Title: Assessment of the influence of clinical, functional, immunological and genetic factors on the severity of the course of coronavirus infection with SARS-CoV-2 and Post Covid syndrome

NCT number: BR10965164

Date: 19.04.2021

1. Annotation

The first case of pneumonia of unknown origin was registered in Wuhan, China in December 2019 [1]. Shortly after the first case was discovered, the disease was reported outside of China. On January 30, 2020, the World Health Organization (WHO) announced the disease was inscribed on the International Health Emergencies List (PHEIC), which meant that it posed a threat to the entire world and required a coordinated international emergency response [1, 2]. Already on February 11, 2020, the World Health Organization assigned the name COVID-19 to the disease [1]. On the same day, the International Commission on Virus Classification announced that the new coronavirus had been classified as type 2 coronavirus, severe acute respiratory syndrome (SARS-CoV-2) [1]. The rapid spread of this infection in the first months of 2020 reached pandemic proportions. To date, more than 100 million cases of COVID-19 and more than 2 million deaths have been recorded in the world [3].

COVID-19 is not the first outbreak of severe respiratory illness caused by a virus from the coronavirus family. In the past two decades alone, coronaviruses have caused three outbreaks: COVID-19, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) [4]. Although the mortality rate from SARS-CoV-2 is lower than from two previous outbreaks of coronavirus, its lightning-fast spread, which led to the development of a pandemic, has caused catastrophic consequences for the world. That is why a quick and comprehensive study of the pathogenesis of SARS-CoV-2 is so important.

Currently, the world is actively studying the immune pathogenesis of COVID-19, the search for measures to prevent and develop drugs against SARS-CoV-2 infection based on previous progress in SARS-CoV and MERS-CoV research. Interestingly, COVID-19 can cause a wide range of diseases, ranging from asymptomatic to severe respiratory distress syndrome and respiratory failure. However, the role of humoral and cellular immunity in the mechanism of the “cytokine storm” and various factors leading to a high incidence of complications and mortality from COVID-19 is still not fully understood.

According to a report in the Lancet, Acute Respiratory Distress Syndrome (ARDS) is the main cause of mortality in COVID-19, in the mechanism of which a cytokine storm plays an important role - a fatal uncontrolled systemic inflammatory response resulting from the release of large amounts of pro-inflammatory cytokines and chemokines [5, 6]. That is why, often in the treatment of COVID-19, the use of antiviral agents is not enough and it is necessary to carry out complex treatment with steroids and other medications aimed at suppressing the immune response [6,7]. There is already evidence that the development of a cytokine storm, and, accordingly, respiratory complications is largely associated with genetic factors [7]. However, in order to determine the exact data, it is necessary to carry out additional, complex research.

2. Explanatory note

1. General information

1.1. Name of the topic of the scientific, scientific and technical program: "Assessment of the influence of clinical, functional, immunological and genetic factors on the severity of the course of coronavirus infection with SARS-CoV-2 and Post Covid syndrome"

1.2. A strategically important state task for the solution of which a program has been developed - the Message of El Basy dated January 10, 2018 "New development opportunities in the context of the fourth industrial revolution", in terms of the development of personalized medicine; Clause 5.5 Improving the provision of medical care of the State Program for the Development of Healthcare of the Republic of Kazakhstan for 2020 - 2025:

Measures to combat the main diseases leading to mortality are diseases of the circulatory system (strokes, heart attacks and others), respiratory diseases, oncological diseases, injuries, health care for children and mothers, neurological diseases, and in other clinical services includes: transfer and introduction of new and innovative technologies for the diagnosis and treatment of diseases within the framework of public and private initiatives

1.3. Place of implementation of the program: NJSC "National Research Center for Cardiac Surgery"

1.4. Estimated start and end date of the program, its duration in months: 2021 - 2023, 36 months.

1.5. Organization-applicant of the program: NJSC "National Research Center for Cardiac Surgery"

1.6. Executors of the program:

Private institution "National Laboratory Astana", Nazarbayev University

School of Medicine, Nazarbayev University

Joint Stock Company "National Center for Neurosurgery"

1.8. Key words characterizing the industry and the direction of the program for the selection of independent experts: Coronavirus infection, Post Covid syndrome, COVID-19, pneumonia, cardiology, genetic research, immunology.

2. The general concept of the program.

2.1. Introductory part.

Principal Investigator - Bekbossynova Makhabbat, Doctor of Medical Sciences, Acting Chairman of the Board of the NJSC "National Research Center for Cardiac Surgery ".

Responsible person - Tauekelova Ainur Tulepbergenovna, cardiologist, head of the admission department, NJSC "National Research Center for Cardiac Surgery".

On the basis of the NJSC "National Research Center for Cardiac Surgery" an infectious hospital has been deployed in June 2020 for hospitalization of persons with confirmed cases of severe coronavirus infection. By order of the Ministry of Health of the Republic of Kazakhstan No. 763 dated November 24, 2020, the country's first postcovid center was created. In December 2020, on the basis of the postcovid

center, 312 adult patients and 100 children were examined after suffering a coronavirus infection with a confirmed COVID-19 diagnosis.

