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EPIDEMIOLOGY. INFECTIONS. SOCIETY.
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POST-COVID SYNDROME. Thromboinflammation and its consequences

Essays And Research

Edited by
RAS Academician V.I. Starodubov,
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Central Research Institute
of Epidemiology
of Rospotrebnadzor

SCIENCE IN THE SERVICE OF YOUR HEALTH

Moscow 2026

Department of Medical Sciences of the Russian Academy of Sciences
Central Research Institute for Epidemiology of Rospotrebnadzor

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The monograph presents the research results of the authors, as well as data from the analysis of Russian and foreign literature on the issue of post-COVID syndrome. The publication details the epidemiology of the coronavirus infection (COVID-19), the characteristics of pulmonary, cardiovascular, neurological and certain other manifestations of post-COVID syndrome, and presents a classification of its clinical forms. There is particular focus on the main aspects of the pathogenesis of post-COVID syndrome, including the mechanisms of thrombophlebitis.

The publication examines potentially promising preventive strategies aimed at the early diagnosis of post-COVID syndrome and dispensary observation of patients during the convalescence period of COVID-19.

The monograph is intended for doctors of various specialties, researchers, healthcare organizers, and can also be recommended as additional educational material for students, interns and graduate students of medical universities.

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Preface

Throughout the history of humankind, infectious diseases have always been and remain the focus of attention. The issue of biological safety is extremely relevant for all countries of the world due to the expansion of the range of real and potential threats caused by the impact of biological agents that are dangerous to the health and welling of society and the environment. The world of microorganisms is becoming increasingly aggressive towards humans, as it was clearly demonstrated by the pandemic of the novel coronavirus infection (COVID-19), which has had a comprehensive impact on all aspects of people's lives, going far beyond the sphere of healthcare. In this regard, the leading role in ensuring biological sovereignty is played by the need to effectively counter biological challenges that could lead to the emergence and spread of epidemics, epizootics, epiphytotics, and mass poisoning, as well as the ability to prevent and promptly eliminate such biological risks.

The main biological threats in modern conditions include risks associated with the emergence of new infections caused by unknown pathogens; the overcoming of interspecies barriers by microorganisms in combination with changes in the genotype and phenotype of human and animal organisms caused by environmental influences; the spread of antimicrobial resistance, etc.

The COVID-19 pandemic has clearly demonstrated to the world that epidemic and epizootic outbreaks of new and re-emerging infectious diseases, most of which are characterized by sudden onset, high mortality, lack of specific diagnostic and treatment methods, and significant costs for anti-epidemic and anti-epizootic measures, pose a serious threat to national security.

While studying the novel coronavirus infection (COVID-19), it has become clear that it is not enough to describe the clinical picture of the acute phase of the infection. An important component of the infectious process is the convalescence period, which in the case of COVID-19 is characterized by the development of a symptom complex in more than half of patients, including damage to various organs and systems.

In foreign scientific literature, this symptom complex is terminologically referred to as long COVID, which lasts 4–12 weeks after SARS-CoV-2 infection, or post-COVID syndrome, in which complaints persist for more than 12 weeks. In Russian scientific circles, these definitions are combined into a single concept of the convalescence period of coronavirus infection as the time between the disappearance of the symptoms of the acute period of infection and the patient's full recovery.

The pathogenesis of post-COVID syndrome has not been fully studied. However, studies have shown that the pathogen triggers a cascade of pathogenic processes in the human body, affecting all organs and tissues and leading to the development of systemic multi-organ pathology of varying severity. The leading pathophysiological mechanisms of systemic pathological response during the convalescence period of coronavirus infection are thromboinflammation and endothelial dysfunction. These two processes are interrelated and characterized by mutual reinforcement of their damaging effects. Pathomorphologically, this is expressed by the formation of multiple microthrombi, the development of tissue hypoxia and a systemic inflammatory response, which are exacerbated by immune dysregulation with the production of pro-inflammatory mediators and the activation of autoreactive immunity.

The clinical manifestations of post-COVID syndrome are caused by systemic pathogenetic processes and are characterized by the development of pathologies in the cardiovascular, respiratory, nervous, and other systems of the human body. However, the lack of clear diagnostic criteria for post-COVID syndrome means that in practical healthcare, this problem is often masked by other nosologies, which complicates the implementation of diagnostic and preventive measures for this category of patients.

Approaches to the treatment of post-COVID syndrome have not yet been fully developed. However, research by Russian scientists demonstrates the effectiveness of correction of immune disorders, primarily immunosuppression, recorded during the convalescence period of coronavirus infection.

Early detection is key to preventing post-COVID syndrome, which necessitates a scientifically based system for monitoring patients during convalescence and identifying risk groups vulnerable to developing post-COVID syndrome.

Extensive scientific discussions at general meetings and sessions of the Presidium of the Russian Academy of Sciences have contributed to the formation of modern views on the problem of post-COVID syndrome in Russia. The possibility of implementing an interdisciplinary approach to solving this problem allows for a comprehensive approach to the issues of diagnosis, therapy, and prevention of post-COVID syndrome.

This collective scientific work is devoted to issues of pathogenesis, clinical manifestations and their classification, as well as approaches to the prevention and treatment of post-COVID syndrome.

Academician of the Russian Academy of Sciences
V.I. Starodubov

Chapter 1

COVID-19 Pandemic: Epidemiology and Unresolved Issues

V.G. Akimkin, A.A. Ploskireva

ABSTRACT

The theoretical and practical basic principles of epidemiology as a science were laid in Russia, and their development found practical application in the formation of an epidemiological surveillance system in our country, which demonstrated its effectiveness during the COVID-19 coronavirus pandemic. The "Theory of Self-Regulation of Parasitic Systems," developed in the 20th century by the Russian school of epidemiology by Academician of the USSR Academy of Medical Sciences V.D. Belyakov, clearly demonstrated its practical effectiveness during the pandemic. Long before the advent of modern molecular genetic methods, Academician of the USSR Academy of Medical Sciences V.D. Belyakov demonstrated the basic patterns of epidemic development, consisting of the mutual adaptation of the pathogen population and humanity as a whole. A study of the epidemic development patterns of a novel for the humanity coronavirus infection has revealed key trends in the pathogen's genetic variability and, consequently, dynamic changes in the manifestations of the epidemic process and the clinical picture of the disease in both the acute and convalescent periods. The obtained scientific data allowed not only to track ongoing changes in the pathogen's genetic structure but also to predict the characteristics of the infection process and the patterns of pandemic development.

EPIDEMIOLOGY OF COVID-19

The novel, highly contagious viral disease COVID-19, caused by coronavirus SARS-CoV-2, which emerged at the turn of 2019–2020, has had a catastrophic impact on the demography and economy of countries worldwide [1]. On March 11, 2020, the World Health Organization (WHO) declared a pandemic, which has had a devastat-

ing impact on public health and the global economy. By the end of May 2025, over 777 million confirmed cases of COVID-19 and more than 7 million related deaths had been registered worldwide, of which the Russian Federation accounted for over 24 million and 400,000 cases, respectively, making it the most serious global health crisis in modern times [2] (Figure 1).

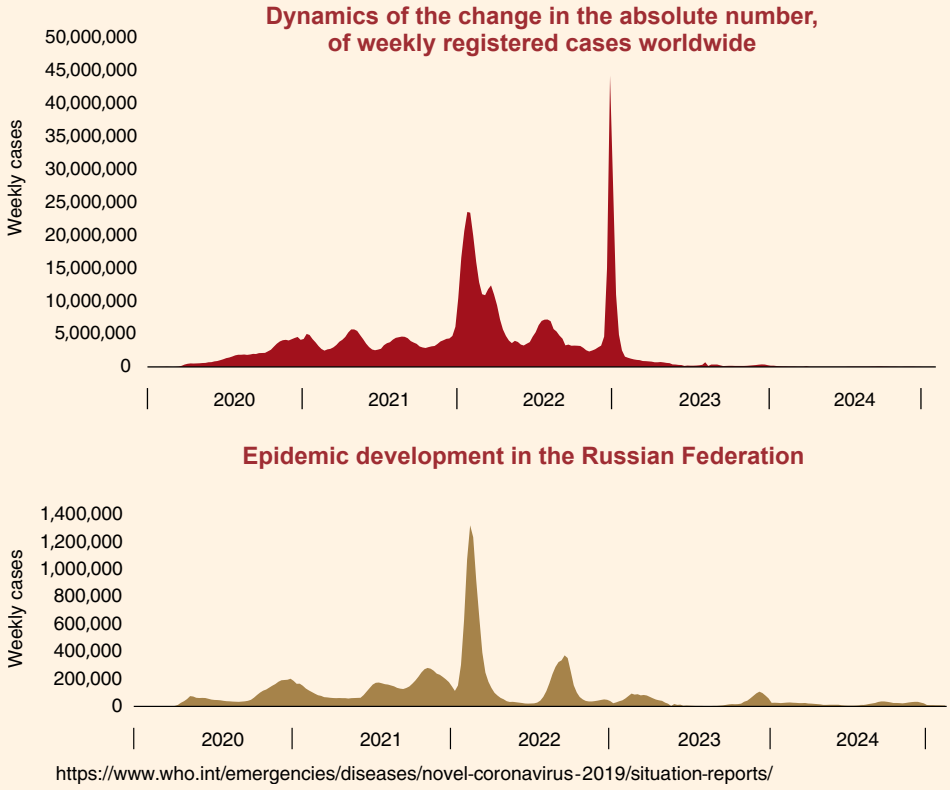


Figure 1. Dynamics of the novel coronavirus pandemic: current situation as of February 2025

According to the World Bank, global GDP fell by 3.4% in 2020, resulting in a loss of over $2 trillion [3, 4]. Globally, direct medical costs due to COVID-19 accounted for 2.7% of healthcare expenditure and 0.25% of GDP, indirect costs caused by COVID-19 accounted for 10.5% of global GDP, and total COVID-19 expenditure accounted for about 86% of healthcare expenditure and 9.13% of GDP [5]. The total economic damage caused by COVID-19 in the Russian Federation in the healthcare sector for 2020–2022 amounted to at least 3.6 trillion rubles, of which direct medical costs accounted for about 1.7 trillion rubles, and indirect losses to the economy — about 1.9 trillion rubles [6].

The causative agent of COVID-19 is the SARS-CoV-2 virus, which belongs to the *Coronaviridae* family (Figure 2). Representatives of two genera of this family (alphacoronaviruses and betacoronaviruses) are capable of causing diseases in humans and animals [7]. SARS-CoV-2 is an enveloped RNA virus, therefore, to diagnose the

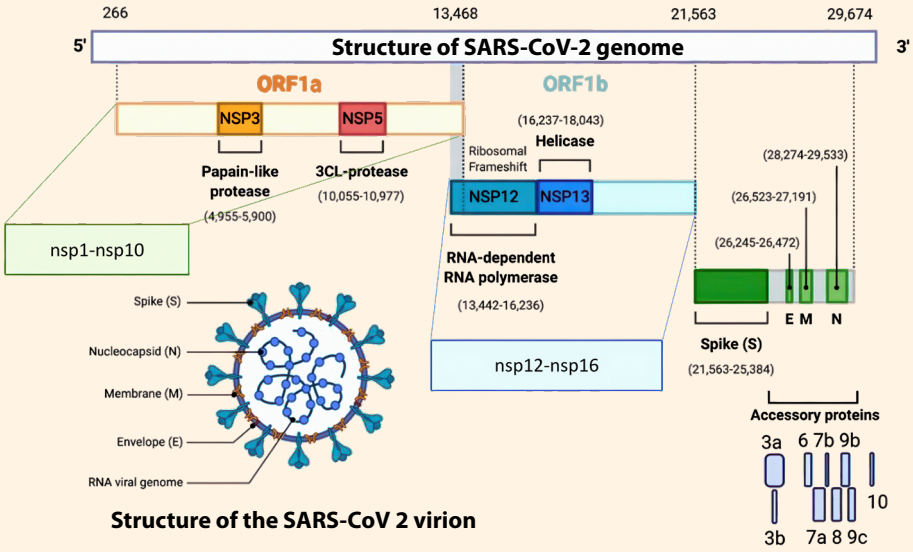


Figure 2. SARS-CoV-2 coronavirus, the causative agent of COVID-19

causative agent of the new coronavirus infection, both molecular methods for detecting viral RNA (PCR or LAMP), and methods for detecting viral antigens (N or S proteins) were used. Serological studies were also actively used to monitor the level of herd immunity, which contributed to the improvement of the organization of epidemiological surveillance for this infection.

Before the major outbreak of atypical pneumonia (SARS) — a severe, highly lethal infection caused by the betacoronavirus SARS-CoV — emerged in Asia in 2003–2004, coronaviruses were considered to cause only mild forms of acute respiratory viral infections in humans. Since 2012, cases of another severe disease, Middle East respiratory syndrome (MERS-CoV), caused by the novel betacoronavirus MERS-CoV, began to be reported in the Arabian Peninsula and adjacent countries. Finally, in 2019, SARS-CoV-2, the virus that caused the COVID-19 pandemic, spread through a population. All three of these betacoronaviruses (SARS-CoV, MERS-CoV, and SARS-CoV-2) originated in bats. Moreover, if in the case of the Middle East syndrome people were infected with MERS-CoV from camels, then the question of the intermediate host for the SARS-CoV and SARS-CoV-2 viruses remains controversial.

Although closely related strains have been found in different animals (for example, in civets or pangolins), recent studies indicate that SARS-like viruses are found in bat populations that do not require additional adaptation in an intermediate host, and the transition of these viruses into the human population could have occurred directly from bats [8, 9].

The biological factor driving the development of the epidemic process is associated with genetic variability and other multifocal characteristics of the pathogen (Figure 3). The etiologic agent of the new coronavirus infection SARS-CoV-2, adapting to its new hosts — humans, is subject to genetic evolution, which leads to mutations in the viral genome that can change the pathogenic potential of the virus (Table 1). Since the

Table 1.

Dynamics of the emergence of "significant" genetic variants of the SARS-CoV-2 virus

Genetic variant	Sublineages	Date of emergence	Unofficial names
—	Multiplicity	December 2019	"Wuhan"
"Alpha"	B.1.1.7	September 20, 2020	"British"
"Beta"	B.1.351	May 2020	"South African"
"Gamma"	B.1.1.248	January 2021	"Brazilian"
"Delta"	B.1.617.*+ AY*	October 2020	"Indian"
"Omicron"	B.1.1.529+ BA.1/2/3/4/5	November 8, 2021 – presently	BA.2 "Stealth" VA.2.75 "Centaur" VA.5.2 "Triton" VQ.1.1 "Cerberus" HVV "Griffon" HVV.1.5 "Kraken" EG.5 "Eris"

preservation of the pathogen as a biological species is impossible without evolutionary development, during the pandemic, the range of heterogeneity of the coronavirus population expanded due to the circulation of both low-virulent and virulent variants, followed by stabilizing selection and the establishment of an epidemic variant.

Numerous mutations that RNA viruses are susceptible to against the background of high reproductive activity have led to significant adaptive variability of the SARS-CoV-2 population, which is highly contagious but moderately virulent, which is due to the implementation of a survival strategy by the pathogen under the pressure of the growing immunity of the host population during the epidemic process [7]. Evidence of

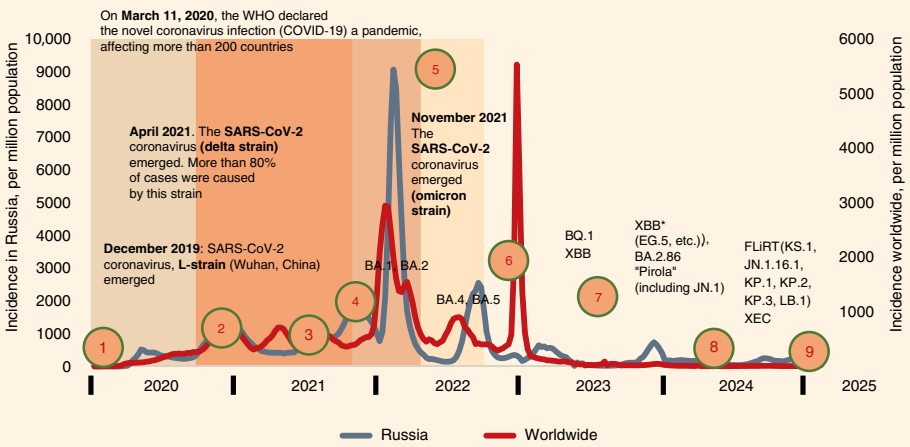
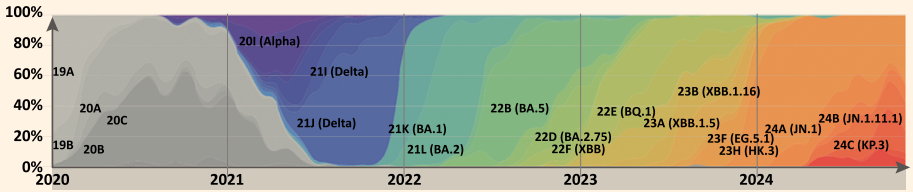


Figure 3. Dynamics of COVID-19 incidence globally and in the Russian Federation (per million population) and the evolution of the SARS-CoV-2 virus

this statement is the change in the indicators of contagiousness and mortality depending on the emergence of "significant" SARS-CoV-2 genetic variants in the territory of the Russian Federation for 2020–2025 (Figure 4).



Strain	Contagiousness index	Mortality
"Wuhan"	2–4	2.3%
"Delta"	5–8	1.8%
"Omicron" (BA.1, BA.2)	6–9	0.5%
"Omicron" (BA.4, BA.5)	7–10	0.4%

Figure 4. Changes in the biological and genetic properties of coronavirus genetic variants

The development of the pandemic was significantly influenced by the demographic characteristics of the regions (population size, national traditions, age structure, living conditions), population activity (migration, mass cultural and sport events), as well as the presence of somatic complications in patients with comorbid pathology [1].

The population groups most susceptible to COVID-19 in 2021–2023 were 30–49 years old (33.7%) and 50–64 years old (24.0%) [9, 10]. Significant risk factors for severe clinical course of the new coronavirus infection included immunosuppression, chronic cardiovascular diseases, obesity, diabetes, pregnancy, orphan diseases, etc. [11–13].

Retrospective epidemiological analysis of data at various stages of the epidemic from 2020 to 2022 showed an increase in the proportion of children aged 0–17 years from 10.4% in 2020 to 17.9% in 2022, and the dynamics of changes in the structure of forms of severity of coronavirus infection in 2020–2022 were characterized by a decrease in the severity of the infection — the proportion of severe forms decreased from 3.6% in 2020 to 0.5% in 2022 [9, 13] (Table 2, Figure 5).

The Global Genomic Surveillance Strategy 2022–2032 developed by WHO, based on previous experience and lessons learned from the COVID-19 pandemic, addresses the role of genomics in public health and aims to mobilize efforts in the field of genomic surveillance for all pathogens posing a pandemic or epidemic threat by strengthening all laboratories performing genomic sequencing and uniting them into a single global network [14]. The goal of the genomic surveillance strategy is to develop a unified concept for using genomics as a powerful complementary tool to address public health challenges in preparedness and response to a wide range of pandemics and epidemics. Genomic surveillance enables disease monitoring and pathogen control, the implementation of interventions and recommendations for the population, the development of protective measures such as vaccines, and the eradication of diseases.

Table 2.

Comparative characteristics (dynamics) of the manifestations of the COVID-19 epidemic process, taking into account the evolution of the pathogen

Manifestations of the epidemic process	"Wuhan" strain	"Delta" strain	"Omicron" strain
Incidence per 100,000 population	8.0–20.4	17.2–28.2	139.1
Proportion of severe forms of infection (%)	3.6	3.0	0.5
The proportion of coronavirus circulation among the relatively healthy population, (%)*	10–12	13–16	30–37
The proportion of children among those infected, (%)	10.7	10.3	17.9

Note: *According to data from the city of Moscow and the Moscow region (n=2,411,220).

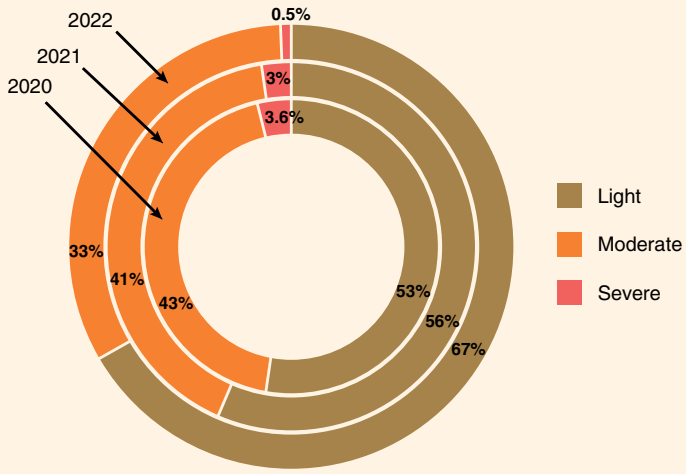


Figure 5. Dynamics of changes in the structure of severity forms of coronavirus infection in 2020–2022

The Russian Federation is one of the leading countries in the implementation of this scientific direction. In accordance with the Resolution of the Government of the Russian Federation No. 448, of March 23, 2021 "On approval of the Temporary procedure for providing data on the decoding of the genome of the causative agent of a new coronavirus infection (COVID-19)", to ensure a rapid assessment of the spread dynamics of known and new SARS-CoV-2 genetic variants circulating in the country, specialists from the Central Research Institute for Epidemiology of Rospotrebnadzor developed and implemented the Russian platform for aggregation of virus genome data (Virus Genome Aggregator of Russia — VGARus), which contains information on the nucleotide sequences of coronaviruses and their mutations [15].

The software integrated into the VGARus platform allows you to analyze sequencing results, determine the probable virus strain, generate standardized reports, and upload samples intended for further sequencing (Figure 6).

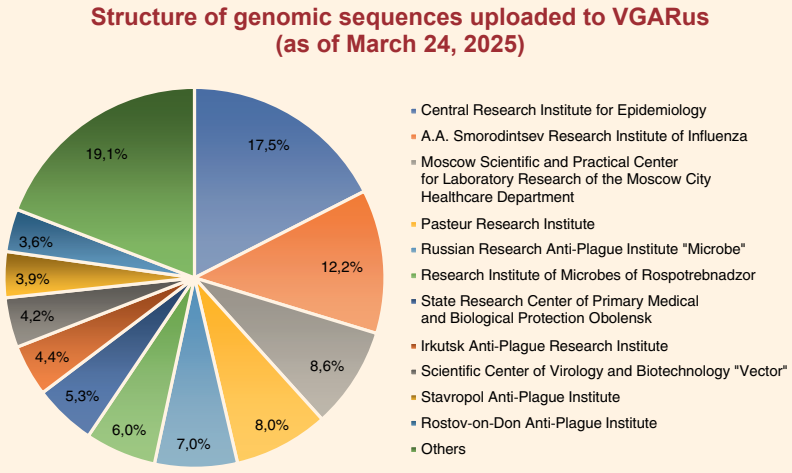


Figure 6. Monitoring changes in the properties of infectious disease pathogens is a key area of epidemiological surveillance

The VGARus database, which contains a large set of SARS-CoV-2 sequences, currently represents an invaluable scientific resource for tracking and deciphering the development of the epidemic situation for more than 80 pathogens, including SARS-CoV-2. As of May 2025, more than 420 thousand genomes (250 thousand — complete) of infectious disease pathogens have been uploaded to the VGARus database; including SARS-CoV-2 — more than 360 thousand (230 thousand — complete genomes) (Figure 7).

Considering the biological factor as the driving force of the epidemic process, SARS-CoV-2 has undergone genetic evolution and the emergence of new genetic variants of the virus. Since the beginning of the COVID-19 pandemic, a rich diversity of different SARS-CoV-2 lineages was observed for almost the entire 2020. However, they did not demonstrate significant evolutionary advantages. In December 2020, UK authorities informed the WHO of the discovery of a new SARS-CoV-2 lineage, named VOC-202012/01 [16]. The lineage had numerous mutations in its genome and was initially called the “British” lineage, but was later renamed Alpha. A combination of new mutations affected the virus’s ability to infect cells and evade the host immune response, thus allowing it to spread more effectively. This same variant was detected in Russia in late 2020 and persisted into early 2021, coinciding with an increase in new cases (Figure 8).

Following the identification of the Alpha variant, the Beta variant was detected, with prevalence in the Russian Federation significantly lower than in South Africa, where it initially emerged. In the summer of 2021, the Delta variant emerged, rapidly gaining dominance and correlating with a significant increase in morbidity and hospitalizations [17].

After a relatively favorable epidemiological situation, the Omicron variant was discovered in December 2021, leading to a significant increase in cases in Russia. However, an equally rapid decline in infection rates soon followed.

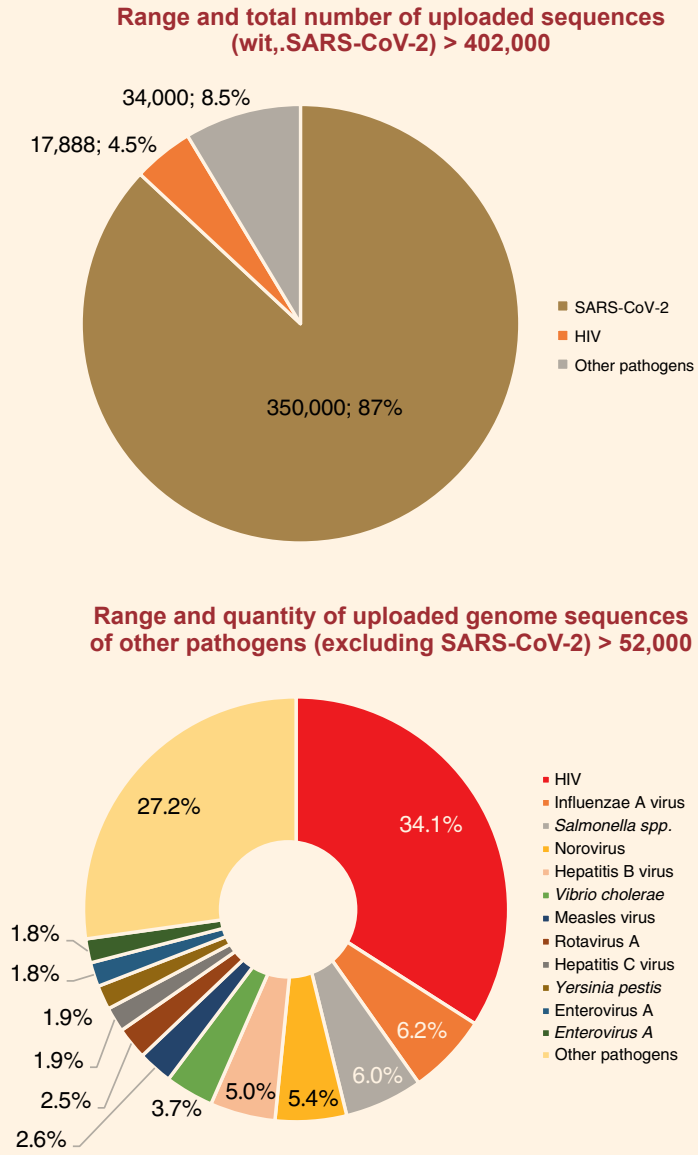


Figure 7. VGARus — uploaded genome sequences of various pathogens (2021–2025)

Despite the low number of COVID-19 cases detected in the spring of 2022, the emergence of Omicron subvariants BA.4 and especially BA.5 led to an increase in cases that continued until the end of October 2022.

In late 2022 and early 2023, highly contagious variants such as BQ.1 emerged. Such shifts in dominant lineages illustrate the ever-changing and complex nature of SARS-CoV-2 evolution. In November 2023, the BA.2.86 coronavirus variant, informally named Pirola, began to spread rapidly in several countries, including Russia. It was notable for a higher number of mutations in its genome compared to earlier

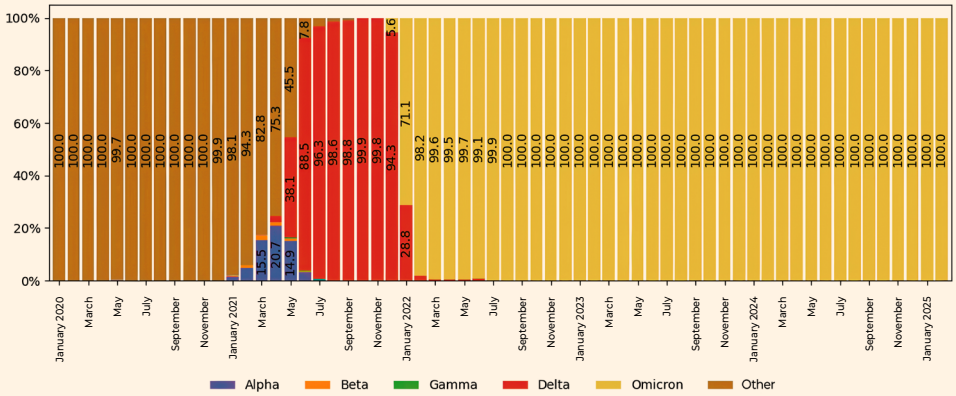


Figure 8. Dynamics of detection of SARS-CoV-2 variants in the Russian Federation (2020–2025)

lineages and by the end of 2023 had become the dominant lineage of the virus, and by early 2024, its JN.1 sublineage was almost completely dominant in most countries of the world. It was replaced by the recombinant XEC variant in late 2024, the share of which is gradually increasing worldwide (Figure 9).

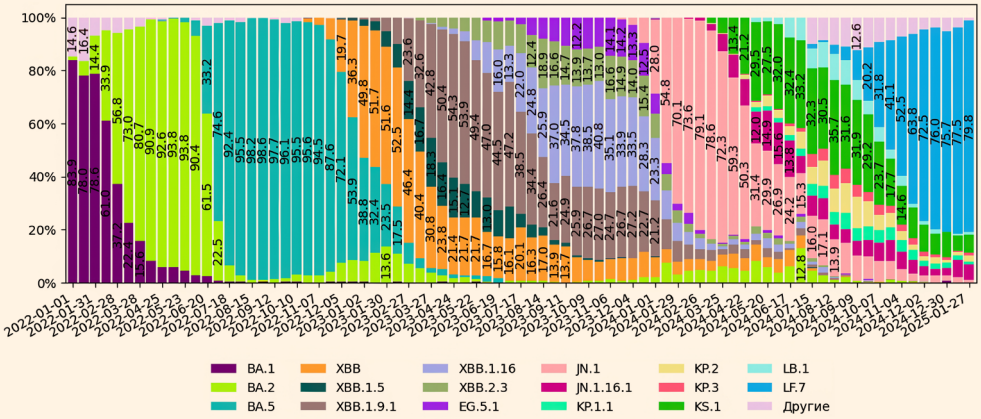


Figure 9. Representation of various sublineages of the Omicron variant in the Russian Federation (2022–2025)

Thus, the risk of the emergence and spread of a new pandemic in the future remains constant — this is "not a theoretical risk", but an "epidemiological reality", stated WHO Director-General Tedros Adhanom Ghebreyesus, speaking on April 7, 2025, at the opening of the 13th session of the Intergovernmental Negotiating Body preparing an agreement on the prevention of pandemics [18].

The following measures are required to prevent future pandemics:

- studying the genetic properties of known viruses;
- monitoring and searching for new pathogens that cause human infectious diseases;

- improving diagnostic methods, capabilities, and quality;
- introducing and implementing genomic epidemiological surveillance;
- developing modern vaccines;
- studying the characteristics and role of humoral and cellular immunity;
- studying the human genome and searching for genetic, epigenetic, and cellular mechanisms to combat infections.

Many unresolved scientific and practical questions remained after the end of the COVID-19 pandemic, in particular:

- high evolutionary potential of the SARS-CoV-2 population: variability of genetic variants and the number of sublineages, changes in the biological properties of the infectious agent, directly affecting the manifestations of the epidemic process;
- the significance and role of immunity in COVID-19 morbidity: the duration of post-infection and post-vaccination immunity, the importance of humoral and cellular immunity, the role of T cells, specificity and dependence on genetic changes (genetic variants);
- heterogeneity of the human population: ACE2 receptors, risk groups (age), risk factors (obesity, diabetes, chronic diseases, pregnancy, orphan diseases, etc.) influencing the development of COVID-19;
- the role and significance of the animal population and the ecology of the pathogen: natural reservoirs, interspecies transitions, reassortment of viruses influencing the evolution of pathogens of viral etiology.

In addition, as a separate section of the study of the problem of long-term persistence of complaints and symptoms of damage to various organs and systems after a coronavirus infection, it is necessary to note the development of a complex of conditions called post-COVID syndrome.

CONCLUSION

The novel coronavirus pandemic has demonstrated the global healthcare system's unpreparedness to fully respond to threats of this magnitude.

Currently, WHO requires focusing efforts on preparing for a hypothetical pandemic, improving the efficiency and reliability of the epidemiological surveillance system and developing preventive measures to counter the potential threat.

The evolution of the pandemic over time has demonstrated the importance of the biological factor as the main driving force behind the development of the epidemic process, which is associated with genetic variability and other polydeterminate characteristics of the pathogen, which is consistent with the theories of the development of the epidemic process of leading Russian epidemiologists.

The analysis of the patterns of the novel coronavirus epidemic development revealed key trends in the evolution of the pathogen's genetic variability and its impact on the manifestations of the epidemic process. The resulting scientific data allowed us to improve epidemiological surveillance, elevating it to a fundamentally new molecular genetic level, which has become a new, modern tool for epidemiological monitoring and forecasting.

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Chapter 2

Post-COVID Syndrome. Basic Concepts and Classification

V.G. Akimkin, A.A. Ploskireva

ABSTRACT

Following a coronavirus infection, a significant proportion of patients seek medical attention due to long-term health problems. The medical community most often refers to this symptom complex as "post-COVID syndrome," encompassing a range of clinical manifestations that occur in patients following a coronavirus infection. The term "Long COVID," used in scientific literature, describes a period of four weeks or more following infection. However, to date, there is no definitive, generally accepted concept describing the complex of clinical manifestations following infection. The Russian school, led by Academician of the Russian Academy of Sciences V.G. Akimkin, proposes a more universal approach, viewing this period as the recovery period of coronavirus infection. This approach allows for a classification of the main clinical manifestations, taking into account the pathogenesis of the acute period of infection and developing approaches to the diagnosis and prevention of this condition. The classification of clinical manifestations of post-COVID syndrome, developed at the Central Research Institute for Epidemiology of Rospotrebnadzor, is based on knowledge of the pathogenesis of acute and recovery periods of coronavirus infection and allows practitioners of various specialties to more accurately differentiate the symptoms observed in patients following infection.

POST-COVID SYNDROME. BASIC DEFINITIONS

Coronavirus infection (COVID-19) remains relevant to this day, not only due to the lack of etiotropic therapy, but also because a number of patients continue to experience symptoms during the period of convalescence, leading to reduced working capacity and a deterioration in their quality of life.

The problem of prolonged complaints and symptoms affecting various organs and systems after coronavirus infection has previously been observed in patients after severe acute respiratory syndrome caused by the SARS-CoV-1 coronavirus, which spread in 2002–2003. Studies conducted at that time showed that for a long time after atypical pneumonia, patients continued to complain of fatigue, sleep disturbances, myalgia, and muscle weakness. These patients also reported symptoms of mild to moderate depression [1]. The symptom complex described as chronic fatigue remained clinically significant in these patients for 4 years after recovery and became a cause of disability [2].

In general, post-COVID syndrome can be defined as a consequence of COVID-19 infection characterized by the prolonged presence of symptoms in patients after the infection (asthenia, shortness of breath, loss of smell and taste, pain syndrome, sleep disorders, etc.) that persist after the end of the acute period of infection and cannot be explained by an alternative diagnosis [3–5].

The set of symptoms observed during the recovery period from coronavirus infection is terminologically differentiated in foreign scientific literature, depending on the duration of the patient's health problems, into Long COVID, which lasts 4–12 weeks after SARS-CoV-2 infection, and post-COVID syndrome, in which complaints persist for more than 12 weeks [6]. The Russian scientific school uses these definitions to combine the concept of the convalescence period from coronavirus infection as the time between the disappearance of the symptoms of the acute period of infection and the onset of complete recovery. It includes:

- early convalescence period (gradual disappearance of acute infection symptoms);
- late convalescence period (clinical recovery, gradual restoration of morphological changes, formation of post-infectious immunity). The duration of the convalescence period is determined by the severity of the infection, the patient's condition, therapeutic tactics, and other clinical aspects [7].

The International Classification of Diseases, 10th Revision, provides for a separate code for post-COVID syndrome: U09.9 (unspecified condition following COVID-19). This code is not recommended for use in cases where the SARS-CoV-2 virus is still detectable in the human body. It is assumed that the symptoms are observed in the patient after recovery from the pathogen [8].

In the International Classification of Diseases, 11th Revision (this classification has not been adopted in Russia), the code "RA02. Condition following COVID-19" is recommended for diagnosing post-COVID syndrome. This implies that the patient has a condition following a COVID-19 coronavirus infection, which "occurs in individuals with a probable or confirmed history of SARS-CoV-2 infection, usually 3 months after the onset of COVID-19, with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. Common symptoms include fatigue, shortness of breath, cognitive dysfunction, and others, and typically affect daily functioning. Symptoms may appear for the first time after initial recovery from an acute episode of COVID-19 or persist after the initial illness. Symptoms may also change or recur over time" [9].

Post-COVID syndrome is not a unique condition specific to coronavirus infection. The symptoms that develop after the main symptoms of infectious diseases have been

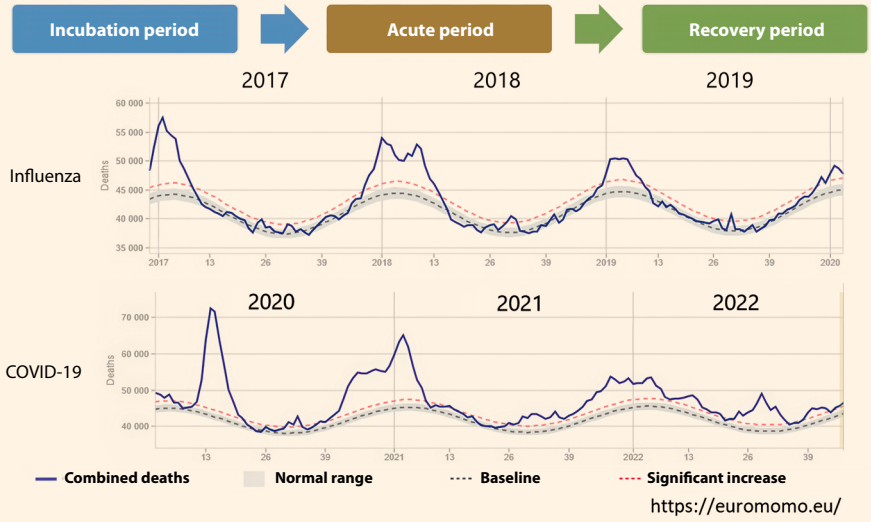

Figure 1. Excess mortality as a phenomenon of the post-COVID period

relieved are characteristic of any infection and are part of the concept of convalescence. Pathophysiologically, this period of the infectious process is characterized by the gradual restoration of impaired body functions, the relief of inflammatory symptoms, and the formation of post-infectious immunity. As a rule, the main complaint of patients during convalescence is weakness and rapid fatigue. However, in a number of patients, usually immunocompromised, this period may be accompanied by the development of complications, which increases the likelihood of death within 30 days after discharge from the hospital [10]. At the population level, this is reflected in a seasonal increase in excess mortality during periods of increased incidence of acute respiratory infections and influenza [11] (Figure 1).

PREVALENCE OF POST-COVID SYNDROME

According to current estimates, the number of people living with post-COVID syndrome worldwide has exceeded 65 million [12]. However, the actual figures are higher, as without clear diagnostic criteria, this problem is often masked by conditions of other nosology.

According to various authors, the proportion of patients who experience post-COVID syndrome after recovering from coronavirus infection varies widely, from 10% to 82.1% [4, 13]. A study conducted at the Central Research Institute for Epidemiology of Rospotrebnadzor showed that 66.8% of patients who had undergone coronavirus infection experienced some manifestations of post-COVID syndrome during the convalescence period [14]. At the same time, the frequency of post-COVID syndrome development was statistically indistinguishable both in the outcome of the primary infection and after repeated infection (67.6% and 66.1%, respectively). It was also found that the frequency of post-COVID syndrome development does not depend on the genetic variant of the pathogen.

Post-COVID syndrome can develop in patients of any age and with any severity of infection. According to foreign researchers, most patients diagnosed with post-COVID syndrome were between the ages of 36 and 50 [15]. Similar data were obtained by the Federal Research Center for Epidemiology of the Federal Service for Supervision of Consumer Rights Protection and Human Wellbeing: patients with post-COVID syndrome aged 31 to 45 accounted for 36.5% of all registered cases, and those aged 46 to 69 accounted for 37.4% [14] (Figure 2).

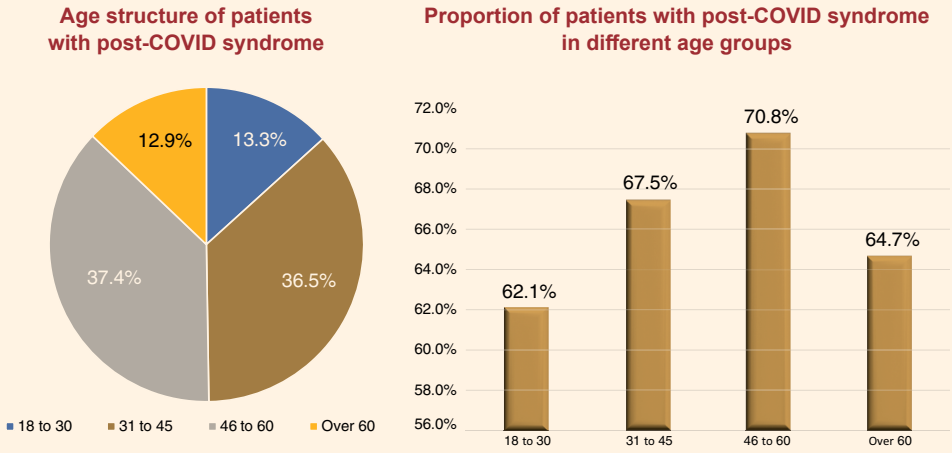

Figure 2. Age characteristics of patients with post-COVID syndrome [14]

Studies have also shown that post-COVID syndrome was observed in patients who had a mild form of coronavirus infection that did not require hospitalization [15–17]. The latter fact underscores the importance of active diagnostic efforts aimed at identifying post-COVID syndrome regardless of the severity of COVID-19.

PATHOGENESIS OF POST-COVID SYNDROME DEVELOPMENT

Currently, there is no single concept explaining the pathogenesis of post-COVID changes associated to coronavirus infection. It is believed that the SARS-CoV-2 virus triggers a cascade of pathogenic processes affecting all organs and tissues and leading to the development of systemic multiple organ pathology of varying severity, which can persist for a long time and clinically manifest itself in the development of post-COVID syndrome (Figure 3).

Pathomorphological changes characteristic of post-COVID syndrome were first described in a joint study conducted by the Central Research Institute for Epidemiology of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing and the S.P. Botkin Multidisciplinary Scientific and Clinical Center in Moscow. This study showed that during the COVID-19 convalescence period, the most pronounced pathomorphological changes were observed in the lung tissue and were characterized by structural disorganization of the lung parenchyma with changes in normal histoarchitecture due to fibrosis (Table 1).

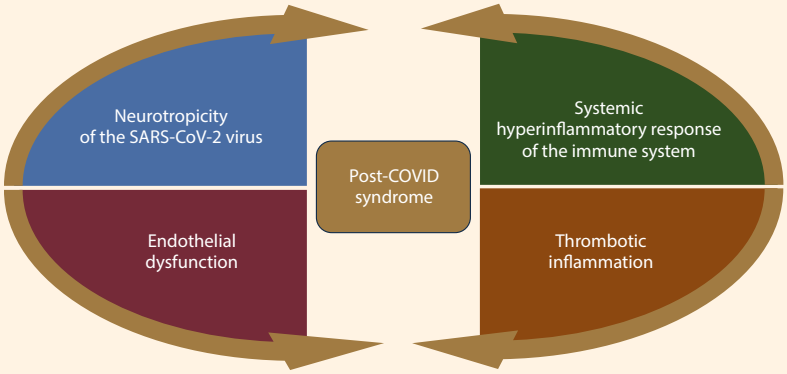

Figure 3. Diagram of the pathogenesis of post-COVID syndrome

Table 1.

Pathomorphological changes during the convalescence period of coronavirus infection

Criterion	Acute cerebrovascular accident with COVID-19 convalescent (%)	Acute cerebrovascular accident without COVID-19 (%)	*p*
Type 2 myocardial infarction	86.7	60.0	>0.005
Cardiac hypertrophy	73.3	40.0	>0.05
Diffuse pneumofibrosis	0	50	<0.05
Arteriolar wall hyalinosis	6.7	10	>0.05
Vascular congestion	40.0	20	>0.05
Ischemic cerebral infarction	33.0	10	>0.05
Intracerebral hemorrhage	15.7	0	>0.05
Infectious endocarditis	6.7	10.0	>0.05
Hematoxylin balls in brain tissue	40.0	20.0	>0.05
Hyaline globule in alveolar lumen	33.0	10.0	>0.05
Early signs of atherosclerosis	15.7	0	>0.05

In addition to lung tissue, morphological changes were detected in the brain, kidneys, liver, and heart, characterized by systemic changes in the blood vessels of the microcirculatory bed. Such commonality of pathomorphological changes in various organs and tissues during the convalescence period of coronavirus infection is the main cause of the polymorphism of clinical manifestations of post-COVID syndrome.

CLINICAL MANIFESTATIONS OF POST-COVID SYNDROME

The duration of recovery from COVID-19 coronavirus infection can range from 1 month to several years.

The main clinical manifestations can affect various body systems, which results in polymorphic clinical symptoms. Some authors describe up to 200 symptoms observed in patients with post-COVID syndrome, affecting virtually all organ systems [12].

Data obtained from the Central Research Institute for Epidemiology of Rospotrebnadzor show that the most frequently reported symptoms during the convalescence period of COVID-19 were asthenic syndrome (weakness, increased fatigue, etc.), cognitive impairment ("brain fog," inability to remember certain words, etc.), and memory impairment (Figure 4). The presence of symptoms such as:

- damage to the pulmonary system;
- damage to the gastrointestinal tract (development of irritable bowel syndrome, colitis, gastritis);
- pathology of the nervous system (sleep disorders, myalgic encephalomyelitis, chronic fatigue syndrome);
- smell disorders, etc.

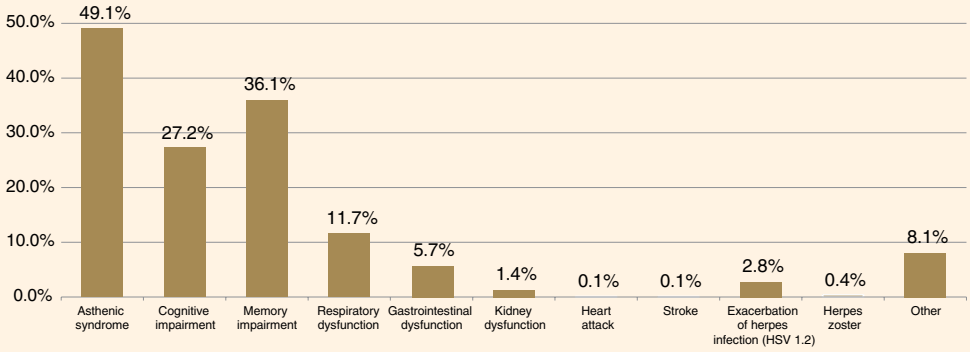

Figure 4. Structure of post-COVID symptoms during the COVID-19 convalescence period [14]

Cognitive impairment (cognitive dysfunction) during recovery from coronavirus infection is the most socially significant problem. Cognitive dysfunction includes mental health disorders that primarily affect learning, memory, perception, and problem solving, anxiety disorders, mood disorders, and "brain fog," the inability to remember certain words or concentrate, which ultimately leads to disruption of one's usual lifestyle and loss of work.

It was also found in a study by the Central Research Institute for Epidemiology of Rospotrebnadzor that during the convalescence period of coronavirus infection, 4% of patients experienced an exacerbation of herpes infections (Herpes simplex virus types 1 and 2, Herpes zoster), which, given the characteristics of the immunopathogenesis of the convalescence period, can be considered as manifestations of post-COVID syndrome.

The structure of post-COVID syndrome symptoms was stable throughout the pandemic and did not depend on the pathogen's gene variant.

CLINICAL CLASSIFICATION OF POST-COVID SYNDROME

Specialists from the Central Research Institute for Epidemiology of Rospotrebnadzor proposed a classification of clinical manifestations of post-COVID syndrome [14].

It includes four main blocks of clinical manifestations of the convalescence period of coronavirus infection:

1. Virus-associated manifestations:
- lung damage (consolidation foci, fibrosis);
- cognitive impairment, sleep disorders;
- secondary immunodeficiency;
- acute cerebrovascular disorders, acute myocardial infarction, and other vascular catastrophes;
- autoinflammation (arthritis, hair loss);
- gastrointestinal tract damage (diarrhea, constipation);
- exacerbation of herpes infections;
- smell disorders;
- recurrent acute respiratory infections.

2. Iatrogenic:
- antibiotic-associated syndrome;
- toxic hepatitis, nephritis, etc.

3. Genetically determined:
- manifestation of autoimmune diseases (rheumatoid arthritis, thyroiditis, diabetes, etc.);
- manifestation of cardiovascular pathology.

4. Exacerbation of chronic somatic pathology (cardiovascular diseases, oncological diseases, etc.).

Virus-associated manifestations of post-COVID syndrome during the convalescence period of coronavirus infection are associated with the systemic effect of the pathogen on the human body during the acute phase of infection, which manifests itself in damage to lung tissue, the central nervous system, vascular endothelium, and other systemic damage. Regeneration and restoration of functions impaired by the virus are observed during the convalescence period of coronavirus infection. Accordingly, the speed and completeness of these processes will determine the duration of post-COVID syndrome symptoms.

Iatrogenic causes may underlie the genesis of some symptoms during the convalescence period of coronavirus infection. Given the irrational use of antibacterial drugs, especially in the first year of the pandemic, the leading iatrogenic manifestation of post-COVID syndrome is antibiotic-associated syndrome, which is primarily characterized by the development of diarrheal syndrome [18].

Any infection can lead to the manifestation of a genetic predisposition to various diseases (mycoplasma, rhinosyncytial and metapneumovirus infections are associated

with the manifestation of bronchial asthma, enterovirus infection with diabetes mellitus, shigellosis with juvenile arthritis, etc.) [19]. Autoimmune diseases are most often recorded after an infectious disease. The Central Research Institute for Epidemiology of Rospotrebnadzor found that the presence of a genetic predisposition to arterial hypertension (the risk factor is the presence of the rs1937506-A and rs662-G alleles in the genotype) and increased thrombus formation (the presence of the GG genotype of the rs1937506 locus) may be a risk factor for the development of acute vascular catastrophes [20].

At the end of a coronavirus infection, as with other infectious diseases, an exacerbation of chronic somatic pathology may be observed, associated with a weakening of the body's defenses against the background of the infectious process [21]. To prevent the progression of comorbid conditions in patients in risk groups, active dispensary observation is necessary during the convalescence period of coronavirus infection.

The proposed classification is based on the pathogenesis of the development of clinical manifestations of post-COVID syndrome and may be useful in practical application by physicians of various specialties.

Thus, post-COVID syndrome is a symptom complex characteristic of the convalescence period of coronavirus infection, including polymorphism of clinical manifestations caused by damage to various organs and systems. It can be observed in more than half of patients during the recovery period from coronavirus infection, including patients who had a mild form of the disease.

The leading groups of clinical manifestations of post-COVID syndrome are virus-associated and iatrogenic manifestations, the manifestation of diseases with a genetic predisposition, and the exacerbation of chronic somatic pathology.

There is currently no treatment for post-COVID syndrome; symptomatic therapy is indicated. The most important aspect of preventing complications during the recovery period from coronavirus infection is the active identification of risk groups, primarily patients with chronic somatic pathologies, and the provision of dispensary observation for patients who have had coronavirus infection.

CONCLUSION

Post-COVID syndrome is a symptom complex characteristic of the convalescence period of coronavirus infection, including polymorphic clinical manifestations caused by lesions of various organs and systems. It can be observed in more than half of patients during the recovery period from coronavirus infection, including patients who had a mild form of the disease.

The proposed classification of clinical forms of post-COVID syndrome allows for the differentiation of manifestations observed in patients according to pathogenetic groups: virus-associated, iatrogenic manifestations, manifestation of diseases with genetic predisposition, and exacerbation of chronic somatic pathology. This approach makes it possible to identify risk factors and groups for an unfavorable course of convalescence from coronavirus infection.

There is currently no treatment for post-COVID syndrome; symptomatic therapy is indicated. The most important aspect of preventing complications during the recovery

period from coronavirus infection is the active identification of risk groups, primarily patients with chronic somatic pathologies, and the provision of dispensary observation for patients who have had coronavirus infection.

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Chapter 3

Long COVID, Hypercoagulability, Endothelial Dysfunction, and Future Directions

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ABSTRACT

Long COVID is a major global public health concern with significant social and economic consequences affecting millions of people worldwide. This chapter explores the multifaceted nature of Long COVID, with a particular focus on key pathophysiological mechanisms: persistent hypercoagulation, endothelial dysfunction and thromboinflammation. SARS-CoV-2 infection has been shown to initiate a cascade of immune and endothelial activation that can persist long after the acute phase of the disease. The increased risk of both venous and arterial thrombotic complications in the post-acute period, as well as gender differences in susceptibility to Long COVID, are emphasized. The chapter discusses diagnostic challenges due to the lack of standardized criteria and validated biomarkers, and analyzes recent research findings indicating persistent changes in the hemostatic system.

INTRODUCTION

Long COVID has become a significant global health challenge with wide-ranging socioeconomic impacts representing a complex multisystem disorder that persists well beyond the acute phase of SARS-CoV-2 infection. This review examines long COVID's multifaceted nature through three interconnected perspectives: (1) its socioeconomic burden, (2) underlying pathophysiological mechanisms, and (3) clinical management challenges, with particular focus on endothelial dysfunction and hypercoagulability as central drivers of its pathophysiology and clinical manifestations.

LONG COVID: SOCIO-ECONOMIC BURDEN

The population-level impact of long COVID syndrome is undeniable, with surveillance data indicating that it affects millions worldwide (Figure 1) and presents as a multisystem disorder involving neurological, psychiatric, cardiovascular, and pulmonary complications. Approximately 7% of adults and over 1% of children — equivalent to around 15 to 20 million Americans and more than 60 million people globally — have experienced long COVID. The condition significantly impairs daily activities, physical functioning, and both professional and personal life. Since July 2021, long COVID has been officially recognized as a disability in the United States. In a study of 15,308 individuals aged 18–69 years, 12% were found to be unemployed. Currently, an estimated 16 million working-age Americans (18 to 65 years old) are living with long COVID, with 2 to 4 million unable to work because of it. The annual wage loss associated with long COVID is estimated at $170 billion and could rise to $230 billion. Long COVID has three principal societal impacts: reduced quality of life, loss of income, and increased healthcare needs. Approximately 70% of affected patients report reduced work hours or job loss, contributing to a net income loss of nearly $1 trillion. In addition, annual medical expenditures in the U.S. for direct and indirect healthcare services related to long COVID are estimated at $528 billion. Altogether, the combined economic burden — including lost quality of life, lost income, and medical costs — reaches an estimated $3.7 trillion in U.S. (reviewed in [1, 2]).

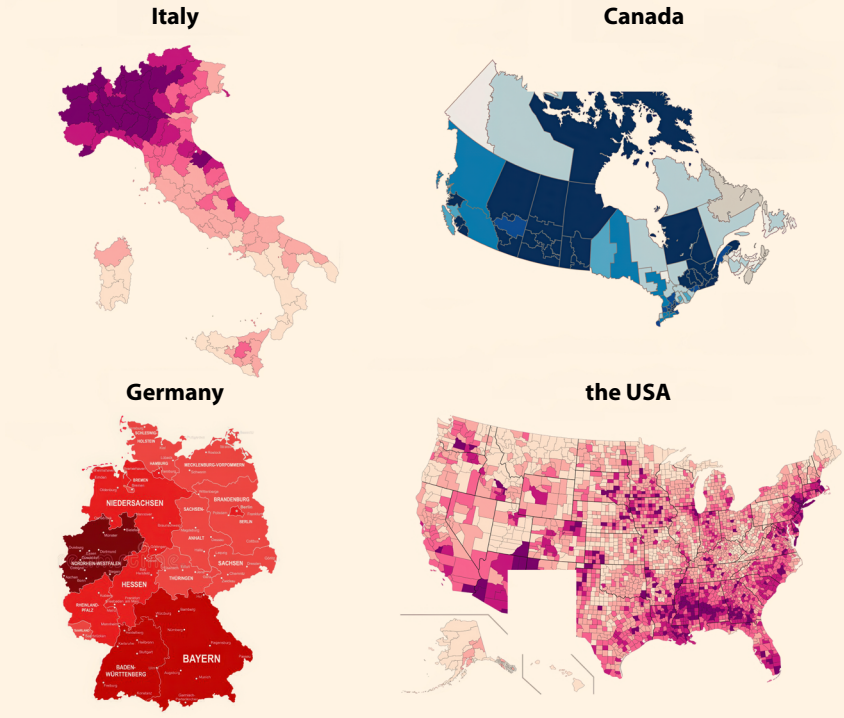

Figure 1. Number of confirmed COVID-19 cases as of October 2020 in different countries (according to OECD, 2020). Darker areas indicate higher numbers of cases

LONG COVID: DEFINITIONS AND CHALLENGES

In addition to the term "long COVID" other names frequently used in the scientific literature include "post-COVID conditions" (PCCs), "chronic COVID-19," and "post-acute sequelae of COVID-19" (PASC).

