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Research Article

Immune response – genesis, duration, and strength in patients with moderate and severe coronavirus infection of different age groups

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Abstract

Acquired (adaptive) immunity is a major factor determining effective immune response against a few infectious diseases.

The immune response during recovery from COVID-19 is complex, involving both cellular and humoral adaptive immunity.

The purpose of the study is to determine the intensity and effectiveness of the immune response at the end of the second year after discharge from the hospital in patients who have suffered from moderate and severe forms of coronavirus infection. A study among 2683 patients who suffered from moderately severe and severe coronavirus SARS-CoV2 infection with recorded complications which have not received a vaccine against SARS-nCoV-2 was performed. In the studied group of patients there were no deaths. In the whole cohort, the share of underlying prehospital comorbidity was also analyzed. The immune response induced because of moderate and severe infection with COVID-19 could serve as source of protection from recurrent severe infection for patents of different ages with various comorbidities.

Keywords

Immunity, duration, severity, COVID-19, anti-SARS-CoV-2 IgG, anti-SARS-CoV-2 IgTotal, immunogenesis, vaccination, booster doses, comorbidity, age, relapse, essential oils, vector of infection

Introduction

The human immune system is essentially an extraordinary achievement of Evolution. It is a complex biological system that functions on the basis of dynamic equilibrium and self-control. The continuous and complex interaction of a number of specific factors determines its effectiveness. These factors are the result of the functioning of a complex physiological mechanism made up of cells, tissues, mediators, cytokines, membrane receptors and molecules, which together represent the overall picture of immune homeostasis, constantly responding to all changes related to age, diseases, gender and a number of external effects on the human body.

Scientists investigate in depth all manifestations of the human immune response in a number of conditions, seeking answers to important questions related not only to the treatment, but also to the prevention of a wide range of diseases. This knowledge is constantly filling in missing pieces of the

large and complex puzzle of immunity. For example, cells of acquired immunity are thought to be unique „memory" cells, until the discovery of „immunocompetent" cells of innate immunity, similar to cytokine-secreting T cells (Varadé et al. 2021). The last decade has been characterized by the rapid development of new techniques and methods for studying the immune system, with exceptional precision. The ability to study „up close" and in detail the immune response to a number of vaccines, oncological diseases, as well as viral infections such as COVID-19, is due to high-throughput „omics" technologies that calculate the influence of genes, mRNA (transcriptomics), proteins (proteomics), cells (mass spectrometry) and epigenetic modifications (ATAC-sequencing). The processing of the data from these studies is carried out on the basis of specific analyses (Pulendran and Davis 2020). The human immune system is a complex syncytium between the two main functional units of the immune response – innate (non-adaptive) and acquired (adaptive) immunity. These structural units of the immune system ensure the body's protection against pathogens recognized by it as foreign structures for the specific homeostasis. They react adequately to any „encroachment" on the human body in absolute interdependence and in interaction with the existing structural (anatomical) and biochemical barriers in the human body. An adequate immune system unites four main principles: 1) the ability to detect and fight against an infection; 2) the ability to recognize its own cells as „its own" and thereby protect them from harm; 3) memory of previous infections and previous encounter with specific pathogens; 4) ability to limit the immune response once the pathogen has been eliminated. These „functional" principles on which the essence of the adequately functioning immune system and the physiologically functioning network of interacting organs, tissues and cells are based, provide an environment of adequate protection of the body**.** In turn, a dysregulated immune response to various stimuli could be self-destructive for the individual (Lentz and Feezor 2003). Innate (non-adaptive) immunity is the basic immunological mechanism to fight against various pathogens. Innate immunity is a rapid immune response initiated minutes to hours after the „attack" on the body. Innate immunity is devoid of mechanisms for building immune memory. (Turvey and Broide 2010) Acquired (adaptive) immunity, on the other hand, is antigen-dependent and antigen-specific. Its outstanding property is immune memory. This phenomenon of „brandishing the foreign" allows the body to react faster and more adequately in the event of a subsequent encounter with the same or similar antigen (Bonilla and Oettgen 2010).

A dynamic balance and synergistic interaction exists between the two main components of the immune system. Possible defects both in innate immunity and in the mechanisms of acquired immunity can provoke an inadequate immune response with the clinical manifestation of autoimmune diseases, immunodeficiency states, reactions of hypersensitivity, uncontrollable immune response (cytokine storm) (Marshall et al. 2018).

Innate immunity could be considered as including four main types of protective barriers – anatomical (skin and mucous membranes), physiological (body temperature, low pH, chemical mediators..), cytolytic and phagocytic, and inflammatory. The functional systems that are part of the anatomical barriers responsible for the effectiveness of innate immunity, as well as the protective processes within this framework, have been well studied and described over the years. The innate immune response is the result of the function of so-called specific receptors – PRRs (pattern recognition receptors), which allow a limited number of immune cells to recognize and react to a wide range of antigens that have similar structures – for example, PAMPs (pathogen associated molecular patterns).

Examples of similar structures that are components of the bacterial cell wall such as protein structures, lipopolysaccharides, double-stranded RNAs are synthesized by target cells during various viral infections. An important function of innate immunity is the rapid cell migration to the site of infection (doorway, target cells and tissues) and the subsequent inflammatory response generated by the production of cytokines and chemokines. The innate immune response is realized through the interaction of a large number of cells – macrophages, neutrophils, activated phagocytes, mast cells, basophils, eosinophils, NK-cells, etc. (Marshall et al. 2018). Acquired immunity is the basis of effective immunization against a number of infectious diseases. Cells of the acquired immune response are antigen-specific T-cells that are activated and directed to proliferate by antigen-presenting cells, as well as B-cells specialized in differentiated antibody production. (Bonilla and Oettgen 2010) The purpose of the immunological memory is to protect the macroorganism from reinfection, to control a persistent one, including through the maternal antibodies (the mechanism of passive immunity) to protect the immunologically immature organism of the newborn from primary infections (Welsh et al. 2004). How long this memory could be maintained, in the absence of reinfection, is the subject of ongoing debate. Recent studies of the immunity created after smallpox vaccination demonstrate that T-cell memory declines steadily with a half-life of 815 years, while antiviral antibodies in survivors remain stable for more than 75 years (Slifka 2004).

