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Review

IMMUNE STATUS AND CYTOKINE SPECTRUM AS PREDICTORS OF THE RISK OF SEVERE DISEASE AND PERFORMANCE INDICATORS OF INTENSIVE THERAPY IN PATIENTS WITH CORONAVIRUS INFECTION COVID-19

V.F. Sadykov[1], R.A. Poltavtseva[1], A.V. Chaplygina[2], N.V. Bobkova[2]

[1]National Medical Research Center for Obstetrics, Gynecology, and Perinatology the name of Academician V.I. Kulakov, 4 Akademika Oparina Str., Moscow, 117997, Russian Federation
[2] Russian Academy of Sciences, Institute of Cell Biophysics – a Separate Division of Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences, 3 Institutskaya Str., Pushchino, Moscow region, 142290, Russian Federation

The pandemic caused by a new strain of the SARS-CoV-2 coronavirus has swept the whole world but effective methods for treating this severe pathology have not yet been created. It has now been established that a risk of a severe course of COVID-19 is not so much a patient's age itself, but so-called age-related diseases; the renin-angiotensin system (RAS) is directly or indirectly involved into their development. The SARS-CoV-19 virus interacts with one of the main regulatory elements of this system, ACE2, and disrupts the balance between the two RAS branches. This ultimately manifests itself in an increase in levels of angiotensin II, which, through binding to the angiotensin type 1 receptor (AT1R), causes a number of pathological conditions, including hypertension, atherosclerosis, and cardiovascular diseases, enhances cell proliferation, apoptosis, death of vascular endothelial cells, etc. This process has been described in many reviews by Russian and foreign authors [1, 2]. However, cells of innate and adaptive immunity are another less well-described but no less important target of angiotensin II. The consequences of this interaction are analyzed in detail in this review. With COVID-19, dendritic cells are activated, macrophage proliferation and neutrophil infiltration increase with further involvement of CD4-lymphocytes and other cellular elements of the adaptive immunity in this process. Hyperactivation of the immune system is accompanied with the release of a large amount of pro-inflammatory cytokines, which can lead to the occurrence of a cytokine storm. The picture is aggravated by the inhibitory effect produced by the virus itself on the synthesis of signaling interferons at initial stages in its internalization into the cell. A separate section in the review addresses the problem how to predict a risk of a developing serious condition and search for its predictors by analyzing the state of the RAS and ratios of key cellular elements in the immune system. This is extremely important for making decisions concerning the amount of necessary medical care and strategies for subsequent treatment.

Keywords: *COVID-19, SARS-CoV-2, cytokine profile, cytokine storm, immune cells, immunodysregulation, predicting factor, immune status, renin-angiotensin system (RAS).*

The SARS-CoV-2 is an RNA-containing coronavirus. It is highly contagious and is spread both by sick people and asymptomatic carriers with a positive PCR-test with their share varying between 15 and 25 % [3]. The spike (S) protein in the coronavirus allows it to bind to angiotensin-converting enzyme 2 (ACE2) on target cell surfaces in an area with protease activity. The virus then penetrates a cell by endocytosis [4]. To enter a cell, the SARS-CoV-2 virus also employs the host's type II transmembrane serine protease, which

Valentin F. Sadykov – anesthesiologist-resuscitator (e-mail: valentinsadykov@yandex.ru; tel.: +7 (910) 468-63-98; ORCID: http://orcid.org/0000-0002-3511-5292).

Rimma A. Poltavtseva – Candidate of Biological Sciences, Leading Researcher of the Laboratory for Clinical Immunology (e-mail: rimpol@mail.ru; tel.: +7 (916) 549-01-68; ORCID: http://orcid.org/0000-0001-8625-9205).

Alina V. Chaplygina – Junior Researcher at the Laboratory for Cellular Mechanisms of Memory Pathology (e-mail: shadowhao@yandex.ru; tel.: +7 (925) 927-63-14; ORCID: http://orcid.org/0000-0002-6351-1997).