2. 2. The purpose of the program.

To determine the clinical, functional, immunological and genetic factors affecting the severity of the course of acute coronavirus infection COVID-19 and PostCovid syndrome, in order to develop management tactics for such patients to reduce the risk of complications and disability.

2.3. Objectives of the program:

1. To determine the clinical and functional characteristics of patients with varying degrees of the course of the acute phase of COVID-19 and Post Covid syndrome.

1.1. To study the features of neurological disorders in patients with varying degrees of the course of the acute phase of COVID-19 and Post Covid syndrome.

2. To study the immunological profile of patients with varying degrees of the course of the acute phase of COVID-19 and Post Covid syndrome.

3. To study the genetic profile of patients with varying degrees of the course of the acute phase of COVID-19 and Post Covid syndrome.

4. Identify potential predictors of COVID-19 severity.

5. To determine the markers that allows predicting the development of the Post Covid syndrome.

6. Based on the selected markers, develop a COVID-19 outcome scale to determine the tactics of patient management to prevent the development of Post Covid syndrome.

3. Scientific innovativnes and significance of the program.

Immunological research has largely become central to the study of the pathogenesis of COVID-19. Today, all aspects of the immune system are being studied in the world in order to better understand the causes of complications and, possibly, to develop targeted treatments to avoid them. The recent publication in the journal Immunology was the first work that helped to systematize scientific data on this issue [8]. According to this study, an important feature of COVID-19 is its ability to evade an innate immune response [8]. Thus, in patients with a severe form of coronavirus, a significant violation of the expression of interferon (IFN-I), which plays an important role for antiviral protection, was noted in relation to patients with moderate and mild forms [8]. Noted,

Another feature of SARS-CoV-2 is its complex effect on all the germs of the immune system. Many studies have shown that coronavirus can lead to a decrease in natural killer (NK) levels, which also correlates with the severity of the disease [8]. In a more detailed study of the role of NK cells in the pathogenesis of COVID-19, it was suggested that their stimulation can help eliminate infection and

prevent the development of ARDS [8]. In addition to NK cells, studies are continuing on the role of the T-cell response in patients with coronavirus infection. It was noted that lymphopenia (especially a decrease in the level of CD8 T cells) can serve as a prognostic factor indicating a more severe course of the disease [8]. Moreover, the authors note that a violation of the activity of T cells can lead to immunopathology of the lungs and, therefore, to the development of complications in patients with COVID-19 [8].

Humoral immunity undoubtedly plays a key role in neutralizing the virus. In disease, antibodies are usually found that bind the internal N-protein SARS-CoV-2 and the external glycoprotein S [8]. The receptor binding domain (RBD) of protein S is highly immunogenic, and antibodies that bind this domain are likely to have a high neutralizing ability to block COVID-19 when it binds to human cells [8]. An important issue in the study of the consequences of coronavirus infection is the identification of immunity after an illness. It is noted that B-cell memory is key in this issue, however, it is still not clear how long it can persist in the human body. Long-term protection is achieved through the production of long-lived plasma cells and memory B-cells, but the life cycle of these cells after suffering COVID-19 is still not fully understood [8].

There are also suggestions about the role of antigen presenting cells (APCs) in the pathogenesis of COVID-19. They are important in the development of antiviral immunity in the body and are recognized by virus-specific cytotoxic T-lymphocytes (CTL) [9]. Today it is becoming clear that COVID-19 can cause a complex systemic reaction between various components of the immune system [10]. Therefore, additional research on this topic will help us understand the pathogenesis of COVID-19 and the role of humoral and cellular immunity in the development of complications.

There are still controversial issues in the diagnosis of COVID-19. To date, the standard for diagnosis of COVID-19 is reverse transcription polymerase chain reaction (PCR), which includes the detection of viral nucleic acid in sputum or nasopharyngeal swab. But as practice has shown, this test has several drawbacks, ranging from expensive cost to low sensitivity, which can affect a truly positive result. In addition, the occurrence of conflicting results between chest CT and PCR, described in some studies, lead us to the conclusion that the results of the PCR test should always be interpreted in a broader context, taking into account other diagnostic markers and the clinical picture [11,12].

Various complications of COVID-19 have become one of the significant and dangerous outcomes of coronavirus infections. Respiratory failure caused by pneumonia, acute respiratory distress syndrome, diabetes mellitus, severe heart damage with a significant increase in the level of troponin in the blood and heart failure became the causes of high mortality.

In particular, the reported mortality rates for COVID-19 are 10.5% in patients with cardiovascular diseases, 7.3% in patients with diabetes and 6.0% in patients with arterial hypertension [13]. This is higher than the mortality rate observed globally in patients without concomitant diseases, which is 3-4%

[13]. Finally, the increased incidence of unwanted cardiovascular and other complications after recovery from COVID-19 may also play a role in the increased mortality rate of patients with coronavirus infection.

