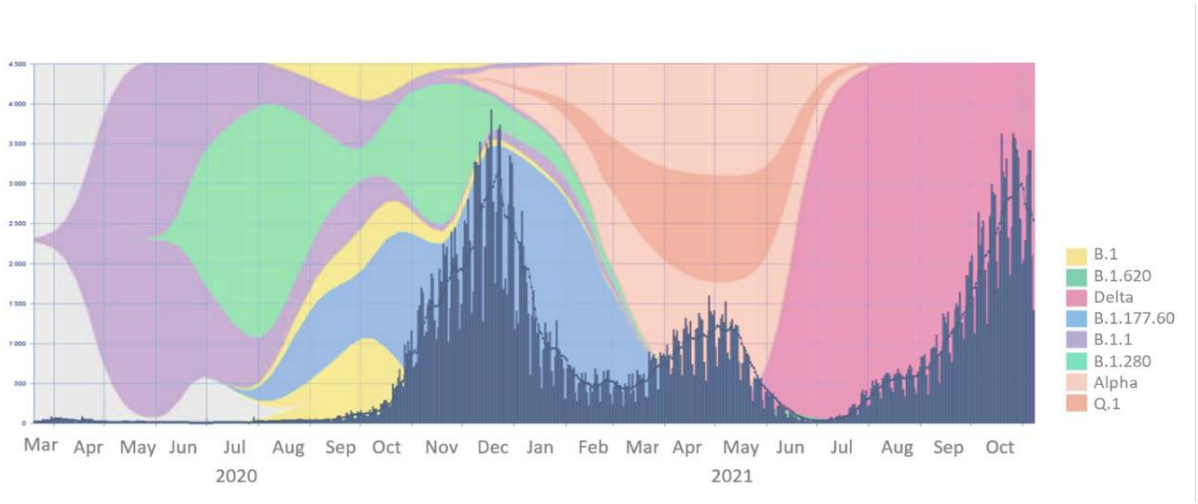

Figure 1. SARS-CoV-2 strains in Lithuania.

Information about the characteristics of the "Anykščių" strain (B.1.620) which circulated in Lithuania has been published in the scientific journal Nature Communications[1]. More

information on the coronavirus strains that circulated in Lithuania can also be found in the article in the Lithuanian Encyclopedia written by medical biology specialists at the VUH SK[2].

In 2021, the SARS-CoV-2 genome was frequently tested in Lithuania, with between 5 and 15% of all positive COVID-19 cases additionally evaluated by sequencing – this can be regarded as a good indicator in the context of the EU. This initiative was noticed by European scientists and VUH SK was invited to participate in the HORIZON 2020 call for pandemic management projects. **VUH SK, together with 17 partners from all over the world, prepared the EuCARE project and was awarded almost €10 million from the prestigious European Union HORIZON 2020 fund.** The project will bring together researchers from different disciplines from around the world to provide robust data-based evidence to tackle coronavirus strains and help to manage the COVID-19 pandemic, with a focus on hospitalized patients, vaccinated healthcare workers and educational institutions.

Development of novel COVID-19 testing strategies

The global COVID-19 pandemic has revealed that healthcare facilities have limited tools and capacity to detect infectious diseases rapidly and with great sensitivity, leading to delays in some diagnostic or treatment procedures. This problem was particularly relevant at the beginning of the pandemic.

Currently, the most common method used to diagnose such diseases is quantitative polymerase chain reaction (qPCR), which is sensitive but time-consuming and requires special equipment. SARS-CoV-2 diagnostics consists of several steps: first, viral RNA is purified from nasopharyngeal samples, then reverse transcription is performed to obtain complementary DNA (cDNA) from the viral RNA, which is amplified by qPCR and the data are analyzed. The whole process takes more than two hours. The method requires expensive equipment, which leaves samples in a queue in case of its shortages.

Pooled sample testing

Preventive testing is an important tool for managing the pandemic and therefore methods that are simple, cheap, sensitive and specific need to be introduced into the routine practice. After assessing these criteria, it was decided to validate the pooled sample testing method in the VUH SK. Instead of the routinely used nasopharyngeal swab, it was decided to use a swab of the anterior nasal cavity. **This sample is very easy to take, can be done individually, does not require the assistance of a nurse or cause any unpleasant sensations.** Several (maximum six) patient samples are placed in one tube and then tested together. If a positive pooled sample test result is found, all patients with a sample from the one tube are retested individually.

Such testing is an excellent preventive measure, and when the prevalence of the virus is low, it can prevent the spread of infection in schools, workplaces and medical institutions. It is for these reasons that Vilnius City Municipality was the first to introduce pooled sample testing in some schools in the capital of Lithuania. The strategy was then applied in other cities. Most of the VUH SK staff are vaccinated, so preventive testing using the pooled sample approach was also suitable as a simple way to manage COVID-19 outbreaks among staff and patients.

PRECISE

Novel solutions are being explored to preserve the sensitivity of qPGR but reduce the time from sample collection to virus detection. The nucleases encoded by the CRISPR-Cas system are able to accurately recognize nucleic acid sequences, thus, their utility for rapid diagnosis of COVID-19 is being investigated worldwide. Methods are being sought that are sensitive and provide rapid detection of the virus without the need for special or expensive equipment, or training. The development of a CRISPR-Cas-based method would make it very easy to adapt to the detection of

new pathogens as well. Some of the new CRISPR nucleases are also available in Lithuania and could be used to develop new DNA detection tests.

The CRISPR nuclease-based method is being developed at VUH SK with biotechnology leaders "CasZyme". **Together with partners, the aim is to develop an accurate and rapid method for the detection of SARS-CoV-2, which would facilitate and expand the testing of patients for COVID-19.** The PRECISE project is funded by the European Union and the call was organized by the Lithuanian Research Council in accordance with the themes provided by the Ministry of Health of Lithuania.

Mutation detection by PCR

Sequencing of SARS-CoV-2 mutations is a longer and more expensive method that is well suited for searching for new mutations, identifying prevalent strains, monitoring strain dynamics and assessing socially accepted constraints. However, for epidemiologists, it is important to quickly identify already known dangerous viral mutations, to locate and investigate the individual's contacts. By developing specific primers, targeted SARS-CoV-2 mutations can also be detected by qPGR. Such a simple and rapid method has been developed in the Molecular Medicine Laboratory of the VUH SK for the detection of dangerous mutations (E484K, N501Y, del69-70). This search for targeted mutations, which started at VUH SK, was soon extended nationwide by order of the Minister of Health. This is another new tool to manage and prevent the spread of dangerous SARS-CoV-2 mutations quickly and efficiently.

Virus integrity testing

SARS-CoV-2 virus detection by conventional cDNA synthesis involves the use of random hexamers that can adhere to remaining fragments of the viral genome. This means that results of a SARS-CoV-2 test can be positive for up to four months after infection, or even longer. **To distinguish whether the nasopharyngeal sample contains only residues of the virus that have not yet degraded, or whether it also contains full-length viral genome molecules, researchers at VUH SK, together with partners from Princeton and Cambridge universities, have developed a method that detects the presence of full-length RNA molecules in a sample**. Instead of random hexamers, which bind any part of the viral genome, oligo(dT)20 primers are used, which bind only the 3' end of the RNA molecule, thereby separating degraded RNA fragments from full-length RNA molecules. The detection of full-length viral RNA molecules in the sample suggests that the virus is still actively replicating in the patient's body and that the individual requires isolation and/or treatment. Initial results supporting this approach were presented at the Baltic Hematology Conference[3].

Investigation of the immunogenicity, safety and efficacy of SARS-CoV-2 vaccines in the immunosuppressed population: the HemVac/HemReVac trial

Researchers from the Hematology, Oncology and Transfusiology Center (HOTC) at Vilnius University Hospital Santaros Klinikos (VUH SK) have conducted a comprehensive analysis of the results of the SARS-CoV-2 vaccination among blood cancer patients. After vaccinating more than 1300 patients with malignant blood disorders at VUH SK, information on the effectiveness and safety of the SARS-CoV-2 vaccines was collected. The findings will help clinicians around the world to better plan the most appropriate timing for vaccination and to better predict the effectiveness of the vaccines in this group of patients. The value of the VUH SK study is reflected by a publication in "The Lancet Haematology" [4]. The study is ongoing and data on the incidence of COVID-19 after vaccinations well as the durability of the resulting immunity are still being collected.

Why is this study important?

People with malignant blood disorders are at risk of severe COVID-19, with a mortality rate up to 20 times higher than in the general population: both the blood disorder and its treatment inhibit the immune system, which normally fights against SARS-CoV-2. These patients are also often

excluded from vaccine clinical trials, therefore, the efficacy and safety of the vaccines in this vulnerable patient group was unknown.

At the end of 2020, the European Medicines Agency registered the first vaccine against SARS-CoV-2 and vaccination of priority groups was initiated based on a decree of the Minister of Health of Lithuania. As patients with malignant blood disorders were included in a priority group, their vaccination started in January 2021.

Once SARS-CoV-2 started to spread, the uncertainty and lack of evidence-based guidelines from European and Western countries, which are usually the basis for medical care in our country, were disturbing. This is one the major reasons why this study is of particular importance. More than 1300 patients with malignant blood diseases have been vaccinated at the VUH SK by May 2021.

According to the results of the study, four in five patients with malignant blood disorders develop immunity. The incidence and nature of adverse effects of vaccination do not differ significantly from the general population. Although the study also identified various factors associated with a poor immune response, the efficacy of vaccination remains high in the immunosuppressed population, with only one percent of the study participants diagnosed with COVID-19 disease at a median follow-up of 94 days.

The study led to conclusions that patients treated with Bruton tyrosine kinase inhibitors, JAK inhibitors, BCL2 inhibitors or anti-CD20 immunotherapy have the poorest immune response. Although the patients‘ immunity is not equivalent to the one of individuals in the general population, the majority of high-risk patients were still able to develop immunity and reduce their risk of developing severe COVID-19 at a time when the prevalence of the disease was probably highest in Lithuania. These findings will help clinicians around the world to better plan the most appropriate timing for vaccinating patients with malignant blood disorders.

With the introduction of the booster dose for immunosuppressed individuals from August 2021, data on the immunogenicity (both humoral and cellular), safety and efficacy of the third (booster) dose of the vaccine among oncohematological patients are being collected further.

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Clinical trials of acute leukemia

RNA sequencing of single acute myeloid leukemia cells

In collaboration with the biotechnology company “Droplet Genomics”, the Hematology, Oncology and Transfusiology Center (HOTC) is conducting the study "Single Cell RNA Sequencing Analysis of Bone Marrow Samples from Patients with Refractory or Relapsed Acute Myeloid Leukemia (AML) Treated with Venetoclax". The aim of the study is to identify new and specific mechanisms

of resistance to targeted therapies in acute myeloid leukemia at the single cell level, which will allow the selection of optimal life-saving therapies for patients in the future.

Pre-clinical studies on drug synergy in acute myeloid myeloma

Specialists at HOTC conduct translational studies of targeted drugs, investigate synergistic chemotherapy effects. AML cell lines and *ex vivo* cells from relapsed/refractory AML patients are used. Such studies are aimed to identify the most effective drug combinations that can be used to plan further clinical trials and to individually adjust optimal treatment for each patient. The project "Development of innovative therapies and prognostic tools for the treatment of chemotherapy-resistant acute myeloid leukemia" is also a collaboration with the Institute of Biochemistry of the Life Sciences Centre of Vilnius University.

Monitoring patients with relapsed/refractory acute myeloid leukemia

During the observational study "The clinical course and medical management of patients with treatment-resistant oncological and blood disorders" patients with relapsed/refractory AML are monitored to evaluate the efficacy and safety of new target-based combination therapies and determine the prognostic factors for patient survival, response to treatment and time in remission. In 2021, four papers were published on this topic and the results of the study were presented at the European Hematology Association's largest annual congress (EHA 2021)[1-4]. For the first time in the history of hematology in Lithuania, the HOTC study will also be presented at the American Society of Hematology annual congress (ASH 2021), the most important global event for hematology professionals (results will be published in “Blood”, one of the most impactful journals in the field)

Collaboration with the HOVON Study Group in the treatment of acute myeloblastic leukemia

HOTC is participating in multicenter, large-scale clinical trials in collaboration with the HOVON Research Centre in the Netherlands, which provide patients with AML with access to the most advanced treatments developed to date[5-6]. Two clinical trials are currently ongoing at HOTC. HOVON HO150 AML is a Phase 3, randomized, placebo-controlled trial evaluating the safety and efficacy of the combination therapy of enasidenib or ivosidenib as target therapy and standard chemotherapy in patients with AML with IDH1 or IDH2 mutations. HOVON HO156 AML is a Phase 3, randomized trial comparing the safety and efficacy of target therapy midostaurin or gilteritinib with standard chemotherapy in patients with acute myelogenous myeloid leukemia (AML) in case of a FLT3 mutation.

Collaboration with the NOPHO study group in the treatment of acute lymphoblastic leukemia

HOTC's long-standing collaboration with the NOPHO (Nordic Society of Paediatric Haematology & Oncology) study group has enabled to implement the modern ALLTogether protocol for the treatment of acute lymphoblastic leukemia at VUH SK. Participation in the ALLTogether clinical trial is planned soon.

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Advanced therapy in oncology and hematology

Virus-specific T lymphocytes

Viral infections and viral reactivation are one of the main causes of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). In such occasions, the first-line treatment is antiviral drugs, but they may be toxic or ineffective for some patients. According to published data from clinical trials, cell therapy with virus-specific T lymphocytes (VST) is one of the alternatives. However, it is not yet available to patients in Lithuania.

The COVID-19 pandemic, which started in 2020, has led to a search for effective treatments for this infectious disease. The 60-100% efficacy of virus-specific T lymphocyte therapy in the treatment of viral infections caused by cytomegalovirus, Epstein-Barr virus, and others has led to the assumption that **VST therapy may also be effective in the fight against SARS-CoV-2.**

In 2020, Vilnius University Hospital Santaros Klinikos (VUH SK) received approval from the Vilnius Regional Biomedical Research Ethics Committee to conduct the biomedical study "Production of virus-specific T lymphocytes against SARS-CoV-2 and CMV, EBV, ADV, BK, and investigation of their specificity and cytotoxicity" (VST-CM-2020) – the production and characterization of virus specific T lymphocytes was initiated with the aim to develop effective advanced therapeutic drugs for treatment of viral infections.

In 2021, the Hematology, Oncology and Transfusiology Center (HOTC) at VUH SK won a grant in the Lithuanian Research Council's call "Short-term (essential) research (in health, social and other areas), analysis and implementation of diagnostics (in agreement with the Ministry of Health) related to COVID-19" and started the project "Management of COVID-19 with advanced therapeutic methods". This project will help develop a prototype of a VST-based advanced therapeutic, evaluate the potential for the amplification of VST against SARS-CoV-2, and assess the safety and specificity of these cells using techniques of molecular biology and flow cytometry. The prototype developed will pave the way for further clinical trials and likely to be used in routine practice.

HOTC's experience in the production of virus-specific T lymphocytes was presented at the International Scientific and Practical Conference "Baltic Hematology Congress "Sharing

Experience"" on 2 October 2021. A detailed characterization of virus-specific T lymphocytes is currently being carried out and their culture conditions are being optimized.

Mesenchymal cells

Mesenchymal cells are one of the first and most widely used cell therapy products in regenerative medicine. One of the main indications for the use of these cells is graft-versus-host disease (GVHD), especially its acute form. In 2009, the first successful case of mesenchymal cell therapy for acute GVHD was published, but to date there is no registered treatment for steroid resistant acute GVHD in Europe. Mesenchymal cells are used as one of the potential treatments in transplantation centers due to their favorable safety and efficacy characteristics.

In 2013, 4 years after the first case report and in line with the latest global trends, mesenchymal cell therapy for GVHD was launched at VUH SK as part of the Hope Program. Cell production started with state-of-the-art equipment – bioreactors, which significantly reduce the workload of the staff, while ensuring fully automated and safe cell cultivation in a "closed system" and enabling the production of sufficient cell volumes (Figure 1).

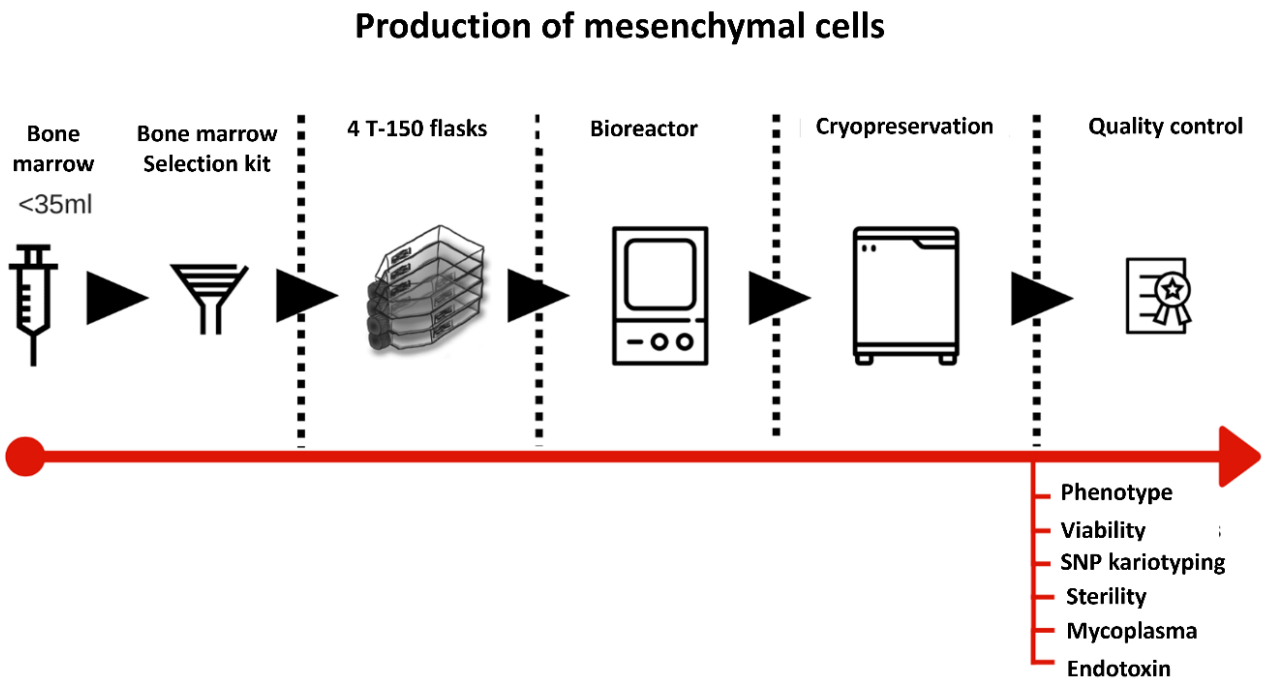

Figure 1. A scheme of mesenchymal stromal cell production at VUH SK.

In 2015, the Vilnius Regional Biomedical Research Ethics Committee granted permission to conduct the "Study of the clinical course of graft-versus-host disease and its medical care" (TPSL-LTU-2015). The clinical experience of VUH SK was presented at the annual European Blood and Bone Marrow Transplantation (EBMT) conferences in 2016 and 2018. In 2018, together with foreign colleagues, a paper was published evaluating and summarizing mesenchymal cell production data from various transplant centers.

The experience of the last few years with mesenchymal stromal cells for the treatment of patients with severe GVHD was presented at the International Scientific and Practical Conference "Baltic Hematology Congress "Sharing Experience"" in 2021. At the end of this year, it is estimated that

more than 70 patients with acute steroid-resistant GVHD (>45 of them with severe GVHD) were treated at VUH SK.

Optimization of mesenchymal cell production processes and cell characterization studies are ongoing at VUH SK. Data analysis is believed to help further improve the treatment of GVHD.

Genomics and data science in oncology and hematology

Next-generation sequencing

Next-generation sequencing (NGS) technology enables the simultaneous analysis of vast amounts of genetic information. It is one of the most effective tools for the diagnosis of rare diseases, allowing the assessment of changes in all human genes.

In 2019, based on the best practices of European genetics centers, the Human Whole Exome Sequencing service was launched at the Hematology, Oncology and Transfusiology Center (HOTC). The exome makes up approximately 1% of the human genome - these are the regions that code proteins. Most of the disease-causing mutations are in these protein-coding regions. A single NGS test identifies from 60 000 to 100 000 individual exome sequence variants, from which only a few must be selected to explain the patient's disease. Whole exome sequencing is now successfully used to diagnose acute myeloid leukemia, myelodysplastic syndrome, primary immunodeficiencies, bleeding disorders and other oncohematological diseases.

Bioinformatics analysis and data science

Understanding which variations among the thousands of possible variants are clinically significant is perhaps the greatest challenge in assessing human genetic information. To facilitate this task, HOTC applies tools that automatically screen for variations that may be associated with disease.

One of the tools that facilitates the search for significant mutations, DeNovoCNN, has been developed in collaboration with foreign colleagues and uses neural network technology[1]. DeNovoCNN simulates parts of the process that a specialist would go through when evaluating new mutations, thus, reducing human workload.

HOTC is currently searching for new genetic mechanisms involved in rare oncohematological diseases. This research focuses on new clinical cases that remain unresolved even after whole exome sequencing. Some of these cases can be explained by structural changes in the DNA, which are often only observed when genomic data from different individuals are compared with each other. HOTC is developing methods that allow simultaneous comparison of NGS data from several hundred individuals.

For a proportion of individuals, an additional whole genome analysis is performed. In genome sequencing, a disease variant has to be selected from several million possible choices, which requires extremely high computational power. HOTC is currently developing a methodology to facilitate the evaluation of whole genome sequencing data and to select the most relevant variations detected in non-coding DNA.

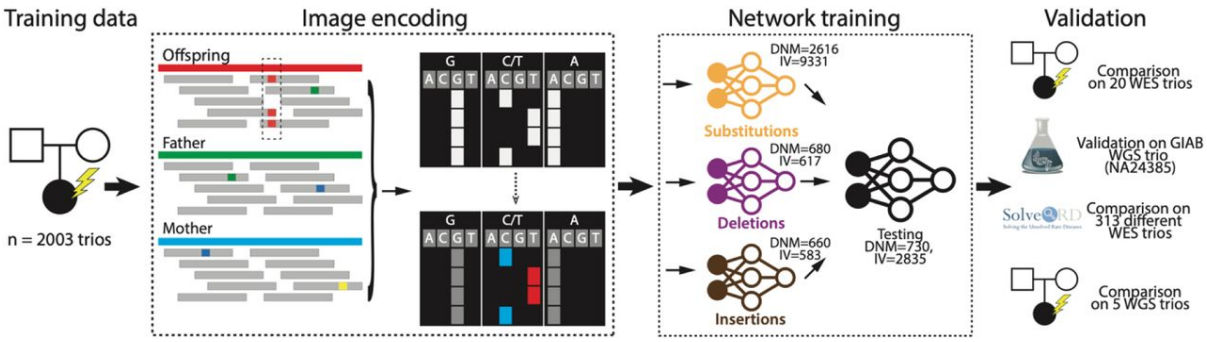

Figure 1. A flowchart of bioinformatic analysis of genetic data.

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Research projects with samples and health data from Biobank

A biobank is a dynamic collection of biological material and related health information that is continuously updated and used in biomedical research. It helps to bridge the gap between biomedical research and healthcare services by rapidly providing researchers with high-quality human biological samples they need for basic, comparative, drug candidate or even personalized medical research.

The second year of the pandemic has brought many challenges as well as new opportunities for the Biobank at Vilnius University Hospital Santaros Clinikos (VUH SK) (Figure 1). The spread of the SARS-CoV-2 virus has stimulated collaboration between specialists from different fields - medicine biotechnology, basic research and information technology. In 2021, the Biobank launched 10 innovative research projects with 7 new partners.

The public has also started to better understand the importance of Biobanks, with 4,359 new participants joining our Biobank in 2021, reaching a total of 7762.