Antibodies play a key role in limiting viral proliferation during viral infection. However, they are not capable of eliminating completely the virus after the onset of infection. When an infection has already occurred, cell-mediated mechanisms are of greatest importance for the body's defense against most intracellular pathogens. (Schroeder and Cavacini 2010) Cell-mediated immunity targets both those microorganisms that survive in phagocytizing cells and those that infect other cells. This type of immune response is effective in eliminating virus-infected and neoplastic cells, but also participates in the immune defense against fungi, protozoa, intracellularly developing bacteria (Marshall et al. 2018).

Respiratory viral infections are often the cause of serious diseases of varying severity – from mild and medium-severe infections of the upper respiratory tract to severe bronchitis and pneumonia, which evolve into chronic obstructive pulmonary disease. Common viral infections caused by influenza, respiratory syncytial virus, rhinoviruses and corona viruses under certain conditions cause significantly increased morbidity and mortality. The lungs are exposed

to the action of external foreign agents continuously and, as a target of respiratory viruses, have extremely precisely built mechanisms for antiviral protection, including a complex network of interactions between acquired and innate immunity. Immediately after infection, a wide range of proinflammatory cytokines, chemokines and interferons generate an inflammatory immune response that could be seen as a "double-edged sword" – on the one hand, it aims to completely eliminate the viral pathogen, but on the other hand, a prolonged and violent response to the infection can lead to a chronic course of the inflammatory process, complicate the course of the respective disease and generate severe damage in the target organs and functional systems.

The course of inflammatory diseases of the respiratory system is largely determined by the cells of innate and acquired immunity, as well as by the cells of the respiratory tract, which have the general task of dealing with the control of infection and creating immune memory at the local and systemic levels. This immune memory is key to preventing reinfection. This immune memory is extremely important for the control of the disease, given the many mechanisms of its complication, for example, in a setting predisposing to bacterial superinfection (Reijnders et al. 2021). For most viral diseases, there are currently no effective antiviral medicines, nor effective vaccines. This is also true for most respiratory viruses. (Kikkert 2020) This fact determines the importance of attempts to thoroughly understand the interaction between the macroorganism and the pathogen virus, the development of an immune response, the duration of immune memory, as this would contribute to optimizing strategies for the effective treatment and limitation of a number of viral diseases (Asha et al. 2021).

The effective etiological treatment of most acute viral diseases, including the „new" coronavirus disease, is reduced to the application of monoclonal antibodies, which in the conditions of modern medicine are the means of choice and applied in the indicated periods of rounding, lead to an extremely good result.

There are still many unclear details regarding the duration of protective immunity after exposure to most respiratory viruses. This immunity is implemented by different mechanisms, but neutralizing antibodies provide optimal protection against acute infections, also mediating vaccination immunity (Plotkin 2020), especially if they are presented at the local (mucosal) level (IgA antibodies for example). When most seasonal respiratory viral infections are relapsed, the body produces neutralizing antibodies that protect against recurrent diseases (Fujimoto et al. 2012).

Generated immunity depends on a number of factors – severity of the disease, comorbidity of the patient, and there are differences in the effectiveness of immunity after re-infection and after vaccination. For example, with the influenza virus, it was found that a year after relapse, patients with a severe course of the disease maintain high levels of humoral immunity as well as the presence of a T-LyCD4+ cell population, in contrast to patients with a mild and medium-severe form of the disease, as well as the vaccinated, in which cellular immunity prevails, as evidenced by the high levels of TCD4+ cells activated by gamma interferon released during degranulation in the mucosal tissues (Bonduelle et al. 2014). Certain viral infections, such as influenza, generate the production of neutralizing antibodies and circulating specific B and T lymphocytes, mediating immune memory, which may persist for years. A number of other viruses, such as respiratory syncytial virus, do not generate robust, lasting immunity. Reinfections with this virus, which cannot be explained by antigenic variations, are most likely due to significantly weakening immunity over time. Nevertheless, subsequent reinfections with homologous viruses always occur relatively milder, which speaks of residual immunity against the respective group of viruses (Siggins et al. 2021). Even outside of pandemic conditions, respiratory viral diseases cause seasonal outbreaks with large numbers of cases. The role of induced population immunity is central for the control of these diseases, a fact that we have had to be reminded of during the COVID-19 pandemic.

The highly contagious, „new" corona virus – SARS-CoV-2, which causes a complicated moderate and severe acute respiratory disease „COVID-19", was the reason for the declaration of a pandemic. The scale of the infection seriously hampered the global health system and lead almost the entire a world to face a serious health, socio-economic and political crisis, and also brought negative impact on the life of the „ordinary taxpayer". A number of in-depth studies are aimed at revealing the close interactions between the immune system of the sick and the causative agent of COVID-19. When suffering from a corona virus infection, macrophages, antigen-presenting phagocytes, Nk- cells, CD8+ T-cells, Th1-, Th17-, Tfh-cells and effector B-cells are involved in the antiviral defense. In the case of dysfunction of the immune response, over-activation of the inflammatory process, development of lung damage (development of respiratory distress syndrome) and multiple organ failure are provoked (Zhu et al. 2022).