Although there is still no officially clinically proven specific antiviral agent for the treatment of SARS-CoV-2 in the world, symptomatic treatment, including oxygen therapy, anticoagulants, and the use of broad-spectrum antibiotics for the treatment of secondary complications, remains important in the treatment strategy [14].

It is not yet known whether SARS-CoV-2 infection leads to sustained protective immunity, by what mechanism this can occur, and whether the vaccine-induced immune responses will protect without causing harm [5]. According to studies of the molecular mechanisms of the SARS-CoV-2 genomes, there are currently several potential targets for effective therapy, but nevertheless, there are more and more open questions requiring detailed study [15,16].

There is already evidence that COVID-19 can lead to long-term consequences and exacerbation of chronic diseases [17, 18]. The so-called longcovid or ongoing symptomatic COVID-19 is characterized by symptoms lasting from 3 to 12 weeks after suffering COVID-19 [18,19]. If complications last more than 12 weeks, the syndrome becomes postquoid [19]. Symptoms vary greatly from person to person and further study is necessary to develop management strategies for these patients. The world community has already noted that further study of long and postcovid syndromes is necessary to prevent the development of even greater disorders [20].

As of today, the WHO has updated the ICB-10 database on COVID-19 coding. Code U09.9 now encodes "Condition after COVID-19", which includes loncoid and Post Covid syndromes [21]. However, the timing of the course of this pathology, symptoms, risk factors and other data have not yet been determined and need more detailed study. The study of the consequences of the postponed coronavirus infection continues in order to predict the epidemiological situation in the world. The situation is aggravated by the fact that the course, severity of the disease and complications in patients are very different. Thus, studying the genetic epidemiology of patients with COVID-19 in a complex of factors influencing the course of the disease and outcomes will help develop measures to combat the epidemic of the virus.

Thus, the above data served as the purpose of this research work, which for the first time in Kazakhstan will become work on the study of pathogenetic mechanisms, immunological and genetic markers for the development of effective treatment methods, determination of prognostic factors and management of patients with COVID-19 and Post Covid syndrome to prevent the development of subsequent complications.

For the first time, a comprehensive assessment of the severity of the course and outcomes of coronavirus infection will be carried out in the context of clinical, functional, immunological and genetic factors:

1. The relationship between the immune response, determined by genetic status, and the severity of the course in patients with coronavirus infection will be investigated
2. Risk factors for the development of long / Post Covid syndromes will be identified.
3. Genetic characteristics of patients with long / Post Covid syndrome were determined to identify potential predictors of severity, predict the course and outcomes of the disease.
4. The immunological profile of patients after coronavirus infection will be studied in dynamics to assess its role in the development of long / Post Covid syndromes.

The identified predictors will be used to develop a scale of COVID-19 outcomes for predicting and determining various course options, which will allow introducing a treatment algorithm to prevent the development of Post Covid syndrome, complications and disability in patients who have undergone coronavirus infection.

4. Research methods and ethical issues.

This study is multicenter, cohort controlled, comparative and prospective. The selection of patients will be carried out among patients who have undergone COVID-19 admitted to the National Research Center for Cardiac Surgery and Republican Diagnostic Center of the University Medical Center corporate fund using laboratory-functional tests in accordance with standard clinical practice.

The objects of the study will be: patients who have undergone COVID-19.

Sample size 300 patients

Materials and methods:

Inclusion criteria

- Age over 18 years old
- Patients with a history of coronavirus infection COVID-19 confirmed by PCR analysis
- Patients who signed informed consent to participate in the study

Exclusion criteria:

- Refusal to undergo diagnostic procedures determined by the research protocol.

- Evidence for preexisting interstitial lung disease.
- Participation in another study.

The study will include patients with a positive PCR test for COVID-19. Patients in the acute phase of the course of the disease are monitored and treated in accordance with the republican COVID-19 treatment protocol. After signing the informed consent, the patient will be included in the study. The collection of the necessary materials for subsequent analyzes (clinical-functional, genetic, immunological) will be carried out in accordance with this protocol. Subsequently, patients are observed within one year from the moment of illness in accordance with the study protocol and with the collection of all necessary materials.

Further, on the basis of complex data, the severity of the course of the disease will be determined, followed by distribution and analysis (Figure 1).

