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Research Article

Immunogenesis in patients with medium and severe coronavirus infection – dynamics in different age groups

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Abstract

The results of a one-year prospective study, during which the process of immunogenesis in patients over 18 years of age with moderate and severe coronavirus infection was monitored and analyzed in clinical and paraclinical (clinical laboratory) aspects, are summarized and presented.

The study included 2683 patients, all treated in the Clinic of Internal Diseases at the University Multiprofile Hospital for Active Treatment and Emergency Medicine "N. I. Pirogov" EAD, Sofia for the period from April 2020 to December 2020. Patients were followed for one year after recovering from moderate to severe coronavirus infection. Patients are grouped into four age categories as follows: 18–45 years; 46–65 years; 66–80 years and over 80 years.

The results of our study show that during the study period in 97% of patients the level of anti-SARS-CoV2, rose and in the remaining three percent it was flat or followed by subsequent waning (in less than 1% of patients), but does not reach critically low levels (i. e. below the positivity conditional threshold). The level of IgG reached a peak and then waned, but on the other hand, as mentioned above, the amount of Ig-Total tested shows a significant increase. This trend is observed in all age groups, with a difference in the level of IgG and Ig-Total depending on age.

The results of the additional screening in the target period in terms of virulence and virus segregation, categorically rule out the suspicion of the presence of "silent spreader". During the follow-up period, no patients were re-hospitalized due to recurrence of Coronavirus infection (re-infection and illness).

Keywords

COVID-19, anti-SARS-CoV2, anti-SARS-CoV2-IgG, immunogenesis, vaccination, vaccine, age, waning antibody levels

Introduction

Over the last 20 years, SARS-CoV, MERS-CoV and SARS-CoV-2 coronavirus infections, which have become more common in the human population, pose a threat to public health, as they can lead to the development of severe acute respiratory syndrome (SARS), (Santiesteban-Lores et al. 2021). "Coronavirus disease 2019" (COVID-2019) is caused by a new strain of the human population of the Coronavirus family, known as SARS-CoV-2), identified in Wuhan, China (Chen et al. 2020; Xu X et al. 2020). The spread of the disease was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (Astuti and Ysrafil 2020). Until October 10, 2021 the number of infected is 238 378 962, as all deaths in the world are 4 863 187, the number of survivors is 215 508 640. In Bulgaria so far confirmed cases with Covid 19 are 520 241, the number of deaths is 21 616 and 448 224 are survivors (https://apps.who.int/gb/COVID-19/pdf_files/2021/18_02/EPI-WIN.pdf).

SARS-CoV-2 infections result in highly heterogeneous clinical outcomes, ranging from the absence of any symptoms to severe disease and death (Jagannathan and Wang 2021). The incubation period of the disease was from 1 to 12 days with a median of 4 days (Peng et al. 2003).

The first report of a new type of upper respiratory disease among chickens in North Dakota (USA) dates back to 1931 (Schalk and Hawn 1931). The causative agent was recognized as a virus in 1933 and together with the disease were recognized as unique, completely different from any previously known viral disease. The causative agent of the disease is gaining popularity within scientific circles as "infectious bronchitis virus" (IBV), and after the isolation of the pathogen in later years, it is called "avian coronavirus" (Boursnell et al. 1987; Tyrrell and Fielder 2002; Lalchhandama 2020).

In 1947, a new brain disease in mice, murine encephalomyelitis, was described at Harvard Medical School in Boston (Theiler 1937; Ceever and Daniels 1949; Boursnell et al. 1987). Three years later, a "new" mouse hepatitis with an etiological cause called "mouse hepatitis virus" (MHV) was reported. (Mc Intosh 1974; Fehr and Perlman 2015). In the following years, more and more information about the Coronavirus family is accumulating, mainly in the field of veterinary science and practice.