Natalia V. Bobkova – Candidate of Biological Sciences, Head of the Laboratory for Cellular Mechanisms of Memory Pathology (e-mail: nbobkova@mail.ru; tel.: +7 (903) 184-52-77; ORCID: http://orcid.org/0000-0002-4114-687X).

acts as S-protein activation co-factor, as well as splitting ACE2 thereby facilitating the merger of virus cells with membranes. It is noteworthy that a receptor responsible for ACE2 binding to the coronavirus is a vital component in the renin-angiotensin system (RAS) functioning. This system plays the key role in maintaining proper homeostasis in the body through regulation of interactions between the cardiovascular and respiratory systems, water-salt and carbohydrate metabolism and blood pressure regulation [5]. The SARS-CoV-2 virus creates considerable imbalance in functioning of this vital system by interacting with the ACE2 receptor. We should not neglect the fact that the virus not only starts to reproduce actively after it has penetrated a cell but also produces certain effects on a response given by a cell to its invasion. By now, these two aspects in effects produced by the virus on the host body have been described in many reviews by Russian and foreign authors; however, there are hardly any works that concentrate on analyzing a close interrelation between these two systems. Therefore, the aim of this review was to analyze outcomes of the RAS impairments caused by the SARS-CoV-2 virus for the elements in the inborn and adaptive immunity as well as to estimate ability to predict a clinical course of the disease considering changes in them.

The RAS physiology and its relation with the immune system. In its normal conditions, the RAS supports proper homeostasis by regulating interactions between the cardiovascular and respiratory systems, water-salt and carbohydrate metabolism and blood pressure regulation [5].

The classic RAS consists of two branches. The first one includes angiotensin / renin / angiotensin I / angiotensin-converting enzyme (ACE) / angiotensin II (ANG II) and its receptors AT1R and AT2R. Interaction between ANG II and AT1R is accompanied with elevated blood pressure, vascular constriction and growing ADAM17 [6] activity. All this leads to occurring circulating TNF-a with subsequent induction of pro-inflammatory cytokines, more intense proliferation, infiltration and apoptosis [5, 7]. At present, it is a well-established fact that not a patient's age itself is a risk factor of severe COVID-19 but so-called age-related diseases that develop due to either direct or indirect involvement of the 1st RAS branch activation and high ANG II levels [8].

The second RAS branch has been discovered only recently. It includes ACE2/ANG 1-7/Mas receptor (Mas R) [9]. Its main function is to inhibit the 1st branch hyperactivity. ANG-(1–7) interacts with Mas receptor thereby inhibiting NF-κB pathway and consequently producing anti-inflammatory and anti-apoptotic effects [10] as well as activating the inborn immunity. ACE2 is a linking component in these two RAS branches and a key enzyme that converts ANG II (1-8) – ligand AT1R into ANG (1-7) with subsequent MasR activation [11]. Obviously, the virus induces a decline in ACE2 receptor density and this leads to imbalance between activity of two RAS branches together with a decrease in ANG 1-7 levels and elevated ANG II activity with all the following negative outcomes. This underlies a more severe clinical course of the disease in elderly people with concomitant pathologies who typically have initially lower ACE2 receptor expression [12]. Naturally, elevated ACE/ACE2 ratio is a predictor of developing acute respiratory distress syndrome that can be prevented by ANG 1-7 administration [13]. However, wide use of ANG 1-7 in clinical practice is limited due to its rapid degradation in the body and, probably, difficulties in delivering it to lung and brain tissues by intravenous introduction. Recently new data have become available that MasR can be activated not only by ANG 1-7 but also by Neuropeptide FF, Alamandine, Angiotensin III and IV as well as by Angioprotectin and other

MasR agonists. Their development is a promising trend in anti-COVID-19 therapy. At present, more than 7 compounds are being clinically tested, which either activate or enhance MasR expression, as well as agonists of this type of receptors [14–19]. As we can see from literature review, it is advisable to rely on using persistent ANG-1-7 analogues that activate MasR to correct any RAS impairments when treating patients with COVID-19 and other viral infections caused by viruses that use ACE2.

AT1R is localized on membranes of many cells. Cells that are included into the inborn and adaptive immunity are no exception. Figures 1 and 2 provide schemes that describe events happening in a patient infected with COVID-19 against elevated angiotensin II levels.