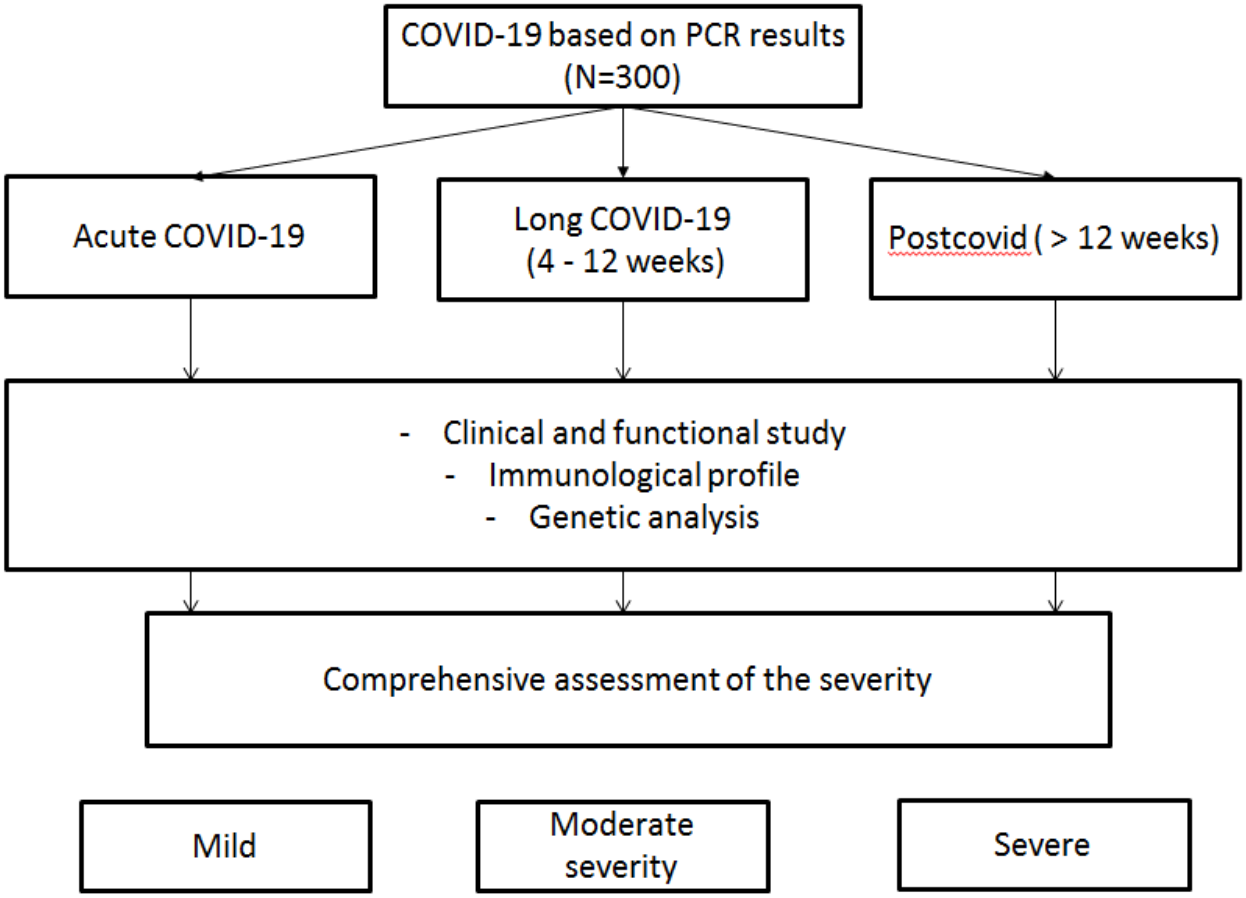

Figure 1. Dividing patients into groups

For the study, clinical-functional, immunological and genetic research methods will be used in accordance with the republican and international recommendations for the treatment of patients with

COVID-19.

Clinical and functional analysis:

Detection of RNA of the COVID-19 virus using PCR analysis. Conducting complex laboratory studies in accordance with table 1.

General blood analysis	Complete blood count on an analyzer with differentiation of 5 classes of cells, the ratio of neutrophils to lymphocytes
Blood chemistry	ALT, AST, total bilirubin, direct, LDH, CRP, alpha-amylase, creatinine, urea, glucose, ferritin, glycosylated hemoglobin, vitamin 25 - OH vitamin D, vitamin B12
Coagulogram	D-dimers, fibrinogen, INR, APTT
Other	NT-pro BNP, Homocysteine, IL 6, Troponin, blood group determination
Linked immunosorbent assay	Determination of IgG and IgM antibodies to SARS-CoV-2 coronavirus (COVID-19) in blood serum, RBD
Functional diagnostics	• ECG • EchoCG + strain • CT scan of the lungs • Holter • SMAD • Kidney ultrasound • Ultrasound • Doppler ultrasonography of veins and arteries • Chalder Scale, EQ Questionnaire
Table 1. Clinical and functional analysis	

Neurological disorders:

1. Neurological examination with the isolation of neurological syndromes (motor, cognitive impairments, sleep disorders, asthenic-depressive syndromes, etc.).