For the first time, Coronaviridae strains causing SARS in humans ("human coronaviruses") were isolated and described by English scientists Tyrrell and Bynoe in 1965. In a study of ARI students in 1960, the authors adapted the putative pathogen into epithelium. cells of organ culture from the trachea of human embryos. One year later, Hamre and Procknow cultivated a pathogenic strain of primary cell cultures from a human embryo. From then until now, these "human" coronaviruses have been monitored and studied. Since then, there has been a great deal of systematic scientific information (rigorous scientific information) on morphology, antigenic structure, resistance, cultivation, pathogenicity to experimental animals (and possibly humans), clinic, pathogenesis (Fehr and Perlman 2015; Zheng et al. 2020), epidemiology (Peng et al. 2003; Kampf 2021), microbiological diagnosis (Guo et al. 2020), immunogenesis (Lau et al. 2006; Sloots et al. 2006; Poland et al. 2020; Favresse et al. 2021; Henss et al. 2021), immunoprophylaxis and immunotherapy in diseases caused by these strains (Boursnell et al. 1987; Lalchhandama 2020).

Human coronaviruses are from family Coronaviridae (Payne 2017) and imclude: NL63 (HCoV-NL63) (Huynh et al. 2012; Kahn and McIntosh 2005), OC43 (HCoV-OC43), 229E (HCoV-229E) (Lau et al. 2006), HKU1 (CoV-HKU1) (Esper et al. 2005, 2006; Kahn and McIntosh 2005; Lau et al. 2006; Sloots et al. 2006; Vabret et al. 2006). Severe acute respiratory syndrome (SARS)-like CoV was identified in the Chinese horseshoe bat (Rhinolophidae) in 2005 This is the evidence supporting a zoonotic origin of human coronavirus strain NL63 (Huynh et al. 2012). HCoV-HKU1 circulates in the United States, and the strain identified in New Haven is similar to the original strain described from Hong Kong (Esper et al. 2006).

Nevertheless, the new strain of SARS-CoV-2 has become, intentionally or unintentionally, an enigma that has instilled much fear in society and worldwide. This fear logically reflects in the organization of the diagnosis and treatment of the "new" old disease, as well as in the antiepidemic measures, often organized spontaneously and applied inconsistently in most countries, even worldwide. But the most disastrous result of all, that is happening is that a biological phenomenon is becoming an instrument for trampling on ancient human rights, and the main responsibility in this sense lies with the ruling elite.

Nearly 2 years after the beginning of the fight against modern coronavirus infection, it became clear, that the attention of specialists should be focused on understanding the immune response to the causative agent, the mechanism of generated immunity, it's duration and intensity. Monitoring the immune response in patients with Covid-19 is a key point not only for a better understanding of the disease, but also for assessing all possibilities for controlling it's pandemic course. Monitoring of naturally acquired immunity is essential to refine the indications for vaccination and to compare the postvaccination immune response.

A number of scientists, directly and/or indirectly monitoring the course of the pandemic, believe that the ability to quickly control and control it is hampered by a lack of detailed knowledge of SARS-CoV-2 / host interactions, mainly a lack of in-depth knowledge of viral biochemistry, viral morphology, and host immune response (Boechat et al. 2021).

Characteristic of the morphology of SARS-CoV-2 are four major structural proteins - spike glycoprotein (S), envelope (E), membrane glycoprotein (M) and nucleocapsid glycoprotein (N), as well as several additional proteins. Spike glycoprotein (S) is a transmembrane protein with a molecular weight of about 150 kDa, found on the outer side of the virus. The S protein forms homotrimers that protrude into the viral surface and mediates the binding of viral adhesive structures to host cells, involving

angiotensin-converting enzyme 2 (ACE2), expressed in lower respiratory tract cells. This glycoprotein is lysed by the furin-like protease of the host cell of 2 subunits – S1 and S2. Oligomer S1 is responsible for determining viral range for host target cells as well as the strength of cellular tropism in the receptor binding domain, while S2 mediates "viral penetration", i. e. the transmission of viral information to the cellular replication process (Astuti and Ysrafil 2020).

The N protein is the major protein represented in the nucleocapsid. It is the structural component of SARS-CoV-2. It is involved in the processes multiplying the viral genome, the viral replication cycle and the cellular response of host cells to viral infection (Schoeman and Fielding (2019). The N protein is thought to be the "culprit" for increasing the affinity of viral RNA for nonviral RNA (Fehr and Perlman 2015).

Another important structural and biochemical component of this virus is the membrane protein (M), which is the best structured protein, plays a key role in determining the shape of the viral envelope. This protein can bind to all other structural proteins.