Not only dendritic cells become active, and macrophage proliferation and neutrophil infiltration occur in the inborn immunity. Another effect is intensified synthesis of pro-inflammatory cytokines against elevated sensitivity of membrane Toll-like receptors, subtypes 2, 4, 7 and 9, responsible for recognizing microbial and viral pathogens. Similar events happen to the adaptive immunity cells (Figure 2). T-helpers additionally activate elements in this system through releasing multiple pro-inflammatory cytokines and this happens against weaker inhibitory effects produced by T-regulatory cells and a decline in the released quantity of anti-inflammatory cytokine IL-10.

The SARS-CoV-2 virus was established to be able to penetrate the brain and interact with ACE2 receptors localized on astrocyte and microglia membranes [20] where multiple AT1R and TLRs receptors can also be found [21]. Figure 3 provides the scheme showing outcomes of interactions between the virus and ACE2 and TLR against elevated angiotensin II levels in the brain. Microglia acquires a pro-inflammatory phenotype corresponding to neural inflammation,

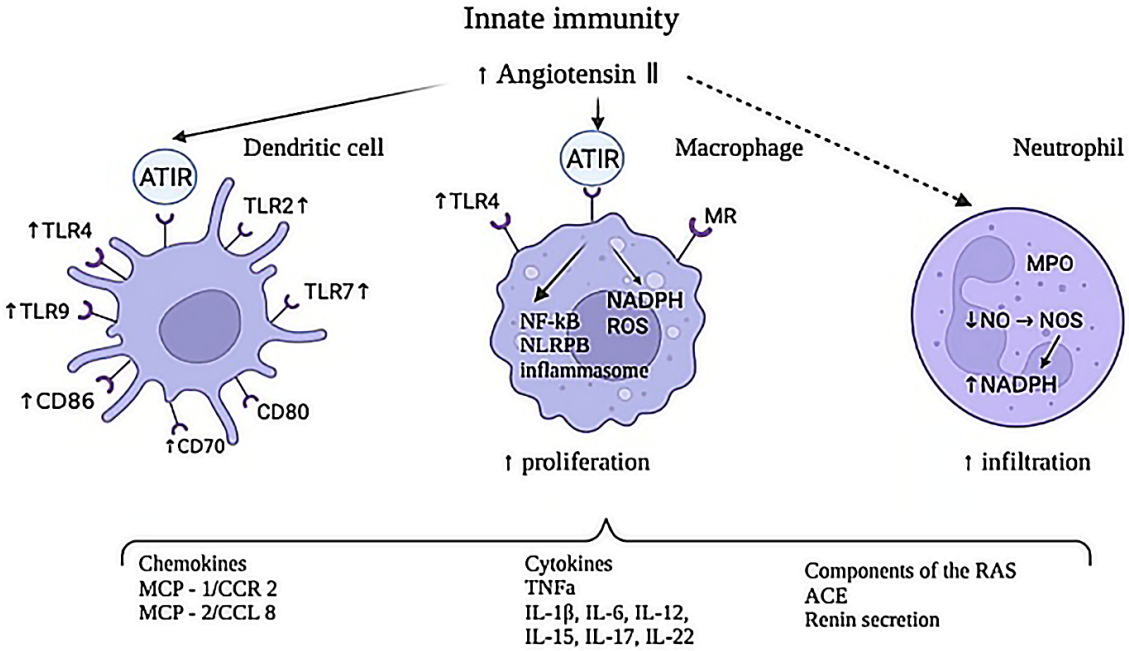

Figure 1. Angiotensin II excess influences inborn immunity cells through ATIR and TLR receptors. Dendritic cells become more active, macrophage proliferation and ROS and NF-kB production grow. Although neutrophils do not have a direct receptor for interaction, they still become able to infiltrate and increase NADPH production