2. Neuropsychological methods - research on the scales of anxiety and depression, MMSE, etc.

3. Instrumental method - EEG, polysonography, ultrasound of the neck vessels, CT perfusion.

4. Laboratory research methods:

a. The study of antibodies to some neurospecific antigens - myelin basic protein (MBP), neurospecific enolase (NSE).

b. Study of cellular immunity (CD3 +, CD4 +, CD8 +) and general indicators of humoral immunity (IgG, IgA, IgM, circulating immune complexes).

Immunological analysis:

A comprehensive immunological analysis will be carried out to determine the level of the immune response. Calculation of the level of CD4 +, CD8 + and NK cells. The level of antibodies of the IgG and IgM classes to the proteins of the coronavirus S1, RBD and N was determined

Multiplex Immunoassay. For evaluation of immunological parameters, samples are diluted in 200 µl of phosphate buffer, centrifuged and the supernatant analyzed using the manufacturer's protocol. The MILLIPLEX MAP human cytokine / chemokine magnetic bead panel will be used for the analysis of multiple cytokines and chemokines / immunoglobulins, and the Milliplex® magnetic bead panel (HGAMMAG-301K-06, EMD Millipore Corp., Billerica, MA) will be used for immunoglobulin isotyping. Samples will be analyzed on Bioplex BIO-RAD for the following indicators: sCD40L, EGF, Eotaxin / CCL11, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL -1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF- AA, PDGF-AB / BB, RANTES, TGF-α, TNF-α,

Immunophenotyping of T-cell and B-cell subpopulations using the flow cytometry method. Subpopulations of B- and T-lymphocytes will be examined by staining peripheral blood mononuclear cells (PBMCs) isolated from whole peripheral blood with monoclonal antibodies conjugated to fluorochromes. PBMCs can be isolated from EDTA-treated whole blood using Ficoll density gradient centrifugation or special erythrocyte lysis buffers. It is preferable to analyze isolated PBMCs directly on the day of blood collection, which gives more reliable results. PBMC viability will be assessed using LIVE / DEAD ™ fixed dead cell kits or 0.4% trypan blue and propidium iodide solution. After lysis of erythrocytes and incubation with monoclonal antibodies, the stained cells are resuspended in a staining

medium and examined using a MoFlo Astrios flow cytometer (Beckman Coulter, USA). The resulting data will be analyzed using Summit (Beckman Coulter) and FloJo (Tree Star) software.

Forward and side scatter will be used to distinguish the lymphocyte population in addition to the signal from specific fluorochromes. Distribution CD3-, CD5 +, CD19 + (total number of B-lymphocytes), CD5-, CD19 +, CD27 + (memory B-cells), CD19 + CD27- (naive B-cells), CD19 + CD27 + CD38 + IgD - (Class-Switched Memory B-Cells) CD19 + CD27 + CD38 + IgD + (Unswitched Memory B-Cells) will be analyzed on the general lymphocyte population. The distribution of markers CD3, CD4 and CD8 will be analyzed in the pool of T-lymphocytes. To analyze the differentiation status of T cells, cells are additionally stained with anti-CCR7, anti-CD45RO antibodies. Antibodies will be purchased from Invitrogen ™ unless otherwise noted.

Genetic analysis:

Isolation (extraction) of DNA will be performed from whole blood using commercial kits according to the manufacturer's instructions. To analyze a large number of genetic markers, it is planned to carry out genome-wide sequencing followed by analysis of genetic polymorphisms of candidate genes encoding coronavirus receptors and immunological factors.

Sequencing will be performed using high-throughput next generation sequencing platforms Illumina NovaSeq6000 (Illumina), method validation using traditional capillary sequencing - ABI 3730XL ™ DNA Analyzer (Life Technologies), real-time PCR.

*Bioinformatic data analysis.*Bioinformatics sequencing data will be analyzed. The software packages for bioinformatic analysis of sequencing data (GATK, bwa, bowtie, bowtie2, VarScan etc) will be used. The sequencing data will be compared with the publicly available data from the world's international databases of genomic research (https://www.covid19hg.org/, ExAC, HGMD (Human Gene Mutation Database), ESP, GeneBank, NCBI, ESP6500, 1000Genomes, SNPDb130, Ensembl, ClinVar, SNPedia, etc.). Differences in the type and frequency of genomic variation among the surveyed groups will be determined.

To classify the detected genetic variants, in silico models will be used (SIFT_score / pred, Polyphen2_HDIVscore / pred, Polyphen2_HVAR_score / pred, LRT_score / pred, MutationTaster_score / pred, MutationAssessor_score / pred, FATHMM_score / pred, Radial / MetaRVM_score pred). The classification of clinically significant genetic variants will be carried out according to the international ACMG / AMG criteria.

Statistical analysis:

Statistical analysis will be carried out using version R 3.6.2. Quantitative data, including clinical, biochemical, molecular genetic parameters, will be checked for normality using the Shapiro-Wilks test and recognized as parametric in distribution. Comparison of mean differences will be performed using one-way ANOVA, and subsequent pairwise comparison will be performed using Tukey's special test. Within-group mean differences will be performed using the paired sample t-test. The graphs will be executed using the ggplot2 R package. Statistical analysis will be performed for the multiplex analysis results using the R psych package and standard t-tests.