The viral genome of SARS-CoV-2 encodes four phenotypes (viral proteins): nucleocapsid – (N)protein surrounded by an envelope containing three membrane proteins as follows: membrane (M), envelope (E) and spike (spike S). In turn, the spike protein consists of two functional units – S1 and S2 (Gaebler et al. 2021). The receptor binding domain (RBD) of the S1 subunit of the viral S-protein interacts directly with the cellular angiotensin-converting enzyme 2 (ACE2) receptor, thereby mediating pathogen entry into the target cell (Hoffmann et al. 2020; Lan et al. 2020; Wrapp et al. 2020; Zhuang et al. 2021; Liu et al. 2022).

Virus-specific antibodies against the main viral immunogens S and N are found in patients who have recovered from COVID-19 infection. Although knowledge of the immune response to SARSCoV-2 virus infection is continuously increasing, the definition of protective immunity as well as the determination of target antibody titers against the virus remain a challenge (Hodgson et al. 2021). There is evidence of a positive correlation between available antibodies and their neutralizing activity against SARS-CoV-2, especially upon re-encounter with the virus (Uprichard et al. 2022). Specific immunoglobulins (IgG) directed against protein S, nucleoprotein N, and the receptor-binding do-

main RBD develop 6–15 days after the onset of symptoms (Grandjean et al. 2021). The speed of the immune response is different and correlates with the severity of the COVID-19 infection, but in most cases the body produces antibodies detected in serum (Long et al. 2020b) and saliva (Isho et al. 2020) up to 4 weeks after the development of infection. Neutralizing activity of the immune response was recorded in all convalescent patients, including asymptomatic children and adults (Siggins et al. 2021).

Assumptions about the lifetime of protective antibodies against SARS-CoV-2 virus can be based on studies of antibody titers against SARS-CoV-1, which was originally thought to have a relatively short half-life (Cao et al. 2007). It has been found that, despite the lack of re-exposure to the virus, in about 90% of individuals who survive SARS-CoV1 viral infection, neutralizing antibodies are detected up to 3 years after encountering the virus, as specific IgG antibodies, in some patients prove even in the thirteenth year after infection. And in this case, as with a number of other viral infections, there is a drop in antibodies in the first two years after an illness, and their stabilization in the following years (Siggins et al. 2021). Studies of patients with corona virus infections have demonstrated that adult volunteers who were followed up maintained high levels of antibodies that remained significantly elevated one year after infection (Chia et al. 2020). These facts also correlate with the results of our studies in this direction. In addition, there is evidence that any reinfection with the same coronavirus is milder or even asymptomatic, especially in elderly patients, which proves that functional immunity remains stable in the period between individual infections (Juno et al. 2020; Peng et al. 2020; Hicks et al. 2021).

A significant number of SARS-CoV-2 specific memory B cells showed a steady increase in the following months after infection, and were also recorded six months after illness, indicating that B cell immune memory to SARS-CoV-2 is likely continuous (Dan et al. 2021). Our experience strongly indicates that the patients followed in this study did not relapse within the two-year study period. Comparing the values of S- and RBD-specific memory B-cells, in patients hospitalized due to COVID-19 and those with a mild course of the disease, shows a significantly higher activity of the immune response in a severe course of the infection, which highlights the importance of viral load on the strength of the humoral immune response generated (Christian Gaebler et al. 2021).

Circulating CD4+ and CD8+ T cells against SARS-CoV-2 were observed in the majority of convalescent individuals, regardless of the severity of infection, and these cells persisted up to 8 months post-infection (Zuo et al. 2021). Most CD4+ T cells possess a typical antiviral TH1 phenotype or TFH phenotype (Rydyznski Moderbacher et al. 2020). The presence of TFH further enhances available T cells in their interaction with specific B-cells and differentiation in the germinal centers, so as to realize the production of potent and time-stable antibodies (Siggins et al. 2021).

Neutralizing antibody titers directly correlate with protection from SARS-CoV-2 virus and the development of a severe form of COVID-19 (Cromer et al. 2022), as direct evidence of this statement is the successfully applied treatment with monoclonal antibodies. Not only the humoral immune response is important for protection against the new coronavirus. Cellular immunity is also an extremely important factor. Determining the effectiveness of the cellular immune response is the functional activity of CD4+ CD8+ T-cells, especially in the vaccination immune response. Adequate cellular immune mechanisms guarantee effective protection against an unfavorable outcome of the disease.

With the emergence of constantly changing new variants of the SARS-CoV-2 virus that manage under certain conditions to evade neutralizing antibodies, the role of Tcells that are directed against the relevant epitopes should not be underestimated (Wherry and Barouch 2022). The interplay between cellular and humoral immune responses has been demonstrated by kinetic assays demonstrating rapid activation of CD4+ and CD8+ cell lines. This activation precedes the serological rise of neutralizing antibodies (Cromer et al. 2023). Long-term follow-up of S-specific T-cell populations generated by repeated vaccinations and re-encounters with the virus demonstrated the ability of these cells to realize their protective functions over a period of one to two years, without reducing the levels and specificity of their action (Koutsakos et al. 2023).

Research and comprehensive analysis of immunity against the SARS-CoV-2 virus is extremely important because of the much-sought answers to vital questions that have arisen in the conditions of an epidemic crisis. Understanding the degree and characteristics of protection against reinfections, against the development of severe forms of COVID19, the complications associated with the experience of moderate and severe forms of the disease and the eventual disability associated with the already accepted pathological phenomenon – „prolonged post-covid syndrome“ is a main goal of the current studies of the immune response in patients who survived coronavirus infection.

This knowledge is critical to solving important problems related to the risks of future epidemic outbreaks, including those caused by a new pathogen. This knowledge guarantees the correct planning, structuring and implementation of policies and restrictions related to migration processes in society, and at last but not least to provide patients with a real informed choice in the matter of vaccination and revaccination.