The last component is a representative of the shell - the so-called protein, which is the smallest protein in the structure of SARS-CoV. It plays a role in the copying and biochemical maturation of the virus (Tai et al 2020).

Human angiotensin converting enzyme 2 (ACE2) is the receptor for SARS-CoV-2. Upon penetration into the host cell, SARS-CoV2 binds to the recipient's ACE2. The enzyme is strongly expressed in target cells of the nasal epithelium (Sungnak et al. 2020), the lower respiratory tract, cells in the upper esophagus, enterocytes from the ileum and colon, cholangiocytes, myocardial cells, cells of the proximal tubules of the kidneys and urothelial cells of the bladder (Xu H et al. 2020). Therefore, patients who are infected with this virus not only experience respiratory problems such as pneumonia leading to acute respiratory distress syndrome (ARDS), but also suffer from disorders of cardiac, biliary-hepatic, renal function, and digestive tract function (Astuti and Ysrafil 2020).

The entry of coronavirus into host cells is mediated by the transmembrane spike glycoprotein, which forms homotrimers exiting the viral surface. The S protein is represented by two functional subunits responsible for binding to the host cell receptor (S1 and S2). SARS-CoV-2 and some other members of the SARS family (SARSr-CoV) interact directly with angiotensin converting enzyme 2 (ACE2) upon entry into target cells. Because glycoprotein S is superficially exposed and mediates host cell entry, it is a major target of neutralizing antibodies (Abs) in infection and is the focus of the immunomodulatory design of most vaccines currently developed (Walls et al. 2020).

Immunity is a complex, dynamic functional system, that plays a major role in maintaining homeostasis. The immune system itself functions on the basis of a precisely balanced dynamic balance. The essence of human immunity is to protect the body (human body) from infections, but with this basic biological task it's functions are far from exhausted. From an anatomical and physiological point of view, human immunity can be classified as humoral (complement system, coagulation-fibrinolytic enzyme systems, soluble precipitating proteins, that recognize cell surface biochemical structures, interferons, chemokines, naturally produced circulating antibodies) and cellular immunity (NK cells, immunocompetent cells, cells representative of the monocyto-macrophage system). Defects (congenital and / or acquired) in this complex defense system mediate uncontrolled viral replication in the airways and the inability to respond adaptively. Severe forms of coronavirus infection are definitely due not only to direct viral effects on target tissues, but also to inadequate self-regulation of the immune response and the subsequent uncontrolled inflammatory process. Of interest in this regard are antiglycan antibodies. They are found naturally in serum, ie they exist in the absence of prior immunization, similar to naturally circulating ABO antibodies. They mainly belong to the IgM class. Physiological IgM concentrations appear to reflect some of the clinical severity patterns in COVID-19. They decrease significantly with age (> 40 years) and are found in lower concentrations in men (Boechat et al. 2021).

The complement system is another important functional unit of humoral immunity. It is activated during a viral infection and plays an important role in the effectiveness of innate and acquired immunity. A number of authors, emphasizing the protective functions of the complement, especially the C3 fraction, agree that it's role in coronavirus infections seems contradictory. For example, during SARS-CoV-2 infection, complement may control viral infection in asymptomatic patients or in those with mild severity. At the same time, it may exacerbate local and systemic damage in some patients with severe infection, due to it's potent proinflammatory effect. In such extreme conditions, activation of the complement system intensifies the cytokine storm and worsens the prognosis. Experimental treatment with complement inhibitors has been the subject of intensive research in search of promising adjunctive therapy in patients with severe COVID-19 (Santiesteban-Lores et al. 2021).

The interferon (IFN) response is also a key element in humoral antiviral protection. Up-to-date data suggest possible dysregulation of IFN type I and type III in SARS-CoV-2 infection (Deng et al. 2020). Non negligible stidies report impared response of IFN type I in patients with severe or critical SARS-CoV-2 infection, accompanied by high viral load and uncontrolled inflammatory response generated by NF-κB (Lu et al 2011). This type of immune response is associated with elevated levels of tumor necrosis factor (TNF) and interleukin (IL)-6 (Hadjadj et al. 2020). Recent analyzes of results from animal studies of SARS-CoV-2 infection suggest that IFNs type I and III contribute to limiting local (type III) and systemic (type I) viral spread (Heymann and Shindo 2020).