Much of the definition challenge is in how the clinical manifestations may be. Thus, in the large retrospective cohort study by Subramanian et al. (2022), published in *Nature Medicine*, the authors investigated the heterogeneity of long COVID symptoms among non-hospitalized adults using data from over 486,000 individuals in the UK's primary care electronic health records. By applying a matched cohort design, the study identified 62 symptoms significantly associated with a history of SARS-CoV-2 infection persisting 12 weeks post-diagnosis. These symptoms were classified into distinct clusters, revealing a high degree of heterogeneity in long COVID presentation; including respiratory, neurological, gastrointestinal, and musculoskeletal manifestations. The most frequently reported symptoms included anosmia, hair loss, sneezing, ejaculation difficulty, and reduced libido. Symptom profiles varied substantially by demographic and clinical factors, with women and younger individuals more likely to report fatigue and neurological symptoms, while older adults more often reported respiratory and cardiovascular symptoms, highlighting the complexity and individualized nature of post-acute sequelae [3].

To harmonize this problem, several definitions have been proposed by international health authorities. According to the World Health Organization (WHO), Long COVID is defined as a post-COVID condition occurring in individuals with a history of probable or confirmed SARS-CoV-2 infection, typically within three months of symptom onset, with symptoms lasting at least two months and not explained by another diagnosis.

The Centers for Disease Control and Prevention (CDC) define long COVID as a chronic condition occurring after SARS-CoV-2 infection and persisting for at least three months. The U.S. National Academies of Sciences (NAS) emphasize that long COVID is an infection-associated chronic condition that begins after SARS-CoV-2 infection and continues for at least three months, presenting as continuous, relapsing and remitting, or progressive disease involving one or more organ systems.

According to the Russian Ministry of Health long COVID is defined as a complex of symptoms persisting for more than 12 weeks after the acute phase of SARS-CoV-2 infection, not explained by another diagnosis, including persistent fatigue, dyspnea, chest pain, cognitive impairment ("brain fog"), joint pain, and autonomic disturbances.

In France, the Haute Autorité de Santé (HAS) defines long COVID using three criteria:

1. An initial symptomatic COVID-19 episode confirmed by PCR, antigen test, serology (for unvaccinated individuals), or typical clinical presentation (sudden anosmia/ageusia, CT-proven pneumonia), or probable COVID-19 with ≥3 suggestive symptoms in an epidemic context.
2. Persistence of symptoms beyond four weeks (either unresolved initial symptoms or new ones).
3. Absence of an alternative medical explanation.

This definitional heterogeneity impedes standardization of diagnostic criteria, limits comparability across studies, and delays the development of effective treatment strategies. Despite differing criteria, all major definitions of long COVID converge on two points:

- SARS-CoV-2 infection, not the severity of acute illness, is the trigger for long COVID-19;
- the time frame is defined as three months from symptom onset, except in the French definition (one month).

However, our current understanding faces three major limitations that complicate diagnosis, prognosis evaluation, and treatment:

1. Lack of clear, standardized diagnostic criteria.
2. Absence of definitive clinical, biological, or imaging profiles.
3. No validated biomarker panels to aid in diagnosis, risk assessment, or targeted therapy.

Despite its wide-ranging impact, our understanding of the underlying molecular mechanisms and pathways remains limited. Identifying circulating and tissue biomarkers is essential to improve risk stratification, guide prognosis, and facilitate the development and application of targeted therapies.

In the light of these challenges, and to respond to the need for a rationalized strategy in diagnosis and treatment of long COVID we propose to re-evaluate some key figures of the mechanisms leading to COVID-19 and worsening symptoms.

LONG COVID: THE CONJUNCTURE OF HYPERCOAGULABLE STATE, ENDOTHELIOPATHY AND THROMBOINFLAMMATION

SARS-CoV-2 initiates a cascade of immune and endothelial activation, leading to a hypercoagulable state and thromboinflammation. In some patients, SARS-CoV-2 inflection triggers an intensive inflammatory reaction (cytokine storm) that further amplifies hypercoagulable state and endotheliopathy. Thromboinflammation in patients with moderate or severe COVID-19, triggers pulmonary intravascular coagulation. Immuno-thrombosis and vascular occlusion in the microcirculation of the lungs as has been documented by autopsies studies. This immune-thrombotic axis is central to disease severity and sequelae. SARS-CoV-2 disrupts host immune and vascular homeostasis through the targeted cleavage of NF-κB Essential Modulator (NEMO) by its main protease, 3CLpro. NEMO is a critical scaffold protein required for the canonical activation of the NF-κB pathway [4], which regulates inflammatory responses and the expression of endothelial-protective and anticoagulant mediators. Structural studies have demonstrated that 3CLpro binds and cleaves NEMO at Gln231, effectively silencing NF-κB signaling and dampening antiviral defense mechanisms. This proteolytic interference impairs endothelial integrity and contributes to a pro-inflammatory, pro-thrombotic phenotype. In the context of COVID-19, this mechanism likely underlies key features of COVID-associated coagulopathy, including microvascular thrombosis, platelet activation, and dysregulated cytokine signaling. Moreover, cleavage of NEMO in brain endothelial cells may contribute to the neuroinflammation and

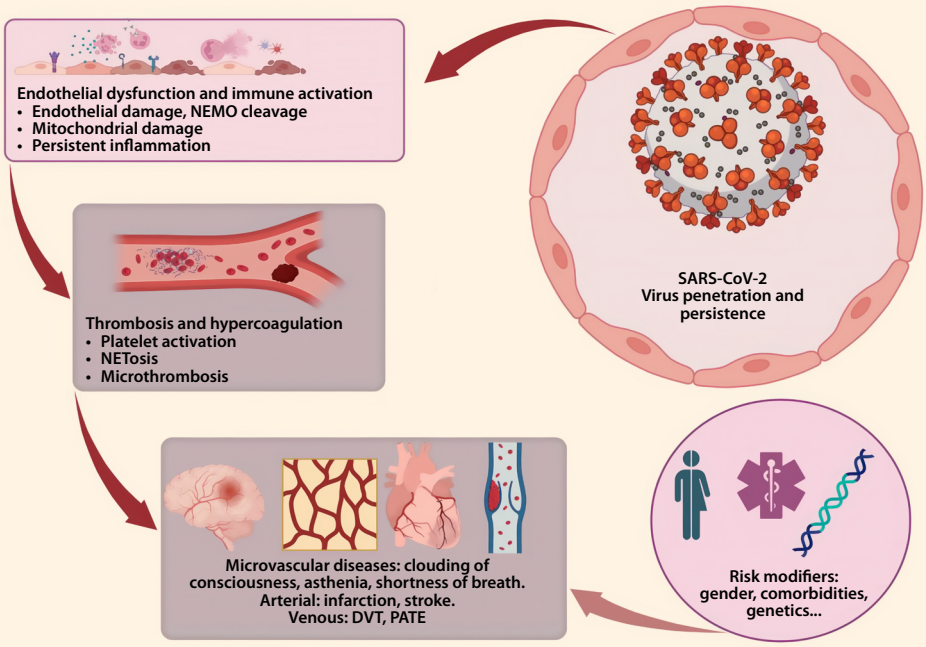

Figure 2. SARS-CoV-2, endothelial dysfunction and thromboinflammation

microthrombi observed in long COVID, highlighting a mechanistic link between viral persistence, immune evasion, and sustained thromboinflammatory complications [5].

Individuals carrying or exposed to the above-mentioned risk factors respond very frequently to both conditions (i) Activated endothelium and (ii) sustained hypercoagulable state. Both conditions contribute to the amplification of thromoinflammation triggered by SARS-CoV-2 infection [6, 7] (Figure 2).

Of particular interest is the presence of "ghost vessels" in the brain. Studies on postmortem human brain tissue as well the carotid body tissue showed cell nuclei highlighting a blood vessel in which vascular endothelial cells express SARS-CoV2 genetic material [8, 9]. Autopsy studies have documented the presence of SARS-CoV-2 in at organs and tissues at distance from le infected lungs such as the oesophagus, the spleen, the appendix, the adrenal gland, the ovairies, the testis, the prostate, or the endometrium [10].

LONG COVID: VASCULAR HEALTH IN PATIENTS WITH COVID-19 AND RISK OF POST-ACUTE SEQUELAE

Delayed arterial or venous thrombosis post COVID-19 is an emerging health issue associated with long COVID-19 [11–14].

VTE in acute COVID-19 survivors. Numerous studies have shown that symptomatic venous thromboembolism (VTE) is one of the main clinical manifestations of COVID-19. In a meta-analysis of 47 studies (n=6459 patients) published in 2022, where all patients were subjected to imaging diagnostic evaluation for PE/DVT, the

prevalence of pulmonary embolism (PE) and deep vein thrombosis (DVT) in hospitalized patients with COVID-19 was up 32% and 27%, respectively. Importantly, a twofold increased risk for death was demonstrated in patients with VTE compared to those without VTE [15, 16]. This confirmed a meta-analysis published in the early course of the pandemic, including more than 8000 patients with COVID-19 (21% of whom developed VTE), indicating a 74% higher odds of mortality when venous thromboembolism occurred concomitantly with COVID-19 (odds ratio (OR) 1.74, 95% confidence interval (CI) 1.01–2.98). SARS-CoV-2 infection, has been associated with a higher risk of cerebral venous thrombosis [14]. As a result, about 10% of survivors of acute severe COVID-19 experience long-term complications related to VTE.

In the NIH RECOVER-Adult cohort study, Shah D.P. et al. (2025) demonstrated a clear sex-specific difference in long COVID risk, which may extend to thrombotic sequelae including venous thrombosis [17]. The study prospectively followed 12,276 adults across 83 sites in the U.S., using a symptom-based scoring algorithm to identify long COVID at least six months post–SARS-CoV-2 infection. After rigorous propensity score matching to adjust for sociodemographic variables, clinical comorbidities, and infection characteristics, female sex was independently associated with a significantly increased risk of developing long COVID (relative risk (RR) 1.44; 95% CI 1.17–1.77 in the reduced model). Notably, the increased risk persisted across age groups and was most pronounced in women aged 40–54 years, with or without menopausal status. These findings suggest that sex-based biological mechanisms — such as hormonal influences and immune modulation — may also influence the differential risk of VTE observed in long COVID patients.

Arterial thrombosis in COVID-19 survivors. The available data indicate that, in addition to the risk of VTE, SARS-CoV-2 infection is associated with an increased long-term risk of arterial thrombosis. An analysis of national registry data from the Netherlands, Italy, Spain, the UK, and Germany, covering the period from September 2020 to July 2021 and including 909,473 COVID-19 cases (32,329 of whom were hospitalized), showed that within 90 days following COVID-19 diagnosis, the cumulative incidence of VTE ranged from 2 to 8 per 1,000 patients, and the incidence of arterial thrombosis ranged from 1 to 8 per 1,000 patients. However, significant inter-country variations were observed, reflecting differences in healthcare system capacities and responses [18].

A nationwide cohort study from the Swedish Intensive Care Registry evaluated one-year cardiovascular and thromboembolic risks in patients with severe COVID-19 treated with mechanical ventilation and discharged between March 1, 2020, and June 8, 2021. Population-based controls were matched by age, sex, and district of residence. Multivariate analysis showed a twofold increase in the risk of major adverse cardiovascular events (MACE), a 49-fold increase in pulmonary embolism, and a 16-fold increase in deep vein thrombosis [19].

In a large cohort study the long-term cardiovascular and thromboembolic risk after SARS-CoV-2 infection was assessed over a 3.5-year follow-up [20]. The study included 56,400 patients with COVID-19 and 1093904 contemporary controls without COVID-19 from the Montefiore Health System (inclusion period: March 11, 2020, to July 1, 2023). The primary outcome was the incidence of major adverse cardiovascular

events (MACE) occurring between 30 days and up to 3.5 years after the index date. The results showed a significantly higher incidence of MACE in the COVID-19 group (14%) compared to the control group (9%), yielding a RR of 1.56.

Data from the UK Biobank, including over 10,000 confirmed COVID-19 cases and more than 200,000 controls, were analyzed to assess the long-term risk of MACE over a follow-up of approximately 3.5 years. Using proportional hazard models, the study showed that hospitalized COVID-19 patients with pre-existing cardiovascular disease ($COVID^{+}/CVD^{-}$ had the highest cumulative incidence of myocardial infarction, stroke, or death. Individuals with COVID-19 but no prior cardiovascular disease ($COVID^{+}/CVD^{-}$) had an intermediate risk, while those without either condition ($COVID^{+}/CVD^{-}$) had the lowest risk. Comparisons with propensity score–matched controls confirmed these findings, highlighting COVID-19 as an important long-term cardiovascular risk factor, particularly in those with underlying cardiovascular disease [21].

The ensemble of the clinical and epidemiological data, part of them presented here in support the concept that cardiovascular risk factors and vascular diseases are major morbidities that constitute a particular burden of long COVID. This concept if further supported by a large epidemiological study conducted in Africa countries, that enrolled 9.5 million confirmed COVID-19 cases and showed that the prevalence of long COVID-19 in African populations raises up to 41% (95% CI 26–56%). Major clinical predictors for high risk of long COVID are cardiovascular risk factors (hypertension, obesity, hyperlipidemia, diabetes mellitus), cardiovascular disease (ischemic heart disease, arterial or venous thrombosis, HIV infection, chronic obstructive pulmonary disease/asthma, active cancer, tuberculosis, renal disease and psychiatric diseases [22].

LONG COVID: THE CHALLENGE OF HYPERCOAGULABILITY AND ENDOTHELIAL DYSFUNCTION

Data from prospective longitudinal observational studies show that persistent hypercoagulability and endothelial cell activation after the recovery of the acute phase of the disease are associated with long COVID symptoms.

The prospective observational study ROADMAP-postCOVID-19, enrolled COVID-19 survivors (n=208) and 30 healthy individuals. Survivors showed significantly higher levels of D-dimer, fibrin monomers (FM), TFPI, soluble thrombomodulin (sTM) and heparanase as compared to the control group. Survivors had significantly shorter the procoagulant phospholipid-dependent clotting time (PPL-ct). Elevated sTM and FM levels were observed in about 9% of survivors. Elevated levels of D-dimer, heparinase, and TFPI was found in about 25% of the survivors. Procoag-PPL ct was shorter than the lower normal limit in 8% of the survivors and was associated with female gender. Elevated FM was also associated with female gender whereas increase in heparanase was independently associated with male gender [23].

The persistent endothelial activation in patients with long COVID-19 is further supported by the decrease levels of the ADAMTS-13. The implication of neutrophil activation in patients with long COVID-19 is indicated by the CD10 low-density hyperreactive phenotype and increased PLA formation with consequent pulmonary dysfunction [24].

A prospective study on 50 COVID-19 survivors, showed that two months following SARS-CoV-2 infection, ongoing endotheliopathy is a common finding, independent of the severity of the acute phase. Thrombin generation was increased and the levels of factors VIII, and von Willebrand and soluble thrombomodulin remain persistently elevated in a about 20% of patients following apparent resolution of acute COVID-19. Persistent endotheliopathy appeared to occur independently of ongoing acute phase response or NETosis and is associated with enhanced thrombin generation potential [25].

Nevertheless, there are no evidence establishing a link between critical COVID-19 and the presence of antiphospholipid antibodies [26].

The large prospective epidemiological study conducted in African populations showed that patients with long COVID had higher levels of micro clot and platelet-poor plasma viscosity, serum A-Amyloid, α-2 antiplasmin, platelet factor 4 von Willebrand Factor (VWF), endothelial-leukocyte adhesion molecule 1 (E-selectin), platelet endothelial cell adhesion molecule-1 (PECAM-1) [17].

A longitudinal, prospective, single-center observational study on 60 patients with severe COVID-19 admitted to the ICU and 25 patients with mild COVID-19 treated at home, showed that after 4 weeks from the acute phase, extracellular vesicles expressing platelet antigens (CD41A), endothelial antigens (CD31) and TF were significantly increased in about 10% and 20% of patients with mild COVID-19 or severe COVID-19 respectively [27]. These findings build on a growing body of evidence highlights the importance of platelet activation in PASC. In a sequential multi-omics study of 117 hospitalized COVID-19 patients, Wang K. et al. used cytokine, proteo-

Table 1.

Prevalence of risk factors and other comorbidities related to disease worsening in patients hospitalized with COVID-19

Comorbidities	Severe COVID-19. %	Critical CODIV-19. %	Odds ratio for disease worsening (95% CI)
Current smoking	4.2–6.1	3.9–5	0.71 (0.19–2.68)
Cancer	1–6	1.5–10	1.6 (0.81–3.18)
Diabetes	6–25	14–60	2.13 (2.68–5.1)
Chronic renal disease	7	19	2.92 (1.04–6.09)
Hypertention	7–39	15–64	3.34 (1.72–5.47)
Cardiovascular diseases	1–10	9–40	5.19 (3.25–8.29)
Respiratory disease	1–8	5–10	5.15 (2.51–10.5)
Obesity	8	31	5.4 (2.77–10.67)

mic, and metabolomic profiling at acute illness and six-month convalescence to uncover biological drivers of long COVID [28]. Through unsupervised clustering using autoencoder-based dimensionality reduction, three distinct molecular phenotypes were identified. Cluster A, comprising 48.7% of participants, showed minimal molecular changes and few PASC risk factors, while cluster B was defined by elevated triglycerides and organic acids. Notably, Cluster C was enriched in female patients and characterized by persistent inflammation, platelet degranulation markers, and activation of the HIF-1α signaling pathway — implicated in sex-differentiated hypoxic responses. Elevated levels of thrombospondin-1, soluble CD40L, and serotonin indicated sustained platelet activation during convalescence, even among individuals reporting symptom resolution. These findings suggest that hyperreactive platelet states and sex-specific metabolic adaptations may underlie the vascular vulnerability and symptom heterogeneity in long COVID.

Current data suggest that cardiovascular events following COVID-19 may be linked to persistent immune dysregulation, endothelial cell activation, and hypercoagulability. Patients experiencing prolonged symptoms often exhibit elevated levels of proinflammatory molecules, including tumor necrosis factor-α, interferon-γ, and interleukins 2 and 6. This immune dysregulation can trigger the activation of the coagulation pathway, leading to the formation of extensive micro- clots during both acute COVID-19 and long COVID-19. These micro-clots are likely a significant mechanism underlying persistent symptoms and cardiovascular complications [29].

The European Society of Cardiology suggested that the evaluation of endothelial function, in addition to myocardial injury and respiratory function markers in COVID-19 survivors may represent possible means for early detection of vascular sequelae post-COVID-19 [30].

LONG COVID: PROFILE, PREVENTION AND TARGETED PERSONALIZED TREATMENT

Given the substantial public health impact of long COVID-19, there is an urgent need for a comprehensive strategy that focuses on:

- Profiling COVID-19 patients at risk of developing long COVID. The identification of panel of biomarkers of hypercoagulability, endothelial cell activation, thromboinflammation as well as the identification of genetic polymorphisms predisposing to long COVID are expected to be of major clinical value.
- Prevention of long COVID-19 through targeted community interventions. The identification of the most vulnerable individuals — including those with accumulated cardiovascular risk factors or established vascular disease — is essential. In addition, implementing territorial and environmental strategies tailored to specific geographic regions and social groups can help reduce exposure risks, improve access to care, and support early intervention. Together, these measures offer a proactive approach to preventing long COVID and mitigating its long-term health and socioeconomic impacts.
- Targeting hypercoagulablity and endothelial cell dysfunction. It is important to intensify research on therapeutic agents that can downregulate the

hypercoagulable state and inhibit the endothelial cell activation in patients with long COVID. In this context, evaluating the efficacy and safety of antithrombotic agents — such as direct oral inhibitors of activated factor X — and endothelial cell-targeting drugs like sulodexide, which has shown promising results in pilot studies in selected patient groups (including those with cardiovascular disease, cardiovascular risk factors, cancer, or HIV infection), represents an attractive strategy [1, 31, 32]. These approaches warrant assessment in prospective clinical trials.

CONCLUSION

In an effort to improve diagnostic tools, biomarker panels and personalized therapeutic strategies for patients with long COVID, particularly regarding sustained hypercoagulability and endothelial cell activation, international multidisciplinary collaboration among vascular and thrombosis specialists is of major importance. Responding to this need, the VAS — European Independent Foundation in Angiology/Vascular Medicine (https://www.vas-int.net/) has played a key role since the early phase of the COVID-19 pandemic.

VAS is uniquely positioned to lead, building on its early contributions to vascular guidance during the pandemic [6, 33, 34]. VAS was one of the first international institutions — alongside the International Society of Thrombosis and Haemostasis, the European Society of Cardiology, the American Heart Association, the American Society of Hematology, the Chinese Association of Chest Physicians, the Russian Academy of Sciences, the WHO, and the CDC — to coordinate guidance on managing COVID-19-related vascular complications and advocate for a balanced, integrated outbreak response strategy.

VAS has also been instrumental in amplifying critical messages to citizens, policymakers, and governments, including:

- advocating for equitable management of the pandemic;
- highlighting the role of antithrombotic treatments in preventing disease progression in COVID-19 patients;
- underscoring the impact of social inequalities and geographic barriers on the risk of SARS-CoV-2 infection and adverse outcomes, particularly in patients with cardiovascular disease, cardiovascular risk factors, or cancer;
- calling attention to the need for special care for vulnerable populations, such as low-income individuals, those living in environmentally degraded areas, and people with limited access to healthcare.

Moreover, VAS has emphasized the importance of strengthening public health systems, expanding primary healthcare, and leveraging modern technologies in the fight against the pandemic.

In facing the new challenge of long COVID, VAS is well-positioned to continue playing a leading role in promoting research, clinical guidance, and advocacy. For managing the long COVID syndrome, VAS and other international groups for the harmonization of management approaches at the global level.

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Chapter 4

Thrombogenic Risks in COVID-19 and in the Post-COVID Period

N.P. Mitkovskaya, E.A. Grigorenko, A.A. Pleshko

ABSTRACT

Systemic infection caused by SARS-CoV-2 is associated with proven development of coagulopathy and DIC syndrome, which determines the severity of the disease and the high risk of adverse outcomes. Post-COVID syndrome can be explained by the combined effects of the formation of hidden reservoirs of SARS-CoV-2, chronic hypoxia, persistent inflammatory response, endothelial dysfunction, and thrombosis. Understanding the mechanism of coagulation abnormalities in Long COVID-19 may help to more effectively inhibit thrombosis and prevent the progression of damage to various organs and systems.

INTRODUCTION

Systemic infection caused by SARS-CoV-2 is associated with the development of coagulopathy, which determines the characteristics and severity of the disease, the risk of adverse outcomes, and prolongation of the disease. Coagulopathy in coronavirus infection is clinically manifested by a high frequency of development in patients with severe COVID-19 venous, arterial, and microvascular thrombosis.

Morphologically, coronavirus infection causes intracellular and interstitial edema in the cardiovascular system; thrombosis (Figure 1), microthrombosis, and thromboembolism; increased vascular permeability; damage and necrosis of cardiomyocytes; infiltration of inflammatory cells or macrophages; collagen deposition with the formation of interstitial fibrosis and scarring as a result of direct cardiotoxicity against the background of virus localization directly in the myocardium [25].

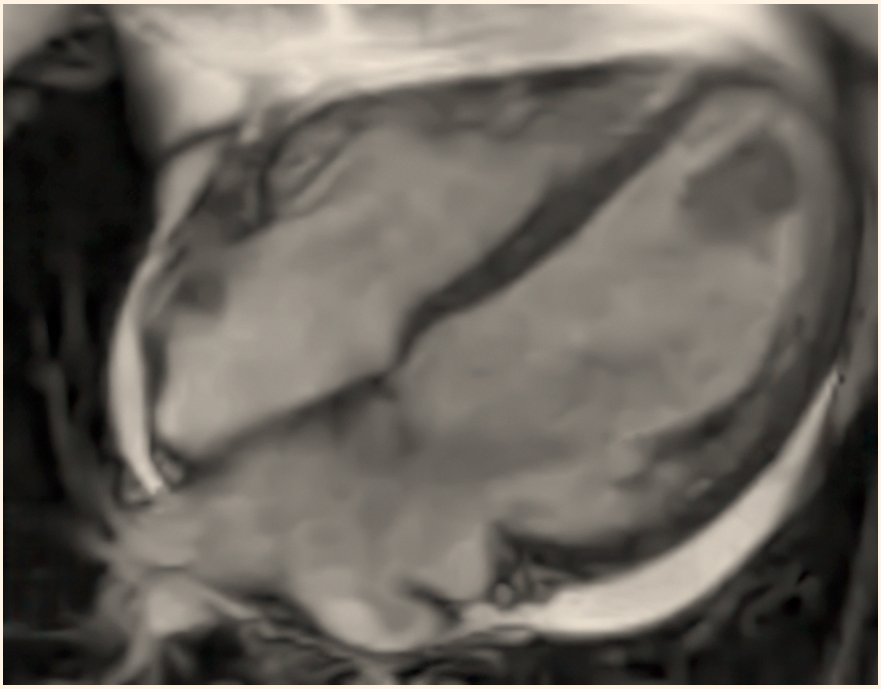


Figure 1. Cardiac MRI, thrombus in the left ventricular cavity, authors' own data

Increased troponin levels in patients with COVID-19 may be due to type 1 and type 2 myocardial infarction (in 45% of patients in intensive care units (ICUs) and in 6.1% of all infected patients), myocarditis, Takotsubo cardiomyopathy, arrhythmia, heart failure [22], rethrombosis, cerebral circulation disorders, pulmonary artery and coronary artery thromboembolism, which makes it difficult to interpret changes in this indicator during differential diagnosis in some cases, may be accompanied by overdiagnosis of acute coronary syndrome (ACS), and should be carried out taking into account the entire clinical and instrumental picture of the disease [5, 34].

Myocardial infarction with coronary thrombosis was diagnosed in 11% of patients in a study conducted by the authors based on coronary angiography (CAG) data. Of these, coronary artery (CA) thrombosis was detected within one month of the onset of the disease in 72.2% of individuals, in 16.7% of individuals — up to 6 months after the acute period of COVID-19, and in 11.1% of individuals — more than 6 months after COVID-19. Coronary artery thrombosis developed in patients with SARS-CoV-2 infection and ACS against a background of higher levels of interleukin (IL)-6, IL-1β, Lp-FLA2, and D-dimer [6].

The respiratory system in COVID-19 is characterized by changes that are not typical for bacterial pneumonia and H1N1 influenza (Figure 2): desquamation of pneumocytes; lymphocytic and neutrophilic infiltration in the interstitial space against the background of the absence of exudation typical for pneumonia; high frequency of venous and arterial thromboembolic complications (35% in ICU patients and 2.6% among all infected patients) [4, 32] (Figure 2).

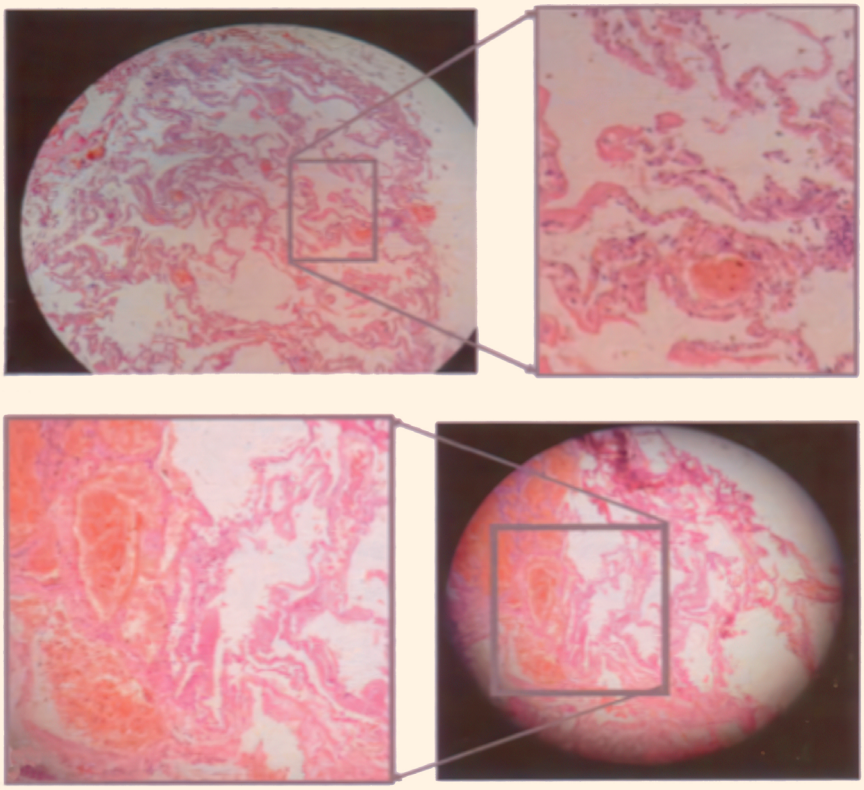


Figure 2. Microscope slide of the lung of a patient who died from COVID-19, authors' own data

The SARS-CoV-2 virus binds to ACE2 receptors, primarily on type 2 alveolar cells, enters the target cell, replicates within it, causing inflammation and necrosis of the alveoli, and pulmonary capillary endothelial cells, provoking microvascular thrombosis, which contributes to oxygenation disorders in patients with COVID-19 [29]. In individuals with SARS-CoV-2 infection who died from acute respiratory distress syndrome (ARDS), microthrombosis of the alveolar capillaries was 9 times more common compared to data from patients who died from ARDS associated with influenza A virus (ITA (H1N1)) [7, 32].

Examination of autopsy material from the livers of COVID-19 patients reveals microvesicular steatosis, focal necrosis of hepatocytes, and microthrombi in the sinusoids. The proportion of individuals with liver damage among patients with severe coronavirus infection is significantly higher than among those with mild disease [4]. Acute kidney injury in coronavirus infection is associated with a 3.9-fold increase in the risk of in-hospital death, and a 3.5-fold increase in the presence of baseline elevated creatinine and urea levels at admission [12].

The severe manifestations of coronavirus-induced lesions of the central nervous system include acute necrotic encephalopathy; "hemorrhagic shock-encephalopathy" syndrome, "hemiconvulsion-hemiplegia-epilepsy," "encephalopathy-biphasic seizures

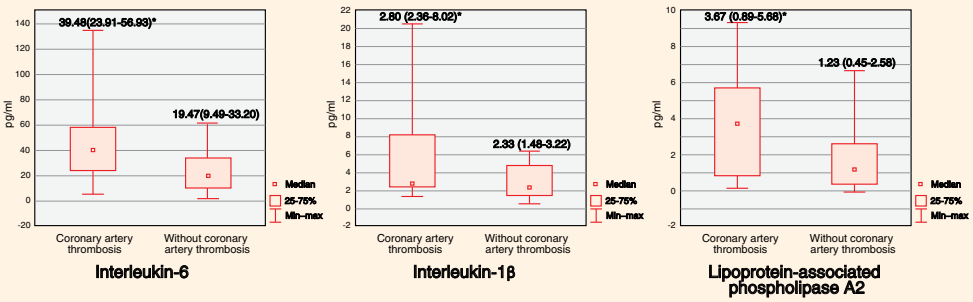


Figure 3. Levels of interleukin-6, interleukin-1β, and lipoprotein-associated phospholipase A2 in patients with and without thrombosis against the background of coronavirus infection [5].

*$p<0.05$ in comparison with patients without coronary artery thrombosis

with reduced diffusion in the hemispheres" syndrome, or non-herpetic non-paraneoplastic limbic encephalitis [31].

Such a diverse clinical picture is caused by complex pathogenetic reactions occurring in the body of a patient with coronavirus infection: the pathological picture develops as a result of a cytokine storm, developing endothelial dysfunction, endotheliitis and apoptosis of the endothelium of the pulmonary microvascular bed, hypoxia, coagulopathy with the development of thrombosis and microthrombosis.

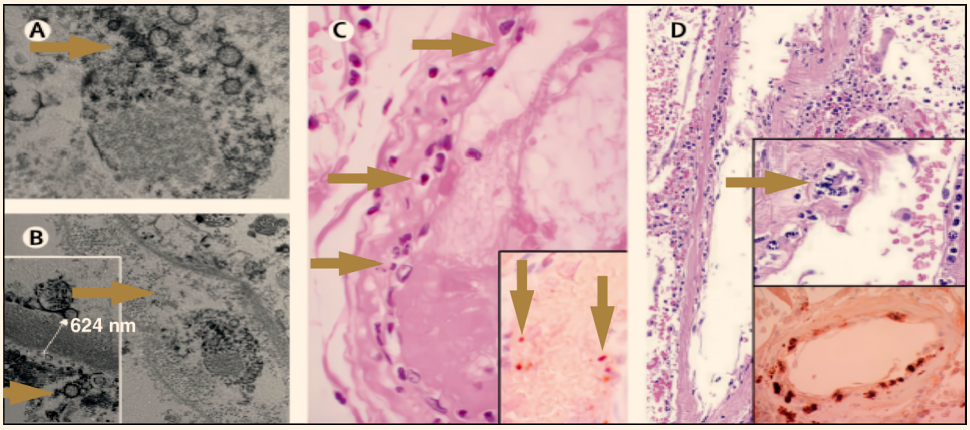


Figure 4. Endothelial cell infection and endotheliitis in COVID-19.

(A, B) Electron microscopy of kidney tissue shows viral inclusions in the peritubular space and viral particles in the endothelial cells of the glomerular capillary loops. Aggregates of viral particles (arrow) have a dense round surface and a transparent center. The asterisk in panel B indicates the peritubular space corresponding to the capillary containing viral particles. The inset in panel B shows the glomerular basement membrane with an endothelial cell and a viral particle (arrow; approximately 150 nm in diameter). (C) Small intestine resection specimen from patient 3, stained with hematoxylin and eosin. The arrows indicate predominant mononuclear cell infiltrates in the intima along the lumen of many vessels. The inset in panel C shows immunohistochemical staining for caspase 3 in small intestine samples from the serial tissue section described in panel D. The staining patterns corresponded to apoptosis of endothelial cells and mononuclear cells observed in hematoxylin and eosin-stained sections, indicating that apoptosis is induced in a significant proportion of these cells. (D) A postmortem lung specimen stained with hematoxylin and eosin showed thickened pulmonary septa, including a large arterial vessel with infiltration of mononuclear cells and neutrophils (arrow in upper inset). The lower inset shows immunohistochemical staining for caspase 3 on the same lung specimen; these staining patterns corresponded to the apoptosis of endothelial cells and mononuclear cells observed on hematoxylin and eosin-stained sections. Adapted from Varga Zsuzsanna, et al. [35]

Table 1.

Laboratory tests reflecting hemostasis in patients with COVID-19-related coagulopathy. Adapted from Gasecka A. et al. [20]

Laboratory indicator	Dynamics, %	Comments
D-dimers	4,2–6,1	Significantly increased, 3–4-fold increase associated with high mortality
Fibrin degradation products	1–6	Increased
Fibrinogen	6–25	Increased Decreasing trend if the patient's condition progresses to a consumption coagulopathy phenotype (e.g., DIC)
APTT	7	Within normal limits, or slightly increased
PTT	7–39	Downward trend if the patient's condition progresses to a consumption coagulopathy phenotype (e.g., DIC)
Platelets	1–10	Within normal limits or moderately increased Within 100–150 × 10^9 cells/L in 70—95% of patients with severe COVID-19, platelet counts < 100 × 10^9 cells/L were found in approximately 5% of patients with severe COVID-19. The indicator may be slightly increased (based on limited data obtained in small cohorts)
Plasma viscosity	1–8	On average, a 2-fold increase
Activity	8	Increased
Factor VIII		Increased
von Willebrand factor		Moderately decreased
Antithrombin activity		Moderately decreased
Free S-protein		Moderately decreased
C-protein		Moderately decreased

The concentration of proinflammatory cytokines at the time of death could significantly exceed normal levels (IL-1β, IL-1RA, IL-6, IL-8, IL-15, IL-16, MIR-1a, MIR-1b, MCR-1, M-CSF, MIF, IP-10, GRO-a, Eotaxin), which is confirmed in our study [6] (Figure 3), and it is this phenomenon, called a "cytokine storm," that is a powerful factor in endothelial damage, creating conditions for the virus to enter endothelial cells.

An important mechanism of microvascular thrombosis specific to COVID-19 is the affinity of the virus to ACE2 and its expression on endothelial cells with the development of endothelial dysfunction and endotheliitis, which are accompanied by apoptosis of endothelial cells, microvascular damage, thrombosis, and multiple organ failure [35] (Figure 4).

Destruction and infection of endothelial cells cause thrombotic microvasculitis, plasma hypercoagulation, and cell apoptosis [35]. Apoptosis of the pulmonary microvascular endothelium triggers the dissemination of pathogens in the blood. Platelet precursor cells and bone marrow endothelial cells expressing ACE2 receptors become infected. Infected megakaryocytes reproduce defective platelets that potentiate thrombosis. SARS-CoV-2 can activate platelets and cause an inflammatory response characterized by the synthesis of a wide range of immunomodulatory cytokines, chemokines, and other mediators.

Coagulopathy caused by SARS-CoV-2 infection has its own characteristics: a unique pathological picture with diffuse microthrombosis and hemorrhages, along with a large number of intravascular megakaryocytes in all major organs, including the lungs, heart, kidneys, and liver. Microvascular thrombosis is a clinical diagnostic challenge because microthrombi are hard to see due to their small size (often ≤10 μm) and there aren't any specific biomarkers to detect them. Clinically, microvascular occlusion leads to ischemia, the consequences of which vary from changes in plasma blood coagulation markers to severe multiple organ failure [11].

The procoagulant profile in severely ill patients with coronavirus infection revealed increased D-dimer levels and hyperfibrinogenemia, as well as increased clot strength (CS) due to the contribution of platelets and fibrinogen to its formation (Table 1). The increase in D-dimers predicts the severity of the clinical picture of COVID-19 and may persist in patients after discharge from the hospital. An increase of D-dimers in the blood after vaccination against COVID-19 may indicate an increased risk of vaccine-induced thrombocytopenia and thrombosis [28].

LONG COVID-19 / POST-COVID-19 SYNDROM

Long COVID-19 (post-COVID) syndrome refers to a long-term multisystem syndrome observed in patients who have survived coronavirus infection and is defined by the US Centers for Disease Control and Prevention and the National Institutes of Health as a continuation of the disease that lasts more than 4 weeks after the initial infection [13]. People who remain infected with SARS-CoV-2 for a long time develop structural and functional disorders of various systems: respiratory, cardiovascular, hematological, neurological, urinary, gastrointestinal, musculoskeletal, and endocrine systems, which explains the multifaceted clinical picture of post-COVID syndrome (Table 2).

The prevalence of ACE2 cell membrane receptors, which mediate SARS-CoV-2 cell entry, explains the long-term complications of viral infection resulting from massive damage to organs and tissues. SARS-CoV-2 infection is characterized by accelerated replication in the acute phase with a rapid decrease in viral load after the first week. However, autopsy results in critically ill patients with COVID-19 showed that

Table 2.

Studies of persistent symptoms in post-COVID syndrome. Adapted from Wang C., (2022) [38] with modifications

References	Population	Evaluation time	Symptoms (% of patients)
Groff, D., et al. (2021) [21]	57 studies involving 250,351 COVID-19 survivors	1 month after acute COVID-19; 2 and 5 months after infection; 6 months after COVID-19	Generalized anxiety disorder (29.6%); general functional disorders (44.0%); fatigue or muscle weakness (37.5%); difficulty concentrating (23.8%); memory impairment (18.6%), cognitive impairment (17.1%); dysgeusia (11.2%); anosmia (13.4%); headache (8.7%); shortness of breath (29.7%); cough (13.1%); decreased mobility (20.2%); decreased exercise tolerance (14.7%); joint pain (10.0%); flu-like symptoms (10.3%); general pain (32.4%); persistent fever (0.9%); muscle pain (12.7%); chest pain (13.3%); palpitations (9.3%); gastrointestinal disorders (9.3%)
Alkodaymi, M.S., et al. (2022) [9]	63 studies involving 257,348 patients with COVID-19	3–<6 months, 6–<9 months, 9–<12 months, and ≥12 months	Fatigue, shortness of breath, sleep disturbances, and difficulty in concentration (32%, 25%, 24%, and 22%, respectively, when observed for 3–<6 months); intolerance to physical exertion, fatigue, sleep disturbances, and shortness of breath (45%, 36%, 29%, and 25%, respectively, when observed for 6–<9 months); fatigue (37%) and shortness of breath (21%) after 9–<12 months, and fatigue, shortness of breath, sleep disturbances, and myalgia (41%, 31%, 30%, and 22%, respectively, when observed for >12 months)
Kamilova U.K. et al. (2023) [3]	Belarusian-Uzbek project involving 10,908 patients	3 months and 6 months	3 and 6 months: weakness (31.8% and 24.1%, respectively); shortness of breath (28.6% and 17.9%, respectively); increased blood pressure (18.1% and 18.3%, respectively); heart palpitations (11.6% and 5.2%, respectively); cough (8.2% and 3.7%, respectively); chest pain (4.1% and 2.8%, respectively); loss of taste and smell (2.3% and 0.8%, respectively)

viral RNA can be detected many months after death, suggesting that prolonged presence of the virus in the body is associated with adverse outcomes [15].

Specific symptoms of post-COVID syndrome may include: absence of lung damage despite the presence of airflow obstruction; changes in the microstructure of the white matter of the brain and hypoperfusion of the gray matter detected by magnetic resonance imaging (MRI) with arterial axial sectioning; skin biopsy results indicating axonal degeneration associated with peripheral neuropathy and thrombotic vasculopathy; anosmia indicating prolonged neurological post-COVID syndrome associated with microvascular damage to the olfactory analyzer; development of male infertility due to a previous coronavirus infection with excessive generation of active oxygen species associated with mitochondrial activity, which is abundant in testicular tissue; retinal vein occlusion, etc. [8].

Studies of the pathophysiological mechanism of Long COVID-19 indicate a multifactorial mechanism of its potentiation: the presence of hidden reservoirs of SARS-CoV-2, damage to the vascular endothelium during long-term viral infection, dysregulation of the immune system, chronic hypoxia, and inflammatory response, which initiate coagulation and microthrombosis, leading to various systemic functional disorders and clinical events, especially in patients with comorbidities [5] (Figure 5).

The development of post-COVID syndrome is facilitated by the formation of hidden SARS-CoV-2 reservoirs against the background of reduced antiviral immunity, which is confirmed by the lower incidence of post-COVID syndrome in vaccinated individuals. It is assumed that hidden reservoirs of SARS-CoV-2 in various tissues are sources of direct infection of endothelial cells, release of soluble viral products, infection of monocytes, and activation of other viruses, such as Epstein–Barr (EBV), which can transmit the virus to microvascular endothelial cells, causing cytopathic effects involving autoantibodies. SARS-CoV-2 can be transported to distant tissues and organs [10], with the mechanism and means of virus movement playing an important role in the activation of coagulation [33].

One potential mechanism for COVID-19 recurrence may be the transfer of viruses by nanoscale vesicles (exosomes), which, as a universal means of intercellular communication, are formed inside dendritic cells, neutrophils, monocytes, macrophages, lymphocytes, thrombocytes, adipocytes, neurons, epithelial and endothelial cells during endocytosis, and then released into the extracellular environment and, interacting with other cells, deliver their cargo [39, 16, 10]. The reappearance of viral RNA in patients who have recovered from COVID-19 with a recurrence of clinical symptoms in various organs and systems is associated with the insidiousness of the “Trojan horse” story.

Various signs of hidden SARS-CoV-2 reservoirs underlying vascular pathology in Long COVID-19 have been described: prolonged viral shedding in feces, circulating SARS-CoV-2 RNA fragments, detection of viral RNA by polymerase chain reaction (PCR) in various tissues at autopsy more than 7 months after acute coronavirus infection; detection of viral RNA in the corpus cavernosum of the penis in men with erectile dysfunction; the ability of SARS-CoV-2 S and N proteins to induce prothrombotic factors in vitro; association with suboptimal humoral and cellular immunity against SARS-CoV-2, as well as an increase in the concentration of autoantibodies, some of which, by analogy with acute COVID-19, can cause endothelial damage; reduction

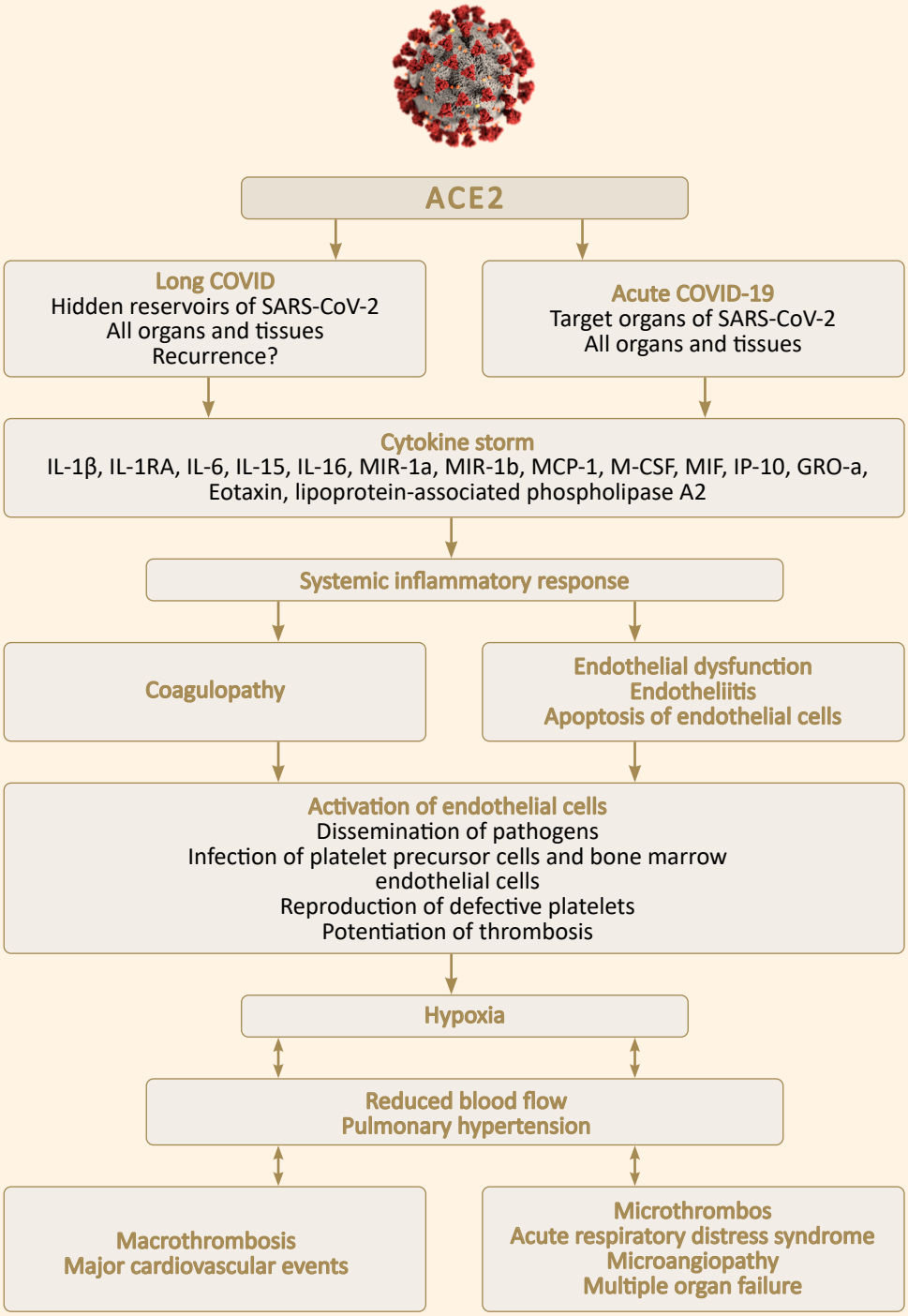


Figure 5. Schematic representation of the pathophysiological sequence of thrombotic events in patients with severe COVID-19 and recurrence in the post-COVID period. Adapted from Joly B.S., et al. with modifications [24]

in the manifestations of post-COVID syndrome after effective vaccination against SARS-CoV-2 [8, 19]. Prolonged viral presence, hypoxia, and inflammatory reactions lead to permanent damage to the endothelium, extensive endotheliitis, and thrombosis.

DIAGNOSTIC TESTS FOR LONG COVID-19

A simple 4-millimeter skin biopsy of normal-looking skin is a diagnostic method that has been used to investigate thrombotic microangiopathies associated with atypical hemolytic uremic syndrome and hematopoietic stem cell transplantation, as well as in acute COVID-19.

Direct biopsy of other accessible tissues, including the lungs and peripheral nerves, is a diagnostic method that can provide evidence of vascular damage, the presence of microthrombi, and direct SARS-CoV-2 infection.

Viral particles in stool and peripheral blood, as well as hematological/immunological abnormalities associated with post-COVID syndrome, can also be tracked over a long period of time [8].

Endothelial dysfunction is an independent risk factor for post-COVID syndrome [18]. Endothelial cells (ECs) regulate blood flow and coagulation, initiate and amplify inflammation, and maintain vascular tension, structure, and homeostasis. SARS-CoV-2 infection causes severe endothelial damage and endotheliitis, capillary inflammation, thrombosis, and new abnormal angiogenesis [27]. Damage to the endothelium increases vascular permeability and leukocyte adhesion while weakening the anticoagulant properties of cells due to a decrease in antithrombin III, an inhibitor of the tissue factor pathway and protein C. Damage to the ECs and microcirculation system caused by inflammation can lead to a massive increase in the concentration of plasminogen activator, which is consistent with high D-dimer levels in severe COVID-19 patients. Thus, endothelial trauma, persistent endothelial dysfunction, and increased permeability of the pulmonary capillary bed are the most important components in the formation of post-COVID syndrome [27].

Increased permeability of the pulmonary capillary endothelial layer contributes to plasma entering the alveolar cavity and the formation of hypoxia, which leads to the activation and apoptosis of ECs, reducing their anticoagulant properties [14]. Damage to the endothelium by viruses, inflammation, and hypoxia reduces blood flow velocity, causing platelet aggregation and thrombosis [7]. In addition, pathological angiogenesis, which is observed in various organs in deceased patients with COVID-19, is one of the manifestations of endothelial dysfunction [30].

Immune system dysregulation in post-COVID syndrome is characterized by increased levels of interferon-g and IL-2, pathological changes in lymphocytes, monocytes, and B lymphocytes. Increased oxidative phosphorylation and inflammatory reactions associated with reactive oxygen species displace inflammatory reactions caused by TNF-α and IL-6, forming persistent symptoms and progression of post-COVID syndrome [17].

Inflammation caused by hypoxia can further exacerbate capillary dysfunction and promote thrombosis, which, against a background of hypoxemia, can lead to increased

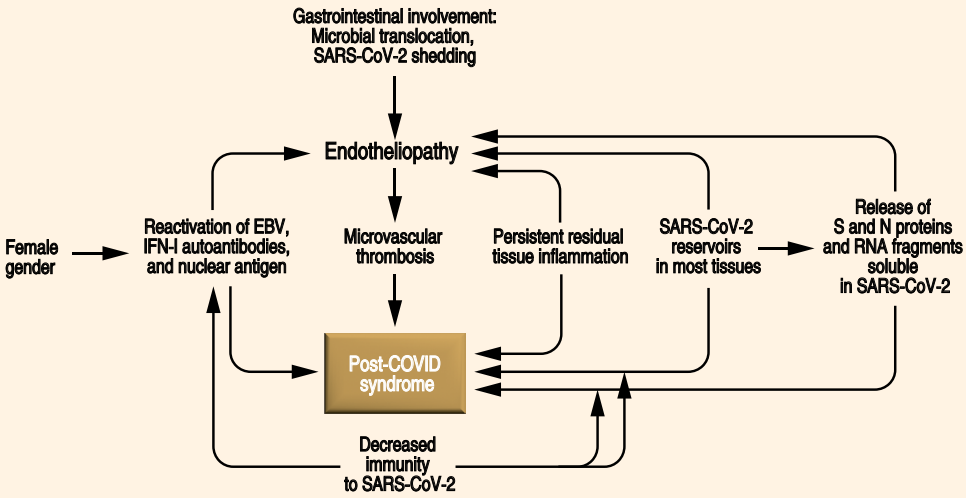


Figure 6. Potential mechanisms underlying Long-term COVID-19. Adapted from Ahamed J., et al. (2022) [8]

levels of metabolic toxins, energy deficiency, extensive cell damage, cell death, and multiple organ failure.

Due to the persistence of SARS-CoV-2 infection, chronic inflammation in post-COVID syndrome may be a mechanism that stimulates platelets and other inflammatory cells, contributing to the activation of procoagulant factors and destroying the protective function of the vascular endothelium, thereby causing abnormal coagulation. Chronic persistent inflammation in post-COVID syndrome may stimulate ECs, platelets, and other cells, contributing to the enhancement of procoagulant factors and destroying the protective function of the vascular endothelium, causing abnormal coagulation (Figure 6) and forming a feedback loop where inflammation causes thrombosis, and the resulting thrombi contribute to the inflammatory process. Thrombosis can exacerbate vasculitis, which is consistent with autopsy findings confirming that post-COVID syndrome is essentially a thrombotic continuation of acute viral infection [36] (Table 3).

Both acute SARS-CoV-2 infection and post-COVID syndrome have their own characteristics associated with the presence of comorbidities in patients. In our joint study with Uzbek colleagues, 22.3% of individuals had at least one concomitant cardiovascular disease (CVD), 17.3% of patients had a combination of coronary heart disease and arterial hypertension, 12.8% had a combination of three or more CVDs, and 35.9% of convalescents had three or more cardiovascular risk factors, which was associated with an increase in the level of humoral markers of endothelial dysfunction and a more severe course of viral infection [3, 4].

Various studies have shown the frequency of thrombosis in patients after discharge [3] (Table 3). SARS-CoV-2 infection increases the risk of thromboembolic complications not only in the acute phase of the disease. One month after recovery, there is an increased risk of venous thrombosis and, as a result, life-threatening pulmonary embolism. Early prophylactic anticoagulant therapy for COVID-19 can quickly remove

Table 3.

Research on thrombosis and thromboembolism in Long COVID-19. Adapted from Wang C. (2022) [39] with modifications

References	Population	End points/goals		Conclusions
Giannis et al. (2020) [20]	*n*=4906	Thromboembolic outcomes and mortality after discharge	Venous thromboembolism (VTE) was diagnosed in 1.55% after discharge and included 0.90% cases of deep vein thrombosis, 0.85% cases of thromboembolism of pulmonary artery (TEPA)	VTE, arterial thromboembolism (ATE), and all-cause mortality frequently occurred after hospitalization for COVID-19. Antithrombotic therapy (AT) after discharge reduced the risk by 46%
von Meijenfeldt et al. (2020) [18]	*n*=52	Hemostatic status of patients who have had COVID-19 infection	Plasminogen activator inhibitor 1 (PAI-1) levels were higher in patients than in the control group both at admission and at the 4-month follow-up	Patients with COVID-19 showed persistent prothrombotic changes and decreased plasma fibrinolytic potential 4 months after discharge from the hospital
Kamilova U.K., et al. (2023) [3]	*n*=152	Factors that increase cardiovascular risk after COVID-19 infection	Three or more factors increasing cardiovascular risk were identified in 35.9% of convalescents. In patients with comorbid cardiovascular disease who had COVID-19, the frequency of risk factors and the clinical course of the disease were associated with increased levels of humoral markers of endothelial dysfunction	A retrospective analysis of the medical records of 10,908 hospitalized patients with COVID-19 showed that thromboembolic complications developed in 6.0% of cases

Table 4.

Recommendations for the prevention of thrombosis and thromboembolism. Adapted from Wang C. (2022) [38] with modifications

Guidelines	Groups of patients with indications for antithrombotic therapy after discharge	Recommendations for antithrombotic therapy after discharge
ASH	Suspected or confirmed venous thromboembolism (VTE) or other indications for antithrombotic therapy	Outpatient antithrombotic prophylaxis is not used in discharged patients with COVID-19 without suspected or confirmed VTE or other indications for antithrombotic therapy. Undesirable consequences may outweigh the desired results
SSC-ISTH	Hospitalized COVID-19 patients with high-risk criteria for VTE (including advanced age, hospitalization in the intensive care unit, oncological pathology, history of VTE,	Extended thromboprophylaxis after discharge should be considered for all hospitalized COVID-19 patients who meet the criteria for high risk of VTE
ACC	Patients at increased risk of VTE (including patients with limited mobility and a history of VTE or active malignancy)	After discharge, long-term prophylaxis with low molecular weight heparin or direct oral anticoagulants (DOACs) may reduce the risk of VTE but increase the incidence of bleeding, including major bleeding. Although there are no data specific to COVID-19, it is reasonable to use individual risk stratification for thrombosis and bleeding and then consider extending prophylaxis (up to 45 days) for patients at increased risk of VTE
ACF	Patients at increased risk of VTE (e.g., elderly people, cancer patients, obese patients, pregnant women, patients with congestive heart failure or a history of VTE)	Extended VTE prophylaxis is not necessary for all discharged COVID-19 patients. A multidisciplinary discussion should be held at or shortly before discharge to determine whether patients have persistent VTE risk factors, whether long-term post-hospital VTE prophylaxis may be beneficial, and to ensure that VTE prophylaxis is available
Belgian clinical guidelines	Patients at increased risk of VTE (e.g., patients hospitalized in intensive care units, with known thrombosis, obesity, taking high doses of estrogen, immobilization, heart failure, respiratory failure, aged 70 years and older, with active cancer, with a personal or family history of VTE, and/or who have undergone surgery within the last 3 months)	If other risk factors for VTE are present, it is recommended to extend prophylactic treatment to 4-6 weeks after discharge

various procoagulant substances, thereby protecting the blood system and surrounding tissues and organs from damage by suppressing the initiation of coagulation, thrombosis, and its complications [1].