In the last year, 35 000 new samples have been added to the Biobank repositories. The Biobank now has a total of almost 80 000 biological samples ready for research. The Biobank contains a wide range of samples from patients with hematological, oncological, infectious, genetic, rare and chronic non-infectious diseases: blood serum and plasma, bone marrow, nasopharyngeal swabs, saliva, purified ribonucleic acid (RNA), deoxyribonucleic acid (DNA), viable cells or other residual samples left over from diagnostic testing. Samples may also be accompanied by medical information which is stored separately in the Electronic Medical Record system of the VUH SK.

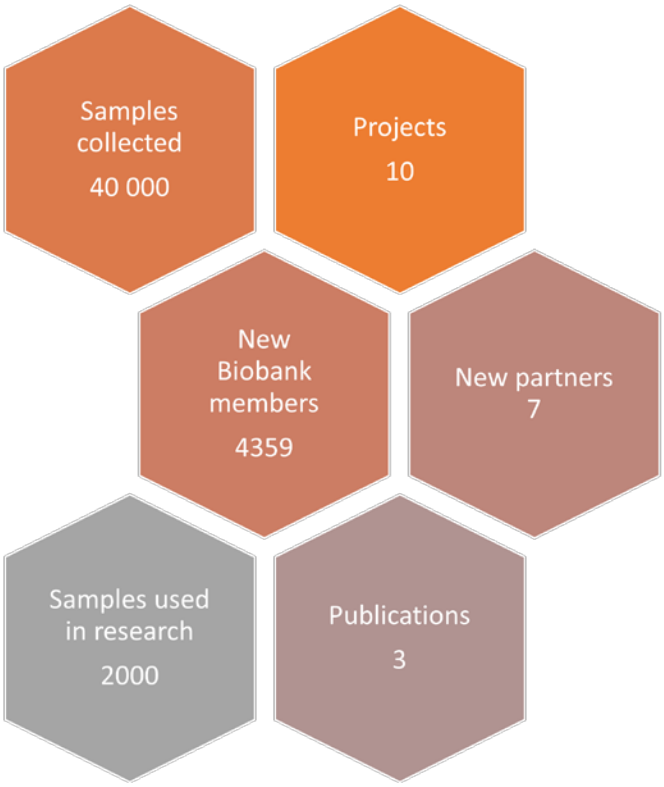

Figure 1. VUH SK Biobank's performance in 2021.

The samples and health information stored in the Biobank were used in different research projects in 2021, as shown in Table 1.

Partners	Studies
UAB „Thermo Fisher Scientific“	Analysis of SARS-CoV-2 variants
UAB „Droplet Genomics“	Bone marrow analysis of AML patients receiving Venetoclax-based therapy using single-cell RNA sequencing
UAB „Thermo Fisher Scientific“	Investigation of molecular tools for the detection of SARS-CoV-2
VUH SK	Investigating the immunogenicity, safety and efficacy of SARS-CoV-2 vaccines in an immunosuppressed population
Vilnius University	Molecular studies of hematopoietic cells

Table 1. Studies using biological samples and health information from the VUH SK Biobank in 2021.

Most of the research that required biological samples and health information collected by the Biobank in 2021 was related to the management of the COVID-19 pandemic (e.g., analyzing the disease course of COVID-19 patients, selecting the best treatment strategies, developing and applying new diagnostic and vaccination strategies). The results of these studies have also been directly translated into everyday clinical practice. The Biobank has made it possible to conduct such research because of rapid access to novel data and the opportunity to continuously apply new knowledge: once the necessary samples and information have been collected in the Biobank, experiments or data analysis can be carried out immediately, without the need for extensive and time-consuming sample and data collection procedures.

Biobank's collection of biological samples and health information is also being used in cancer research: analysis of the effects of drugs on cancer cells, investigation of advanced drug therapies, and studies of immunosuppressed individuals.

Biobank's mission is not limited to the transfer of samples and information to research teams - the staff at the VUH SK's Biobank participate in research as well and engage in projects funded by the Lithuanian Research Council, HORIZON2020, collaborate with specialists in the field ("Thermo Fisher Scientific", "Droplet Genomics" and "CasZyme"), and publish their results[1,2].

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Competence Center for Hemophilia and Coagulation Disorders

Experience and expertise in the care of patients with coagulation disorders and state-of-the-art treatment

The Competence Center for Hemophilia and Coagulation Disorders of Vilnius University Hospital Santaros Klinikos (VUH SK) (**coordinator – pediatric oncologist and hematologist Dr. Sonata Šaulytė Trakymienė**) is a certified and officially recognized center for comprehensive hemophilia care in Europe (https://eahad.org/european-haemophilia-centres-certification/). Such a certificate is obtained by those centers that care for patients with life-long coagulation disorders from birth throughout their lives, guarantee them full diagnosis, long-term monitoring, apply modern treatment and can effectively combat the main complications of these diseases. The Competence Center for Hemophilia and Coagulation Disorders is a member of the European Reference Network EuroBloodNet (http://eurobloodnet.eu).

The center has more than 10 years of experience of clinical research in the development of novel hemophilia therapeutics[1-4]. Today, most of the hemophilia community in Western Europe, including patients in Lithuania, are treated with these drugs. There has been a breakthrough in the treatment of patients with severe hemophilia A in 2021: subcutaneous non-factor therapy has been introduced, raising the goal of treating hemophilia to concepts of "zero bleeds" and a "hemophilia-free mind". Now there is an opportunity to better control hemophilia patients with inhibitors, and improve the quality of life of patients with severe hemophilia without inhibitors. Today, about 36% of patients under 18 years are treated with this innovative therapy.

In 2021, a new clinical trial of subcutaneous non-factor therapy was initiated at the Center ("Efficacy and Safety of Concizumab prophylaxis in patients with hemophilia A or B without inhibitors (explorerTM8)", principal investigator at the Center for Pediatric Oncology and Hematology – **Dr. Sonata Šaulytė Trakymienė**, principal investigator at the Center for Hematology, Oncology and Transfusion Medicine – **Lina Kryžauskaitė**). This study will look at the effectiveness of the new medicine concizumab in patients with hemophilia A or B without inhibitors. The aim is to show that concizumab can protect against bleeding and is safe to use.

The Center is actively involved in clinical trials investigating aspects of thrombosis in children and young adults with acute lymphoblastic leukemia (ALL). Since 2008, prospective thromboembolic events have been registered in Nordic and Baltic patients with ALL treated with the ALL2008 protocol of the Nordic Society of Hematology and Oncology. The data collected during the study were analyzed in different aspects and published. The latest study was aimed to assess the incidence, treatment, and outcome of asymptomatic right atrial thrombosis detected at routine echocardiography of children after acute lymphoblastic leukemia treatment in the Nordic and Baltic countries[5]. Eleven (2.7%, 95% confidence interval: 1.4 to 4.9) of 406 patients had asymptomatic right atrial thrombosis, ranging from 10 to 25 mm at detection. Three patients were treated with anticoagulation. None of the thromboses affected cardiac function, and they showed neither sign of progress nor spontaneous or treatment-related regress at follow-up

In 2021 the Center was chosen as a training center for countries that had the goal to improve hemophilia care in their countries. Colleagues from Azerbaijan and Sakartvelo underwent their training in Lithuania, at the Competence Center for Hemophilia and Coagulation Disorders.

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Center for Pediatric Oncology and Hematology

Skills to improve care for children with malignant tumors

In 2021, Vilnius University Hospital Santaros Klinikos (VUH SK) launched an international HORIZON2020-funded collaboration project **"Twinning in Research and Education to improve survival in Childhood Solid Tumors in Lithuania (TREL)"** [1].

This is a particularly significant initiative of the Center for Pediatric Oncology and Hematology of VUH SK, as its specialists are participating in the project as the main coordinators (**the project leader is Assoc. Prof. Jelena Rascon**), and one of the objectives of the project is to improve the reputation of VUH SK as a research institution, which is expected to increase cooperation with potential project partners.

The TREL project focuses on molecular-genetic studies of the most common pediatric solid tumors (CNS, neuroblastoma and kidney tumors) and resistant cancers. To achieve this goal, the project includes as many as 8 research centers and hospitals in different countries around the world, and involves 46 staff members from VUH SK.

In 2021, a multidisciplinary team of VUH SK specialists had the opportunity to get acquainted with the work organization of the project partners' units conducting early phase clinical trials with children, to improve their skills in monitoring drug concentrations, and to learn about the principles of a centralized pharmacy (Figure 1). Particular attention is paid to improving the project management and administration skills at VUH SK by adopting good management practices from partners (e.g. improving intellectual property protection skills)

Figure 1. Training of VUH SK specialists while implementing the project: in the pediatric onco-hematology and bone marrow transplantation wards of the Rigshospitalet (Copenhagen), and in a centralized pharmacy (left, center); in the Innovation and Technology Transfer Unit of the Gustave Roussy Institute (Paris) (right).

One of the project's work areas is developing scientific skills among young scientists. In 2021, a series of seminars on scientific methodology was organized, which was attended by over 200 participants, and "Guidelines on Scientific Methodology" were developed for use by young scientists conducting research at VUH SK[2].

The TREL project helps to exchange information and share best practices, launch new clinical and scientific research, and discuss complex patient cases. Scientific knowledge gained during the project as well as the network of specialists it has created will strengthen the scientific potential of VUH SK and contribute to improvements in treatment outcomes for children with oncological diseases.

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The Survivor Passport – an innovative tool for survivors of childhood cancer

In March 2021, the project "Study on the development and implementation of the PanCare Digital Survivorship Passport to improve the care of childhood cancer survivors (PanCareSurPass)" was launched at Vilnius University Hospital Santaros Klinikos (VUH SK). Its aim is to develop a system using information technology to transfer the monitoring recommendations of childhood cancer survivors into an electronic health system, so that they are visible and accessible to peripheral healthcare facilities. The project is implemented by the Center for Pediatric Oncology and Hematology at VUH SK (**project leader – Assoc. Prof. Jelena Rascon**) and the Information Systems Unit of the Center for Informatics and Development (**leader – Dr. Justas Trinkūnas**).

Several initiatives funded by the European Commission have led to the development of the Survivorship Passport (SurPass), an online platform that generates recommendations for follow-up of a cancer survivor based on data of the disease and its previous treatment. For example, it is known some treatment choices may increase the risk of future conditions, such as breast cancer and endocrine complications. The increased risk of late complications varies depending on the specific diagnosis and the intensity of treatment. The SurPass platform assesses these risks based on patient data and generates patient-specific monitoring recommendations.

So far, SurPass has been used in Italy and Austria and is only available to specialists at specific hospitals – those in which the recovered person was treated. The PanCareSurPass project aims to roll out SurPass in six EU countries (including Lithuania) and to use information technology and e-health capabilities to digitize SurPass more widely, i.e., to make it available to other healthcare facilities the recovered person may be visiting. In this way, information about the previous illness its treatment and, most importantly, follow-up recommendations will be available to all healthcare professionals working with the patient. This specific information can only be made available with the consent of the person who has recovered.

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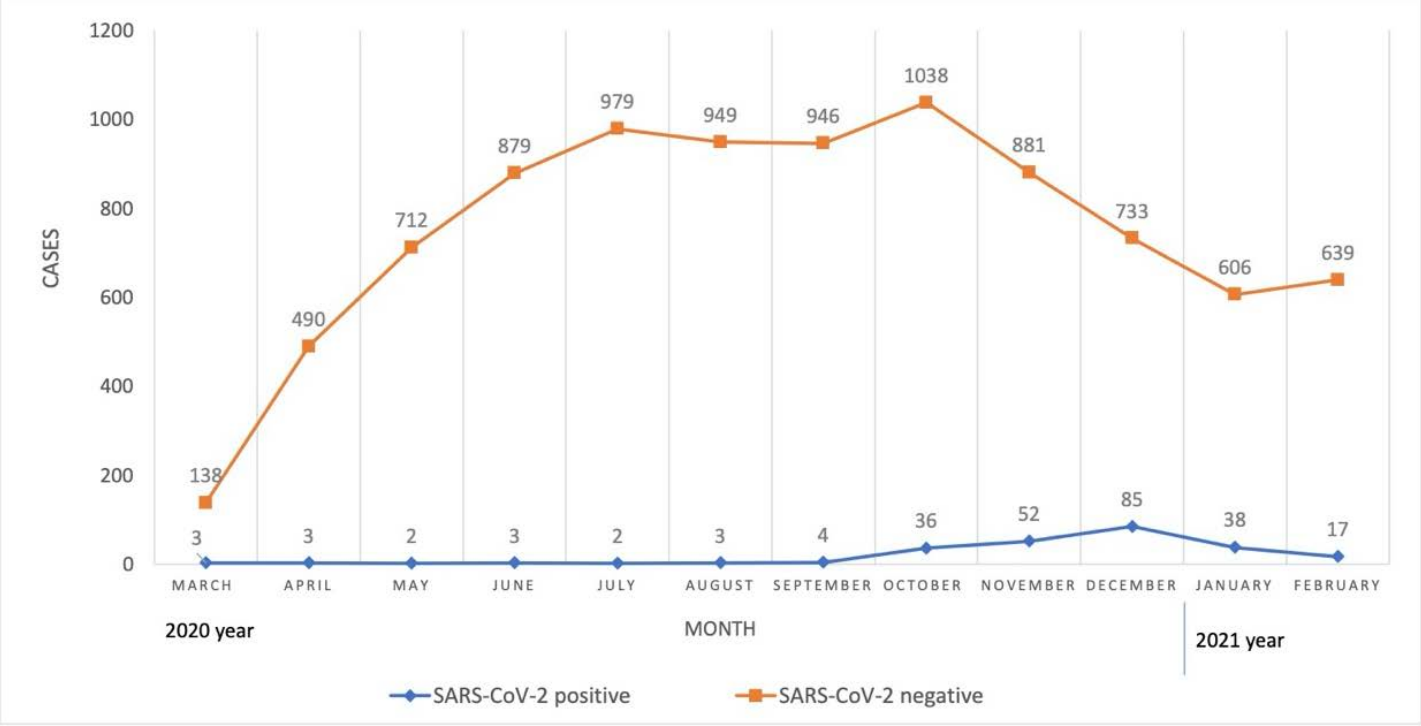
Center for Pediatrics

COVID-19 infection in children during different pandemic phases

With the onset of the COVID-19 pandemic, most data were available about adults and data regarding children was lacking, especially from European countries. In Lithuania, according to the recommendations of the Minister of Health, many children have been tested for SARS-CoV-2 infection since the beginning of the pandemic (e.g., children arriving at the emergency department for both acute and chronic diseases)

A retrospective study was conducted at Vilnius University Hospital Santaros Klinikos (VUH SK) during the first wave of the pandemic. Patients under 18 years, tested for COVID-19 at the pediatric emergency department from March 1 to May 31, 2020, were enrolled in the study. The study revealed that screening for COVID-19 in children is exceptionally challenging due to the diverse and non-specific symptoms of their infection. Testing strategies should not only focus on typical COVID-19 symptoms, such as fever or cough, but also include other symptoms, especially gastrointestinal symptoms. The greatest attention should be paid to known exposure to SARS-CoV-2, especially in family clusters. Screening of asymptomatic children with unknown exposure should be weighed for medical necessity and cost-effectiveness.

After the first successful publication[1], the duration of the study was extended to February 28, 2021. Almost ten thousand children (n=9238) of different age groups were tested for COVID-19 during a one-year period (Figure 1). The study was one of the few that compared COVID-19 data in children during different phases of a pandemic (Phase I: quarantine during the first wave (March-May 2020); Phase II, released restrictions as numbers of cases decreased (June-September 2020); and Phase III, the second wave (October 2020-February 2021))[2].



1 pav. The distribution of SARS-CoV-2-positive and SARS-CoV-2-negative cases monthly.

SARS-CoV-2 PCR tests were positive for 2.7% of all tested children. Although high numbers of testing were maintained throughout the year, the positive test results were significantly higher during the Phase III (5.5%) compared with the Phase I (0.6%, $p<0.001$) and Phase II (0.3%, $p<0.001$). Comparison of SARS-CoV-2-positive subjects during Phases I–III is shown in Table 1.

Characteristics	**Phase I, n=8 (2020.03-2020.05)**	**Phase II, n=12 (2020.06-2020.09)**	**Phase III, n=228 (2020.10-2021.02)**	**Total, n=248 (2020.03-2021.02)**
Male	3 (37.5)	8 (66.7)	117 (51.3)	128 (51.6)
Female	5 (62.5)	4 (33.3)	111 (48.7)	120 (48.4)
Median age (IQR)	4.0 (0.5-11,25)	11.0 (3.0-16.0)	3.0 (0.25-12.0)	4.0 (1.0-12.0)
< 1 year	2 (25)	2 (16.7)	57 (25)	61 (24.6)
1-2 years	1 (12.5)	0 (0)	46 (20.2)	47 (19)
3-6 years	2 (25)	2 (16.7)	33 (14.5)	37 (14.9)
7-11 years	1 (12.5)	3 (25)	34 (14.9)	38 (15.3)
12-17 years	2 (25)	5 (41.7)	58 (25.4)	65 (26.2)
With known exposure	8 (100)	9 (75)	135 (59.2)	152 (61.3)
Without known exposure	0 (0)	3 (25)	93 (40.8)	96 (38.7)
With known COVID-19 symptoms	7 (87.5)	7 (58.3)	195 (85.5)	209 (84.3)
Without known COVID-19 symptoms	1 (12.5)	5 (41.7)	33 (14.5)	39 (15.7)

Table 1. Comparison of SARS-CoV-2 positive subjects in Phases I-III.

The study also evaluated the possible predictors of COVID-19. Infants and teenagers (12-17 years) accounted for a larger proportion of COVID-19 patients (24.6 and 26.2%, respectively) compared to other age groups. There were more COVID-19 cases among children with a known SARS-CoV-2 exposure compared to those who did not declare any contact (18.2 vs. 1.1%). When symptoms were adjusted for age, gender and known exposure to SARS-CoV-2, univariable logistic regression analyses showed that fever, pharyngitis, headache and anosmia / ageusia were the most significant predictors.

The research team was pleased to present worldwide the first clinical and epidemiological COVID-19 data in children from our country[1,2].

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Center for Children's Surgery, Orthopedics and Traumatology

Cryptorchidism and infertility: pathogenesis, treatment and molecular biology

Now for a decade, physicians at the Center for Children's Surgery, Orthopedics and Traumatology of Vilnius University Hospital Santaros Klinikos (VUH SK) in collaboration with the Cryptorchidism Research Institute under the guidance of Prof. F.Hadziselimovic (Switzerland), conduct not only clinical but also basic research to elucidate the causes of cryptorchidism and infertility. Specialists of the Center have already defended two dissertations on the topic at the Faculty of Medicine of Vilnius University ("The timing of surgery for cryptorchidism and fertility prediction based on the evaluation of spermatogenesis in testicular biopsies" – Dr. Vytautas Bilius, 2015, and **"Correlation between histology and gene expression in cryptorchidism" – Dr. Beata Vincel, 2021**)[1]. In 2021, the results were presented at international conferences as well[2-3].

Undescended testes are one of the most common urogenital conditions in newborn boys. In Lithuania, the overall incidence of cryptorchidism is 5.7%, it ranges between 2 and 9% worldwide. The etiology of cryptorchidism is multifactorial – the development of undescended testes is influenced by anatomical, hormonal, genetic and environmental factors. The etiology of undescended testes is mostly unknown. Hypogonadotropic hypogonadism is referred as one of the main causes of cryptorchidism-related infertility, because in 70% of the cases undescended testes display the signs specific for hypogonadotropic hypogonadism, such as lack of germ cells, impairment of gonocyte transition to Ad spermatogonia, Leydig cell atrophy. Cryptorchidism is associated with infertility and a 5 to 10 times greater risk of developing testicular tumors. Up to date, orchidopexy during the first year of life is the recommended treatment of undescended testes. However, surgical treatment itself does not prevent all formerly cryptorchid men from developing non-obstructive infertility. Decreased fertility potential is associated with impaired endocrine equilibrium because of underexpressed genes that control the hypothalamic-pituitary-gonadal (HPG) axis and are involved in germ cell development. In her dissertation work, B.Vincel determined that adjuvant hormonal treatment with gonadotropin-releasing hormone analogue (GnRHa) induces gonocyte transition to Ad spermatogonia and has positive effect on restoring male fertility potential (Figure 1)[1].

By continuing the collaboration with the Cryptorchidism Research Institute, further investigations of the impact of different genes on spermatogenesis and testicular descent are planned. Moreover, a research project titled "Influence of maternal infectious diseases and vaccinations during pregnancy on the development of cryptorchidism" is being prepared. One of the hypotheses to be tested is that an increase in estradiol levels during pregnancy because of viral infections is a possible risk factor for the development of cryptorchidism.

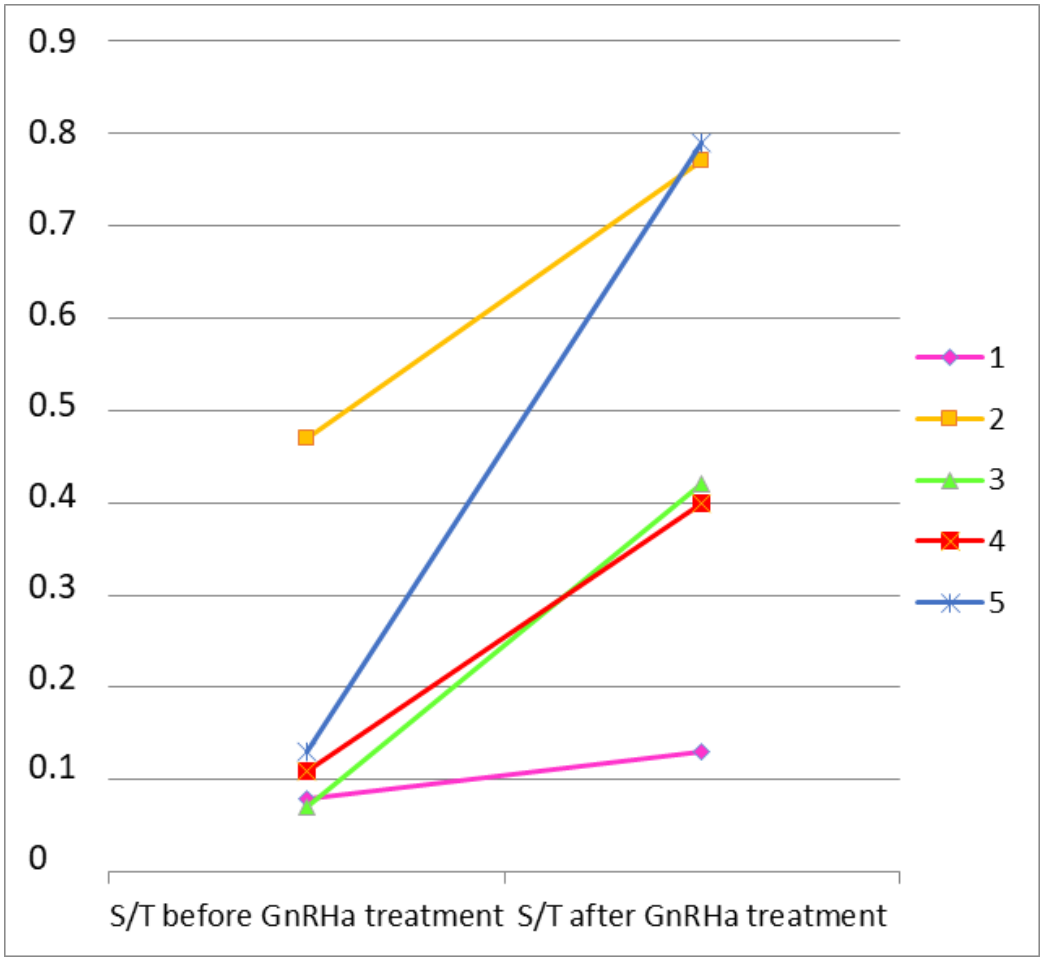

Figure 1. S/T (spermatogonia count per tubule) changes in boys with bilateral cryptorchidism before and after adjuvant hormonal treatment. Every color represents one patient.