IL-1, as part of humoral immunity, is another important cytokine associated with inflammatory reactions in the human body. It is secreted mainly by activated mononuclear phagocytes and may induce other cytokines such as IL-6 and TNF. IL-1 activated by SARS-CoV-2 stimulates the secretion of IL-6 and TNF in a complex that

can cause a cytokine storm with fatal lung and systemic effects (Conti et al. 2020; Boechat et al. 2021).

Experimental data confirm the active involvement of cytokines in the pathogenesis of COVID-19. SARS-CoV-2 infection induces a number of other chemokines, such as CXCL2 (GRO) and CXCL8 (IL-8), whose secretion is also responsible for the granulocyte cell population. This hypothesis is consistent with the characteristic peripheral neutrophilia observed in patients with severe COVID-19 infection (Chen et al. 2020). Neutrophilia (granulocytosis) is a poor prognostic sign and the ratio of neutrophils to lymphocytes is an independent risk factor for severe disease, which is confirmed by our observations (Henry et al. 2021).

Since SARS-CoV-2 is a new strain of the human population, specific antibodies to it's S glycoprotein are not detected in the early stages of infection: before generating an adaptive immune response (Okba et al. 2020). Most serological studies to date have analyzed the acute stages of infection and the results show, that IgM antibodies appear between days 8 and 12 and disappear by week 12. Usually after the third month their levels tend to zero. Immunoglobulin G (IgG) antibodies to the spike protein appear later – around the 14th day and persist for a long time: over 6 months The intensity of the IgG response appears to be related to both viral load and clinical course severity (Guo et al. 2020). The degree and duration of protection due to this immune response are subject to continuous monitoring and subsequent correct analysis.

Recent findings raise concerns that humoral immunity to SARS-CoV2 may not be long-lasting in people who have had mild illness (Joffe AR 2021). According to our data, this statement is also speculative at the current stage of monitoring. A number of studies have shown that severe COVID-19 is characterized by intense proliferation of metabolically hyperactive plasmoblasts (PBs) and a relative reduction in specialized B-lymphocytes. These changes coincide with the severity of inflammation and slowly reduce the entry of convalescence (De Biasi et al. 2020; Tay et al. 2020; Kuri-Cervantes et al. 2021).

The T-cell response is a key component of the adaptive immune response to viral infections (Tay et al. 2020). Intense reactions have been observed in individuals recovering from severe COVID-19, and lower-intensity reactions have been observed in patients with mild disease. A similar cellular reaction (lower intensity) has been observed even in family members exposed to the virus, sometimes in the absence of anti-SARSCoV-2 specific antibodies. In the acute phase of the disease, SARS-CoV-2-specific T cells show a highly activated cytotoxic phenotype, while cells in the various stages of the convalescent phase are multifunctional and show a phenotype characteristic of cellular representatives of immune memory (Boechat et al. 2021).

Human ACE2 is the receptor for SARS-CoV-2. The expression of ACE2 in the bronchial and nasal epithelium is localized mainly in the goblet and mucociliary cells (Xu et al. 2020). Angiotensin-converting enzyme 2 acts as a binding site or receptor for viral spikes (S-proteins S) present on the outer surfaces of beta-coronaviruses. The immune response (congenital and / or acquired) is activated by SARS-CoV-2. and are activated immune memory factors as T cells (Kuri-Cervantes et al. 2021): CD4^{+} T cells, and CD8^{+} T cells and memory B cells (Dan et al. 2021; Lee and Oh 2021). The CD4 + fraction of T lymphocytes stimulates B cells to produce specific antiviral antibodies, including class G and M immunoglobulins. The CD8 + fraction of T lymphocytes has a direct cytotoxic effect on virus-contaminated cells (Boechat et al. 2021). T-helpers produce cytokines, mediating inflammation to mediate the physiological activity of other immunocompetent cells. On the other hand, SARS-CoV-2 can block the immune defense of the host by suppressing T cell function by inadequately triggering cellular apoptosis. The adequate immune response is actually the result of the balanced function of cellular factors, complement fractions of complement factors C3a and C5a and antibodies. Only a stable immune system, functioning in the conditions of dynamic balance, is a guarantee for the effective fight of the organism against the viral infection (Dan et al. 2021).

