COVID-19 RESEARCH IN LATVIA: SCIENCE TO BE USED IN PRACTICE AND PRACTICE TO BE USED IN SCIENCE

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SARS-CoV-2 emerged in the human environment very suddenly. This created many problems, but at the same time contributed to medical science research. Within a short period of time, it became evident that previous beliefs that had been held as standards regarding the course, pathogenesis and prognosis of infectious diseases have not be accurate. Similarly, beliefs about the relationship between viruses and macroorganisms have also been affected. Comprehensive studies have been launched worldwide and in Latvia in order to find out the course of the new acute respiratory virus, and what needs to be done to minimise its effects.

In most cases, acute respiratory diseases (ARDs), including viral aetiology (ARVI), are well known, since active studies have been carried out for decades. It is possible to treat and control them effectively through carefully designed vaccines that have been tested in the long term, as in the case of influenza. However, SARS-CoV-2 has brought many revelations. Due to the rapid spread of SARS-CoV-2 and COVID-19 researchers have not had the time to conduct their studies more slowly, and each finding has had to be put into practice immediately.

We – a group of researchers and medical practitioners – participated in the call for tender on COVID-19 research that took place in the summer of 2020. We set out a clear medical and practical research aim, defined by the National Research Programme (NRP) as follows:

> Clinical, biochemical, immunogenetic paradigms of COVID-19 infection and their correlation with socio-demographic, etiological, pathogenetic, diagnostic, therapeutically and prognostically important factors to be included in guidelines.

Our group was aware that we should present evidence-based scientific knowledge on the course of COVID-19 and its effects, and that our study should have a fundamental approach, thus contributing to international science.

To achieve the aim of the study, we set 11 objectives. Among them were objectives that involved identifying properties and facts related to SARS-CoV-2, the course of the COVID-19 disease and certain stages of pathogenesis. We studied, and continue to study, the effects of immunogenetic factors and oxidative stress on COVID-19 that could play a key role in the development and course of the disease. We studied and analysed the consequences of COVID-19 through a targeted, modern and comprehensive radiological examination of the internal organs and a broad biochemical examination, including apoptosis and fibrosis markers, which could play a key role in patient disability.

I will outline the details of the findings that are already important for practical medicine.

When launching the study, there were indications that SARS-CoV-2 causes a disease with a pathogenesis

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that differs from other ARDs despite its symptoms bearing a similarity to other ARVI. An example of this is the surprisingly hyperactive involvement of the immune system in the process with unexpected clinical turning points. This results in needing to take a different approach to pathogenetic therapy, which is quite often only possible in an inpatient setting, for example, for patients to be able to receive oxygen therapy.

More than 970 indicators were analysed in order to identify specific or non-specific disease symptoms, factors that contribute to a more severe condition, and to identify the effects of the disease in detail.

Thanks to the enormous amount of work that has been done in a short period of time, all of us, including practitioners, have gained a better understanding of the virus. We are now able to predict and influence the course of COVID-19. The facts and findings that we have obtained, along with data collected by researchers from other countries, have been included in COVID-19 recommendations, developed for medical practitioners through the NRP. It is of the utmost importance to inform all medical practitioners of these findings on how to lessen the effects of COVID-19 in order for them to immediately be able to use what is necessary for more efficient healthcare, in diagnostics, prognosis, and treatment. Thus, within a few months, it was possible to identify, analyse and evaluate the spectrum of the clinical manifestations of COVID-19, and to calculate and highlight a group of symptoms. Based on this information it is now possible to get an indication on **whether the course of the disease will be potentially severe, as well as to recognise the factors that indicate a deadly prognosis.** With this information, it is possible to get ahead of a deadly outcome and apply the necessary treatment methods. Well-designed and evidence-based guidelines for diagnosing and treating COVID-19 patients have been created based on these findings. The guidelines have been introduced to and are used by the project's largest partner – Rīga East Clinical University Hospital. The guidelines can be improved further as more information is discovered. As we have now been living with COVID-19 for almost a year, many things already seem self-evident, for example that ferritin and troponine T (which are not used in regular ARVI practice as they usually remain unchanged) are among the vitally important laboratory examinations to evaluate the new ARVI. These findings, for example, were only discovered in the summer of 2020. At first, this seemed strange and not immediately comprehensible in practice, yet it was supported by scientific evidence. Now, it already has pathogenic explanations that have been included in the guidelines. Practice shows that researchers' findings and recommendations are useful and can often save patients' lives.