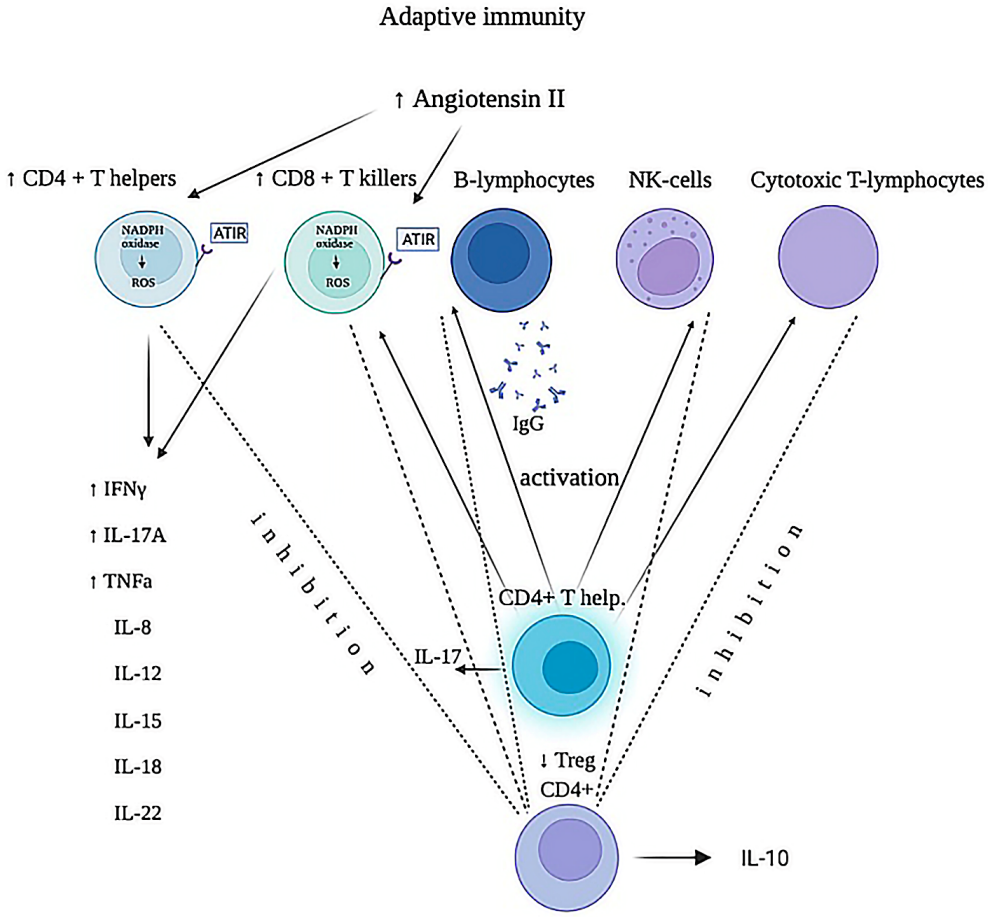

Figure 2. Angiotensin II excess activates CD4-lymphocytes, which induce the further reaction cascade. The quantity of regulatory T-cells with inhibitory effects goes down; as a result, all the cells become hyperactive and a cytokine storm occurs