A number of studies have confirmed protection against reinfection after relapse, before the appearance of the omicron variant, as stable and sufficient, lasting at least 40 weeks after illness. Protection against omicron BA1 is considered to be much less reliable and short-lived. Regardless of the variant of the virus, it is absolutely certain that recovery protects against a severe course of COVID-19 (Chemaitelly et al. 2022).

Immunity after illness should be considered in relation to immunity generated after vaccination. The preparation of vaccination recommendations would be most adequate based on an assessment of the individual immune status, especially of health workers, as well as on an assessment of the collective immunity in different regions of the world.

Although post-infection immunity probably wanes over time, the level of protection of the "naturally" generated immune response against reinfection, development of symptomatic disease, and severe COVID-19 appears to be more durable and effective than the level of protection provided by of two doses of mRNA vaccines against the original, alpha, delta and omicron BA1 variants. This fact has been demonstrated by studies comparing naturally acquired immunity with that after vaccination. Such „natural" immunity lasts at least one year for the listed variants. (Pilz et al. 2022; Pulliam et al. 2022), and our experience shows that immunity acquired after relapse persists efficiently well into the second year after relapse.

Is this a proof that natural immunity is superior to vaccination or not? Does the immunization policy against SARS-CoV-2 hove to be re-evaluated and targeted at certain groups of people at high risk of severe COVID-19 infection, taking into account factors such as age, comorbidity of the patient, as well as epidemiological data on the prevailing virus variants causing severe types of the disease?

As already noted, the immune response during a relapse from COVID-19 is complex, including both functional units of the immune system – cellular and humoral immunity (Shrotri et al. 2021; Turner et al. 2021). It targets not only the spike protein antigens, but also other viral proteins, suggesting the presence of a stable immunity protecting against the different variants of SARSCoV-2 the virus, in the structuring of which mucosal immunity with its barrier function plays significant role (Le Bert et al. 2020; Gaebler et al. 2021). So-called „secretory" or mucosal immunity, in addition to a barrier function, also has an extremely important, perhaps main, function – anti-epidemic. Mucosal immunity is the factor determining the exclusion of the sick as a vector of infection.

There is a growing number of studies whose aim is to make an adequate assessment of immunity after recovery from COVID-19, its duration, the factors that influence its formation and its effectiveness. The main drawback of most studies in this direction is their relatively short duration – most cover a period of 1 year or 18 months. Data from a large-scale survey conducted in the Faroe Islands highlight interesting highlights on the most frequently asked questions. In this study, it was found that circulating antibodies, IgG-isotype, were registered in 94% of the relapsed patients up to 15 months after infection, and in 92% virus-neutralizing antibodies were also demonstrated.

It is characteristic of IgG antibodies that a biphasic curve is observed, with an initial decrease in titers followed by a stable plateau after about 7 months. Virus-neutralizing antibodies remained relatively stable throughout the period. The strength of the immune response generated by antibodies has been shown to be dependent on smoking and hospitalization of patients – lower levels of IgG antibodies in smokers compared to non-smokers, and hospitalized patients have higher levels of antibodies against COVID-19 compared with the non-hospitalized (slightly ill in an outpatient setting).

The results of these studies correlate with the data from our experience. A longer immune response is associated with male gender and older age, and in these groups of patients, higher antibody titers at the beginning of the immune response were found, but with a more significant decline in the curve for the studied period (Petersen et al. 2023). Data from studies over a longer period of time, although scarce, are quite promising – in pre-diseased patients, the generated humoral immunity could have a protective role against the delta variant of COVID-19 and its other variants, possibly with the exception of the omicron (BA.1, BA.2 and BA.4/5) variant, which could to some extent „bypass" this immunity (Wang et al. 2023).

Data from pandemic waves of the omicron variant in the year 2022 allow for the possibility of reinfection with the particular viral variant, with significant differences in the reporting of reinfection depending on the age groups, the location, the particular viral wave (Keeling 2023). Serious problems for clinicians in this period were caused by the use of „rapid antigen tests" of low quality, that yielded a positive result when reacting with carbonated drinks, coffee, citrus juices, including tap water. Unfortunately, such tests results are accepted for use for making the diagnosis of coronavirus infection in many countries, including Bulgaria. The paradoxical lack of response (regardless of a number of regularly sent reports about rapid antigen tests of inadequate quality) from the local health authorities deserves special attention.

Of note, studies of immunity against COVID-19 in healthcare workers, for whom all the factors for the creation of strained immunity are present (they inevitably encounter the virus much frequently, the viral load is high) are in favor of the stability of naturally built immunity and its duration. Santibodies are observed up to 200 days after infection in 95% of those studied, suggesting that antibodies will persist up to 465 days and longer after infection.

In a study of healthcare workers with positive serologic tests, none required hospitalization due to re-infection, moreover, higher antibody titers were found in the medics who were severely ill with COVID-19 and a longer immune response is expected (Grandjean et al. 2021). There are studies from the last two years (Juno et al. 2020; Peng et al. 2020; Hicks et al. 2021) that point to the possibility that previous contact with other types of corona viruses, to which pediatricians are particularly frequently exposed, may contribute to building lasting protection against the SARS-CoV-2 virus (Kissler et al. 2020).