Not less important, particularly from an epidemiological point of view, is the study's objective to establish which **pathways transmit SARS-CoV-2** and the dynamics of antibody formation in infected and sick patients. This part of the study was led by Assoc. Prof. Modra Murovska (Rīga Stradiņš University, RSU) and brought some surprises. Nasopharyngeal swabs, blood plasma, peripheral blood mononuclear cells, faeces and urine were examined for traces of SARS-CoV-2. Traces of

SARS-CoV-2 were present in the faecal samples of almost 50% of patients. Additionally, these traces could be found for an extended period of time and, in some cases, the trace amounts were greater than those found in the other materials that were examined.

Analysing the **dynamics of patient-specific antibodies to SARS-CoV-2**, it was found that IgM antibodies **could often once again be found in high titres** more than two months after the characteristic period of reduction during recovery, or even disappear after an acute infection. This could be explained by the persistence of SARS-CoV-2, or by reinfection. It is important to find this out, because it might be necessary to develop a different approach to evaluate the health of COVID-19 patients, or to setting up treatment.

The presence of respiratory bacteria and persistent/chronic virus infections –7 bacteria and 12 viruses – was identified in COVID-19 patients with a multiplex diagnosis in real time with PCR. More than a third of patients have been found to **have one of the tested co-infections.** This could affect both the clinical course of COVID-19 and add co-infection symptoms to COVID-19 symptoms, in this way creating the illusion that the other symptoms that are characteristic to bacterial or viral diseases belong to COVID-19.

An **immune response regulating gene analysis** was conducted by *Dr. med*. Jeļena Eglīte and Oksana Koļesova in order to evaluate the genetic factors that may, or may not, affect whether a patient contracts SARS-CoV-2. Four more common and three less common HLA Class II gene alleles have been identified in Latvian COVID-19 patients. These might contain the answer to whether a patient is at a higher risk of contracting, or of not contracting COVID-19. This has resulted in practical recommendations that will supplement public epidemiological recommendations.

Our research also focused on the subject of oxidative stress and selenium. It is known that selenium deficiency can lead to changes in the virus' genome, increased virulence, problems with the host's immune system, inflammatory processes and to an increase in oxidative stress, which is of particular importance during COVID-19. Under the leadership of Prof. Andrejs Šķesters (RSU), a group of researchers evaluated levels of selenium and selenoprotein in patients who had recovered from COVID-19 and determined the level of oxidative stress in their plasma. Levels of selenium in patients who had recovered from COVID-19 were close to the lowest acceptable norm. In some patients the concentration of selenium was dramatically low. The material is now being supplemented and the findings require detailed analysis and interpretation.

Whereas in relation to oxidative stress the results obtained were three times over the norm. These could be the effects of post-infection or a continuation of inflammatory processes in the body, including the "cytokine storm" which is typical to COVID-19.

On the basis of previously proven facts, researchers recommend the use of organic selenium in physiological doses for both prevention and recovery.

The aftereffects of COVID-19 are also being examined within the framework of the project. We conducted in-depth medical examinations of people who had recovered from COVID-19 by creating a biochemical profile, identifying apoptosis and fibrosis factors (as mentioned above), identifying immunological indicators, and conducting a modern special radiological investigation. The findings of the examinations show that most people still had health complaints three or more months after the acute period of the disease. These were characterised by breathing problems, lingering symptoms in the nervous system and the cardiovascular system. The examinations discovered changes in the biochemical markers of the liver, cardio markers and immunological parameters, as well as high levels of oxidative stress. It was found that approximately 6% of people experienced severe changes in the number of T lymphocytes after COVID-19, indicating a state of immunodeficiency.