Current guidelines and recommendations indicate that extending venous thromboembolism prevention beyond hospital discharge may be helpful, but the benefit is limited by the high risk of thromboembolism in the context of COVID-19 [38] (Table 4). Understanding the mechanism of coagulation abnormalities in post-COVID syndrome may help to inhibit thrombosis more effectively and prevent the progression of pathological processes.

ANTITHROMBOTIC THERAPY FOR PATIENTS WITH COVID-19, RECOMMENDED AT THE OUTPATIENT STAGE OF TREATMENT

In individual patients hospitalized with COVID-19, prophylactic doses of rivaroxaban after discharge for 30 days may be considered to reduce the risk of venous thromboembolism.

Patients with respiratory failure, as well as patients with a high cardiovascular and thromboembolic risk of complications (≥3 points on the risk factor table) are transferred to therapeutic doses of anticoagulants [23].

In patients who have had COVID-19, risk factors for thrombosis must be taken into account: the presence of active cancer (patients with regional lymph node metastases or distant metastases who have received chemotherapy or radiation therapy within the last 6 months), venous thromboembolism/history of thrombosis of any location (except superficial vein thrombosis), immobilization (planned bed rest [with the ability to use the bathroom/toilet] due to reduced patient mobility or doctor's recommendations for ≥3 days), diagnosed thrombophilia (antithrombin, protein C or S deficiency, antiphospholipid syndrome, factor V Leiden gene mutation, prothrombin *G-20210A* gene mutation) [23].

POTENTIAL LONG-TERM CONSEQUENCES OF SARS-CoV-2 INFECTION ON THE FUNCTIONING OF THE CARDIOVASCULAR SYSTEM

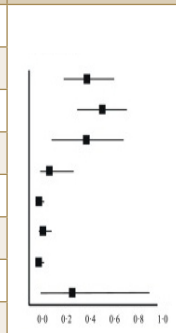
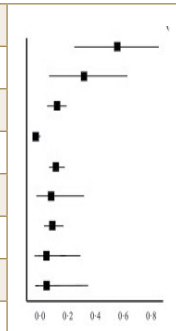
INCREASE in the long-term clinical manifestations of coagulopathies: recurrent myocardial infarctions, cerebral infarctions, stent thrombosis, thromboembolism.

A dynamic study of the cardiovascular system in patients who have had COVID-19 is NECESSARY.

Unfortunately, there is currently no effective treatment for Long COVID-19. Therefore, prevention is essential to reduce the extent of thrombotic damage and potentially mitigate long-term consequences, reducing the burden of post-COVID syndrome on patients and healthcare systems. In acute COVID-19, the importance of controlling viral replication and preventing inflammation is beyond doubt. However, early removal of procoagulant substances and protection of the vascular endothelium may be the best means of preventing long-term thrombotic risks in the post-COVID period.

Table 5.

Meta-analysis of changes identified in asymptomatic patients. Adapted from Kronbichler A., (2020) [26]

Laboratory test results	Total number of patients examined	Number of patients with changes	Percentage of patients with changes (total), %	Percentage	Percentage of patients with changes according to meta-analysis, % (95% CI)	Heterogeneity *P*, %	Percentage of patients with changes (95% CI)
Normal	62	34	54.84		61.74 (30.12–88.62)	80.98 (p=0.0219)	63.41 (46.81–80.00)
Abnormal	62	28	45.16		38.26 (11.38–69.88)	80.98 (p=0.0219)	36.60 (20.00–53.19)
Leukopenia	102	17	16.67		17.15 (10.56–24.96)	0 (p=0.3411)	16.38 (12.77–20.00)
Leukocytosis	102	1	0.98		1.45 (0.06–4.63)	0 (p=0.3620)	0.91 (0–1.82)
Lymphocytopenia	117	19	16.24		16.79 (10.67–23.97)	0 (p=0.3831)	20.00 (10.64–20.00)
Increased LDH	102	15	14.71		13.24 (0.88–36.70)	88.01 (p=0.0039)	13.95 (4.26–23.64)
Increased SRB	122	17	13.93		14.48 (8.85–21.22)	1.30 (p=0.3631)	15.00 (8.51–18.18)
Increase in procalcitonin	122	8	6.56		9.54 (0.02–33.23)	88.08 (p=0.0002)	10.00 (0–26.09)
Increase in E-dimers	62	5	8.06		9.87 (0.20–39.96)	81.23 (p=0.0210)	10.87% (0–21.74)
Normal	135	51	37.78		43.34 (22.77–65.20)	83.12 (p=0.0001)	43.90 (6.67–100)
Abnormal	135	84	62.22		56.66 (34.80–77.24)	83.12 (p=0.0001)	56.10 (0–93.33)
The “frosted glass” effect	80	36	45.00		43.09 (13.42–75.86)	88.13 (p<0.0001)	34.51 (0–93.33)
Shadow spots	80	15	18.75		11.86 (1.26–31.02)	75.46 (p=0.0067)	5.00 (0–31.71)
Consolidation	80	1	1.25		2.510 (0.27–6.91)	0 (p=0.9162)	0 (0–2.44)
Shadow stripes	80	5	6.25		5.03 (0.57–13.56)	40.57 (p=0.1683)	0 (0–12.16)
Bronchitis	80	1	1.25		2.51 (0.27–6.91)	0 (p=0.9162)	0 (0–2.44)
Signs of pneumonia	96	40	41.67		31.25 (3.96–96.65)	98.41 (p<0.0001)	36.67 (2.44–70.91)

Inadequate diagnosis and/or inadequate thromboprophylactic therapy for potentially preventable micro- and macrovascular thromboses and their cardiovascular complications (Table 5) may underlie the high frequency of non-ARDS-associated deaths in patients with SARS-CoV-2 infection.

A study conducted by Belarusian authors in immunocompetent patients with acute cytomegalovirus infection (ACVI) demonstrated a 7% probability of developing thromboembolic complications (TEC) of various locations in adult patients, which requires stratification of modifiable and non-modifiable risk factors and monitoring of CRP and D-dimer levels, which are prognostically unfavorable laboratory indicators of the risk of TEC development in ACVI [2]. The results obtained support the assumption that not only SARS-CoV-2 infection, but also other viral infections have their own mechanisms for increasing the risk of TEC [29].

Diagnostic tests to verify thromboembolic events are not currently performed systematically, which means that the prevalence of thrombosis and PATE in patients with post-COVID syndrome may be underestimated. Given the large number of studies signaling the presence of disabling consequences in COVID-19 convalescents and the need for subsequent drug and non-drug rehabilitation, it is important to search for new biomarkers, including coagulation, fibrinolysis, and endothelial activation associated with the course, early outcomes, and late complications in patients with SARS-CoV-2 coronavirus infection, and the development of clinical protocols specifying the prevention and management of patients with early and late complications of SARS-CoV-2 coronavirus infection, comorbid pathology, positioning an individual approach to the prevention and treatment of this category of patients.

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Chapter 5

Post-COVID Syndrome in Therapeutic Practice

O.M. Drapkina, A.Yu. Gorshkov, S.A. Berns

ABSTRACT

The coronavirus pandemic has demonstrated the relevance of the proper organization of therapeutic services. Currently, chronic non-infectious diseases account for the majority of visits to therapists worldwide, who during the pandemic have encountered situations where the course of infection in this category of patients can lead to decompensation of the underlying disease. This is because coronavirus infection has a systemic effect on the human body — it is impossible to identify any organ or system that is not involved in the infectious process. The development of infection in patients with chronic non-infectious diseases was often accompanied by a high risk of severe forms of infection, complications, and adverse outcomes. Studying the pathogenetic, immunological, and clinical characteristics of coronavirus infection in patients with chronic non-infectious diseases helps prevent adverse outcomes of the infection. A global view of the patterns of pandemic development against the backdrop of a steady increase in the number of patients with chronic non-infectious diseases can be defined as a syndemic. This approach allows us to determine strategies for the healthcare system during the pandemic, taking into account not only the characteristics of the epidemic process, but also the risk factors for chronic non-communicable diseases, which will reduce the serious consequences and negative impact of the COVID-19 pandemic.

INTRODUCTION

Since the beginning of the 21st century, despite ongoing technological progress, humanity has already faced a number of epidemics that have challenged the global healthcare system.

On May 5, 2023, following a decision by the Coronavirus Disease 2019 (COVID-19) Committee, the head of the World Health Organization announced the end of the COVID-19 pandemic, as the coronavirus disease no longer constituted a public health emergency of international concern. At the same time, experts believe that uncertainty remains due to the potential variability of SARS-CoV-2. Today, COVID-19 can definitely be considered a persistent health problem in many countries.

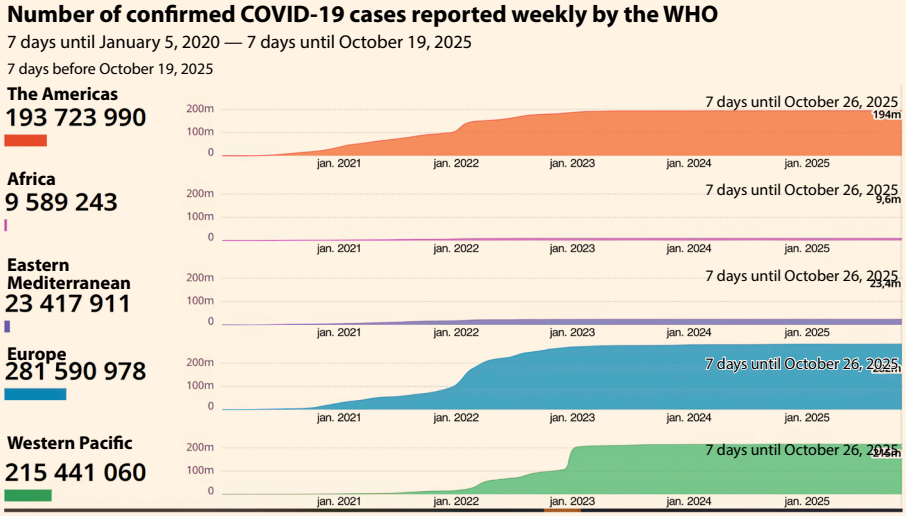

Figure 1. Confirmed cases of COVID-19 as of February 9, 2025 [3]

As of February 9, 2025, there were more than 770 million confirmed cases of COVID-19 (Figure 1).

Ways to "improve" SARS-CoV-2 include:

- SARS-CoV-2 may improve its transmissibility — its ability to infect as many people as possible. This is estimated by the average number of people infected by one person before they are isolated.
- Virulence — the severity of disease symptoms — may also increase.
- In addition, the coronavirus may become better at "evading the immune response" — antibodies and other defense mechanisms, such as T cells. In fact, this has been observed in the human coronavirus 229E.

Chronic non-infectious diseases (CNIDs), in turn, can also be considered in the context of the pandemic, as the number of people suffering from CNIDs is steadily increasing, placing a significant burden on healthcare systems in most countries around the world. Today, there are serious consequences and negative interactions between COVID-19 and CNIDs, which allows these conditions to be classified as a syndemic with a certain degree of assumption.

SYNDEMIC

Syndemic — the interaction between an epidemic and the presence of CNIDs, exacerbated by social, economic, and regional inequalities of many kinds. It is impossible to rely solely on strategies to reduce virus transmission routes in a syndemic, as it is also necessary to address the risk factors for CNIDs [1, 2].

The situation is further aggravated by the fact that there is a certain similarity between the risk factors for CNIDs and the infectious process. Indeed, risk factors for infectious diseases, such as poor eating habits, smoking, alcohol consumption, acute and chronic stress, and physical inactivity, are traditionally also considered risk factors for CNIDs.

In addition, if we recall the localization of angiotensin-converting enzyme 2 (ACE2) receptors, their highest expression is observed in the heart, adipose tissue, and lungs, which explains the more serious negative consequences of COVID-19 in patients with obesity, Chronic Obstructive Pulmonary Disease (COPD), diabetes mellitus, and cardiovascular pathology [4]. Thus, there is a phenomenon of mutual aggravation.

Several studies [5, 6] have reported the persistence of subclinical and/or symptomatic SARS-CoV-2 infection for up to three months after infection. Other researchers [7, 8] found SARS-CoV-2 shedding from both lungs for 4 months and from the gastrointestinal tract for 2 months.

Turning to the terminology of pathologies associated with COVID-19, it is worth paying attention to some of them [9].

POST-COVID SYNDROME

Thus, according to the International Classification of Diseases, 10th Revision, post-COVID syndrome has been assigned code U09.9, “Unspecified condition following COVID-19.” Post-COVID syndrome occurs in individuals after coronavirus infection with confirmed SARS-CoV-2 infection, or in individuals with suspected coronavirus infection, usually 3 months after the onset of COVID-19, with symptoms that last at least 2 months and cannot be explained by an alternative diagnosis.

A period of persistent symptoms lasting 4 weeks or more is referred to as “Long-COVID,” with chronic persistence of the virus in the body.

Since the beginning of the pandemic, millions of people have experienced post-COVID syndrome [10]. According to numerous estimates, post-COVID syndrome develops in 6 out of 100 people who have had COVID-19. These are mainly people who had COVID-19 at the beginning of the pandemic, with the prevalence of the syndrome being extremely variable [11].

Post-COVID syndrome affects virtually all organs and systems (Figure 2). Post-COVID syndrome is characterized by heterogeneous and multi-organ symptoms, which is explained by the peculiarities of its pathogenesis and requires multidisciplinary interaction between specialists in the management of these patients [12].

The pathogenesis of post-COVID syndrome involves several interrelated mechanisms that affect various systems of the body:

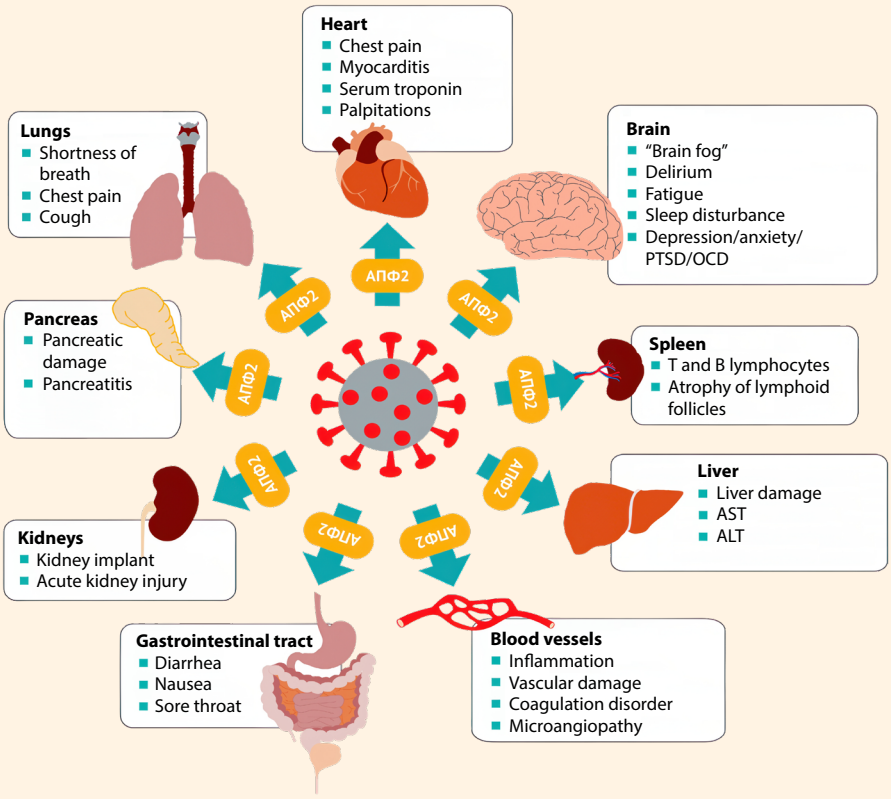

Figure 2. Manifestation of post-COVID syndrome

- chronic thrombovasculitis, which mainly affects the nervous system (central, peripheral, autonomic), lungs, kidneys, and skin. SARS-CoV-2 infects the vascular endothelium, causing direct damage and disrupting its anticoagulant properties. This creates conditions for the formation of microthrombi in the microcirculatory bed and ischemia of organs and tissues;
- in addition, there may be an immune complex response associated with the deposition of immune deposits in the vascular walls of internal organs and activation of the complement system, causing autoimmune inflammation. Persistence of the virus in the body leads to chronic inflammation. Hyperproduction of pro-inflammatory cytokines (interleukin (IL)-6, tumor necrosis factor-α) and an imbalance of Th1/Th17 and B cells causes systemic inflammation. The release of neutrophil deoxyribonucleic acid (neutrophil extracellular traps — NETs) contributes to thrombus formation;
- neurotropicity of the SARS-CoV-2 virus, which, entering the central nervous system perivascularly and transneurally (through the olfactory nerve), directly affects the hypothalamus, limbic complex, cerebellum, and stem structures. This causes disturbances in thermoregulation, sleep, cognitive disorders, and anxiety.

Post-COVID syndrome may progress in conjunction with the development of mast cell activation syndrome. Persistent inflammatory responses, autoimmune mimicry,

and pathogen reactivation, combined with changes in the host microbiome, may contribute to the development of post-COVID syndrome. Systemic inflammatory response syndrome may be a potential cause of organ dysfunction and tissue damage in post-COVID syndrome. Due to the excessive immune response and high pro-inflammatory reaction in COVID-19, relative immunosuppression develops to maintain immunological homeostasis [14, 15], which, in turn, can lead to catabolic syndrome and the development of post-COVID syndrome [16].

Furthermore, Russell B. et al. (2020) showed that transforming growth factor-β (TGF-β), which is an immunosuppressive, profibrotic, and anti-inflammatory cytokine, increases during and after SARS-CoV-2 infection to attenuate excessive pro-inflammatory responses [17].

Prolonged SARS-CoV-2 infection activates T-cells through antigen presentation by antigen-presenting cells [18]. An example of T-cell immune stimulation in post-COVID syndrome is the development of autoimmune thyroid dysfunction [19]. In addition, B-cell activation and the production of antiphospholipid autoantibodies were detected in 52% of patients with post-COVID syndrome [20]. Similarly, autoantibodies were detected in 50% of patients with COVID-19 and post-COVID syndrome, indicating a link with the development of autoimmune diseases, including systemic lupus erythematosus [21].

Lymphopenia has been shown to be associated with persistent SARS-CoV-2 infection and hyperimmune inflammation in patients with post-COVID syndrome [22, 23].

Thus, immune dysregulation, hyperinflammatory reactions, autoimmune mimicry, and pathogen reactivation, combined with changes in the host microbiome, may contribute to the development of post-COVID syndrome.

It has also been proven that the progression of post-COVID syndrome is associated with the development of mast cell activation syndrome [24, 25].

Indeed, post-COVID syndrome was associated with an imbalance between T helper cells and regulatory T cells, as well as an increase in the number of CD8+T cells, which led to the development of an autoimmune reaction that persisted for a long time [26]. An association was found between the imbalance in the T-cell immune response and pulmonary complications, as mature T cells are capable of producing granzyme B, the level of which is elevated in people who have had COVID-19 [27].

Persistent lymphopenia and prolonged high levels of proinflammatory cytokines correlated with the development of headaches, arthralgia, and fatigue — the main symptoms of chronic fatigue syndrome [28]. In turn, chronic fatigue syndrome leads to systemic effects affecting the cardiovascular, respiratory, nervous, and musculoskeletal systems.

In general, the pathogenesis of immune thrombosis in SARS-CoV-2 infection is complex and involves virus-induced damage to endothelial cells along with activation of the coagulation cascade. As described above, against the background of virus-induced activation of inflammatory processes in monocytes and/or macrophages, proinflammatory cytokines (IL-1 and IL-18) are released, activating the cellular link of immunity (neutrophils and platelets). Neutrophils release neutrophil extracellular traps, activate factor XII, and initiate contact-dependent coagulation pathways. Neutrophil extracellular traps can also bind to von Willebrand factor, recruit platelets, and

contribute to coagulopathy. Activated platelets can release proinflammatory cytokines and hypoxia-induced transcription factors that promote thrombus formation [29].

The combination of pro-inflammatory and immunothrombotic pathological processes appears to mediate cardiovascular symptoms in long-term COVID-19. In addition, functionally active autoantibodies against G-protein-associated receptors in long COVID may act as agonists of beta2-adrenoreceptors, alpha1-adrenoreceptors, AT1 angiotensin II receptors, nociceptin-like opioid receptors, or antagonists of muscarinic M2 receptors to exert positive or negative chronotropic effects on the cardiovascular system [30].

Table 1 shows the correlation between symptoms and mechanisms of damage to various systems in post-COVID syndrome.

Table 1.

Correlation between symptoms and mechanisms of damage to various systems in post-COVID syndrome

System	Mechanisms	Manifestations
Nervous	Neurotropism of the virus, autoimmune reactions, hypoxia	Headaches, brain fog, depression, sleep disturbances
Cardiovascular	Endotheliitis, microthrombosis, autonomic nervous system dysfunction	Tachycardia, arrhythmias, orthostatic hypotension
Respiratory	Pulmonary fibrosis, bronchial remodeling	Shortness of breath, feeling of "incomplete inhalation"
Immune	Cytokine storm, Th-cells imbalance, autoimmune reactions	Chronic inflammation, vasculitis
Digestive	Damage to the gastrointestinal tract, dysbiosis, malabsorption	Diarrhea, nausea, abdominal pain

In the process of in-depth study of post-COVID syndrome, certain clinical patterns are identified. For example, some symptoms occur in combination with each other, such as imbalance, severe palpitations, dizziness when standing, and intolerance to exertion (due to postural orthostatic tachycardia syndrome), symptoms of post-exertional malaise, or chronic fatigue syndrome [31].

Indeed, when analyzing the biological basis of post-COVID syndrome and chronic fatigue syndrome, researchers found many similarities between them [32] (Figure 3).

Chronic inflammation in the brain and neuromuscular junctions can lead to prolonged fatigue.

In skeletal muscles, damage to the sarcolemma, fiber damage and atrophy, as well as a number of psychological and social factors can contribute to the development of fatigue.

One of the main manifestations of cardiovascular decompensation is shortness of breath, which requires careful differential diagnosis with respiratory system pathology. The mechanism of shortness of breath involves the continuous production of pro-inflammatory cytokines and active forms of oxygen, damage to the endothelium, and the development of hypercoagulation (Figure 4).

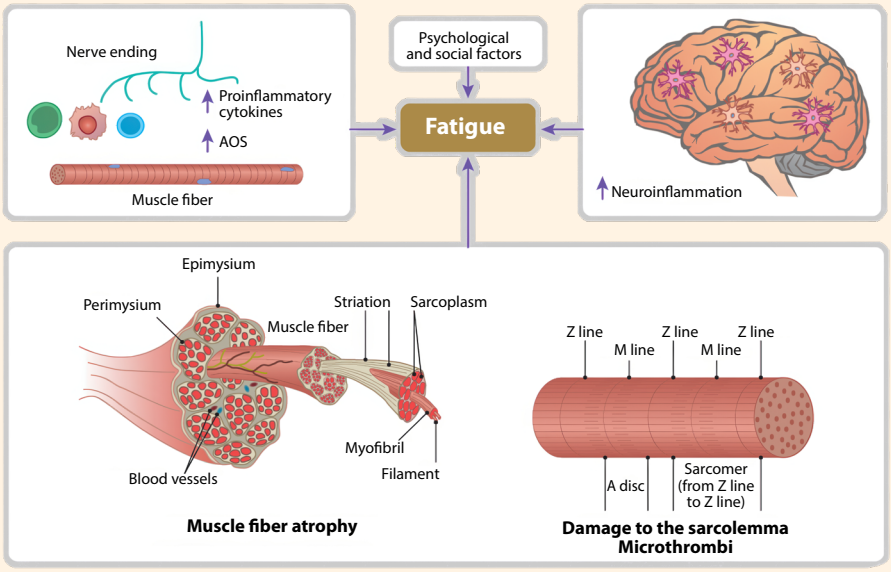

Figure 3. Mechanisms of chronic fatigue syndrome [13]

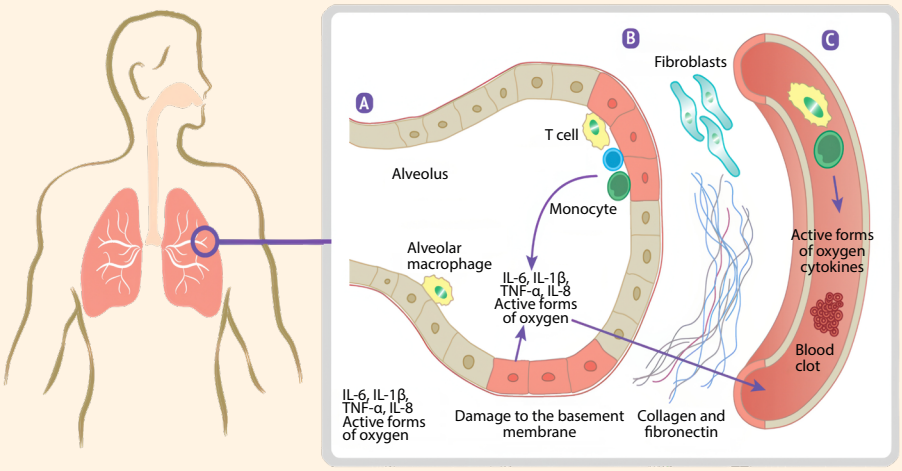

Figure 4. Possible mechanisms of shortness of breath [13]

Damage to the endothelium triggers the activation of fibroblasts, which deposit collagen and fibronectin, leading to fibrotic changes.

Endothelial damage, complement activation, interactions between platelets and leukocytes, release of proinflammatory cytokines, disruption of normal blood coagulation pathways, and hypoxia lead to the development of prolonged hyperinflammatory state and hypercoagulation.

The mechanism of heart damage (Figure 5) involves the production of proinflammatory cytokines and the development of chronic inflammation, fibrosis, and impaired function of the afferent autonomic nervous system.

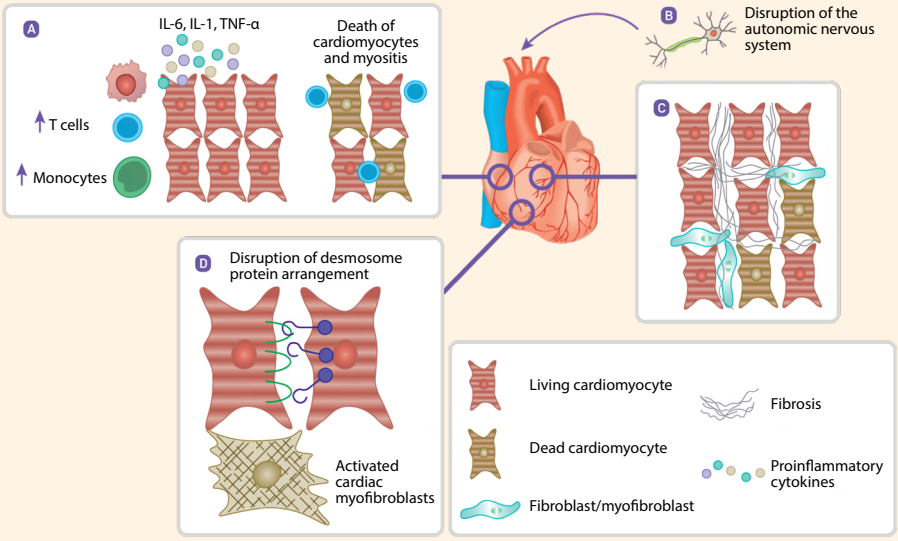

Figure 5. Possible mechanisms of heart damage [13]

Chronic inflammation of cardiomyocytes can result in myositis and cardiomyocyte death. Fibrotic changes are accompanied by an increase in the number of cardiac myofibroblasts, while damage to desmosomal proteins leads to a weakening of intercellular contacts. Prolonged inflammation and cell damage cause fibroblasts to begin intensively secreting extracellular matrix molecules, including collagen, which leads to fibrosis.

Disruption of the afferent autonomic nervous system can cause complications such as postural orthostatic tachycardia syndrome.

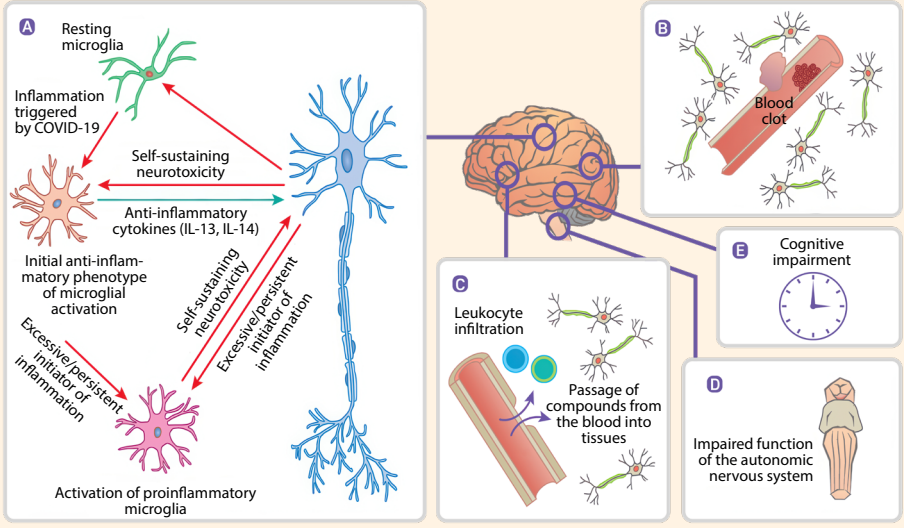

Figure 6. Possible mechanisms of damage to the central nervous system [13]

Chronic damage to neurons, pathological permeability of the blood-brain barrier, and dysfunction of the autonomic nervous system play a role in the mechanism of damage to the central nervous system (Figure 6). A prolonged immune response activates glial cells, contributing to chronic damage to neurons.

Damage to the blood–brain barrier and disruption of its regulation lead to pathological permeability, allowing blood-borne compounds and lymphocytes to penetrate the brain parenchyma. Hyperinflammation and hypercoagulation increase the risk of thrombotic events. Cognitive impairment in post-COVID syndrome is a common phenomenon, affecting up to 45% of people who have had COVID-19. It manifests itself in the form of decreased memory, concentration, and thinking speed, and is often accompanied by asthenic and psychoemotional disorders.

COVID-19 has the potential to affect the gut microbiome, including enrichment with opportunistic pathogens and depletion of beneficial commensals. The ability of the gut microbiota to alter the course of respiratory infections (gut-lung axis) has been recognized previously in influenza and other respiratory infections. Research is currently underway to assess the long-term effects of COVID-19 on the gastrointestinal tract, including irritable bowel syndrome and dyspepsia.

One of the characteristics of post-COVID joint syndrome is that exposure to coronaviruses is generally more associated with the development of arthralgia and myalgia than with clinical arthritis [33].

In one of the studies by Ciaffi J. et al. (2020), the frequency of arthralgia (2.5% of patients) was reported specifically as a symptom of COVID-19, with the researchers clearly differentiating arthralgia from myalgia [34].

A cohort study conducted in the United States included 153,848 patients who survived the first 30 days after SARS-CoV-2 infection. Psychiatric morbidity was compared with individuals without SARS-CoV-2 infection (n=5,637,840). Patients who had COVID-19 were found to have an increased risk:

- anxiety disorders by 35%;
- depression-related disorders by 39%;
- neurocognitive decline by 80%;
- sleep disorders by 41%.

It is important to note that the increase in the incidence of psychiatric disorders was characteristic of both patients who required hospitalization and those who were receiving outpatient care [35].

Experts from the World Health Organization emphasize that the symptoms of post-COVID syndrome may change from time to time, either weakening or exacerbating.

It cannot be ruled out that some individuals may develop a chronic form of immune system defects as part of post-COVID syndrome, which may persist for several years.

There are also musculoskeletal manifestations of post-COVID syndrome. The activation of pro-inflammatory cytokines (IL-6, IL-1, tumor necrosis factor-α) plays an important role in their development).

The pathogenesis of post-COVID joint syndrome is based on an autoimmune component, which is represented by the hyperproduction of pathogenic antiphospholipid and antinuclear autoantibodies. Partially overlapping clinical, pathological, and se-

rological manifestations reflect a certain similarity between the immunopathological mechanisms of COVID-19 and immunoinflammatory rheumatic diseases [36–39].

Di Filippo L. et al. (2023) demonstrated that low vitamin D levels increased the risk of developing post-COVID syndrome. In a multiple regression analysis, low vitamin D levels were the only variable associated with post-COVID syndrome. In patients with this condition, 25-hydroxyvitamin D levels were 13% lower than in people without post-COVID syndrome. Among patients with vitamin D deficiency and post-COVID syndrome, 25-hydroxyvitamin D levels were 16.5% lower than among participants without post-COVID syndrome. Significantly lower levels of 25-hydroxyvitamin D were also found in patients with neurocognitive symptoms, which included headache and mental confusion [40].

As mentioned earlier, post-COVID syndrome is characterized by heterogeneous and multi-organ symptoms, which requires multidisciplinary interaction between specialists in the treatment and rehabilitation of this category of patients.

The role of the therapist/general practitioner in the management of patients with post-COVID syndrome cannot be overestimated. The scope of care for such patients includes comprehensive diagnosis, development of individualized therapy, coordination of interdisciplinary care, and prevention of complications.

Today, the medical community recognizes that there is no specific drug treatment for post-COVID syndrome.

Against the backdrop of developed post-COVID syndrome, the exacerbation of CNID dictates drug correction of the existing chronic disease in order to compensate for it in accordance with current clinical protocols for diagnosis and treatment:

- diabetes mellitus;
- chronic heart failure;
- arterial hypertension;
- coronary heart disease;
- chronic obstructive pulmonary disease, bronchial asthma, etc.

In December 2020, the National Institute for Health and Care Excellence (MCE) published guidance on managing the long-term effects of COVID-19. A multidisciplinary approach (addressing physical, psychological, and psychiatric aspects) to the rehabilitation of patients with post-COVID-19 syndrome was considered [41].

On January 25, 2024, the latest update to the above recommendations was released, which remained largely unchanged.

Back in the 10th version of the Temporary Methodological Recommendations "Prevention, Diagnosis, and Treatment of the Novel Coronavirus Infection (COVID-19)," a new chapter appeared on dispensary observation of patients with COVID-19.

The 14th version of the Temporary Methodological Recommendations "Prevention, Diagnosis, and Treatment of the Novel Coronavirus Infection (COVID-19)" dated December 27, 2021, there is an extremely important chapter entitled "Features of Dispensary Observation and In-Depth Dispensary Care for Citizens Who Have Had the Novel Coronavirus Infection COVID-19."

The measures included in in-depth medical examinations allow for the timely detection of changes in the functioning of various organ systems, as well as possible complications after suffering from the novel coronavirus infection, i.e., post-COVID manifestations (Table 2).

Table 2.

Studies within the framework of an in-depth medical examination program for individuals who have had a novel coronavirus infection

Research method	Comments
Stage 1 of medical examination	
Blood oxygen saturation at rest (saturation)	All citizens with a saturation level of 94% or less are recommended to undergo CT and echocardiography as part of the second stage of medical examination
6-minute walk test	It is performed when the initial blood oxygen saturation is above 94% in combination with the patient's complaints of shortness of breath and edema that have appeared for the first time or have increased in intensity. When walking a distance of less than 550 meters, an echocardiogram is indicated as part of the second stage of medical examination
Spirometry	All citizens
Chest X-ray	To be done if not done earlier in the year
General (clinical) blood test	All citizens
Biochemical blood test: total cholesterol, low-density lipoproteins, C-reactive protein, ALT, AST, creatinine	All citizens
Determination of D-dimer concentration in blood	It is performed on persons who have suffered from moderate to severe cases of the new coronavirus infection with an increase in D-dimer levels by more than 1.5–2.0 times relative to the upper limit of normal. Duplex scanning of the veins of the lower extremities is indicated
Stage 2 of medical examination	
Duplex scanning of the veins of the lower extremities	It is performed when the D-dimer level in the blood increases by more than 1.5–2.0 times relative to the upper limit of normal
Computed tomography of the chest organs	It is performed if the saturation level at rest is 94% or less
Echocardiography	It is performed if the saturation level at rest is 94% or less, as well as based on the results of a 6-minute walk test

CONCLUSION

Thus, despite the end of the COVID-19 pandemic, the problem caused by various consequences of post-COVID syndrome continues to persist in real clinical practice. Given the diversity of clinical manifestations of this condition, as well as the more frequent development of post-COVID syndrome in predisposed individuals, particularly those suffering from CNIDs, it is therapists and general practitioners who are the first to encounter the phenomena of post-COVID syndrome.

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Chapter 6

COVID-19 and Interstitial Lung Diseases

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ABSTRACT

Damage to lung tissue can be attributed to the leading pathogenetic process in COVID-19 coronavirus infection. Currently, the evolution of the pathogen has led to changes in the clinical picture of the infection, but questions of differential diagnosis of interstitial lung damage of infectious and non-infectious origin, which manifest themselves in similar clinical data, remain important for practical healthcare. Also significant for practical healthcare is the identification of the specific effects of COVID-19 coronavirus infection on the course of interstitial lung diseases.

INTRODUCTION

Interstitial lung diseases (ILD) are a heterogeneous group of diseases characterized by the predominance of inflammation or the development of fibrosis in the lung parenchyma. There are many potential etiological factors that contribute to the development of ILD, including systemic connective tissue diseases (SCTD), occupational or environmental exposures, medications, radiation therapy, and viral infections [1].

COVID-19 (CoronaVirus Disease 2019) is an infectious disease caused by the SARS-CoV-2 (Severe Acute Respiratory Syndrome CoronaVirus 2) coronavirus, which predominantly affects lung tissue. At the beginning of the COVID-19 pandemic, the disease was associated with a high risk of mortality for a significant number of patients, but after three years of fighting SARS-CoV-2, treatment strategies and vaccines have been developed, which has made COVID-19 a manageable disease.

However, since a number of patients with COVID-19 experience severe forms of the disease with the development of acute respiratory distress syndrome (ARDS), often contributes to the progression of chronic respiratory diseases and can lead to per-

manent changes in the lung parenchyma, it should be recognized that COVID-19 still requires close attention from pulmonologists [1].

In the context of the COVID-19 pandemic, it has become clear that there are certain difficulties in the differential diagnosis between ILD and viral pneumonia caused by SARS-CoV-2. It is particularly difficult to differentiate between COVID-19 and certain types of newly developed or exacerbated pre-existing ILD [2]. In addition, it should be noted that viral infections are considered to be trigger factors for the development of ILD and the risk of its exacerbation [3].

DIFFERENTIAL DIAGNOSIS OF COVID-19 AND INTERSTITIAL LUNG DISEASES

Nonproductive cough and shortness of breath are the main clinical symptoms in patients with ILD, while fever and rapid progression of symptoms are common in organizing pneumonia and acute interstitial pneumonia [4, 5]. In addition, patients with ILD associated with SCTD (SCTD-ILD) often experience fever with extrapulmonary manifestations, such as skin, musculoskeletal, and renal involvement [6]. The differential diagnosis of these diseases from COVID-19 is significantly complicated by the presence of the above clinical manifestations in ILD.

It is currently believed that COVID-19 causes acute lung damage and inflammatory changes in the pulmonary interstitium, with possible subsequent development of pulmonary fibrosis (PF). There is also discussion of the widespread prevalence of post-COVID syndrome, which is characterized by the prolonged persistence of symptoms such as weakness, shortness of breath, and persistent cough [7].

Therefore, according to a large prospective cohort study by Munblit D. et al. analyzing the condition of patients who had COVID-19 (n=2,649), it was found that 6–8 months after discharge from the hospital, approximately 50% had persistent symptoms, the most common of which were chronic weakness (25%) and respiratory problems (17.2%), which can mimic the picture of ILD and lead to difficulties in differential diagnosis [8].

The likelihood of a patient having COVID-19 increases with a corresponding epidemiological history, acute onset of the disease, and symptoms such as fever, general weakness, and myalgia, as well as signs of upper respiratory tract and gastrointestinal tract involvement. In addition, leukopenia, lymphopenia, and significantly elevated levels of C-reactive protein, D-dimer, and ferritin are typical for COVID-19 [2].

From the point of view of laboratory diagnostics, it is important to determine the level of autoantibodies to verify SCTD-ILD, but it should be noted that elevated levels of SCTD biomarkers have been described in a number of patients with severe COVID-19 [6]. In particular, according to a prospective study by Gagannis D. et al., antinuclear antibody titers ≥1 : 320 and/or immunoblots of extractable nuclear antibodies were detected in 84.6% of patients with COVID-19 and ARDS, as well as in 11.1% of patients with COVID-19 without ARDS (p=0.002) [6].

It should also be remembered that many SCTD cases are characterized by elevated C-reactive protein levels and abnormalities in clinical blood tests. From an imaging

diagnostics perspective, a number of difficulties often arise, as COVID-19 and ILD can have similar patterns. In typical cases, changes in high-resolution computed tomography (HRCT) findings in COVID-19 are represented by bilateral multilobar areas of "ground glass" with peripheral and/or posterior basal distribution. In the later stages of the disease, thickening of the intralobular and interlobular septa, the "cobblestone" symptom, areas of consolidation, and bronchial dilatation are also common. In some patients, pleural effusion, lymphadenopathy, and the "halo sign" may occur [9–12].

HRCT changes such as widespread areas of "ground glass," reticular changes, areas of consolidation, and symptoms such as the "halo sign," "cobblestone pattern," and signs of fibrosis are typical changes in many ILDs. For this reason, it is not always possible to differentiate between COVID-19 and lung changes associated with previous COVID-19 infection and ILD based on the HRCT pattern alone, without data on medical history, clinical presentation, and laboratory parameters.

Thus, a combination of factors plays a key role in the differential diagnosis of COVID-19 and ILD: characteristics of the onset of the disease, clinical manifestations, HRCT changes pattern, as well as laboratory data and SARS-CoV-2 identification [2].

THE IMPACT OF COVID-19 ON THE COURSE OF INTERSTITIAL LUNG DISEASES

The consequences of COVID-19 for patients with ILD are not limited to delayed diagnosis and obstacles to treatment due to isolation measures. Taking into account the characteristics of course of chronic ILD and COVID-19, it can be assumed that patients with pre-existing ILD may be more susceptible to SARS-CoV-2 infection. According to many studies, previous ILD had a consistently negative impact on the clinical course of COVID-19 [1].

There are several possible reasons for the poor prognosis of COVID-19 in ILD. First, it is assumed that patients with ILD have a worse prognosis due to reduced lung reserve and impaired gas exchange. In addition, increased expression of angiotensin-converting enzyme 2 (ACE2) genes, as well as synthesis of interleukin (IL)-6 and type 1 interferon in cells, has been reported in patients with ILD.

Finally, in patients with ILD, especially idiopathic pulmonary fibrosis (IPF), high levels of avb6 integrins are observed in the alveolar epithelium, which is associated with a poor prognosis; avb6 integrins also include a binding site for the SARS-CoV-2 virus [13]. These data may also explain the more unfavorable prognosis of ILD exacerbations associated with previous COVID-19. Moreover, many patients with COVID-19 show signs of ARDS in the form of acute respiratory failure developing against the background of a respiratory viral infection, which requires respiratory support, which also contributes to the poor prognosis of the disease [14].

According to a large national study conducted in Korea involving patients with confirmed COVID-19 (n=8070), the proportion of ILD is significantly higher than that in the cohort of patients with confirmed COVID-19 (0.8% vs 0.4%; odds ratio — 2.02; 95% confidence interval (CI) 1.54–2.61; p<0.001).

At the same time, patients with COVID-19 and concomitant ILD more often had severe COVID-19 compared to those without ILD (47.8% vs. 12.6%), as well as higher mortality (13.4% vs. 2.8%) (for all indicators — $p<0.001$) [15].

Higher mortality rates among patients with ILD and severe COVID-19 are also shown in a study by Pruneda A.K.S. et al.; a statistically significant difference in COVID-19 mortality was found between patients with pre-existing ILD and those without such a diagnosis (63% vs. 33%; $p=0.007$) [16]. Cilli A. et al. confirmed that patients with IPF are a high-risk group for COVID-19. The study included patients with IPF and COVID-19 ($n=46$), 24 (52.1%) of whom required hospitalization, 16 (66.6%) were admitted to the intensive care unit, 10 (41.6%) underwent invasive mechanical ventilation, and 13 (28.2%) died from complications of COVID-19. Risk factors for fatal outcomes included a decrease in the ratio of carbon monoxide diffusion capacity (DLCO) to alveolar ventilation volume (VA), prolonged oxygen therapy, and the detection of consolidation on HRCT of the chest organs ($p<0.05$) [17].

A large study involving patients with COVID-19 and a diagnosis of ILD ($n=133,526$) investigated the association between different types of ILD and the risk of fatal outcome in COVID-19. The most common ILD in the study was IPF ($n=74,783$), followed by Sjögren's syndrome ($n=47,327$) and systemic scleroderma ($n=5,639$) with lung involvement. It was found that the risk of death was increased for all ILD subtypes (IPF, hypersensitivity pneumonitis, ILD with rheumatoid arthritis), with the exception of ILD associated with Sjögren's syndrome, in which the overall mortality rate was lower than that in the comparison group. The most pronounced trend toward an increased risk of death was observed in IPF and ILD in systemic scleroderma [18].

It should be noted that viral infections are one of the possible causes of ILD exacerbations, significantly worsening the prognosis for patients, especially in the presence of a pattern of usual interstitial pneumonia [19, 20]. At the same time, due to the similarity of clinical manifestations and radiological changes, severe viral pneumonia is difficult to distinguish from exacerbation of ILD [21, 22].

According to a multicenter observational study ($n=137$) conducted in Spain, a significantly higher incidence of clinical deterioration requiring hospitalization was observed in patients with ILD and COVID-19 infection compared to patients without COVID-19 (55% vs. 11%, respectively; $p=0.002$). In addition, the adjusted risk of death was the highest in patients with IPF compared to SCTD-ILD and other ILDs [23].

Another retrospective study involving 102 patients showed that after COVID-19, there was a significant increase in the prevalence of HRCT changes (as assessed by Warrick's semi-quantitative method) and progression of fibrotic changes, especially in the group of patients with IPF [24].

A multicenter retrospective study conducted in Japan analyzed data from patients with exacerbated ILD hospitalized in 134 hospitals. No difference in the total number of hospitalizations was found between 2019 ($n=894$) and 2020 ($n=854$), but it was shown that in 2020 exacerbations associated with COVID-19 had a significantly worse prognosis compared to exacerbations not associated with COVID-19 in terms of both 30-day ($p=0.0071$) and 90-day ($p<0.0001$) mortality [13].

INTERSTITIAL CHANGES IN THE LUNGS AFTER COVID-19

It is well known that ARDS can be followed by non-progressive PF with persistent functional and radiological changes [25], including ventilator-associated lung injury [26]. Risk factors include age, severity of viral pneumonia, and duration of mechanical ventilation. A distinctive feature of the post-COVID condition is that a history of ARDS or mechanical ventilation is not necessary for the development of fibrosis [27].

Although various respiratory viral infections are considered a risk factor for the development of PF, the molecular mechanisms underlying the formation of PF after viral infections have not yet been fully studied [1].

When SARS-CoV-2 enters the respiratory tract, the spike proteins on the surface of the virus bind to the ACE2 receptors of the host cells. Type 2 alveolar epithelial cells are one of the cell types that express ACE2 receptors and become target cells for SARS-CoV-2 infection. ACE2 receptors are regulators of the renin-angiotensin system. ACE2 converts angiotensin I (AT-I) to angiotensin II (AT-II), which has pro-inflammatory and profibrotic properties by activating various signaling pathways, such as transforming growth factor beta (TGF-β), IL-1β, tumor necrosis factor alpha (TNF-α), IL-6, and IL-8 [28, 29].

In addition to ACE2 receptors, SARS-CoV-2 can infect host cells through other receptors, such as integrins αvβ3 and -6. Integrin αvβ6 promotes the differentiation of fibroblasts into myofibroblasts and TGF-β1-mediated epithelial-mesenchymal transition, which plays a central role in the pathogenesis of IPF [30]. Thus, binding of SARS-CoV-2 to these integrins may trigger fibrogenesis.

TGF-β is one of the profibrotic mediators secreted by damaged and endothelial cells and activated inflammatory cells, and plays a central role in the pathogenesis of PF. It promotes the migration and accumulation of fibroblasts in the damaged area, the recruitment of circulating fibrocytes, and the differentiation of epithelial and endothelial cells through the endothelium-mesenchymal transition. TGF-β also causes the activation and proliferation of fibroblasts, their differentiation into myofibroblasts, and the deposition of extracellular matrix, leading to damage to the basement membrane and abnormal reparation [31].

Oxidative stress is considered another cause of alveolar epithelial cell damage in the context of COVID-19. Hyperoxia promotes the production of reactive oxygen species in mitochondria. Reactive oxygen species activate TGF-β, which in turn triggers fibroblast differentiation. On the other hand, hypoxia can also cause PF through epithelial-mesenchymal transition induced by hypoxia-inducible factor-1α [32]. Thus, excessive oxygenation, especially during mechanical ventilation, as well as hypoxia caused by pneumonia in severe COVID-19 patients, may contribute to the formation of PF.

When analyzing the morphological pattern of changes in the lungs after COVID-19, according to the results of a study by Konopka K.E. et al., the most common pattern was ordinary interstitial pneumonia. According to the morphological conclusion, in some cases, desquamative interstitial pneumonia, acute and organizing bronchopneumonia, or no morphological abnormalities were observed [33].

According to some studies, the earliest CT signs that are characteristic of PF are detected 3 weeks after COVID-19. The severity of the changes varies depending on the severity of the acute phase of the disease [34, 35].

In 55 (71%) patients who had COVID-19, Zhao Y.M. et al. identified reticular changes in the lungs [36]. According to Hu Q. et al., 35% of patients had reticular changes 2 weeks after the onset of COVID-19 [37]. The same study provides data on patients who showed a significant reduction in the severity of changes according to HRCT data within 1 month after COVID-19. However, 54% of patients had visualizable interstitial changes in the lungs one month after clinical recovery, among which the most common were areas of reticular changes alone and/or in combination with focal/multifocal areas of "ground glass" [37, 38].

According to Wang Y. et al., the most typical CT pattern among patients with post-COVID-19 was organizing pneumonia combined with reticular changes, which most often regressed spontaneously over time [39]. It is reported that these changes are not pathognomonic for lung damage in COVID-19 and are found in other viral pneumonias. Thus, a similar picture can be observed in viral pneumonia caused by influenza A subtype H1N1 [40].

According to a prospective longitudinal study by Han X. et al., 40 (35%) of 114 surviving patients with severe COVID-19 had fibrosis-like changes in their lungs during the 6 months of follow-up after the onset of the disease [41]. During one year of observation, 9 of 62 (15%) participants experienced shortness of breath during exercise, 7 (78%) of whom had fibrotic changes according to HRCT data of thoracic organs. Thirteen of 53 (25%) had decreased DLCO, especially among patients with signs of PF according to HRCT (11 (85%) of 13) [42].

According to a number of studies, CT images of residual changes in lung tissue were evaluated at specific intervals after severe COVID-19 pneumonia. Thus, Baratella E. et al. noted that 3 months after discharge from the hospital, the most common findings on HRCT were linear atelectasis (84%), areas of "ground glass" (75%), reticular changes (34%) with symmetrical distribution mainly in the lower lobes of the lungs. In particular, during one year of observation, the majority of patients showed improvement in radiological indicators [43].

More severe COVID-19 was associated with abnormalities on HRCT after 3 months. However, signs of PF were observed in only a small number of patients. There was improvement in the radiographic picture in most cases during the 1-year follow-up period [44].

CT characteristics of residual changes in the lungs 5-7 months after severe COVID-19 pneumonia were evaluated in a retrospective cohort study involving (n=405) patients who survived severe COVID-19 pneumonia. In 225 (55.6%) patients, no pathology was detected on HRCT, while in 152 (37.5%) patients, non-fibrotic changes were detected, in 18 (4.4%) — fibrotic changes, and in 10 (2.5%) — post-ventilation changes (cicatricial emphysema and bronchiectasis in the anterior sections of the upper lobes). Among non-fibrotic changes, the most common were areas of "ground glass" resembling non-fibrotic nonspecific interstitial pneumonia with or without signs of organizing pneumonia. The most common signs of the fibrotic process were subpleural reticular changes, traction bronchiectasis, and areas

of "ground glass" resembling the fibrotic pattern of nonspecific interstitial pneumonia [45].

The UKILD study analyzed the frequency of lung damage in the post-COVID period. It was found that in 79.4% of patients, residual changes in the lungs exceeded 10% on average 113 days after discharge. According to HRCT data, "ground glass" areas predominated, accounting for an average of 25.5% of cases, while reticular changes were detected in 15.1% of cases. A repeat CT scan was performed on 33 patients at least 90 days later: upon repeat imaging, the prevalence of reticular changes and areas of "ground glass" did not change significantly. The risk of residual lung abnormalities was higher in men and individuals over 60 years of age, as well as in patients with severe COVID-19, a decrease in DLCO <80% of the normal level, and the presence of changes in lung tissue according to X-ray examination of the thoracic organs [46].

Huang Y. et al. showed that 75% of patients who had suffered from severe COVID-19 during a 30-day observation period experienced a decrease in respiratory function indicators, primarily DLCO. The decrease in DLCO was positively correlated with changes in the pulmonary parenchyma according to HRCT data and depended on the severity of the acute phase of COVID-19 [47].

Longer-term studies may help track the dynamics of changes in the development of ILD associated with post-COVID-19. The duration of observation in one such prospective study involving patients who had COVID-19 (n=83) was 12 months. Most patients showed improvement in lung function, but after 12 months, 1/3 of them had a DLCO of <80%, and 11% had a forced pulmonary vital capacity (FPVC) of <80%. After 3 months, residual CT changes were observed in 65% of patients, with 78% of cases showing areas of "ground glass," 34% showing thickening of the septa, and 33% showing reticular changes. After 9 months, 20% of patients still had changes according to HRCT data, but none of them had a confirmed diagnosis of fibrotic ILD or progression of changes. The predominant pattern of changes remained areas of "ground glass," and no further improvement was observed between the 9th and 12th months [48].

Lee J.H. et al. conducted a meta-analysis of 30 out of 18,062 studies devoted to investigating the prevalence of pulmonary implications of COVID-19, taking into account functional indicators and ILD of thoracic organs, conducted at least 6 months after the initial infection. A significant number of people who survived COVID-19 experienced chronic pulmonary complications in the post-COVID period. The most common abnormality was DLCO impairment: the overall prevalence was 35% (95% CI 30–41%), 6 months after COVID-19, DLCO decreased by 39% (95% CI 34–45%), and after 12 months, it was ≤31% (95% CI 21–40%), with the difference not being statistically significant (p=0.115). Restrictive pulmonary function impairment, manifested by a decrease in FPVC, was less common (total prevalence — 8%; 95% CI 6–11%), but its prevalence was lower at 12 months of follow-up compared to 6 months of follow-up (95% CI 3–7%) vs. 13% (95% CI 8–19%); p=0.006). During subsequent ILD of thoracic organs, the overall prevalence of persistent changes in the form of "ground glass" areas and CT signs of PF was 34% (95% CI 24–44%) and 32% (95% CI 23–40%), respectively, and this prevalence did not decrease over time [49].

A high prevalence of lung pathology according to ILD of thoracic organs data within 1 year after COVID-19 was established based on the results of a meta-analysis

of 21 studies and data from 1,854 patients. The most common pattern (2.4–67.7%) was the presence of "ground glass," while the prevalence of traction bronchiectasis was 1.6–25.7%, and "honeycomb lung" was even less common (0–1.1%) [50].

It should be noted that, at present, it is impossible to confirm the irreversibility of changes after COVID-19 due to the lack of data on long-term follow-up of patients after discharge from the hospital. It has also been shown that ILD changes in the affected areas in the acute phase of COVID-19 are subject to significant regression over time [51, 52]. The basis for classifying CT changes after COVID-19 should probably be the dominant CT pattern: predominance of "ground glass," a combination of "ground glass" and fibrotic changes, predominance of fibrotic changes (reticular changes, traction bronchiectasis, formation of "honeycomb lung") [53].

Figures 1, 2 show ILD results from the authors' personal archive. The images clearly illustrate the heterogeneity of interstitial changes in the lungs 3–6 months after COVID-19.