Inspection frequency:

Patients are followed up for 12 months from the date of illness. Disease detection corresponds to the baseline (day 0). At the time of diagnosis, materials are taken for clinical, functional and immunological diagnostics. After that, the sampling is carried out every month for the next year from the moment of the disease in accordance with the study protocol (Figure 2). The collection of materials for genetic analysis is carried out once.

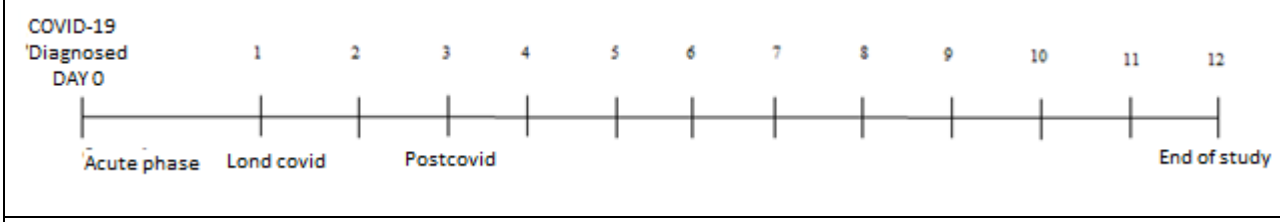

Figure 2. Frequency of visits and sample collection (in months)

Visits

Month / Analysis	0	1	2	3	4	5	6	7	8	9	10	11	12
Visit - doctor's consultation	1	1	1	1	1	1	1	1	1	1	1	1	1
Written agreement	1												
Blood tests: KLA, Neutrophil to lymphocyte ratio	1	1	1				1						1
Blood type	1												
ALT, AST, total and direct bilirubin, LDH, CRP, alpha-amylase, creatinine, urea, glucose,	1	1	1				1						1
Ferritin	1	1	1				1						1
Glycohemoglobin	1						1						1
25 - OH vitamin D	1						1						1
vitamin B12	1						1						

D-dimer, fibrinogen, INR, APTT	1	1	1				1						1
ICL IgM / IgG, RBD, S1, N	1	1	1	1	1	1	1	1	1	1	1	1	1
NT - pro BNP	1						1						1
Homocysteine	1						1						1
Troponin	1												
HR, NPV, BP, t C, Saturation	1	1	1	1	1	1	1	1	1	1	1	1	1
ECG	1	1					1						1
EchoCG + Strain	1						1						1
CT Abdominal	1			1									1
HMECG	1												
SMAD	1												
Abdominal ultrasound	1												
Ultrasound of the kidneys	1												
Doppler ultrasonography of veins and arteries	1			1									
Antibodies to myelin basic protein (MBP), neurospecific enolase (NSE)	1												
EEG, CT - perfusion, polysonography	1												
Genetic analysis	1												
Evaluation of New LongCovid Symptoms, PostCovid	1	1	1	1	1	1	1	1	1	1	1	1	1
6-minute walk test	1						1						1
Chadler's questionnaire	1	1	1	1	1	1	1	1	1	1	1	1	1
EQ questionnaire	1			1			1						1
Anxiety and Depression Scale	1	1	1	1			1						1
Summary Mental Status Evaluation (MMSE)	1						1						1

Immunofetnotyping of a subpopulation of T and B lymphocytes CD3, CD4, CD5, CD8, CD19, CD27, CD38, IgD, anti-CCR7, anti-CD45RO, NK - cells	1	1	1	1	1	1
Multiplex immunoassay sCD40L, EGF, Eotaxin / CCL11, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70) , IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB / BB, RANTES, TGF-α, TNF-α, TNF-β, VEGF, IgA, IgG1-G4, IgM.						

Research materials:

1) blood
2) deoxyribonucleic acid (DNA) samples

The study will be conducted in accordance with international standards GCP (Good Clinical Practice) in compliance with the principles of scientific ethics, as well as in accordance with the Order of the Minister of Health of the Republic of Kazakhstan dated December 14, 2020 No. 248 "On approval of the Rules for conducting biomedical experiments, preclinical (non-clinical) and clinical trials , as well as requirements for preclinical and clinical bases ".

Patient safety procedures will be strictly followed in accordance with the requirements of the Joint Commission International (JCI). Each patient will be introduced to the nature of the study. The doctor - researcher will explain in detail the essence, benefits and risks of this study. If the decision is positive, the patient will sign an informed consent for this study.

The main composition of the research group:

The qualification of the program group allows for extensive and versatile systematic research in the chosen direction. In addition, the program participants have sufficient work experience and professional qualifications, which allows obtaining reliable data using modern equipment. The project executors have repeatedly participated in competitions.