Molecular analysis performed in Switzerland revealed that differential gene expression significantly differs between high and low infertility risk groups. The genes analyze are involved in hormonal control of the hypothalamic-pituitary-gonadal axis and fertility, affect loci involved in canonical and alternative testosterone synthesis pathways. Moreover, non-coding ribonucleic acids (RNAs) that may play an important role in the regulation of spermatogenesis were analyzed. It was determined that adjuvant hormonal treatment helps restore fertility potential. Based on the results adjuvant hormonal treatment with GnRHa is recommended for cryptorchid boys with a histologically confirmed high risk of infertility.

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Center for Children's Surgery, Orthopedics and Traumatology, Center for Urology

Pathogenesis, diagnosis and treatment of the obstructive uropathies

Urinary tract obstruction is a common problem. Its symptoms depend on the level of obstruction, which may occur in different levels – from the urethra up to kidneys. Specialists from both the Center for Urology and the Center for Children's Surgery, Orthopedics and Traumatology are involved in the European Reference Network for Urorectogenital Diseases and Complex Conditions, eUROGEN (**Ramunė Žilinskaitė-Tamašauskė, Aivaras Grybas, Vytis Kazlauskas, Arūnas Želvys, Gilvydas Verkauskas)** are investigate clinical and basic etiopathogenesis of fibrosis.

Urethral stricture is the lowest site of obstruction and may be either congenital or acquired. The pathogenesis of this process is not entirely clear and better prevention and treatment options are being sought.

Recently, in cooperation with researchers from the Life Sciences Centre of Vilnius University (V.Bukelskienė, D.Baltriukienė, EŠimoliūnas, P.Barasa, E.Baltrukonytė, N.Krestnikova, M.Alksnė, I.Rinkūnaitė) a project "Artificial urethra in the treatment of hypospadias and urethral strictures" was started with the aim to create a functional urethral tissue which could be used to replace the defected one along with a possibility to evaluate potential genetic risk factors for scarring and inhibiting them in the bioengineered tissue. Two reports of the project were presented in 2021 at the Congress of the European Association of Pediatric Surgeons (EUPSA) in Athens[1] and the conference "Life Sciences Baltics 2021".

A comprehensive diagnosis of obstruction is essential in reducing the risk of terminal renal damage. Elimination of the obstructive component effectively prevents the worsening of kidney function. The most challenging part of the diagnosis is the identification of obstruction in cases of a "grey zone" when a proper diagnosis can be made just after multiple examinations. Among the disadvantages of such an approach is time loss that may lead to a further deterioration of renal function, diagnostic modalities with exposure to X-rays, the requirement for peripheral venous access and general anesthesia in small children. These reasons substantiate research of new minimally invasive or non-invasive diagnostic modalities.

Between 2019 and 2021, a prospective study was performed at the Center for Children's Surgery, Orthopedics and Traumatology of VUH SK by collecting urine from pediatric patients with evidence of obstructive uropathy[2]. The same children underwent standard diagnostic workup for obstructive uropathy. Subsequently evaluation of ultrasound data and biochemical urinary markers was evaluated for association with clinical and radiological data.

The latter study also resulted in the initiation of an international collaboration in the AGORA (Aetiologic research into Genetic and Occupational/environmental Risk factors for Anomalies in children) project, particularly cooperating with the Radboudumc Amalia Children's Hospital (the Netherlands) to investigate the etiopathogenesis of congenital posterior urethral valves. The aim of this study is to perform genome sequencing and look for potential mutations which can cause the development of posterior urethral valves. Parents of the patients are also asked to fill out questionnaires concerning potential environmental risk factors for the development of posterior urethral valves.

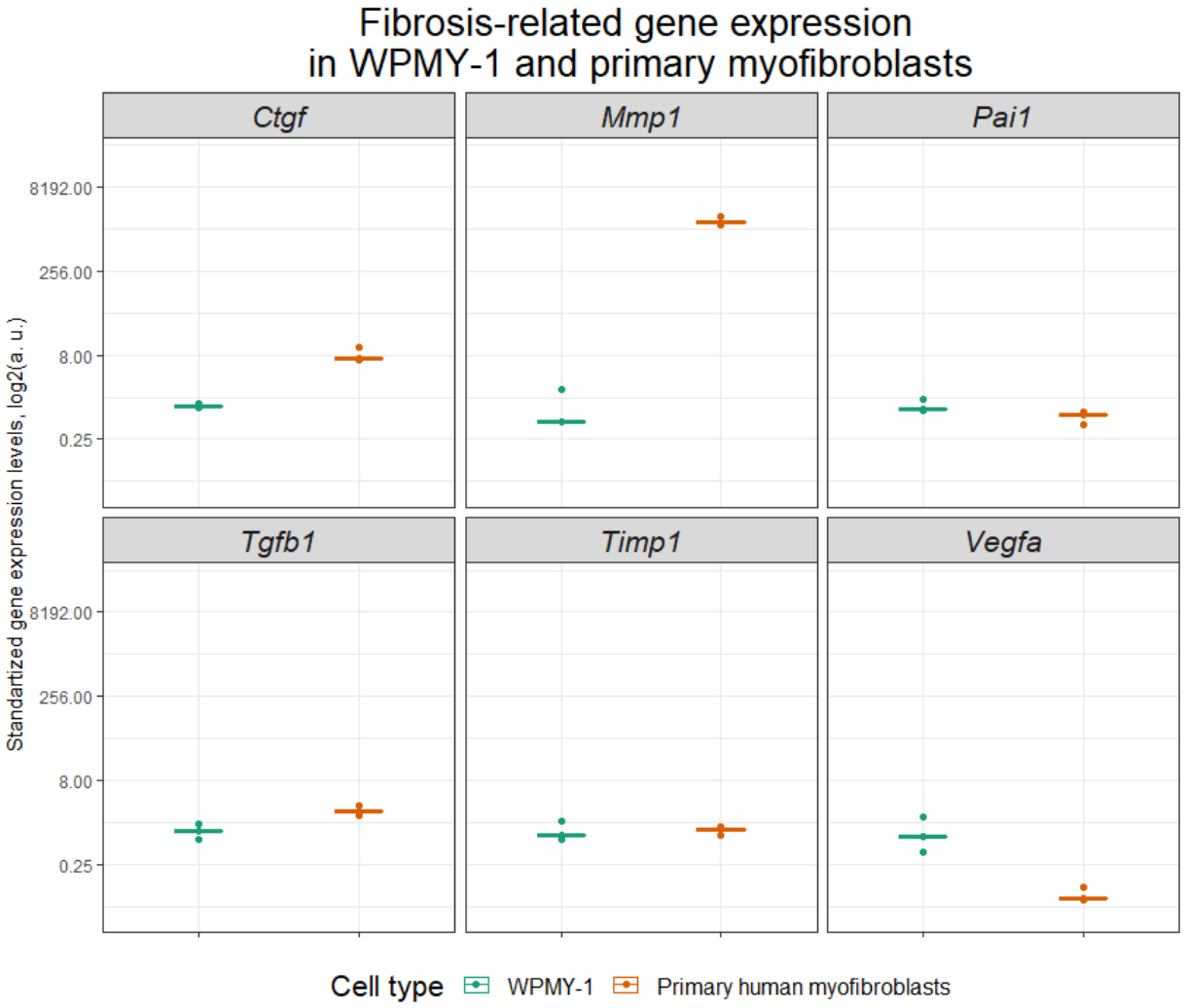

Figure 1. Analysis of gene expression. Fibrosis-related gene expression WPMY-1 (ATCC commercial myofibroblast cell line) and human cell myofibroblast isolates (qPCR).

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Center for Obstetrics and Gynecology

Noninvasive Immunological Analysis of Amniotic Fluid in Preterm Birth

In 2019, specialists of the Center of Obstetrics and Gynecology at Vilnius University Hospital Santaros Klinikos (VUH SK) received a grant from the Research Council of Lithuania for a study titled **"Noninvasive Immunological Analysis of Amniotic Fluid in Preterm Birth"** at Vilnius University. **From the six members of the research team, four are doctors at VUH SK: Prof. Diana Ramašauskaitė, Assist. Prof. Ingrida Pilypienė, Greta Balčiūnienė and Violeta Gulbinienė.**

Preterm birth is birth up to 37 weeks of pregnancy. One of the most common causes of spontaneous preterm birth is chorioamnionitis, which is detected in 40-70% cases of all preterm births. This infection is dangerous for women because of an increased risk of uterine atony and postpartum bleeding, endometritis, peritonitis, and sepsis. Chorioamnionitis also leads to fetal inflammatory response syndrome, which is associated with higher rates of neonatal morbidity (e.g., cerebral palsy, intracranial hemorrhage, sepsis, respiratory distress syndrome, necrotizing enterocolitis, and neurodevelopmental disorders) and mortality.

Histological, microbiological, biochemical, and clinical criteria can be used to define chorioamnionitis. Clinical criteria for the diagnosis of chorioamnionitis have low sensitivity, microbiological examinations require several days to be completed, and histological examination is possible only after delivery. Because of these reasons, there is no universally accepted method for the diagnosis of chorioamnionitis. More trials are being performed each year to validate a method that would be suitable for the early diagnosis of chorioamnionitis, could be performed rapidly and easily, and would have high specificity and sensitivity.

Many studies have been conducted to identify amniotic fluid markers to allow the earlier diagnosis of chorioamnionitis. Most of these studies have analyzed the results of amniotic fluid immunological markers from amniotic fluid obtained by amniocentesis. Amniocentesis has been shown to be a safe procedure to collect amniotic fluid, but it is more complicated in cases with preterm premature rupture of the membranes (PPROM) because of a low amount of residual amniotic fluid.

The aim of the study at VUH SK was to analyze amniotic fluid that is obtained non-invasively. The non-invasive collection is easy to perform, does not require special skills and does not have additional risks

The main findings:

1. The leukocyte count, widely used and included in the clinical diagnostic criteria for chorioamnionitis, is not a sufficiently reliable diagnostic parameter for chorioamnionitis. Neutrophil lymphocyte ratio has higher diagnostic values than the leukocyte count.
2. Interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), matrix metalloproteinase-8 (MMP-8) and soluble urokinase plasminogen activator receptor (suPAR) in non-invasive amniotic fluid are statistically significant markers of the histological diagnosis of chorioamnionitis (Figure 1).
3. Evaluation of maternal blood neutrophil lymphocyte ratio in combination with MMP-8 in non-invasive amniotic fluid had statistically significantly higher diagnostic value than evaluation of blood inflammatory markers alone.
4. The analysis of immunological markers of non-invasively collected amniotic fluid had sufficiently high diagnostic values for histological chorioamnionitis and could be an alternative method to invasive amniocentesis.

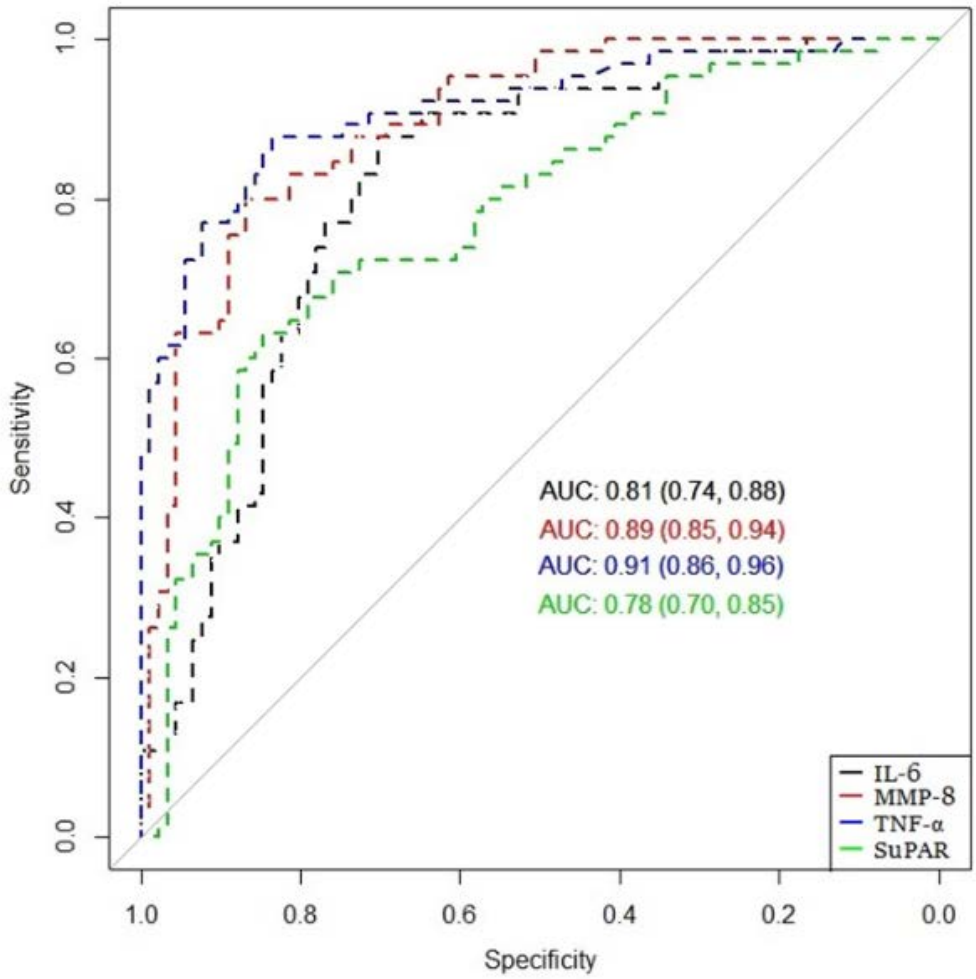

Figure 1. The diagnostic accuracy using different biomarkers. IL-6 – Interleukin-6, 6, MMP-8 – matrix metalloproteinase-8, SuPAR – soluble urokinase plasminogen activator receptor, TNF-α – tumor necrosis factor alpha.

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Center for Hepatology, Gastroenterology and Dietetics

International collaboration and research at the Center for Hepatology, Gastroenterology and Dietetics

Since 2015, specialists from the Center for Hepatology, Gastroenterology and Dietetics (HGD) at Vilnius University Hospital Santaros Klinikos (VUH SK) have been collaborating with the Center for Diseases Analysis Foundation (CDAF), a research center in the United States, to carry out research on the epidemiological surveillance of viral hepatitis B and C. The CDAF involves around 200 research centers from around the world. Developments in the epidemiology of viral hepatitis are publicly available on the Polaris Observatory website (https://cdafound.org/polaris/). The Center for HGD regularly provides CDAF analysts with information on the epidemiological situation of viral hepatitis B and C in Lithuania. The Center for HGD is a full member of Polaris Observatory (Polaris Observatory Collaborator), therefore, the specialists at the center are actively involved in the preparation of international agreements, guidelines and publications.

Based on the results of long-term surveillance and several studies on the modulation of the prevalence of viral hepatitis, the CDAF and the World Health Organization (WHO) have initiated a challenge to eradicate viral hepatitis (Figure 1). In 2021, the preliminary achievements of the challenge to eliminate hepatitis C virus (HCV) by 2030 were summarized[1]. Recommendations were published emphasizing the need to adapt viral hepatitis elimination strategies according to the specific epidemiological situation of each country.

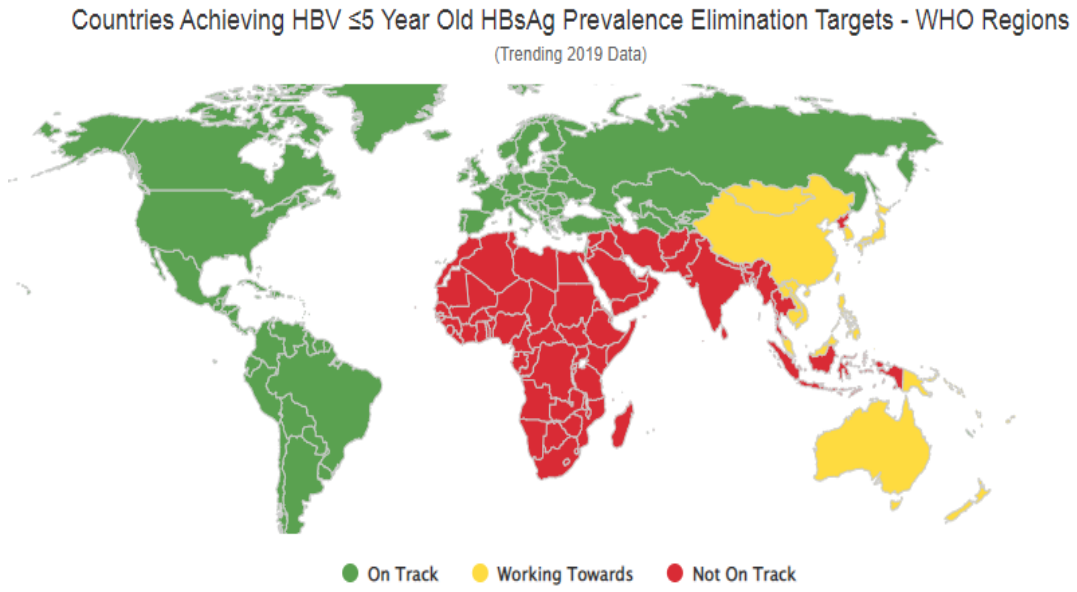

Figure 1. Countries that have achieved hepatitis B virus elimination targets.

The Center for HGD is also part of the international Nonalcoholic fatty liver disease (NAFLD) study group. Clinical experts from VUH SK contributed to the development of the NAFLD consensus guidelines, which were published in prestigious scientific journals in 2021[2-3].

Another focus at the Center for HGD is the assessment of the prevalence, interactions, healthcare services, use of various medications and clinical outcomes of chronic liver and gastrointestinal diseases in Lithuania. The aim of the ongoing research is to examine the treatment and care of patients with chronic liver and gastrointestinal diseases in real-life settings and the interactions between chronic diseases. This will help to provide recommendations to reduce the burden of chronic diseases on the health system[4].

The Center for HGD is also a research base for PhD students from the Faculty of Medicine of Vilnius University[5]. Rare and complex clinical cases are being observed with resident doctors: 3 clinical cases and one literature review on the challenges of treating the rare Flood syndrome during the COVID-19 pandemic were published in 2021[6-8]. Finally, doctors at the Center for HGD Centre are actively involved in clinical drug trials. The main therapeutic areas are inflammatory bowel diseases, small bowel diseases and liver diseases.

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Center for Abdominal and Onco-surgery

New diagnostic and therapeutic approaches in abdominal and onco-surgery

The Center for Abdominal and Onco-Surgery at Vilnius University Hospital Santaros Klinikos (VUH SK) (head – Prof. Kęstutis Strupas) is home to Hepatopancreatobiliary and Transplantation (HPB+Tx), Gastrointestinal-Metabolic-Endocrine, and Colorectal surgery working groups.

HPB+Tx surgery

The number of HPB+Tx liver and pancreas surgeries performed while using minimally invasive techniques is increasing in the Center for Abdominal and Onco-Surgery at VUH SK. After various 3D imaging reconstructions, individualized surgical tactics are selected and multi-stage liver surgery is performed, where the bulk of the hepatic parenchyma is first dissected by ligation of the portal and hepatic venous system, and the affected part of the liver is removed once the hepatic parenchyma is sufficiently enlarged[1]. In 2021, a unique and less traumatic modification of this operation was performed, where sufficient liver augmentation was achieved without liver separation and the intrahepatic circulation was occluded with a tourniquet.

In 2021, an international program for medical students, lasting eight months, was initiated by doctors of the Center for Abdominal and Onco-Surgery at VUH SK. The educational program "From Cell Biology to Surgery" is based on a cooperation agreement signed in 2018 with partners from Kansai Medical University (Japan). International HPB+Tx Surgery Meetings are held monthly and composed of a discussion of novel literature in the field (organizer from VUH SK – Dr. Aistė Kielaitė-Gulla).

The Center also carries out basic research, such as the development of liver organoid models (Fig. 1) or in vitro evaluation of anticancer drug efficacy[1-2].

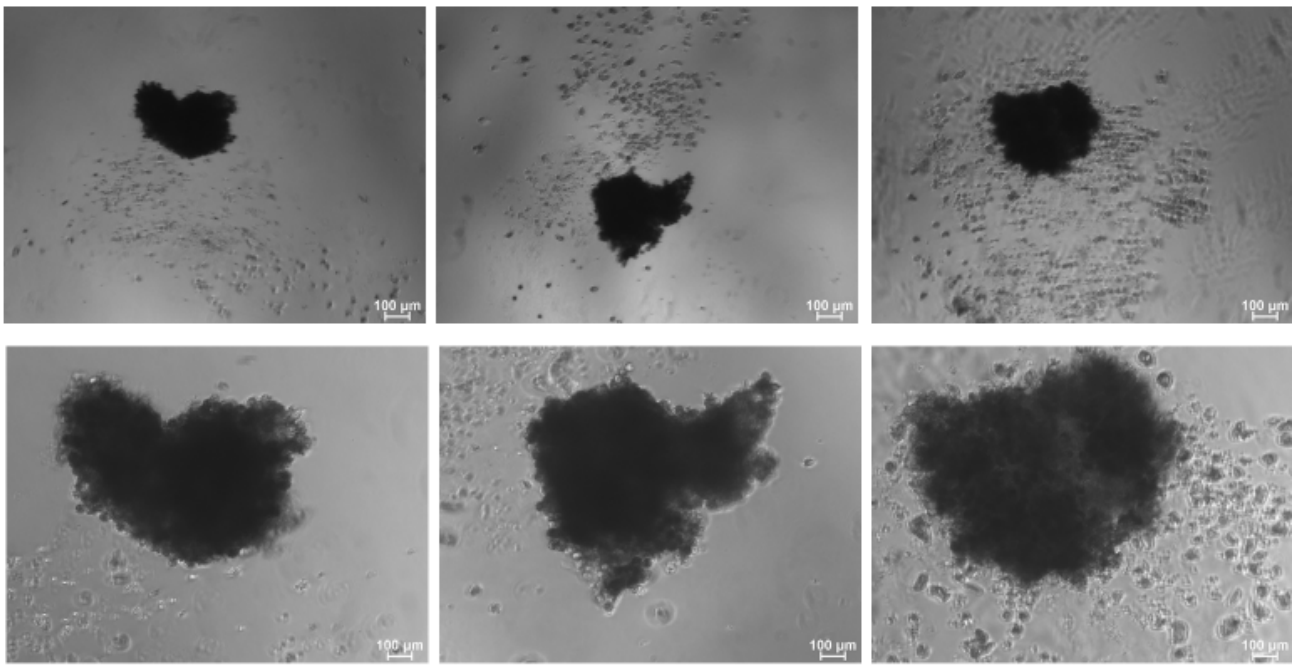

Figure 1. The formation of hepatic organoids

CEACAM6 – a promising biomarker for pancreatic cancer

In 2021, a team of researchers led by Prof. Audrius Šileikis **completed a study that looked for a new advanced biomarker for pancreatic cancer, sought to assess its clinical characteristics, and find ways to personalize the care of pancreatic cancer patients**[3]. Due to the heterogeneous nature of the disease, the lack of early diagnostic tools and resistance to chemotherapy, the survival of patients with pancreatic cancer has remained stable for several decades. Between 2013 and 2020, 267 patients were enrolled (Figure 2). Surgical pancreatic tissue samples were analyzed using high-resolution mass spectrometry. Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) was identified as a promising biomarker for pancreatic cancer. The predominance of CEACAM6 in pancreatic cancer tissue was confirmed using antibody-antigen interaction-based methods. To complete the clinical evaluation of the disease, serum blood samples were also assayed for carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) levels.