As the pandemic caused by SARS-CoV-2 so as the appearance of other dangerous pathogens, representatives of the "viral kingdom", highlighted the need for functional analysis of antibodies to monitor and responsibly analyze humoral immunity over time. Antibodies directed against the SARS-CoV-2 spike (S) protein are an important component in assessing the immune response to coronavirus infection. Namely this immune response is still the subject of debates sometimes bordering with dishonesty and professional nihilism (Niu et al. 2018).

Specific anti-SARS-CoV2 IgM, IgA and IgG have been identified in almost all patients with Coronavirus infection at different times since infection, suggesting that the antibodies mediate protective immunity to SARS-CoV-2 (Chen et al. 2020; Conti et al. 2020).

The overall kinetics of the antibody response to SARS-CoV-2 is similar to that of SARS-CoV-1, characterized by expressed seroconversion (IgM / IgG) 7–14 days after the onset of symptoms and antibody concentrations persisting weeks to tens of months after infection (Henry et al. 2020), A prospective study evaluating the kinetics of glycoprotein-specific antibodies in patients with COVID-19 found that IgA antibodies were produced early (in the first week) and peak at day 20–22, while IgM antibodies reached high titers at 10–12th day, which subsequently decreases, usually 18 days after the onset of symptom (Sariol et al. 2021). Our results show that at the end of the third month after illness, IgM titers tend to zero.

One of the earliest studies to characterize humoral responses to SARS-CoV-2 infection used viral material based on HIV-1 virions pseudotyped with the SARS-CoV-2 spike protein (SARS-CoV-2 pseudovirus). The aim was to evaluate the response of human antibodies to SARS-CoV-2 in 149 individuals recovering from COVID-19 of varying severity. Plasma neutralization of SARS-CoV-2 pseudovirus by convalescent patients with COVID-19 infection collected on average 39 days after the onset of symptoms has variable half-maximal neutralizing

titers (NT50) (Sariol et al. 2021). It should be noted that these are conclusions made on the basis of analysis of results obtained from in vitro laboratory methods.

Understanding immune memory to SARS-CoV-2 is crucial for improving the diagnosis and treatment of infection, but this understanding plays a key role in disease prevention, ie the development of effective vaccines. The clear idea of the intensity and duration of naturally acquired immunity (after illness) is the key to accurately assessing and predicting the likely future course of the COVID-19 pandemic. Referring to a comprehensive analysis, a number of authors report that the titer of anti-Spike-protein-specific IgG is relatively stable within 6 months. Spike-specific B cells were more abundant at 6 months than at 1 month after onset of symptoms. SARS-CoV-2-specific CD4 + T cells and CD8 + T cells decrease with a half-life of 3–5 months. A number of studies have shown that B-cell, CD4 + T-cell and CD8 + T-cell populations, as separate components of SARS-CoV-2 immune memory, show different kinetics (Sariol et al. 2021).

The severity of COVID-19 increases with age and male patients face more serious problems during the course of the disease compared to female patients. The severity of the disease is related to the amount of anti-SARS-CoV-2-specific IgG and IgA, as well as the neutralizing activity of the antibodies. The amount of anti-SARS-CoV-2-specific IgG decreases over time after permanent negation of PCR samples in specific patients, as the antibodies directed against the nucleoprotein decreasing more rapidly than antibodies directed against the spike protein (Henns et al. 2021) Especially for the treatment with antibodies and vaccine development, the humoral immunity against SARS-CoV-2 has been extensively studied, although there are still many unknowns and expectedly conflicting data (Lee and Oh 2021).

In SARS-CoV-2 infection, as in a number of other infections, the human body responds by activating cellular and humoral immunity. A major component of this immune response is the production of specific antibodies, which are also a major presentor of so-called humoral immunity. The antibodies produced provide protection against future infections by the specific pathogen that provoked the immune response and persist in the human body for a period of time – from months to years, and sometimes for life after the infection. Part of these antibodies can block the entry of the virus into cells and its replication. These antibodies are called neutralizers. The presence of these antibodies can be determined now by a number of tests, and developments in this direction aim to create an accurate quantitative methodology for determining the titer of virus-neutralizing (Dan et al. 2021).