NJSC "National Research Center for Cardiac Surgery"

1. Tauekelova Ainur - head of the admission department of the National Research Center for Cardiac Surgery
2. Shestak Elena - cardiologist, National Research Center for Cardiac Surgery.
3. Dalbekova Marzhan Kairatovna - cardiologist, National Research Center for Cardiac Surgery.
4. Seitkasym Sholpan Kanatkyzy - cardiologist, National Research Center for Cardiac Surgery.
5. Litvinova Lia - Clinical Pharmacologist, National Research Center for Cardiac Surgery.
6. Ivanova-Razumova Tatyana - Cardiologist, National Research Center for Cardiac Surgery
7. Saylybayeva Aliya Ibaydullaevna, PhD, Head of Science Department, National Research Center for Cardiac Surgery
8. Daniyarova Gulnur - Scientific Secretary, National Research Center for Cardiac Surgery
9. Khamitov Sadyk - Chief Specialist of the Science Department, National Research Center for Cardiac Surgery
10. Vacancy - accountant of National Research Center for Cardiac Surgery
11. Vacancy - lawyer of the National Research Center for Cardiac Surgery

12. Ayapbergenova Roza - economist, National Research Center for Cardiac Surgery

13. Vacancy - resident doctor of National Research Center for Cardiac Surgery

14. Vacancy - resident doctor of NJSC National Research Center for Cardiac Surgery

School of Medicine, Nazarbayev University

15. Dimitri Poddige, Associate Professor of the Faculty of Medicine of Nazarbayev University, Director of the Clinical Academic Department of Pediatrics at the University Medical Center (UMC).

From 2007 to 2009, Dr. Dimitri Poddige worked as an Immunology Research Fellow at the Boston Children's Hospital at Harvard University in Boston, Massachusetts, USA. Since 2010, Dr. Dimitri Poddige has worked as a general practitioner in the pediatric departments of several hospitals in Lombardy (Italy), including the Fondazione IRCCS Policlinico San Matteo (Pavia), in collaboration with the University of Pavia, where he worked as a teacher / instructor in the Department of Pediatrics since 2015.

16. Kuanysh Dosybaeva, M.Sc. Molecular Medicine, Research Assistant, School of Medicine, Nazarbayev University.

17. Vacancy, Doctoral student, School of Medicine Nazarbayev University.

Joint Stock Company "National Center for Neurosurgery"

18. Jamantaeva Botagoz Daukimovna, JSC " NCN " Head of the Department of Neurosurgery and Neuropathology.

19. Sakhipova Aiym Garipullayevna, JSC "NCN", Chief specialist of the Republican Coordination Center for Stroke Problems.

20. Guldana Zhumabayeva, JSC "NCN", leading specialist of the Republican Coordination Center for Stroke Problems.

6. Research environment.

NJSC "National Research Center for Cardiac Surgery" has a sufficient material and technical base.

Participating organizations are equipped with modern equipment and apparatus for conducting clinical and laboratory, instrumental and specific methods according to the profile. The declared equipment for research is available and has passed the technical inspection with calibration. Also, there are standardized protocols for research methods.

The program will involve young scientists, doctoral students, undergraduates and residents.

The program is carried out on a permanent basis at the National Research Center for Cardiac Surgery, while molecular genetic methods and specific diagnostic methods will be carried out in profiling laboratories. As the temporary full-time team of each organization becomes busy, it is planned that employees will participate in scientific work on general tasks and sections, which will increase efficiency and reduce work time.

Laboratory of Genomic and Personalized Medicine and the Laboratory of Bioinformatics and Systems Biology of the Center for Life Sciences have the necessary equipment, material and technical base, equipment that is standard for world-class genomic laboratories, as well as necessary for high-throughput sequencing. For analysis of full genome sequencing data and storage of information, there are servers and computer stations. For storage of biological samples, there are freezers -80 C. The list of the main units of equipment and technology is given in the table, except for laboratory small-sized equipment, which is necessary for the project.

9. Expected results of the program.

Patients who have undergone a coronavirus infection and have received a number of complications that affect the quality and duration of life are one of the urgent problems in the world. The situation is aggravated by the fact that the issue is still not fully understood due to the novelty of the disease, which poses an even greater serious threat to humanity.

Nevertheless, understanding and studying the problem plays a decisive role not only in health care, but in general will affect the economic, social consequences in the world, as well as its safety.

The implementation of this project, for the first time in the Republic of Kazakhstan, will allow studying the main mechanisms of infection, its consequences not only for the body, but also for society as a whole.

The results of this project will contribute to improving public awareness of the causes, prevention of infection and complications of coronavirus infection. Physicians in clinical practice will be able to rely on the developed criteria for diagnosis and treatment. Medical staff will receive a more detailed picture of the most acute and important measures for the management and care of patients with coronavirus infection to prevent the frequency of complications and the development of post-ovarian syndrome, and will also be able to provide appropriate assistance to patients to adapt to a new lifestyle.