CEACAM6 was found not to be suitable as a diagnostic biomarker. Its serum levels were higher in one of the control groups (patients with chronic pancreatitis) than in pancreatic cancer patients. On the other hand, Kaplan-Meier analysis (disease-free survival and overall survival) revealed that shorter overall survival was statistically significantly associated with increased serum CEACAM6 levels (17.0 vs. 12.6 months, with or without CEACAM6 detection, respectively, p=0.017) in patients who underwent radical treatment and adjuvant chemotherapy (Figure 3). There was no correlation between elevated CEA or CA19-9 levels and patient survival. Thus, CEACAM6 has been identified as a promising new biomarker for pancreatic cancer with important implications for prognosing chemoresistance and leading to improved individualized treatment.

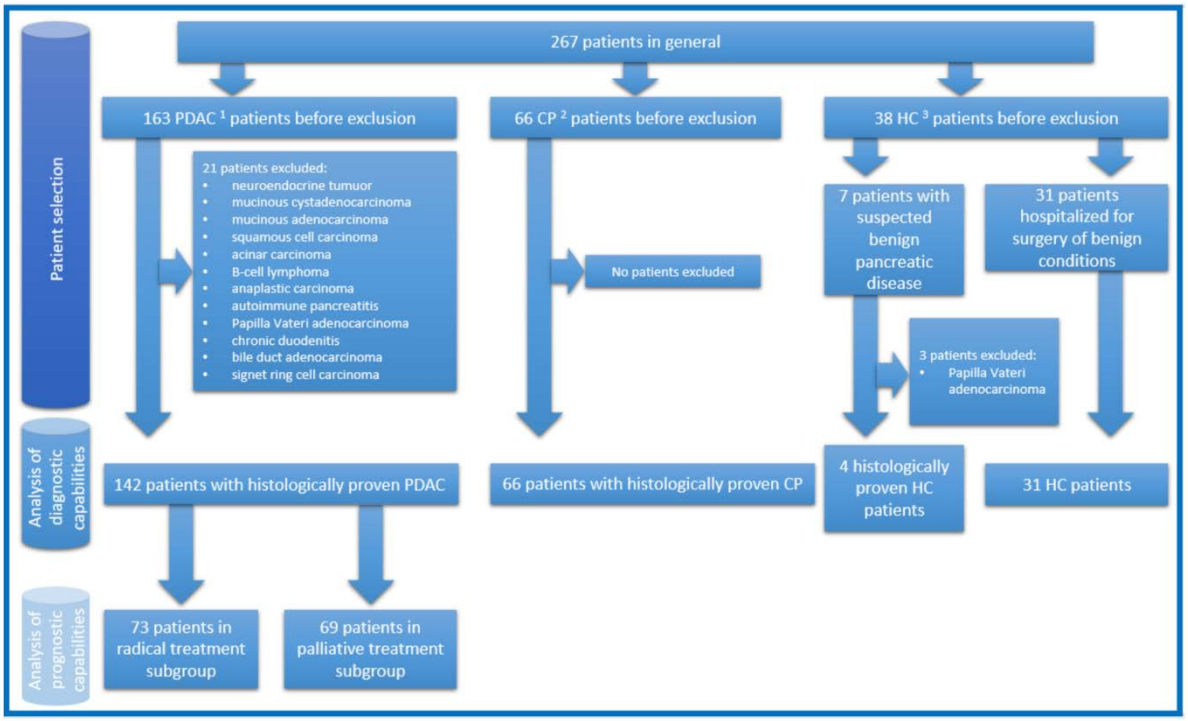

Figure 2. The study design, numbers of patients in each analyzed group, and reasons for exclusion. 1 Pancreatic ductal adenocarcinoma; 2 chronic pancreatitis; 3 healthy controls.[3]

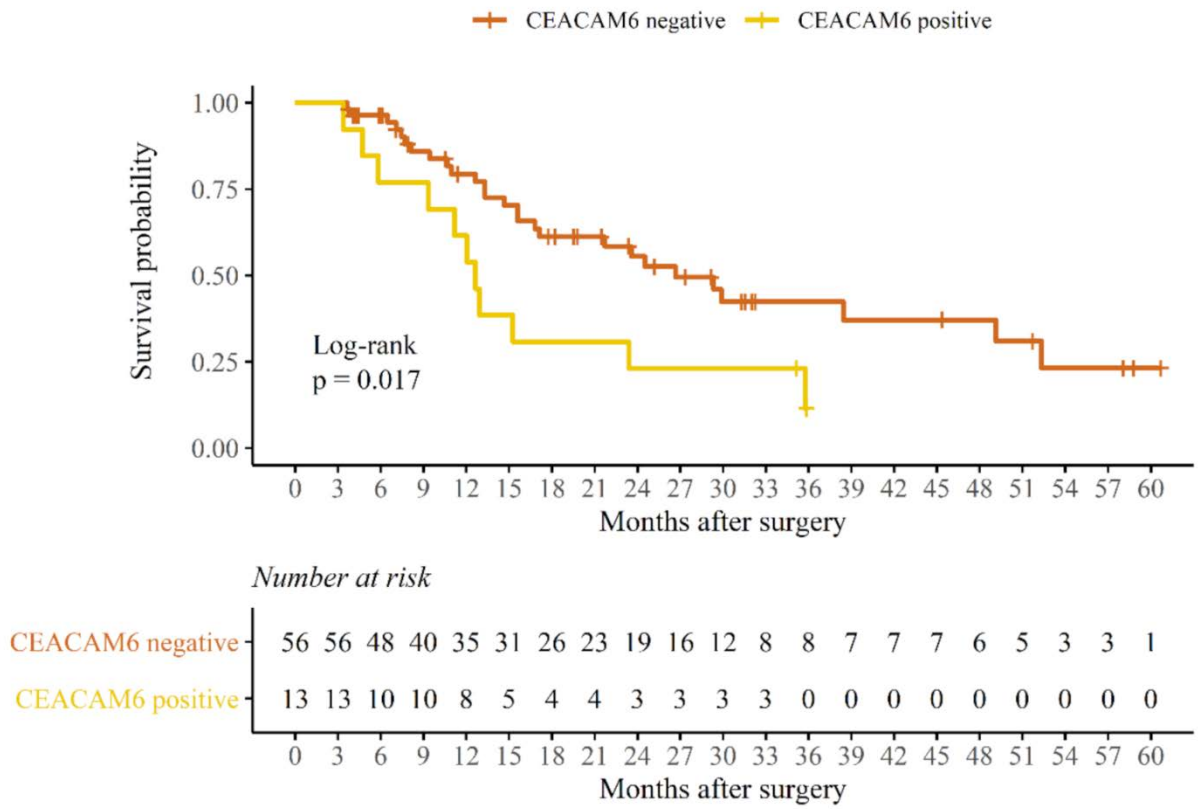

Figure 3. Kaplan-Meier curve representing the dependency of CEACAM6 blood serum concentration values on overall survival in PDAC patients after radical treatment.[3]

Gastrointestinal-Metabolic-Endocrine surgery – focus on stomach cancer

In recent years, upper gastrointestinal surgery has improved rapidly, especially considering minimally invasive surgery for gastric cancer. In these operations, the stomach or its part, together with the surrounding tissues and regional lymph nodes, is removed by making only a few small incisions in the abdomen (about one centimeter in length), thus, the patient recovers more rapidly and, if necessary, can begin additional cancer therapy earlier. Attention is also paid to pre-operative care, with a study published in 2021 showing that a maximum of 30 days between neoadjuvant chemotherapy and gastrectomy can be expected to result in a better pathological response[4]. A clinical trial has been launched with the National Cancer Institute to assess the impact of a new treatment concept – specific patient preparation ("pre-operative rehabilitation") – on treatment outcomes. This new and innovative approach is expected to significantly reduce the incidence of post-operative complications and speed up recovery after major surgery.

Unique endocrine surgery

When performing thyroid and adrenal gland surgery, it is particularly important to choose a treatment method that is the safest for the patient. In 2021, a modern minimally invasive transaxillary thyroid surgery was launched at VUH SK, with its main advantage being that incisions in the neck are avoided (Figure 4). A neuromonitoring system has also been introduced in daily practice to ensure maximum patient safety during thyroid and parathyroid surgery (Figure 5).

VUH SK remains the only center in the region that performs retroperitoneal adrenalectomies where the adrenal gland is removed without entering the abdominal cavity. Modern thermal ablation procedures (microwave ablation, radiofrequency ablation, laser ablation) have also been introduced among endocrine organ surgery techniques. An experimental-clinical study has been initiated to evaluate the use of laser ablation for the treatment of thyroid cancer. The Center for

Abdominal and Onco-Surgery of VUH SK has already become a training center for endocrine surgery, where, in cooperation with foreign partners, colleagues from Lithuania and abroad come to improve their skills.

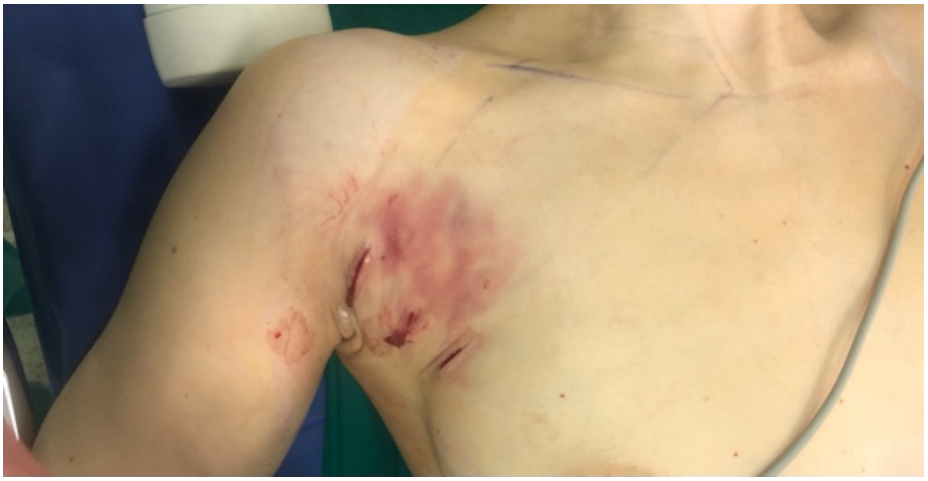

Figure 4. Transaxillary thyroid surgery

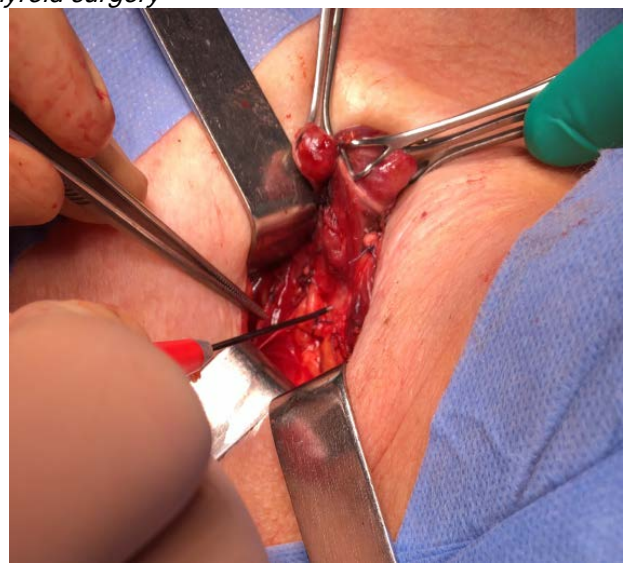

Figure 5. Neuromonitoring system.

An improved diagnostic algorithm for acute appendicitis

In 2021, colorectal surgery specialists (led by Prof. Tomas Poškus), together with representatives of the VUH SK Center for Radiology and Nuclear Medicine, have published findings of a prospective clinical trial seeking to optimize the diagnosis of acute appendicitis5. The study showed that, in cases when acute appendicitis is suspected, but results of abdominal ultrasound are unclear, abdominal computerized tomography can optimize the use of radiological resources, while achieving high diagnostic sensitivity and specificity. This clinical study also confirmed that the routine use of abdominal magnetic resonance imaging in pregnant patients with an unconfirmed ultrasound diagnosis of acute appendicitis significantly reduces the number of unnecessary surgical interventions. **All these findings have led to an adjustment of the previously used algorithm for the diagnosis of acute appendicitis and to a reduction of unnecessary surgical interventions with no additional risk for the patients.**

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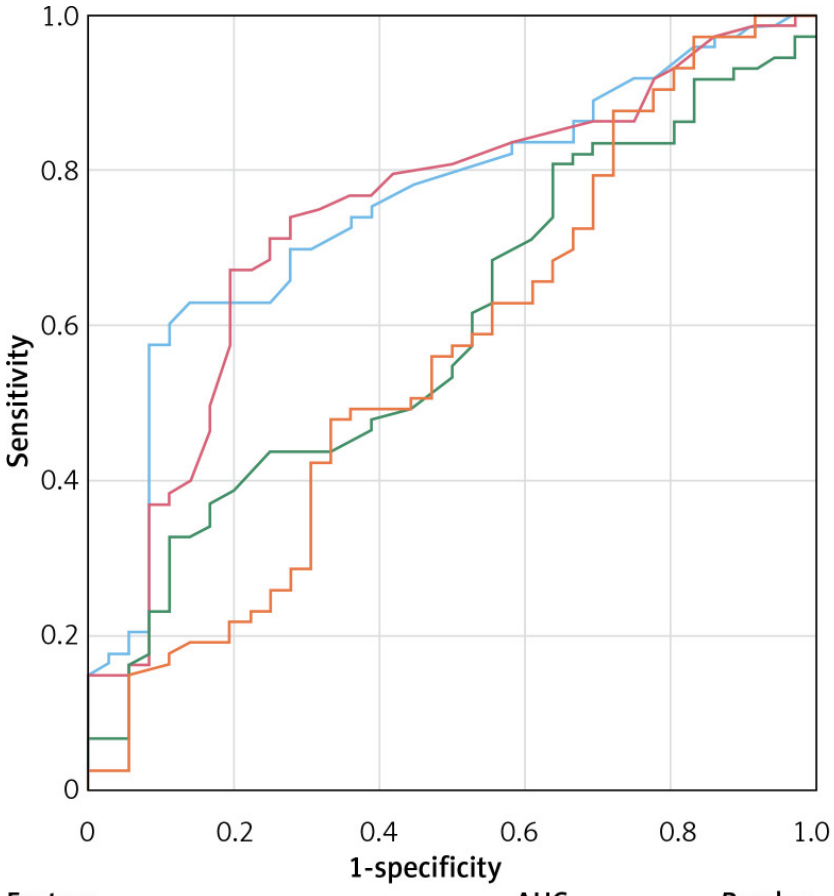
Center for Urology

Clinical and basic research in urology

In 2021, studies on extracorporeal lithotripsy were continued at the Center for Urology at Vilnius University Hospital Santaros Klinikos (VUH SK). Over the last decade, the need for urological services for people with kidney stones has increased by ten percent, which has encouraged the development of minimally invasive lithotripsy procedures. **Marius Snicorius, Dr. Arnas Bakavičius, Dr. Albertas Čekauskas, Prof. Marius Miglinas, Gediminas Platkevičius and Assoc. Prof. Arūnas Želvys**, evaluated data of patients treated with extracorporeal shock wave lithotripsy (ESWL) between January 2015 and December 2019, looking for clinical features of renal calculi that are associated with a more effective course of procedure[1]. Of the 109 individuals included in the study, 73 (67.0%) had a successful procedure. Failure was associated with a higher volume of kidney stones, a longer distance to the target, and lower energy used during the procedure ($p<0.05$). Stone size was found to be the most important factor associated with poorer procedural outcomes (Figure 1). The above results have provided more information on the use of ESWL in the treatment of renal calculi and will allow for a more efficient planning of therapeutic procedures in patients with renal calculi, providing the latter with more accurate information.



Factors	AUC	*P*-value
Stone volume	0.754	**< 0.01**
Stone surface area	0.742	**< 0.01**
Skin to stone distance	0.594	0.10
Mean stone attenuation value	0.561	0.31

Figure 1. Factors influencing extracorporeal shock wave lithotripsy efficiency for optimal patient selection. ROC analysis results for ESWL failure.[1]

In 2021, specialists of the Center for Urology at VUH SK closely collaborated with researchers from the Life Sciences Centre and the Faculty of Physics of Vilnius University and the National Cancer Institute by conducting basic oncology research in urine and tumor tissue samples in search of new biomarkers related to the course and prognosis of prostate, kidney and bladder cancer[2-4]. **The study titled "The significance of novel prostate-specific antigen isoforms for the early diagnosis of prostate cancer" by Marija Barisienė was completed as well.** Prostate cancer is the second most common cancer in the world and the most common cancer in Lithuania. Prostate-specific antigen (PSA) is the most common serum molecular marker used in the diagnosis of prostate cancer. It is characterized by low specificity, when its serum levels are below 10 ng/ml. The aim of the aforementioned study was to evaluate the role of the serum molecular marker [-2]proPSA and its indices %p2PSA, Prostate Health Index (PHI) and Prostate Health Index Density (PHID) in the early diagnosis of prostate cancer in men with serum PSA levels between 2 and 10 ng/ml who do not have prostate cancer-specific abnormalities upon digital rectal examination. A total of 210 patients participated in the study. Prostate biopsy material revealed prostate cancer in 112 patients (53.3%). Based on Epstein criteria and the 2014 ISUP classification, clinically significant prostate cancer was detected in 72.3% and 35.7% of patients, respectively, and 51 (24.3%) patients underwent radical prostatectomy. Based on the final pathological diagnosis, clinically significant prostate cancer was diagnosed in 74.5% of patients. PHI and PHID had the best combination of sensitivity, specificity, positive and negative predictive value, and the highest diagnostic accuracy for the detection of prostate cancer and its clinically significant forms. PHI statistically significantly improved the diagnostic accuracy of a model consisting of demographic, clinical parameters, and blood molecular markers for the detection of clinically significant prostate cancer as assessed by ISUP score ≥2.

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Center for Organ Transplantation Coordination

Unique pancreatic islet implantations and renal transplants from a donor with COVID-19

In collaboration with specialists from Uppsala and Karolinska Universities (Sweden), the first pancreatic islet transplantation procedure was performed at Vilnius University Hospital Santaros Klinikos in 2021 (head of the Center for Organ Transplantation Coordination at VUH SK – Dr. Aistė Kielaitė-Gulla). It is the first operation of its kind in the Baltic States and was performed on a patient with diabetes and living with a kidney transplant. The surgery required a multidisciplinary team of endocrinologists, nephrologists, abdominal transplant surgeons and interventional radiologists. The explantation of the pancreas was performed according to the same principles as the explantation of the pancreas-kidney complex. This procedure was successful and significantly reduced the patient's insulin requirement. In addition, the implanted pancreatic cells prolong the function of the transplanted kidney and help to control fluctuations in blood glucose levels, especially by preventing sudden drops in glucose levels. The latter, in a patient who has been living with a well-functioning donor kidney for 16 years, recurred up to five times a day and were a direct threat to the patient's life. It is hoped that after pancreatic islet transplantation, the patient will no longer experience life-threatening hypoglycemia, will have a reduced need for insulin and better control of diabetes.

In 2021, a multidisciplinary team at VUH SK prepared and successfully transplanted two kidneys from a patient with COVID-19 disease for the first time[1]. The donor was diagnosed with COVID-19 infection in his nasopharynx after brain death, while both recipients were on dialysis had a history of COVID-19. Based on adequate levels of recipients' protective anti-SARS-CoV-2 antibodies and isolated reported cases of kidney transplantation in COVID-19 patients worldwide, the physicians at VUH SK took the decision to perform the necessary operations. Although this was the first operation of its kind in the world, it is likely that in the long term the possibility for COVID-19 survivors to become organ donors or recipients will remain relevant.

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Center for Eye Diseases

Structural and functional glaucoma progression after trabeculectomy

Glaucoma is the leading cause of irreversible blindness worldwide. To date, elevated intraocular pressure is proven to be the principal manageable risk factor for the development and progression of glaucoma. The lamina cribrosa is implicated to be the principal site of glaucomatous damage. The biomechanical paradigm of glaucoma postulates that elevated intraocular pressure causes compression, stretch, and shear of the lamina cribrosa, which lead to lamina deformations and remodeling, damage of the retinal ganglion cell axons, and corresponding visual field loss. Thus, all morphologic parameters describing the biomechanics of lamina cribrosa in relation to intraocular pressure, as well as subsequent changes in neural tissues of retina and optic nerve head, and visual field are of great importance to provide insights into the fundamental mechanisms of glaucoma pathogenesis, diagnostic biomarkers and treatment.

Since 2020, a prospective observational study of factors associated with glaucoma progression after glaucoma surgery is being carried out at the Center for Eyes Diseases of Vilnius University Hospital Santaros Klinikos (VUH SK) (**research group Ernesta Jašinskienė, Dr. Aistė Kadziauskienė, Assist. Prof. Rimvydas Ašoklis, Prof. Leopold Schmetterer**). The aim of this ongoing longitudinal study is to determine the risk factors related to the structural and functional changes of glaucoma progression in postoperative eyes. By using spectral domain optical coherence tomography and optical coherence tomography angiography, the structural parameters of peripapillary nerve fiber layer, optic nerve head, retina, and their vascular density as well as morphologic parameters of lamina cribrosa are assessed. The results of the study are yet to be established.

The study of glaucoma progression associated factors following ocular surgery is a continuation of long-lasting collaboration with the Singapore Eye Research Institute (Singapore National Eye Center). Scientific collaboration of specialists from the Center for Eye Diseases of VUH SK with the team of Prof. L. Schmetterer results in joint international publications.

The primary aim of our prospective longitudinal study was to evaluate the morphologic changes of the lamina cribrosa in glaucoma-affected eyes following trabeculectomy as well as to assess their relationship to biometric and clinical ocular parameters. To our knowledge, this was the first study to assess the response of the overall shape of the lamina cribrosa and its actual curvature to the reduction of intraocular pressure in glaucomatous eyes (Kadziauskienė ir kt., *Ophthalmology, 2018)*. 3D morphology of lamina was reconstructed and its parameters of depth, global shape index and curvatures in the main meridians were calculated using the semi-automated software Morphology 1.0. The results of the study revealed that in most eyes, trabeculectomy resulted in a long-term flattening and shallowing of the lamina cribrosa, associated with greater reduction of intraocular pressure, younger age, and advanced glaucoma. Greater morphologic changes of the lamina cribrosa after trabeculectomy were significantly related to the structural and functional progression of glaucoma with the relationship being influenced by the severity of the disease (Figure 1)[1].

The second stage of this study included analysis of structural and functional glaucoma progression in glaucoma patients undergoing trabeculectomy. Analysis of data from 100 patients revealed that measurable changes occur in both visual field and retinal nerve fiber layer over a 1-year follow-up period after trabeculectomy. In the early phase, we observed an improvement in visual field that was more likely to occur in patients with mild-moderate disease. From the third month, a decline in visual field was observed. Risk factors for the deterioration of the visual field were increasing post-operative intraocular pressure, late-stage disease, thinning of retinal nerve fiber layer, and

absence of visual filed improvement in the first three months (Figure 1). Our data also indicate that even in late-stage glaucoma, structural progression can be monitored with the measurements of retinal nerve fiber layer thickness using spectral-domain optical coherence tomography. Later analysis of the data showed that characteristics derived from the baseline structure-function relationship were also strongly associated with postoperative visual filed outcomes.[2] These findings suggested that the structure-function relationship could potentially have a role in predicting VF progression after trabeculectomy.