The radiological examinations were performed by two associate professors at RSU – Ardis Platkājis and Maija Radziņa. The examinations show that some patients remained affected by changes in the lungs and had abnormalities in their liver parameters potentially associated to COVID-19. Levels of fibrosis and apoptosis markers in serum, which indirectly imply potential disability, confirmed an increased tendency in connective tissue formation in these organs following acute COVID-19 infection.

Through various serological tests a group of researchers, led by Prof. Dace Gardovska (RSU), **identified an immune response to SARS-CoV-2 infection in children with selected chronic diseases**. The study's findings show that the presence of antibodies, i.e. the

seroprevalence of SARS-CoV-2, is low at 1.7% in blood samples from children with chronic diseases. Thus, it is possible to draw a preliminary conclusion that children suffering from chronic diseases are more susceptible to SARS-CoV-2.

Prof. Mārcis Leja (University of Latvia, UL) took the lead in ensuring **participation in the Solidarity Trial**. This project is mainly related to studying different drugs and evaluating them in the treatment of COVID-19 patients. This part of the project is closely linked with the World Health Organisation (WHO) and involves cooperating with many countries worldwide. It was thanks to the Solidarity Trial that Remdesivir could be used in Latvia so quickly. Participating in the Solidarity Trial together with Rīga East Clinical University Hospital and Pauls Stradiņš Clinical University Hospital is strategically important on a national level as it demonstrates the ability to cooperate with the WHO and also allows for the staff involved in the study to raise their qualifications by participating in trainings and following the course of the study on an international level. The trial's findings will affect international guidelines for pharmacological COVID-19 treatments.

The project launched studies in a number of directions aimed at improving COVID-19 diagnosis by using modern and adequate technologies. Thus, a group of researchers at the Latvian Biomedical Research and Study Centre, led by Vita Rovīte PhD, introduced and optimised **a completely new technique for sequencing one-cell transcriptomes**. The study revealed a number of factors controlling inflammatory blood cell processes that need to be further analysed in detail in order to allow them to be used in disease prognosis or as targets for more effective therapy.

Another group of researchers from the Latvian Biomedical Research and Study Centre, led by Prof. Aija Linē (UL), developed **a new type of antibody test** that enables antibodies to be detected against 30 SAR S-CoV-2 protein fragments simultaneously. This test is currently being used to analyse the profile of antibodies in serum from COVID-19 patients, and to understand its relationship with the severity of the disease.

Led by Prof. Leja, specialists from the UL Institute of Clinical and Preventive Medicine have developed another type of **sensor technology** together with specialists from Rīga East Clinical University Hospital and the company JLM Innovation GmbH. This technology can be used to monitor changes in various chemicals in the exhaled air of patients who are connected to artificial respiration systems. This method could allow doctors to predict the course of how the disease is developing in patients with severe COVID-19. Measurements of respiration sensors have already been performed on the first patients.

As part of the project, **recommendations for COVID-19 diagnostics and treatment** have been drawn up in accordance with scientific and practical information and recommendations from the WHO, the Centres for Disease Control and Prevention (CDC) and specialists from the European Union and the USA.

The recommendations have been developed by more than 30 specialists from a variety of fields. Among them are infectologists, laboratory and intensive care specialists, general practitioners, pneumologists, paediatricians, radiologists and others. Advisers with extensive and varied experience also participated in drafting the recommendations. The final version of the recommendations consists of 20 chapters on epidemiology, pathogenesis, clinical manifestations, complications, diagnostics and the treatment and prevention of COVID-19. It is intended to be used by all medical practitioners involved in the care for COVID-19 patients, and by medical students and residents. Presumably, for now, the recommendations can be seen as one of the best practical materials the NRP has produced. The contribution is both scientific and practical. The most important practical sections are aimed at clarifying facts about the disease agent, the pathogenesis, approaches to treatment, and other essential disease-related problems.

It should be emphasised that mathematical processing and analysis of the material that has been obtained from patients is ongoing, as a large part of the material was obtained during the study's second phase when there was a significant increase in the number of COVID-19 patients in Latvia.

We are sure that we are going to gain much more scientific knowledge and recommendations for medical practitioners and patients. The scientific facts that were obtained during the unique initial stage of COVID-19 will serve as an outstanding platform on which to base many future studies.