In addition, the results of the proposed study will influence the further development of research initiatives such as the development of preventive vaccinations, the development of new drugs and the study and implementation of new strategies in the treatment of coronavirus complications.

Along with this, the developed and implemented scale of infection outcomes will significantly improve the prognosis and survival of this category of patients and affect the epidemiological situation.

The target consumers of the results obtained will be patients who have undergone COVID-19, as well as the entire population of the Republic of Kazakhstan, which are currently the main value of the country.

At least 2 (two) articles and (or) reviews will be published in peer-reviewed scientific journals in the scientific direction of the program, included in 1 (first), 2 (second) or 3 (third) quartiles in the Web of Science database and (or) having percentile by CiteScore in the Scopus database not less than 50 (fifty). To popularize science, a separate website will be created, which will provide complete information on the progress of the program. For each scientific publication within the framework of the program, information will be published on the website and / or in social networks.

In general, the expected efficiency and effectiveness of the proposed project will make a significant contribution to the development of not only health care, but will also have a positive contribution to the country's economy as a whole. In addition, the effective distribution of budgetary will maximize the concentration of the expenditure side of the implementation of this project to achieve the designated goals and objectives.

This project is aimed at achieving the indicators of the State Program for Healthcare Development 2020-2025. an increase in average life expectancy up to 75 years and a decrease in the risk of premature mortality from 30 to 70 years from cardiovascular diseases.

1. Bibliography

1. Coronavirus Disease (COVID-19) - events as they happen [Internet]. Who.int. 2021 [cited 12 February 2021]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen
2. A pneumonia outbreak associated with a new coronavirus of probable bat origin. P. Zhou et al. Nature (2020), 10.1038/s41586-020-2012-7.
3. Coronavirus Update (Live): 106,685,660 Cases and 2,327,308 Deaths from COVID-19 Virus Pandemic - Worldometer [Internet]. Worldometers.info. 2021 [cited 8 February 2021]. Available from: https://www.worldometers.info/coronavirus/
4. SARS and MERS: recent insights into emerging coronaviruses E. de Wit et al. Nat. Rev. Microbiol., 14 (2016), pp. 523-534, 10.1038/nrmicro.2016.81

5. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. C. Huang, et al. Lancet (2020), 10.1016/S0140-6736(20)30183-5
6. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. Frontiers in Immunology. 2020;11.
7. Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clinical Rheumatology. 2020;39(7):2085-2094.
8. Vabret N, Britton G, Gruber C et al. Immunology of COVID-19: Current State of the Science. *Immunity*. 2020;52(6):910-941. doi:10.1016/j.immuni.2020.05.002
9. Novel immunodominant peptide presentation strategy: a featured HLA-A∗2402-restricted cytotoxic T-lymphocyte epitope stabilized by intrachain hydrogen bonds from severe acute respiratory syndrome coronavirus nucleocapsid protein J. Liu et al. J. Virol., 84 (2010), pp. 11849-11857, 10.1128/JVI.01464-10
10. Henderson L, Canna S, Schulert G, Volpi S, Lee P, Kernan K et al. On the Alert for Cytokine Storm: Immunopathology in COVID -19. Arthritis & Rheumatology. 2020;72(7):1059-1063.
11. Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing Xingzhi Xie, Zheng Zhong et al Jingjing Liu. Published 2020 Medicine Radiology
12. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology 2020:200642. doi:
13. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese. Center for Disease Control and Prevention. JAMA. 2020;2019:3–
14. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China C. Huang et al. Lancet (2020), 10.1016/S0140-6736(20)30183-5
15. Molecular immune pathogenesis and diagnosis of COVID-19. Xiaowei Li at al. Journal of Pharmaceutical Analysis Volume 10, Issue 2, April 2020, Pages 102-108.
16. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding R. Lu, … +2 … , et al. Lancet (2020), 10.1016/S0140-6736(20)30251-8
17. Halpin S, O'Connor R, Sivan M. Long COVID and chronic COVID syndromes. Journal of Medical Virology. 2020;93(3):1242-1243.
18. Mendelson M, Nel J, Blumberg L, Madhi S, Dryden M, Stevens W et al. Long-COVID: An evolving problem with an extensive impact. South African Medical Journal. 2020;111(1):10.
19. Overview | COVID-19 rapid guideline: managing the long-term effects of COVID-19 | Guidance | NICE [Internet]. Nice.org.uk. 2021 [cited 16 February 2021]. Available from: https://www.nice.org.uk/guidance/ng188
20. Vink M, Vink-Niese A. Could Cognitive Behavioural Therapy Be an Effective Treatment for Long COVID and Post COVID-19 Fatigue Syndrome? Lessons from the Qure Study for Q-Fever Fatigue Syndrome. Healthcare. 2020;8(4):552.

21. ICD-10 Version:2019 [Internet]. Icd.who.int. 2021 [cited 12 February 2021]. Available from: https://icd.who.int/browse10/2019/en#/U07