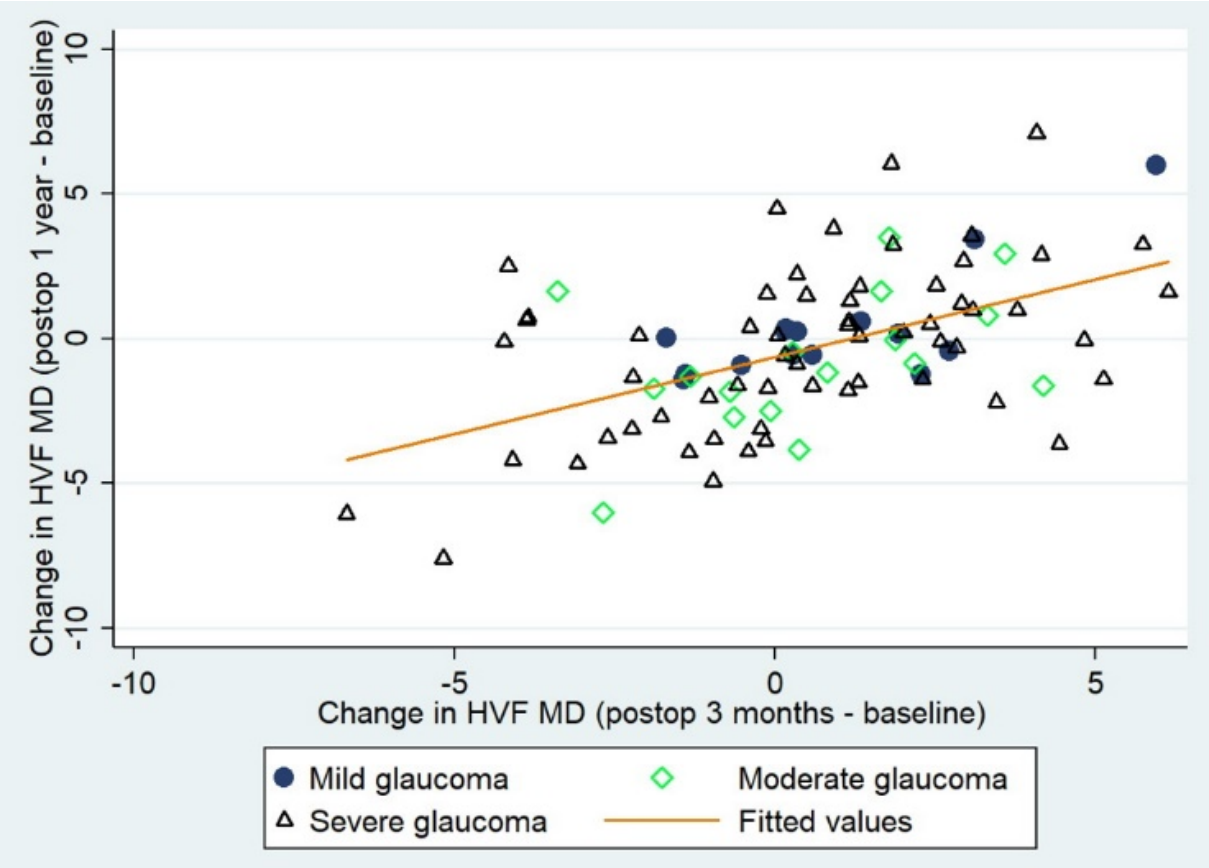

Figure 1. Scatterplot of the change in visual field mean deviation (VF MD) from baseline to third month follow-up versus the change in VF MD from 1 year after surgery compared with baseline, stratified by glaucoma severity. Eyes having mild glaucoma were indicated with dark filled circle, those having moderate glaucoma in green hollow diamond and severe glaucoma in black hollow triangle.[1]

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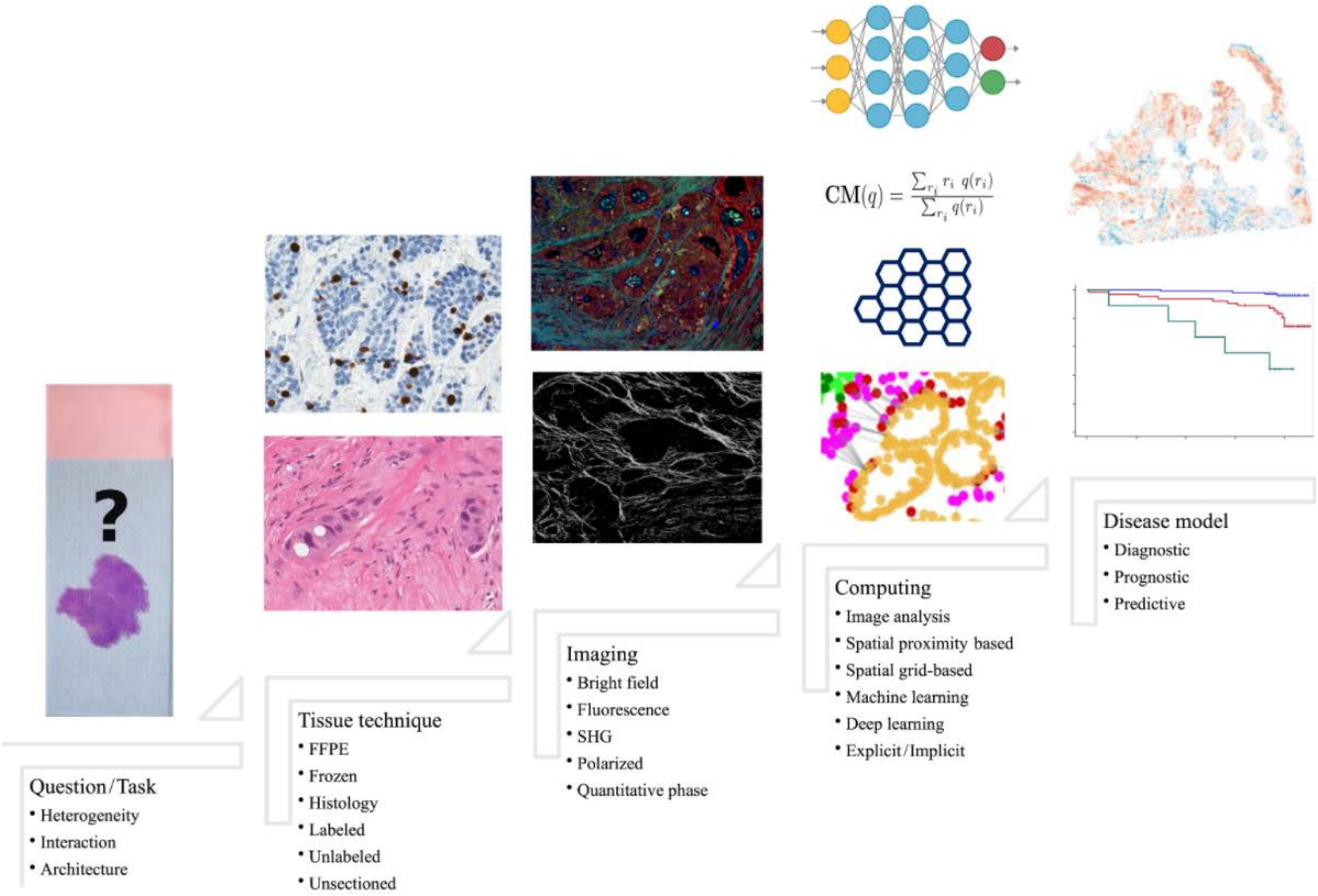
National Center of Pathology

Digital Pathology for Artificial Intelligence

In 2021, the National Center of Pathology (**NCP, director Prof. Arvydas Laurinavičius**) continued its digital pathology research using the hexagonal grid analysis technique, which allows to automatically determine the boundary between a tumor and healthy tissue and to assess the propensity of immune cells to enter the tumor tissue (immunogradient). The tumor-stroma interface immunogradient has been updated and validated in independent patient cohorts. In addition to breast, colorectal and colon cancer, there is a growing body of research in patients with lung cancer, hepatocellular carcinoma and glioblastoma[1-5]. In addition, deep learning artificial intelligence techniques are being used in studies where non-cancerous pathological changes are identified, for example, in kidney allograft biopsies.

Also in 2021, a team of urologists, pathologists and bioinformaticians at Vilnius University started a project funded by the Research Council of Lithuania entitled "Artificial intelligence-driven prediction of BCG immunotherapy response in patients with non-muscle invasive papillary urothelial carcinoma". It primarily aims at discovery of independent AI-driven pathology features to predict clinical outcomes in non-muscle invasive bladder cancer patients treated with BCG immunotherapy (Figure 1).



1 pav. Principle steps for machine-learning applications to extract tumor microenvironment features from digital pathology images.[2]

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Center for Neurology

Changes in Prehospital Stroke Care and Stroke Mimic Patterns during the COVID-19 Lockdown

The substantial strain imposed by the pandemic on the medical systems worldwide caused significant concern regarding the potential ramifications on acute stroke care. A decreasing number of stroke admissions observed during the ongoing COVID-19 pandemic raised the concern of suboptimal prehospital identification and referral of acute illnesses.

Therefore, a team of neurologists from the Center of Neurology at Vilnius University Hospital Santaros Klinikos (VUH SK) (**Rytis Masiliūnas, Aleksandra Ekkert, Prof. Dalius Jatužis,)** together with colleagues from Harvard Medical School (USA) aimed to compare stroke care patterns before and during a state-wide lockdown in Lithuania by analyzing prospective data of stroke alerts referred to our stroke center 15 weeks before and 13 weeks during the first state-wide lockdown declared in Lithuania on 16 March 2020.

In this prospective study in an academic stroke center with a large urban catchment population (VUH SK), we found a significant decrease in prehospital stroke triage quality and longer delays from symptom onset to hospital arrival during a state-wide COVID-19 lockdown. We also found fewer stroke alerts and stroke admissions during the lockdown. In addition, serious neurological conditions, such as seizures and intracranial tumors, were encountered more often as stroke mimics.

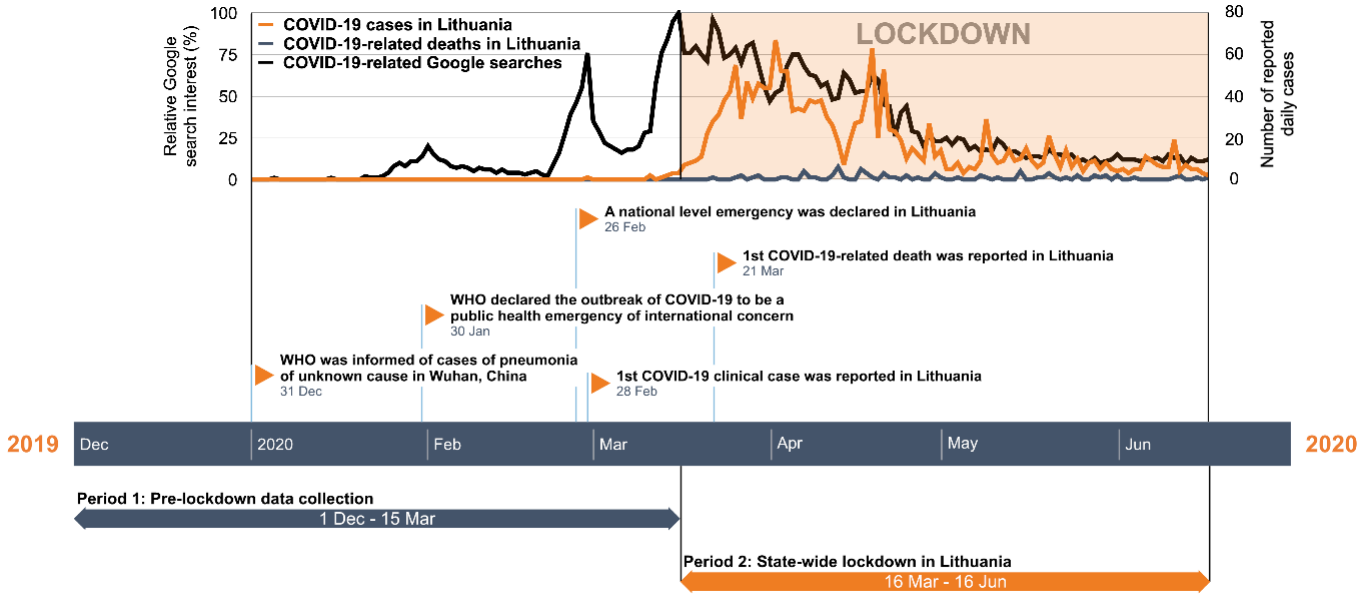

Figure 1. COVID-19 public concern and study timeline. Timeline of data collection periods overlapped with normalized data from COVID-19 related Google searches in Lithuania (100—high interest; 0—no or insufficient interest data) and COVID-19 daily incidence.[1]

Our findings provided novel knowledge on the impact of the state-wide lockdown on prehospital stroke care in low COVID-19 incidence settings and could help guide care delivery strategies during public health emergencies. We suggest that improved strategies are required to maintain optimal neurological care during public health emergencies.

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International recognition in the field of stroke

On 27 August 2021, **Dalius Jatužis, a neurologist at the Center for Neurology at Vilnius University Hospital Santaros Klinikos (VUH SK), a Professor at the Faculty of Medicine of Vilnius University and the president of the Lithuanian Stroke Association, was awarded the prestigious "Nordic Stroke Award"** for his profound contribution in the development of a national network of stroke-ready hospitals and creation of a comprehensive national policy leading to huge improvement in the availability of reperfusion therapy for acute stroke patients in Lithuania, and for his leadership in promoting local and international clinical and scientific cooperation. The award comes during the Nordic Stroke Congress, which takes place every two years, for the lifelong merits of stroke research, prevention and treatment.

It is also a recognition of the entire VUH SK Stroke Service, which was awarded a „Diamond Status" in 2021 by the European Stroke Organization, the highest among Lithuanian stroke centers this year.

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2. https://www.angels-initiative.com/angels-awards/winners

New insights into the cognitive function and causes of death among people with epilepsy

Epilepsy and cognitive functions

On 10 December 2021, **Arminas Jasionis,** a neurologist at the Center for Neurology of Vilnius University Hospital Santaros Klinikos (VUH SK), **defended his PhD thesis "Relationship of emotions, cognitive and social functions of people with epilepsy and their demographic and clinical characteristics" (supervisor Prof. Rūta Mameniškienė).** The study revealed that many cognitive functions (attention, working memory, verbal fluency, memory, executive functions, social cognitive functions) are worse in people with epilepsy (PWE) than in controls. The results of the present study are among the first to show that social cognitive functions (emotion perception and

mentalization) are related to education, work and quality of life among PWE (Figure 1), but not to their marital status[1]. During the preparation of this thesis, instruments to assess social cognitive functions and adverse effects of medication were adapted and validated, which provides more opportunities for research based on psychometric tools in Lithuania[2].

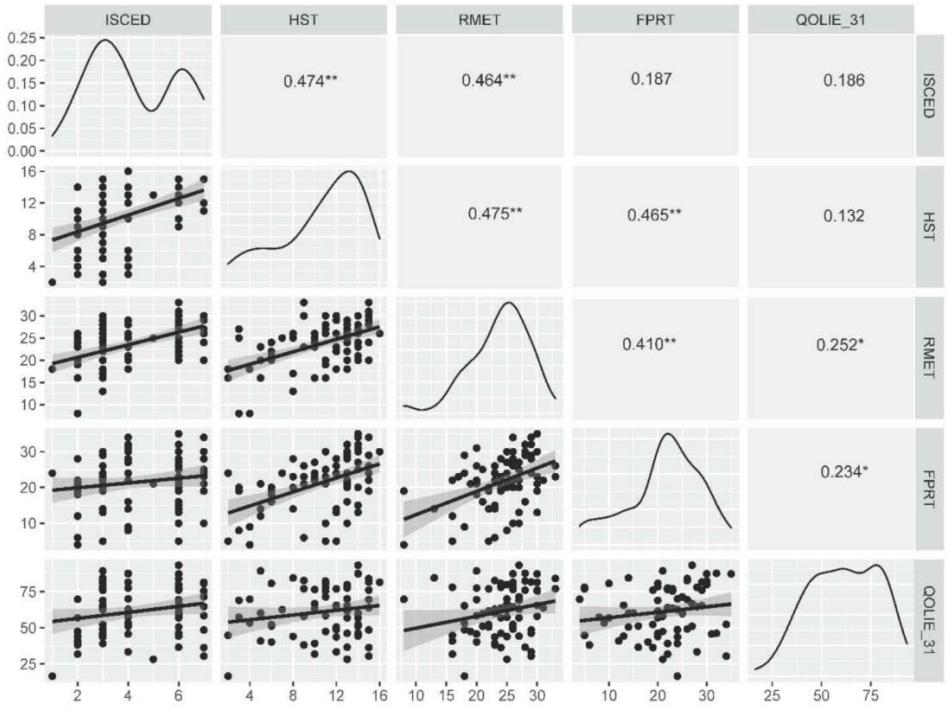

*Figure 1. Correlations between the level of education, ToM and ER tasks and the measure of quality of life. *—p < 0.05, **—p < 0.0001. FPRT—faux pas recognition test (total score), HST—the Happé strange stories test, ISCED—The International Standard Classification of Education, RMET—Reading of the Mind in the Eyes test, QOLIE-31—The 31-item Quality of Life in Epilepsy inventory (total score).[1]*

Continuous research on cognitive functions of PWE at the Center for Neurology of VUH SK has led to the first evidence of accelerated long-term forgetting (ALF) in adults with genetic generalized epilepsy (GGE). ALF is a phenomenon where PWE recall information as well as controls for the first 30 minutes but forget it more rapidly afterwards[3]. Over time, it has become clear that ALF is not unique to epilepsy and can be found in patients with dementia, brain injury or stroke, but it had not been shown to exist in adults with GGE. Further research on ALF should provide more information on memory impairment in PWE, its possible association with seizures or subclinical epileptic activity observed on electroencephalogram (EEG). The interaction between cognitive performance and EEG results has also been investigated in real time at the Epilepsy and Sleep Disorders Unit of VUH SK. Patients performed cognitively demanding tasks during the EEG – such a modification of the test has been shown to increase the sensitivity of EEG in selected subgroups of patients with focal or generalized epilepsy[4].

Mortality among people with epilepsy in Lithuania

An analysis of PWE deaths and their causes was done in 2021: it was found that between 2016 and 2019, PWE mortality in Lithuania was about three times higher than in the general population, with sex disparities in mortality (male mortality being four times higher than in the general population, and female mortality being about two times higher)[5]. Although the most common causes of death in epilepsy were the same as in the general population (cardiovascular diseases, oncological diseases and external causes of death), **PWE are also at increased risk of dying from a variety of medical conditions,** often not directly related to epilepsy (Figure 2). **Additional attention to suicide, drowning and poisoning prevention is likely to reduce the risk of PWE deaths from preventable, often socially determined, causes.**

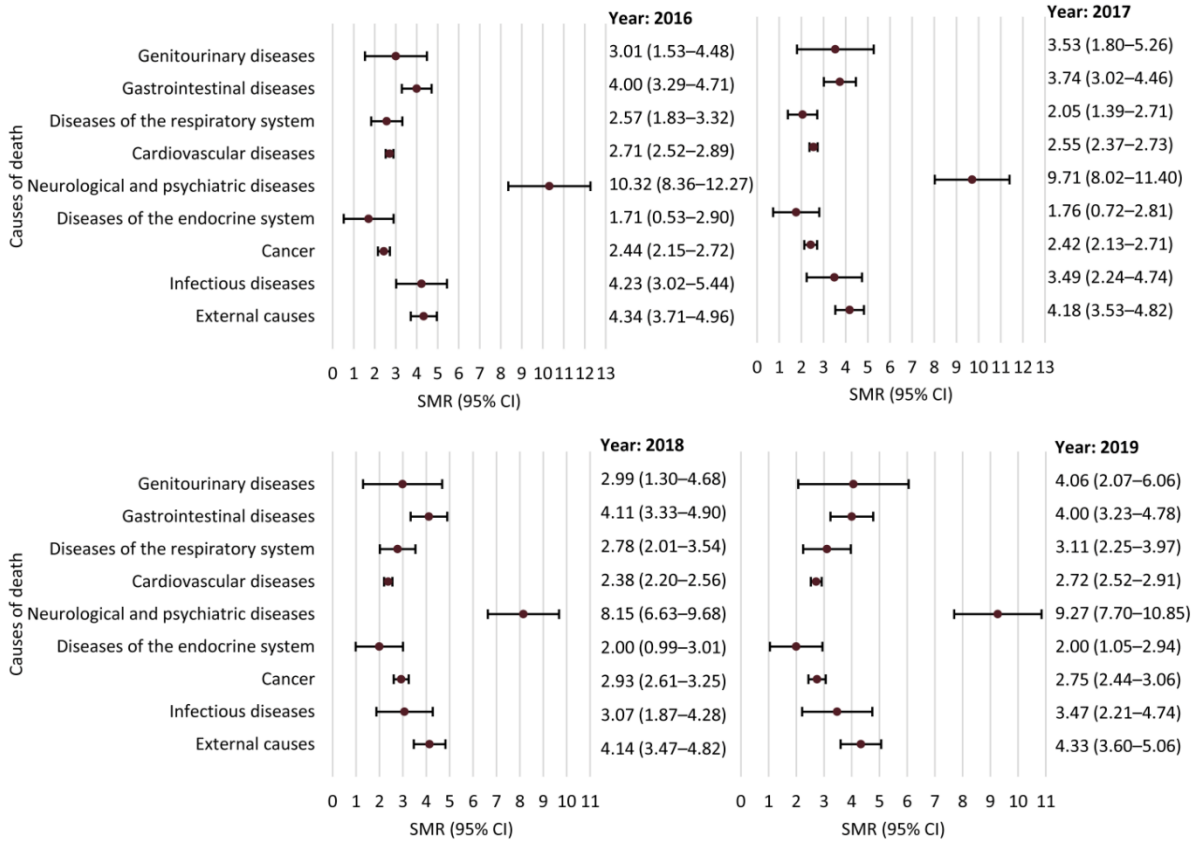

Figure 2. Standardized mortality ratios (SMRs) and 95% confidence intervals (CIs) for different groups of causes of death.[5]

International cooperation

With colleagues from Denmark, Brazil, Guatemala, Turkey and Uruguay, epileptologists at VUH SK continued multicenter research on epilepsy and migraine modulating factors[6]. Using a standardized questionnaire, it was found that 83% of respondents with epilepsy and 92% of respondents with migraine could name the factors that provoke their episodes. The identification of disease-modulating factors by the patient not only provides important information on the possible origin of epileptic and migraine attacks, but also becomes important in daily practice, allowing the professional to make personalized recommendations that potentially reduce the number of disease-specific episodes.

In 2021, the results of a study by the Epi25 Collaboration Network were also published (representative from VUH SK – Prof. Rūta Mameniškienė), in which the use of whole genome sequencing methods helped to substantiate the biological causal link between the oscillations observed in the EEG and the seizures in genetic generalized epilepsy[7]. Finally, a multicenter survey of European epilepsy centers highlighted the ethical and legal difficulties in collecting demographic and clinical data on PWE living in different countries when conducting international retrospective epidemiological studies[8].

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Center for Pulmonology and Allergology

A Diversified view on care for pulmonary diseases and allergic disorders

Risk factors for complicated community-acquired pneumonia course in patients treated with b-lactam monotherapy

Community-acquired pneumonia (CAP) is the deadliest communicable disease and a major cause of morbidity and mortality worldwide. The objective of a study by specialists at the Center for Pulmonology and Allergology at Vilnius University Hospital Santaros Klinikos (VUH SK) was to investigate CAP treated with b-lactam monotherapy, 30-day mortality and risk factors predicting a complicated CAP course[1]. The results of the study revealed that in a population with low *Streptococcus pneumoniae* resistance, CAP treatment with b-lactam monotherapy results in a relatively low mortality rate. Neuromuscular disease, multilobar opacities, and clinically unstable condition (as evaluated using Halm's criteria) predict a complicated CAP course (Table 1).