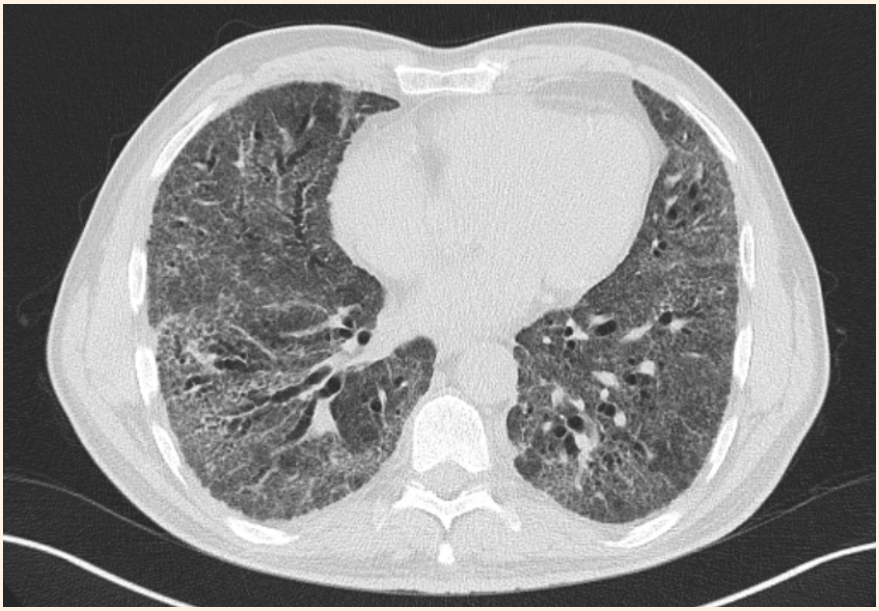

Figure 1. Female patient, born in 1952, 4 months after suffering from severe COVID-19. Computed tomography of the chest organs, axial section: areas of "ground glass" combined with reticular changes

The main predictors of interstitial changes in the lungs after COVID-19 include advanced age, concomitant severe chronic diseases, prolonged hospitalization and the need for respiratory support, as well as a history of alcohol abuse and long-term smoking [54]. The severity of interstitial changes in the lungs and the likelihood of developing PF directly depended on the extent of damage to the lung parenchyma and the severity of the systemic inflammatory response in the acute phase of COVID-19 [55]. Thus, high IL-6 levels in the context of COVID-19 may serve as a predictor of LF development during subsequent observation [56].

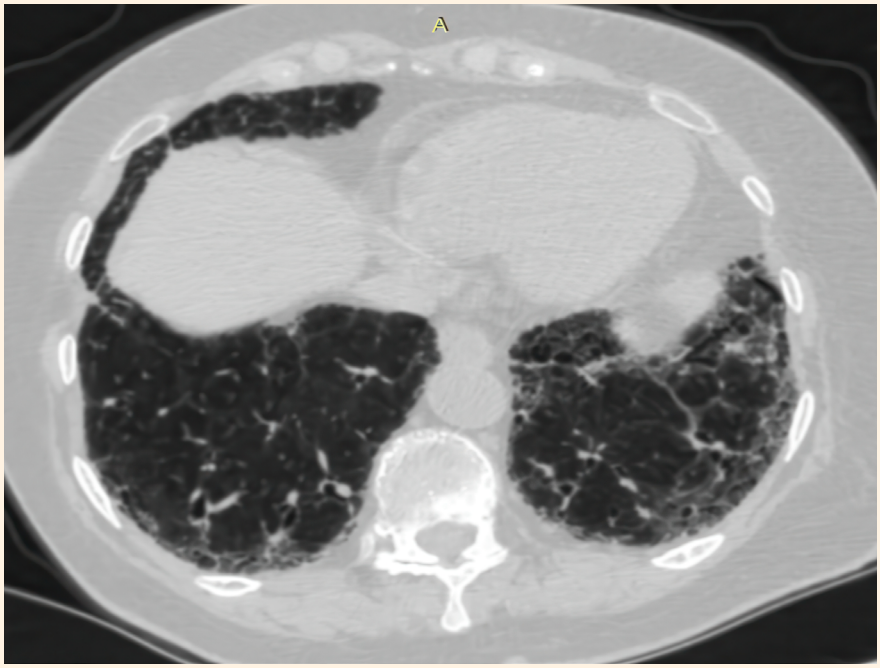

Figure 2. Female patient K., born in 1960, 6 months after suffering from severe COVID-19. Computed tomography of the chest organs, axial section: reticular changes, traction bronchiectasis

The following important question remains unanswered at this time: to what extent can the results of observations of the first patients infected with more virulent strains be extrapolated to new COVID-19 patients? Given the significant reduction in the severity of clinical manifestations and the risk of fatal outcomes of COVID-19 in recent times, it is likely that the prevalence of ILD associated with COVID-19 will decrease significantly.

CONCLUSION

The issue of ILD and COVID-19 remains extremely relevant at the moment, given the difficulties of differential diagnosis of the acute phase of COVID-19, changes in the post-COVID period, and various nosological forms of ILD, as well as the significant impact of COVID-19 on the course of the underlying disease in patients with an established diagnosis of ILD. When differentiating between ILD and viral pneumonia caused by SARS-CoV-2, it is important to consider a combination of various characteristics: the features of the onset of the disease, clinical data, changes in HRCT data, as well as laboratory data and identification of SARS-CoV-2. Most often, after COVID-19, HRCT shows reticular changes and areas of “ground glass” in the lungs. To date, it is impossible to say that the changes after COVID-19 are irreversible, but a number of studies have shown that HRCT changes in the affected areas during the acute phase of COVID-19 are subject to significant regression over time.

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Chapter 7

Neurological Manifestations of COVID-19

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ABSTRACT

Damage to the nervous system associated with COVID-19 is one of significant manifestations of this infection. Neurological symptoms described in COVID-19 include those arising from the central nervous system, the most common being stroke symptoms. Symptoms involving peripheral nerves include taste and smell disorders as well as involvement of neuromuscular system. Knowledge of the pathophysiology of early nervous system involvement as well as factors affecting outcomes of the disease help improve early diagnosis and prevention of nervous system dysfunction in COVID-19.

INTRODUCTION

With the increase in the number of patients suspected of having COVID-19 at the beginning of the pandemic, hospitals in the United States opened specialized wards for this group of patients, and in some cities with the highest prevalence, such as New York, all traditional wards were repurposed for the treatment of COVID-19 patients. Doctors from different specialties were also regrouped for the purpose of treating COVID-19 patients. It is worth noting that the number of patients with neurological symptoms who sought help at that time decreased, probably due to fear of being admitted to hospitals overflowing with infected patients, since at the beginning of the pandemic, mainly patients with more severe symptoms continued to be admitted to hospitals.

Hospitalized patients were tested for COVID-19, and with improved diagnostics, our and other research teams were able to describe the spectrum of neurological man-

Table 1.

Neurological symptoms described in COVID-19

Localization	Symptoms
Central nervous system	Headache Dizziness Stroke symptoms* Seizures Clouding of consciousness Agitation Delirium Stupor Coma
Peripheral nerves	Disturbance of taste Disturbance of smell General weakness
Neuromuscular system	Myalgia Weakness

*Note: According to unpublished data from a research group in Memphis, USA, 7% of all hospitalized COVID-19 patients in the first year of the pandemic were admitted with primary stroke symptoms even before pulmonary complications were detected.

ifestations in the early stages of the COVID-19 pandemic [1–3]. These symptoms and their localization are shown in Table 1.

PATHOPHYSIOLOGY OF EARLY LESIONS OF THE NERVOUS SYSTEM

Just like the heart, lungs, and intestines, the brain and skeletal muscles express angiotensin-converting enzyme 2 (ACE2), which may increase their susceptibility as potential targets for viruses that cause severe acute respiratory syndrome, including COVID-19 [3, 4]. Proposed neurotropic mechanisms include viral access to the central nervous system (CNS) via the systemic bloodstream or through the cribriform plate of the ethmoid bone, leading to symptoms of smell and taste disorders (hyposmia and hypogeusia). It is also suggested that viral neuroinvasion and subsequent central neuronal damage contribute to acute respiratory distress syndrome in patients with COVID-19 [5]. Moreover, SARS-CoV-2 virus adhesion to ACE2 receptors is particularly significant in cases of intracerebral hemorrhage due to receptor inactivation and subsequent dysfunction in blood pressure regulation [2]. In severely infected patients, coagulopathy and prolonged prothrombin time due to disseminated intravascular coagulation may contribute to an increased risk of secondary intracranial hemorrhage.

In cases of ischemic stroke, potential mechanisms include hypercoagulation associated with inflammation, activation of the endothelium and platelets, dehydration, and cardioembolism due to viral damage to the heart [6, 7].

Table 2.

Probable mechanisms leading to cerebrovascular pathology in COVID-19, according to research data [11–19]

Mechanism	Stroke	Hemorrhage	CVT
Inactivation of ACE2	Dysregulation of blood pressure and endothelial function		
Coagulopathy	Thrombosis in situ	Long-term use of anticoagulants	Thrombosis in situ
CNS — vasculitis and endotheliitis	Vascular stenosis	Fragility of blood vessels	
Viral heart disease	Cardioembolism		

Hypoxemia and the development of intrapulmonary vasodilation, leading to right-to-left shunting of blood, may further exacerbate neuronal damage [8]. This shunting was detected in 83% of patients with severe pulmonary COVID-19 using intravenous contrast and robotic transcranial Doppler ultrasound [8].

Additional mechanisms have been identified that may explain why acute cerebrovascular accident develops along the path of ischemic stroke, cerebral hemorrhage, or cerebral venous thrombosis (CVT) (Table 2).

There was an increase in the number of patients with multiple foci of ischemic stroke and unusual forms and locations of intracranial hemorrhages due to various mechanisms, which led clinicians to suspect COVID-19.

Previously rare cases of CVT became more frequent with COVID-19 infection, and clinicians began to detect CVT in patients with headache or clouding of consciousness, especially with high D-dimer levels (>2.0 ng/ml) [9].

INITIAL OBSERVATIONS OF THE NATURE OF NEUROLOGICAL DISORDERS

Although peripheral nerve lesions were among the first symptoms to be recognized (loss of smell), the most widely studied manifestations of the disorder are neurological manifestations of CNS disorders, particularly severe symptoms mainly associated with ischemic stroke, cerebral hemorrhage, and cerebral venous thrombosis [9].

An initial decline in hospitalizations of stroke patients and in the number of treatments with intravenous thrombolysis and thromboextraction was observed only in the first two months of the pandemic in the United States [10]. After this period, there was an increase and return in the number of stroke patients admitted [10]. The difference between epidemiological studies of stroke development in all patients admitted with COVID-19 [9, 10] and our observations (data from 0.5, 1.48, 5% versus 7% in our clinic) may be due to insufficient diagnostic accuracy at the beginning of the pandemic and the repurposing of most hospitals at a time when our group in Memphis continued to operate uninterruptedly as a multidisciplinary stroke treatment center.

Despite an initial decline in hospitalizations for stroke, some trends were observed in the predisposition of SARS-CoV-2-infected patients to develop acute cerebrovascular disorders (ACVD) [9, 20–25]:

- increase in the proportion of young patients;
- increase in the proportion of patients with occlusions of the major cerebral vessels;
- more frequent or severe development of ACVD in Latinos and African Americans, as well as in patients with diabetes mellitus and obesity;
- increased mortality compared to ACVD patients without COVID-19;
- increase in the proportion of men diagnosed with CVT.

FACTORS AFFECTING THE OUTCOME OF DISEASES

Patients with acute ischemic stroke who were hospitalized during the COVID-19 restrictions required urgent reperfusion therapy and mechanical thrombectomy. Initially, it was believed that patients were reluctant to seek medical help for stroke symptoms due to fear of contracting COVID-19, so they subsequently arrived at the emergency department with a significant delay, outside the time window allocated for emergency reperfusion therapy [26, 27]. This worsened the outcome of the disease.

In the US and other countries, various cohort studies have evaluated the treatment of patients with acute stroke during the COVID-19 restrictions compared to a control group that received treatment during the same periods before the pandemic. Some of these studies highlighted the negative impact of quarantine on the treatment of ischemic stroke, as evidenced by a decrease in the number of hospitalizations for stroke, the total number of thrombolysis and/or thrombectomy procedures, and a significant increase in the time from symptom onset to treatment initiation [28–32].

In addition to observing that COVID-19 is a risk factor for mortality in stroke patients, it has also been shown that patients with COVID-19 who suffered an acute stroke during infection had significantly lower survival rates than patients without stroke [33]. In addition, a history of previous stroke was an independent risk factor for severe pneumonia leading to critical condition, the need for mechanical ventilation, and high mortality in patients with COVID-19 [34, 35].

In patients with cerebral hemorrhage, the course of COVID-19 was negatively associated with prognosis. Higher mortality rates were observed in patients with COVID-19 compared to both contemporary and historical negative control groups with hemorrhages of comparable severity [36]. In another cohort, all patients with COVID-19 with hemorrhagic lesions on magnetic resonance imaging of the brain suffered from acute respiratory distress syndrome and were hospitalized in intensive care units [37]. A total of 20% of patients with hemorrhagic lesions died during hospitalization, compared with 6% of COVID-19 patients with other non-hemorrhagic lesions [37].

CONCLUSION

Despite the fact that COVID-19 has a more pronounced tropism for lung tissue, damage to the nervous system is inextricably linked to the pathogenetic features of this

infection. The SARS-CoV-2 virus triggers a cascade of pathological reactions leading to thrombus formation and systemic vascular inflammation, which contributes to the development of neurological manifestations not only in the acute phase of infection, but also in the longer term. Timely identification of risk groups and factors that increase the risk of developing neurological pathology, primarily acute disorders of cerebral circulation, can reduce the number of nervous system lesions associated with COVID-19.

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Chapter 8

Post-COVID Syndrome in Neurology

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ABSTRACT

The neurological consequences of the novel coronavirus infection (both acute and long-term) are an extremely heterogeneous group of syndromes: cerebrovascular pathology, demyelinating and neurodegenerative disorders, smell and taste disorders, neurocognitive dysfunction, etc. Current data, including our own data on post-COVID syndrome in neurology, are presented.

INTRODUCTION

The novel coronavirus infection (COVID-19) has been one of the most significant challenges to healthcare in the early 21st century, leading to a huge number of cases (>770 million confirmed cases) in a relatively short period of time and accompanied by high levels of hospitalizations (>28 million) and mortality (>7 million) [1]. However, the accumulation of clinical experience and observational study data has revealed a significant prevalence of symptoms in patients in the early and late post-infection periods. This made it possible to identify the so-called "post-COVID syndrome" as a separate nosological entity, defined by the WHO as "the persistence or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting more than 2 months and not explained by other causes." It is listed in the International Classification of Diseases ICD-10 under the heading "Post COVID-19 condition" U09.9, and has other synonyms: post-acute sequelae of SARS-CoV-2 infection (PASC), post-COVID syndrome, Long COVID.

The most common and defining symptoms of post-COVID syndrome are asthenia (37.2%) and neurocognitive disorders (31.3%) [2], and the severity of neurological

Table 1.

The spectrum of neurological disorders associated with post-COVID syndrome [7]

Lesion of the brain parenchyma	Hemorrhagic/ischemic stroke Thrombosis of cerebral venous sinuses Encephalitis Seizure syndrome Encephalopathy
Lesion of the cerebral meninges	Meningitis Meningoencephalitis Headache
Spinal cord lesion	Transverse myelitis
Lesion of the peripheral nervous system	Guillain–Barré syndrome Polyneuropathies
Lesion of the cranial nerves	Hyposmia/anosmia Ophthalmoparese Hypogeusesthesia/ageusia
Neuromuscular syndromes	Rhabdomyolysis Myalgia Pathological fatigue

symptoms/manifestations varies from 7.7 to 100% [3], which allows us to consider post-COVID syndrome as a neurologically associated condition. The conditional spectrum of neurological states/syndromes in patients in the post-COVID period is presented in Table 1.

Given the significant clinical polymorphism, epidemiological assessment of the prevalence of post-COVID syndrome is expected to be difficult: according to a 2024 meta-analysis, it may be as high as 42% [4], while the estimated frequency of neurological or psychiatric diagnosis within 6 months after acute COVID-19 is 34% [5].

Olfactory, leukocytic, hematogenous, and transsynaptic pathways are identified among the possible common mechanisms of SARS-CoV-2-associated neurological disorders development [6]; certain neurological disease groups (e.g., cerebrovascular pathology, demyelinating states, etc.) may have other pathogenetic aspects.

Many authors point to the long-term effects of COVID-19 on the nervous system, observed six months or more after the acute infection, referring to this condition (along with other non-neurological symptoms) as “long haul” COVID-19 or post-COVID-19 syndrome. According to some data, more than half of patients who have had COVID-19 continue to experience neurological and/or neuropsychological symptoms for several months after recovery [8].

SARS-CoV-2 RNA and proteins were detected in various tissues, including the brain, weeks and months after COVID-19 infection in autopsy and biopsy studies [9], suggesting the presence of a viral reservoir in tissues. Confirming the long-term persistence of SARS-CoV-2 in the body, the literature presents the results of numerous

studies in which viral antigens (full-length spike protein, S1- or N-proteins) were detected in the blood plasma of patients suffering from post-COVID syndrome months (up to 17) after infection, compared to asymptomatic patients. In addition, SARS-CoV-2 proteins have been detected in the blood plasma of patients suffering from post-COVID syndrome, not only in free form, but also as part of extracellular vesicles, including those of neuronal and astrocytic origin.

COVID-19-ASSOCIATED CEREBROVASCULAR DISEASES

COVID-19-associated cerebrovascular diseases (prevalence 1–6%) represent a heterogeneous group of ischemic and hemorrhagic disorders of cerebral circulation, with the risk of ischemic stroke being several times (up to 7.6 times) higher in COVID-19 than in influenza [10]. A large cohort study (>150,000 patients and >5 million controls) demonstrated a continued increased risk of cerebrovascular disease in the post-COVID period — 1.5 times higher within 12 months after COVID-19 [11]. Subsequent reports confirmed this association, as well as a steady trend toward an increased risk of ischemic stroke — even 49 weeks after infection, the likelihood of its occurrence is twice as high [12]. At the same time, such cerebral circulation disorders are accompanied by a host of additional associations, in particular, hemorrhagic complications, including hemorrhagic transformation of cerebral ischemia (Figure 1).

The mechanisms leading to cerebrovascular complications in COVID-19 are likely to be diverse. First, patients with COVID-19, especially those with severe disease, often have comorbidities that increase the baseline risk of thromboembolism. These include dehydration, prolonged immobilization, chronic cardiovascular risk factors or diseases (e.g., coronary heart disease, cerebrovascular disease, and chronic kidney disease), and hereditary thrombophilia. It has also been shown that SARS-CoV-2

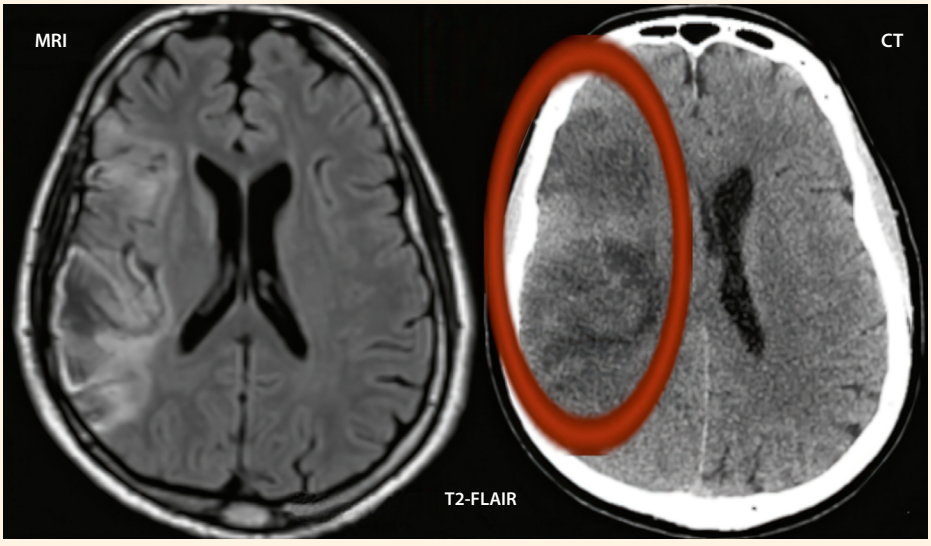

Figure 1. MRI and CT scans of a patient with a cerebral infarction with a hemorrhagic component in the post-COVID period

increases the risk of cardiovascular disease and affects all three factors that make up Virchow's triad (endothelial damage, stasis, and hypercoagulation), which ultimately contributes to thrombosis.

As a result of vascular endothelial inflammation, whether systemic or in cerebral microcirculation, patients with COVID-19 may experience coagulopathy with an increased risk of in situ thrombosis [13]. In particular, endothelial release of pro-inflammatory cytokines (cytokine storm) is associated with a state of hypercoagulation, as evidenced by abnormal levels of von Willebrand factor, D-dimer, fibrinogen, and factor VIII, as well as suppression of ADAMTS13 function; all of which contribute to thrombosis through a process similar to thrombotic microangiopathy. These processes increase the risk of both arterial and venous thrombosis with or without paradoxical embolism, involving cerebral circulation.

Thrombo-inflammation is one of the most important mechanisms in the development of COVID-associated coagulopathy and increased risk of thrombosis, including cerebrovascular complications. A large cohort study by C.J.J. Tartari et al. showed that

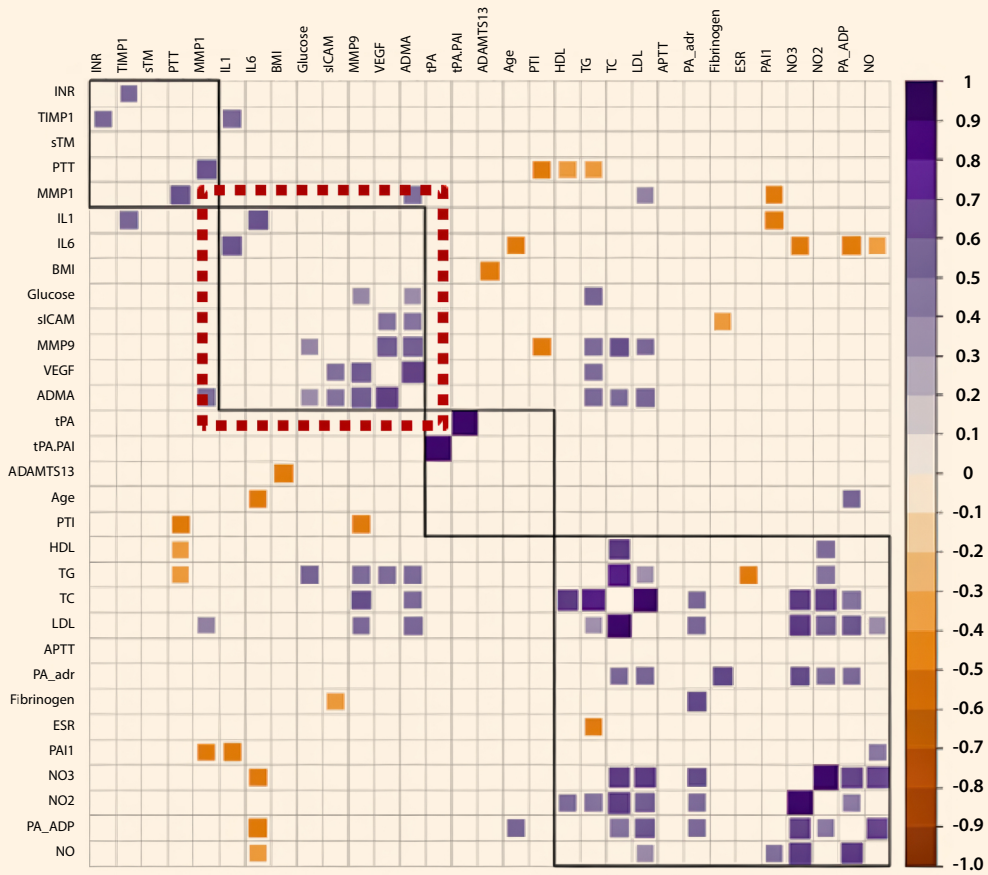

Figure 2. Correlation analysis of a wide range of hemorheology and hemostasis indicators in patients with post-COVID syndrome; the cluster of endothelial dysfunction markers is highlighted in red dotted lines

recovered patients had endothelial dysfunction and hemostasis system disorders for up to 9 months after COVID-19 [14].

Taking into account the presence of endotheliopathy and pathology of hemorheology and hemostasis as one of the probable pathogenetic aspects in infection with SARS-CoV-2, a study was conducted by the Russian Center of Neurology and Neurosciences that showed an increase in prothrombogenic activity in the blood of patients with cerebrovascular diseases against the background of cerebral atherosclerosis within 18 months after COVID-19. This manifested itself in a persistent increase in platelet aggregation, pronounced alterations of the morpho-functional characteristics of red blood cells (a significant increase in amplitude (11.0 *vs.* 7.7) and aggregation index (64 *vs.* 52.3), acceleration of the formation time of "coin stacks" and three-dimensional aggregates (2.4 and 15.0 *vs.* 3.5 and 20.8, respectively), a decrease in red blood cell deformability (0.43 *vs.* 0.51), as well as a significant increase in the level of soluble thrombomodulin. In addition, a new cluster of endotheliopathy markers (in particular, ADMA, VEGF-A, sICAM) has been identified, which may provoke/induce prothrombogenic changes in post-COVID patients and contribute to the persistence of post-COVID neurological pathology (Figure 2).

COVID-19-ASSOCIATED AUTOIMMUNE AND INFLAMMATORY DISEASES OF THE NERVOUS SYSTEM

In addition to endothelial tropism and increased prothrombogenic status, the novel coronavirus infection can lead to autoimmune and inflammatory diseases of the nervous system in a small proportion of patients. Since the beginning of the pandemic, the Russian Center of Neurology and Neurosciences has been participating in a large registry of neurological complications after COVID-19 under the auspices of the European Academy of Neurology and has accumulated data on a wide range of cases that debuted after COVID-19. These include both "classic" forms of demyelinating diseases (e.g., multiple sclerosis [MS]) and rare pathologies, such as conditions caused by antibodies to myelin oligodendrocyte glycoprotein (MOG) (Figure 3) or myelitis. Existing data indicatepotential common mechanisms in the pathogenesis of MS and COVID-19-associated conditions: dysregulation of innate and adaptive immunity, neuroinflammation and thrombosis, and inadequate activation of the complement system [15]. Symptoms of post-COVID syndrome may overlap with the course of the underlying disease in patients with MS (12.4% of patients continue to experience symptoms after COVID-19 for more than 12 weeks), which requires differential diagnosis of these conditions and personalized treatment. The following factors are associated with unfavorable outcomes of COVID-19 in patients with MS: high level of disability, primary or secondary progressive course of the disease, and use of a number of drugs that alter the course of MS (in particular, anti-CD20 therapy, e.g., rituximab, ocrelizumab) [16]. The Russian Center of Neurology and Neurosciences has developed new diagnostic algorithms, in particular for immune-mediated neuro-ophthalmic lesions and myelitis associated with COVID-19.

A characteristic lesion of the peripheral nervous system in COVID-19 is Guillain–Barré syndrome (GBS), which is a group of acute disimmune neuropathies. The most

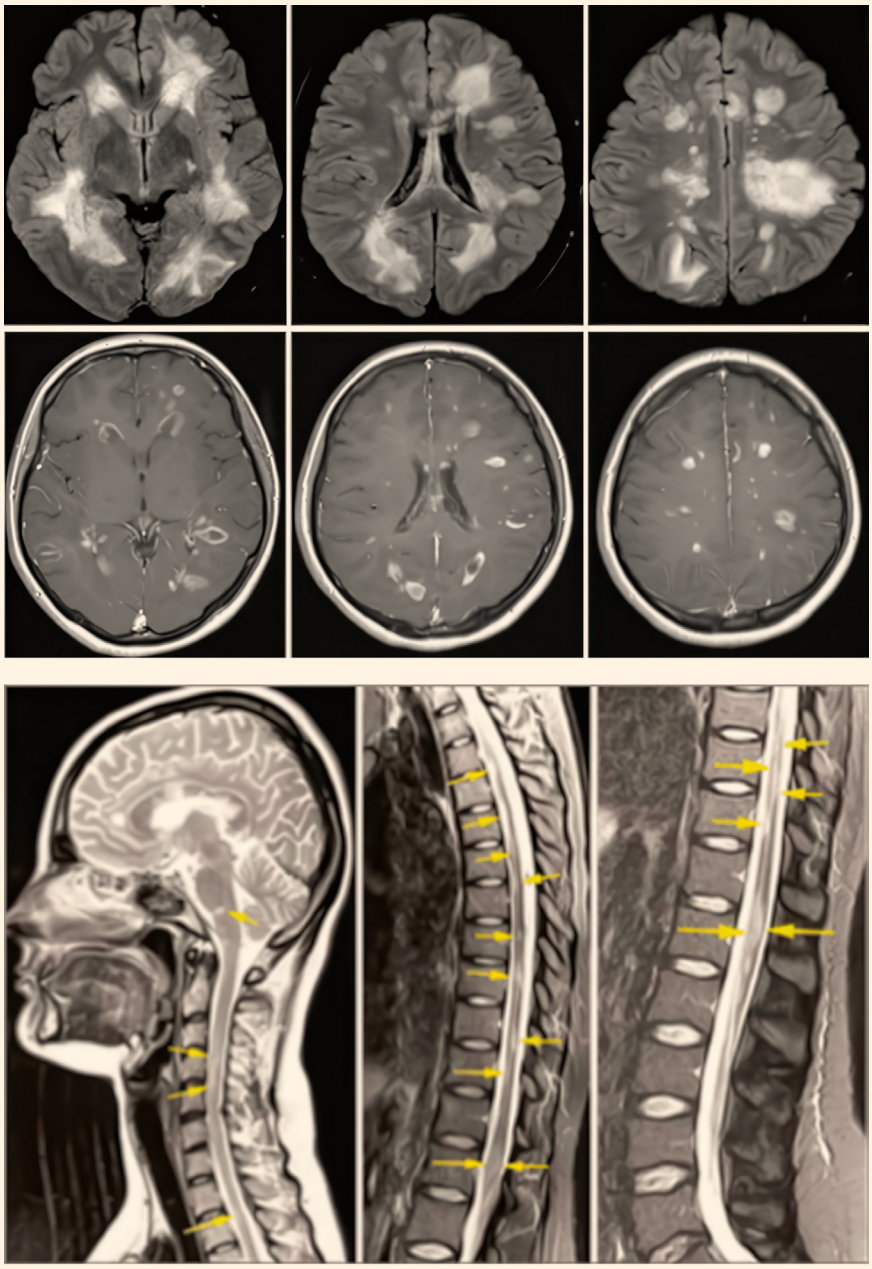

Figure 3. MRI of the brain (upper half) and spinal cord (lower half) of a female patient with MOG-associated encephalomyelitis against the background of COVID-19

common forms of GBS in COVID-19 are acute inflammatory demyelinating polyneuropathy, Miller–Fisher syndrome, and Bickerstaff's brainstem encephalitis. A number of epidemiological studies have demonstrated a possible link between the development of GBS and SARS-CoV-2 infection, as well as vaccination against COVID-19.

NEURODEGENERATIVE DISEASES APPEARING IN THE POST-COVID PERIOD

Since the beginning of the pandemic, there have been scattered reports of cases of neurodegenerative diseases appearing in the post-COVID period. We observed a small cohort of patients at the Russian Center of Neurology and Neurosciences whose characteristic complaints were temporally associated with COVID-19: from Alzheimer's disease to a spectrum of frontotemporal dementia (including variants of progressive aphasia) and to synucleinopathy, represented by dementia with Lewy bodies. This relationship is also confirmed by proteomic studies: SARS-CoV-2 infection is associated with the accumulation of beta-amyloid in older patients [17]. In the case of SARS-CoV-2 infection in patients with neurodegenerative diseases, there is often a marked decompensation of clinical symptoms. This may be due to impaired cerebral metabolism of neurotransmitters, changes at the receptor level, the direct toxic effect of endotoxins, or other mechanisms [18]. Clinical practice and the initial data from epidemiological studies available to date are consistent with the view of COVID-19 as a driver of neurodegenerative processes and indicate a certain increase in the incidence of some forms of neurodegenerative diseases following infection with SARS-CoV-2. This is most convincingly demonstrated for Alzheimer's disease. A large retrospective North American study evaluated a cohort of 6,245,282 elderly individuals (>65 years) and found that those who had contracted the novel coronavirus infection had a significantly higher risk of developing Alzheimer's disease within 360 days of their initial COVID-19 diagnosis (risk ratio 1.69) [19]. It is important to note that previously diagnosed neurodegenerative diseases (Alzheimer's disease and other dementias, Parkinson's disease) are a risk factor for more severe COVID and higher mortality.

A phenomenon closely related to neurodegeneration and highly relevant to post-COVID syndrome is the impairment of smell and taste, which occurs in 17–98% of patients in the acute phase of COVID-19 and persists for more than a year in one-third of patients [20]. The mechanisms of this phenomenon are diverse and include damage to the olfactory epithelium and death of olfactory neurons (sensoneural type), damage to supporting and stem cells of the olfactory epithelium, and the formation of calcium microthrombi in the glial vessels around the olfactory neurons. Post-COVID anosmia is associated with structural reorganization of key brain structures: reduced connectivity between the amygdala and entorhinal cortex [21]. We conducted a pilot study of the phenomenon of anosmia using functional magnetic resonance imaging (fMRI) at different stages of smell recovery in patients who had COVID-19. We described a pattern of brain activation in response to olfactory stimuli without involvement of the orbitofrontal region (Figure 4). The absence of activation on fMRI in a patient with subjective normosmia may indicate delayed mechanisms of smell recovery [22].

PAIN AND COGNITIVE DISORDERS IN THE POST-COVID PERIOD

Pain is one of the most common nonspecific symptoms of COVID-19. The extremely wide range of pain (chest pain, pain along the gastrointestinal tract, musculo-

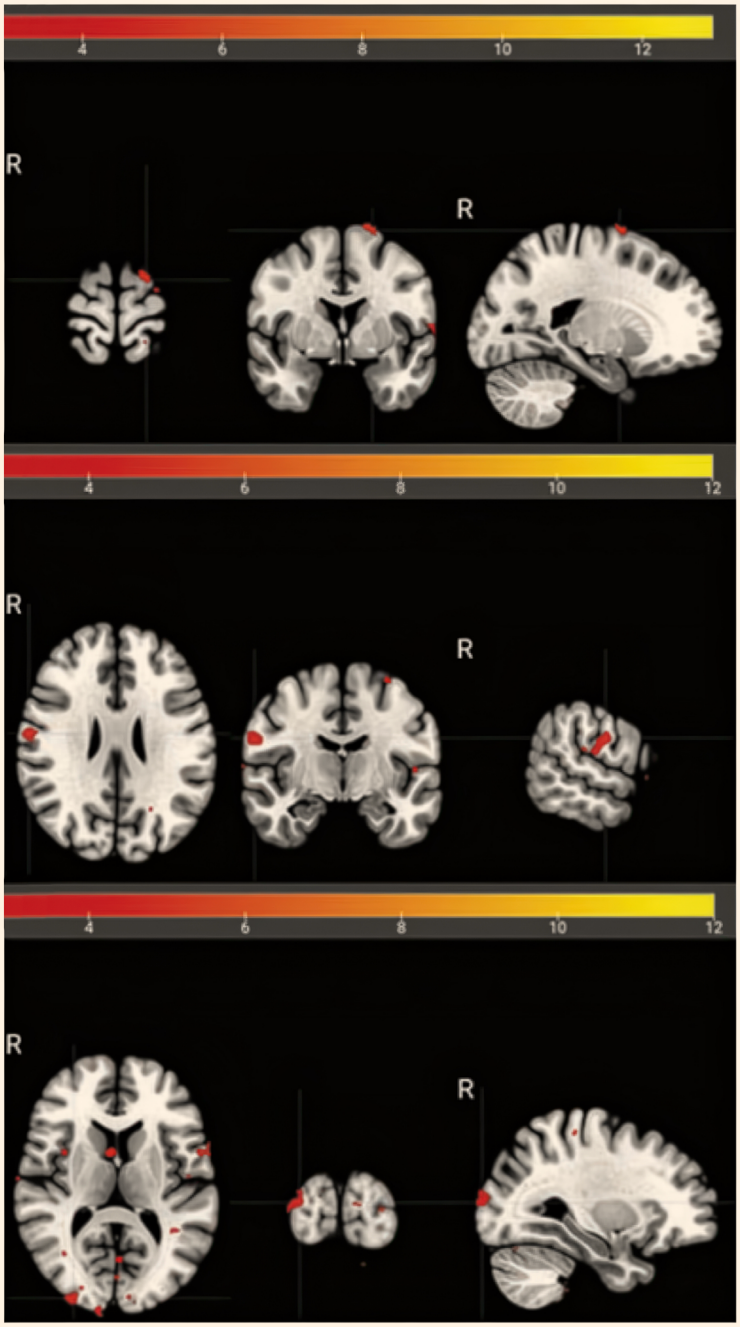

Figure 4. Functional MRI with an original olfactory paradigm:
a — activation of the left superior frontal gyrus in response to an olfactory stimulus (p_{corr}=0.001);
b — activation of the right postcentral gyrus (Brodmann area 4) in response to an olfactory stimulus (p_{corr}<0.001); *c* — activation of the right middle occipital gyrus in response to an olfactory stimulus p_{corr}=0.001)

skeletal pain, joint pain, body pain, headache, neuralgia) is caused by both exogenous and endogenous factors.

The prevalence of the most common pain syndrome — headache — one year after the onset of COVID-19 is 12%, and the mechanisms are represented by activation of the trigeminovascular and/or immune systems, a history of headache, and changes in functional connectivity. According to a study involving 465 patients with post-COVID syndrome an average of 71 weeks after infection, 58% of patients met the diagnostic criteria for fibromyalgia [23]. In 8–15% of patients who have had SARS-CoV-2 infection, chronic headaches (>6 months) are observed [24].

The prolonged course of post-COVID syndrome leads to cognitive impairment (memory, perception, speech, intelligence, ability to recognize, analyze, and assimilate information). Despite the growing scope of clinical data, the mechanisms of post-COVID cognitive impairment (CI) remain insufficiently studied. Their multifactorial nature is being discussed: virus persistence, complement activation and platelet aggregation leading to microthrombosis; "silent" brain infarcts; "fusion" of neurons and glial cells, compromising neuronal activity; neuroinflammation, impaired neurogenesis; vagal signaling dysfunction caused by low serotonin levels [25]. Compared to participants who did not have COVID-19 (control group), cognitive impairment comparable to a 3-point decrease was found even in patients who had mild COVID-19. Participants with persistent symptoms (post-COVID syndrome) showed a 6-point decline, and those who were admitted to the intensive care unit showed a 9-point decline [26]. Cognitive impairment in post-COVID syndrome correlate with the presence of morphological substrate — these patients have areas of statistically significant thinning of the cerebral cortex [27].

MRI with various modalitites is one of the most important methods for assessing damage to various brain structures as a basis for the development of CI. Currently, the most promising method is functional MRI (fMRI), which allows for the assessment of the functional properties of the brain, including the activation, deactivation, and connectivity of various brain structures in response to certain stimuli. In COVID-19, fMRI has revealed changes in functional activity and connectivity in several key neural networks, including the passive brain mode network (default mode network), the frontal-parietal network, and the executive network. PET scans have revealed areas of pronounced hypometabolism in the pons, hippocampus, and cerebellum in patients with post-COVID syndrome and cognitive disorders [28].

ASTHENIC SYNDROME AND CHRONIC FATIGUE SYNDROME

Asthenic syndrome is one of the leading clinical manifestations of post-COVID condition. It is a painful condition manifested by increased fatigue and exhaustion, accompanied by mood swings, loss of self-control, intolerance, restlessness, sleep disturbances, loss of physical and mental capacity, and poor tolerance to noise, light, smells, etc. Up to a third of all people who have had the novel coronavirus infection, regardless of the severity of the disease, experience symptoms of this syndrome, which can bother patients for months after recovery from the acute infection [29]. Complaints

of so-called “brain fog” are common among patients, occurring in approximately 32% of cases of post-COVID syndrome; it manifests itself as a feeling of sluggish thinking, a sense of vagueness or distraction, which affects a person’s ability to think and concentrate. This syndrome mainly affects cognitive functions such as attention, fluency of speech, speed of information processing, as well as executive functions and memory. Such disorders negatively affect the quality of life of patients with post-COVID syndrome, their socioeconomic and psychological well-being, which determines the need for further study of the problem and the search for effective methods of treating cognitive disorders in such patients.

According to a population study conducted in 2022, the occurrence of “brain fog” is associated with the following factors: female gender, the onset of respiratory symptoms at the onset of the disease, the severity of the disease (namely, the need for intensive care), and a previous anxiety disorder [30]. The role of vaccination administered before the first case of a novel coronavirus infection or after its resolution is not yet clear.

The closest thing to cognitive impairment in post-COVID syndrome is probably chronic fatigue syndrome, or myalgic encephalomyelitis, a condition in which the aforementioned “brain fog” has previously been described [31]. In chronic fatigue syndrome, decisive importance is attached to ongoing neuroinflammation, which is often difficult to assess objectively.

The prevalence of myalgic encephalomyelitis/chronic fatigue syndrome after SARS-CoV-2 infection was 2.66 per 100 person-years, while in the control group it was 0.93, corresponding to a risk ratio of 4.93 [32]. The proportion of patients with criteria for chronic fatigue syndrome after COVID-19 was 4.5%, and in the uninfected group, it was 0.6%. At the same time, 88.7% of patients with chronic fatigue syndrome met the criteria for a diagnosis of post-COVID syndrome. Up to a quarter of patients with post-COVID syndrome suffer from chronic insomnia and excessive fatigue: those who have had COVID-19 have a 92% (i.e., almost twice) higher risk of developing insomnia compared to those who have had the flu [33].

The causes and pathogenesis of this condition are not precisely known at present, but there are many mechanisms by which SARS-CoV-2 infection can cause or exacerbate existing cognitive impairments [34]. The following mechanisms can be identified among those leading to asthenic disorders in the presence of SARS-CoV-2 infection: direct damaging effect of the virus, neuroinflammation (including that associated with damage to the blood-brain barrier), hypoxia, and cerebrovascular pathology. A number of researchers have found neuroanatomical changes and signs of neurodegeneration, damage to microcirculation[35], metabolic disorders (including areas of hypometabolism in the brain regions responsible for motivation, in particular the dorsolateral prefrontal cortex) [36].

As part of a pilot study, we at the Russian Center of Neurology and Neurosciences analyzed the phenomenon of post-COVID asthenia using fMRI techniques. It turned out that in the group that had COVID-19, compared to the group of healthy volunteers, there was significantly higher activation of certain areas of the brain: the supramarginal gyri, the posterior cingulate cortex, the opercular parts of the precentral gyri, and the posterior lobe of the cerebellum bilaterally [37] (Figure 5).

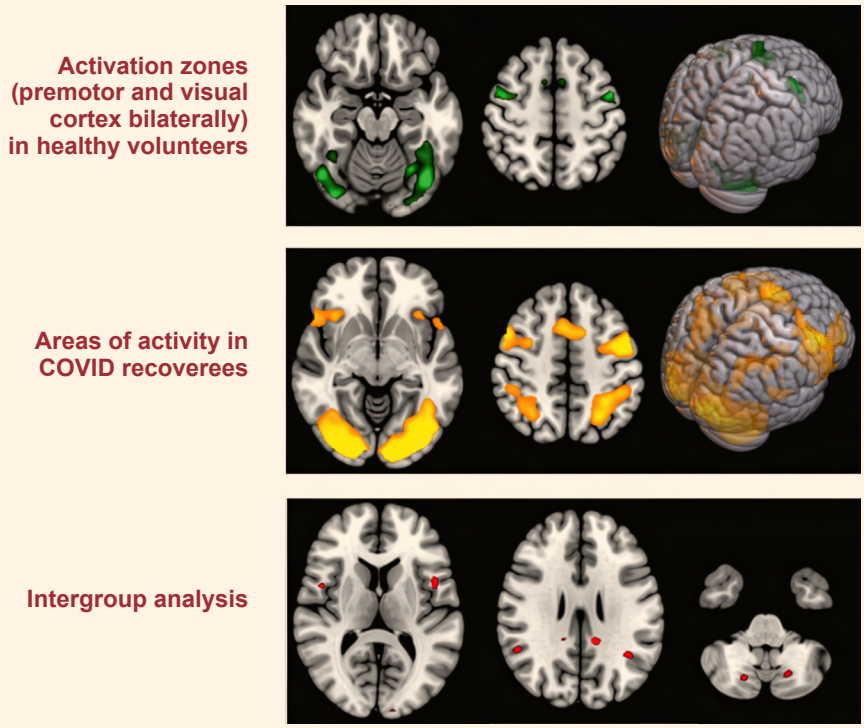

Figure 5. fMRI in groups of patients with post-COVID syndrome and healthy volunteers

This may indicate the need to involve a larger neural substrate to perform cognitive tasks — it is precisely functional disturbances in the neural activity of the brain that may underlie post-COVID asthenia.

The correction of the main symptoms of post-COVID syndrome remains a complex issue, primarily neurocognitive disorders. B. Whitaker-Hardin et al. (2025) believe that therapeutic approaches such as cognitive training, neuromodulation, physical exercise, and targeted pharmacological intervention are promising in reducing the severity of cognitive dysfunction in post-COVID syndrome, but larger clinical studies are needed [38].

We also conducted a double-blind, placebo-controlled study of an original drug with potential neuroprotective properties in patients with post-COVID asthenia: in the active drug study group modulation of activation was noted in a number of cognitive "zones" simultaneously: activation decreased in the visual cortex, in the supramarginal and angular gyri bilaterally. This correlated with the clinical effect of reduced asthenic symptoms, decreasing the areas required to perform cognitive tasks (in the supramarginal and angular gyri), and improving the brain's control functions associated with language processing (strengthening of the connection between the left dorsolateral prefrontal cortex and the upper temporal gyrus) [39].

The polymorphism of neurological manifestations of post-COVID syndrome leads to difficulties in the assessment of the true clinical picture and can lead to a prolonged

differential diagnostic search. However, modern research methods allow to reveal the underlying mechanisms of nervous system damage and, accordingly, to adapt preventive measures in a timely manner.

CONCLUSION

Neurological consequences of the novel coronavirus infection can be observed both in the acute phase and during convalescence from coronavirus infection. They are characterized by a high degree of heterogeneity of symptoms and damage to the nervous system of varying severity. The most common conditions reported after coronavirus infection are cerebrovascular pathology, demyelinating and neurodegenerative disorders, as well as disturbances of smell, taste, and neurocognitive dysfunction.

The polymorphism of neurological manifestations of post-COVID syndrome creates certain difficulties for practicing physicians in assessing the clinical picture and developing management strategies. However, the data obtained on the pathogenesis of neurological disorders in coronavirus infection, as well as modern research approaches, make it possible to identify the fundamental mechanisms of damage to the nervous system and to prescribe effective treatment in a timely manner.

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Chapter 9

Long-Term Effects of the Novel Coronavirus Infection (COVID-19) Depending on the Presence of Cardiovascular Diseases

N.V. Pogosova

ABSTRACT

The publication presents data from the world literature and our own studies results aimed at investigating the relationship between acute infectious pathology — the novel coronavirus infection (COVID-19) — and cardiovascular diseases. The data presented indicate a long-term negative effect of the novel coronavirus infection on cardiovascular health indicators.

INTRODUCTION

In December 2019, an outbreak of pneumonia of unknown origin occurred in the city of Wuhan, People's Republic of China (PRC) [1]. The novel disease, known as COVID-19 (COronaVIrus Disease 2019), spread rapidly, becoming a pandemic and a threat to the entire world. The causative agent of the disease, SARS-CoV-2 (Severe Acute Respiratory Syndrome CoronaVirus 2), is highly contagious and can cause complications in the form of bilateral COVID-19-specific pneumonia, in some cases with the development of acute respiratory distress syndrome, thrombosis, acute respiratory, cardiac, and multiple organ failure, and death [2]. The rate of spread of COVID-19 has been high: in the first six months since the first reports of the outbreak, the number of infected people exceeded 10 million, and the number of deaths exceeded 500,000 [3].

COVID-19 is a disease that the medical community has only been familiar with for five years. However, it quickly became clear that there are a particularly vulnerable groups of people who are at risk of developing severe forms of the infection and suf-

fering adverse outcomes. These are elderly people and people with chronic diseases, primarily cardiovascular diseases (CVD) [2].

During the first wave of the pandemic, both practicing physicians and researchers focused primarily on approaches to etiotropic and pathogenetic treatment of lung tissue damage and correction of respiratory system functions in acute respiratory distress syndrome, whereas later more often was discussed the link between viral infection and CVD and their pathogenetic mechanisms, in particular endothelial dysfunction, increased platelet activity, and a high risk of thrombosis.

Numerous studies and meta-analyses have established a high correlation between severe COVID-19 infection requiring hospitalization and a history of CVD [4, 5]. The presence of hypertension, coronary heart disease, atrial fibrillation, and chronic heart failure in patients with COVID-19 significantly increases the risk of non-fatal complications and death [5, 6].

As it turned out, the novel coronavirus infection can significantly worsen cardiovascular mortality rates, which was clearly demonstrated in our country. Since 2003, a steady positive trend in cardiovascular mortality has been registered in Russia, except for the period when the country faced COVID-19 pandemic [7]. The mortality rate from circulatory system diseases in 2020 exceeded the same indicator in 2019 by 13%. Only the tremendous efforts of the medical community and the expansion of the subsidized drug provision program for cardiological patients made it possible to overcome the negative trend associated with COVID-19 and achieve a further reduction in cardiovascular mortality in subsequent years.

As it is well known, any infectious process, and COVID-19 in particular, is accompanied by the activation of immunobiological defense system and onset of an inflammatory response. At the same time, it is important to remember the role of inflammation, both acute and chronic, in the mechanisms of formation and progression of various pathogenetic continuums, the end point of which is the development of acute or decompensation of chronic CVD.

Like many other federal centers, the National Medical Research Center for Cardiology named after Academician E.I. Chazov, of the Russian Ministry of Health, was repurposed as a “COVID hospital,” which made it possible to create a hospital registry and conduct a comprehensive analysis of factors associated with fatalities during patients’ hospital stays.

The mortality rate among hospitalized patients with COVID-19 was 7.7%. A univariate regression analysis identified the main factors associated with death during hospitalization. These were age over 55 years, a National Early Warning Score of more than 4 points, oxygen saturation of less than 92.0%, plasma glucose levels of more than 5.4 mmol/L and highly sensitive C-reactive protein of more than 25.7 mg/L, as well as creatinine clearance of less than 72.0 mL/min. Moreover, the risk increased as the severity of each of these factors increased. According to multivariate regression analysis, the three most significant predictors of a hard endpoint — death from all causes during hospitalization — were: more than a 5-fold increase in aspartate aminotransferase and/or alanine aminotransferase activity compared to normal values (relative risk (RR) 16.8; 95% confidence interval (CI) 5.0–56.3; $p<0.001$), significant changes in lungs confirmed by computed tomography (CT) — CT-4 (OR=13.4; 95%

CI 3.9–45.5; $p<0.001$) and myocardial infarction or unstable angina during hospitalization (RR=11.3; 95% CI 1.4–90.6; $p=0.023$). Chronic obstructive pulmonary disease, reduced renal function (Cockcroft-Gault creatinine clearance <60.0 mL/min), type 2 diabetes mellitus, cancer, and dementia also significantly increased the likelihood of death [8].

Signs of acute heart damage with increased troponin levels appear in patients with COVID-19 several days after the onset of fever, indicating myocardial damage associated with viral infection. The mechanisms of myocardial damage caused by SARS-CoV-2 remain unclear, but may be partly related to the direct effect of SARS-CoV-2 on cardiomyocytes or to increased levels of angiotensin-converting enzyme 2 in the heart and coronary vessels. Respiratory failure and hypoxia, typical of COVID-19, can also have damaging effects on the myocardium. Immune mechanisms of myocardial inflammation may also play a significant role. Thus, damage to the heart leads to activation of innate immune response with release of pro-inflammatory cytokines, as well as to activation of adaptive autoimmune-type mechanisms through molecular mimicry [9].

Although initial studies suggested that the novel coronavirus infection (COVID-19) could cause direct inflammatory damage to the heart with development of myocarditis, more recent global clinical experience has caused doubt on the existence of a convincing link between SARS-CoV-2 infection and the development of myocarditis. Although the presence of the virus has been demonstrated in the hearts of patients who died from COVID-19, a definitive diagnosis of myocarditis can only be based on the results of endomyocardial biopsy or autopsy using established histological and immunohistochemical criteria [10]. Our own data and the results of other researchers allow us to conclude that myocarditis is a rare complication of SARS-CoV-2 infection.

It is much more important to note that the vascular endothelial dysfunction and coagulopathy develop in patients with severe COVID-19, and thrombosis may develop with the presence of antibodies to phospholipids and a clinical picture resembling catastrophic antiphospholipid syndrome. Clinical and pathological changes are difficult to differentiate from multiple organ thrombosis developing in disseminated intravascular coagulation syndrome and thrombotic microangiopathy. Cytokine storm in COVID-19 usually leads to the development of acute respiratory distress syndrome, multiple organ failure, and can be fatal [9].

The COVID-19 pandemic and related epidemic restrictions have significantly affected people's lifestyles, including a decrease in physical activity, unhealthy changes in eating habits (including "comfort eating" due to increased anxiety and stress), and a reduction in social contacts and social support. The pandemic has had a negative impact on the availability and accessibility of cardiac care. The Russian data and international analysis conducted in a number of countries have shown that the intensity of cardiac care use during the active wave of the pandemic decreased by 60–100% [11, 12].

Thus, the pandemic had both direct effects on mortality among patients with CVD (due to an increase in the number of deaths in patients with a history of CVD) and indirect effects (increased mortality from CVD due to insufficient use of cardiac care resources: reduced access to primary health care, reduced demand for emergency care,

delayed hospitalisations in emergency situations, and a decrease in the number of planned hospitalisations, particularly for chronic heart failure).

Scientific research has established the pathophysiological mechanisms of cardiovascular effects of COVID-19, which can be divided into three main categories: direct damage to the myocardium (damage to myocytes and impaired electrical conductivity); damage to the pulmonary system, leading to respiratory failure and development of systemic inflammation; damage to the entire vascular system (micro- and macrovascular dysfunction).

Scientific data indicate that the third block of pathophysiological mechanisms describing the relationship between COVID-19 and CVD is the most significant. Specific virus-induced and cytokine storm-induced endothelial damage, known as SARS-CoV-2-associated endothelial dysfunction, endotheliitis, and hypercoagulation syndrome, is the basis of the thrombotic microangiopathy characteristic of COVID-19, which is observed in various organs (myocardium, lungs, brain, kidneys, etc.), as well as thrombosis of large arteries and veins, often accompanied by thromboembolism. The lesion of the microcirculatory bed plays a key role in the pathogenesis of COVID-19.

Based on autopsy studies and clinical presentation, various clinical and morphological manifestations of COVID-19 can be identified (all involving lung damage), including cardiac, cerebral, intestinal, renal, hepatic, diabetic, thromboembolic (in pulmonary embolism), septic (in the absence of bacterial or mycotic sepsis), and cutaneous [10].

Studies show that COVID-19 can cause not only acute myocardial damage, but also increase the risk of adverse long-term consequences of the disease for cardiovascular system by activating the cytokine system, renin-angiotensin system dysregulation, destabilization of atherosclerotic plaques, and coagulation system disorders [11, 13].

It quickly became clear that COVID-19 is not only a life-threatening acute infection, but also a disease with long-term consequences. It turned out that 10–20% of patients experience symptoms that persist for 3 or more months and cannot be explained by other diagnoses. This condition has been named post-COVID syndrome [14]. It is important to note that this syndrome is characterized by a wide range of clinical symptoms (more than 200 symptoms of post-COVID syndrome have been described).

Studying the delayed effects of COVID-19 in form of post-COVID syndrome, its clinical manifestations, and pathophysiological mechanisms remains one of the important tasks of modern healthcare. In May 2023, due to a significant decrease in the number of people infected with the SARS-CoV-2 virus, the World Health Organization announced the end of the coronavirus pandemic. However, the number of people with long-term effects of COVID-19 (with post-COVID syndrome) is quite large. After recovery, some people continue to experience or develop a wide range of symptoms of varying intensity, which does not always depend on the initial severity of the disease.

Our own data, obtained from remote monitoring of COVID-19 patients included in the hospital registry, confirm that some of them continue to experience a variety of symptoms. The four most common symptoms are shortness of breath, weakness, chest pain, and a feeling of irregular heartbeat [15] (Figure 1).

Three to seven months after recovering from COVID-19, cases of newly diagnosed arterial hypertension were recorded in the study sample of 240 patients: in the group without a history of CVD in 8 (16%) patients, and in the group with CVD, in 1 (0.6%). Patients in the group with CVD noted a distinct deterioration in their condition, which led to hospitalization of every tenth patient (17; 10.5%), an increase in the degree of arterial hypertension (1; 0.6%) and decompensation of chronic heart failure (10; 4.7%), a transition of paroxysmal atrial fibrillation to a permanent one (1; 0.6%), new cases of coronary heart disease (4; 1.9%), acute cerebrovascular accident (1/0.6%), recurrence of angina pectoris (6; 2.8%), and three patients required myocardial revascularization.

Patients with CVD, compared with those without CVD at a long-term stage after COVID-19, were more likely to have elevated D-dimer levels (17.8% *vs.* 6.1%, p=0.045), troponin I (8.6% *vs.* 0%, p=0.044), and NT-pro-BNP (39.9% *vs.* 10%, p<0.001) [16] (Table 1). Although chronic heart failure was observed in only 12.4% of patients, elevated levels of brain natriuretic peptide NT-pro-BNP were detected in 39.9% of patients with CVD, which may indicate the presence of hidden heart failure, especially considering the left ventricular diastolic dysfunction in 83.0% of patients in this group.

Patients with CVD were more likely than patients without CVD to show ECG signs of *ST* segment displacement at long term after COVID-19 (18.5% *vs.* 6%, p=0.033). No significant differences were found between the compared groups in terms of spirometry and chest computed tomography data.

Cognitive impairment (less than 26 points on the International Cognitive Assessment Questionnaire) was detected in 38% of patients, sleep disorders according to the Pittsburgh Sleep Quality Index were detected in more than 60% of patients, with no differences between groups.

Fifty-seven percent of patients reported increased stress levels at long term after COVID-19, and 29% reported high stress levels, with no differences between groups.