A large body of recent evidence supports the fact that S- and RBD-antibodies with virus-neutralizing activity persisted in unaltered titer for at least 90–150 days after infection with SARS-CoV-2 virus (Iyer et al. 2020; Ripperger et al. 2020). The data reported so far contradict the initial assumptions of a number of studies (Crawford et al. 2020; Ibarrondo et al. 2020; Long et al. 2020a, b; Seow et al. 2020; Crawford et al. 2021) for a short-term immune response, as they demonstrated antibody losses within 3 months after infection. The new fact can be explained by relapse of a mild form of the disease, protective antibodies with different kinetics are found, even in asymptomatic or patients with a mild form of COVID-19 of different age groups. The immune protection lasts at least up to one year in children. (Di Chiara et al. 2022)

Whether any of the rapidly emerging vaccines against the new corona virus could provide competitive efficacy and duration of immunity against COVID-19, remains an expectation in the future. The assessment of the immune response against SARS-CoV-2 during vaccination is important not only for controlling the severity and spread of the disease, but also for determining target groups for vaccination, the need to administer so-called booster doses, to compare the vaccination and naturally acquired immunity. It can also resolve questions about the need of a mass vaccination and its significance to the maintenance and enhancement of immunity. Interesting facts are found in the comparison of patients vaccinated with two doses of mRNA vaccines (either BNT162b2, Pfizer–BioNTech or mRNA-1273, Moderna) who have recovered from COVID-19 with those who have not encountered the virus. The both groups were found to increase the antibody titer against the SARS-CoV-2 virus in response to the first vaccine dose. The group with a history of prior COVID-19 disease reached higher titer of antibodies against the spike protein and higher titer of neutralizing antibodies. No significant increase in antibody titers and neutralizing activity in response to a second dose of vaccine was found in patients who had previously suffered from corona virus infection (Uprichard et al. 2022). These results bring up the question of the need of booster dose for the patients with previous a corona virus infection to further strengthen their immunity. (Manisty et al. 2021; Prendecki et al. 2021; Saadat et al. 2021) Also, interesting results were obtained from the comparison of the dynamics of the IgG response of patients who recovered from COVID-19 and have received two doses of the BNT162b2 vaccine (Pfizer-BioNTech), with those who recovered from the infection but received no subsequent vaccination. Notably, the vaccine has a „booster" effect in those who have been sick and increased the levels of IgG antibodies 161 times, but this effect is relatively short-term. Conversely, the unvaccinated patients who have been sick have maintained an unaltered high level of antibodies for the entire period of the study (Dehgani-Mobaraki et al. 2023). It has been found that compared to older unvaccinated patients who survived COVID-19 infection (Chvatal-Medina et al. 2021; Cohen et al. 2021), patients over 50 years of age vaccinated following infection have lower titers of antibodies against the SARS-CoV-2 virus (Uprichard et al. 2022). AntiSARS-CoV-2 antibody titers measured by ELISA have been shown to correlate with virus neutralizing titers. (Amanat et al. 2020; Salazar et al. 2020) In summary, in accordance with a number of studies (Abu Jabal et al. 2021; Goel et al. 2021; Krammer et al. 2021; Stamatatos et al. 2021), the full course of vaccination of recovered COVID-19 patients does not contribute to a significant increase in both antibody titers and neutralizing activity. Then, do we need to vaccinate patients in the first six months after experiencing infection? Besides, the rate of recurrent infection and hospitalization in patients who recovered from COVID-19 was lower compared to that of patients who were vaccinated against SARS-CoV-2 virus. Our experience is in conformity with and support all the conclusions drawn by the aforementioned studies.

The vaccination of survivors of corona virus infection does not provide additional protection in the months after infection, while in the long term a single booster dose probably increases antiviral protection, or at least with respect to the development of symptomatic COVID-19 infection (Shrestha et al. 2022).

If antibodies produced as a result of corona virus infection or vaccination are maintained at high enough titers, they are expected to have a protective role and be a major factor in ending the pandemic and preventing future epidemic outbreaks. Provided the fact that vaccination is presumed to be the safer method of achieving herd immunity (Jones and Helmreich 2020; Rasmussen 2020), there is a growing need for additional data independent of vaccine manufacturers and additional independent studies on the development and the duration of immunity generated after SARS-CoV-2 vaccination.

The analysis of the entire „pandemic" period shows how serious a health problem of this type could be for Bulgarian and global healthcare. The SARS-CoV-2 virus is highly contagious and this is the main reason for the startling scale of this disease spread and the need for urgent measures to prove the causative agent, treat the infection and control the number of infected patients. The laboratory tests approved in Bulgaria for making the diagnosis COVID-19 infection are the rapid antigen tests and RT PCR, with the number of patients proven with a positive RT PCR test for SARS-CoV-2 as of June 14, 2023 being 1,299,024. Through „COVID", the unit of University Emergency Hospital „Pirogov", during this period 10,083 patients have been examined and diagnosed with COVID-19 infection. Of these, 9,184 patients were admitted with diagnosis moderate or severe type of coronavirus infection. This high rate of moderately severe and severe type of the disease in patients in our hospital, is explained both by the specifics of the work, namely the emergency admission and it is a tertiary care hospital.

Material and method

The patients included in this study were 2,683 and represented 88.81% of the total of 3,021 patients hospitalized for the target period. Each had a confirmed RT PCR for SARS-CoV-2 in naso/oropharyngeal swab material and serum. All patients were hospitalized and treated in the Covid ward of the Clinic of Internal Diseases of the University Emergency Hospital „Pirogov", Sofia April 1, 2020 to December 31, 2020. All patients were admitted with diagnosis moderate and severe corona virus infection and were followed up for two years after the hospital discharge. The patients treated in the Covid ward are over 18 years old, and they are grouped into four age groups, as follows: from 18 to 45 years; from 46 to 65 years; from 66 to 80 years and over 80 years.

The degree of severity of the corona virus infection is determined: according to combined assessment computed tomography score (CT-score); clinical and laboratory markers of inflammatory activity; presence of organs

insufficiency and comorbidities worsening the course of viral pneumonia.