Table 3. Adjusted odds ratio (OR) and 95% onfidence intervals (CI) for independent predictors of community-acquired pneumonia (CAP) complication risk

Predictors of CAP complication risk	OR	95% CI	P-value
Neuromuscular disease	20.440	3.026–138.083	0.002
Multilobar opacities (on chest X-ray or CT)	7.028	2.068–23.888	0.002
Clinically unstable (as evaluated using Halm's criteria)	5.422	1.082–27.174	0.040

CAP — community-acquired pneumonia; CT — computed tomography

Table 1. Adjusted odds ratio (OR) and 95% confidence intervals (CI) for independent predictors of community-acquired pneumonia (CAP) complication risk.[1]

Tuberculosis treatment and services during COVID-19 pandemic

The effects of the COVID-19 pandemic on tuberculosis (TB) disease and TB services became known in the beginning of 2020. Several reports suggested that the COVID-19 pandemic significantly affected TB services. The current study aimed to describe the effects of the COVID-19 pandemic on TB services and TB-related activities during the entire first year of the pandemic in 2020 compared to 2019[2].

The study showed a severe impact of the COVID-19 pandemic on TB services across most participating countries. Therefore, there is an urgent need to reprioritize resources to manage an expected TB resurgence in future (Figure 1)[3].

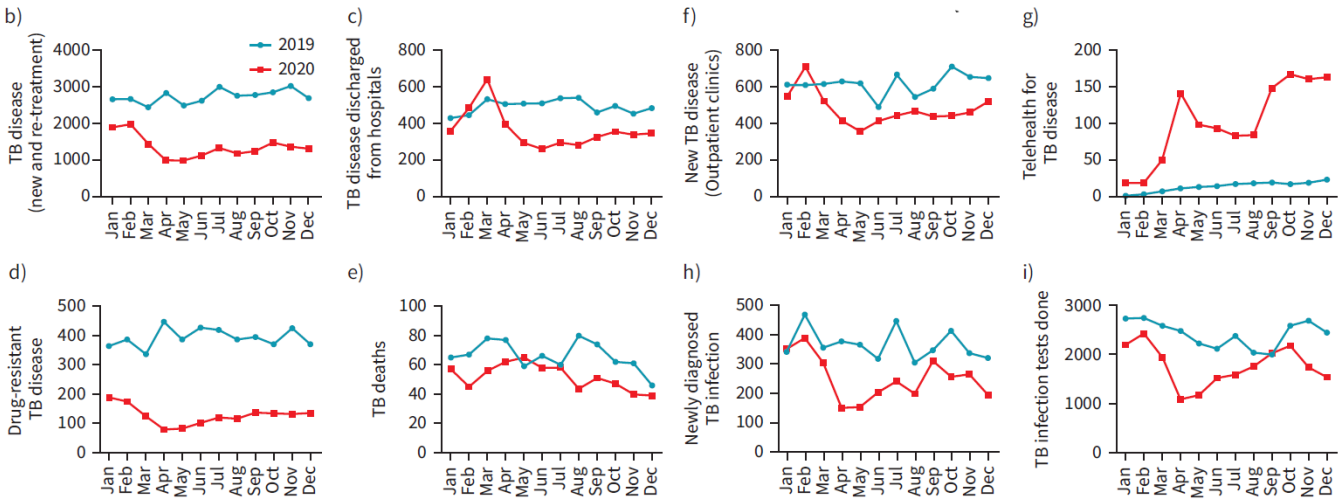

Figure 1. Tuberculosis (TB) disease and infection trends in 2019 and 2020. b) TB disease; c) TB disease discharged from hospital; d) drug-resistant TB disease diagnosed; e) TB deaths; f) newly diagnosed TB disease in outpatient clinics; g) telehealth use for TB disease in outpatient clinics; h) newly diagnosed TB infection across centers; i) latent TB tests comprising of tuberculin skin tests and interferon-γ release assays performed.[3]

CD31⁺, CD38⁺, CD44⁺, and CD103⁺ lymphocytes in peripheral blood, bronchoalveolar lavage fluid and lung biopsy tissue in sarcoid patients and controls

The mechanisms driving the transition from inflammation to fibrosis in patients with sarcoidosis are poorly understood; knowledge regarding prognostic features is lacking as well. This study aimed to establish associations in simultaneous of lymphocyte subset profiles in the blood, bronchoalveolar lavage fluid (BALF), and lung biopsy tissue in the patients with newly diagnosed sarcoidosis.

It was revealed that CD3+CD4+CD38+ in BALF and blood and CD3+CD4+CD44+ in BALF may be markers of the acute immune response in patients with sarcoidosis. CD4+CD103+ T-cells in BALF and in blood are markers of the persistent immune response in sarcoidosis patients and are potential prognostic features of the chronic course of this disease (Figure 2)[4].

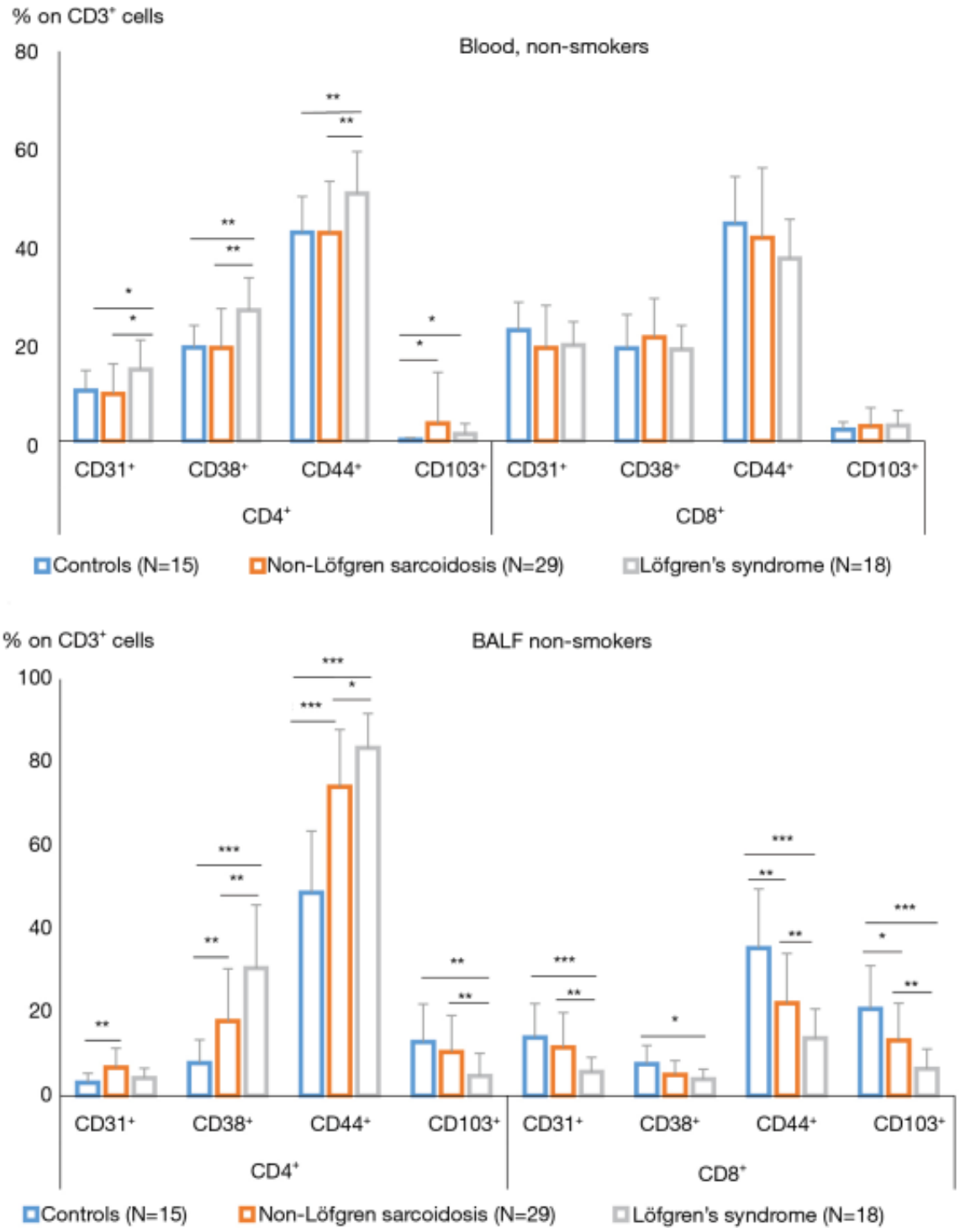

*Figure 2. BALF and blood lymphocyte subsets in non-Löfgren sarcoidosis and Löfgren syndrome non-smokers patients. Data shown are mean ± standard deviation. *p<0.05, **p<0.01, or ***p<0.001.*[4]

Allergology and Clinical Immunology: New Allergens and Exclusive Therapies

In 2021, results of multicenter international studies, in which specialists of allergology and clinical immunology from VUH SK searched for new allergens in collaboration with colleagues, were published. One of the studies showed that allergens from natural sources differ from those used for allergy diagnostics, therefore, reducing the possibility to make a diagnosis of allergy. Propolis, which is commercially tested in China or North America, is positive in only 48% and 35% of patients with a propolis allergy, respectively. At VUH SK, new allergens (one of which was tribenozide,

Figure 3) are also being discovered and reported. Other fields of interest include the determination of the effective concentration of a substance for skin sampling and skin effects of common contact allergens in patients of different professions[5-7].

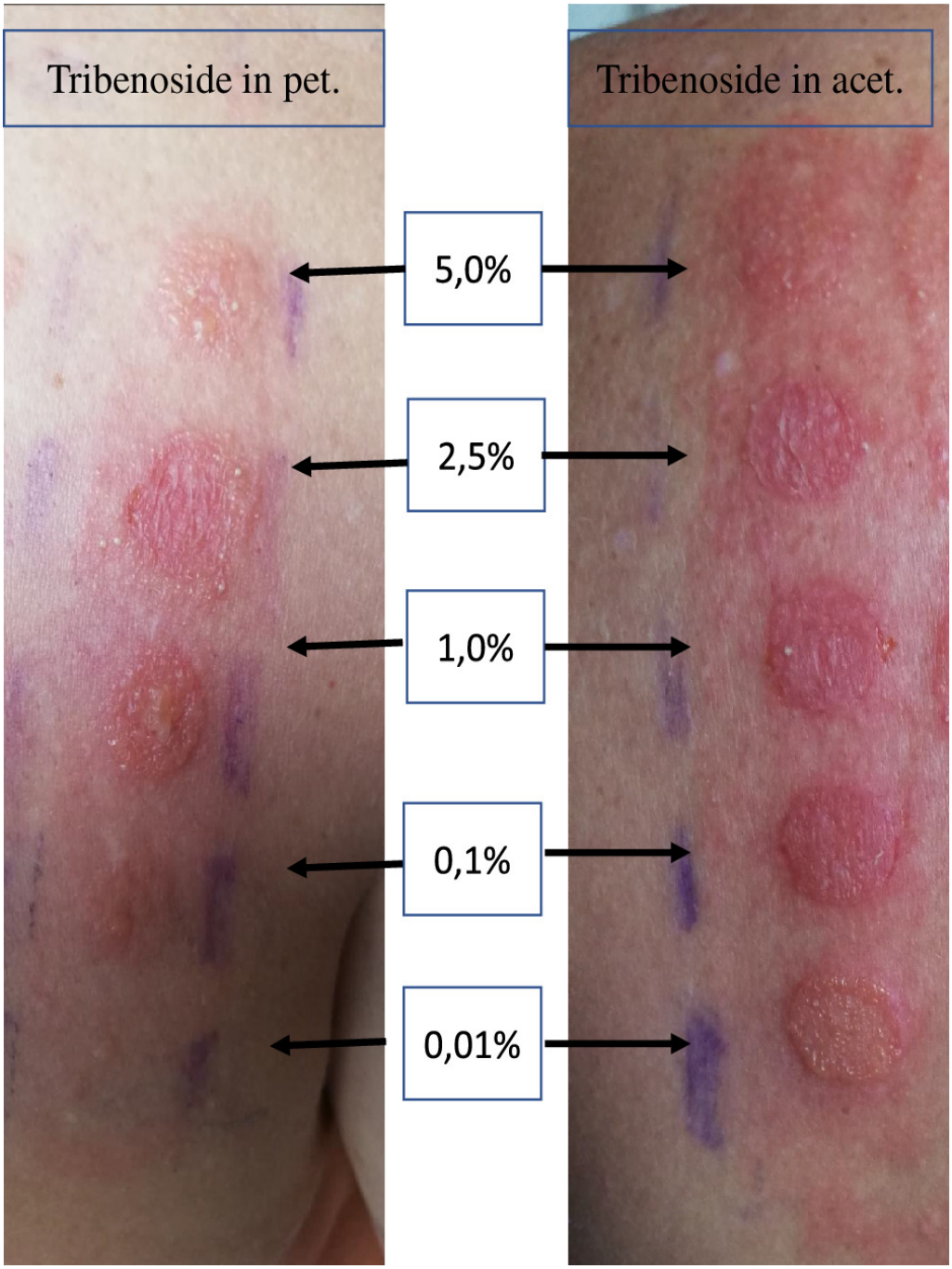

Figure 3. Positive skin patch reactions with tribenoside at different concentrations and in different solvents.[5]

Moreover, the results of a cohort study were published in 2021 – 98 patients with chronic spontaneous urticaria were treated with plasmapheresis: the efficacy of this method was evaluated (Figure 4). **To date, no other study with such a large sample size has been published evaluating the efficacy of plasmapheresis for chronic urticaria[8].**

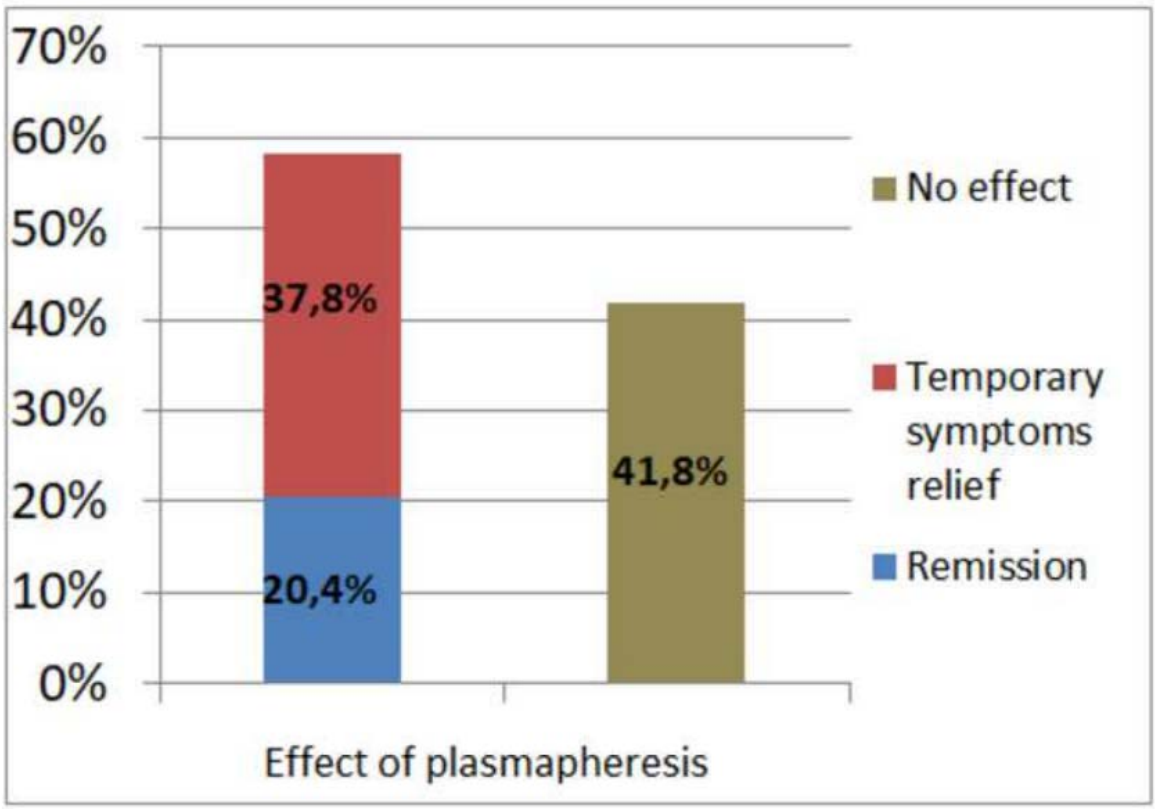

Figure 4. Effect of plasmapheresis in the treatment of chronic spontaneous urticaria.[8]

The Center for Pulmonology and Allergology of VUH SK is the only in Lithuania to perform provocation samples with Hymenoptera to evaluate the effectiveness of specific venom immunotherapy and specific nasal provocations with allergens to diagnose allergic rhinitis by rhinomanometry and to evaluate the effects of specific immunotherapy. VUH SK also has a Center for Primary Immunodeficiencies, which collaborates with the European Society of Immunodeficiencies (ESID) Registry.

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Center for Cardiology and Angiology

The effectiveness of innovative myocardial revascularization methods

One of the main areas of research at the Center of Cardiology and Angiology of Vilnius University Hospital Santaros Klinikos (VUH SK) (**head – Prof. Giedrius Davidavičius**) is the implementation and evaluation of innovative methods of myocardial revascularization. The first experience in this field was gained in 2002 during G.Davidavičius' fellowship at the Gasthuisberg Hospital of the University of Leuven (Belgium), when he was working in an international group (head – by G.Sutherland), which investigated the use of parameters of a myocardial ultrasound to describe the systolic function of the left ventricle. Thus, clinical and scientific experience in the use of myocardial revascularization techniques has been accumulated for about 20 years. In 2017, Arvydas Baranauskas defended a doctoral thesis titled "The effectiveness of myocardial revascularization in patients with diffuse coronary atherosclerosis" at the Faculty of Medicine of Vilnius University. The PhD study showed that the effectiveness of percutaneous coronary intervention (PCI) in the treatment of diffuse coronary artery disease is limited. Therefore, it was then questioned whether intravascular ultrasound during PCI could improve the functional outcome of this procedure. Studies are now ongoing to answer whether the use of intravascular ultrasound could improve the functional outcome of PCI in terms of fractional flow reserve while treating a diffusely damaged coronary segment and potentially reduce the rate of adverse cardiovascular events.

In 2021, the New England Journal of Medicine published the first results of the international multicenter FAME3 study[1]. The aim of the study was to investigate whether PCI of coronary vessels selected by fractional cardiac blood flow testing leads to similar outcomes as coronary-artery bypass grafting in patients with three-vessel coronary artery. The study compared the results of PCI and coronary-artery bypass grafting while PCI was guided by measurement of fractional flow reserve. It was concluded that coronary-artery bypass grafting remains preferable in patients with three-vessel coronary artery disease. A total of 1500 participants were included in the study, 104 of whom were treated at VUH SK.

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Studies of patients with degenerative aortic valve stenosis

According to the Institute of Hygiene of Lithuania, the incidence of valvular heart disease in Lithuania has increased fivefold over the past few decades (Figure 1). Degenerative aortic valve stenosis is the most common valvular heart disease both worldwide and in Lithuania.

In recent years, the availability of new, minimally invasive treatment options for degenerative aortic valve stenosis has made timely diagnosis of this pathology and its accurate assessment essential for planning interventional or surgical treatment.

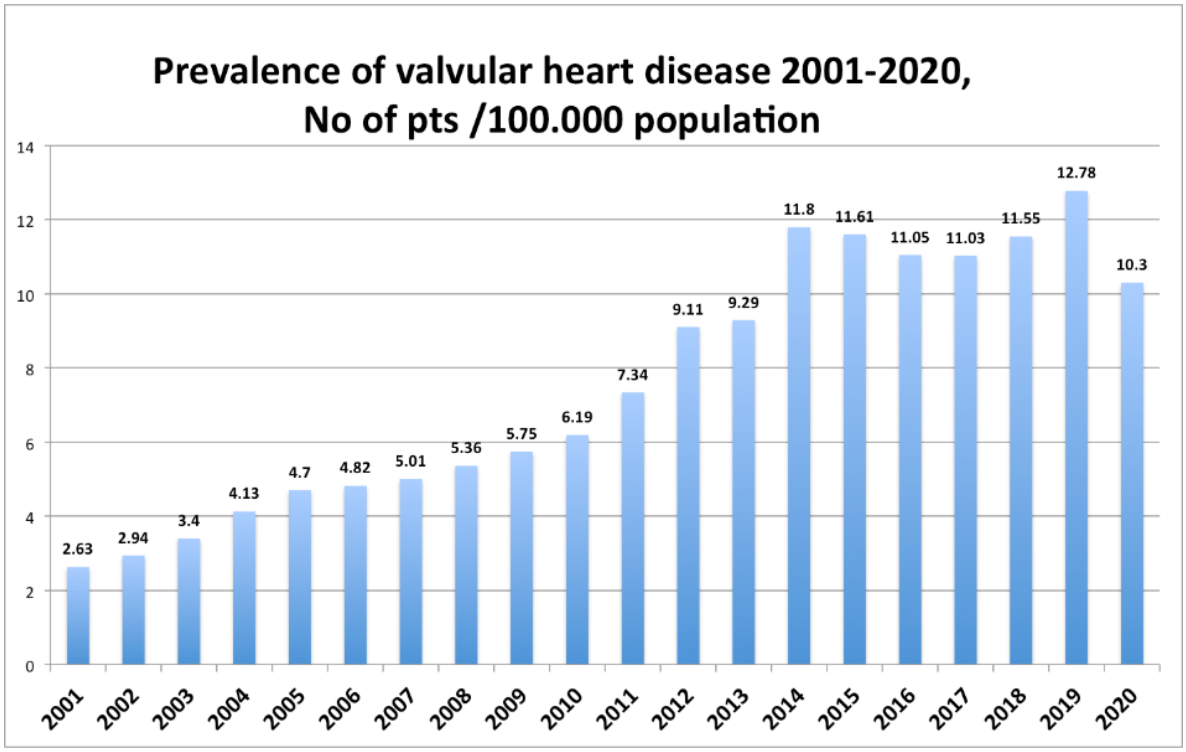

Figure 1. Incidence of valvular heart disease (disease codes I34-I39) in Lithuania from 2001 to 2020, number of patients per 100 000 population (data from the Institute of Hygiene of Lithuania).

Myocardial fibrosis in patients with high-grade degenerative aortic valve stenosis – the FIB-AS study

In 2018, the Center for Cardiology and Angiology of VUH SK, together with the Clinic of Cardiovascular Diseases of the Faculty of Medicine of Vilnius University, started a project funded by the EU Structural Funds entitled "Identification of myocardial structural changes and their prognostic value in patients with high-grade aortic valve stenosis" (led by Prof. Sigita Glaveckaitė). The project, in collaboration with the Center for Radiology and Nuclear Medicine (Prof. N.Valevičienė, Dr. D.Palionis, Dr. A.Samulis), purchased and installed a new parametric imaging sequence (Myomaps) in a magnetic resonance imaging (MRI) scanner (1.5T Siemens Aera) – this allows to obtain T1, T2, and T2* myocardial maps.

The T1 map allows the quantification of various diffuse pathological processes in the myocardium. The aim of the study is to identify the structural and functional changes in the myocardium caused by degenerative aortic valve stenosis and to assess their impact on the prognosis of the disease. As of 2019, 83 patients have been enrolled in the study and 79 have undergone aortic valve replacement surgery. Their myocardial status was evaluated by both non-invasive methods (echocardiography with longitudinal strain analysis, cardiac MRI with T1 mapping) and invasive methods, such as histological analysis of fibrosis in myocardial biopsy taken at the time of the operation (Figures 2 and 3).