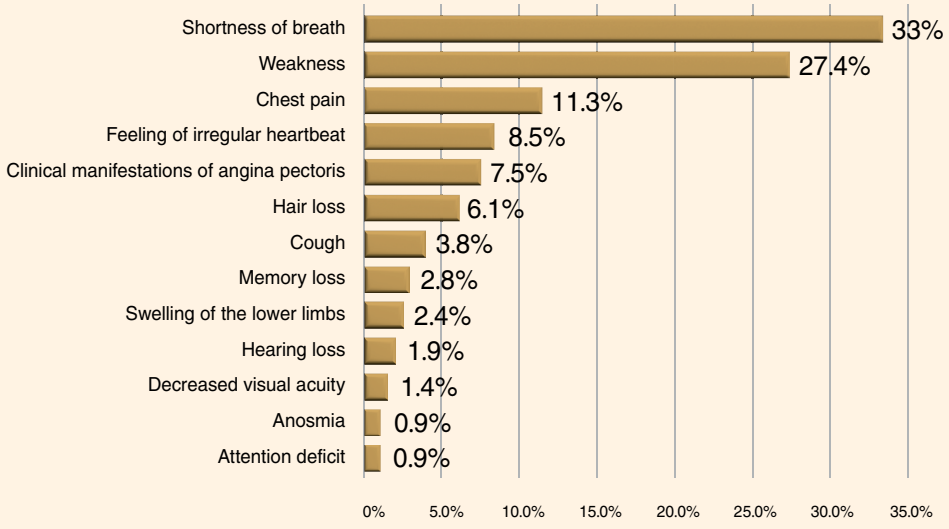

Figure 1. Symptoms of COVID-19 3–7 months after hospitalization [16]

Anxiety symptoms were identified in 22.6% (13.7% subclinical and 9% clinically significant), and depressive symptoms in 17.9% of patients (9.9% subclinical and 8% clinically significant). The frequency of anxiety and depressive symptoms in groups of patients with and without CVD did not differ significantly. At the same time, quality of life indicators in the group of patients with CVD were significantly worse — this applies to both the total score on the European Quality of Life Questionnaire (p=0.027) and its individual domains, such as mobility, self-care, daily activities, and pain/discomfort (Table 2).

A higher mortality rate has been established in the presence of deep vein thrombosis both in the acute phase of COVID-19 and at long term.

According to univariate regression analysis, the initial presence of any CVD was associated with adverse outcomes 3–7 months after COVID-19. However, the associations were age-related. At the same time, a history of myocardial infarction, atrial fibrillation, and chronic heart failure were independent predictors of adverse outcomes at long term. Thus, myocardial infarction increased the likelihood of fatal outcomes more than 3 times (odds ratio (OR) 3.33; p=0.03). Atrial fibrillation increased more than fivefold the risk of death (OR=5.38; p=0.001) and the combined endpoint of death and hospitalization for cardiovascular reasons (OR=5.07; p <0,001).

In cases of decompensated chronic heart failure, the risk of death increased 3.5 times (OR=3.45; p=0.027). The values are adjusted for age and gender (Table 3).

According to multivariate regression analysis, the most unfavorable CVD in terms of long-term prognosis after COVID-19 is atrial fibrillation, which is an independent predictor of adverse outcomes such as all-cause mortality (OR=5.41; p=0.002), and composite endpoint (death and hospitalizations for cardiovascular causes) (OR=4.92; p=0.001). The significance of cardiac arrhythmias in the context of adverse outcomes of COVID-19 has also been established in a number of other studies [17].

In addition, a significant increase in the risk of myocardial infarction, cerebral strokes, venous thromboembolism, and cardiovascular death in patients who had COVID-19, according to long-term follow-up (3 or more months after recovery), was established not only in cases of severe COVID-19 but also in relatively mild cases [16, 17]. Multivariate regression analysis clearly showed that presence of any history of CVD in patients with COVID-19 increased the risk of death during hospitalization by more than 3 times. A particularly clear correlation was established in relation to ischemic heart disease, myocardial infarction, atrial fibrillation, and chronic heart failure.

Our findings are consistent with the results of other studies and meta-analyses, in particular, the large meta-analysis by Xu J. et al., which included 203 studies and more than 24 million patients with COVID-19 [5].

COVID-19 is a serious challenge for global health. CVD aggravates the course of COVID-19 and is associated with a worse prognosis. COVID-19 can lead to cardiovascular complications both in the acute phase of the disease and in the long term. The presence of multiple symptoms and worsening of CVD at long term after COVID-19 indicates the need for rehabilitation measures and active cardiovascular prevention, especially secondary prevention, in patients with CVD in order to achieve optimal control of key indicators.

Table 1.

Selected biochemical indicators 3-7 months after COVID-19 [16]

Indicator	All patients (*n*=212)	Patients with CVD (*n*=162)	Patients without CVD (*n*=50)	*p*
NT-pro-BNP, pg/mL	70.5 (34.8; 175.3)	102.3 (39.7; 224.0)	41.4 (20.2; 71.7)	<0.001
NT-pro-BNP >125 pg/mL, *n* (%)	70 (32.9%)	65 (39.9%)	5 (10%)	<0.001
Wp-CRP, mg/L	2.0 (1.0; 3.9)	2.2 (1.2; 3.9)	1.8 (0.8; 3.9)	0.068
Increased level of wp-CRP, *n* (%)	35 (16.4%)	26 (16%)	9 (18%)	0.732
Glucose, mmol/L	5.46 (5.01; 6.17)	5.59 (5.05; 6.36)	5.25 (4.78; 5.50)	0.002
Creatinine, μmol/L	78 (68; 87)	79 (69; 87)	74 (66; 86)	0.111
CKD according to the CKD-EPI formula, mL/min/1.73 m^2	87 (77; 97)	84(71;94)	96 (87;105)	<0.001
Decrease of CKD <60 mL/min/1.73 m^2, *n* (%)	16 (7.5%)	15 (9.2%)	1 (2%)	0.126
AST, U/L	22 (18; 27)	22 (18; 27)	21 (17; 25)	0.468
ALT, U/L	22 (17; 33}	23 (17; 33)	22 (16; 33)	0.528
LDH, U/L	193 (171; 218)	195 (174; 221)	184 (162; 207)	0.021
Total cholesterol, mmol/L	5.65 (4.62; 6.46)	5.64 (4.53; 6.34)	5.80 (5.19; 6.72)	0.129
Triglycerides, mmol/L	1.46 (0.98; 2.01)	1.47 (1.03; 2.07)	1.36 (0.89; 1.88)	0.188
LDL cholesterol, mmol/L	3.59 (2.61:4.11)	3.40 (2.53; 4.01)	3.78 (3.15; 4.45)	0.027
HDL cholesterol, mmol/L	1.34 (1.17; 1.59)	1.33 (1.12; 1.58)	1.35 (1.24; 1.62)	0.228
Target values of LDL cholesterol, *n* (%)	14 (6.6%)	11 (6.7%)	3 (6%)	1.0
D-dimer >243 ng/mL or >0.50 μg/mL, *n* (%)	32 (15.1%)	29 (17.8%)	3 (6.1%)	0.045
Wp-troponin 1 >34.2 pg/mL in men and >15.6 pg/mL in women, *n* (%)	14 (6.6%)	14 (8.6%)	0	0.044

Table 2.

COVID-19 long-term follow-up: quality of life indicators [16]

Indicator	All patients (*n*=212)	Patients with CVD (*n*=162)	Patients without CVD (*n*=50)	*p*
Quality of life according to VAS	75 (60; 85)	70 (60; 85)	80 (70; 90)	0.027
Quality of life according to the EQ5D questionnaire				
Mobility, *n* (%):				
I have no difficulty with walking	135 (63,7)	97 (59,9)	38 (76)	0.038
I have some difficulty with walking	77 (36,3)	65 (40,1)	12 (24)	0.038
I am bedridden	0	0	0	–
Personal care, *n* (%):				
I have no difficulty with personal care	182 (85.8)	135 (83.3)	47 (94)	0.059
I have some difficulty with washing and dressing	29 (13.7)	27 (16.7)	2 (4)	0.023
I am unable to wash or dress myself	1 (0,5)	0	1 (2)	0.236
Daily activities, *n* (%):				
I have no difficulty with my usual daily activities	141 (66.5)	101 (62.3)	40 (80%)	0.021
I have some difficulty with my daily activities	67 (31.6)	57 (35.2)	10 (20)	0.044
I am unable to perform in my usual daily activities	4 (1.9)	4 (2.5)	0	0.575
Pain/discomfort, *n* (%):				
I have no pain or discomfort	120 (56.6)	84 (51.9)	36 (72)	0.012
I have moderate pain or discomfort	87 (41)	73 (45.1)	14 (28)	0.032
I am suffering from extreme pain or discomfort	5 (2.4)	5 (3.1)	0	0.594
Anxiety/depression, *n* (%):				
I do not have anxiety or depression	126 (59.4)	94 (58)	32 (64)	0.512
I have moderate anxiety or depression	81 (38.2)	63 (38.9)	18 (36)	0.713
I am extremely anxious or depressed	5 (2.4)	5 (3.1)	0	0.594

Medical rehabilitation of patients with COVID-19, especially those with comorbid CVD, should begin in intensive care units and continue after discharge. Our clinical experience shows that starting of rehabilitation in the intensive care units as early as possible leads to significantly faster stabilization of patients' condition and shorter recovery times.

After an infection, it is not uncommon to see a worsening of CVD, so it is recommended to continue rehabilitation measures as part of the second stage of medical rehabilitation (in medical rehabilitation departments for patients with somatic diseases and conditions) and the third stage of medical rehabilitation (in medical rehabilita-

Table 3.

Frequency of adverse events at long term after COVID-19 depending on presence or absence of CVD [16]

Indicator	*n*	All patients (*n*=240)	Patients with CVD (*n*=189)	Patients without CVD (*n*=51)	*p*
Death from all causes, *n* (%)	240	27 (11.3%)	26 (13.8%)	1 (2%)	0.018
Hospitalizations, *n* (%)	212	17 (8%)	17 (10.5%)	0	0.014
Combined endpoint 1 (death + hospitalizations), *n* (%)	239	44 (18.4%)	43 (22.9%)	1 (2%)	0.001
Hypertension, *n* (%)	212	9 (4.2%)	1 (0.6%)	8 (16%)	<0.001
CAD, *n* (%)	212	4 (1.9%)	4 (2.5%)	0	0.575
Resumption of angina pectoris clinic, *n* (%)	212	6 (2.8%)	6 (3.7%)	0	0.339
PCI, *n* (%)	212	3 (1.4%)	3 (1.9%)	0	1.0
dCHF, *n* (%)	212	10 (4.7%)	10 (6.2%)	0	0.122
SF, *n* (%)	212	1 (0.5%)	1 (0.6%)	0	1.0
ACE, *n* (%)	212	1 (0.5%)	1 (0.6%)	0	1.0
Combined endpoint 2 (death + hospitalizations + CAD + exertional angina + hypertension + SF + dCHF + PCI + ACE), *n* (%)	239	64 (26.8%)	55 (29.3%)	9 (17.6%)	0.097

tion departments of day hospitals, outpatient medical rehabilitation departments for patients with somatic diseases and conditions of medical organizations), as well as at home using telemedicine technologies [10].

CONCLUSION

The COVID-19 pandemic has had a significant impact on mortality among patients with CVD, both directly, by increasing the number of deaths among patients with a history of CVD, and indirectly, by increasing mortality due to reduced access to specialized cardiac care as a result of prolonged hospitalization for acute conditions, including those related to patients' fears of infection in healthcare facilities, and a reduction of planned hospitalizations.

A past coronavirus infection may lead to destabilization of patient's existing cardiovascular pathology, for example, due to the progression of chronic heart failure. The establishment of a dispensary observation system and the continuation of rehabil-

itation measures in medical rehabilitation departments for patients with somatic diseases and conditions, in day hospitals or outpatient medical rehabilitation departments for patients with somatic diseases and conditions, can prevent the progression of cardiovascular pathology in patients after coronavirus infection.

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Chapter 10

Post-COVID and Endocrinopathies

N.G. Mokrysheva

ABSTRACT

We are currently observing a clear increase in endocrine pathology associated with COVID-19 infection. To date, more than 700 million confirmed cases of COVID-19 have been reported worldwide. The SARS-CoV-2 virus exhibits tropism to human cells via the angiotensin-converting enzyme 2 (ACE2) receptor. ACE2 is expressed in multiple tissues, including the pancreas, thyroid gland, hypothalamus, pituitary gland, adrenal glands, and gonads, which may explain the broad spectrum of endocrine disorders seen in patients with COVID-19. The severity of endocrine manifestations depends on the density of expression of these receptors and the presence of pre-existing conditions. Nevertheless, the pathophysiological mechanisms clinical characteristics and long-term outcomes of SARS-CoV-2–induced endocrinopathies remain insufficiently understood.

INTRODUCTION

Five years after the emergence of the novel coronavirus infection that entered human life after 2019, we need to carefully reassess the knowledge and evidence accumulated over recent years. The burden that COVID-19 has placed on the human population cannot be overstated.

POST-COVID AND CARBOHYDRATE METABOLISM DISORDERS

More than 420 million people worldwide suffer from diabetes mellitus. It is precisely this patient population that is at the highest risk when confronted with an infectious process, including COVID-19. Numerous data indicate that SARS-CoV-2 infection and diabetes mellitus (DM) have multidirectional interactions, which leads to the accumulation of pathogenic effects on the human body. DM is one of the main diseases

that aggravates the course of COVID-19, increasing the frequency of hospitalization in intensive care units, and is therefore associated with a high risk of death from the novel coronavirus infection. On the other hand, SARS-CoV-2 infection itself can provoke pancreatic pathology, including the development of pancreatitis, ketoacidosis, and the onset of diabetes mellitus [1].

The expression of canonical and alternative SARS-CoV-2 receptors on pancreatic cells explains their susceptibility to SARS-CoV-2 infection. Numerous studies indicate that SARS-CoV-2 infection impairs the survival and function of pancreatic cells. Autopsy data indicate transdifferentiation of pancreatic cells, leading to decreased insulin secretion and increased expression of alpha and acinar cell markers, including glucagon and trypsin 1 [2] (Figure 1). However, for a clearer and more comprehensive understanding of the underlying molecular processes, it is necessary to continue the research initiated over the past five years during the COVID-19 pandemic.

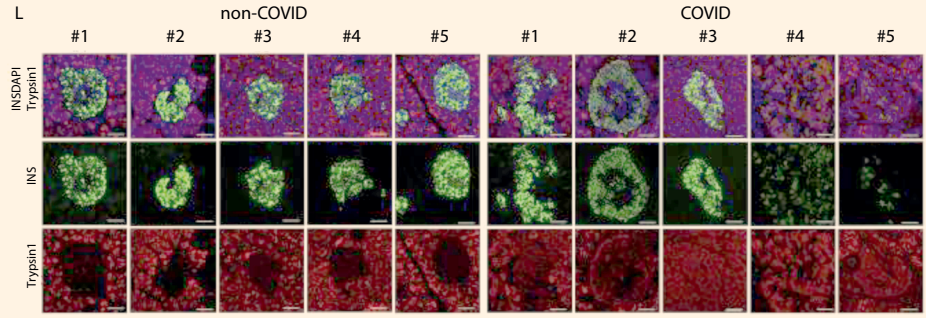


Figure 1. Confocal microscopy of pancreatic cells.
INS — quantitative assessment of relative expression intensity, red color — trypsin 1, green — insulin, blue — DAPI (cell nucleus) (adapted from Xie Yan et al., 2021)

Risk factors for disturbances of carbohydrate metabolism and the development of diabetes mellitus in the post-COVID period include viral load itself, pre-existing prediabetic conditions, metabolic syndrome, excess weight and obesity, the use of antiviral and anti-inflammatory drugs, and others [3] (Figure 2).

When infected with SARS-CoV-2, human islet cells activate the mitogen-activated protein kinase (MAPK) signaling pathway and the integrated stress response pathway (Figure 3). Activation of these pathways leads to apoptosis, transdifferentiation, and changes in the secretory profile of beta cells, which causes a decrease in insulin levels in beta cells and a decrease in glucose-stimulated insulin secretion.

Early reports from 2020 pointed to a rising incidence of diabetes mellitus in several population groups. Later studies demonstrated that the risk of developing diabetes in association with Long COVID increases and is higher than in the general population. Research that began immediately after the onset of the pandemic has continued, and we see that over time, the risk of developing not only type 2 diabetes but also type 1 diabetes increases in the most vulnerable population of children and adolescents [2, 5]. During the period analyzed, the risk of developing type 1 diabetes ranged from 19 to 27 cases per 100,000 children and subsequently showed an upward trend [6].

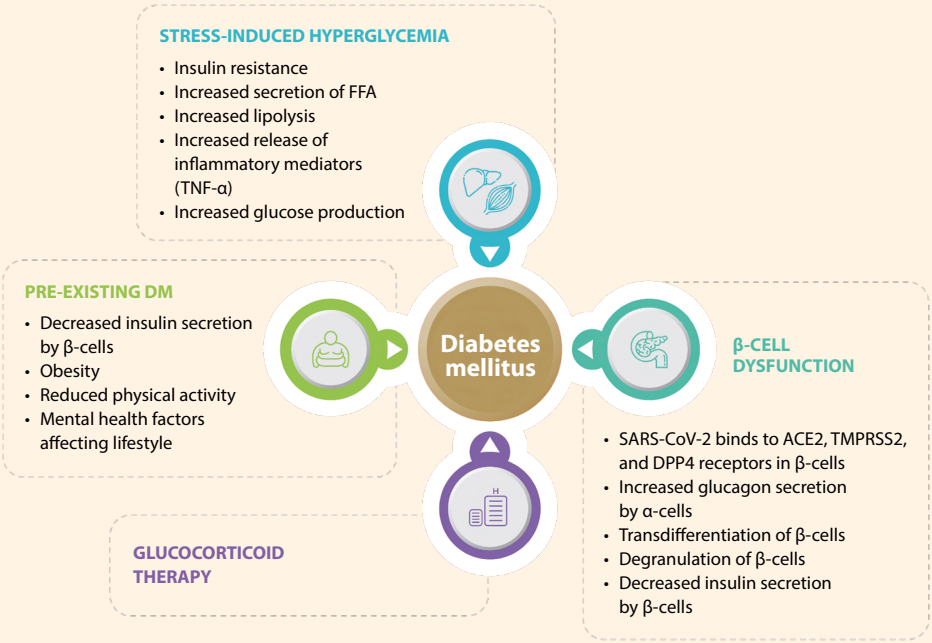


Figure 2. Risk factors for diabetes mellitus in individuals who have had COVID-19

Although the endocrinology community still lacks a complete understanding of these processes, it is clear that the pathogenetic basis of type 1 diabetes — autoimmune pancreatic damage and a cascade of infection-triggered mechanisms — will draw increasing attention to the relationship between type 1 diabetes and the novel coronavirus infection, as well as infectious processes more broadly. The team at the Endocrinology Research Centre, established a COVID ward in Moscow subsequently observed a large number of hospitalized patients.. In addition, we analyzed the dynamics of their condition both during hospitalization and after discharge. The initial data indicated that patients receiving insulin therapy, as well as those treated with sulfonylurea drugs, experienced a more severe course of COVID-19 and were significantly more likely to be admitted to the intensive care unit or die [7].

Obviously, the endocrinology community views these data critically: this is not a direct cause-and-effect relationship, but it characterize the process that we routinely analyze. Insulin therapy and sulfonylurea agents are typically prescribed to patients who have greater difficulty to achieving glycemic control in the context of diabetes mellitus [8]. This represents the most severe category of patients in terms of carbohydrate metabolism disorders, and they had the poorest prognosis for the course of COVID-19.

We assessed the relationship between carbohydrate metabolism indicators upon admission with various carbohydrate metabolism disorders. The first group consisted of patients with a history of diabetes mellitus, while the second group consisted of patients who had not previously been diagnosed with diabetes mellitus. We divided the second group of patients into three categories based on their glycated hemoglobin (HbA1c) levels: normal values (less than 6%); levels from 6.1% to 6.4%; and HbA1c

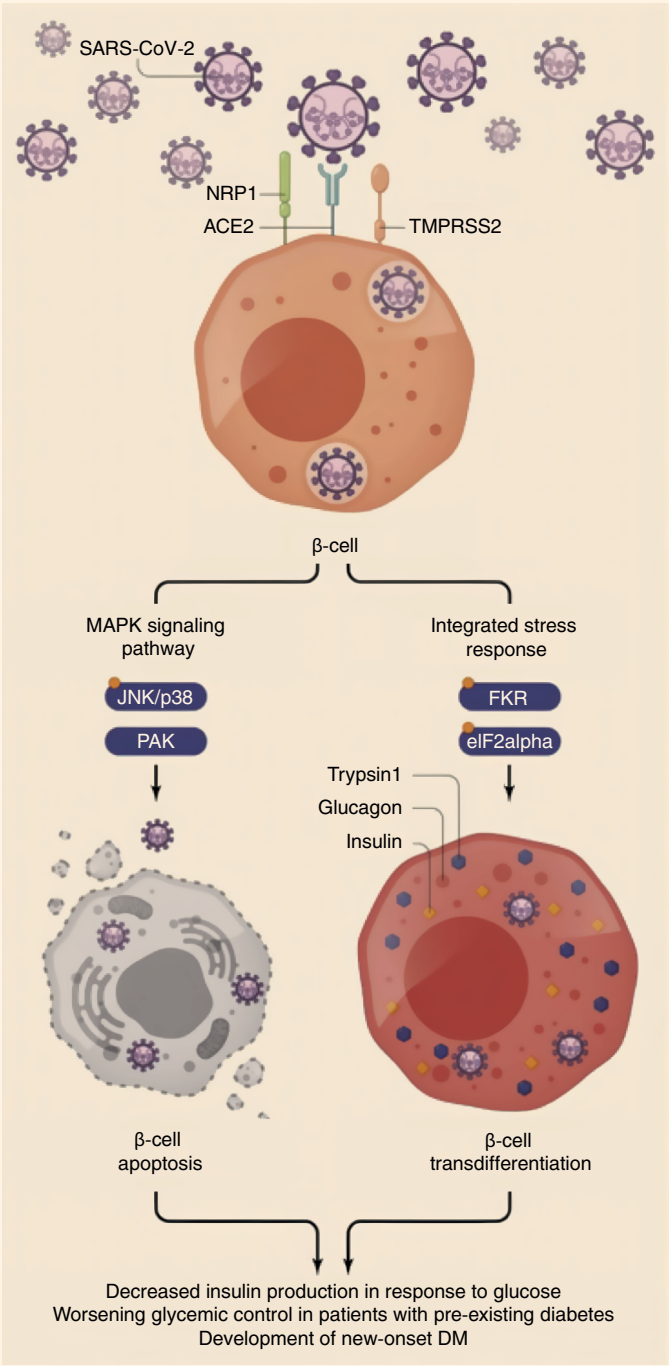


Figure 3. Mechanism of transdifferentiation under the influence of SARS-CoV-2.
MAPK — mitogen-activated protein kinase; JNK — c-Jun N-terminal kinase; PAK — p21-activated kinase; PKR — protein kinase R; eIF2alpha — eukaryotic translation initiation factor 2 alpha (adapted from [4])

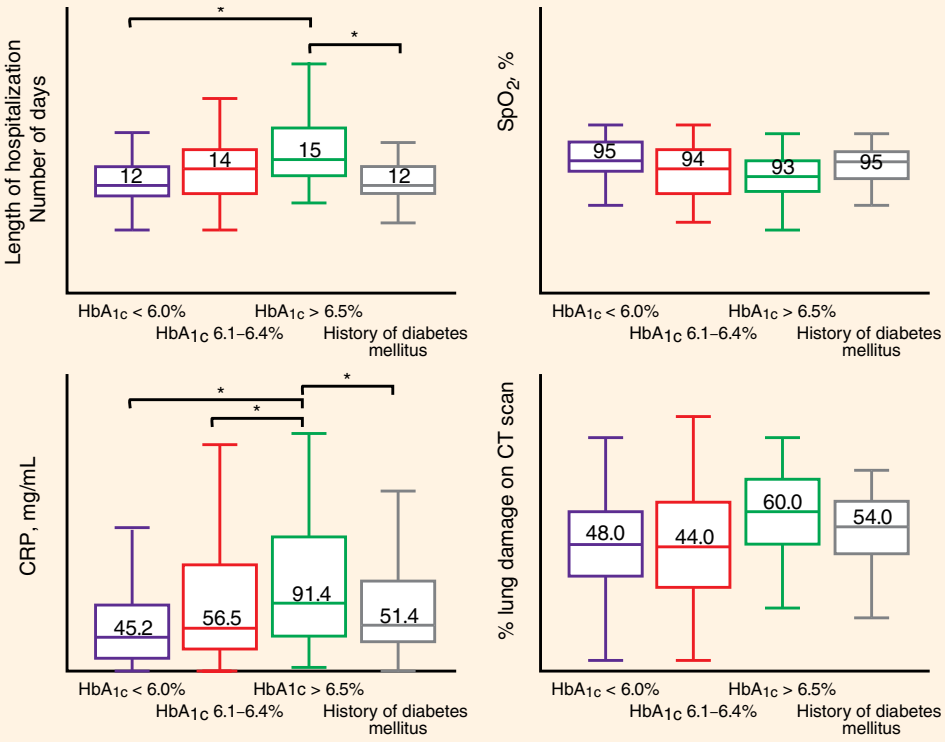


Figure 4. Severity of COVID-19 and carbohydrate metabolism (data of the Endocrinology Research Centre, Moscow, Russia)

levels above 6.5% at the time of hospitalization. Among the groups studied, patients with HbA1c levels above 6.5% and without previous diabetes had the worst prognosis in terms of dynamics and lung condition; according to the results of computed tomography, they had the lowest degree of blood oxygen saturation and the highest level of C-reactive protein. The length of hospitalization required for them to reach a stable condition was also the highest in this subgroup [9] (Figure 4).

The incidence of pre-existing diabetes mellitus in hospitalized patients in the acute phase of COVID-19 was 17%; newly diagnosed diabetes mellitus at the time of hospitalization was observed in 5% of patients; 35% of patients with no history of diabetes mellitus had an isolated increase in glycated hemoglobin above 6% with normal fasting blood glucose levels upon admission to the COVID ward.

We analyzed the dynamics of the condition of patients without previously diagnosed diabetes in the early stages after discharge from the COVID hospital and six months later. After 6–8 weeks, during an oral glucose tolerance test, 40% of patients were found to have prediabetic carbohydrate metabolism disorders, and 3–14% of patients were diagnosed with diabetes. In 45% of patients, no disturbances of carbohydrate metabolism were detected after recovery, and the normalization of HbA1c levels in most — despite the absence of factors that could influence carbohydrate metabolism or HbA1c — was particularly noteworthy. In other words, the increase in HbA1c levels observed during or in the context of coronavirus infection in this group was transient.

At the 12-month follow-up, patients with a history of DM had HbA1c levels that were, as expected, higher than their glycemia levels. The transient decrease in HbA1c was confirmed one year after discharge in almost all groups of patients who had experienced an increase in HbA1c against the background of COVID-19 (Figure 5).

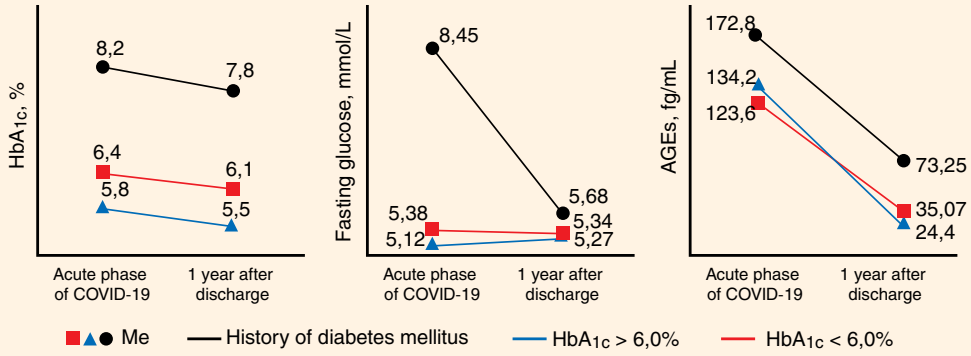


Figure 5. Dynamics of glycated hemoglobin levels 12 months after COVID-19 (data of the Endocrinology Research Centre, Moscow, Russia)

A more detailed analysis revealed a decrease in HbA1c levels after one year in 76% of patients. Other possible causes of the increase and subsequent decrease in transient HbA1c dynamics were excluded during our analysis.

Patients with initially increased HbA1c levels but without a prior diagnosis of diabetes were re-examined after one year and underwent a glucose tolerance test. Two patients from this group, who were diagnosed with prediabetes on the 6th–8th day after discharge, had a verified diagnosis of diabetes after one year. Two patients who had no disturbances of carbohydrate metabolism 6–8 weeks after hospitalization also had prediabetes one year later and all the indicators associated with it.

Thus, the detection of increased HbA1c levels against the background of a new coronavirus infection in the acute phase of COVID-19 in individuals without previous diabetes is an incentive to analyze and study carbohydrate metabolism indicators after the post-COVID period in order not to miss both the manifestation of DM and the negative dynamics of carbohydrate metabolism indicators, up to the state of prediabetes.

Insulin secretion and insulin resistance indicators were quite variable throughout the observation period: from the acute phase of COVID-19 to full recovery after 12 months. Overall, there was an increase in insulin resistance and immunoreactive insulin secretion with elevated levels of C-peptide, proinsulin, and proinsulin/insulin ratio in the acute phase of the disease, with a decrease in indicators after one year (Figure 6).

This pattern may be associated with both the direct effect of the virus and the development of physiological insulin resistance in patients with standard DM progression. We searched for predictors of carbohydrate metabolism disorders in patients who had COVID-19. Our analysis data allow us to record and consider proinsulin levels below 1.1 mU/L in the acute phase of COVID-19 as a protective factor for car-

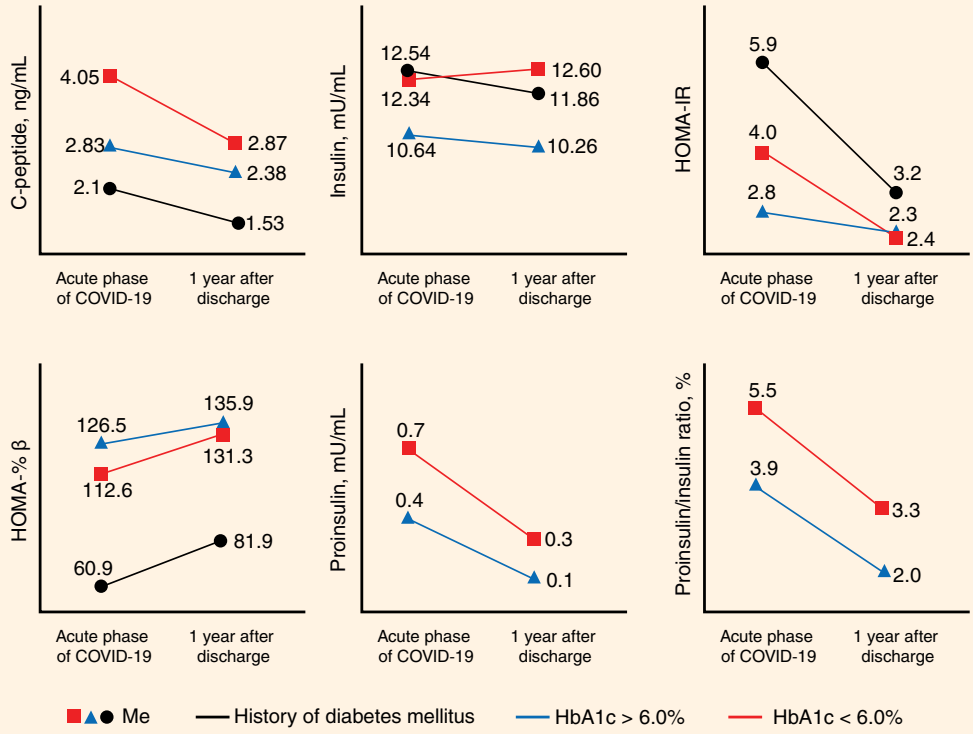
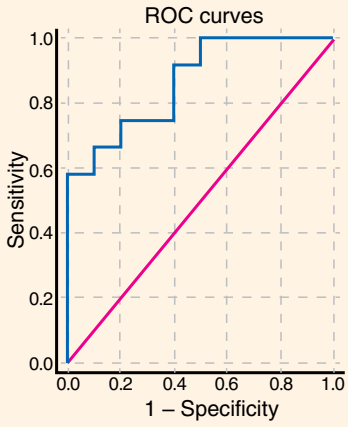


Figure 6. Increased insulin resistance and secretion of immunoreactive insulin in the acute phase of the disease, with a decrease in indicators after 1 year (data of the Endocrinology Research Centre, Moscow, Russia)



Feature	Median	Area under the curve (95% CI)	*n*	Standard error	*p*
Proinsulin, mU/mL	1.10	0.906 (0.784–1.000)	22	0.062	<0.001

Value (95% CI), %	Значение (95% ДИ), %
Sensitivity	69.2 (48.2–76.5)
Specificity	88.9 (58.4–99.4)
The predictive value of a positive result	54.4 (14,1–77.2)
The predictive value of a negative result	93.8 (83.5–99.6)
Odds ratio	18.0 (1.7–196.3)

Figure 7. Predicting carbohydrate metabolism disorders after COVID-19 (data of the Endocrinology Research Centre, Moscow, Russia)

bohydrate metabolism after COVID-19 (Figure 7). This is consistent with data on the transient nature of the increase in proinsulin levels in the acute phase of COVID-19 in our patients.

POST-COVID AND THYROID PATHOLOGY

In the acute phase of COVID-19, various autoimmune processes are observed, including in the endocrine organs. Atypical and subacute thyroiditis, secondary hypothyroidism are quite common pathologies in the novel coronavirus infection [10]. In addition, autoimmune diseases of the thyroid gland could debut in a delayed period after SARS-CoV-2 infection.

Currently, COVID-19 is asymptomatic in most cases. However, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can cause dysfunction in multiple vital organs including the thyroid gland, which the virus enters via angiotensin-converting enzyme 2 (ACE2). Atypical thyroiditis has emerged as a new thyroid pathology associated with SARS-CoV-2 coronavirus infection. It debuts directly during the acute phase of COVID-19 and accompanies clinical symptoms of respiratory disorders. According to our observations, one of the characteristic manifestations of atypical thyroiditis associated with SARS-CoV-2 infection is the development of thyrotoxicosis during the acute phase of the disease. Unlike classic subacute thyroiditis, antithyroid antibody levels in these patients are typically negative. A notable feature is the predominance of painless forms of thyroiditis in the context of COVID-19 [11].

In the cohort of patients hospitalized at the COVID-19 unit of the Endocrinology Research Centre, the incidence of thyrotoxicosis during the acute phase of COVID-19 was 4%. Patients with low levels of thyroid-stimulating hormone were found to have normal or high-normal levels of thyroid hormones, confirming the development of thyrotoxicosis due to SARS-CoV-2 infection and atypical thyroiditis, rather than euthyroid syndrome, in which thyrotropin levels decrease following low free T3 and free T4 levels. Evaluation of these patients demonstrated that the identified thyroid disorders were solely the result of the mandatory diagnostic screening we performed in the COVID hospital to detect endocrine pathology. Patients with low levels of thyroid-stimulating hormone were found to have normal or high-normal levels of thyroid hormones. Another component of our study focused on the pathogenesis of SARS-CoV-2–associated atypical thyroiditis. Binding of the SARS-CoV-2 spike (S) protein to ACE2 triggers complex molecular disturbances within the renin-angiotensin-aldosterone and kallikrein-kinin systems. Suppression of ACE2 activity during COVID-19 reduces the enzyme's capacity to hydrolyze angiotensin II, resulting in elevated levels of this peptide. The accumulation of angiotensin II activates inflammatory pathways, including the transcription of pro-inflammatory cytokines, such as interleukin-6, interleukin-1-beta, tumor necrosis factor, and others [12]. At the 6-month follow-up (from the onset of COVID-19), hypothyroidism was observed in 9% of patients.

Subclinical hypothyroidism was observed in 7 patients. Symptomatic hypothyroidism was developed in 2 cases. We also detected a statistically significant increase in the concentration of antibodies to thyroperoxidase during the 6 months after the onset of COVID-19. The correlations we identified between the thyroid profile and the levels of cytokines involved in the development of autoimmune thyroiditis confirm the pathogenetic relationship between them.

Negative correlations were found between changes in thyrotropin concentration and proinflammatory cytokines: macrophage inflammatory protein-1 beta and tumor

necrosis factor-alpha. Macrophages and monocytes activated by macrophage inflammatory protein-1 beta release inflammatory mediators involved in the pathogenesis of autoimmune thyroid disease, tumor necrosis factor-alpha induces an inflammatory process associated with the development of autoimmune damage and autoimmune processes.

An interesting discovery from our point of view is the identified negative correlation between changes in interferon-gamma levels and free T3 levels (Figure 8).

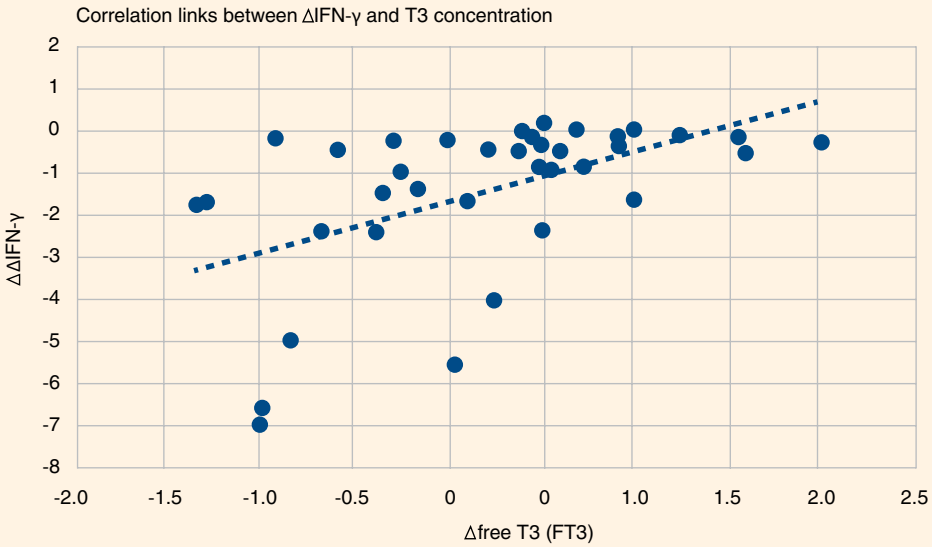


Figure 8. Thyroid dysfunction and proinflammatory cytokines (data of the Endocrinology Research Centre, Moscow, Russia)

Interferon-gamma promotes immune cell activation and subsequent thyrocyte injury, which may lead to the destruction and dysfunction of thyroid cells characteristic of autoimmune-mediated damage.

The inverse correlation between changes in interferon-gamma and free triiodothyronine suggests impaired synthesis or altered metabolism of thyroid hormones, a pattern characteristic of autoimmune-mediated thyroid dysfunction.

POST-COVID AND MINERAL METABOLISM DISORDERS

Against the backdrop of novel coronavirus infections of varying severity, we also observed disturbances in mineral metabolism. It is important to note that the most severely ill patients were characterized by hypermagnesemia, hypocalcemia, and severe vitamin D deficiency [13]. Hypocalcemia is considered an independent risk factor for hospitalization with COVID-19. According to our data and reports from international colleagues, its incidence during hospitalization averaged 60 to 80% of cases. According to the literature, hypocalcemia is more common in patients with more severe coronavirus infection, more pronounced lung damage, longer hospitalization, and a higher likelihood of transfer to the intensive care unit and use of mechanical ventilation. It is

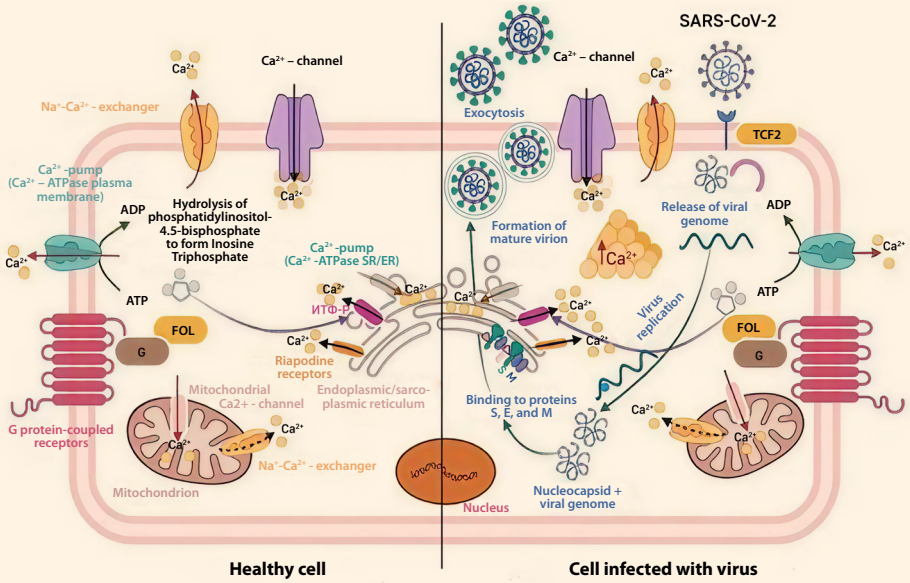


Figure 9. Calcium exchange in a healthy cell and when infected with a virus (adapted from Chen Xingjuan et al. [22])

also associated with an increased risk of multiple organ failure, the need for prolonged hospitalization, and a high risk of death [12–20].

According to our data, blood calcium levels below 2 mmol/L are associated with an increase in the 28-day mortality rate in patients with COVID-19 [21]. This association may be explained by the role of calcium ions in facilitating the entry of viral particles into human cells. In addition, activation and proliferation of lymphocytes require an increase in intracellular calcium concentration within immunocompetent cells.

Subsequent excessive release of intracellular calcium leads to increased permeability of mitochondria and cell membranes and an influx of extracellular calcium. In turn, "local" hypocalcemia contributes to cell necrosis and apoptosis. This protective mechanism ensures the destruction of virus-infected cells, preventing the spread of the virus. Calcium controls antibody formation and is also involved in the production of pro-inflammatory cytokines, tumor necrosis factor-alpha, and interleukin-1 beta (Figure 9).

High intracellular calcium levels lead to hypercytokinemia or cytokine storm, which is characteristic of coronavirus infection.

Elevated intracellular calcium levels can contribute to excessive cytokine release ("cytokine storm"), a hallmark of severe COVID-19. It is also known that viral particles use calcium ions for replication, which may manifest as marked hypocalcemia during the acute phase of the disease. In mild forms of COVID-19, when low-virulence strains of the virus enter the cells of the ciliated epithelium of the nose and oropharynx, the primary immune response helps to contain the spread of the virus. At high viral loads or in the setting of an impaired immune response, viruses exploit various host cells for replication, leading to cellular dysfunction accompanied by abnormally

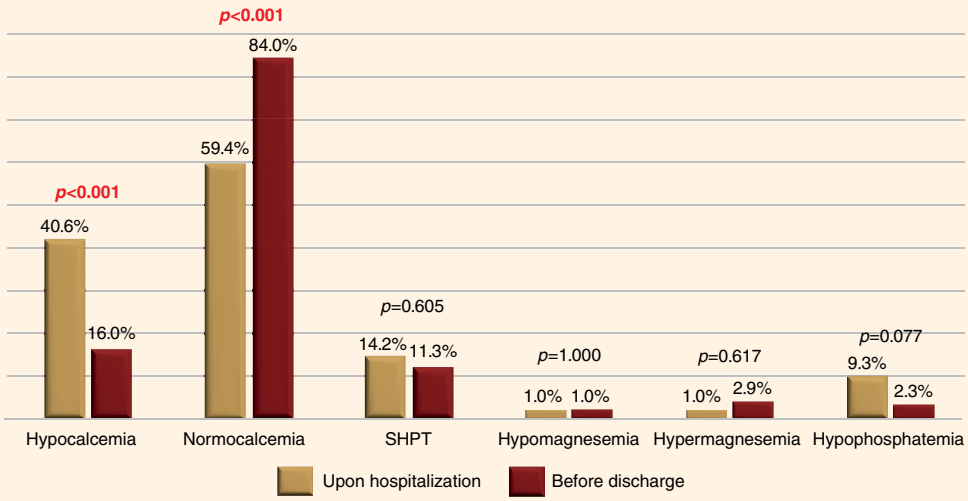


Figure 10. Dynamics of phosphorus-calcium metabolism indicators in patients who have had COVID-19 (data of the Endocrinology Research Centre, Moscow, Russia)

elevated intracellular calcium concentrations, subsequent cell death, and the development of severe hypocalcemia. By the end of hospitalization in the group we examined, regression of hypocalcemia was observed in 25% of cases without the administration of calcium and vitamin D supplements (Figure 10). This finding further supports the association between infection burden and disturbances in mineral metabolism.

The median 25(OH)D level upon admission to the hospital was 12.50 ng/mL, with deficiency detected in 81.1% of cases and insufficiency in 14.2%.

Optimal vitamin D concentrations were observed in only 4.7% of patients. Notably, despite the high prevalence of hypocalcemia and vitamin D deficiency, secondary elevations in parathyroid hormone were detected in only 14.2% of cases. Previously published studies likewise report a high prevalence of vitamin D deficiency (67.9%), hypocalcemia (based on ionized calcium in 70.5% of cases), and a low frequency of secondary hyperparathyroidism (20.5%) among patients with COVID-19 [23]. These observations suggest other possible causes of increased parathyroid hormone levels in COVID-19. One possible explanation for the lack of the expected response of parathyroid cells to hypocalcemia in this case may be its rapid development against the background of an acute inflammatory process at the onset of the disease, followed by spontaneous normalization of blood calcium levels during clinical recovery. We separately analyzed a cohort of patients with extremely severe COVID-19, characterized by the development of cytokine storm and subsequent transfer to the intensive care unit. When comparing the frequency of mineral metabolism disorders, we found that, in addition to hypocalcemia based on total calcium, hypermagnesemia occurred significantly more often in the subgroup with fatal outcomes. In addition, these patients were characterized by hyperphosphatemia. The pathogenesis of these alterations in COVID-19 remains incompletely understood. It is assumed that hypermagnesemia may result from SARS-CoV-2 cell damage, which is accompanied by the release of magnesium ions into the extracellular space. The data obtained show that magnesium,

in addition to calcium, is an important marker of severity and adverse outcome in COVID-19 [24].

The decrease in disease activity was reflected by changes in the main inflammatory markers observed in these patients, consistent with the dynamics of mineral metabolism indicators. Before discharge, all patients with phosphorus-calcium metabolism disorders were prescribed vitamin D and calcium therapy. In some cases, calcium supplements were required in large doses for a long period of time. The dynamics of all indicators during the six months after the coronavirus infection were positive. While we observed a regression in the frequency of hypocalcemia as early as the 3rd and 7th days of hospitalization, all other disturbances in mineral metabolism required a longer period for normalization of parathyroid hormone, phosphorus, and magnesium levels.

CONCLUSION

The impact of SARS-CoV-2 on the endocrine system is being actively studied. The data obtained, including those from the Endocrinology Research Centre, indicate an increase in the incidence of endocrine pathology with this infection, as well as clinical mimicry of the identified disorders. Active study of these processes and attentive care for patients allow for the timely diagnosis of pathological changes in the endocrine glands and thus significantly improve the condition of each patient. Correction of endocrine and metabolic disorders is crucial for restoring organ function and should be an integral component of the comprehensive rehabilitation of patients recovering from COVID-19.

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Chapter 11

Long COVID, Thromboinflammation and Cancer: Exploring the Hidden Links

Elalamy I.

ABSTRACT

This chapter is devoted to analyzing the complex and multifaceted relationships between post-COVID syndrome (Long COVID), thromboinflammation, and oncological diseases. The COVID-19 pandemic has led to a significant number of cases of Long COVID, which is characterized by persistent symptoms affecting various organ systems. Cancer patients are particularly vulnerable, with a prevalence of Long COVID reaching 50-60%. The paper examines in detail the pathophysiological mechanisms of Long COVID, including endothelial dysfunction, microthrombosis, immune dysregulation, chronic inflammation, and persistence of viral components. The oncogenic potential of SARS-CoV-2 is discussed, including the disruption of tumor suppressors (p53, pRB), the induction of angiogenesis and metastasis, and the weakening of antitumor immune surveillance. Data from epidemiological and molecular studies, including Mendelian randomization, are presented, indicating a potential causal relationship between COVID-19 and an increased risk of developing certain types of cancer (breast tumors, gastrointestinal tract tumors, thyroid tumors).

INTRODUCTION

Both COVID-19 and Cancer play a *Game of Thrones* within an Inflamed microenvironment [1]. COVID-19 pandemic accounts for more than 750 million infections and more than 7 million deaths worldwide. SARS-CoV-2 infection causes a highly complex form of inflammation both acutely and chronically. Long COVID affects 10–20% of SARS-CoV-2 infected individuals who share persistent, chronic symptoms and conditions with Post-Acute COVID-19 Syndrome (PACS) [2]. Currently, it is estimated that 85 million people worldwide have a PACS.

Factors including comorbidities, age, gender, ethnicity and treatment received play a role in the profile of these symptoms. The WHO defines long COVID as the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months without other explanation (Figure 1).

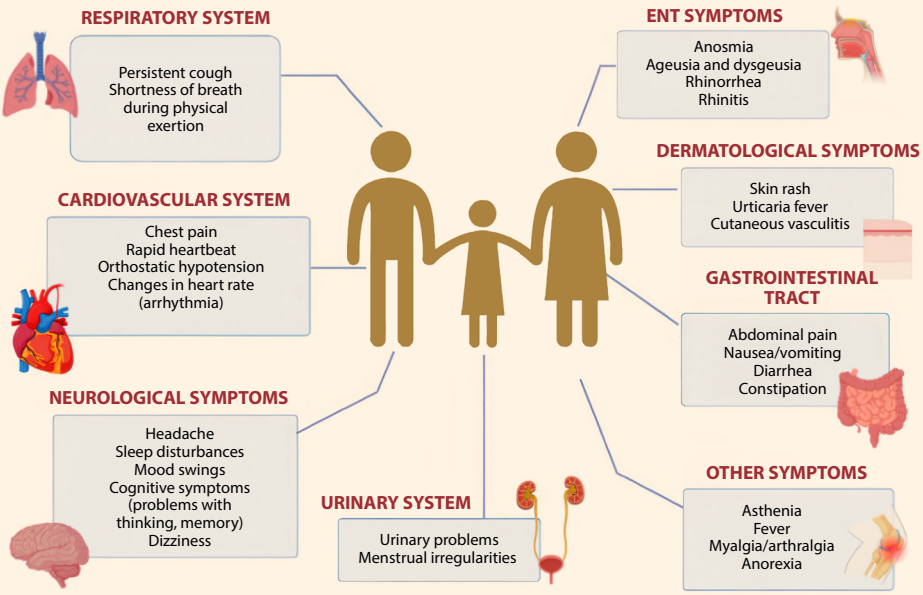

Figure 1. Long COVID syndrome

Long COVID affects 50–60% of SARS-CoV-2 infected cancer patients [3]. The prevalence of Long Covid is remarkably higher in infected cancer patients (50% to 60%) impacting more severely their Quality of Life. This fact can be linked to their compromised immune system and weakened physiological reserve. (Figure 2).

Cancer is a complex inflammation-induced and immune-editing disease. Certain studies propose that long-term effects of SARS-CoV-2 infection can have oncogenic potential in terms of oncogenesis and/or tumor progression. The potential importance and the unclear outcome of an orchestrated immune response against a developing tumor, under a SARS-CoV-2-driven chronic inflammatory status, should be stressed out.

Whether SARS-CoV-2 infection and derived "long lasting inflammatory status" frequently observed might affect the cancer immunosurveillance mechanisms and their risk of developing cancer, as well as the tumor and immune cell behaviors within the inflamed microenvironment.

Multiple Damage to endothelial barriers contributes to long COVID but also to thrombosis. Cancer is one main condition associated with so called "catastrophic thrombosis": a severe characterized by a hypercoagulable tendency leading to multiple thromboembolic events in different blood vessels, usually within a short time frame.

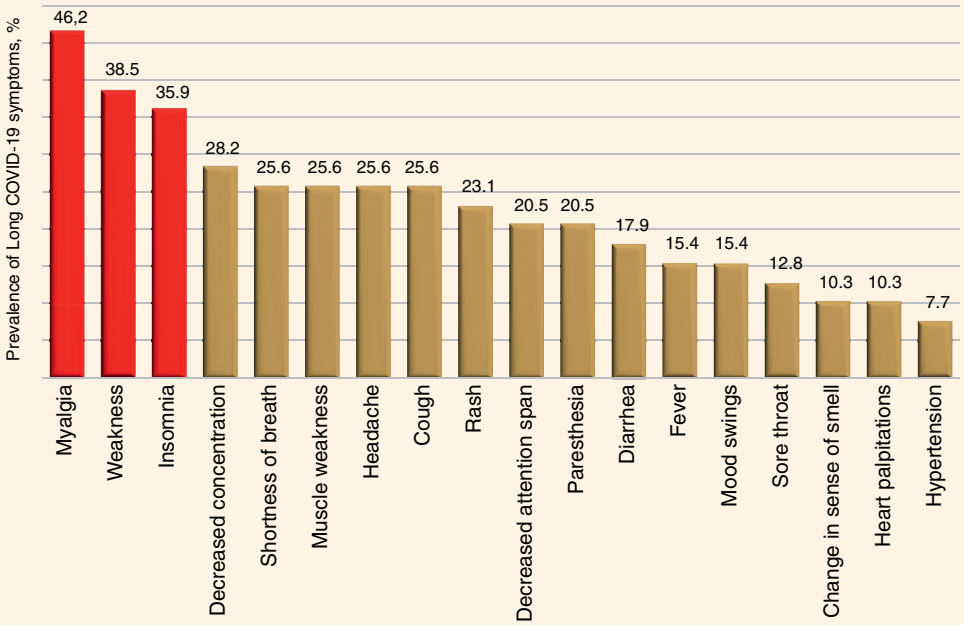

Figure 2. Long-term effects of COVID-19 pandemic for patients with cancer [3]

This particular incendiary profile is also reported in COVID 19 or vaccine induced thrombotic thrombocytopenia [4].

MECHANISMS INVOLVED IN LONG COVID OR PACS

Physiopathological mechanisms leading to PACS include vascular dysfunction and formation of micro-clots promoting thrombosis, immune dysregulation with increased pro-inflammatory response and autoreactive immunity driven by molecular mimicry and by stander activation of lymphocytes, the persistence of viral replication and SARS-CoV-2 proteins circulation, and reactivation of human latent herpes viruses [5]. Multiple blood biomarkers are candidates to identify Long COVID status [6] (Figure 3). Upon entry of SARS-CoV2, a possible cascade of acute inflammatory pathways in the alveolar lumen is displayed. SARS-CoV2 employs its Spike protein to bind with ACE2 receptor and membrane-bound serine protease TMPRSS2. SARS-CoV-2 also interacts with hyaluronic acid of the glycocalyx layer. SEM (Spike, Envelope, Membrane) pseudovirus particles or possible shedding of spike proteins also cause direct infection in alveolar dendritic cells followed by major histocompatibility complex II presentation and activation of CD4+ T helper 1 cells [5]. Subsequent production of interferon γ and nuclear factor-kB lead to productions of inflammatory cytokines and chemokines commonly known as cytokine storm. T helper 1 cell-mediated severe activation of macrophages and microglia might also cause non-specific phagocytosis of myelin. Possible activation of B cells produces autoantibodies. Active virus particles, T cells, and inflammatory mediators spread through distant organs across blood brain barrier, and triggering a severe cell-based inflammatory response with demyelinating

Table 1.

Long COVID blood markers [6]

Parameters	Status with Long COVID	Cytokines	Status with Long COVID
Erythrocytes	↓	IL-6	↑
MSU (ESR)	↑	TNF-α	↑
MCHC	↑	IL-1β	↑
Lymphocytes	↓	IL-2	↓
Hemoglobin	↓	IL-17	↓
Platelets	← →	IFN-γ	↓
D-dimers	↑	IFN-λ1	↑
Ferritin	↑	IFN-β	↑
C-reactive protein	↑	IL-10	← →
Lactatdehydrogenase	← →	IL-4 and IL-8	↓

Legend: ↑ — increased level; ↓ — decreased level; ← → — with no significant changes.
MCHC — mean corpuscular hemoglobin; IL — interleukin; TNF — tumor necrosis factor; IFN — interferon.

effects. Impaired nerve signal causes muscular fatigue frequently observed in Long COVID. SARS-CoV2 directly infects mitochondria, manipulates mitochondrial gene synthesis machinery, and alters mitochondrial metabolomes. The impairment can be linked to the release of pro-apoptotic molecules such as Bax, Bad, and cytochrome C; reversal of membrane potential; downregulation of β-oxidation and electron transport mechanism causing impaired ATP synthesis; induction of mitochondria-independent cytosolic glycolysis resulting in increased lactate synthesis. Thus, all these events trigger mitochondrial loss, fatigue and other manifestations of the PACS disease.

MECHANISMS IN FAVOR & AGAINST LONG COVID-INDUCED CARCINOGENESIS

The relationship between COVID-19 and cancer development is a complex interaction of immune suppression, chronic inflammation, genetic and epigenetic changes, and possible direct oncogenic viral effects [7]:

- Immunosuppression with lymphopenia, natural killer (NK) cell reduction, exhausted NK and CD8+ cells, diminished IFN response, decreased MHC-1, autophagy alterations.
- Hyperinflammatory and protumoral responses with oxidative stress and cytokine storm, DNA damage, direct ongenic impact.
- Dowregulation of tumor-suppressing proteins (p53, Retinoblastoma).
- Reactivation of oncogenic viruses such as human papilloma virus and Epstein–Barr virus.