CT-score a method based on the percentage involvement of the lung parenchyma at CT study. The method was widely used during the Covid pandemia to quantify the affected lung volume (Francone et al. 2020; Fu et al. 2020; Yang et al. 2020; Abdel-Tawab et al. 2021). Patients with lung parenchymal involvement below 25% were only 8.3%. In 34% of the patients 26–50% involvement of the lung parenchyma was detected; approximately 45% have lung parenchymal involvement 51–75%. Patients with CT evidence of atypical viral pneumonia with more than 75% lung involvement were about 12.7% (Fig. 1). CT scan of a patient with mild CT-score (<25%) pulmonary parenchymal involvment (Fig. 2). CT scan of a patient with moderate CT-score (Fig. 3) (26%–50%) pulmonary parenchymal involvement. CT scan of a patient with severe CT-score 4) (51%–75%) pulmonary parenchymal involvement (Fig. 4). CT scan of a patient with very severe CT-score 5) (>75%) pulmonary parenchymal involvement (Fig. 5).

The patients included in the study were analyzed according to their co-morbidities (the cardiovascular, the pulmonary and the endocrine comorbidities convey the greatest risk of COVID-19 infection with complicated course). These results are presented in Fig. 6.

One or more than one cardiovascular disease was the most common comorbidity in patients admitted with COVID-19 infection in this study. They affect 31% of patients. The proportion of patients with more than two accompanying diseases was 72% of all included in the study.

Table 1 shows the absolute number of patients included in the study, divided into three groups as follows: Patients without comorbidities, patients with one comorbidity, and patients with two or more comorbidities.

At each patient visit during the follow-up, a thorough clinical examination, RT PCR for SARSCoV-2 in naso/

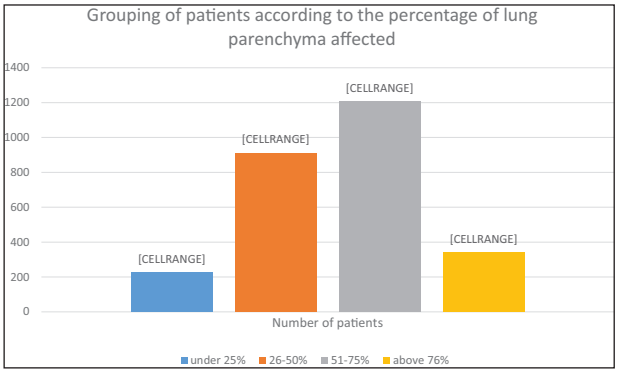

Figure 1. Analysis of the severity of lung involvement according (CT-score).

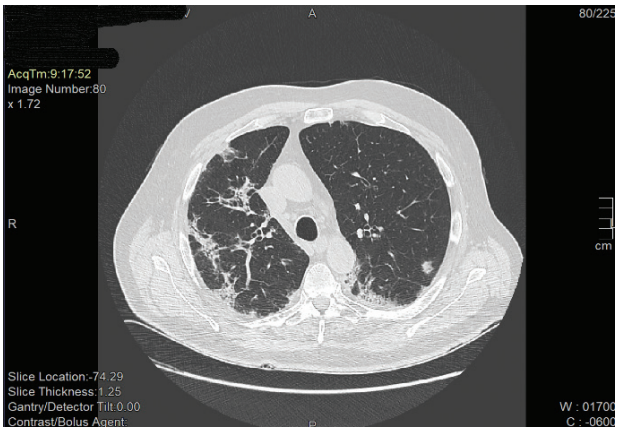

Figure 2. CT scan of a patient with mild CT-score (<25%) pulmonary parenchymal involvment.

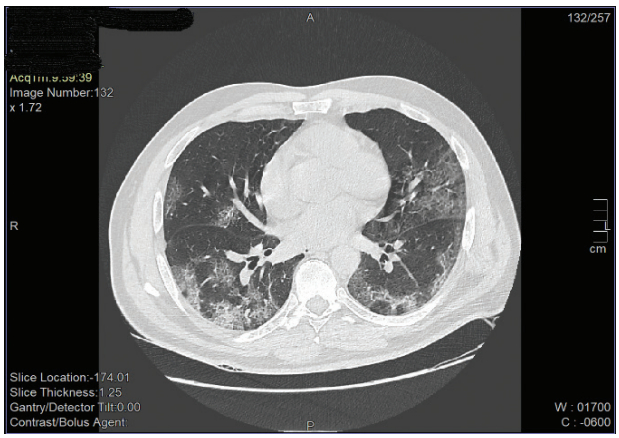

Figure 3. CT scan of a patient with moderate CT-score (26%–50%) pulmonary parenchymal involvement.

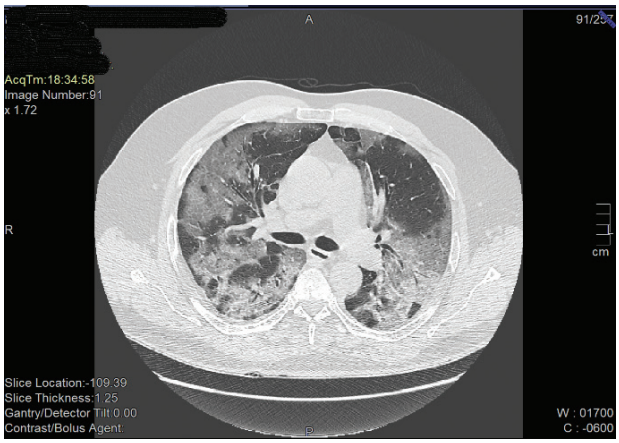

Figure 4. CT scan of a patient with very severe CT-score (51%–75%) pulmonary parenchymal involvement.