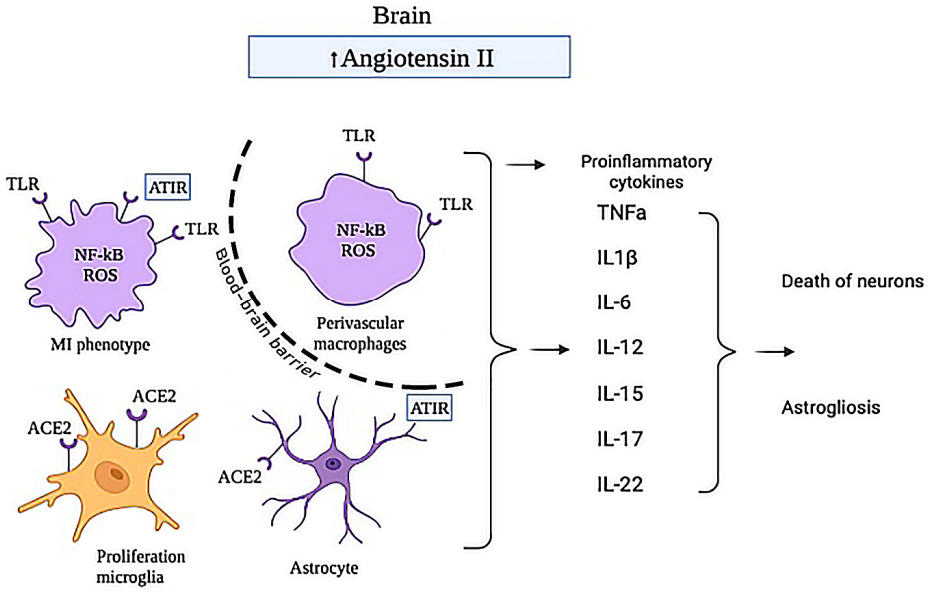

Figure 3. Angiotensin II accumulation in the brain in case ACE2-receptor is blocked by the SARS-CoV-2 virus produces adverse effects on AT1R and TLR receptors. This leads to pro-inflammatory way activation, formation of reactive oxygen species, synthesis of multiple pro-inflammatory cytokines and death of neurons against astrogliosis.

the blood-brain barrier becomes more permeable, neurons die and astrogliosis occurs. Macrophages in the vascular bed are activated and ROS and pro-inflammatory cytokines are released thereby making the blood-brain barrier more permeable and leading to death of neurons. In future, this will become obvious through varied neurological complications after recovery.

It is noteworthy that when soluble extramembrane sACE2 was used as a medication to bind the virus, there was no significant improvement in COVID-19 patients. This was probably due to formation of such immune complexes as 'recombinant sACE2+SARS-CoV-2', which induced an autoimmune response in patents with formation of antibodies not only against the virus but also against parts of the ACE and ACE2 receptors with subsequent damage to all organs and tissues where these receptors were expressed. This only exacerbated the disease [22].

How the immune response to the SARS-COV-2 infection is impaired. Superficially, the SARS-CoV-2 interactions with the host body hardly seem different from other viruses: first, the virus binds to endosomal Toll-like receptors, 3rd and 7th types, and cytoplasmic RNA-receptors. Then a cascade of inborn immunity reactions is induced, NF-κB and IRF pathways are activated, interferons, IL-1 and IL-6 are produced, the adaptive cellular immunity and, consequently, the humoral immunity are activated. However, the SARS-CoV-2 virus induces an inflammatory cascade without letting the body to form a proper immune response; this is accompanied with intensive release of pro-inflammatory cytokines thereby exacerbating inflammatory reactions and leading to massive lesions in various tissues done by immune complexes.

If we analyze the issue in greater detail, we can see that the coronavirus is able to activate synthesis of alpha- and gamma-interferons in the host cells; the latter can interact with a regulatory site located in the *Ace2* gene promoter. This leads to intensified ACE2 synthesis thereby prolonging a situation when the virus is likely to spread further in an infected body [23]. Therefore, when the interferon system activated by the virus is overloaded with immune stimulators, this may lead to an inverse effect; namely, induce a 'cytokine storm' and the necessity to use immune suppressors [24] including antibodies to pro-inflammatory cytokines or their receptors, such as IL-6 and tumor necrosis factor alpha (TNF-α) or their receptors. It is assumed that a 'cytokine storm' develops due to step-by-step involvement of some intracellular pathways in the host body. Virus RNA that enters a cell is recognized by host endosomal RNA receptors, TLR3 and TLR7, as well as by RIG-I/MDA5, cytosolic RNA sensor. This activates NF-κB and IRF3 pathways together with translocation of these transcription factors into the nucleus and stimulation of type I interferon expression (alpha- and gamma-interferons) with their subsequent release into the extracellular space. Interferons interact with IFNAR membrane receptors and activate the JAK-STAT system. Next, there is phosphorilation of STAT1 and STAT2 proteins that penetrate the nucleus due to forming unified complexes with IRF9. There they stimulate certain genes (ISGs) with the IRSE element located on their promoter. This regulates the response intensity. Therefore, the coronavirus disrupts formation of protection responses rather crudely against the background interferon synthesis and at the stage when CTAT1 phosphorilation occurs. Initially delayed ISGs stimulation is then realized through hyperactivity of pro-inflammatory cytokine expression [24]. Bearing in mind that patients infected with the new COVID-19 infection typically have their immune system reacting too strongly to it, we can assume that the immune status of a given patient plays a significant role in making early predictions of how severe the disease will be.