The first line of host defense against SARS-CoV-2, innate immunity, is the response against the virus by recognizing Damage or Pathogens Associated Molecular Patterns (PAMPs/DAMPs) through transmembrane or intracellular pattern recognition receptors (PRRs). Recognition of viral components leads to the activation of immune cells and transcription factors that lead to the production of different cytokines, chemokines, and anti-viral proteins. promoting activation of adaptive immune response that consists of three major cell types (B cells, CD4+ T cells, and CD8+ T cells). After pathogen elimination, adaptive immunity regulates innate immunity to avoid unnecessary host cell damage. Unbalanced response and immune system overactivation can cause collateral damage to host tissues and exacerbate disease severity [8].

"Does immune dysregulation predispose individuals to cancer?" is a key question.

SARS-CoV2 genome encodes structural and non structural proteins that hijack host cell regulatory pathways. SARSCoV2 has the potential to cause severe disruption of homeostatic mechanisms that protect cells against neoplastic transformation. Thus, IL-6/JAK/STAT signaling pathway is also abnormally activated in many types of cancer and plays a fundamental role in tumorogenesis and immunosuppressive tumor microenvironment regulating the growth, survival, invasiveness, metastasis, cancer development and associated with a poor clinical prognosis. Moreover, NFκB Pathway is also often altered in both solid and hematopoietic malignancies promoting tumor-cell proliferation and leading to uncontrolled cell proliferation, differentiation, and apoptosis, as well as metastasis, and treatment resistance. Lastly, IFN-I signaling are involved in the development of innate and adaptive immune responses against both cancer and infectious diseases and a possible role of impaired INF-I signaling induced by SARS-CoV-2 infection with an inefficient antitumor response, which leads to tumor progression [8]. Besides immune dysregulation, chronic inflammation and oxidative stress, genetic and epigenetic changes induced by SARS-CoV-2 influence gene expression through DNA methylation and histone modification impacting oncogenes and tumor suppressor genes expression [9]. The viral proteins may also trigger carcinogenesis. Angiotensin-converting enzyme 2 (ACE2) increasing angiotensin II levels promotes tumor growth and angiogenesis through angiotensin II receptor-1 (ATGIIR1) and Hypoxia-Inducible Factor 1-alpha (HIF-1α) activation [9, 10]. Transmembrane serine protease 2 (TMPRSS2) degrades extracellular matrix proteins facilitating cancer cell invasion and metastasis through Jun N-terminal Kinase (JNK) signalling activation [9, 10] (Figure 3).

There is a real interplay between the virus SARS-COV-2 and cancer biology molecular interactions between the two diseases. COVID-19 is first driven by the entrance and invasion of SARS-CoV-2, as well as complement activations, intense apoptosis and pyroptosis following inflammatory stimuli which often involve inflammatory mediators. There is a sharply increasing number of neutralizing antibodies, which are involved in preventing viral spreads, featuring the second phase — yet, they are able to exacerbate the inflammatory cascades, leading to further lung and organ injury [11]. Similarly, cancer affects one's immune system and physiology through higher D-dimer, lower levels of albumin, longer prothrombin time, and higher neutrophil counts, for example in case of a hepatic involvement. Viral infection is involved in the development of cancer through epigenetic mechanisms

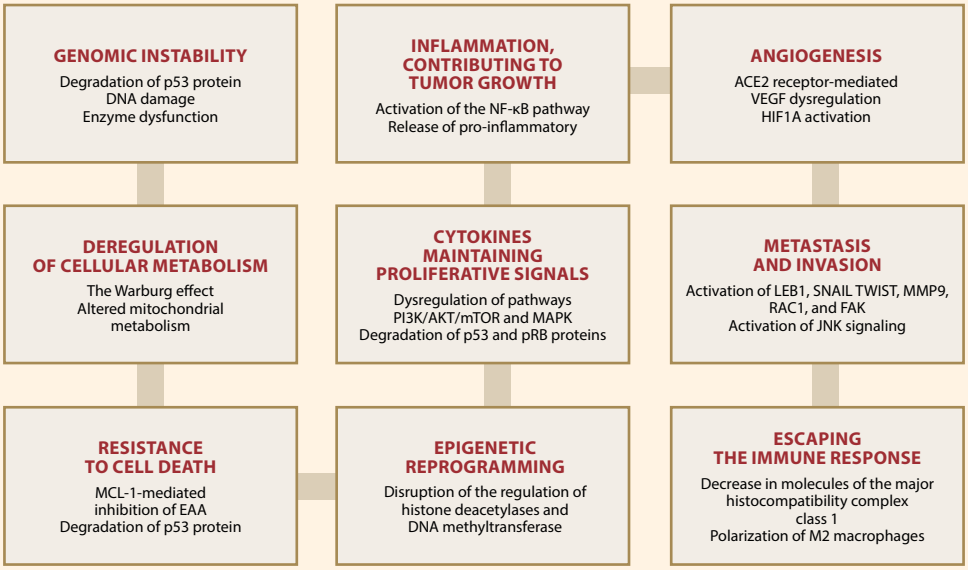

Figure 3. Oncogenic potential of SARS-CoV-2: targeting hallmarks of cancer pathways [10].

MCL-1 — myeloid cell leukemia 1, a protein of the Bcl-2 family that suppresses apoptosis (anti-apoptotic protein); BAK — Bcl-2 homologous antagonist/killer — a pro-apoptotic protein that, when activated, triggers cell death; PI3K/AKT/mTOR — phosphoinositide-3-kinase / protein kinase B / mammalian target of rapamycin; MAPK — mitogen-activated protein kinase; VEGF — vascular endothelial growth factor; HIF1A — hypoxia-inducible factor 1-α; ZEBI, SNAIL, TWIST — transcription factors that are key inducers of epithelial-mesenchymal transition; MMP9 — matrix metalloproteinase 9; RACI — a protein that regulates cytoskeletal reorganization; FAK — focal adhesion kinase; JNK — c-Jun N-terminal kinase)

leading to a dysfunctional immune response. SARS-CoV-2 infection with immune system suppression andimmunosuppression creates an optimal tumourigenic environment for pre-malignant, malignant, and dormant cells [12]. Thus, there is a clear interconnected biology of Neutrophil Extracellular Traps (NETS), Reactive Oxygen Species (Ros), immunosuppressive mediators, persistence of neutrophil stimulation induced in COVID-19 leading to chronic inflammation, autoimmunity and thrombosis feedback loops seen in long COVID and therefore overlapping biology with cancer [13, 14].

POSSIBLE CANCER-CAUSING CAPACITY OF COVID-19: IS SARS-CoV-2 AN ONCOGENIC AGENT?

European study Mendelian randomization (MR) is an epidemiological method to assess the potential causal association between exposure and outcome. MR can minimize the conventional confounding and reverse causation because genetic variation is randomly distributed during meiosis, independent of environment, disease onset, and progression. The aim of this study is to reveal the causal associations between COVID-19 and cancer diseases [15]. They found that COVID-19 had suggestive causal associations (genetic predisposition) with the risk for several cancers: HER2-positive breast cancer (OR=1.0924; p=0.0116), Esophageal cancer (OR=1.0004; p=0.0226),

Colorectal cancer (OR=1.0010; p=0.0242), Stomach cancer (OR=1.2394; p=0.0331), Colon cancer (OR=1.0006; p=0.0453). They are some limitations. First, the results were primarily based on Europeans only reducing the racial influence. Second, they assessed only genetic liability to COVID-19 and cancers, and the confounding factors smoking, body mass index, and alcohol intake frequency might not be completely ruled out. Thirdly, the lifelong average effects of genetic variants cannot be fully interpreted in the brief-period of this conventional observational study [15].

A recent retrospective cohort analysis of surgical, biopsy and autopsy archival material has shown the detection of SARS-CoV-2 persistence in endothelium and macrophages as well as in tumor cells of benign and malignant cardiac neoplasms [16]. The increase in the number of these tumors, especially cardiac myxomas, was significant after the pandemic by 2023. Immunohistochemical study in the control group did not reveal the expression of SARS-CoV-2 spike protein, while the virus protein was detected in tumor cells and macrophages in almost all mixomas [16].

Inflammation connects COVID-19 and cancer with pulmonary diseases. Inflammation leads to pulmonary manifestations exemplified by ARDS and COPD, which are observed in COVID-19 as well as lung cancer patients. A number of key cell surface proteins and enzymes play parallel roles in lung cancer progression and SARS-CoV-2 infection (ACE2, TMPRSS2, FURIN, PAI-1, CD147) [17]. A majority of them seem to play a role in the entry of SARS-CoV-2 into host cells while also being reported to be elevated in metastatic lung cancers.

Papillary thyroid cancer (PTC) trends were reported in the wake of the COVID-19 pandemic with a shift toward a more aggressive entity [18]. There was a significant increase between pre-pandemic and post-pandemic in the aggressive PTC variants (3% *vs.* 11.5%, p=0.001), increased poor prognostic factors such as bilateral multifocality (10.8% *vs.* 32.4%, p=0.001), as well as increased capsule — vascular tumor invasion (19.8% *vs.* 27%) [18]. A great rise in unfavorable prognostic markers and aggressive subtypes of PTC was seen post-pandemic in thyroidectomy patients.

Gut microbiota plays a crucial role in health and has been linked to the development of colorectal cancer (CRC). Investigations have shown that SARS-CoV-2 infection and long lasting impact of SARS-CoV-2 on gut dysbiosis cause changes to the gut microbiota, including an overall decline in microbial diversity, enrichment of opportunistic pathogens such as *Fusobacterium nucleatum bacteremia*, and depletion of beneficial commensals, such as the butyrate-producing bacteria [19]. Further, these changes lead to increased colonic inflammation, which leads to gut barrier disruption, expression of genes governing CRC tumorigenesis, and tumor immunosuppression, thus further exacerbating CRC progression [20].

Should we expect an increase in the number of cancer cases in people with long COVID? The potential long-term effects of SARS-CoV-2 infection on oncogenesis, immune surveillance, chronic inflammation, oxidative stress, cell cycle dysregulation, potential viral genome integration, epigenetic alterations and genetic mutations, reactivation of dormant cancer cells must be taken into account for cancer development and its progression [21, 22]. So, all patients should be regularly screened for cancer after SARS-CoV-2 infection, as the virus has been shown not only to affect cancer progression but also to induce oncogenesis and cancer recurrence [23].

CONCLUSION

Many questions remain for this cross talk between SARS-CoV-2 infection and cancer. The expression of some SARS-CoV-2 proteins has oncogenic effects but this does not necessarily imply that they promote cancer development. We need to monitor lung cancer patients for the appearance of new comorbidities but other cancers with an increased incidence are reported. It is essential to identify biomarkers that could allow us to assess the impacts of cancer and evaluate possible therapeutic interventions. It would be interesting to study the potential role of SARS-CoV2 vaccination in the pathways that potentially promote cancer. It is imperative to continue to conduct studies, to develop animal models to provide clarity on this issue.

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Chapter 12

Long COVID and Women's Health

Bitsadze V.O.

ABSTRACT

The COVID-19 pandemic is associated with increased maternal and neonatal risks, an increase in the frequency of thromboembolic complications, and dysregulation of immune processes in pregnant women. Currently, the pathogenetic mechanisms of Long COVID, the effect of hormone therapy and assisted reproductive technologies on thrombophilic conditions are being studied. The data obtained within the RECOVER project allow us to systematize modern ideas about this problem.

In the period of the COVID-19 pandemic, key issues in obstetrics have become the assessment of maternal and neonatal morbidity and mortality, the study of the mechanisms of transmission of the SARS-CoV-2 virus and maternal antibodies to the fetus, as well as their possible role in ensuring the health of the newborn. According to Servante J. et al., the risk of thromboembolic complications in pregnant women with COVID-19 is almost three times higher than in uninfected women, with the frequency of hemostasis disorders increasing tenfold [1]. SARS-CoV-2 infection during pregnancy is generally associated with increased risks for both the mother and the fetus, including the likelihood of adverse outcomes such as maternal and perinatal mortality. Moreover, the results of a systematic review by Conde-Agudelo A. et al. show that COVID-19 significantly increases the risk of serious obstetric complications, including preeclampsia, fetal growth restriction, premature placental abruption, and premature delivery [2].

These complications are usually caused by endothelial dysfunction, microcirculation disorders, activation of the coagulation cascade, and a pronounced inflammatory response, which adversely affects placental function and uteroplacental blood flow.

Among the adverse neonatal outcomes in children born to mothers who had COVID-19, there were cases of stillbirths, neonatal asphyxia, intrauterine infection, pneumonia, and increased neonatal mortality. The results of a multinational cohort study conducted by Villar J. et al. demonstrated a significant increase in the frequency of severe complications in both mothers infected with SARS-CoV-2 and their newborns, including severe forms of respiratory failure and septic conditions [3]. In addition, according to the Royal College of Obstetricians and Gynaecologists (RCOG, 2020), the risk of premature birth in pregnant women with COVID-19 more than doubles, especially in late pregnancy [4] (Figure 1).

Thus, COVID-19 should be considered a significant risk factor for severe obstetric and neonatal complications, which necessitates careful dynamic monitoring of the condition of pregnant women and the timely application of preventive and therapeutic measures to reduce the likelihood of adverse outcomes. When managing pregnant women with COVID-19, it is particularly important to comprehensively assess the functional state of the placenta, the severity of the systemic inflammatory response, and factors contributing to the development of placenta-associated complications, as these indicators largely determine the prognosis for the course of pregnancy.

Given the leading role of immune responses in the pathogenesis of SARS-CoV-2, studies devoted to the activity of maternal immune cells and their effect on the vas-

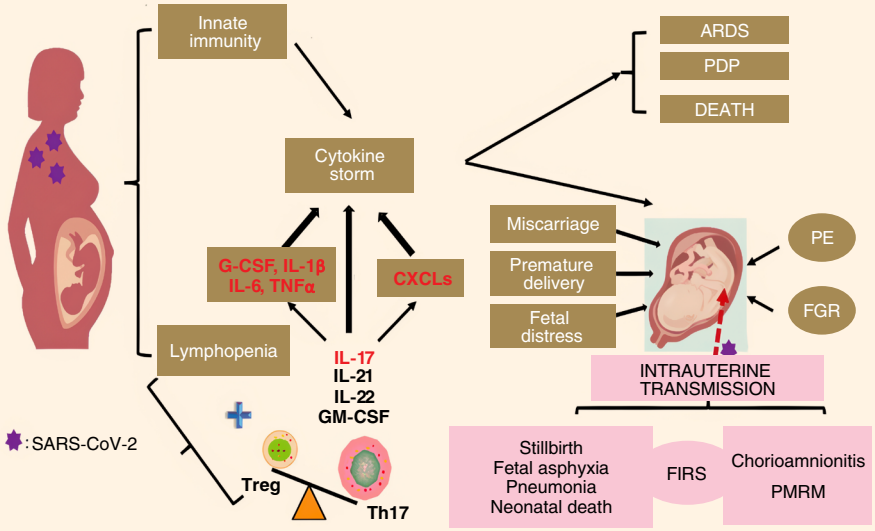

Figure 1. Effects of SARS-COV-2 during pregnancy.

The SARS-CoV-2 virus causes significant changes in the immune system of pregnant women, activating innate immunity, imbalance, and dysfunction of regulatory T cells (Treg) and T lymphocytes that produce pro-inflammatory interleukin-17 (Th17), towards a Th17 pro-inflammatory response with the development of a cytokine storm. COVID-19 increases risks for both the mother and the fetus, including the risk of death and placenta-associated complications: preeclampsia (PE), fetal growth restriction (FGR), premature detachment of a normally located placenta (PDP), and premature delivery. Adverse neonatal outcomes: stillbirths, neonatal asphyxia, pneumonia, neonatal death may be the result of both the development of chorioamnionitis with premature rupture of membranes (PMRM) and fetal inflammatory response syndrome (FIRS), and in some cases, intrauterine transmission of the SARS-CoV-2 virus; ARDS — acute respiratory distress syndrome; GM-CSF — granulocyte-macrophage colony-stimulating factor; CXCLs — chemokine ligands; TNF-α — tumor necrosis factor-α; IL — interleukin

cular system of the mother and fetus are of particular interest. The inflammatory response in COVID-19 largely replicates the pathogenetic mechanisms characteristic of preeclampsia, including endothelial dysfunction, cytokine cascade activation, and microcirculation disorders. These processes can exacerbate the negative impact on fetal neurovascular development, increasing the risk of adverse perinatal outcomes.

A study by Sullivan K.S. et al. demonstrated the key role of $CD4^+$ T cells obtained from women with preeclampsia and/or previous SARS-CoV-2 infection in the development of hypertension, cerebral blood flow disorders, and cognitive dysfunction in both mothers and offspring. These data confirm that maternal immune cells can have a long-term impact on the neurovascular health of offspring, even in the absence of changes in body weight, blood pressure, and growth rates in the early postnatal period. The pathogenic mechanisms of such changes are associated with hyperactivation of $CD4^+$-T lymphocytes, leading to an imbalance of proinflammatory cytokines (interleukin, tumor necrosis factor-α), endothelial dysfunction, and impaired regulation of vascular tone and microcirculation [5].

COVID-19 causes a systemic hyperinflammatory response (cytokine storm), which exacerbates the prothrombotic state and endothelial damage, which is also characteristic of preeclampsia. These conditions have overlapping pathophysiological mechanisms, including activation of the renin-angiotensin-aldosterone system via angiotensin-converting enzyme 2 receptors and dysregulation of angiogenic factors. According to Conde-Agudelo A. et al., women with COVID-19 have a 33–62% higher risk of developing preeclampsia compared to uninfected pregnant women, which correlates with the changes observed in the model by Sullivan K.S. et al. [2, 5]. In addition, systemic immune changes caused by SARS-CoV-2 may be associated with impaired fetoplacental blood flow, increased vascular permeability, and changes in neurovascular interaction, which exacerbates the risks of cognitive impairment in offspring.

Recent reviews also confirm the importance of inflammatory and immune cascades in COVID-19. Rad H.S. et al. (2021) point to significant damage to the placenta during infection, associated with syncytiotrophoblast dysfunction and immune cell activation [6]. Furthermore, data from Kozłowski P. et al. (2024) confirm the contribution of persistent inflammation, autoimmune reactions, and cytokine activation to the disruption of neurovascular homeostasis [7]. These observations highlight the importance of further research into the immune response in the combination of COVID-19 and preeclampsia, as well as the search for therapeutic strategies aimed at modulating $CD4^+$ T-cell activity and reducing the risks of long-term consequences for offspring. The totality of these observations points to the need for further study of the immune response in the combination of COVID-19 and preeclampsia, as well as the promise of finding therapeutic strategies aimed at modulating $CD4^+$ T-cell activity and reducing the risks of long-term consequences for the mother and offspring.

In this context, the mechanisms of vertical transmission of SARS-CoV-2 from mother to fetus are of particular interest, as they are largely associated with damage to placental structures and immune disorders. Potential transmission routes include direct damage to the placental villi with disruption of the protective layer of the syncytiotrophoblast, induced by viral apoptosis and damage to the vascular network. Additionally, mechanisms such as viral penetration through the maternal endothelium to

the extravillous trophoblast, transfer of viral particles by maternal immune cells, and transplacental cell transport are described. In addition to hematogenous routes, ascending (vaginal) infection is possible, including infection through swallowed or aspirated amniotic fluid, which is particularly relevant in the peripartum period.

The results of the study by Rad H.S. et al. confirm these hypotheses: analysis of morphological and immunohistochemical changes in the placenta in COVID-19 revealed signs of syncytiotrophoblast damage, activation of the inflammatory cascade, and possible transplacental spread of the virus [6]. Although morphological and immunohistochemical data demonstrate the possibility of such transmission routes, systematic reviews and meta-analyses have not found convincing evidence of intrauterine transmission of SARS-CoV-2. In particular, a meta-analysis of 39 studies and 1,316 pregnant women showed that vertical transmission of SARS-CoV-2 is extremely rare.

Data on transplacental transfer of antibodies are of particular importance. A study by Flannery D.D. et al., conducted on a cohort of 1,471 mother-newborn pairs, it was shown that 87% of seropositive mothers (72 out of 83) had effective transfer of IgG antibodies to SARS-CoV-2 into the newborn's bloodstream, confirming the ability of the placenta to form passive immunity in the fetus [9]. These results demonstrate that, despite the rarity of vertical transmission of the virus, the placental barrier functions effectively as an immune filter, transmitting protective antibodies.

These observations suggest that SARS-CoV-2 is capable of triggering persistent immune and inflammatory changes that are not limited to the acute phase of infection. It is likely that these mechanisms may form the basis for long-term post-COVID conditions in both mothers and children, affecting their neuroimmune and metabolic health.

Such data are reflected in the broader context of studying post-COVID complications, including Long COVID (LC) syndrome. This multisystem syndrome is characterized by marked heterogeneity of clinical manifestations, including chronic fatigue, headaches, cognitive impairment, hair loss, myalgia, and shortness of breath. To date, more than 200 different symptoms of LC have been described, highlighting its complex pathogenesis and the involvement of systemic inflammatory and immune mechanisms [10].

Current understanding of the pathogenesis of LC points to its multifactorial nature. Key mechanisms include viral persistence and the formation of viral reservoirs that sustain chronic inflammation, as well as molecular mimicry that contributes to the development of autoimmune processes. Systemic inflammation with activation of proinflammatory cytokines, endothelial dysfunction, and immunothrombosis play a significant role, leading to microcirculation disorders and organ damage [11]. Important factors include microbiome dysbiosis, which exacerbates immune imbalance, as well as the reactivation of latent viruses such as Epstein–Barr virus (EBV) and human herpesvirus type 6 (HHV-6), which are associated with chronic fatigue and cognitive impairment. Persistent autoimmune reactions can maintain a pathological inflammatory background even after the virus has been eliminated, leading to long-term complications [12–20] (Figure 2).

In this regard, the priority task is to establish clear diagnostic criteria for this multisystem syndrome, which will allow for a deeper understanding of its pathogenetic mechanisms and the development of personalized therapeutic strategies.

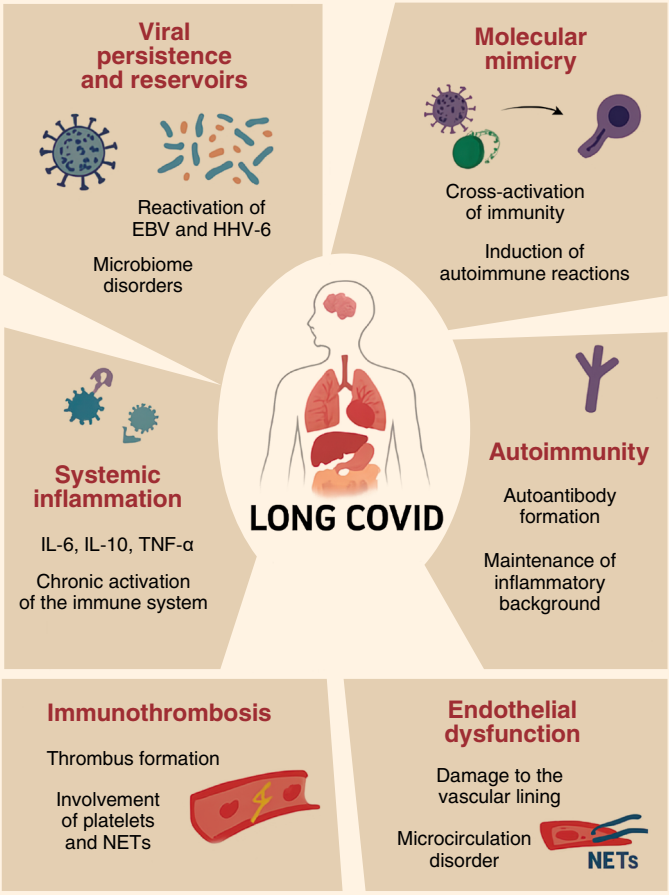

Figure 2. Theories of Long COVID pathogenesis

In patients with LC, laboratory tests often show persistently high levels of D-dimer, proinflammatory cytokines, particularly interleukin-6 (IL-6), markers of hypofibrinolysis, high levels of plasminogen activator inhibitor, von Willebrand factor as possible markers of endothelial damage, as well as procalcitonin, ferritin, C-reactive protein, and, accordingly, brain natriuretic hormone — protein) [21].

The prevalence of LC is approximately 20–30% of the population infected with COVID-19, or more than 200 million people. SARS-CoV-2 affects twice as many women as men and may disproportionately affect transgender people. Women in perimenopause have an increased risk of developing LC, suggesting a key role for sex hormones in the development of this condition [22].

The likelihood of developing post-acute effects of COVID-19 increases in people who have had severe or moderate COVID-19, especially if they were not vaccinated before infection. The presence of additional risk factors also increases this likelihood [23]. COVID-19 can reveal existing health problems and hidden diseases that were not previously clinically apparent, and exacerbate the course of known diseases. The

existence of post-COVID complications has prompted many countries to study this syndrome as a matter of national importance.

Researchers in the United States are working with patients, doctors, and communities across the country to identify strategies for preventing and treating the long-term effects of COVID-19, including LC. Furthermore, a dedicated website has been created to highlight and publish the latest data obtained from the RECOVER study. The first longitudinal studies of neurocognitive development in children born to SARS-CoV-2-infected mothers show a significant decrease in psychomotor development scores [24]. The RECOVER initiative also conducted a study to assess the prevalence of post-acute effects of COVID-19 infection after infection during pregnancy, with an assessment of risk factors.

According to the analysis, 61% of SARS-CoV-2 infections were recorded during the period when the Omicron variant was dominant (December 2021 and later), with 51% of patients having received a full course of vaccination prior to infection. Risk factors included obesity (adjusted odds ratio (aOR) 1.63, 95% CI 1.13–2.44), pre-existing depression or anxiety disorder (aOR 2.64, 95% confidence interval (CI) 1.80–3.87), economic difficulties, and the need for oxygen therapy during illness (aOR 1.88, 95% CI 1.01–3.50) [25].

The prevalence of LC 6 months after SARS-CoV-2 infection during pregnancy is 9.3% (95% CI 7.9–10.9%), which is lower than the result published in the NIH RECOVER-Adult cohort, 23%, which included adult men and women, so it was not very comparable. This study was followed by the following retrospective cohort study, which used electronic medical record data from 19 healthcare systems in the United States of America. The aim of the study was to assess the association between SARS-CoV-2 infection during pregnancy compared to SARS-CoV-2 infection outside of pregnancy and the development of LC symptoms [26]. The study covered more than 88,000 women aged 18 to 49 who had laboratory-confirmed SARS-CoV-2 infection between March 2020 and June 2022. Of these, more than 83,000 were infected with the virus outside of pregnancy, and 5,397 were infected during pregnancy.

Non-pregnant women with COVID-19 infection were more likely to be older and have comorbidities. It is noteworthy that pregnant women who had COVID-19 were more likely to develop certain conditions commonly associated with post-COVID syndrome, namely cardiac arrhythmia (adjusted risk ratio (aHR) 1.67, 95% CI 1.43–1.94), abdominal pain (aHR 1.34, 95% CI 1.16–1.55), and thromboembolism (aHR 1.88, 95% CI 1.17–3.04). However, the likelihood of developing symptoms such as malaise and fatigue (aHR 0.35, 95% CI 0.27–0.47), pharyngitis (aHR 0.36, 95% CI 0.26–0.48), and cognitive problems (aHR 0.39, 95% CI 0.27–0.56) was, on the contrary, reduced [26].

Thus, SARS-CoV-2 infection during pregnancy was associated with a lower risk of developing post-acute COVID-19 symptoms within 30–180 days after infection, which was 25.5% versus 33.9% of women who developed these symptoms during non-pregnancy [26].

The low prevalence of LC in pregnant women after COVID-19 may be due to the immunomodulatory effect of pregnancy. It is assumed that the high physiological concentrations of estrogen and progesterone characteristic of pregnancy influence the immune response. In particular, high physiological concentrations of 17β-estra-

diol suppress the production of pro-inflammatory cytokines IL-6, IL-1β, TNF-α) and chemokine CCL2 by macrophages, thereby preventing the migration of neutrophils and monocytes to the site of inflammation [27].

When discussing LC, it is important to note that this syndrome can affect women in menopause, as well as those who use hormonal contraception or are preparing for in vitro fertilization (IVF) and assisted reproductive technologies. Therefore, the issue of using hormonal drugs in patients with LC remains extremely relevant and is currently being actively studied (Figure 3).

Given the potential anti-inflammatory benefits of hormones, hormone therapy could theoretically be beneficial, but menopausal hormone therapy (MHT) is known to be associated with an increased risk of thrombosis. Studies show that MHT can increase the risk of venous thromboembolism by 2–4 times. This risk depends on many factors, including the type and dose of the drug, the method of administration, the patient's age, and the presence of factors predisposing her to thrombosis. Therefore, it is important to carefully evaluate all risks and benefits before starting MHT [28].

Straczek C. et al. showed that women with thrombophilia who take MHT, especially those containing estrogens, have a significantly increased risk of venous thromboembolism. For example, the likelihood of developing thrombosis in such patients increases 25-fold [29]. It is important to note that transdermal forms of MHT demonstrate a more favorable safety profile with regard to thrombosis, approaching the risks observed in non-thrombophilic patients receiving MHT [30].

Thus, the safest option for MHT for menopausal women, in terms of the risk of venous thromboembolism, is transdermal estrogens, and micronized progesterones. At present, there is insufficient data to assess the risk of thrombotic complications in patients with LC. The possibility and safety of hormone therapy in this group, even under the cover of anticoagulants, has not been studied. All these issues require further in-depth research.

Combined oral contraceptives (COCs) are associated with a 3–6-fold increase in the risk of venous thromboembolism (VTE) and an approximately 1.7-fold increase in the risk of arterial thrombosis and ischemic stroke [31]. In light of these data, special attention is paid to the use of COCs in women with a predisposition to thrombotic complications.

The use of hormone therapy in high-risk groups remains controversial. Despite the potential anti-inflammatory effects of hormone therapy, it should be prescribed with extreme caution. According to the World Health Organization's criteria for medical acceptability, combined hormonal contraceptives are contraindicated in the acute phase of VTE, as well as in women with a history of VTE, even if they are receiving anticoagulant therapy [32].

The recommendations of the International Society on Thrombosis and Haemostasis allow for the use of COCs in patients receiving anticoagulants, provided that therapeutic doses of anticoagulants are capable of counteracting the prothrombotic effects of hormone therapy [33]. However, in patients with LC, the question of the safety of hormonal contraceptives remains open and requires further research.

It is important to emphasize that the discussion concerns only patients with VTE, but not those with arterial thrombotic events. Patients with antiphospholipid syndrome

and thrombotic complications against its background constitute a special risk category. It is known that both hormonal contraceptives and new oral anticoagulants are contraindicated in this group, with vitamin K antagonists, in particular warfarin, remaining the standard of care [34].

Thus, the question of the possibility of using hormonal contraceptives in patients with LC remains unresolved and requires further clinical research.

There is a tendency in Russian medical practice to expand the use of anticoagulant therapy in patients at risk of thrombosis. This is due to the fact that women receiving anticoagulants are at risk of gynecological complications, such as ovarian apoplexy and severe menorrhagia. Schulman S. et al. note that abnormal uterine bleeding is more common in patients taking rivaroxaban compared to groups receiving enoxaparin or warfarin [35].

In this context, hormonal methods of contraception can be used not only to prevent pregnancy, but also as a therapeutic tool for the prevention of hormonal disorders in gynecological practice. When used in this way, the risk-benefit ratio may shift towards the benefits of hormonal contraceptives, but each clinical situation must be assessed individually.

It is important to pay special attention to the use of assisted reproductive technologies in patients with post-COVID syndrome. According to a study by Henriksson R. et al., the incidence of venous thromboembolism in women after IVF is higher than

Figure 3. Risk factors for Long COVID

in women with spontaneous pregnancies [36]. The risk is the highest during the first trimester of pregnancy. Some studies, such as the work of Olausson N. et al., have shown that the transfer of cryopreserved embryos is associated with a lower risk of venous thrombosis in the first trimester [37]. However, ovarian hyperstimulation syndrome significantly increases the likelihood of venous thromboembolism — almost 100 times. The RIETE registry has indicated that ineffective IVF protocols are also associated with a high risk of thrombotic complications [38].

The topic of reproductive disorders in women with LC has not been sufficiently studied to date. A recently published article notes complications such as menstrual cycle disorders, premature ovarian failure, endometriosis, vulvodynia, and dyspareunia [39].

Previously, similar long-term consequences were studied in patients after severe thrombotic conditions, including catastrophic antiphospholipid syndrome and thrombotic thrombocytopenic purpura. In particular, a study by Vesely S.K. focused on the consequences of thrombotic thrombocytopenic purpura [40].

Studying the long-term effects of COVID-19 is a priority in modern medicine, especially given their potential impact on women's health and reproductive systems. The RECOVER project, initiated by the US National Institutes of Health, is conducting a comprehensive assessment of the post-acute sequelae of SARS-CoV-2 infection (PASC — Post-Acute Sequelae of COVID-19, "long COVID"), including analysis of clinical, laboratory, and biological data from patients in various cohorts: adults, pregnant women, and children [41]. A key feature of the RECOVER approach is the use of multi-omic technologies, including genome, epigenome, transcriptome, proteome, metabolome, and microbiome research, which allows for a deeper understanding of the pathogenesis of the disease and the identification of new biomarkers, predictors of complications, and potential therapeutic targets.

PASC is a collection of chronic conditions associated with a wide range of symptoms and significant healthcare costs. The clinical manifestations of PASC are highly heterogeneous and likely include several molecular subtypes, but their pathophysiological mechanisms remain poorly understood. This hinders the development of rationally based therapeutic strategies. As part of the RECOVER initiative, an interdisciplinary OMICS group was formed, bringing together clinicians, pathologists, molecular biologists, and data analysis specialists. The main task of this group was to create standardized protocols for the use of advanced systems biology methods in the study of PASC. Over a period of 14 months, published data were regularly evaluated and comprehensive recommendations for research design were formulated [41].

An important achievement was the decision to conduct longitudinal multi-omic studies on a single platform with centralized sample processing, which will minimize interlaboratory variability and increase the reliability of the data obtained. The collected multidimensional molecular datasets will be correlated with clinical phenotyping, social determinants of health, lifestyle characteristics, and comorbidities. This systematic approach will enable the identification of molecular subtypes of PASC, as well as the search for potential biomarkers and therapeutic targets for personalized treatment [41] (Figure 4).

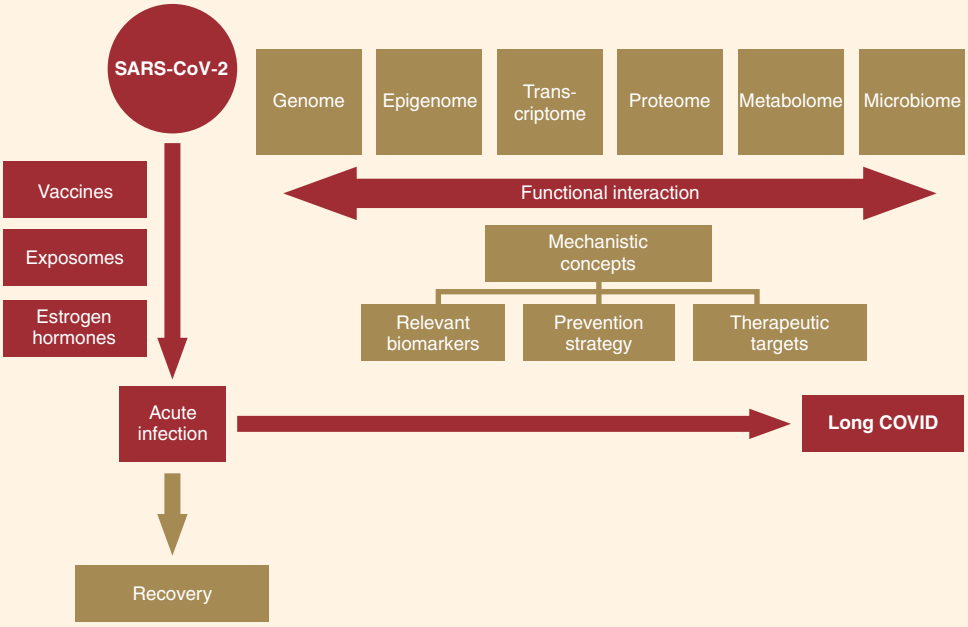

Figure 4. Comprehensive multiomic approaches to the study of SARS-CoV-2

The use of multiomic methods to study the impact of COVID-19 on the reproductive system is of particular importance. Pregnancy is a condition accompanied by physiological immunosuppression and hormonal changes, which can increase susceptibility to viral infections and contribute to the development of long-term complications. Multiomic studies allow us to assess not only immunological and metabolic shifts in women after COVID-19, but also to identify potential markers of placental dysfunction, endothelial dysfunction, and inflammatory reactions associated with LC. This approach is important for predicting obstetric complications, developing preventive measures, and personalized therapy.

The use of multiomics technologies opens up new opportunities for studying the pathogenetic mechanisms of LC associated with reproductive health. Genomic analysis allows identifying genetic predispositions to thrombophilia, autoimmune reactions, and endothelial dysfunction.

Epigenomic and transcriptomic studies help us understand how SARS-CoV-2 alters the expression of genes that regulate immune response and placental function. Proteomic profiles provide insight into the imbalance of blood coagulation proteins, cytokines, and angiogenesis factors, which may be key in the development of preeclampsia and placental pathologies. Metabolomic studies allow us to assess changes in energy metabolism and oxidative stress, which may be associated with impaired neurovascular development of the fetus.

Thus, the multiomic approach can be an effective tool for early prediction of obstetric complications and post-COVID reproductive disorders. It opens up opportunities for the implementation of personalized treatment strategies aimed at modulating the immune response and preventing adverse outcomes. The use of multiomic tech-

nologies allows not only to identify molecular markers associated with the risk of complications, but also to develop individual therapeutic solutions based on a deep understanding of the pathogenetic mechanisms of the disease.

An important area of such research is the study of women who have had COVID-19 during pregnancy, with the aim of assessing the long-term consequences for both the mother and her offspring. It has been shown that SARS-CoV-2 can cause changes in the immune and regulatory systems that persist after childbirth and affect fetal development. In particular, data on reduced neurodevelopmental outcomes in children born to mothers with COVID-19 underscore the need for long-term follow-up of this patient group [25]. In addition, the RECOVER results confirm that not only biological but also social factors influence the clinical manifestations of PASC, including virus variants, vaccination rates, socioeconomic determinants, and comorbidities.

Pregnant women represent a special risk group, since SARS-CoV-2 infection during this period is accompanied by pronounced changes in the immune response, similar to the pathogenetic processes in preeclampsia, including endothelial dysfunction, cytokine cascade activation, and microcirculation disorders. These mechanisms may increase the likelihood of both acute and long-term complications, including the development of LC symptoms.

CONCLUSION

The COVID-19 pandemic has significantly changed approaches to obstetrics and perinatal medicine. Studying post-COVID complications, including PASC, is particularly important for women who have had COVID-19 during pregnancy, given the potential long-term consequences for the mother and fetus. The application of a multiomic approach within RECOVER opens up prospects for the creation of personalized prevention and treatment strategies aimed at reducing the risk of complications and improving the quality of life of patients.

The study will be conducted in accordance with the principles of good clinical practice and ethical standards.

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Chapter 13

Neonatal Thrombosis as One of the Manifestations of Immune and Thromboinflammatory Consequences of COVID-19

A.D. Makatsariya

ABSTRACT

This chapter analyzes immune and infectious variants of thrombosis in newborns, including antiphospholipid syndrome (APS), its catastrophic form (CAPS), thrombosis associated with SARS-CoV-2 infection, and conditions resembling vaccine-induced thrombotic thrombocytopenia (VITT). Particular attention is paid to de novo production of antiphospholipid and anti-PF4 antibodies in newborns, which is considered a possible trigger for thromboinflammatory reactions. Epidemiological aspects, pathogenetic mechanisms, diagnostic difficulties, and modern therapeutic approaches are discussed. The importance of rethinking diagnostic algorithms, taking into account the role of immune and inflammatory factors, is emphasized. The need to integrate multidisciplinary and personalized approaches in the management of patients with neonatal thrombosis is highlighted.

INTRODUCTION

Thrombotic complications in the neonatal period represent a complex clinical and diagnostic challenge at the intersection of several disciplines: neonatology, hematology, immunology, and perinatal medicine. Although the frequency of detection of neonatal thrombosis in routine practice remains relatively low, the clinical significance of these conditions is due to the high risk of serious consequences, including disability and death.

In recent years, increasing attention has been paid to the study of immune and infection-mediated mechanisms of thrombosis in newborns. This group includes pathological conditions associated with antiphospholipid antibodies (APA), CAPS, thrombosis occurring against the background of coronavirus infection, as well as rarely recognized cases associated with the formation of anti-PF4 antibodies, pathogenetically similar to VITT.

Particular attention should be paid to the phenomenon of independent, non-maternal APA production in the fetus, *de novo*, which is increasingly considered an independent factor in thrombus formation in newborns, especially in the context of an infectious or inflammatory trigger.

EPIDEMIOLOGY OF NEONATAL THROMBOSIS

According to current epidemiological data, the incidence of venous thromboembolism (VTE) in the neonatal period varies significantly depending on the population studied, the level of medical infrastructure development, and the diagnostic methods used. According to the results of large clinical reviews, the incidence of thrombosis in children in intensive care units reaches 2.4 cases per 1,000 hospitalizations, with even higher rates among premature newborns [1, 2]. The highest incidence of VTE occurs in the early neonatal period — the first 28 days of life and the risk of thrombosis in this age group exceeds that in older children by more than 40 times [3].

The vast majority of thrombotic episodes in newborns are associated with the presence of a central venous catheter, which is described in 90% of clinical cases. However, catheterization alone is not a sufficient condition for the development of clinically significant thrombosis — its manifestation is observed in only about 5% of patients with a catheter [4].

At the same time, VTE is extremely rare in healthy full-term infants, which highlights the key role of additional trigger factors in the pathogenesis of thrombosis [5]. In this regard, particular attention should be paid to identifying immunoinflammatory mechanisms, including circulating APA and anti-PF4 antibodies, congenital forms of thrombophilia, the presence of infectious agents, and systemic inflammatory responses. Inflammation plays an important role in the formation of hemostatic imbalance: activation of neutrophil extracellular traps (NETs), endothelial damage, and secretion of proinflammatory cytokines in response to infectious or stressful stimuli create conditions for thromboinflammation — a pathophysiologic process underlying conditions such as catastrophic APS, VITT, and thrombosis associated with the SARS-CoV-2 virus.

PATHOGENESIS AND CLASSIFICATION OF IMMUNE THROMBOSIS

Immune-mediated thrombosis in newborns forms a special category within the spectrum of neonatal VTE, distinguished by the direct involvement of both innate and adaptive immune mechanisms in the initiation and maintenance of coagulation disorders. The pathogenesis of these conditions is based on immunothrombosis, a process in which components of the immune system activate the hemostasis cascade.

The key pathophysiological mechanisms of immunothrombosis include:

- formation of APA directed against beta2-glycoprotein I, anticardiolipins, and lupus anticoagulant;
- synthesis of antibodies to platelet factor 4 (anti-PF4), capable of inducing platelet activation via the FcγRIIA receptor;
- neutrophil activation with the formation of extracellular traps (NETs), which play a role in enhancing procoagulant activity and endothelial damage;
- the presence of a pronounced pro-inflammatory cytokine profile (TNF-α, IL-1β, IL-6, IL-10, etc.), especially in cases of maternal coronavirus infection transmitted to the fetus through the placental barrier [6] (Figure 1).

Immune forms of neonatal thrombosis can be classified as follows:

- secondary, arising as a result of transplacental transfer of maternal antibodies (including APA and anti-PF4);
- associated with de novo antibody synthesis in the fetus against the background of an infectious or inflammatory stimulus [7–9];
- associated with autoimmune or autoinflammatory activation characteristic of conditions such as CAPS or COVID-19-induced immunoregulatory dysfunction.

Particular pathogenetic significance is attached to the "second strike" hypothesis, according to which the presence of a single sensitizing factor (e.g., antibodies) is insufficient for the development of thrombosis without an additional trigger, whether it is infection, hypoxic condition, or genetically determined features of the immune response. This model is particularly relevant when interpreting cases with *de novo* APA production [7, 10] (Figure 2).

ANTIPHOSPHOLIPID SYNDROME AND ITS NEONATAL FORMS

APS in newborns is a rare but clinically significant form of immune-mediated thrombotic pathology. In pediatric practice, including the neonatal period, APS demonstrates clinical features that differ from those seen in the adult population: in

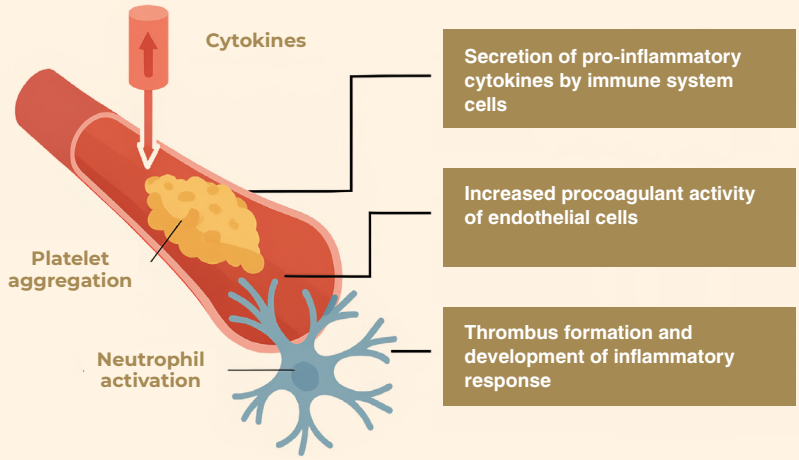

Figure 1. Thrombotic inflammation mechanism

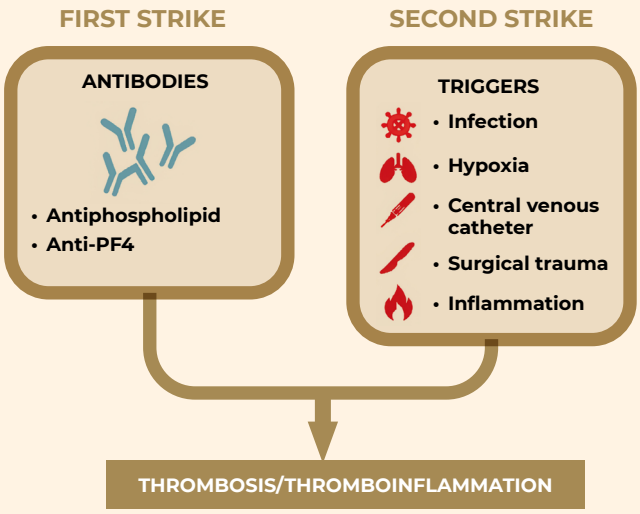

Figure 2. The "second strike" hypothesis

addition to thrombosis, newborns often experience hematological, dermatological, and neurological manifestations.

There are two main forms of neonatal APS:

- acquired transplacental APS, caused by the transfer of maternal 1dC-antibodies through the placental barrier;
- de nov*o* APS, characterized by independent production of APA in the fetus or newborn in the absence of antibodies in the mother.

Analysis of the literature confirms the reality of an autonomous immune response in newborns: in one study, 6 out of 21 APA-positive newborns with thrombosis did not have antibodies, indicating *de novo* synthesis [11], In another observation involving 12 children with cerebral thrombosis, only 2 mothers were found to have APA, while in the remaining 10, antibodies were formed directly in the child [12].

The main proposed mechanisms of *de novo* APA production include:

- intrauterine or early postnatal infectious activation of the fetal immune system;
- development of systemic inflammatory response syndrome;
- exposure of fetal-placental immunity to viral antigens, including SARS-CoV-2;
- presence of genetically determined prothrombotic conditions (FV Leiden mutation, G20210A, antithrombin deficiency, etc.).

It is important to note that *de novo* APS in newborns is more often accompanied by the production of IgM-isotype APA, as opposed to transplacental IgG. These antibodies often demonstrate polyspecificity (simultaneous binding of anticardiolipins and beta2-glycoprotein I) and can persist in serum for up to 6-12 months of life, requiring long-term clinical observation and differential diagnosis with other autoimmune conditions [13] (Table 1).

Currently, there are no verified clinical and laboratory criteria that allow for a reliable diagnosis of APS in pediatric practice. In this regard, the SHARE (Single Hub and Access point for paediatric Rheumatology in Europe) initiative has proposed an

Table 1.

APS forms

Form	Mechanism	Special features
Transplacental APS	Transfer of APA from mother to fetus through the placenta	Newborns passively receive antibodies from their mothers; the risk of thrombosis usually occurs when additional factors are present
De novo APS	Production of APA by the newborn	It is observed when the fetal immune system is activated (e.g., against the background of infection or inflammation)
Secondary APS	It is related to other autoimmune or inflammatory conditions in newborns	Occurs in combination with neonatal infection or severe inflammatory syndrome

adapted paediatric version of the classification criteria, excluding obstetric parameters and placing greater emphasis on thrombotic and non-thrombotic manifestations [14].

Thus, neonatal APS should be considered not only as a derivative of maternal autoimmune status, but also as a potentially independent disease that develops *de novo*, requiring a separate diagnostic approach, long-term observation, and multidisciplinary therapy.

CAPS in newborns is an extremely rare but extremely severe form of APS characterized by the sudden development of multiple organ failure against a background of generalized microvascular thrombosis. In the neonatal population, CAPS is usually observed in conditions of severe systemic inflammation — severe sepsis, congenital infection, or a combination of APA positivity with prothrombotic genetic mutations.

Clinical manifestations of CAPS in the neonatal period include:

- acute onset with fever and multiple organ dysfunction;
- dermatological signs of ischemia (livedo reticularis, necrosis, gangrene);
- thrombosis in the mesenteric, renal, pulmonary, and cerebral vessels;
- severe thrombocytopenia and hypofibrinogenemia;
- high mortality rate, reaching 25–30% [15].

Clinical cases of CAPS in newborns with a combination of IgM APA, positive lupus anticoagulant, heterozygous FV Leiden mutation, and septic shock have been described [16], emphasizing the key role of interaction between the immune, inflammatory, and coagulation systems. This pathology represents the most aggressive form of the concept of immunothrombosis, where the thromboinflammatory cascade reaches its maximum expression (Table 2).

VITT AND VITT-LIKE SYNDROMES IN NEWBORNS

Initially, VITT was considered exclusively as a rare and severe complication observed in adult patients after the use of adenovirus vector vaccines. However, data from recent years allow us to hypothesize the existence of VITT-like conditions in

Table 2.

Clinical manifestations and complications of CAPS in newborns

Form	Mechanism
Central nervous system	Coronary artery stroke, intracranial hemorrhage, seizures
Kidneys	Renal vein thrombosis, acute renal failure
Skin	Purpura, skin necrosis, ulcers
Lungs	Pulmonary embolism, respiratory failure
Cardiovascular system	Thrombosis of large vessels, arterial hypertension
Hemostasis system	Thrombocytopenia, hemolytic anemia

newborns, characterized by the production of antibodies to platelet factor 4 (PF4), accompanied by the development of thrombocytopenia and thrombosis, often in non-standard vascular beds.

In 2025, the New England Journal of Medicine published a clinical case representing the first reported observation of neonatal ischemic stroke associated with transplacental transfer of anti-PF4 IgG from a mother with a history of thrombosis and a heterozygous FV Leiden mutation [17]. The newborn was found to have severe thrombocytopenia (32×10^9/L), markedly elevated D-dimer levels, hypofibrinogenemia, arterial and venous thrombosis in the brain structures, and positive anti-PF4 IgG in both the child and the mother.

The possibility of transplacental transfer of anti-PF4 antibodies, similar to the mechanism described for APA, is of particular importance in this context. However, given the characteristics of neonatal immunity, autonomous (*de novo*) synthesis of these antibodies in the fetus in response to innate activation of the immune system, for example, during infection or hypoxic stress, cannot be ruled out.

The current concept considers VITT-like conditions to be part of a broader group of immune-mediated syndromes associated with the production of anti-PF4 antibodies. This spectrum also includes heparin-induced thrombocytopenia (HIT), "spontaneous" HIT (in the absence of heparin), post-operative anti-PF4 syndromes, and other forms of immune-mediated thrombosis. The common link in the pathogenesis of all these conditions is platelet activation via FcγRIIA receptors, leading to systemic thrombocytopenia and thrombosis in unusual locations, including cerebral venous sinuses and splanchnic vessels [18].

COVID-19 AND IMMUNOTHROMBOSIS IN NEWBORNS

SARS-CoV-2 infection during pregnancy, especially in the third trimester, is associated with an increased risk of adverse perinatal outcomes, including the development of neonatal thrombosis, generalized inflammatory response, and multiple organ dysfunction in newborns [19, 20].

The presumed mechanisms of COVID-associated immunothrombosis in the neonatal period include:

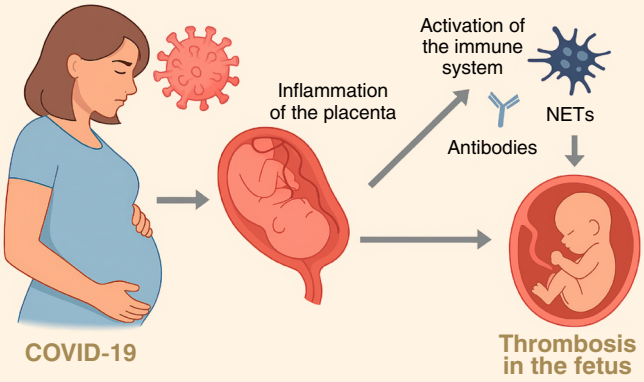

Figure 3. COVID-19 and thromboinflammation

- high levels of proinflammatory cytokines in umbilical cord blood, including IL-1α, IL-6, IL-10, TNF-α, and CXCL10;
- increased activity of myeloperoxidase, an enzyme associated with NETs formation;
- impaired regulatory T cell function combined with hyperexpression of costimulatory molecules CD80 and CD86;
- a decrease in the population of naive CD4+-T lymphocytes with a simultaneous predominance of activated subpopulations with an effector memory phenotype [21] (Figure 3).

The combination of these immunological disorders indicates the formation of a systemic predisposition to thromboinflammatory reactions in newborns exposed to SARS-CoV-2 during the antenatal period.

The clinical spectrum of manifestations varies from limited venous thrombosis to severe forms of multisystem inflammatory syndrome in newborns, phenotypically similar to Kawasaki disease and CAPS [22–24].

It should be emphasized that the pathomorphological picture of placentas in mothers infected with SARS-CoV-2 shows expressed morphological similarities with placental changes in APS. These include perivillous fibrinoid deposits, intervillous inflammation, and thrombosis of the villous chorionic vessels. These findings indicate the implementation of a universal mechanism of thromboinflammation underlying both COVID-associated and immune-mediated thrombotic conditions of pregnancy.

DIAGNOSIS OF IMMUNE THROMBOSIS IN NEWBORNS

Diagnosis of neonatal thrombosis is a clinically and organizationally complex task that requires a high degree of vigilance, especially with regard to immune and infection-mediated forms. These conditions often present with non-specific symptoms or manifest sub clinically, which significantly complicates timely detection. Effective diagnosis is only possible with multidisciplinary collaboration between specialists: neonatologists, hematologists, neurologists, and immunologists.

The algorithm for examining a newborn with suspected VTE includes the following steps:

- thorough clinical examination with mandatory assessment of neurological status, including signs of intracranial lesions;
- ultrasound examination of blood vessels (in cases of suspected thrombosis associated with a central catheter, as well as lesions of the inferior vena cava, renal or portal vessels);
- neuroimaging (MRI or CT) in cases of clinical suspicion of stroke or cerebral venous thrombosis;
- venography – in situations with ambiguous results of non-invasive imaging (Figure 4).

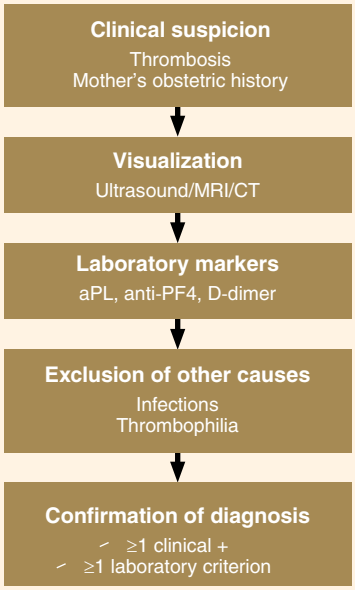

Figure 4. Diagnostic algorithm for neonatal CAPS

Laboratory testing includes:

- general blood test with mandatory platelet count assessment;
- extended coagulation profile: prothrombin time, international normalized ratio, fibrinogen level, D-dimer concentration, antithrombin III, protein C and S levels;
- immunological screening: determination of lupus anticoagulant, anticardiolipin and beta2-glycoprotein antibodies;
- determination of anti-PF4 antibody levels (in the context of differential diagnosis with VITT-like conditions and HITT);
- genetic testing for prothrombotic mutations — FV Leiden, G20210A prothrombin, and others.

A key aspect of diagnosing immune-mediated forms is interpreting the presence of APA or anti-PF4 antibodies. When these antibodies are detected in a newborn, it is necessary to differentiate between transplacental transfer of IgG from the mother and autonomous (*de novo*) synthesis. The latter requires exclusion of APS in the mother, as

well as dynamic monitoring of antibodies in the child during the first 6–12 months of life to assess their persistence and clinical significance.