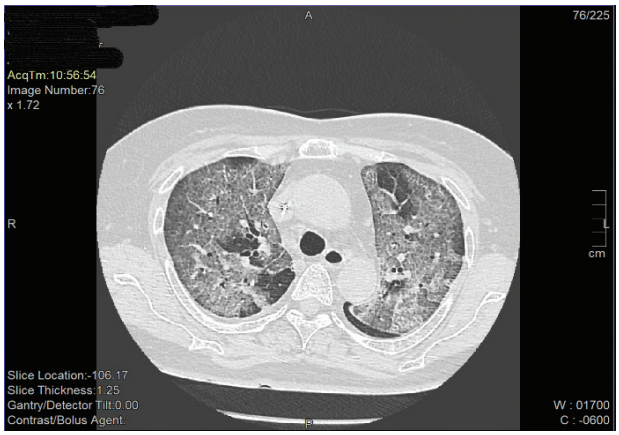

Figure 5. CT scan of a patient with very severe CT-score (>75%) pulmonary parenchymal involvement.

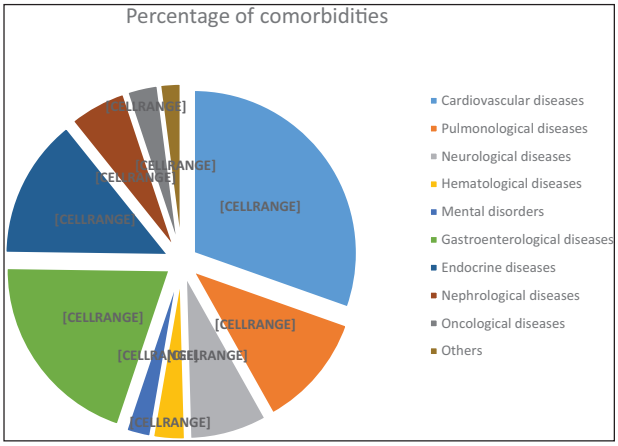

Figure 6. Co-morbid profile of the study group.

Table 1. Co-morbidity of the studied patients.

Comorbidity	N
No	184
One chronic disease	576
Two or more chronic diseases	1923

oropharyngeal swab material and in blood for detailed laboratory tests (peripheral blood with differential leukocyte count, biochemical tests including inflammatory markers, liver and cardiac enzymes, electrolytes, lipid profile, BUN, coagulogram, arterial blood gas) and imaging studies (radiography of lung or CT). In accordance with the obtained results, the need to continue outpatient therapy or hospital readmission was discussed.

Patients who, at the time of discharge, persist even with minimal changes in the control imaging study, remain on home therapy with essential oils and generally strengthening medications. The main class of essential oils that have been administered in therapy during the treatment of corona virus infection and as an ongoing outpatient therapy are eucalyptus, sweet orange, myrtle, lemon, broadleaf lavender. Serazyme, bromelain, quercetin are included in the composition of general strengthening medicines.

In the period of follow-up, the patients were examined with RT PCR, in the presence of symptoms characteristic of respiratory infection. None of these patients had a positive RT PCR for SARSCoV-2, respectively, no patient was readmitted with diagnosed corona virus infection. The humoral immunity in the different categories of patients was monitored at the first month, the sixth month, the first and the second year after the index event. The results of the research up to the first year have been published in our previous article. During the follow-up examination of the second year, after a relapse of corona virus infection, the values of anti-SARS-CoV2-IgG and anti-SARSCoV2-Total were reported.

The laboratory methods that have been used to determine the amount of antibodies are chemiluminescent immunoassay (CLIA) and enzyme-linked immunosorbent assay (ELISA). A chemiluminescent immunoassay (CLIA) is a method for the semiquantitative determination of specific anti-S1 and anti-S2 antibodies of the IgG class to SARS-CoV-2 in human blood serum or blood plasma samples. The test is designed to study the state of the patient's immune system, providing an indication of the presence of neutralizing antibodies of the IgG class against the SARS-Cov-2 virus. Enzyme immunoassay (ELISA) is a method for semiquantitative determination of the inhibitory activity of antibodies on the binding between the RBD-ACE2 receptor, in human plasma and serum.

An analysis in terms of the intensity and the duration of the immune response in the groups with different severity of the corona virus infection was performed. Patients are divided into four age groups, respectively 18–45 years, 46–65 years, 66–80 years and over 80 years of age.

Results

Complete resolution of changes in the lung parenchyma was observed in 89% of patients on control imaging after the first year. In the second year, this percentage is 93%. Comparison of the amount of anti-SARS-CoV-2 IgG and anti-SARS-CoV-2 IgTotal between the first and second year in the 18–45 age group showed a clear increase in anti-SARS-CoV-2 IgTotal by 1, 8 times compared to the studied values of the same in the first year after discharge (Fig. 7).

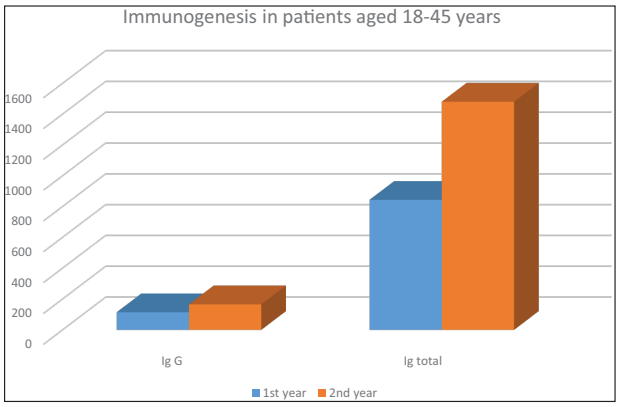

Figure 7. Ig G and Ig total at one year and at two year follow-up in the patients aged 18–45.

The largest group was the group that included patients from 46 to 65 years of age. In this group, the increase in the anti-SARS-CoV-2 IgTotal level at the end of the second year was 1.4 (Fig. 8).