After the administration of a single dose of vaccine in previously infected persons with Covid 19, the humoral response against SARS-CoV-2 is greater with significantly higher neutralizing antibody titer, than the lower response with lower neutralizing antibody titer after administration of a second dose of vaccine in previously uninfected people (Anichini et al. 2021; Gazit et al. 2021). In SARS-CoV-2 infection, the majority of infected individuals develop robust and long-lasting T cell immunity (Jagannathan and Wang 2021). SARS-CoV-2-specific $CD4^+$ T cells and $CD8^+$ T cells declined with a half-life of 3 to 5 months. Spike-specific memory B cells are more abundant at 6 months than at 1 month after symptom onset (Dan et al. 2021). High COVID-19 vaccination rates were expected to reduce transmission of SARS-CoV-2 in populations by reducing the number of possible sources for transmission (Kampf 2021).

Materials and methods

In our study were used:

1. Chemiluminescent Immune Assay (CLIA) for the quantification of specific anti-S1 and anti-S2 antibodies of the IgG class to SARS-CoV-2 in samples of human blood serum or blood plasma. The test is designed to help diagnose COVID-19 and study the condition of the affected patient's immune system, providing an indication of the presence of neutralizing IgG antibodies against SARS-CoV-2.
2. Enzyme-linked immunosorbent assay (ELISA) for the quantification of the inhibitory activity on RBD-ACE2 binding of antibodies to SARS-CoV-2 in human plasma and serum. This test determines the percentage of antibodies that block the virus from binding to receptors and thus prevent it from entering host cells.

The study included 2683 patients, all treated in the Clinic of Internal Medicine of UMHAT "N. I. Pirogov "EAD, Sofia for the period from April 2020 to December 2020. The patients were followed for one year after suffering from moderate to severe coronavirus infection. Patients are grouped into four age categories as follows: 18–45 years; 46–65 years; 66–80 years and over 80 years (Table 1, Fig. 1).

Table 1. Follow-up of patients and their distribution by age.

Age	18–45	46–65	66–80	above 80
Number of the patients	643	1141	764	135

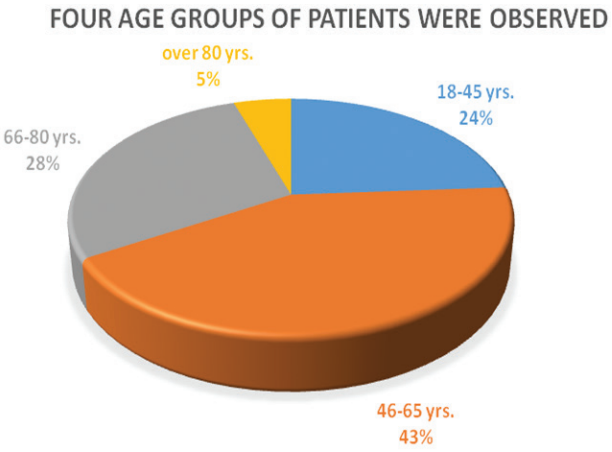

Figure 1. Distribution of patients by age.

Results and discussion

Serological tests of humoral immunity were performed and anti-SARS-CoV2-IgG and anti-SARS-CoV2-Total values were reported, respectively, in the first, sixth and twelfth months of the target period. Assumptions were made, based on the current immunological theory, as well as on the results, obtained from the methods used, for the share of anti-SARS-CoV2-IgM, anti-SARS-CoV2-IgA.

The dynamics of humoral immunity was observed in the target groups in the first, sixth and twelfth month after discharge.

The results of our study show, that within the study period in 97% of patients the total amount of anti-SARS-CoV2-AB increases, and in the remaining three percent it is flat or descending (less than 1%), but not enough critically low levels (ie below the minimum positive conditional unit). The amount of anti-SARS-CoV2-IgG decreases gradually (Figs 2, 3), but on the other hand, as mentioned above, the amount of anti-SARS-CoV2-Total tested, shows a significant increase (Figs 2, 4). This trend is observed in all age groups, with a difference in the absolute number of anti-SARS-CoV2-IgG and anti-SARS-CoV2-Total with age.