Complications and predictors of severe COVID-19. The clinical picture of COVID-19 patients varies from mild to severe; the latter variant is becoming prevalent steadily all over the world together with a growing number of deaths due to the infection. Given that, there is an acute need to have reliable predictors of severe COVID-19 [25]. Having analyzed reviews on relevant predictors, we concluded there were at least 49 variables that would provide valuable predictive information about mortality due to COVID-19 among infected patients or its severe clinical course. These identified variables include socio-demographic indicators, data from medical histories on concomitant diseases, results of a physical examination, laboratory and X-ray data. It is necessary to select the most informative factors at the early stage in order to identify what treatment procedures would be the most effective [26].

Elevated IL-6, IL-10 and C-reactive protein (CRP) levels as well as a lower lymphocyte quantity are the earliest predictors of the disease exacerbation in COVID-19 patients [27]. Thus, it was established that the acute respiratory distress syndrome (ARDS) developed in 3–4 % of patients with bilateral pneumonia; the syndrome was combined with thrombotic coagulopathy in ¾ of such patients. In terminal cases, these complications would lead to thrombosis and a 'cytokine storm' caused by systemic release of pro-inflammatory cytokines such as interleukins IL-2, IL-6, IL-7, granulocyte colony-stimulating factor, C-X-C motif chemokine 10 (CXCL10), chemokine (C-C motif) ligand 2 (CCL2) and tumor necrosis factor alpha (TNF-a). The process is accompanied with leukopenia that indicates the cellular immunity is weak [28].

At present there are no doubts that there is an unbreakable internal link between the immunity and cytokine secretion; their high levels against lower lymphocyte populations were associated with elevated risks of death due to COVID-19. A prediction model was created based on IL-8 levels and quantities of CD4 + T-cells and NK-cells. It yielded good results in predicting death of COVID-19 patients. When a threshold equal to 0.075 was used, the model sensitivity and specificity amounted to 90.20 % and 90.26 %. Neutrophils, IL-6, $CD3,^{-}CD56$, $CD16^{-}$ cells and leucocytes were the most powerful among all the factors in severe cases whereas CD, CD56, $CD16^{+}$ cells, $PD\text{-}1^{+}$ NK-cells, NK-cells, $CD4^{+}/CD8^{+}$ and perforin were the first five variables in milder ones. At the same time, neutrophils made the greatest contribution to this model and this is in line with a stronger inflammatory response in patients with severe COVID-19. Besides, measuring of NK-cell subsets proved to be useful at an early stage in hospitalization for identifying how the disease would develop [29]. Dynamic monitoring of cytokines and lymphocyte subpopulations potentially provides some useful data for more effective management of the disease. Large-scale meta-analyses identified a negative correlation between the declining cellular immunity and growing levels of pro-inflammatory cytokines for predicting transitions from a mild clinical course to a more severe one [28]. In this case, there is a signifiacnt drop in quantities of lymphocytes, monocytes, CD4 + T-cells, CD8 + T-cells, CD3-cells, CD19 cells and natural killers (NK) as well as an increase in interleukin-2 (IL-2), IL-2R, IL-4, IL-6, IL-8, IL-10, TNF-α and gamma-interferon levels in peripheral blood (INF-γ) [30]. Growing plasmin and plasminogen concentrations in blood are also biomarkers of elevated susceptibility to SARS-CoV-2 since plasmin can cut the relevant site in the S-protein of SARS-CoV-2 thereby increasing its virulence [31]. Growing neutrophils to lymphocytes ratios (NLR) and neutrophils to CD8 + T-cells (N8R) ratios are a powerful predictor of severe COVID-19 [32].