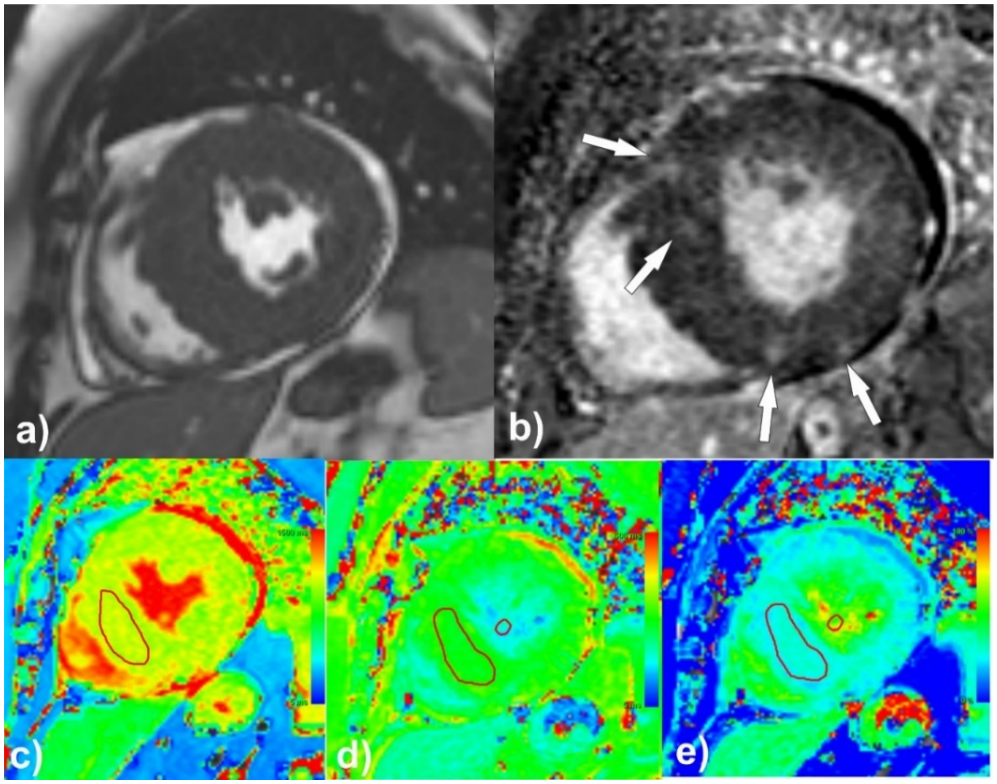

Figure 2. Assessment of focal and diffuse myocardial fibrosis in a 74-year-old patient with high-grade aortic valve stenosis using 1.5T Siemens Aera MRI scanner and NeoSoft SuiteHEART software.

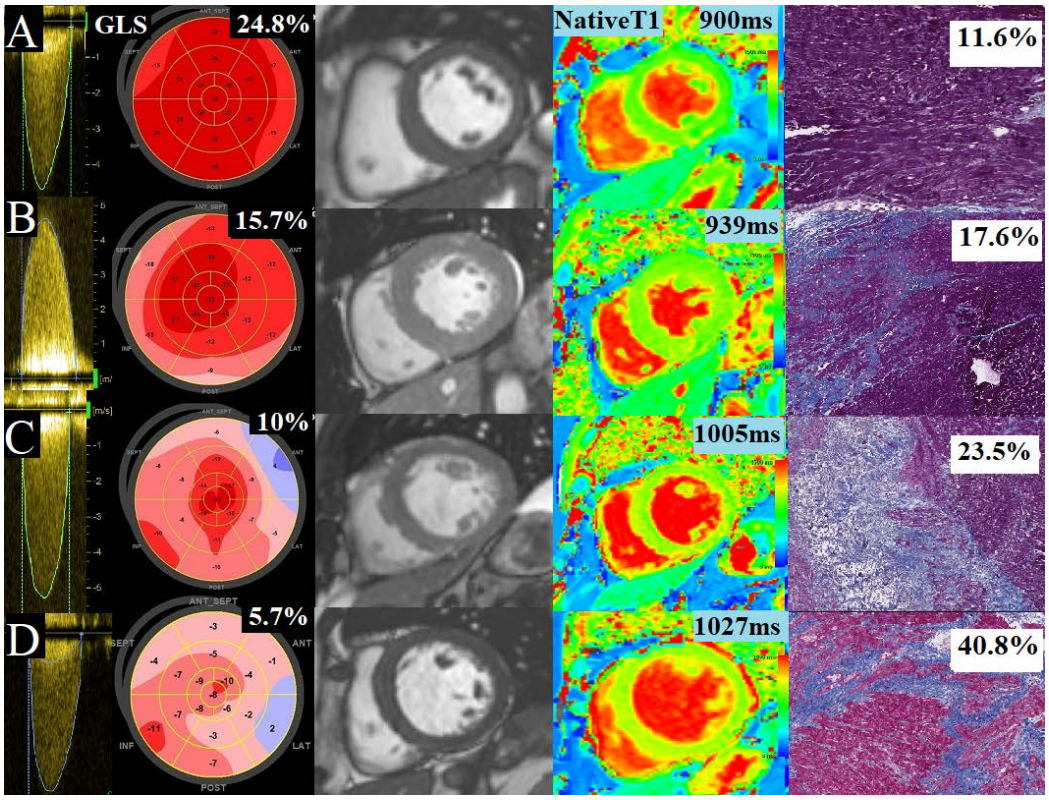

Figure 3: Evaluation of left ventricular myocardium in four patients with high-grade degenerative aortic valve stenosis by echocardiography with longitudinal strain assessment, cardiac MRI with T1 mapping, and histological examination with quantitative analysis of interstitial fibrosis.[1]

The study is led by a multidisciplinary team of radiologists, cardiologists, cardiac surgeons, and pathologists, in collaboration with Aalborg University Hospital (Denmark). The study revealed focal myocardial fibrosis in up to 2/3 of patients on MRI images and interstitial fibrosis of varying degrees during histological examination. The study identified early biomarkers of cardiac muscle damage, such as native T1 time and global longitudinal strain coefficient. Currently, post-operative follow-up and outcome assessment of patients are being carried out, the results of the study are being presented at international conferences and upcoming articles as well as a PhD thesis on the topic are being prepared[1].

Comparison of aortic valve replacement and close follow-up strategies in patients with high-grade asymptomatic aortic valve stenosis of degenerative origin – the AVATAR study

In 2021, the results of the AVATAR study, initiated by international partners in collaboration with the Cardiovascular Diseases Clinic of the Faculty of Medicine of Vilnius University, and conducted at the VUH SK Center for Cardiology and Angiology, were published. The study showed that in asymptomatic patients with high-grade degenerative aortic valve stenosis, early operative treatment of the malformation is associated with a statistically significant reduction in composite primary outcomes (all-cause death and major adverse cardiovascular events) compared with close follow-up up to the onset of symptoms[2].

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Center for Endocrinology

Genetic and epigenetic factors causing complications of diabetes mellitus

Diabetes mellitus (DM) is a global public health problem of epidemic proportions that affects more than 663 million people worldwide. DM is associated with increased morbidity and mortality due to a progressive nature of associated macrovascular and microvascular complications. One of the main mechanisms leading to the development of diabetic complications is oxidative stress caused by hyperglycemia. Biomarkers currently used in clinical practice (e.g., glycated hemoglobin) do not always correlate with the risk of development and progression of diabetic complications. Some patients with good glycemic control develop diabetic complications in the prediabetic or early diabetes stages, while others do not develop complications despite poor glycemic control and long-lasting diabetes. Preliminary scientific data suggest that predisposition to diabetic complications may be due to genetically determined susceptibility to hyperglycemia-associated oxidative stress.

Doctors of Vilnius University Hospital Santaros Klinikos (VUH SK) and researchers of the Faculty of Medicine of Vilnius University together with representatives of the Life Sciences Center of Vilnius University (VU LSC) conduct a joint study titled "Influence of genetic, epigenetic and lifestyle factors on the development of diabetic complications" with the aim to assess individual susceptibility of patients with DM to oxidative damage of deoxyribonucleic acid (DNA), the patients' profile of non-coding ribonucleic acid (RNA), foot microbiota, and to evaluate the associations of these findings with macro-vascular and micro-vascular complications.

The study is carried out by Assoc. Prof. Žydrūnė Visockienė, PhD student Laura Šiaulienė, Ieva Sereikė and nurse diabetologist Violeta Bičkauskienė at VUH SK and Prof. Juozas Rimantas Lazutka, Assoc. Prof. Veronika Dedonytė, Assoc. Prof. Kristina Daniūnaitė and Prof. Eglė Lastauskienė at VU LSC.

There are 144 patients with DM and healthy controls currently recruited into the study. Patients' blood lymphocytes are exposed to a mutagen and DNA oxidative damage is evaluated by formation of micronuclei, chromosome aberrations and sister chromatid exchange (Figure 1). It is expected that patients, who are more sensitive to mutagenic activity and, thus, to oxidative stress, have an increased risk of diabetic complications. The ability to determine the patients' genetic susceptibility to oxidative stress would help to individualize DM care and treatment early at the onset of the disease, achieve optimal disease management goals, and prevent or delay the progression of diabetic complications.

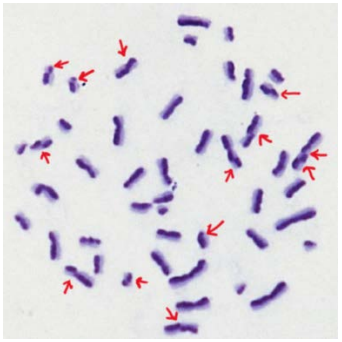
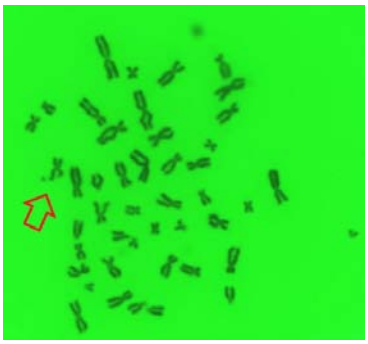
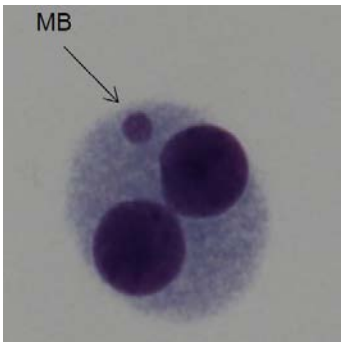
(A)  (B)  (C) 

Figure 1. Sister chromatid exchange (A), chromosome aberrations (B) and micronuclei (C)

Identification of micro-RNA biomarkers for diagnosing papillary thyroid cancer

Specialists at the Center for Endocrinology at Vilnius University Hospital Santaros Klinikos (VUH SK) (**head – Assoc. Prof. Žydrūnė Visockienė**) focus on the diagnosis of thyroid cancer and postoperative care. Thyroid cancer is the most frequently occurring endocrine malignancy, with an increasing rate of incidence over the last three decades. Papillary thyroid carcinoma (PTC) accounts for up to 85% of all thyroid cancers. Generally, PTC has an excellent prognosis with a relatively low mortality rate, but a small portion of PTC patients suffer from an aggressive form of the disease with tumor invasion and metastasis. Lymph node metastasis (LNM) in PTC was shown to be associated with poor prognosis and increased risk of locoregional recurrence after surgery. More recent works have focused on molecular markers in predicting outcome for patients with thyroid cancer. Therefore, there is a need for a reliable biomarker for the prediction of LNM in this type of cancer. MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene translation or degradation, play key roles in numerous cellular functions including cell-cycle regulation, differentiation, apoptosis, invasion and migration. Many miRNAs have been proposed as promising molecular markers of PTC. However, there are limited data on the correlation between miRNA expression, and neck lymph node metastasis.

In collaboration with the Center for Medical Genetics at VUH SK, endocrinologist and Vilnius University PhD student Romena Laukienė (supervised by Prof. Loreta Cimbalistienė) is performing miRNA analysis in patients with confirmed papillary thyroid carcinoma. The aim of the study is to determine and compare miRNA expression profiles of PTC with LNM and PTC without LNM, and to estimate the diagnostic utility of the detection of specific miRNAs in the preoperative assessment of thyroid nodules.

By identifying new biomarkers, it would be possible to predict the progression of thyroid carcinoma prior to the surgery, select the correct extent of surgery or even to avoid the latter overall. This can reduce the number of postoperative complications and improve the quality of life of patients.

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Diabetes risk in women with gestational diabetes mellitus – the DIARIND study

Specialists at the Center for Endocrinology of Vilnius University Hospital Santaros Klinikos (VUH SK) are conducting a study to determine whether women with gestational diabetes mellitus (GDM) and their children are at a higher risk of developing diabetes than those without GDM during the 10-year follow-up period.

The principal investigator of the DIARIND project is Assoc. Prof. Žydrūnė Visockienė and the research team consists of endocrinologists (Gintarė Naskauskienė, Vaiva Šarkovienė, Airida Audronė Bagdžiūnienė), obstetricians-gynecologists (Prof. Diana Ramašauskaitė, Dr. Gintautas Domža, Dr. Jelena Volochovič), neonatologists (Dr. Ingrida Pilypienė, Ieva Navarackaitė),

pediatricians (Galina Mikšo, Daiva Vaičiūnienė) and geneticists (Assoc. Prof. Laima Ambrozaitytė). The study also requires close collaboration with researchers from the Life Sciences Center of Vilnius University.

There were 225 women with GDM and 11 women without GDM as well as 180 biological fathers and 208 neonates included into the study from 2018 to 2021. Throughout the pregnancy and after childbirth all women are consulted by endocrinologists and obstetricians-gynecologists; a lifestyle adjustment program is applied. All women who meet the MODY (Maturity Onset Diabetes of the Young) diabetes criteria are consulted by geneticists. Neonatologists and pediatricians ensure the follow-up of offspring. So far, 180 women and 104 children are under follow-up one year after giving birth and 27 women and 7 children are in their third year of follow-up.

Currently, 132 of the 225 women involved in the DIARIND study met the MODY criteria, thus, 55 of these cases had already been studied to clarify the diagnosis and in 5 (9%) of them MODY diabetes was confirmed.

The initial analysis of female and fetal outcomes and risk factors showed that higher mothers' body mass index (BMI) before pregnancy was associated with insulin resistance and increased triglyceride levels, however, these women gained less weight during pregnancy as compared with women with lower BMI (Figure 1). No significant risk factors affecting the female or fetus outcomes have been identified so far – this is likely due to a relatively small current sample size.

During the follow-up period covering the time from 6 to 12 weeks postpartum, one woman with GDM was diagnosed with type 1 diabetes and 11 – with prediabetes. Following the recommendations provided by nutritionists and physical medicine and rehabilitation doctors, only four women still had prediabetes a year later and two women – three years after giving birth. The DIARIND study is ongoing – enrollment and follow-up both women and offspring is continuing.

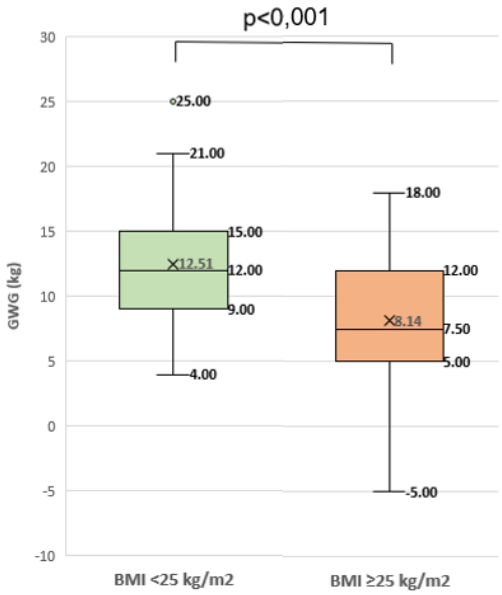

Figure 1. Comparison of gestational weight gain in women with a body mass index (BMI) prior to gestation <25 kg/m^2 (N 86) and those with the BMI > 25 kg/m^2 (n=59).

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Center for Radiology and Nuclear Medicine

New diagnostic and treatment solutions at the Center for Radiology and Nuclear Medicine

The Center for Radiology and Nuclear Medicine of Vilnius University Hospital Santaros Klinikos (VUH SK) (**head – Dr. Artūras Samuilis**) is constantly innovating and conducting research in close cooperation with other centers of VUH SK as well as other Lithuanian and foreign institutions.

Artificial intelligence in radiology: from theory to practice

From February 2021, The "ChestEye Quality" chest radiograph analysis solution (https://oxipit.ai/products/chesteye/) of the Lithuanian artificial intelligence technology company "Oxipit" has been implemented at the Center for Radiology and Nuclear Medicine. This was possible because of the contribution by staff at the Center during testing and development of the system (lead – Bernardas Rimkus). With more than 75 000 chest radiographs performed and evaluated per year at VUH SK, the system aims to ensure that all radiological findings, even the most subtle ones, are detected in chest radiographs. The "ChestEye Quality" solution automatically analyses the radiographs and respective radiological descriptions at VUH SK in real time. It helps to detect examinations with the potential for subtle undescribed radiological findings. These imaging results are returned to the radiologist for additional review – specialists can then verify the AI-identified findings and, if necessary, supplement the final study description (Figure 1).

Suspicious examinations are reported by the AI to the responsible radiologist by email. As the solution operates in real time, it is possible to complete the profile before clinical action is taken in response to the radiological description of the examination

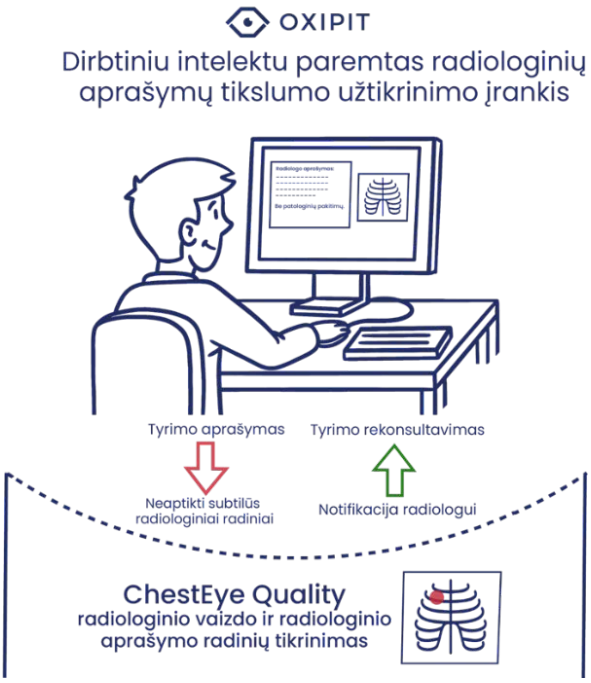

Figure 1. Artificial intelligence solution for the evaluation of chest radiographs is an important tool for radiologists in their daily practice and an excellent learning tool for young radiology residents.

Ultrasound examinations with contrast material in children. Is it really safe?

Andrius Čekuolis and Rasa Augustinienė, echoscopy specialists at the Department of Pediatric Radiology at VUH SK have joined a registry administered by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) and led by Prof. Christoph F.Dietrich (Germany). Together with colleagues from Germany, Poland, the United Kingdom, Italy, Switzerland, China and Hungary, the clinical value and safety of ultrasound examinations with intravenous contrast media in the pediatric population were assessed.

The registry included 46 examinations from Lithuania, which were performed in the Department of Pediatric Radiology at VUH SK. No adverse reactions were recorded in any patient. The result of the project is that ultrasound with intravenous contrast material in pediatric patients is safe, can be recommended to be used, reduces the need for expensive CT or MRI scans, and avoids the use of medical ionizing radiation in young patients. Adverse reactions to intravenous ultrasound contrast material were rare, occurring in 0.9% of all subjects, most of them were mild. The registry data were published in the EFSUMB guidelines[1].

Diagnosis of acute appendicitis. Is there anything new to say?

Accurate diagnosis of acute appendicitis remains a challenge in the 21st century, even though this pathology has been well known and treated for centuries. For a long time, the diagnosis of acute appendicitis has been based on clinical examination, and, to reduce the number of complications, surgical treatment has been considered to be a necessity. Globally, this approach has led to a rate of up to 30% of operations during which an intact appendix was found despite a clinical diagnosis. Such operations are called negative appendectomies (NA). In a retrospective study at VUH SK, the incidence of NAs after basing the diagnosis on clinical examination was found to be up to 23%. Since 2017, a new diagnostic protocol has been introduced at VUH SK (**representative of the Center for Radiology and Nuclear Medicine – Raminta Lukšaitė-Lukštė**), which actively incorporates imaging techniques such as abdominal ultrasonography (US) and abdominopelvic computed tomography (CT). Abdominal ultrasound was the imaging test of first choice, and if this test did not provide a definitive diagnosis but the suspicion of acute appendicitis remained, abdominopelvic CT was ordered. This diagnostic technique, also known as "conditional CT", has reduced the rate of negative appendectomies by up to 4.2%[2].

The diagnosis of suspected acute appendicitis in pregnant patients has been similarly optimized. Until now, pregnant patients with clinically suspected acute appendicitis and an uninformative abdominal ultrasound were treated with a surgical intervention called diagnostic laparoscopy. A new diagnostic testing technique, including abdominopelvic MRI after an uninformative abdominal UG, reduced the number of diagnostic laparoscopies from 55.3 to 5.3% (Figure 2)[3].

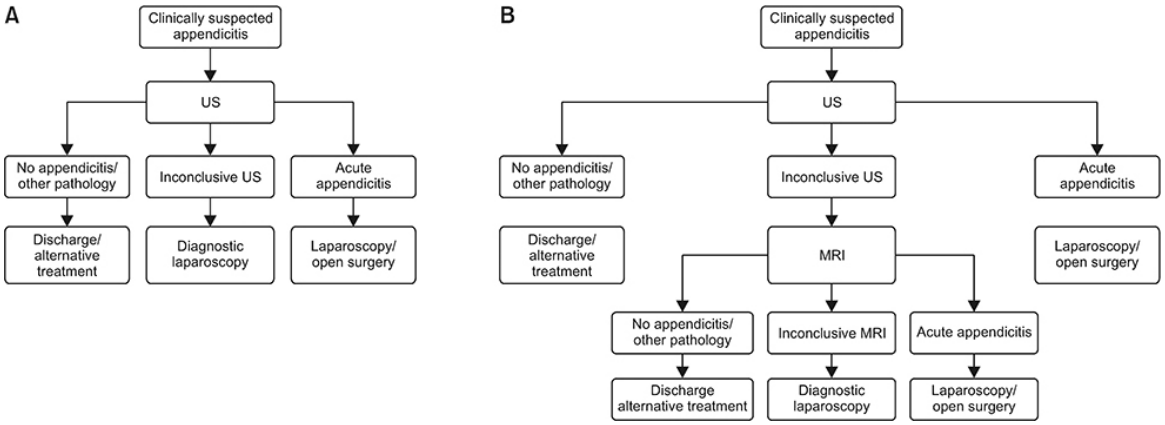

Figure 2. Diagnostic algorithms or pregnant women with suspected acute appendicitis. Before (A) and after (B) introduction of MRI. US, ultrasound.[3]

New hope for cancer patients: electrochemotherapy

In 2021, a new local treatment method – electrochemotherapy (ECT) – was launched at VUH SK in a collaboration between the Center for Radiology and Nuclear Medicine (**representative – Donatas** Jocius) and the Center for Abdominal and Onco-Surgery.

Electrochemotherapy is a minimally invasive local treatment of tumors of various localizations, in which cancerous tissues are exposed to high-voltage and low-duration electrical pulses (1000V/cm, 100 µs, 5kHz). The procedure creates temporary pores in the tumor cell membranes which allows large molecules, including chemotherapeutic agents (e.g., bleomycin, cisplatin), to enter the cell. This electrical pulse effect greatly increases the intracellular concentration of the chemotherapeutic agent (e.g. the intracellular concentration of bleomycin increases by a factor of 1000) and leads to the immediate death of the tumor cell by apoptosis.

Unlike hyperthermic or hypothermic minimally invasive ablative techniques, ECT does not damage critical structures, such as the bowel wall or blood vessels, and can therefore be used in anatomically complex localizations without the risk of damage to healthy tissue. Seven ECT procedures were performed in patients with pancreatic adenocarcinoma, a pancreatic neuroendocrine tumor, intrahepatic cholangiocarcinoma (Figure 3), metastatic colorectal cancer and anal melanoma at VUH SK. During the follow-up period (>6 months), more than half of the patients showed local disease stabilization or complete/partial response to treatment. The literature indicates that a complete or partial response rate to ECT is around 80% in a cohort of mixed oncological conditions. At VUH SK, 4 patients (57%) were observed to have a response to treatment or have the disease stabilized. In almost all cases, ECT was chosen as a last resort when other local therapies (surgical or minimally invasive) were either already used or particularly risky. ECT is now mostly used as a palliative option to prolong survival and quality of life. In the future, with more positive cases with ECT, it should be considered as an initial treatment option for locally advanced tumors.