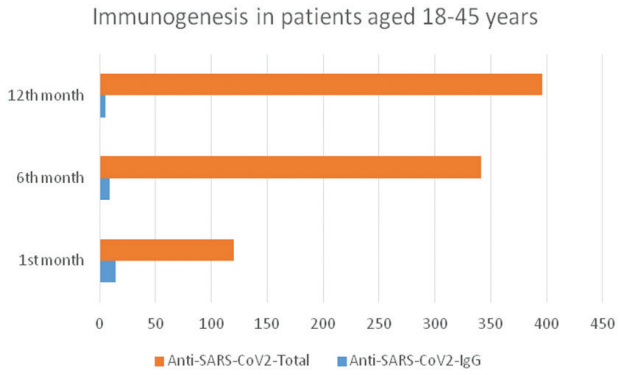

Figure 2. Immunogenesis in patients between 18 and 45 years of age for one year.

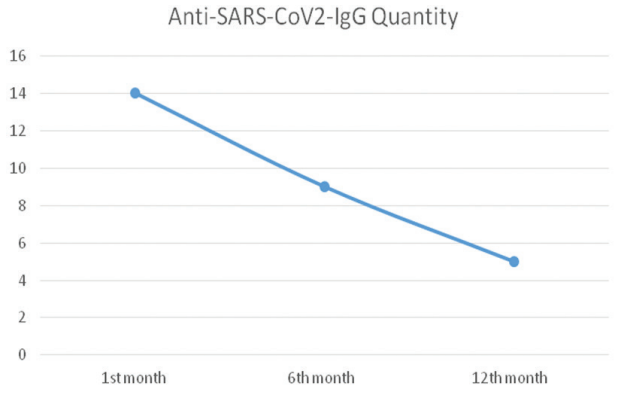

Figure 3. ANTI-SARS CoV2-lgG Quantity.

The difference between the 1st and 6th month is most pronounced both in the amount of anti-SARS-CoV2-IgG and in the amount of anti-SARS-CoV2-Total. Then there is the largest decrease in anti-SARS-CoV2-IgG and

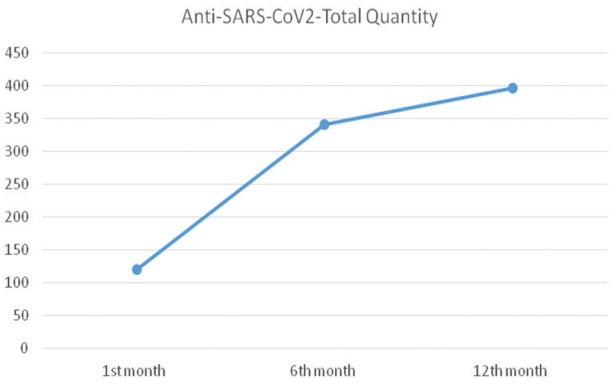

Figure 4. Anti-SARS-CoV2 – Total Quantity.

the largest increase in anti-SARS-CoV2-Total. During the study period, a decrease in anti-SARS-CoV2-IgG values of the order of 2.8 times was observed, and anti-SARS-CoV2-Total values increased 3.3 times in patients in this group.

The most numerous age group is 46–65 years. They demonstrate an identical course of immunogenesis. Lower values of anti-SARS-CoV2-IgG, and higher anti-SARS-CoV2-Total were registered in this group (Fig. 5), but the dependence was again inversely proportional.

The trend is similar in the other two age groups: 66–80 years (Fig. 6), and over 80 years (Fig. 7) –.again it should be noted that the group of patients over 80 years of age is the smallest. As the postmorbid period progresses, the total amount of anti-SARS-CoV2-Total increases and the levels of anti-SARS-CoV2-IgG decrease.

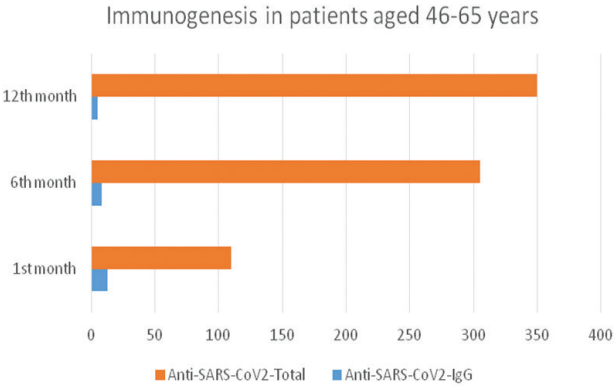

Figure 5. Immunogenesis in patients aged 46–65 years.