THERAPY AND MANAGEMENT STRATEGIES

The therapeutic strategy for neonatal thrombosis is aimed at preventing the progression of the thrombotic process, restoring adequate organ perfusion, reducing the risk of complications, and preventing recurrence. Anticoagulants, mainly representatives of the heparin group, form the basis of drug treatment. Low molecular weight heparins have found the widest clinical application in newborns due to their predictable anti-Xa effect, reduced likelihood of developing HITT, and ease of use in outpatient settings. Alternatively, unfractionated heparin may be used, especially in situations requiring rapid dose adjustment or when there is a high risk of bleeding [25].

In cases of life-threatening thrombotic complications, such as bilateral renal vein thrombosis, massive cerebral venous sinus thrombosis when anticoagulant therapy is ineffective, or when there is a threat of loss of a vital organ or limb, thrombolytic therapy may be considered. However, this approach is associated with a high risk of massive bleeding, especially intracranial bleeding, which requires careful individual consideration of the benefits and potential harms.

Surgical intervention in the form of thrombo-ectomy is used extremely rarely and is considered a last resort in severe arterial or venous thrombosis that cannot be treated with medication, including catheter-induced arterial thrombosis with signs of critical limb ischemia.

Immunomodulatory interventions represent a special therapeutic category that is relevant in cases of suspected immune-dependent platelet activation, including CAPS, VITT-like conditions, and other variants of anti-PF4-associated thrombosis. In such clinical situations, the use of intravenous immunoglobulin may be justified to block FcγRIIA-dependent platelet activation mechanisms, systemic corticosteroids may be used in cases of severe inflammatory response, and plasmapheresis may be used in particularly severe and therapy-resistant cases of CAPS [26].

When asymptomatic thrombosis is detected, in particular non-occlusive portal vein lesions or catheter-associated central vein thrombosis, a wait-and-see strategy is acceptable, with mandatory ultrasound monitoring and laboratory assessment of hemostasis parameters. However, the decision on therapeutic intervention should be made on an individual basis, taking into account the clinical context and the potential risk of thrombosis progression [27].

According to current recommendations from the American Society of Hematology, active anticoagulant therapy is preferred in the following conditions: cerebral venous sinus thrombosis, renal vein thrombosis, pulmonary embolism, occlusive thrombosis of the hepatic vessels.

CONCLUSION

The immune and infection-associated forms of neonatal thrombosis discussed in this review (APS, CAPS, VITT-like conditions, and COVID-19-associated thrombosis) in-

dicate the presence of a common pathogenetic substrate, the key link of which is the phenomenon of thromboinflammation. The latter is the result of synergetic activation of the immune, inflammatory, and coagulation systems, leading to endothelial damage, microvascular thrombus formation, and the development of multiple organ failure.

The specificity of the neonatal period lies in unique immune mechanisms, including transplacental transfer of IgG antibodies and the ability of the fetus to produce immunoglobulins *de novo* in response to antenatal infection or inflammatory stress. The phenomenon of independent APA production in newborns, especially of the IgM class, confirms the existence of an autonomous neonatal variant of APS, requiring a review of current classification approaches and the development of age-specific diagnostic criteria. Even in the absence of signs of autoimmune pathology in the mother, such antibodies can act as primary mediators of severe thrombotic events in the newborn.

Clinical and pathomorphological parallels between CAPS and COVID-19 in newborns, including similarities in cytokine profiles, NET activation, and characteristic changes in placental tissue, confirm the existence of a universal mechanism of thromboinflammation as a key model of vascular catastrophes in the early postnatal period.

In this regard, the modern view of neonatal thrombosis requires a transformation: from the traditional concept of mechanical vascular obstruction to the concept of a multifactorial immunohaematological process. This approach should include a comprehensive assessment of humoral immune markers (including APA and anti-PF4 antibodies), inflammatory cytokines, genetic thrombophilias, and the state of innate immunity. Only an integrative and personalized management model can ensure timely diagnosis, targeted therapy, and prevention of life-threatening complications in one of the most vulnerable categories of patients — newborns.

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Chapter 14

Clinical and Immunological Characteristics of Laboring Women With COVID-19 and Laboring Women Who Had COVID-19 in Different Trimesters of Pregnancy, as Well as Their Newborns

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ABSTRACT

The aim of the study was to assess the health and immune status of laboring women with a positive PCR test for SARS-CoV-2 who had COVID-19 in different trimesters of pregnancy, as well as their newborns. Newborns were examined in 159 mothers who had contact with the virus and in 115 mothers who had no contact with the virus. No data were obtained on the occurrence of severe developmental abnormalities in newborns associated with COVID-19 in the mother.

INTRODUCTION

The emergence of SARS-CoV-2 as a new infection causing severe acute respiratory syndrome accompanied by cytokine storm and pronounced thromboinflammation has challenged healthcare providers to determine the impact of coronavirus infection on pregnancy, since existing experience in studying maternal and neonatal outcomes in recent epidemics caused by viruses such as influenza A (H1N1), SARS-CoV, which caused Middle East respiratory syndrome, and respiratory syncytial virus, dictates the need to revise the management of virus-infected pregnant women.

It has been reported that most pregnant women (<85%) infected with SARS-CoV-2 had mild cases of the disease, with severe cases ranging from 9.3% to 11.1% and

critical cases ranging from 2% to 6.9%, which is close to the rates for the general population [1]. However, data on the impact of SARS-CoV-2 on obstetric and neonatal outcomes, as well as on the likelihood of intrauterine transmission, are contradictory [2, 3].

The clinical and immunological characteristics of newborns are of particular interest at present, on the one hand, in women infected with SARS-CoV-2 during childbirth, and on the other hand, in women who have had coronavirus infection at different stages of gestation, as this will allow us to identify the long-term effects of COVID-19 and characterize their health status as post-COVID. There is insufficient convincing data reflecting the characteristics of the neonatal period and the state of the immune system in these children that could influence the management of the postnatal period. Therefore, given the ongoing circulation of the virus in nature and the lack of methods guaranteed to protect against the impact of emerging new strains with unpredictable epidemiology, it is important to identify the health and immune status of women who test positive for SARS-CoV-2 during childbirth and women who have had COVID-19 in different trimesters of pregnancy, as well as their newborns.

MATERIALS AND METHODS

A prospective study of the health and immune status of women who tested positive for SARS-CoV-2 and their newborns included 27 patients who made up the main group. Pregnant women with signs of acute infectious and inflammatory disease were admitted for delivery to a temporary COVID hospital based at the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after V.I. Kulakov of the Ministry of Health of Russia in April–July 2020. The presence of the SARS-CoV-2 virus at the time of hospitalization was confirmed by the detection of SARS-CoV-2 RNA by RT-PCR in a swab from the mouth and nasopharynx. All women included in the study had mild symptoms: temperature <38°C, cough, weakness, sore throat, and no criteria for severe or moderate infection [4]. Virus elimination was determined by two negative PCR tests within 24 hours. Diagnosis, management, and treatment of pregnant women were carried out in accordance with temporary methodological recommendations [4]. The comparison group (n=45) included patients without coronavirus infection who gave birth at the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after V.I. Kulakov of the Ministry of Health of Russia between November 2019 and July 2020. The absence of SARS-CoV-2 virus at the time of hospitalization during the pandemic was confirmed by PCR analysis. Vaccination against SARS-CoV-2 was not carried out in Russia at that time, and no antibodies to SARS-CoV-2 were detected in the women examined.

A prospective study of the health and immune status of mothers who had COVID-19 at different stages of pregnancy and their newborns included 132 women who gave birth at the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after V.I. Kulakov of the Ministry of Health of Russia. The main group consisted of women (n=62) who had COVID-19 during pregnancy and their newborns (n=62) with a gestational age of 37–41 weeks. In the first trimester, 19 mothers had COVID-19, in the second trimester — 19, and in the third trimester — 24. The com-

parison group consisted of women without laboratory-confirmed COVID-19 during pregnancy (n=70) and their newborns (n=70) at 37–41 weeks.

Peripheral venous blood samples were taken from women in labor upon their arrival at the delivery ward. The total number of leukocytes, lymphocytes, and neutrophils was assessed using a System XS 800i hematology analyzer. Blood samples were taken from children in the main groups on the second day after birth to rule out infectious and inflammatory processes, and from children in the comparison groups as part of the "My First Examination" program.

The studies were approved by the Ethics Committee of the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after V.I. Kulakov of the Ministry of Health of Russia. All patients signed informed voluntary consent to participate in the study and consent to the examination of their children's peripheral blood.

Peripheral blood lymphocytes (T-, B-, and NK cells) were phenotyped using flow cytometry with FITC-, PE-, and APC-labeled monoclonal antibodies produced by Becton Dickinson and eBioscience. The lymphocyte gate was identified using mAb to CD45 (Dako). Neutrophil phagocytic activity (NPA) was determined using the FagoFlowEX Kit (EXBIO). The analysis was performed on a Navios flow cytometer (Beckman Coulter) using the Kaluza software.

Statistical analysis of the obtained data was performed using Microsoft Excel and MedCalc (version 16.8) electronic spreadsheets. To analyze quantitative data in comparison groups, the type of distribution was determined using the Shapiro–Wilk W test. The data are presented as the arithmetic mean and standard deviation (M±SD). When the data distribution deviated from normal, nonparametric statistical methods were used with an estimate of the median and upper and lower quartiles (Me [Q1; Q3]). The Mann–Whitney criterion was used to assess intergroup differences. Differences were considered significant at $p<0.05$. When performing multiple comparisons, the Kruskal–Wallis test was used; when comparing data in four groups, differences were considered significant at $p<0.008$, and for three groups, at $p<0.017$. Qualitative variables are presented as absolute and relative values (abs., %). To assess differences in qualitative variables, Fisher's exact test was used, with differences considered significant at $p<0.05$. For multiple comparisons, Pearson's χ^2 test was used, with differences considered significant at $p<0.017$ or $p<0.008$.

RESULTS

Clinical and immunological examination of laboring women with positive SARS-CoV-2 tests and their newborns. The women in the study groups did not differ in terms of age, parity, or frequency of spontaneous pregnancies (Table 1).

However, women with COVID-19 during childbirth predominantly suffered from gastrointestinal diseases among somatic diseases, among gynecological diseases — external genital endometriosis and ovarian cysts, and had a higher frequency of cesarean sections. The indications for operative delivery in both groups were clinically narrow pelvis, uterine scarring after previous cesarean sections, complicated obstetric history, fetal pathology, and the sum of relative indications. None of the patients included in the study required hospitalization in the intensive care unit (ICU) after delivery.

Table 1.

Clinical characteristics of laboring women with COVID-19

Indicator		Main group (*n*=27)	Comparison group (*n*=45)	*p*
Age, average (years)		30.7	31.3	>0.05
Gestational age (days/weeks)		268 (38 weeks 2 days)	280 (40 weeks)	<0.0001
First-time mothers, abs. (%)		12/27 (44.0)	26/45 (58.0)	>0.05
Repeat mothers, abs.(%)		15/27 (56.0)	19/45 (42.0)	>0.05
Self pregnancy, abs.(%)		23/27 (85.2)	41/45 (91.1)	>0.05
Pregnancy in assisted reproductive technology programs, abs. (%)		4/27 (14.8)	4/45 (8.9)	>0.05
Somatic diseases	organs of vision, abs. (%)	2/27 (7.4)	8/45 (17.7)	0.30
	ENT organs, abs. (%)	3/27 (11.0)	1/45 (2.22)	0.15
	gastrointestinal tract, abs. (%)	6/27 (22.0)	2/45 (4.44)	0.046
	cardiovascular system, abs. (%)	4/27 (15.0)	5/45 (11.1)	0.72
	urinary system, abs. (%)	2/27 (7.4)	1/45 (2.22)	0.55
	endocrine disorders, abs. (%)	6/27 (22.0)	3/45 (6.66)	0.07
Gynecological diseases	Cervical ectopia, abs. (%)	6/27 (22.0)	15/45 (33.3)	0.42
	Infertility, abs. (%)	5/27 (18.5)	2/45 (4.44)	0.09
	External genital endometriosis, abs. (%)	3/27 (11.0)	0/45 (0)	0.049
	Myoma, abs. (%)	3/27 (11.0)	2/45 (4.44)	0.36
	Ovarian cysts, abs. (%)	6/27 (22.0)	2/45 (4.44)	0.046

End of the Table 1.

Indicator		Main group (*n*=27)	Comparison group (*n*=45)	*p*
The course of pregnancy	toxicosis, abs. (%)	4/27 (15.0)	6/45 (13.3)	1.0
	threatened miscarriage, abs. (%)	2/27 (7.4)	4/45 (8.88)	1.0
	threat of premature birth, abs. (%)	6/27 (22.0)	14/45 (31.1)	0.59
	anemia in pregnant women, abs. (%)	13/27 (48.0)	21/45 (46.6)	1.0
	edema in pregnant women, abs. (%)	1/27 (3.7)	2/45 (4.44)	1.0
	acute respiratory viral infection during pregnancy, abs. (%)	4/27 (14.8)	10/45 (22.2)	0.55
Characteristics of childbirth	spontaneous delivery, abs. (%)	14/27 (52.0)	37 (82.0)	0.008
	cesarean section, abs. (%)	13/27 (48.0)	8 (18.0)	0.008
	clinically narrow pelvis, abs.	1/13	2/8	0.53
	uterine scar, abs.	4/13	0/8	0.13
	complicated obstetric history, abs.	3/13	2/8	0.55
	fetal pathology, abs.	3/13	4/8	0.35
	total indications, abs.	2/13	0/8	0.51

Table 2.

Clinical characteristics of newborn infants born to mothers with COVID-19 during childbirth

Indicators	Main group (*n*=27)	Comparison group (*n*=45)	*p*
Premature, abs. (%)	5 (18.5)	0 (0.0)	0.006
Weight (g), Me [Q1; Q3]	3200 [2915; 3629]	3458 [3186; 3708]	>0.05
Length (cm), Me [Q1; Q3]	52 [50; 54]	52 [51; 53]	>0.05
Apgar score at one minute, points, Me [Q1; Q3]	8 [8; 8]	8 [8; 8]	>0.05
Apgar score at five minutes, points, Me [Q1; Q3]	9 [8; 9]	9 [9; 9]	>0.05
Boys, abs. (%)	12 (44,4)	22 (48.9)	>0.05
Girls, abs. (%)	15 (55,6)	23 (51.1)	>0.05
Hospitalization, days	10	3	<0.0001

Newborns in the study groups were comparable in terms of Apgar scores at one and five minutes and weight and height characteristics (Table 2).

At birth, SARS-CoV-2 virus was not detected in children in the main group according to PCR data. 5/27 (18.5%) premature newborns were born in the main group at 35.1 (246 days)±1.1, while all newborns in the comparison group were born at full term (*p*=0.006).

Three premature infants in the main group (60%) required ICU care due to the development of non-infectious respiratory disorders (in particular, transient tachypnea

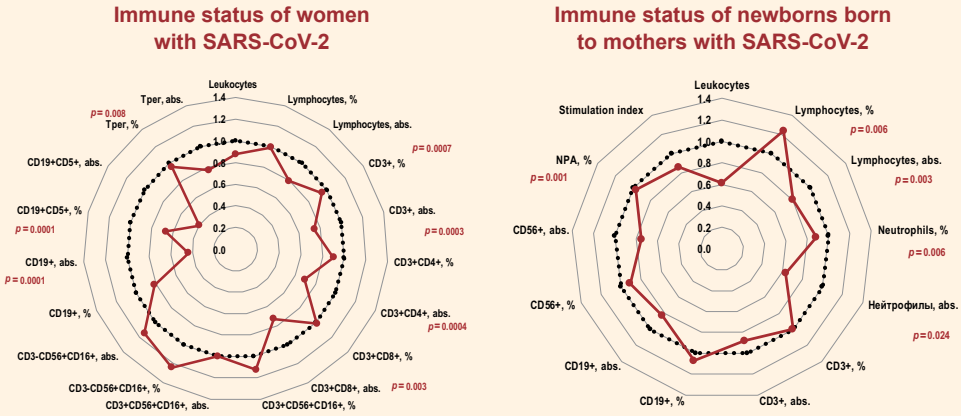

Figure 1. Immune status of SARS-CoV-2-positive mothers and their newborns.

The data are presented as the ratio of the medians of the indicators for patients in the main groups to the medians of the indicators for patients in the comparison groups. The dotted line indicates the level of equality of values. p-values are given for the medians of the indicators studied.

of the newborn) with subsequent transfer to the premature newborn pathology department (PNBPD). Two children were sent directly to the PNBPD after birth.

Figure 1 shows the immune status of mothers with COVID-19 during childbirth and their newborns.

As can be seen from the results presented in Figure 1, the main group of parturients showed a decrease in the absolute lymphocyte count, which was reflected in a decrease in the absolute values of all studied subpopulations, but not exceeding the reference intervals, while the content of NK and NKT lymphocytes did not differ between the groups in either relative or absolute values and also remained within the reference values.

In the main group of newborns whose mothers tested positive for SARS-CoV-2 during childbirth, lower levels of leukocytes, neutrophils, and NPA were detected, as well as lower absolute but increased relative levels of lymphocytes.

Clinical and immunological examination of pregnant women who have had COVID-19 in different trimesters of pregnancy, and their newborns. Women in the main group and the comparison group were comparable in terms of age, frequency of somatic and obstetric-gynecological diseases, timing and frequency of cesarean section, However, during pregnancy, women in the main group were significantly more likely to receive low molecular weight heparin therapy due to hypercoagulation characteristic of coronavirus infection and possible thrombotic changes in the post-COVID period (Table 3).

Newborns in both groups did not differ in anthropometric data, gestational age, Apgar score, and physical development indicators according to the Intergrowth-21 curves (Table 4).

One child from the main group (2%, 1/62) required specialized medical care in the ICU, and six newborns from the main group were monitored and treated in the PNBPD; in the comparison group, one child required such care (p=0.03).

Perinatal infections (pneumonia, rhinitis, otitis), neonatal hyperbilirubinemia, congenital heart defects, namely: ventricular septal defect, atrial septal defect greater than 5 mm - were the reasons for inpatient treatment of children, but their frequency in the groups was comparable (p>0.05), and only the frequency of grade 1 intraventricular hemorrhages in newborns in the main group was higher than in the comparison group (p=0.02).

Table 5 presents the clinical characteristics and diseases of newborns depending on the trimester of pregnancy during which the mother had COVID-19.

As can be seen from the data in Table 5, no significant differences were found between the groups for any of the assessed indicators and diseases. The immune status of newborns is shown in Figure 2. Noteworthy is the lower content of leukocytes and lymphocytes in the main group, the increased content of T-lymphocytes, the low content of B-lymphocytes and NK cells, and the neutrophil content equal to that of the control group with reduced phagocytic activity. However, all values obtained were within the reference range, and only the NK cell content in the main group was 2 times lower, with the lowest NK cell level in children whose mothers had COVID-19 in the first trimester (Figure 2).

Table 3.

Clinical and anamnestic characteristics of mothers who had COVID-19 in different trimesters of pregnancy

Indicators	Main group (*n*=62)	Comparison group (*n*=70)	*p*
Age, years (M±SD)	321±4.7	31.6±4.1	>0.05
Assisted reproductive technologies, *n* (abs., %)	6 (10)	7 (10)	>0.05
History of miscarriage, *n* (abs., %)	10 (16)	14 (20)	>0.05
Hronic arterial hypertension, *n* (abs., %)	4 (6)	3 (3)	>0.05
Hereditary thrombophilia, *n* (abs., %)	3 (5)	3 (3)	>0.05
Lipid metabolism disorder, *n* (abs., %)	25 (41)	20 (29)	>0.05
Threatened miscarriage, *n* (abs., %)	14 (23)	21 (30)	>0.05
Hypothyreoidism, *n* (abs., %)	12 (20)	9 (13)	>0.05
Gestational diabetes, *n* (abs., %)	10 (16)	11 (16)	>0.05
Anemia, *n* (abs., %)	32 (52)	27 (39)	>0.05
Therapy with low molecular weight heparin, *n* (abs., %)	19 (31)	7 (10)	0.017
Isthmic-cervical insufficiency, *n* (abs., %)	5 (8)	6 (9)	>0.05
Spontaneous deliveries, *n* (abs., %)	47 (77)	57 (81)	>0.05
Cesarean section, *n* (abs., %)	14 (23)	13 (19)	>0.05

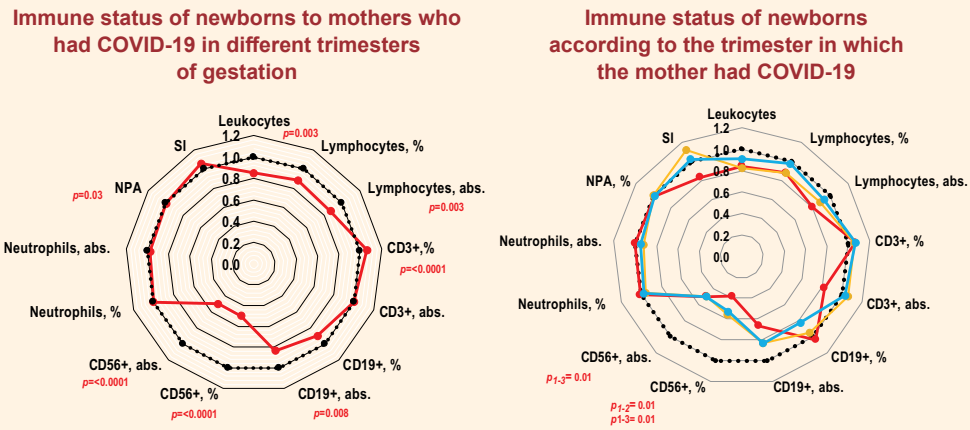

Figure 2. Immune status of the newborns from mothers who had COVID-19 during different trimesters of gestation.

The data is presented as the ratio of the median of the indicator in the main group to the medians of the indicator in the comparison group. The dotted line indicates the level of equality of values. *p*-values are given for the medians of the indicators. On the right, the red line shows the ratios for the first trimester, the blue line for the second trimester, and the yellow line for the third trimester

Table 4.

Clinical characteristics and diseases in newborns whose mothers had COVID-19 during pregnancy

Indicators	Main group (*n*=62)	Comparison group (*n*=70)	*p*
Weight, g (M±SD)	3477±475	3422±412	>0.05
Length, cm (M±SD)	53±2.3	52.3±2.2	>0.05
Apgar score, 1 minute (M±SD)	7.96±0.2	7.98±0.1	>0.05
Apgar score, 5 minutes (M±SD)	8.98±0.1	8.93±0.3	>0.05
Boys, *n* (abs., %)	33 (53.2)	34 (48.6)	>0.05
Girls, *n* (abs., %)	29 (46.8)	36 (51.4)	>0.05
2000–2500 g (abs., %)	1 (2)	2 (3)	>0.05
4000–4500 g (abs,%)	8 (13)	7 (10)	>0.05
More than 4500 g (abs., %)	1 (2)	0 (0)	>0.05
Large for GA, *n* (abs., %)	15 (24)	16 (23)	>0.05
Small for GA, *n* (abs., %)	1 (2	3 (4)	>0.05
Pneumonia, *n* (abs., %)	1 (2)	0 (0)	>0.05
Rhinitis, *n* (abs., %)	3 (5)	0 (0)	0.06
Otitis, *n* (abs., %)	3 (5)	0 (0)	0.06
Hyperbilirubinemia, *n* (abs., %)	1 (2)	0 (0)	>0.05
Anemia, *n* (abs., %)	5 (8)	3 (4%)	>0.05
Atrial septal defect, *n* (abs., %)	3 (5)	1 (1)	>0.05
Ventricular septal defect, *n* (abs., %)	1 (2)	1 (1)	>0.05
Intraventricular hemorrhage 1 degree, *n* (abs., %)	5 (8)	0 (0)	0.02

Note. ASD — atrial septal defect, VSD — ventricular septal defect, IVH — intraventricular hemorrhage

DISCUSSION

The study was conducted to identify the health characteristics of infants born to mothers who had COVID-19 at different stages of pregnancy and to mothers who tested positive for SARS-CoV-2 by PCR upon admission to the maternity ward. No virus was detected in the upper respiratory tract of any of the newborns examined. To date, there is no clear evidence of transplacental transmission of SARS-CoV-2 from an infected mother, but the entry into the fetal bloodstream of various biologically active molecules produced by virus-infected maternal immune cells appears to be a natural process that may influence the maturation of the fetal immune system. The phenomenon of direct infection of killer cells has been established for enveloped virus-

Table 5.

Clinical characteristics of infants born to mothers who had COVID-19 during different trimesters of pregnancy

Indicators	COVID-19 in the first trimester (*n*=19)	COVID-19 in the second trimester (*n*=19)	COVID-19 in the third trimester (*n*=24)	Comparison group (*n*=70)	*p*
Weight, g (M±SD)	3484±492	3490±463	3462±490	3422±412	>0.008
Length, cm (M±SD)	53±2.2	53±2.2	52.9±2.4	52.3±2.2	>0.008
Apgar score, 1 minute (M±SD)	8±0	8±0	7.9±0.2	7.9±0.2	>0.008
Apgar score, 5 minutes (M±SD)	9±0	9±0	8.9±0.2	8.9±0.3	>0.008
Boys, *n* (abs., %)	9 (47.4)	14 (73.7)	10 (41.7)	34 (48.6)	>0.008
Girls, *n* (abs., %)	10 (52.6)	5 (26.3)	14 (58.3)	36 (51.4)	>0.008
2000-2500 g (abs., %)	0	0	1 (4.2)	2 (3)	>0.008
4000-4500 g (abs., %)	1 (5.2)	4 (21.1)	3 (12.5)	7 (10%)	>0.008
More than 4500 g (abs., %)	1 (5.2)	0	0	0 (0)	>0.008
Large for GA, *n* (abs., %)	4 (21.1)	4 (21.1)	7 (29.2)	16 (23)	>0.008
Small for GA, *n* (abs., %)	1 (5.2)	0	0	3 (4)	>0.008
Pneumonia, *n* (abs., %)	0	0	1 (4.2)	0 (0)	>0.008
Rhinitis, *n* (abs., %)	1 (5.3)	0	2 (8.3)	0 (0)	>0.008
Otitis, *n* (abs., %)	1 (5.3)	0	2 (8.3)	0 (0)	>0.008
Hyperbilirubinemia, *n* (abs., %)	0	0	1 (4.2)	0 (0)	>0.008
Anemia, *n* (abs., %)	3 (15.8)	1 (5.3)	1 (4.2)	3 (4)	>0.008
ASD, *n* (abs., %)	0	1 (5.3)	2 (8.3)	1 (1)	>0.008
VSD, *n* (abs., %)	0	1 (5.3)	0	1 (1)	>0.008
IVH 1 degree, *n* (abs., %)	3 (15.8)	1 (5.3)	1 (5.3)	0 (0)	>0.008

Note. Differences in qualitative characteristics, assessed using Pearson's precise χ^2 test, are significant at p <0.008, taking into account the correction for multiple comparisons with control; differences in quantitative characteristics are significant at p <0.008, assessed using the Kruskal–Wallis test with Bonferroni correction.

es, which include SARS-CoV-2 [5]. It is known that NK cell sprouts appear between the 6th and 15th weeks, and killer cells acquire functional properties between the 15th and 22nd weeks of gestation [6]. Children born to mothers who had COVID-19 in the first trimester were found to have dramatically low levels of NK cells. This result raises questions about the consequences of direct infection of maternal immune cells. Infection occurs through the binding of the virus to specific receptors and fusion di-

rectly with the plasma membrane by endocytosis of the viral particle or macropinocytosis (non-specific absorption of extracellular material), via the FcγRIIIA IgG receptor expressed on NK cells, which mediates the binding of the virus to NK cells in the presence of virus-specific antibodies. Antibody-mediated entry has been shown for many enveloped viruses as a mechanism of entry into NK cells in humans who carry sub-neutralizing virus-specific antibodies [7]. Infection of NK cells leads to NK cell depletion through apoptosis. By altering the metabolism and energy exchange of killer cells, viruses contribute to changes in the phenotype of these cells, their production of cytokines and chemokines, and, consequently, changes in their interaction with macrophages and T lymphocytes [5, 8].

The resulting decrease in NK cell content in the peripheral blood of newborns can be attributed to signs of post-COVID syndrome already in newborns. This result was not obtained in children born to mothers with COVID-19 during childbirth, and in general, the anthropometric characteristics of infants born to mothers who had COVID-19 are comparable to those of newborns in the comparison group, and the identified fluctuations in laboratory parameters were within the reference values, which is consistent with the absence of SARS-CoV-2 infection in children, as well as with the established opinion that pregnant women are not at increased risk of severe illness or mortality from COVID-19 compared to the general population, and childbirth does not exacerbate the severity of the disease in women in labor [9], and reports of neonatal mortality associated with COVID-19 are rare [10]. However, even in the absence of severe perinatal outcomes in newborns whose mothers had COVID-19 during pregnancy, our consistent finding of low NK cell counts in their peripheral blood raises questions about the need for consistent follow-up data collection in order to attempt to form an objective opinion about the significance of our findings for the postnatal development of newborns.

CONCLUSION

The study found that all pregnant women with COVID-19 had a mild form of the disease, which is consistent with published global data showing that the vast majority of pregnant women with COVID-19 are asymptomatic or have a mild form of the disease. We did not find any differences in the course of pregnancy in women with coronavirus infection compared to women in the control group, and the higher frequency of premature births and cesarean sections in women with COVID-19 was due to obstetric indications. The decrease in the absolute number of major lymphocyte subpopulations without changes in their relative content, which we identified in women with COVID-19, is due to a decrease in the total number of lymphocytes, while immunological parameters fall within the reference range, which is characteristic of mild COVID-19, while the longer hospitalization period for newborns of mothers with COVID-19 was primarily determined by prematurity and the development of respiratory disorders. It is noteworthy that changes in the immune status of newborns of women with COVID-19 during childbirth are within the reference values, which does not allow us to unequivocally link these changes to the influence of maternal coronavirus infection in the absence of SARS-CoV-2 infection in children.

Thus, the dramatically low level of NK cells in newborns whose mothers had COVID-19 during pregnancy, especially in the first trimester, may be a consequence of post-COVID syndrome. A final conclusion can be made based on observations and follow-up data collection, as well as extended studies of subpopulations and the functional activity of NK cells in this category of newborns.

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Chapter 15

The Impact of COVID-19 Infection and Vaccination on Autoantibody Profiles and Reproductive Outcomes in Women

I.V. Menzhinskaya, D.M. Ermakova, V.V. Vtorushina, N.V. Dolgushina

ABSTRACT

Particular features of the disease course, risk groups for more severe forms, autoantibody profiles in patients and convalescents, in women after COVID-19 and vaccination were studied during the COVID-19 pandemic. The possible negative impact of COVID-19 on the outcomes of ART programs and pregnancy, the need for a time interval between COVID-19 and pregnancy, and autoantibody screening were demonstrated.

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has claimed the lives of more than 20 million people worldwide in three years (from March 2020 to May 2023). In May 2023, the WHO announced its end, which was largely facilitated by the implementation of mass specific immunoprophylaxis. However, to date, more than 23 million cases of SARS-CoV-2 infection and more than 400,000 deaths from COVID-19 have been recorded in the Russian Federation. At the same time, COVID-19 caused by the Wuhan strain and the Delta coronavirus was characterized by a severe course due to the activation of such immunopathological mechanisms in the human body as systemic inflammation, endothelial dysfunction, apoptosis, cytokine storm, hyperactivation of complement and blood coagulation [1]. Given the widespread prevalence of SARS-CoV-2 strains, research into the impact of COVID-19 on human reproductive function is of great scientific and practical importance.

As is known, SARS-CoV-2 penetration into human cells is mediated by the interaction of viral protein S with the angiotensin-converting enzyme 2 (ACE2) receptor, which is present not only in the lungs but also in various tissues of the body, including the ovaries, uterus, and vagina [2]. This interaction with other components of the renin-angiotensin system allows ACE2 to exert a significant influence on the processes of folliculogenesis and oocyte maturation [3]. The expression of ACE2 and its co-receptor, transmembrane serine protease 2, has been detected in the trophoblast cells of the embryo (at the blastocyst stage) [4] (Figure 1). We have obtained data on higher levels and prevalence of antibodies to ACE2 among patients with infertility compared to fertile women [5]. In this regard, both the reproductive organs and the embryo are considered possible targets for the coronavirus, and a history of COVID-19 is considered a risk factor for reproductive failure.

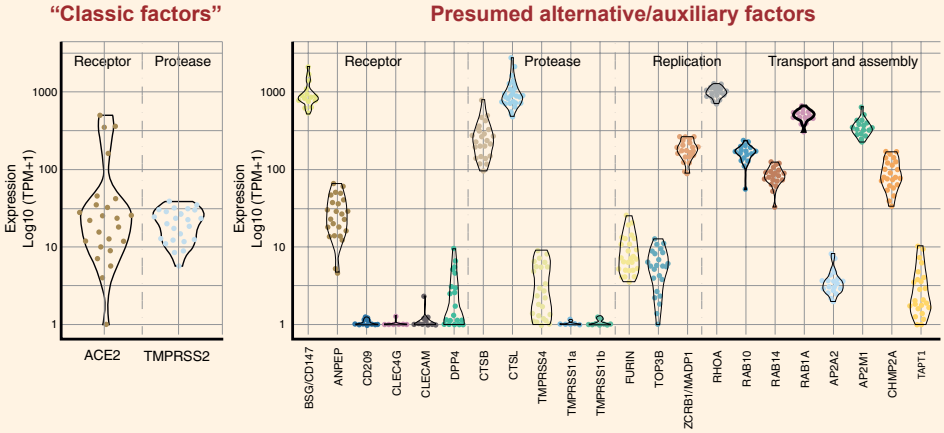

Figure 1. Expression of genes involved in SARS-CoV-2 infection in embryonic cells

Source: Montano M., Victor A.R., Griffin D.K. SARS-CoV-2 can infect human embryos. *Sci. Rep. England.* 2022;12(1):15451. doi: 10.1038/s41598-022-18906-1

SARS-CoV-2 can have a negative impact on organs and systems in the body, both directly and by triggering autoimmune mechanisms. SARS-CoV-2, along with some other viruses, activates autoimmunity: it triggers autoimmune diseases, a pronounced type 1 interferon response, and the formation of autoantibodies in patients with severe forms of infection. The virus contains a superantigen motif in the spike S protein, as well as peptides homologous to fragments of 28 human proteins, which leads to the activation of the molecular mimicry mechanism [6]. In addition, in severe COVID-19, B-lymphocytopoiesis with subsequent differentiation into plasma cells can occur via an extraganglionic pathway lacking tolerance checkpoints, which contributes to the onset of autoimmunity [7]. It has been shown that COVID-19 can cause autoimmune processes in people with a genetic predisposition [8]. The occurrence of immune thrombocytopenic purpura, Guillain–Barré syndrome, and Miller–Fisher syndrome has been described in COVID-19 convalescents [9].

In addition, several autoimmune diseases, including systemic lupus erythematosus, autoimmune thyroiditis, diabetes, and immune thrombocytopenia, have been

reported following vaccination against COVID-19 with mRNA vaccines and adenovirus vector vaccines [10]. The main pathophysiological mechanisms of their initiation were: molecular mimicry, autoantibody formation, and the effect of adjuvants.

The vascular complications observed in COVID-19, such as deep vein thrombosis, stroke, and disseminated intravascular coagulation, were initially associated with the presence of antiphospholipid antibodies (aPL) [9], primarily antibodies to cardiolipin (CL) and β2-glycoprotein-I (β2-GP-I), which are classified as laboratory criteria for antiphospholipid syndrome. However, there are conflicting data in the scientific literature on the prevalence and pathogenetic significance of aPL in COVID-19. According to researchers from China, half of the patients hospitalized with COVID-19 were at least transiently positive for aPL, which showed pathogenicity and enhanced thrombosis in a mouse model [11]. In contrast, researchers from Italy demonstrated a low prevalence of aPL in severe COVID-19 and no association with thrombotic events [12].

We studied the dynamics of the aPL profile in 141 patients with COVID-19 of varying severity: 39 patients with mild infection, 65 with moderate infection, and 37 with severe infection [13]. In the group with severe disease, most patients were male (59.5%), while women predominated in the groups of patients with mild (82.1%) and moderate (63.1%) forms. The older age of patients with severe (63 (53–71) years) and moderate (60 (43–78) years) forms of the disease was noteworthy compared to patients with mild forms (38 (34–54) years; p=0,0001). The likelihood of developing severe COVID-19 was significantly higher in patients who were overweight or obese (OR=3.5; 95% CI 1.46–8.34; p=0.009), as well as in patients with cardiovascular disease (OR=3.7; 95% CI 1.42–9.55; p<0.05), hypertension (OR=3.3; 95% CI 1.28–8.40; p <0.05), and diabetes mellitus (OR=14.1; 95% CI 1.69–116.6; p <0.05). According to the study results, male gender, older age, overweight, and obesity are definite risk factors for severe COVID-19, which is consistent with data from other studies. It has been established that men have higher levels of ACE2 protein expression in alveolar epithelial cells than women [14], and this may be why men experience more severe COVID-19 than women. Data from a meta-analysis conducted by Choi W.-Y. (2021) indicate that being overweight or obese increases the chances of developing severe forms of infection by 2.3 times [15]. This may be due to the frequent combination of obesity with somatic and endocrine diseases, metabolic disorders, and immune disorders [16].

Antiphospholipid antibodies of classes M and G of varying specificity were detected at two measurement points in 41 (29.1%) patients with COVID-19 of varying severity, with no difference in the frequency of aPL detection between the three groups. In 12 (8.5%) patients, aPL was detected only at the first measurement point at the height of the disease, in 20 (14.2%) — only at the second point during convalescence, and in 9 (6.4%) — at both the first and second measurement points. Patients with COVID-19 showed a high frequency of non-criterial antibodies to prothrombin (in 22 (15.6%)) and An V (in 16 (11.3%)), which were detected more often than antibodies to CL (in 10 (7.1%)) and β2-GP-I (in 11 (7.8%)). Harzallah I. et al. (2020) also showed low positivity for IgG/IgM antibodies to CL and β2-GP-I in only 10% of patients [17]. It is suggested that determining the aPL profile, includ-

ing both criterion and non-criterion antibodies, may be useful in assessing the risk of thrombotic events in patients with COVID-19 [18].

It is believed that in most cases, aPL formed during viral infections are transient; however, according to the literature data, the frequency of thrombotic events in patients with virus-associated aPL reaches 71% [19]. It is assumed that some transient aPL have prothrombotic potential. However, low aPL titers are not predictors of thrombotic events in antiphospholipid syndrome. It is important to note that in COVID-19, acute systemic inflammation with complement activation can lead to activation and damage of the endothelium. In this situation, with a high density of accumulation of phospholipid-binding proteins β2-GP-I, annexin V, and prothrombin on the phospholipid surface of the activated endothelium, even low aPL titers can have a pathogenic effect, contributing to thrombus formation.

An increase in aPL levels in patients after COVID-19, regardless of the severity of the disease, indicates that excessive activation of the vascular endothelium with phospholipid externalization and an increase in phospholipid-binding proteins is possible even in mild cases of the infectious process. Apparently, aPL may be involved in the pathogenesis of COVID-19-associated coagulopathy and serve as an additional risk factor for thromboembolic complications not only in patients in the acute phase of the disease, but also in convalescents in the post-COVID period, which was confirmed by the detection of aPL in 29 (20.6%) convalescents.

There are few reports in the scientific literature with conflicting data on the impact of COVID-19 on the female reproductive system. It has been shown that COVID-19 increases the risk of pregnancy complications, such as spontaneous miscarriages and premature births. The incidence of premature birth in pregnant women with COVID-19 reaches 17% [20], and the incidence of early spontaneous miscarriages in pregnant women with COVID-19 is 1.7 times higher than in pregnant women who are not infected with SARS-CoV-2 [21].

There have been single cases of premature ovarian failure in women after COVID-19 [22]. However, the mechanisms underlying these disorders remain unclear. The results of a study by Youngster M. et al. (2022) suggest a negative impact of COVID-19 and a dependence of the number of oocytes obtained in assisted reproductive technology (ART) programs in women with infertility on the time elapsed since the disease [23].

It is known that infection caused by SARS-CoV-2 is accompanied by increased cytokine production, in particular interleukin-6 and tumor necrosis factor-α, which can lead to a cytokine storm, and the negative impact on reproductive function can be realized through the suppression of the hypothalamic-pituitary-gonadal axis [24].

An autoimmune mechanism is one of the possible mechanisms of damage to the reproductive system in women under the influence of COVID-19. Matyushkina D. et al. (2022) showed that people with a specific HLA haplotype are most susceptible to the development of autoimmune processes after COVID-19 [25]. A significant prevalence of autoantibodies of varying specificity, including antinuclear antibodies, antibodies to neutrophil cytoplasm, to CL and β2-GP-I, has been found in patients with COVID-19 [26]. Antibodies to thyroid peroxidase have also been found in post-COVID syndrome [27].

The contribution of autoimmune factor to infertility [28], its influence on fertilization and implantation processes, and placenta formation [29] are well known. The relationship between aPL and infertility is currently the subject of active debate, but the pathogenetic mechanisms remain unclear. Based on the results of a meta-analysis conducted by Chighizola C.B. et al. (2016) concluded that there is a link between infertility and antibodies to β2-GP-I and non-criterial aPL, including antibodies to PE, with 5 of the 18 studies included in the analysis reporting a potentially harmful effect of aPL on the outcome of assisted reproductive technology programs. [30].

One of the important indicators of the state of a woman's reproductive system is ovarian reserve, which is assessed by counting the number of antral follicles in the ovaries based on transvaginal ultrasound examination data and the level of anti-Müllerian hormone. Determining the level of follicle-stimulating hormone is also of important diagnostic significance in the event of menstrual cycle disorders [31]. As a result of studying changes in ovarian reserve parameters and menstrual cycle characteristics in patients of early (up to 35 years inclusive) and late (over 35 years) reproductive age before and after COVID-19, we established a dependence of the degree of reduction in ovarian reserve parameters on the severity of the infection and the age of the patients. Thus, an association was found between moderate COVID-19 in patients of late reproductive age and a decrease in ovarian reserve (OR=5.7; 95% CI=1.2–27.3; $p<0.05$) [32].

We also conducted a prospective study examining a wide repertoire of serum autoantibodies in 135 unvaccinated female patients undergoing ART treatment for infertility who had undergone COVID-19 less than 12 months prior to the ART cycle in mild (n=85) or moderate (n=50) form, and their impact on reproductive outcomes [33]. The main clinical manifestations of the infectious disease in the included patients were: fever in 104 (77%), decreased sense of smell in 100 (74.1%), general weakness in 90 (66.7%), headache in 51 (37.8%), myalgia in 49 (36.3%), cough in 48 (35.5%), runny nose in 39 (28.9%), sore throat in 34 (25.2%), shortness of breath in 20 (14.8%), diarrhea in 15 (11.1%), and nausea in 9 (6.7%). Pneumonia was recorded in 15 patients (11.1%) (Figure 2). Broad-spectrum antibiotics were prescribed to 56 patients (41.5%), interferon inducers and interferon preparations were received by 25 (18.5%) and 19 patients (14.1%), respectively.

The comparison group consisted of 105 patients who had not previously had COVID-19. The average age of all patients included in the study was 34 years (Me COVID-19 "+" [Q25; Q75] = [31; 37 years], Me COVID-19 "–" [Q25–Q75] = [30–36 years]), a third of them were in their late reproductive years. The prevalence of adenomyosis was higher among those who did not have COVID-19, while those who had recovered from COVID-19 had a higher frequency of allergic and otorhinolaryngological diseases, as well as higher body weight and body mass index, especially in patients with more severe infection. The study included the determination of aPL, antibodies to nuclear antigens, thyroid antigens, ovarian antigens, trophoblast antigens, hormones (follicle-stimulating hormone, progesterone).

It has been shown that female patients who had COVID-19 were more likely to have IgG antibodies to annexin V (An V) (8.1%) and phosphatidylethanolamine (PE) (6.7%) compared to patients without a history of COVID-19 (1.9% and

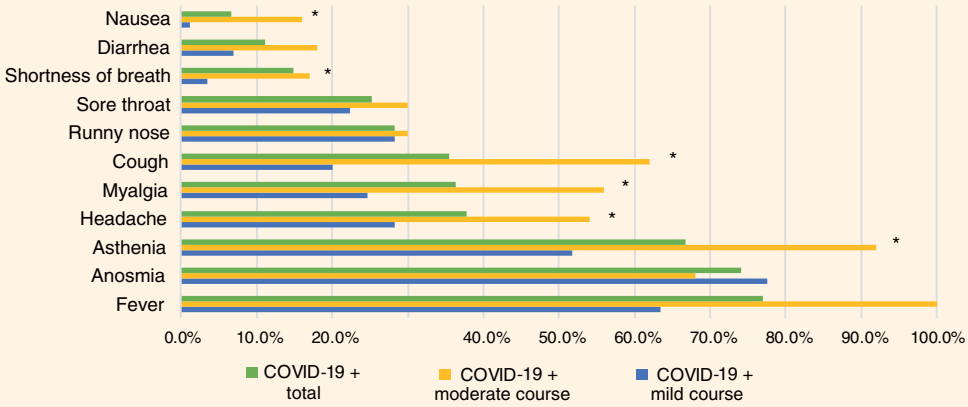

Figure 2. Clinical symptoms of COVID-19 in patients depending on the severity of the disease.

*p<0,05

0.95%, respectively; p<0.05), and higher median levels of IgG antibodies to PE and IgM antibodies to the phosphatidylserine/prothrombin complex were also observed compared to patients without a history of COVID-19. It is known that COVID-19 patients are predisposed to pro-inflammatory and hypercoagulable states and an increased risk of thrombotic events. Increased activation of the vascular endothelium, externalization of phospholipids, and increased levels of natural anticoagulants that bind phospholipids on the surface of damaged endothelium, in particular annexin V, may contribute to the formation of autoantibodies to An V [34].

Our study did not reveal any differences between the parameters of oogenesis, embryogenesis, pregnancy frequency, and live birth in patients with and without a history of COVID-19, which is consistent with the results of other researchers [35]. However, it is important to note that a comparison of embryogenesis parameters between large subgroups of patients with different time intervals from COVID-19 to oocyte retrieval (less than 180 days (n=85) or more than 180 days (n=50)) showed that the proportion of high-quality blastocysts obtained did not differ in these subgroups, while the proportion of low-quality blastocysts was higher in patients in the subgroup with a time interval of less than 180 days (Table 1). Apparently, this unfavorable outcome may be associated with the possible harmful effects of SARS-CoV-2 infection on oogenesis and oocyte quality. Orvieto R. et al. (2021) suggested avoiding ART during the first 3 months after COVID-19 [36]. However, it should be noted that the optimal duration of the necessary interval (3 or 6 months) from recovery from COVID-19 to the ART cycle requires clarification.

It is important to note that we identified an inverse correlation between specific IgG antibodies to SARS-CoV-2 and the parameters of oogenesis and embryogenesis, as well as between IgG antibodies to PL and the number of mature oocytes and zygotes obtained in ART cycles. In addition to aPL, patients who had COVID-19 had a higher frequency of IgG antibodies to the thyroid-stimulating hormone receptor (8.2%) compared to women who did not have COVID-19 (1.9%; p=0.033). The results obtained are consistent with research data demonstrating elevated levels of antithyroid antibodies in patients 3 months after COVID-19 [27]. Since COVID-19

Table 1.

Features of gametogenesis and embryogenesis in ART patients, taking into account the time interval between COVID-19 and transvaginal follicular puncture, Me [Q25; Q75]

Indicator	COVID-19 >180 days, *n*=50	COVID-19 ≤180 days, *n*=85		p_1 (for 2 groups) p_2 (for 3 groups)
		COVID-19 61–180 дней, *n*=65	COVID-19 ≤60 days, *n*=20	
Number of oocyte-cumulus complexes *	9–5 [6; 11]	8 [6; 15]		0.749
		10 [6; 16]	8 [6; 14]	0.937
Number of mature oocytes*	6–5 (49)	7 [5; 11]		0.338
		7 [5; 12]	6.5 [5; 10]	0.629
Ratio of mature oocytes to total number of oocytes*	0–75 (0–60–1–0)	0.83 [0.71; 0.92]		0.249
		0.83 [0.72; 0.92]	0.79 [0.71; 0.94]	0.434
Number of zygotes*		6 (410)		0.194
		6 [4; 10]	6 [4; 9]	0.422
Fertilization rate*	0.9 [0.77; 1.0]	1 [0.8; 1.0]		0.349
		1 [0.80; 1.00]	0.96 [0.80; 1.00]	0.607
Number of blastocysts*	3 [1; 5]	3 [1; 5]		0.456
		3 [1; 6]	3 [1; 4]	0.535
Blastulation level*	0.50 [0.25; 0.68]	0.54 [0.30; 0.71]		0.655
		0.58 [0.33; 0.70]	0.40 [0.25; 0.75]	0.830
Number of excellent quality	1 [0; 2]	1 [0; 2]		0.665
		1 [0; 2]	1 [0; 2]	0.894
Ratio of excellent quality blastocysts to total number of blastocysts*	0.32 [0.0; 0.66]	0.33 [0.0; 0.60]		0.998
		0.33 [0.0; 0.60]	0.50 [0.0; 0.66]	0.598
Number of poor-quality	1 [0; 2]	1 [1; 2]		0.075
		1 [1; 2]	1 [0.5; 1.5]	0.118
Ratio of poor-quality blastocysts to total number of blastocysts*	0.18 [0.0; 0.4]	0.37 [0.14; 0.71]		0.006
		0.37 [0.14; 0.71]	0.35 [0.10; 0.70]	0.021

Note. **Mann–Whitney or Kruskal–Wallis test, *abs. (%), χ^2 test.

can cause autoimmune damage to the thyroid gland, further monitoring of convalescents with thyroid dysfunction is recommended after severe infection.

Herrero Y. et al. (2022) demonstrated the negative impact of SARS-CoV-2 on the microcirculatory bed of ovarian tissue and folliculogenesis, in particular due to changes in the composition of follicular fluid [37]. At the same time, the negative correlation found between the level of IgG to SARS-CoV-2 in follicular fluid and the

total number of oocytes obtained, as well as the number of mature oocytes, confirms that COVID-19 can negatively affect reproductive outcomes. Apparently, oocytes, embryos, and especially late blastocysts have a receptor/protease apparatus and are susceptible to SARS-CoV-2 infection [8]. A lower proportion of high-quality embryos obtained in ART cycles after COVID-19 has been reported [36]. It is assumed that the effect of SARS-CoV-2 infection, which causes systemic inflammation, may impair the quality of developing embryos. Another study observed a decrease in the rate of blastocyst formation in the COVID-19 group, which could be due to the negative effect of oxidative stress on oocyte quality. [38].

When evaluating the clinical outcomes of ART programs in women who had moderate COVID-19, a high incidence of early spontaneous miscarriages (12%) was noted (Figure 3).

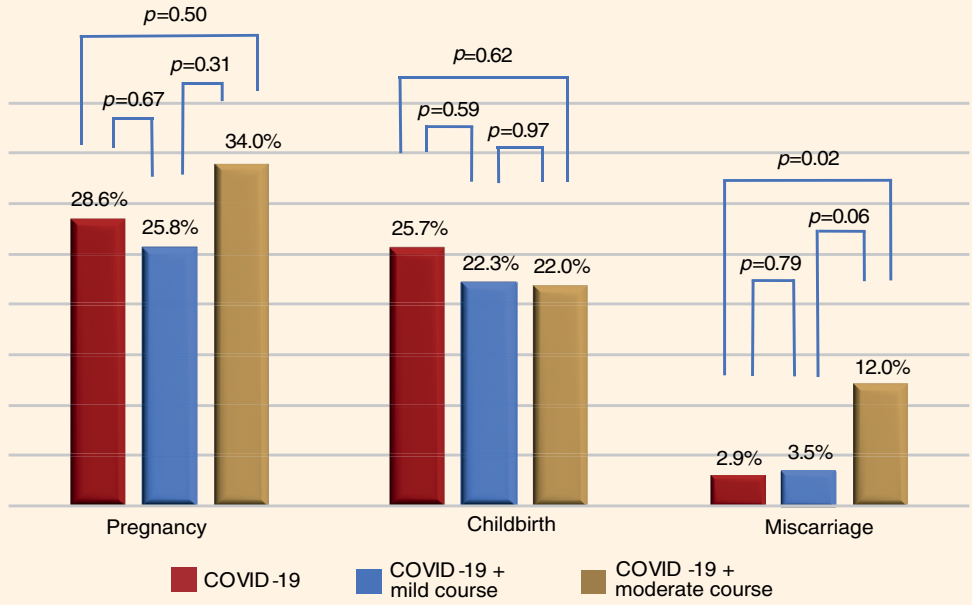

Figure 3. Results of ART programs in the study groups depending on the history of COVID-19 and its course

At the same time, when taking into account gynecological morbidity (adenomyosis, chronic endometritis) as a confounder, an association was found between moderate infection and cases of spontaneous abortion (OR=4.8; 95% CI = 1.10–20.52; *p*=0.031). It should be noted that half of the patients with miscarriage were found to have antibodies to An V and PE, which are possible risk factors for recurrent miscarriage [33].

These results are consistent with data from a meta-analysis, which showed a higher frequency of aPL detection in women with failed ART cycles than in women with successful outcomes [39]. According to this meta-analysis, women who were seropositive for aPL had a higher frequency of miscarriages than seronegative women.

According to our data, patients who had moderate COVID-19 most often had combinations of three or more autoantibodies of different specificities (Figure 4). At the same time, analysis of the clinical outcomes of ART in these patients showed a lower frequency of pregnancy and live birth; the chances of pregnancy were 4.9 times lower, and the chances of childbirth were 5.8 times lower than in women without autoantibodies [33].

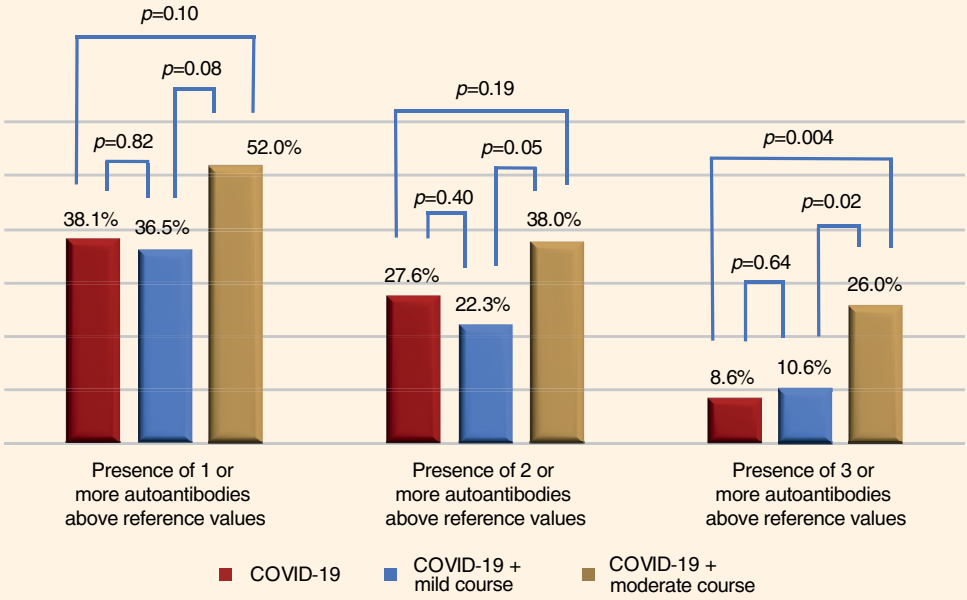

Figure 4. Proportion of female patients with different combinations of autoantibodies with levels above reference values in the study groups

With the advent of COVID-19 vaccines and their widespread use, interest in studying the impact of vaccination on human reproductive health has increased [40–42]. Previous studies in animals and humans have shown the potential negative impact of adjuvant vaccines on reproductive function involving an autoimmune mechanism [43]. According to a meta-analysis, between 2016 and 2019, there were 500 cases of autoimmune/inflammatory syndrome induced by adjuvants (Autoimmune/Inflammatory Syndrome Induced by Adjuvants — ASIA) reported worldwide [44], which was most commonly observed with the use of hepatitis B, influenza, and HPV vaccines [45]. The world's first registered COVID-19 vaccine approved by the Russian Ministry of Health was the Gam-COVID-Vac (Sputnik V), containing an adenovirus vector with an integrated fragment of SARS-CoV-2 genetic material with information about the structure of the virus's S-protein spike, produced by the National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya of the Ministry of Health of the Russian Federation [46]. It is important to note that the Gam-COVID-Vac vaccine does not contain adjuvants. However, in addition to the action of adjuvants, the main mechanisms triggering autoimmunity after vaccination against COVID-19 also include stimula-

tion of the immune system, molecular mimicry, and the production of antibodies to foreign peptides homologous to human peptides with cross-reactivity [45].

To gain a more complete understanding of the effect of COVID-19 vaccination on women's reproductive health, we conducted a prospective study of a wide range of autoimmune antibodies involved in the development of a number of systemic autoimmune diseases in 120 women before and after immunization with the Gam-COVID-Vac (Sputnik V) domestic combined vector vaccine [42]. The criteria for inclusion in the study were age 18 to 49 years, preserved menstrual function, no history of COVID-19, negative PCR test for SARS-CoV-2, and negative IgG and IgM antibody tests for SARS-CoV-2 prior to vaccination, no pregnancy, lactation, and severe somatic diseases. The average age of the patients included in the study was 33.3±7.7 years. All women had normal body weight (mean BMI was 23.1 [20.1; 25.0] kg/m^2). It was found that the most common gynecological diseases in these women were uterine fibroids (10.8%) and endometriosis (10.0%). Allergic diseases were observed in 30.0% of women, chronic gastrointestinal diseases in 21.7%, and otorhinolaryngological diseases in 15.0%.