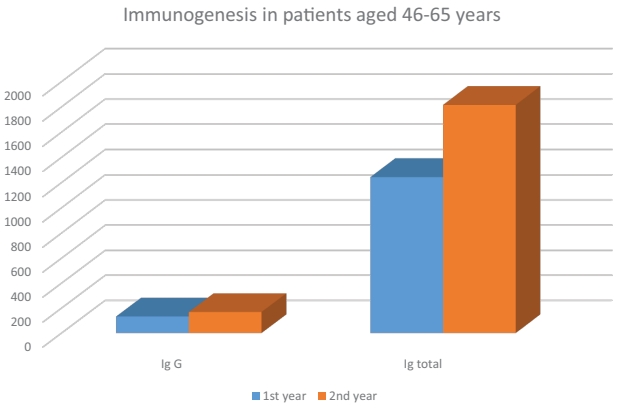

Figure 8. Ig G and Ig total at one year and at two year follow-up in the patients aged 46–65 years.

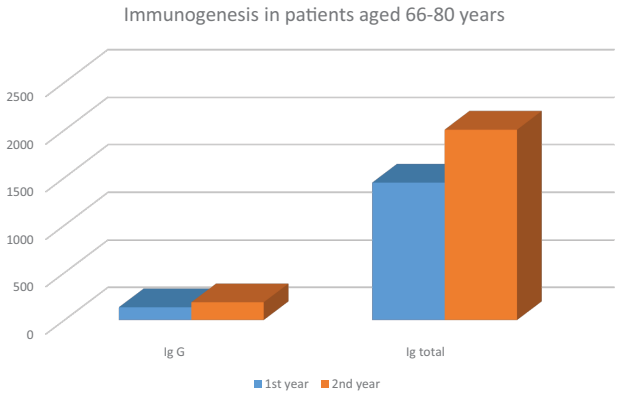

Figure 9. Ig G and Ig total at one year and at two year follow-up in the patients aged 66–80 years.

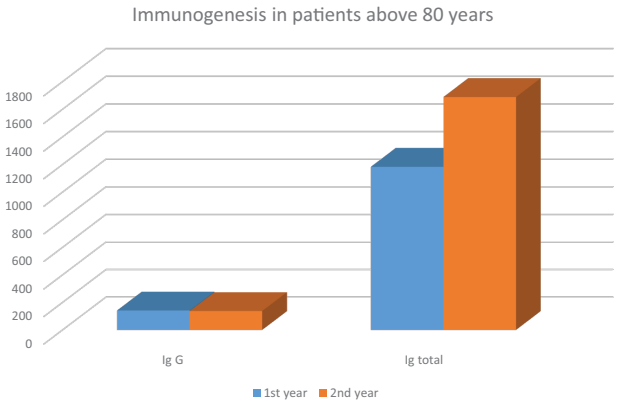

Figure 10. Ig G and Ig total at one year and at two year follow-up in the patients aged 80 years.

The tendency for an increase anti-SARS-CoV-2 IgTotal levels was also preserved in the rest two age groups (Figs 9, 10).

In the group over 80 years of age, 14 patients did not attend a follow-up examination at the end of the second year. When a personal check of the reasons for the non-appearance was made by phone, it was found that all 14 patients are alive and reported no acute or deteriorated chronic disease.

Regardless of a slight differences in the absolute values of anti-SARS-Cov-2-IgG and anti-SARSCov-2-IgTotal, a trend can be outlined. The IgG titers remain relatively unaltered at the end of the second year compared to the values in the first year. The titer of IgTotal continues to grow after the first year, albeit at a slower pace. This means that the immune response of the patients who survived a moderately severe or severe corona virus infection remains extremely strained even after the second year of the index infection.

Discussion and conclusions

The results presented show that there is a correlation of anti-SARS-CoV-2-IgG and anti-SARSCoV-2-IgTotal in patients in the first and second year after COVID infection of mild or moderate severity and confirm the hypothesis of the sufficient protective mechanism of immunity after COVID infection provided mainly at the expense of anti-SARS-CoV2 IgTotal.

A long-term population immunity against the SARS-CoV-2 virus is required for eradication or at least putting under control of the COVID-19 infection. Anti- SARS-CoV-2 spike-binding and neutralizing antibodies showed a biphasic decline curve with a half-life of about 200 days, suggesting generation from long-lived plasma cells. SARS-CoV-2 recurrent infection would likely act as a booster, increasing antibody titers against both SARS-CoV-1 and other common corona viruses. In addition, spike-specific IgG memory B-cells persist, ensuring a rapid humoral immune response upon repeat exposure to the virus or upon „consolidation“ vaccination. Virus-specific CD4+ and CD8+ T-cells are multifunctional and have a half-life of over 200 days. Interestingly, CD4+ T-cells respond equally to several SARS-CoV-2 viral proteins, while CD8+ T-cells have an affinity for nucleoprotein, which highlights the possibility of including the same in future proteinbased vaccines (Chvatal-Medina et al. 2021; Cohen et al. 2021). The role of mucosal immunity in the construction of the complex immune defense after an illness is huge. As our study proves, a major part of IgTotal falls on IgA antibodies, which is logical given the airborne mechanism of spread and thus the entrance door of infection. These summary data on genesis of the immune response against the SAR-CoV-2 virus supports the hypothesis that long-term effective immune protection could be built up after recurrent infections with the coronavirus, and that it is this immunity (naturally acquired) that is the effective immune response limiting the infection.

The infection caused by SARS-CoV-2 is a relatively „new“ disease and the results of the studies on the genesis, the duration and the intensity of immunity including the studies on the protective effect of the mass administration of RNA anti-SARS-CoV-2 vaccines encompass relatively short follow-up periods.

I is known by past experience that the control of an infection of such a scale, necessitates the achievement of collective immunity. Whether this will be done by mass vaccination or through disease outbreaks is still a matter of debate and depends on the contagiousness, virulence and lethality of the respective pathogen.

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