In the course of this study, all patients were monitored regularly (twice monthly) and for viral load, respectively, virus separation with RT PCR test for SARS-CoV2. The results of this additional study, aimed at analyzing RT PCR correlations in survivors of moderate and severe coronavirus infection, will be published in our next article, after the final processing of the findings. We can say with certainty, that none of our patients, who were regularly monitored for anti-SARS-CoV2-AB had a positive PCR test for coronavirus and no causal symptoms were reported during the target period. None of the patients were rehospitalized for recurrence of Coronavirus infection.

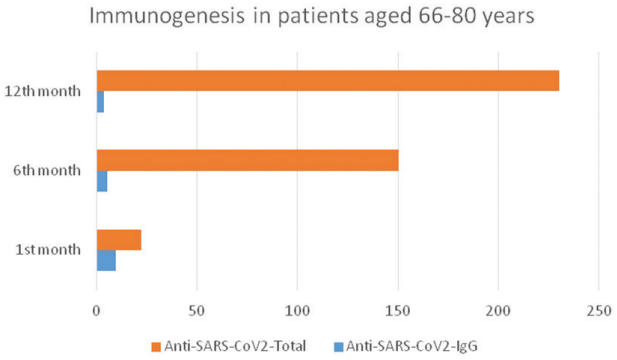

Figure 6. Immunogenesis in patients aged 66–80 years.

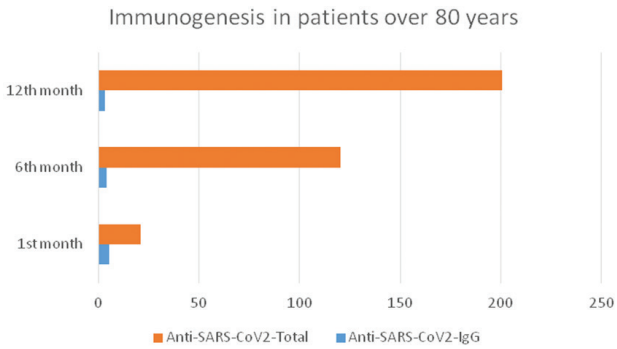

Figure 7. Immunogenesis in patients aged over 80 years.

Conclusions

The conclusions we can draw from the results, obtained in this way, are that the presence of antibodies of class anti-SARS-CoV2-IgG and anti-SARS-CoV2-Total are a sufficient means of protection against re-infection and hospitalization of patients. It is worth noting, that the total fraction (anti-SARS-CoV2-Total) certainly includes anti-SARS-CoV2- IgA, IgG, negligible amounts of IgM – in the early recovery period, and probably also negligible amounts of specific IgE and IgD. In our opinion, the remainder of the total immunoglobulin fraction (anti-SARS-CoV2-Total), after deducting the anti-SARS-CoV2-IgG fraction, is mainly at the expense of anti-SARS-CoV2-IgA. IgA antibodies are likely to provide both basic protection against re-infection and elimination of carrier of infection/spread of infection in the environment. We should not overlook the fact, which it actually is the "gateway" to infection, as well as the main anatomical area, from which the pathogen is released into the environment. The airborne route of transmission is proven to be the main mode of infection. The share of the surface contact path of infection is significantly smaller. Targeted studies on the dynamics of specific IgA in patients with moderate to severe coronavirus infection are also needed to clarify the assumptions discussed above. The results we publish, of course, are only for a period of one year after the illness, but they are a sufficient indicator of the important role of naturally acquired humoral immunity, which in the focus of mass vaccination seems to be deliberately neglected. It is logical to keep in mind, that one of the main approaches to controlling the infection and it's management in a global aspect lies in the long-term follow-up of patients, especially the group with moderate to severe disease, because at this time, they are the most reliable sources for the consequences of Covid 19, for the clinical course of the disease and for immunogenesis, which in turn is the main defense against re-infection. The disease provides the body with natural immunity, sufficient to prevent reinfection. This fact seriously raises the question of whether vaccination is necessary for convalescent patients who have suffered from severe and moderate forms of Covid 19, as well as vaccination of the target group of patients within the first year after the disease.

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