All patients underwent ovarian reserve testing and enzyme immunoassay to determine a wide repertoire of autoantibodies, including aPL, antinuclear, antithyroid, anti-ovarian, antitrophoblastic, and anti-hormonal autoantibodies, before vaccination (1st point) and 90–100 days after administration of the first vaccine component (2nd point).

The high efficiency and safety of the Gam-COVID-Vac (Sputnik V) vaccine has been demonstrated. Specific IgG antibodies to SARS-CoV-2 were produced in 98.3% of vaccinated women, with no serious post-vaccination side effects reported in any of the patients. Menstrual cycle and ovarian reserve parameters did not differ significantly before and after vaccination. No increase in autoantibody levels above reference values was detected after vaccination, with the exception of a transient increase in anti-PE IgM and anti-dsDNA IgG levels. No correlation was found between the level of hormones reflecting ovarian reserve and the level of autoantibodies, which indirectly indicated that autoantibodies had no negative effect on women's reproductive potential. A transient increase in IgM antibody levels to PE after vaccination was observed in 20 (16.7%) cases. At the same time, in 7 (5.8%) seropositive women, aPL appeared for the first time after vaccination (Figure 5).

When examining autoantibodies of other specificities, including antinuclear, anti-thyroid, anti-ovarian, anti-trophoblastic, and anti-hormonal autoantibodies, a high total prevalence of these autoantibodies was observed in the women examined both before and after vaccination (in 70.8% and 75%, respectively) (Figure 6).

It should be noted that vaccinated women at the second point showed a transient increase in IgG antibodies to dsDNA in 18 (15%) cases, with antibodies detected for the first time after vaccination in 14 (11.7%) cases. A repeat blood serum test conducted 3 months later showed a decrease in the level of antibodies to PE and dsDNA to reference values.

After vaccination against COVID-19, ART programs remained highly effective in patients with infertility, with a pregnancy rate of 46.1% and a birth rate of 30.7%, indicating no negative impact of vaccination on reproductive outcomes.

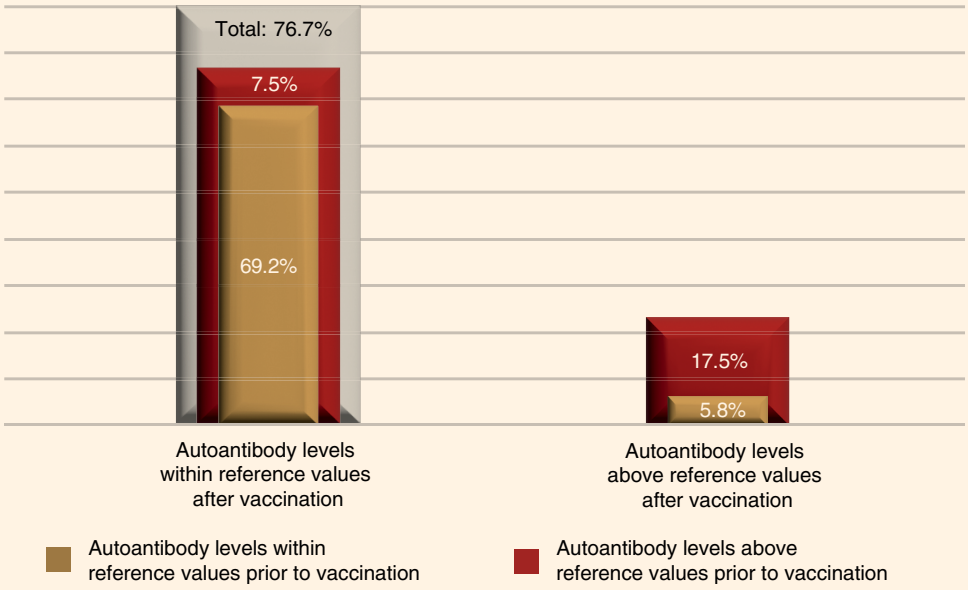

Figure 5. Total frequency of detection and dynamics of aPL levels in women after vaccination against COVID-19

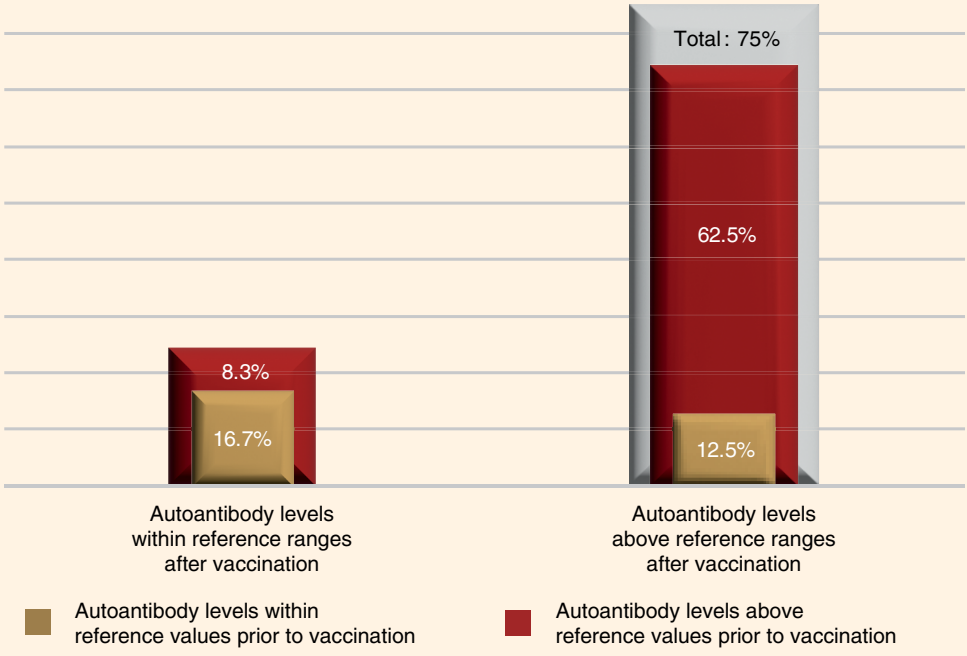

Figure 6. Total frequency of detection and dynamics of autoantibody levels to antigens of the cell nucleus, thyroid gland, tissues and hormones of the reproductive system in women after vaccination against COVID-19

CONCLUSION

Thus, the results of our studies demonstrated:

- the possibility of aPL production in COVID-19 patients, in particular non-criteria antibodies to prothrombin and annexin V, which may be involved in the pathogenesis of COVID-19-associated coagulopathy and serve as a risk factor for thromboembolic complications in both patients and convalescents;
- the possible negative impact of COVID-19, both direct and mediated by autoantibodies, mainly aPL, on the outcomes of ART programs and the course of early pregnancy; the frequency of pregnancy and live birth in patients after severe COVID-19;
- high efficacy and safety of the Gam-COVID-Vac (Sputnik V) combined vector vaccine against COVID-19, the possibility of a transient increase in the level of serum autoantibodies to PE and dsDNA after vaccination, no negative impact of vaccination on women's reproductive health and ART cycle outcomes;
- the need for an individual approach to preparing for ART programs, planning and managing pregnancy, taking into account the possible negative impact of SARS-CoV-2 infection, the time interval after the disease, and the presence of an autoimmune factor.

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Chapter 16

Features of the Antiviral Immune Response in Individuals Who Have Had COVID-19 and Those Vaccinated With the Gam-COVID-Vak Vaccine

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ABSTRACT

The T-cell response was studied in individuals who had recovered from mild to moderate COVID-19 and in those vaccinated with the Gam-COVID-Vak vaccine. The proportion of individuals with a T-cell response to SARS-CoV-2 antigens was 12% in control subjects, 70% in those who had recovered, and 52% in those who had been vaccinated. More than half of vaccinated individuals with a T-cell response had signs of asymptomatic infection.

INTRODUCTION

Intracellular parasitism of viruses significantly limits the ability of the immune system to counteract viral infection of the body through antibodies [1–3]. The ineffectiveness of humoral immunity in preventing infection of epithelial cells by viruses is exacerbated by the inability to maintain a consistently high level of specific antibodies in the main entry points for viral infection — the mucous membranes and their surfaces [4–6]. However, the presentation on the surface of infected cells of epitopes of synthesized viral proteins as part of major histocompatibility complex (MHC) class I molecules [7, 8] allows infected cells to be identified and suppressed by T-cell mech-

anisms of recognition and removal of cells in which foreign or altered proteins are synthesized [9–11].

T-cell immune response occurs after the appearance of cells expressing viral epitopes foreign to the body, with the formation of memory cells [12–14], which increase the rate of growth and strength of the T-cell immune response upon recurrent infection with the same pathogen [15, 16]. Therefore, the presence of T lymphocytes in the body that are specifically activated by epitopes of a particular virus indicates the presence of a necessary component of antiviral immunity, which may be the result of natural (previous infection) [17, 18] or artificial (vaccination) [19, 20] contact of the immune system with the virus or its components.

The production of interferon-γ (IFN-γ) is a distinctive feature of cytotoxic T lymphocytes and ThL T helper cells, which are activated upon interaction with cells whose surface MHC class I and II molecules present foreign epitopes [21, 22]. Therefore, the ability of lymphocytes to produce IFN-γ is widely studied both to expand our understanding of the general principles of the T-cell immune response [23, 24] and to assess the participation of T-lymphocytes in its specific manifestations, including antiviral immunity [25, 26]. The number and characteristics of IFN-γ-producing lymphocytes in peripheral blood, determined by specific stimulation with viral antigens, serve as indicators that should reflect the T-cell component of the body's immune response to viral infection or vaccination [27, 28].

The described features of virus interaction with the host organism and the characteristics of the host immune system's response to viral infection indicate the limited capabilities of vaccines containing only viral protein antigens. The inability of such vaccines to ensure intracellular synthesis of viral proteins and the absence of presentation of viral protein epitopes in the MHC class I leads to the emergence of only a humoral immune response [29, 30]. This disadvantage is absent in vaccines based on live attenuated viruses [31], non-replicating DNA vectors with an embedded virus DNA fragment [32], a plasmid with a built-in fragment of viral DNA [33], and a matrix RNA encoding viral proteins [34]. However, only the first of these vaccines is capable of complete presentation of viral antigens, while the capabilities of the others are limited to one or several proteins and even epitopes [35]. However, the high requirements for the attenuated virus strain and its production mean that vaccines based on the attenuated virus, although classic, are not attractive for emergency development in pandemic conditions [36]. In this regard, the development and implementation of antiviral vaccines based on genetically engineered constructs has been advanced, among which combined adenovirus vector vaccines occupy an important place [37]. The aim of this study is to investigate the characteristics of IFN-γ-producing T cells in patients who have had COVID-19 and in those vaccinated with the combined vector vaccine Gam-COVID-Vak.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of the Russian Ministry of Health (protocol No. 10 of November 5, 2021). Venous blood

samples were studied from individuals who had recovered from COVID-19, individuals vaccinated against COVID-19, and individuals not vaccinated against COVID-19 who did not have a positive PCR test for SARS-CoV-2 in their medical history. The vaccinated group included blood samples from individuals who had been vaccinated against SARS-CoV-2 with a full course of vaccination using the Gam-COVID-Vak combined vector vaccine (the National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya of the Ministry of Health of the Russian Federation). Peripheral blood mononuclear cells (PBMCs) were isolated using a standard method [38] in a density gradient based on a ficoll solution (PanEco). The T-cell immune response was assessed using the "TigraTest SARS-CoV-2" reagent kit (GENERIUM), which is designed to count IFN-γ-secreting T-lymphocytes using the enzyme-linked spot assay (ELISPOT) method. For specific stimulation, two sets of peptides carrying epitopes of different SARS-CoV-2 virus proteins were used in the test. One set (AG1) includes peptides with epitopes of the S-protein spike of the virus envelope, while the other set (AG2) includes peptides with epitopes of the nucleocapsid N-protein, membrane M-protein, and accessory (non-structural) proteins (ORF3, ORF7). The content of antibodies to SARS-CoV-2 proteins in serum was determined by solid-phase enzyme-linked immunosorbent assay. Reagent kits were used in the study to determine IgG antibodies (IgG-S_X) to the receptor-binding domain of the S protein "SARS-CoV-2-IgG-IFA" (HEMA) and IgG antibodies to the S (IgG-S_B) and N proteins (IgG-N_B) of SARS-CoV-2 (SARS-CoV-2-AT Spectrum-IFA-BEST) (Vector Best) were used in the study. The concentration of cytokines in PBMCs culture medium samples obtained during the assessment of the T-cell immune response was determined using a multiplex method with a 17-plex Bio-Plex Pro Human Cytokine 17-plex Assay panel (Bio-Rad). The content of cytokines of the Thl group (IL-1β, IL-2, IL-6, IL-12, IL-17, IFN-γ), Th2 (IL-4, IL-5, IL-10, IL-13, TNFα), chemokines (IL-8, MCP-1, MIP-1β), and growth factors (IL-7, G-CSF, GM-CSF) were determined. Statistical analysis of the results was performed using Microsoft Office Excel 2007 software and the MedCalc Software v. 14.8.1 statistical software package. Bioinformatic analysis was performed using the WOLFRAM MATHEMATICA 13.0 software package.

RESULTS

According to the results obtained, COVID-19 disease and Gam-COVID-Vak vaccination lead to a significant increase in the content of T-lymphocytes in the body, which are responsible for the increased production of INF-γ in response to specific stimulation by both sets of peptides (Figure 1). The predominance of T lymphocytes activated by peptides from set AG2 was observed in subjects who had recovered from the disease.

Significant numbers of lymphocytes activated by peptides from set AG2, whose epitopes should not have appeared in the body during vaccination, were observed in vaccinated subjects with a predominance of T lymphocytes activated by peptides from set AG1. At the same time, among subjects who have had the disease, there are no positive subjects with a significant response only to the antigens of the AG1 set, and

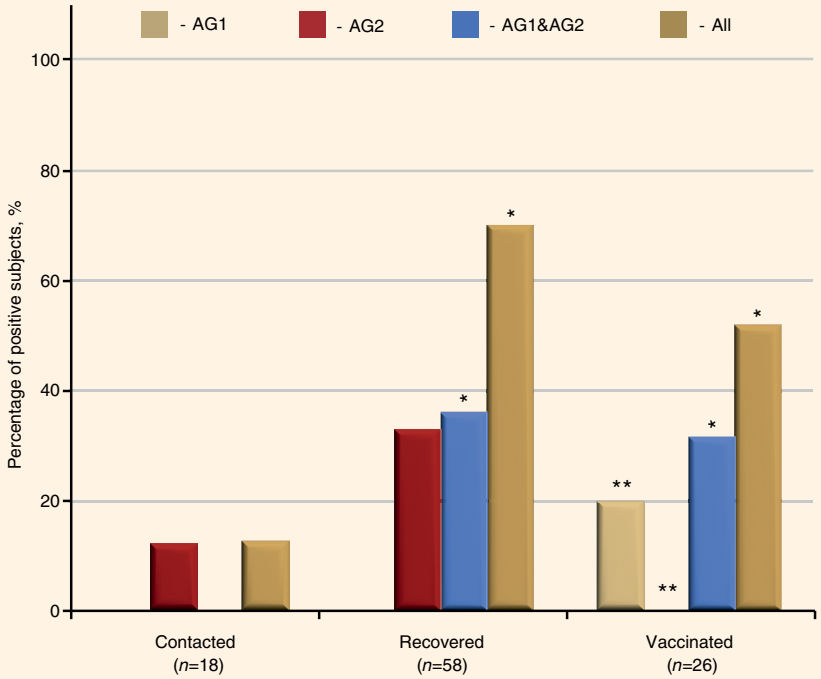

Figure 1. Results of activated T-lymphocyte determination in PBMCs samples. Percentage of subjects with positive test results for the presence of a T-cell immune response to SARS-CoV-2 antigens. AG1 and AG2 — positive response to antigens from only one set, AG1&AG2 — positive response to antigens from both sets simultaneously.

*p<0.01 when comparing the same indicator with the control group.
**p<0.01 when comparing the same indicator with subjects who have recovered

among vaccinated subjects, there are no positive subjects with a significant response only to the antigens of the AG2 set. However, in both of these groups, at least half of the positive subjects simultaneously have both T-lymphocytes that respond to the antigens of the AG1 set and T-lymphocytes that respond to the antigens of the AG2 set. In total, the proportion of subjects with a positive conclusion on the T-cell response to antigens was 12% in the control group, 70% in the group of those who had recovered, and 52% in the vaccinated group.

According to the data obtained on seropositivity (Figure 2), the antibodies sought are detected very weakly in the subjects of the control group, whereas in those who have had the disease and those who have been vaccinated, there is a significantly higher level of antibodies corresponding to the antigenic composition of the antigen source that caused the humoral immune response. According to the data obtained, the majority (more than 90%) of those who had recovered and those who had been vaccinated are seropositive for antibodies to S-protein antigens, while subjects who are seropositive for antibodies to N-protein antigens account for about 90% only in the group of those who had recovered.

Non-specific polyclonal stimulation using OKTZ monoclonal antibodies to the surface marker of T lymphocytes CD3 leads to a significant increase in the content

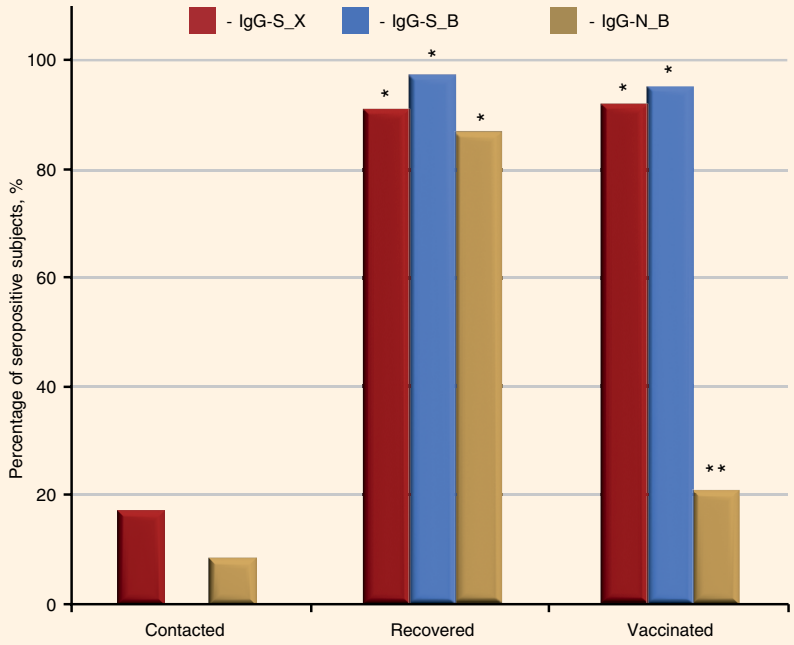

Figure 2. Results of determining antibodies to SARS-CoV-2 protein antigens in the blood serum of the subjects examined. The proportion of seropositive subjects (positivity index >1.1) in the groups of subjects examined for SARS-CoV-2 protein antigens

*p<0.01 when compared with the results of the same method in serum samples from the control group.
**p<0.01 when compared with the results of the same method in serum samples from subjects who had recovered from the disease

of most cytokines in the culture medium with PBMCs of subjects from all groups (Figure 3). At the same time, a greater increase in cytokine content in the medium with nonspecifically stimulated PBMCs compared to unstimulated PBMCs is observed in both recovered and vaccinated individuals. In contrast to non-specific stimulation of OKTZ, specific stimulation with SARS-CoV-2 virus peptides in the form of sets AG1 (Figure 4, *a*) and AG2 (Figure 4, *b*) does not lead to an increase in cytokine content in the PBMCs culture medium of all subject groups. At the same time, cytokine levels are lower in the vast majority of cases in the presence of viral peptides and, in many cases, significantly lower.

Bioinformational analysis with clustering of subjects showed that when using only the indicators of cellular and humoral immunity measured in this study (Figure 5, *a*) each of the three clusters (the number of clusters was selected based on the number of groups in the study) identified by the algorithm with the best clustering includes representatives of all groups. At the same time, despite the impossibility of accepting the hypothesis of a coincidence in the composition of clusters based on the presence of subjects from different clinical groups (p (χ^2)=0.0004 and Cc=0.410 for the corresponding table of feature conjugacy in the insert), the reliable differences obtained do not allow any cluster to be characterized as formed by representatives of a single clinical group.

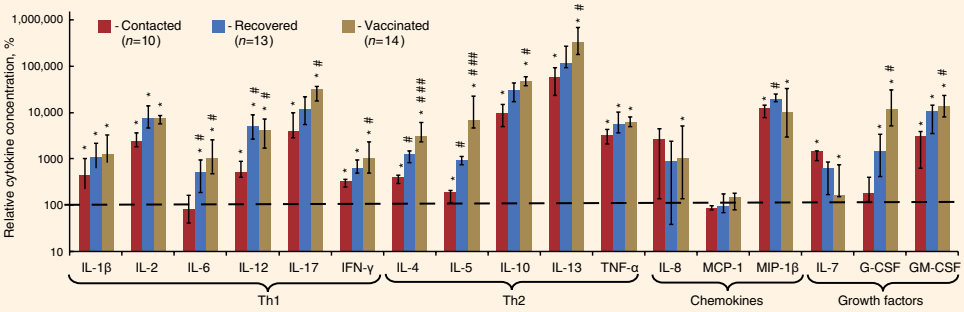

Figure 3. Cytokine content in PBMC culture medium during nonspecific OKT3 stimulation. The relative cytokine concentration is equal to the ratio of the measured true cytokine concentration in the sample to the median of the measurements of the true concentration of this cytokine in samples without stimulation of PBMCs from subjects in this group.

*p<0,01 when comparing samples without stimulation (Me relative cytokine concentration 100%) with samples with nonspecific stimulation within the group, #p<0.01 when comparing samples with nonspecific stimulation between the given and control groups, ##p<0.01 when comparing samples with nonspecific stimulation between the vaccinated and recovered groups. The data are presented as Me [Q1; Q3]

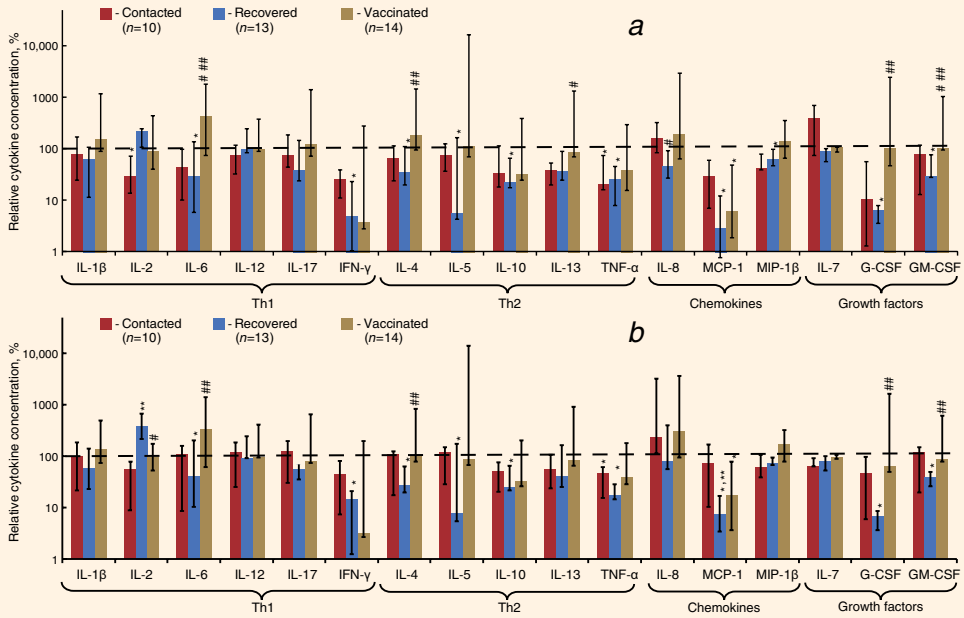

Figure 4. Cytokine content in PBMC culture medium upon specific stimulation with SARS-CoV-2 virus peptides in the form of sets AG1 (*a*) and AG2 (*b*). The relative cytokine concentration is equal to the ratio of the measured true cytokine concentration in the sample to the median of the measurements of the true concentration of this cytokine in samples without stimulation of PBMCs from subjects in this group.

*p<0,01 when comparing samples without stimulation (Me relative cytokine concentration 100%) with samples with specific stimulation within the group, **p<0.01 when comparing samples with specific stimulation by different sets of SARS-CoV-2 peptides within the group, #p<0.01 when comparing samples with the same specific stimulation between the test and control groups, ##p<0.01 when comparing samples with the same specific stimulation between the vaccinated and recovered groups. The data are presented as Me [Q1; Q3]

When subjects are divided into three clusters, including cellular and humoral immunity together with cytokine profile indicators (Figure 5, *b*), the clusters are characterized not only by better correspondence of the table of representation of subjects from different clinical groups (p (χ^2)<0.0001 and Cc=0.732), but also by better alignment with clinical groups. In the cluster, which includes almost all representatives

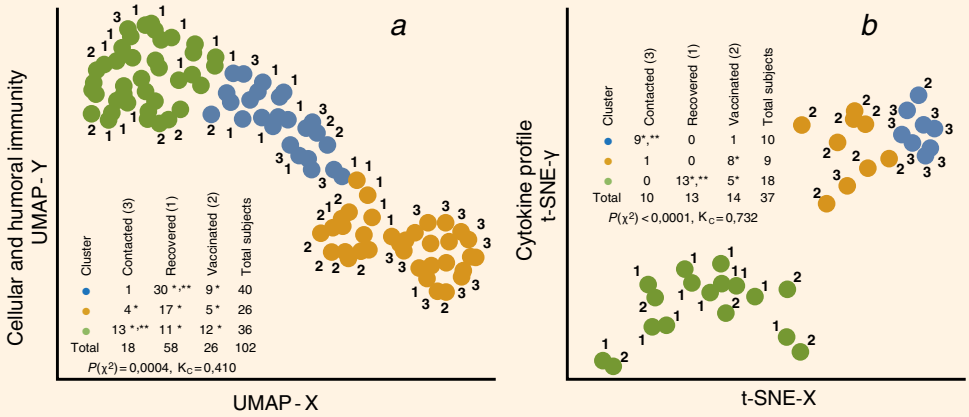

Figure 5. Bioinformatic analysis with clustering of subjects based on cellular and humoral immunity indicators (*a*) and cytokine profiles (*b*). Subjects belonging to the same cluster are marked with circles of the same color in the clustering results images. The numbers next to the circles indicate the subject's clinical group: 1 — subjects who have had the disease; 2 — vaccinated subjects; 3 — control subjects. The contingency tables show the number of representatives of each clinical group in the clusters.

* — the proportion of subjects in the cluster significantly exceeds 0.05 of the number of subjects with the corresponding clinical status (p<0.01), ** — the proportion of subjects in the cluster significantly exceeds the proportion of subjects with the corresponding clinical status in all other clusters (p<0.01)

of the control subjects (9 out of 10), there are no recovered patients and vaccinated patients are insignificantly represented (1 out of 14). All those who have recovered from the disease belong to another cluster, which, as in the case of clustering based on cellular and humoral immunity indicators, is supplemented by a significant number of vaccinated individuals (5 out of 14). The third cluster consists almost exclusively of vaccinated individuals, with no recovered individuals and an insignificant number of control subjects (1 out of 10).

CONCLUSION

Thus, the characteristics of antiviral cellular and humoral immune responses in a sensitized organism are determined by the presentation of all antigens by the full-fledged SARS-CoV-2 virus (disease) and only S-protein antigens by the Gam-COVID-Vak vector vaccine (vaccination). However, the inability to rule out undetected asymptomatic infection during the pandemic appears to be the reason for the detection of a significant T-cell response to antigens unrelated to the S-protein in vaccinated subjects.

If there is a positive conclusion about the presence of a humoral immune response in more than 90% of infected and vaccinated individuals, a conclusion about the presence of a T-cell response can be made for approximately 70% of those who have had the disease and 50% of those who have been vaccinated. The inability to characterize the T-cell immune response as positive in a significant proportion of those who have recovered (30%) and those who have been vaccinated (about 50%) indicates a high probability of the existence of an undetectable weak T-cell immune response to SARS-CoV-2 infection that is not accompanied by clinical manifestations of COVID-19. Although the number of positive T-cell responses among control subjects was insignificant, it is interesting to note that a positive response in this group was observed for antigens in the AG2 set. This brings this group closer to the group of recovered patients and reflects the possibility of developing a weak T-cell immune response during asymptomatic SARS-CoV-2 infection, detectable only by highly sensitive methods [39].

Unlike T-cell immunity, the high level of antibodies in people who have recovered from the disease and those who have been vaccinated fully corresponds to the antigenic composition of the antigen source that triggered the humoral immune response. In people who have had the disease, whose immune systems have interacted with the full SARS-CoV-2 virus, there is a significant increase in antibody levels to both the S and N proteins, whereas in vaccinated individuals, for whom the source of antigens was an artificial virus, high levels are only found for antibodies to the S protein. In terms of antibodies to N-protein antigens, the proportion of seropositive vaccinated individuals is at the level of control subjects.

It is interesting to note that increased production of IFN-γ by individual T cells sensitized by viral antigens, in the case of stimulation of cytokine production by antigenic peptides *in vitro*, is not accompanied by an increase in the content of this and other cytokines in the culture medium, in contrast to nonspecific polyclonal stimulation. The result obtained indicates that, simultaneously with the increase in cytokine production by T-lymphocytes responding to specific stimulation, their ability to utilize cytokines increases to no lesser extent. As a result, a specific immune response creates conditions for maintaining a controlled level of cytokines and preventing the development of a cytokine storm in control subjects.

The low correlation between the composition of the clusters identified in this study and the clinical characteristics of the groups when subjects were clustered according to cellular and humoral immunity indicators is consistent with the recombinant mechanism of the appearance of specific antigen-recognizing molecular structures (receptors) of naive T and B lymphocytes [40, 41, 42]. This mechanism causes the spread of affinity of antigen-recognizing molecules to different epitopes in one subject [43]; it is the cause of different affinity to the same epitopes in different subjects [44, 45], predetermines the presence of polyclonality of T- and B-memory cells in one subject [46, 47], and also leads to different polyclonality and number of antigen-responsive cells in different subjects [48, 49]. This leads to mutual independence of the distributions of specific cellular and humoral immune response indicators in those who have had the disease and those who have been vaccinated, as well as to the presence of a fairly large number of subjects with low immune response indicators in these groups. As a result, there is a significant presence of representatives of different immunostimulated groups

in all clusters and even in the cluster containing the majority of subjects in the control group. The higher correlation with clinical groups in clusters obtained using the cytokine profile of subjects is consistent with the fact that cytokine production by lymphocytes responding to stimuli mediated by antigen-recognizing molecules depends to a greater extent on the metabolic characteristics of the stimulated cells, which they acquire during their maturation during infection or vaccination. As a result, a cluster consisting almost exclusively of control patients (without primary antigenic stimulation), a cluster consisting almost exclusively of vaccinated individuals, and a cluster combining all those who have had the disease and a significant number of vaccinated individuals are reliably distinguished.

Overall, the results obtained once again indicate that the recombinant mechanism of specificity emergence in antigen-recognizing T- and B-cell receptors during the formation of adaptive antiviral immunity leads to the emergence of different combinations of the body's immune system's ability to mount cellular and humoral responses. In most cases, the resulting combinations ensure the body's resistance to viral infection. However, in a number of cases, the resulting combination of components of the immune response is unbalanced [50]. This may result in the inability of the emerging antiviral immunity to completely protect the body from reinfection with the virus and its adverse effects [51], in particular, severe disease in vaccinated individuals and in those who have been infected before [52, 53]. In this regard, it remains equally important for medical countermeasures against infectious diseases caused by SARS-CoV-2 and other respiratory viruses to create conditions for the full provision of medication and equipment for the treatment of patients with symptoms requiring hospitalization [54].

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Chapter 17

Soft Dosage Forms of Recombinant Interferon Alpha-2b in the Treatment of COVID-19 and Post-COVID Syndrome

A.V. Karaulov

ABSTRACT

A significant inhibitory effect of the SARS-CoV-2 virus on the interferon system has been established, as well as the dependence of the severity of COVID-19 on the level of interferon in the patient. A rational strategy would be to create high concentrations of interferon in peripheral blood, especially in the first hours after the onset of the disease, by administering exogenous interferon preparations.

The interferon system is a crucial component of the body's innate non-specific defense against viral, bacterial, and fungal infections and tumor transformations. The main action of interferon (IFN) is the elimination of foreign nucleic acids, suppression of viral replication in cells, sending a signal to other intact cells to increase IFN synthesis to counteract viral aggression, and immunomodulatory effects associated with the activation of various links in the immune system [1].

IFNs were discovered in 1957 at the National Institute for Medical Research in London by virologists Isaac A. and Lindenmann D. while studying a process that was later named virus interference. The researchers discovered that if an organism is already infected with a virus, it cannot be infected with another virus, from which they made the assumption that a certain substance is produced in the cells of the infected host organism that induces the formation of a "state of antiviral resistance" in the cells. This led to the discovery of a protein that Isaacs and Lindenmann named "interferon." Further research has shown that there is a whole group of IFN proteins of various classes, IFN-stimulated genes (ISG) and their receptors, specific cell receptors and enzymes (gc-RNA-dependent 2'-5'-oligoadenylate synthetase and protein kinase),

which are activated when the IFN protein interacts with these receptors, thus constituting the IFN system.

Research on the IFN system, conducted since its discovery, shows that there are a number of factors that can negatively affect IFN production and function. As early as 1981, Levin S. et al. [2] identified congenital and acquired disorders in the IFN defense system associated with low levels of endogenous IFN synthesis. Professor Malinovskaya V.V. and co-authors established that acute viral infection can lead to transient immunosuppression and depletion of the IFN pool in the body, which usually results in secondary infection [3], since there is no interferon depot in the body [4]. Studies of age-related features of the IFN system were conducted under the guidance of Soviet virologist, Academician of the USSR Academy of Medical Sciences V.D. Solovyov, which showed that the lowest titers of leukocyte IFN were found in children under 3 years of age (especially in children under 1 year of age) and in people over 60 years of age [5]. These results were recognized abroad, and subsequently similar studies were carried out in other scientific institutions around the world. It was found that premature babies, newborns, and adults produce IFNs that differ in molecular composition, hydrophobicity, and antiviral properties. Thus, while in adults the activity of the IFN system is directed at fighting viruses, in newborns its main function is to participate in cell differentiation and development. It has been established that a significant amount of this "early" IFN circulates in the blood of newborns, especially in premature babies [6].

Another group with a high probability of severe and complicated viral infections are pregnant women, who are in a state of physiological immunosuppression. All of the above factors place newborns, including premature babies, young children, pregnant women, and elderly patients in a special risk group that can benefit most from the introduction of exogenous IFN drugs into the complex therapy of infectious diseases.

In addition, it has been reliably established that viruses, in the course of their evolutionary phylogenesis, have acquired various mechanisms that allow them to counteract the mechanisms of the innate immune system, including by interfering with the work of the IFN system. Thus, viruses have learned to block IFN synthesis, induce the breakdown of IFN receptors, block IFN signal transmission, and disrupt the functions of IFN-induced proteins [1].

The broad spectrum of anti-infective and immunomodulatory activity of IFN, combined with the development of biotechnology, led to the creation of IFN drugs, which began to be actively researched and used to treat viral infections such as influenza, hepatitis, herpesvirus infections, etc., as well as a number of bacterial and protozoal infections. In 1960, IFN was synthesized in the USSR and in the late 1960s was successfully tested during a flu epidemic, after which leukocyte IFN began to be widely used for the treatment and prevention of infectious diseases.

Currently, recombinant IFNs, created in 1980 using genetic engineering technologies, are most widely used. Research was conducted in Australia, China, and Russia. In 1996, after lengthy development, a recombinant IFN drug was registered in Russia under the brand name Viferon®, developed by a group of scientists at the National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya of the Ministry of Health of the Russian Federation, led by Professor Malinovskaya V.V. At the same time, serial production of recombinant IFN-α-2b in

suppositories and in the form of an ointment for topical application began, and later, in 2005, another domestic dosage form was registered — a gel.

Interest in type I IFN drugs has increased significantly worldwide during the COVID-19 pandemic. From the early days of research into this new disease, it was established that the SARS-CoV-2 virus has a significant inhibitory effect on the IFN system and that the severity of COVID-19 depends on the patient's IFN level. Blocking and suppressing the synthesis of endogenous interferon is a basic biological "survival" strategy for many viruses, including members of the coronavirus family. Research has shown that SARS-CoV-2 coronavirus blocks IFN synthesis in the early stages of infection via the ORF9b protein, which is expressed from the *ORF3b* gene. Sustained replication of the coronavirus is accompanied by dysregulation of type I IFN. This contributes to the accumulation of pathogenic inflammatory monocytes and macrophages, leading to increased levels of inflammatory cytokines/chemokines in the lungs and disruption of virus-specific T-cell responses. It is this mechanism that triggers the so-called "cytokine storm" in the lungs.

A group of scientists from the United States modeled COVID-19 infection in respiratory epithelial cell culture and, through transcriptomic analysis, determined that the coronavirus does not elicit an IFN response in these cells because it does not activate the signaling cascade that induces the expression of type I IFN and IFN-dependent stimulation of certain genes.

Scientists from France have found that the SARS-CoV-2 virus disrupts the function of plasmacytoid dendritic cells, which produce type I IFN, and also sharply reduces the expression levels of five key IFN-stimulating genes: *MX2, ISG15, IRF7, BST2, IFITM2* and *ADAR*.

Danish scientists from Aarhus University studied the induction of IFN expression in lung tissue during SARS-CoV-2 infection and showed that alveolar macrophages in lung tissue do not produce IFN in response to SARS-CoV-2 virus entry and do not mediate the expression of IFN-stimulated genes [1].

It has been established that the coronavirus protein NSP1 blocks the exit channels in the cell nucleus, as a result of which the transcripts of genes responsible for the synthesis of endogenous IFN in the cell cannot leave the nucleus in the form of matrix RNAs, from which the synthesis of the necessary proteins should then occur, as a result of which the synthesis of IFN in the cell is suspended [7].

The lack of sufficient and timely IFN production leads to a disruption in the implementation of further immune mechanisms. IFNs not only trigger the production of active molecules — protein kinase, 2'-5'-oligoadenylate synthetase, MxA (Myxovirus resistance A), which suppress the translation of viral RNA and DNA and inhibit protein synthesis in infected cells, but also, as one of the most important mediators of the immune response, initiate a cascade of immune reactions, including the induction of inflammatory macrophages, the activation of NK cell cytotoxicity, and the formation of effector CD8+ T lymphocytes. It is precisely the imbalance in the IFN system, developing against the background of viral exposure, that is one of the key aspects in the pathogenesis of COVID-19 [8]. Low IFN levels are associated with disease severity and determine the outcome of the disease. It has been shown that serum IFN activity is significantly lower in patients with severe or critical disease compared to patients

with mild and moderate COVID-19, and low levels of IFN-α2 in blood plasma were significantly associated with an increased risk of developing a critical condition [9].

Given the above, a rational strategy would be to create high concentrations of IFN in peripheral blood, especially in the first hours after the onset of the disease, in order to successfully repel the viral attack, eliminate viral particles, and counteract the mechanisms of viral inhibition of IFN synthesis. Intranasal administration of IFN allows for a pronounced antiviral and immunomodulatory effect at the site of infection by acting on local mucosal immunity, but has virtually no systemic effect. Since it is precisely systemic action that can produce a pronounced clinical effect and improve the prognosis, the choice of a suitable dosage form is important. The optimal form of delivery of the drug into the bloodstream is a rectal suppository. Having entered the rectum, the active substance is rapidly absorbed through the lymphatic capillaries into the lymphatic system and then, by passive diffusion, into the blood. In addition, rectal administration protects the drug from the first-pass effect through the liver.

Viferon is such an IFN drug with systemic antiviral and immunomodulatory effects. Its unique feature is the presence of a precisely formulated combination of antioxidants in the suppositories: vitamins C (ascorbic acid) and E (alpha-tocopherol acetate), which not only help to cope with the oxidative stress developing against the background of COVID-19, but also prolong the action of IFN and enhance the specific antiviral activity of IFN-alpha-2b by 12.5 times [1].

It is now well known that the SARS-CoV-2 virus is highly sensitive to the action of exogenous IFN. Lokugamage K.G. et al. showed that SARS-CoV-2 is much more sensitive to type I IFN than even its predecessor SARS-CoV, and in infected but IFN-treated cells, the amount of virus was 1,000–10,000 times lower within a few days than in similarly infected cells that had not been pretreated with IFN [10]. The high sensitivity of the virus to IFN has been demonstrated for the Omicron strain, especially when compared to the Delta strain [11]. Other *in vitro* studies have also confirmed that treating human airway epithelial cell cultures with type I or III IFN has a pronounced prophylactic effect: the introduction of IFN 24 hours before infection reduced the amount of viral RNA by 3 times compared to infected cultures not treated with IFN, and reduced virus replication by 90%. In addition, the viral load decreased even in cases where IFN treatment was performed after infection [12]. These data are confirmed by Russian authors (Isakova-Sivak I.N. et al.), who additionally note the dose-dependent effect of IFN on SARS-CoV-2 replication [13].

It has been established that the timing of recombinant IFN administration is a key factor determining the severity of a patient's condition in COVID-19 [14]. If there is an early and pronounced induction of type I IFN in response to coronavirus infection, the viral load decreases rapidly, an adequate T-cell response and the production of protective antibodies occur, and the disease proceeds in a mild form. This variant of the disease is typical for young people or when the viral load is low. If the IFN system response is delayed or weakens in the early stages of infection, the virus replicates and spreads unchecked. Delayed IFN response, T-cell lymphopenia, and insufficient virus clearance even with normal levels of protective antibodies can lead to severe infection. This form of infection occurs in elderly patients or when infected with a high dose of the virus. If, for one reason or another, type I IFN is not produced at all (for example,

in cases of genetically determined type I IFN deficiency), SARS-CoV-2 replicates unhindered, leading to a severe, life-threatening form of COVID-19. T-cell lymphopenia is observed, and even compensatory activation of humoral immunity is insufficient to control the disease. Early administration of recombinant type I IFN after infection allows for rapid reduction of viral load, resulting in a milder form of the disease [14].

After receiving encouraging experimental data, clinical trials of IFN drugs began. Thus, it was shown that the creation of high concentrations of IFN in peripheral blood in the first hours and days from the onset of the disease is etiopathogenetically justified — in studies conducted in China, high doses of IFN showed high efficacy in the treatment of COVID-19 in adults [15, 16] and children [17, 18]. Positive results in the form of a reduction in the duration of clinical symptoms and faster elimination of the virus were obtained for the drug Viferon in the treatment of COVID-19 in adults [19], including pregnant women [20], as well as in children aged 1 year and older [21]. The high susceptibility of placental cells to COVID-19 and the associated increased likelihood of vertical transmission of the virus from mother to fetus, the high risk of spontaneous preterm birth in pregnant women with COVID-19, and the risk of developing a severe form of the disease allow pregnant women to be identified as a separate risk group. In pregnant patients, a significant effect of high-dose IFN therapy was also an improvement in perinatal outcomes: a 3.6-fold reduction in the incidence of preterm birth, a 2.5-fold reduction in neonatal asphyxia, a 2-fold reduction in the need for cesarean delivery, and no perinatal losses [20].

The therapeutic and preventive efficacy of recombinant IFN preparations has been studied in detail during the pandemic. According to the data from the studies conducted, the use of various forms of IFN has proven to be a reliable method of prevention for patients living in family households [22, 23], as well as among healthcare workers at high risk of COVID-19 infection who work in the “red zone” [24, 25].

IFN preparations, including rectal suppositories, have been included in the Provisional Methodological Recommendations for the Prevention, Diagnosis, and Treatment of the Novel Coronavirus Infection. High doses of IFN (up to 3,000,000 IU per day) are recommended for the treatment of ARVI according to the methodological recommendations of the Federal Medical and Biological Agency “Influenza and other ARVI during the ongoing COVID-19 pandemic: prevention and treatment,” published in 2022. [26], and are also included in the 2022 teaching manual of the Federal State Budgetary Institution “Central Research Institute of Epidemiology” of Rospotrebnadzor with a recommendation to prescribe increased doses of IFN-α-2b with antioxidants (1,000,000–3,000,000 IU twice daily) to children and adults for the treatment of ARVI, including influenza, and COVID-19 during the epidemic season and in pandemic conditions [27].

Unfortunately, the end of the acute phase of infection does not mean a full recovery. Post-COVID syndrome affects a significant proportion of recovered patients and is characterized by a variety of clinical manifestations and immune disorders. According to the World Health Organization, the frequency of post-COVID syndrome varies from 10 to 20%, and according to studies conducted on hospitalized patients, the frequency can reach 80% [28]. This indicator depends on various factors, including the characteristics of the circulating strain and the state of the human immune system.

Long-term inflammation and immune dysregulation play a role in the pathogenesis of post-COVID syndrome. It has been established that in response to SARS-CoV-2 infection, systemic inflammatory response syndrome (SIRS) initially predominates, the severity of which depends on viral exposure, the presence of comorbidities, and the state of the host's immune system. The SARS-CoV-2 virus causes the development of a cytokine storm, accompanied by hyperproduction of pro-inflammatory cytokines: IL-2, -6, -7, -12, -18, etc., TNF-α, IFN-α, and -γ, leading to life-threatening systemic reactions in the body, including multiple organ failure. SIRS is replaced by a prolonged compensatory anti-inflammatory response syndrome (CAIRS), which is accompanied by post-infectious immunosuppression [28]. CAIRS is the body's response aimed at weakening the pro-inflammatory state, preventing multiple organ dysfunction, and restoring immunological balance. If the inflammatory response is too suppressed, the patient may enter a stage of prolonged immunosuppression known as persistent inflammation, immunosuppression, and catabolism syndrome (PIICS). The sequence of these three phases is well known and described in patients with sepsis, but it can also be applied to patients with severe COVID-19. PIICS is one of the presumed causes of persistent post-COVID syndrome [28].

Patients who have had a new coronavirus infection may have persistent post-infectious immune disorders. This theory is supported by reports of reactivation of latent herpesvirus infections (Epstein–Barr virus (EBV), cytomegalovirus, CMV) in this category of patients [29, 30]. The activation of chronic herpesvirus infections is due to the ability of SARS-CoV-2 to suppress the IFN system and enable persistent latent herpesviruses to transition from a state of latency to a state of lytic infection, followed by the onset of clinical manifestations of virus activation [30]. In addition to the mechanisms of immunosuppression described above, the risk of reactivation of latent herpesviruses increases against the background of significant psychological and physical stress accompanying COVID-19, which impairs cytotoxic T-cell surveillance of latently infected neurons, also increasing the likelihood of activation of "dormant" pathogens [31]. It is interesting to note that chronic herpesvirus infections can also be considered one of the causes of post-COVID syndrome. For example, there is evidence that the detection of EBV DNA during acute SARS-CoV-2 infection correlates with the onset of post-COVID syndrome symptoms 30-60 days after recovery [29].

Another immune mechanism that could explain why some immune disorders are developing is the autoimmunity against the body's own tissue antigens, especially against immunomodulatory proteins [32] — for example, the emergence of autoantibodies against components of the IFN system and certain cytokines has been described [33]. Persistent neutralization of the IFN response can lead to insufficient virus clearance and disruption of IFN-dependent immune regulation. The persistence of the SARS-CoV-2 virus, one of the causes of which, along with other factors, may also be the insufficiency of the IFN system, is another potential mechanism of Long COVID pathogenesis [34].

Studies conducted during the COVID-19 pandemic have shown that a decrease in endogenous IFN production is observed not only in the acute phase of the disease, but also persists after its completion. Thus, in the work of Ospelnikova T.P. et al. (2022), it was established that in the acute period, there is a suppression of the biological activity

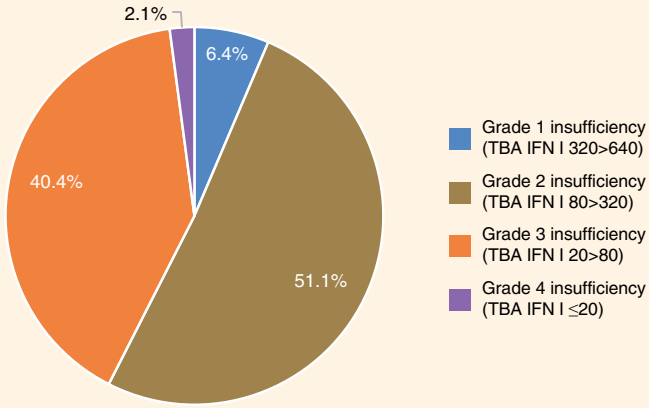

Figure 1. Prevalence of type I IFN production disorders in blood leukocytes in the post-COVID period.

TBA — biological activity titer. Adapted from [35]

of IFN I (20 times) and II (7 times) types, but even in the rehabilitation period, there is no complete restoration of the activity of the IFN system (up to 3 months) [35]. According to the data of the study, during the rehabilitation period after COVID-19, all examined patients showed a decrease in the production of IFN type I by leukocytes of varying degrees of severity — by 50–97% (see Figure 1). The average values of IFN I and II types of the biological activity titers during acute course and rehabilitation period compared to reference data and similar indicators for influenza A/H1N1 are presented in Figure 2. During the 3-month observation period, none of the patients observed showed a return of IFN activity to normal values.

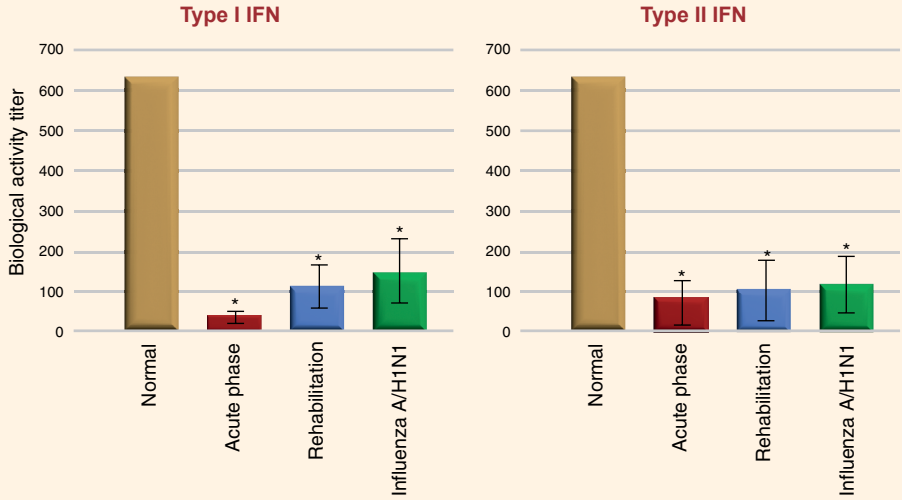

Figure 2. Biological activity indicators of IFN types I and II in COVID-19 compared to influenza.

Adapted from: [35]
License reference: https://virusjour.crie.ru/jour/about/editorialPolicies

Another study [36] examining patients who had suffered from moderate COVID-19 found that even nine months after acute infection, they still had reduced serum IFN (-α, -β, -γ) levels, and one in five patients had increased levels of interleukins IL-4, IL-10 for up to six months, and a persistent impairment of the mucosal immune system throughout all periods of observation in the form of decreased levels of secretory immunoglobulin A (sIgA) in saliva and nasopharynx and an imbalance of antimicrobial peptides. Reduced mucosal immunity can be considered a risk factor for respiratory infections [30, 36], which is important to consider in order to reduce the risk of recurrent infections in the post-infectious period. The authors of the study recommended that patients with a history of COVID-19 use a topical form of recombinant IFN-α-2b based on the data they got. The therapy resulted in a decrease in the frequency of respiratory manifestations of post-COVID syndrome (shortness of breath, dry cough, nasal congestion, and nasal discharge) and headaches. An improvement in serum IFN (-α, -β) levels and stabilization of interleukins-4 and -10 were observed, and normalization of mucosal immunity indicators was established in the form of increased slgA levels in saliva and the nasopharynx.

In addition, as a result of administering the drug twice a day for a month, a decrease in the frequency of acute respiratory infections during the convalescence period after COVID-19 was recorded.

As noted earlier, IFN-α deficiency caused by SARS-CoV-2 virus evasion mechanisms and leading to disruption of antiviral immune defense mechanisms underlies the reactivation of latent pathogens. Nesterova I.V. et al. (2022) developed an immune system rehabilitation program for patients with chronic recurrent herpesvirus co-infections in the acute phase of COVID-19 and in the post-COVID period [30]. The program included prolonged targeted IFN and immunotherapy with the administration of Viferon in gel form to patients to restore the mucosal immunity of the upper respiratory tract by increasing the local level of IFN-α and inducing the interference phenomenon, as well as in the form of high-dose rectal suppositories to restore impaired induced production of IFN-α and IFN-γ, and the number and functional activity of natural killer cells. The course of the post-COVID period in patients in the study group was complicated by the activation of herpesvirus infection. Prolonged targeted IFN and immunotherapy for these patients contributed to the regression of chronic fatigue syndrome, cognitive disorders, fibromyalgia, and arthralgia that developed in the post-COVID period, as well as to the restoration of their ability to work.

CONCLUSION

The use of recombinant IFN-α-2b with antioxidants in high doses for COVID-19 is etiopathogenetically justified and proven by scientific research, the results of which have been published in official medical journals. Studies conducted and clinical experience gained by many authors show that the use of recombinant IFN-α-2b with antioxidants in high doses is an effective and safe method of treatment and prevention of the new coronavirus infection and post-COVID syndrome in the current epidemiological reality.

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Conclusion

Currently, post-COVID syndrome can be classified as a condition that affects a significant portion of society to one degree or another. This monograph demonstrates the importance of studying post-COVID syndrome by specialists from various fields of medical science, since the changes observed during the convalescence period of COVID-19 affect all organs and systems of the patient and require a comprehensive approach to diagnosis, prevention, and treatment.

The monograph presents a detailed description of theoretical and practical issues in the epidemiology of novel coronavirus infection, allowing parallels to be drawn between changes in the genetic characteristics of the infectious agent, the course of the epidemic process, and the clinical manifestations of the infection.

The pathogenetic mechanisms of post-COVID syndrome presented in the monograph, in which thromboinflammation as a phenomenon of a systemic pathological process is a common denominator for damage to the pulmonary, cardiovascular, nervous, and other systems, allow us to form a comprehensive understanding of the cascade of pathological reactions. The course of the infectious process is not limited to the acute phase: during the convalescence period of COVID-19, even after the elimination of the virus, prolonged immunothrombotic dysfunction may be observed, and hypercoagulation and platelet activation may develop. All these links in a single pathological chain have a mutually reinforcing effect on each other, creating a kind of "vicious circle," which is clinically manifested in the development of a symptom complex of post-COVID syndrome.

Based on this, it became possible to create algorithms for the differential diagnosis of post-COVID syndrome, including assessment of the functional state of various organs, monitoring of markers of endothelial dysfunction, hypercoagulation, and immune inflammation. This paves the way for personalized medicine, where the choice of therapeutic and rehabilitation strategies is based on a deep scientific understanding of the mechanisms of the pathological process in a particular patient.

An important aspect presented in the monograph is the significance of early diagnosis of post-COVID syndrome in therapeutic practice, where this state is often observed in patients with chronic non-infectious diseases, in whom the course of infection is often accompanied by risks of adverse outcomes. A systematic approach to assessing the development of the pandemic against the backdrop of a steady increase in the number of patients with chronic non-infectious diseases allows us to distinguish the separate concept of "syndemic," which contributes to the optimization of the healthcare system during the pandemic and reduces the risk of serious consequences of the infection for both the individual patient and the country as a whole.

The main pathological process in COVID-19 is damage to lung tissue, changes in which persist during the convalescence period. Issues important for practical healthcare concerning the differential diagnosis of interstitial lung damage of infectious and non-infectious origin, manifested by similar clinical pictures, as well as the peculiarities of the infectious process of COVID-19 in patients with interstitial lung diseases are presented in detail in the monograph.

The book provides data on the neurological consequences of the novel coronavirus infection, which include a whole range of syndromes: cerebrovascular pathology, demyelinating and neurodegenerative disorders. The authors' detailed analysis of the neurological manifestations of post-COVID syndrome and the data obtained on the pathogenesis of neurological disorders in coronavirus infection help to overcome the difficulties of diagnosis and the development of patient management tactics, enabling practitioners to prescribe effective treatment in a timely manner.

A past coronavirus infection has a negative impact on the course of a patient's existing cardiovascular disease. The approaches to organizing dispensary observation and rehabilitation measures for patients with chronic cardiovascular pathology presented in the monograph help prevent the progression of this pathology in patients after a coronavirus infection.

The book also describes the characteristics of endocrine pathology development in the post-COVID period, demonstrating the risks of developing carbohydrate and mineral metabolism disorders, as well as thyroid pathology.

The monograph devotes special attention to the impact of post-COVID syndrome on women's health. The development of thromboinflammation, pathological immune responses, and endothelial dysfunction has a significant impact on the course of pregnancy. Knowledge of these pathological mechanisms allows for the timely identification of risk groups and opens up new horizons for the creation of personalized approaches to prevention and treatment aimed at reducing the risk of complications in obstetric practice.

In conclusion, it is possible to state with confidence that overcoming the long-term consequences of the COVID-19 pandemic is one of the most significant challenges facing healthcare and medical science as a whole. Success in this area will be determined by a comprehensive approach to the study of post-COVID syndrome, aimed at early diagnosis and prevention of pathological changes in patients who have had COVID-19.

This monograph presents the views of specialists from various fields and highlights the significance of the problem of post-COVID syndrome. Analysis of the current key

findings of research into this issue allows us to outline prospects for further scientific exploration, and this scientific work is intended to serve as a theoretical and practical foundation for future scientific research.

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