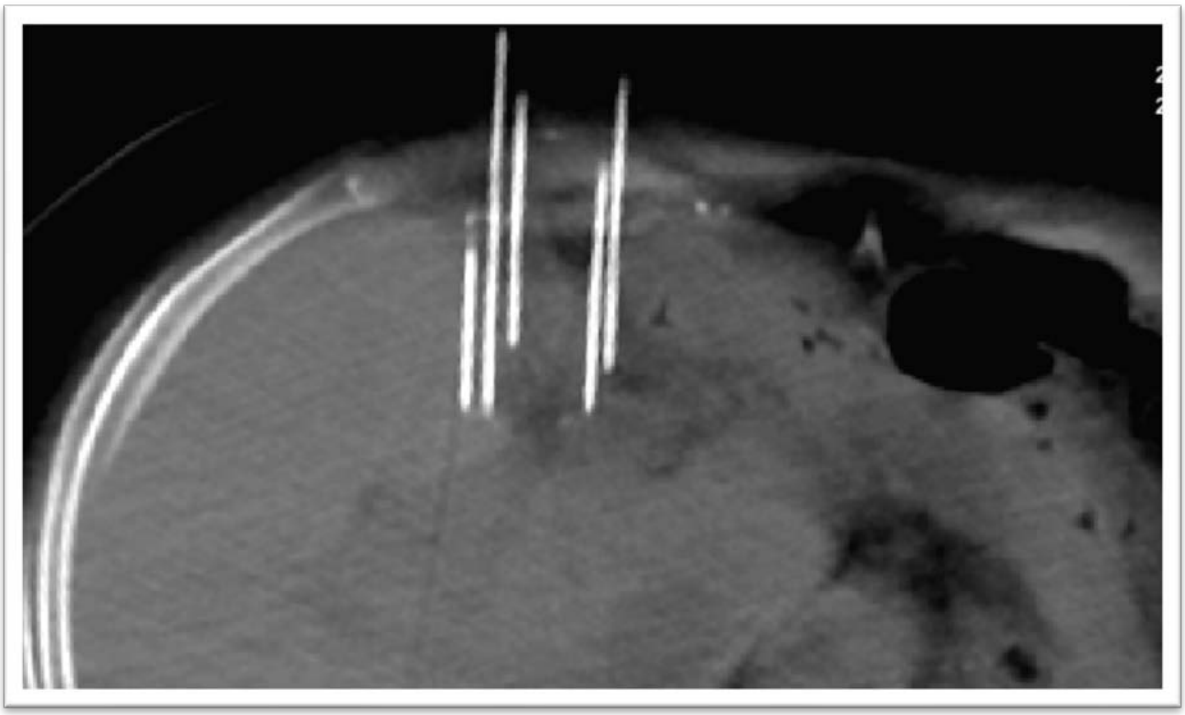

Figure 3. Electrochemotherapy procedure in a patient with intrahepatic cholangiocarcinoma (controlled by computed tomography).

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Clinical Radiation Surveillance Division

Assessment of ionizing radiation exposure – key to more safe future

It is well known that ionizing radiation follows a non-threshold dose for adverse effects meaning that even a very low dose can cause cell mutations and increase the risk of cancer. Thus, special attention must be paid to occupational and patient exposure and optimization studies in order to reduce the number of radiation-induced cancers in the future.

Over 140 000 diagnostic and treatment procedures using ionizing radiation are performed with around 90 different pieces of equipment at Vilnius University Hospital Santaros Klinikos (VUH SK). The number of procedures is growing every year – both at VUH SK and other countries. In collaboration with the International Atomic Energy Agency (IAEA) and other hospitals, the Clinical Radiation Surveillance Division (head – Assoc. Prof. Birutė Gricienė) at VUH SK conducts research related to exposure and risk reduction, image optimization, especially for computed tomography (CT), where doses are hundreds of times higher than in conventional X-ray examination.

CT optimization studies performed since 2012 for pediatric procedures allowed to reduce the dose of radiation during a head CT by 2-4 times, thus, minimize the negative risk of the test[1]. So far, the best result in patient exposure reduction was achieved for CT scans of head deformity in children – they are now done at a dose that is 15 times lower than before. While local diagnostic reference levels (DRLs) do not define a "maximum limit" of radiation doses, they remain an important tool for patient dose optimization. However, until today only a small number of European hospitals have established such reference levels. Since 2012, the Clinical Radiation Surveillance Division has been collecting and evaluating the exposure received by patients at VUH SK. In 2021, new DRLs were established for head and chest examinations of children[1], and for interventional radiology examinations[2].

More than 10 000 interventional radiology and cardiology procedures are performed at VUH SK annually – they can expose patients to a dose of radiation that is significantly higher than during routine X-ray examinations. Doses during standard interventional radiology procedures decreased by 20-80% as compared to 2015 (Figure 1), thus, patients are examined and treated more safely, with a lower risk to their health[2].

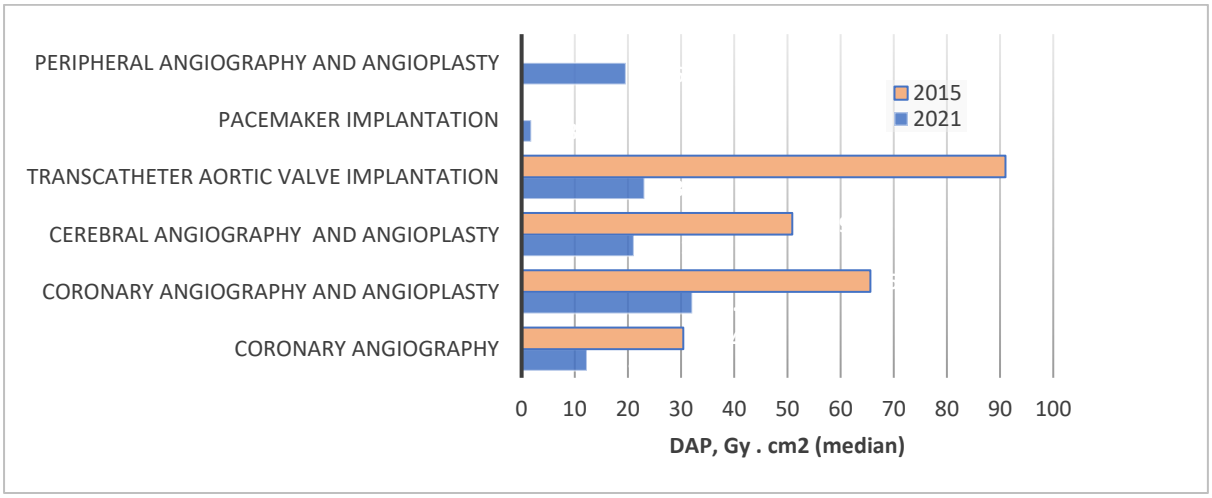

Figure 1. Comparison of exposure doses for interventional radiology cardiology procedures at VUHSK from 2015 to 2021.

In cooperation with the IAEA and the Radiation Protection Center, a human pelvic and abdominal phantom was obtained during the national technical cooperation project (CIRS model 801-P, Figure 2). The phantom is used in diagnostic radiology and radiation therapy to perform image evaluation, optimization, and dosimetry. Using this realistic phantom, exposure parameters of abdominal, pelvic and spinal protocols were assessed using physical parameters, as well as subjective evaluation by physicians[3]. These experimental studies will allow significant patient dose optimization of X-ray protocols for all digital radiography equipment at VUH SK.

Figure 2. Evaluation and optimization of radiological images with the anthropomorphic CIRS phantom.

The assessment of the exposure of nuclear medicine workers to external and internal sources of ionizing radiation is another important task. Previous studies showed that these exposure doses have decreased significantly in recent years and are also several times lower at VUH SK than reported in other countries. The reduction in exposure (especially when working with high-energy radiopharmaceuticals such as 18F-FDG) was due to the hospital radiation safety culture, use of automated and protective equipment (e.g., automatic injectors) and local, national and regional IAEA training. In 2021, studies on the exposure of nuclear medicine personnel extremities were done[4].

Also, harmonization of the bone scintigraphy protocol for three Lithuanian departments of nuclear medicine (the National Cancer Institute, the Hospital of Lithuanian University of Health Sciences and VUH SK) began in 2021. Each department uses different SPECT/CT scanning devices, scanning protocols, reconstruction algorithms and radiopharmaceutical activity. The results of the initiative and resulting new knowledge allowed to have a better understanding of the scanning and reconstruction algorithms (Figure 3), the differences in the image quality between equipment manufacturers as well as to optimize the exposure of patients and to continue the harmonization of other scanning protocols[5].

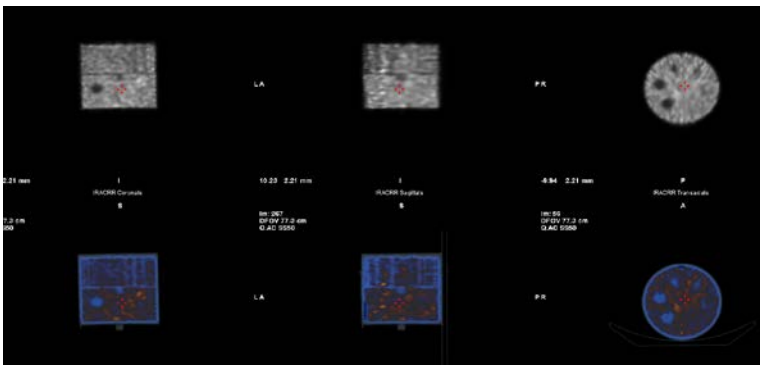

Figure 3. Evaluation of SPECT clinical protocol reconstruction parameters with Jaszczak phantom.

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Children's Physical Medicine and Rehabilitation Center, Center for Rehabilitation, Physical and Sports Medicine

Whole-body motor function and gait assessment in children with cerebral palsy

In 2021, specialists from the Children's Physical Medicine and Rehabilitation Center and the Center for Rehabilitation, Physical and Sports Medicine of Vilnius University Hospital Santaros Klinikos (VUH SK), in collaboration with representatives of the Department of Biomechanical Engineering at Vilnius Gediminas Technical University (VilniusTech), published the results of a study titled "Assessment of Whole-Body Motor Function and Gait in Children with Cerebral Palsy" (principal investigator – Prof. Juozas Raistenskis)[1-3]. The aim of this study was to investigate the biomechanical parameters of gait in children with cerebral palsy (CP) and to use radiological tests to build an individual musculoskeletal model.

Cerebral palsy (CP) is one of the most common motor development disorders in children, resulting in impaired body position and movement. These impairments adversely affect the posture and gait of children with CP, the latter being one of the most important activities of daily and social life, especially for children.

Modern treatments such as medication, rehabilitation, interventional therapy or technical aids can alleviate the course of symptoms and improve the quality of life of children with CP. However, for all these measures to be effective, it is essential to quantify the gait parameters first. This would allow predicting the future course of the disease and the possibility of regaining motor functions. Currently, decisions regarding further treatment are usually based on clinical and diagnostic tests as well as the General Motor Function assessment scale (GMF) for children with CP. However, this is not sufficient to accurately quantify movements and their changes as the disease progresses, or as rehabilitation or other treatment approaches are being applied.

There has been a major advance in computational modelling worldwide: the generic human musculoskeletal model, which is not sensitive enough to detect individual parameter changes, is being replaced by an electromyography (EMG)-based or a personalized musculoskeletal model, which defines individual musculoskeletal geometry using radiological imaging. Such models allow to assess individual physical parameters of each subject much more accurately. Such a model not only provides insight into the most significant gait parameters, allowing clinicians to determine the patient's condition as accurately as possible, but also allows them to select the most appropriate walking aids and to predict the best treatment or rehabilitation methods on each occasion.

The data is collected using innovative motion, ground reaction forces and electromyography (EMG) recording systems:

- A specialized motion recording system, VICON, consisting of eight optical cameras, is used to measure movement (Nexus 2.0 and Polygon 4, software modules for clinical gait analysis, are used to record and pre-process camera data);
- Electromyography (EMG) recorded by the Delsys Trigno system for research purposes (data are recorded by a dedicated EMGworks software);
- The ground reaction force is measured with the BERTEC force plate.

All three measurement systems are synchronized. Finally, Matlab is used to process the data. Classified experimental data and radiological images are all required for the musculoskeletal model, which is built using the AnyBody software package (Figure 1). All these software packages are validated and meet all the highest research requirements.

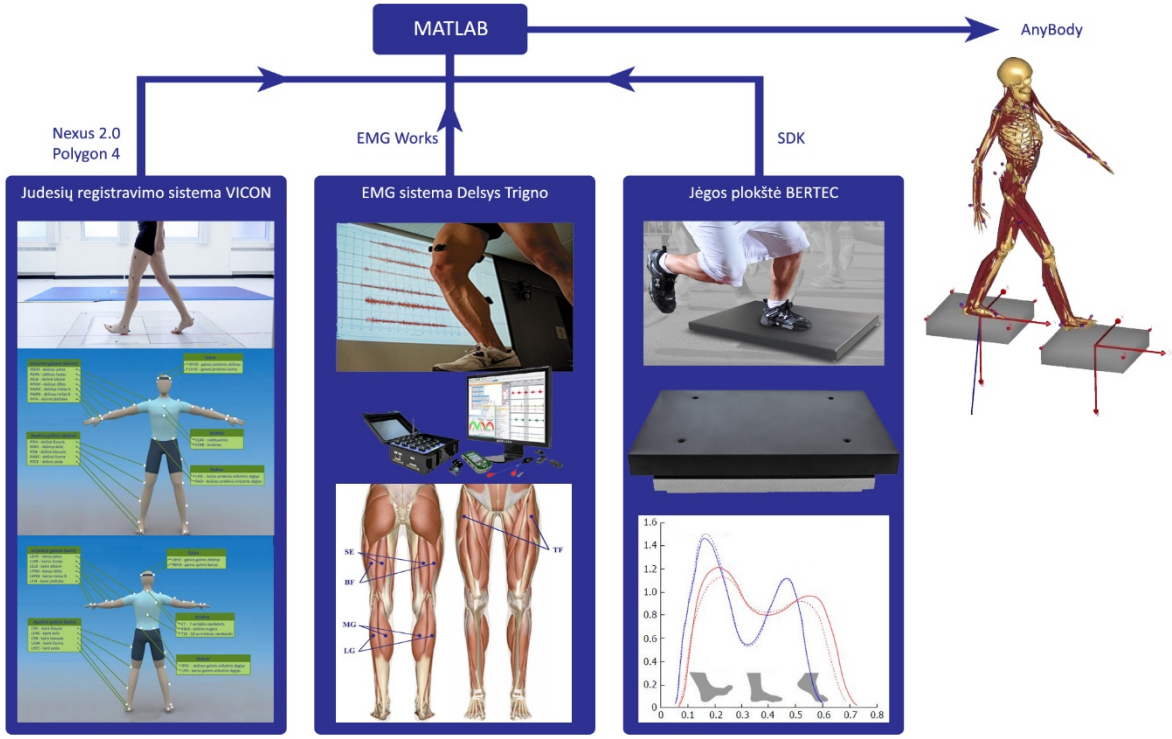

Figure 1. Flowchart of the study.

The scientific benefit of this study is the possibility to develop a new prognostic system for the assessment of gait in children with CP using digital methods – the musculoskeletal model. The introduction of quantitative biomechanical indicators in addition to the usual clinical assessment of CP, which clearly and accurately indicate the subject's real needs to improve his/her quality of life, will eliminate the need for additional and repeated examinations. It is also expected to make the treatment plan and process more understandable for the children and their parents or caregivers and create a favorable psychological climate, which increases motivation to work with the specialists to achieve better treatment outcomes.

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Center for Family Medicine

Hair study to define the risk for cardiovascular disease among women

Dr. Neringa Burokienė, a family physician at the Center for Family Medicine (head – Assoc. Prof. Lina Vencevičienė), took part in a study of the Faculty of Medicine of Vilnius University entitled "Association between hair steroid hormone levels and cardiovascular disease risk in a group of middle-aged and elderly women"[1]. Cardiovascular disease (CVD) accounts for as much as 57% of all deaths in Lithuania. Traditional CVD risk factors do not explain this high prevalence of CVD, and therefore chronic psychosocial stress is thought to play a role in the etiopathogenesis of CVD. Studies examining the impact of chronic stress on women's risk of CVD are needed to confirm this hypothesis.

Increasingly, hair steroid hormone (cortisol, cortisone, dehydroepiandrosterone) assays are being used to objectively assess chronic stress. The use of hair samples as a matrix allows a retrospective assessment of the stress levels experienced by the subjects in the last 1-6 months. The aim of the study was to assess hair steroid hormone levels in middle-aged and older women (50-64 years) enrolled in a CVD prevention program and the association of the hormone levels with chronic stress levels and cardiovascular disease risk. The study included 145 women without acute illness, CVD, diabetes mellitus or chronic renal failure.

The results showed a statistically significant association between hair cortisol levels and the subjects' waist circumference, systolic and diastolic blood pressure, apolipoprotein E levels, subjective perceived stress scale and Pittsburgh Sleep Quality Index questionnaire. Hair cortisone levels were statistically significantly correlated with subjects' body mass index, waist circumference, systolic and diastolic blood pressure, serum glucose and high-density lipoprotein levels. There was also a correlation between DHEA levels and subjects' systolic and diastolic blood pressure. When the subjects were divided into two groups according to the SCORE2 index value, it was found that women with a higher risk of CVD had statistically significantly higher hair cortisone concentrations than those with a low SCORE2 index value.

These results suggest that biomarkers of chronic stress could be important for the assessment of cardiovascular disease risk.

In 2021, doctors of the Center for Family Medicine of VUH SK also continued a very important project titled "Implementation of the Integrated Health Care Model in the Primary Care of Multimorbid Patients" (project leader – Assoc. Prof. Lina Vencevičienė), which aims to improve the quality and accessibility of primary healthcare services for patients with two or more chronic non-infectious diseases in Lithuania, and participated in a biomedical study at the Faculty of Medicine of Vilnius University titled "Analysis of Biomarkers for Suicidal Tendencies and Depression, and Validation of the Prognostic Model in Lithuania" (investigator - Assoc. Prof. Lina Vencevičienė), which is looking for specific biomarkers that may predispose to the risk of suicide attempt.

References

1. Mazgelytė E, Mažeikienė A, Burokienė N, et al. Association between hair cortisol concentration and metabolic syndrome. Open Med (Wars). 2021;16(1):873-881. Published 2021 Jun 16. doi:10.1515/med-2021-0298]

Center for Coordination of Rare Diseases

Personalized care for rare diseases: innovation and international cooperation

The Center for Coordination of Rare Diseases of Vilnius University Hospital Santaros Klinikos (VUH SK) brings together 38 specialized centers of excellence for rare diseases, with teams of rare disease specialists working in different fields (Figure 1). It is the largest center for the diagnosis, treatment, training and research of rare diseases in Lithuania, which offers healthcare services both Lithuanian and foreign patients with rare conditions.

The aim of the Center is to provide high quality, patient-centered, innovation-driven personal healthcare to patients suspected or diagnosed with a rare disease, and to shorten the path towards an accurate diagnosis. At the same time, it aims include raising awareness of rare diseases, supporting and promoting the development of rare disease specialists and expansion of scientific knowledge in this field. This is done by using the modern laboratory and instrumental research infrastructure of VUH SK, laboratory, molecular, pathological, instrumental and radiological diagnostics, molecular karyotyping and next-generation sequencing.

Because of a successful participation of specialists from VUH SK in the European Reference Networks (ERNs), Lithuanian citizens suffering from rare diseases receive international and interdisciplinary healthcare. In addition, physicians consult and share their experience with other European treatment centers, participate in exchange programs and conduct research. By 2020, VUH SK was one of the 30 European hospitals with the most ERN members. In terms of the number of ERN full members per million inhabitants, Lithuania is the regional leader and fourth in the European Union. **In 2021, the Council of Member States of the European Reference Centre Networks approved the full membership of four more Rare Disease Centers of Excellence at VUH SK: GENTURIS, EURACAN, CRANIO, VASCERN.** Currently, from 38 Rare Disease Centers of Excellence operating at VUH SK, 16 have become full members of the ERN.

A survey carried out by the European Organization for Rare Diseases (EURORDIS) showed that patients rate the services provided for rare diseases at VUH SK as 3.6 out of 5 (the overall average rating for all countries participating in the ERN is 3.5, while the average rating for centers not participating in the ERT is 3.0).

Figure 1. Competence centers of rare diseases at VUH SK.

Doctoral dissertations defended by VUH SK employees at Vilnius University in 2021

PhD candidate	Date of defense	Title	Supervisor
Ieva Jūra Paulavičienė	2021 02 12	Changes in human milk macronutrient content of non-breastfeeding mothers after delivery and factors or procedures affecting its composition	Prof. Habil. Dr. Vytautas Usonis
Karolis Ažukaitis	2021 02 14	Arterial Stiffness in Children with Chronic Kidney Disease: Prevalence, Risk Factors and Functional Consequences	Prof. Rr. Augustina Jankauskienė
Beata Vincel	2021 04 22	Correlation between Histology and Gene Expression in Cryptorchidism	Doc. Dr. Gilvydas Verkauskas
Dovilė Žilėnaitė	2021 05 20	Assessing Immunohistochemistry Biomarkers in the Spatial Context of the Microenvironment of Hormone Receptor-Positive Ductal Breast Carcinoma by Digital Image Analysis	Dr. Arvydas Laurinavičius
Aušra Bilotienė-Motiejūnienė	2021 06 04	The problem of hospitalizations during return visits to the hospital emergency department, their impact on the quality to healthcare services and the ways to manage them	Prof. Dr. Rimantas Stukas
Aistė Kielaitė-Gulla	2021 06 17	Heat shock proteins: pathogenic disease factors and potential treatment targets in acute pancreatitis	Prof. Habil. Dr. Kęstutis Strupas
Rolandas Vaicekauskas	2021 06 18	Evaluation of upper endoscopy, endoscopic ultrasound and deep biopsy diagnostic criteria of subepithelial upper gastrointestinal tract lesions	Prof. Habil. Dr. Jonas Valantinas
Marija Barisienė	2021 06 22	Significance of new prostate-specific antigen isoforms for early diagnosis of prostate cancer	Prof. Dr. Feliksas Jankevičius
Agnė Petrulionienė	2021 09 09	Clinical Syndromes and Epidemiological Factors of Lyme Disease in Lithuania	Prof. Habil. Dr. Algirdas Venalis
Aušrinė Pliauckienė	2021 09 13	Non-Invasive Monitoring of Haemodynamics and its Changes During Systemic Infection in Neonatal Patients	Prof. Habil. Dr. Vytautas Usonis

Alfredas Vaitkus	2021 09 15	The value of qualitative sensory testing for diagnostics of chronic lumbosacral radiculopathy with radicular pain and treatment of related chronic pain with transforaminal epidural steroid injection	Prof. Dr. Jūratė Šipylaitė
Aušra Aleknaitė	2021 09 24	The Preoperative Prediction and Management of Choledocholithiasis before Planned Laparoscopic Cholecystectomy based on Individual Risk of Choledocholithiasis	Prof. Dr. Gintaras Simutis
Arminas Jasionis	2021 12 10	Relationship of emotions, cognitive and social functions of people with epilepsy and their demographic and clinical characteristics	Prof. Dr. Rūta Mameniškienė
Daiva Dudutienė	2021 12 10	Use of scientific knowledge in the development of evidence-based safety and health policies for health workers in the context of psychosocial risk management	Prof. Dr. Rimantas Stukas

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