As it was stated above, pathological hyperactivity of the immunity is among key signs of the severe clinical course in COVID-19 patients. The central role in this state belongs to neutrophil activation. Proteomic profiling of plasma in cross-sectional and longitudinal cohorts of hospitalized COVID-19 patients revealed a prominent signature of neutrophil activation, including resistin, lipocalin-2, hepatocyte growth factor, interleukin-8, and granulocyte colony-stimulating factor, which were the strongest predictors of critical illness. A most significant finding was the fact that the signature of neutrophil activation was elevated already on the first hospitalization day in those patients whose state was after exacerbated down to critical levels and they were to be moved to intensive care units. The researchers assumed that high G-CSF levels stimulated emergency granulopoiesis to increase neutrophil production and IL-8 (CXCL8) regulated neutrophil migration into the lungs and, possibly, other tissues. There neutrophils were activated and then they released RETN, LCN2, HGF, MMP8 and other proteins with anti-microbial and other inflammatory functions that also induced considerable side lesions in the lungs, vessels and other organs [33]. NK-cells and T-cells are known to play a vital role in the anti-viral immunity. Studies with participating COVID-19 patients revealed that that the quantity and frequency of $CD4^+$T-cells, $CD8^+$T-cells and NK T-cells was considerably lower in severe cases than in mild ones. Significantly elevated expression of PD-1 and CD244 on $CD8^+$T-cells indicates that T-cells are depleted in COVID-19 patients and there is lower CD27 expression on $CD8^+$T-lymphocytes in severe cases against mild ones. Therefore, it is quite effective to use immune cell profiles and cytokine profiles as risk predictors to identify critically ill patients.

It is extremely vital to be able to predict a risk of a critical condition since this enables making correct decisions on relevant scope of healthcare and future treatment strategies. The current research in the sphere concentrates on several predictors among clinical and laboratory data (4C Deterioration and 4C Mortality Score). However, it should be noted that covariance between 4C Deterioration and 4C Mortality predictions did not differ as per sex or ethnicity and did not get weaker in younger age groups [34]. A research team from the University of Arizona, Harvard University and The National Center for Biotechnology Information (USA) has developed a mathematical model that makes it possible to estimate a probability of a cytokine storm in COVID-19 patients depending on how intensively cytokine production is stimulated by immune cells; the model relies on using multiple indicators. Unfortunately, a probability that the acute respiratory distress syndrome would develop in a COVID-19 patient is estimated in some clinics relying on simpler indicators such as high fever, C-protein levels, lower leucocyte count, developing lesions in the lungs, and lower saturation. Research data indicate that estimation of the disease severity and predictions of a possible fatal outcome should always consider effects produced by immunogenetics of a given patient, in particular, sex-specific immune genetic differences. Thus, for example, at present there is solid evidence that AB0 locus in the HLA genes is associated with susceptibility to the disease and we should remember that these genes regulate expression of DNA sequences coding cytokines and chemokines [35]. In addition, it was established that elderly males were a population group with elevated risks of the severe disease, developing pneumonia with the acute respiratory distress syndrome and death. Age is a key factor in COVID-19 prevalence and mortality; therefore, it is important to get an insight into age-specific immune profiles of patients and the condition of the both RAS branches together with analyzing ACE/ACE2 ratios if we want to select relevant prevention and treatment

strategies. Thus, research results made it possible to identify the leading typical age-specific immune indicator associated with the disease severity, namely, certain circulating factors were determined including CXCL8, IL-10, IL-15, IL-27 and TNF-α. They all had a positive correlation with elderly age, longer hospitalization and a more severe clinical course of the disease [36].

Conclusion. We have analyzed multiple research articles that address variable impairments in the RAS and immune system functioning in COVID-19 patients. This allowed us to conclude there is a close interrelation between these two systems. ACE2 receptor is a binding element here and it plays the major role in the virus internalization in a cell. Elevated angiotensin II levels due to ACE2 receptor blockade and interactions with AT1R receptors localized on several cellular elements in the inborn and adaptive immunity lead to the latter becoming hyper-activated. This is accompanied with synthesis and intense release of multiple variable pro-inflammatory chemokines and cytokines into the extracellular space thereby posing a threat of a developing cytokine storm. Obviously, SARS-CoV-2 has a peculiarity which is its ability to induce an inflammatory cascade before a proper protective immune response occurs due to delayed interferon synthesis. Our analysis of research articles on predictors of the severe disease gave an opportunity to make a certain conclusion that ACE/ACE2 levels ratio and the immune status of a given patient allow estimating a risk of a severe clinical course even at initial stages in the disease development. The closest attention should be paid to changes in neutrophils. Therefore, our analysis of all the materials outlined in this review is of great importance not only for fundamental medicine but also for practical one since it contains data necessary for practical estimation of the disease prognosis and selection of the optimal therapeutic approach.

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