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CELLULAR AND HUMORAL IMMUNITY IN PATIENTS WITH COVID-19 CORRELATED WITH SEVERITY OF CLINICAL PRESENTATION AND SARS COV-2 VACCINATION STATUS

ЦЕЛУЛАРЕН И ХУМОРАЛЕН ИМУНИТЕТ КАЈ ПАЦИЕНТИ СО ПРЕЛЕЖАН COVID-19 ВО КОРЕЛАЦИЈА СО ТЕЖИНА НА КЛИНИЧКА СЛИКА И SARS COV-2 ВАКЦИНАЛЕН СТАТУС

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Abstract

Introduction. The COVID-19 pandemic has changed life across the planet, claiming approximately 7 million lives and more than 776 million cases reported globally, leaving many people with lasting sequelae. Of particular importance is understanding the immune system's defense responses against SARS-CoV-2 infection, with an emphasis on T-lymphocyte cells that modulate both cellular and humoral part.

Aims. To determine epidemiological and clinical features, especially comorbidities in patients who have had severe and mild COVID-19, to determine differences in cellular and humoral immune response and to correlate with vaccination status for SARS CoV-2.

Methods. A 6-month-prospective cohort study was conducted at the University Clinic for Pulmonology and Allergology and the Institute of Immunobiology and Human Genetics at the Faculty of Medicine - Skopje. The study included 88 patients with pre-existing COVID-19 divided into two groups: patients with mild clinical manifestations and patients with severe clinical manifestations requiring hospitalization. The parameters of patients' medical history, number and type of comorbidities, parameters of cellular and humoral immune response, data on vaccination against SARS-CoV-2 were analyzed.

Conclusion. The severity of the clinical presentation correlated directly with the number of comorbidities, and inversely with the vaccination status. Comorbidities were present in 87.7% of patients with a severe clinical course. No correlation was found with the smoking status. The study showed that 97.72% of all patients had positive neutralizing antibodies for SARS-CoV-2. Positive cellular immunity had 54.55% of patients, significantly higher in the group with severe COVID-19 and vaccinated patients. There was a positive correlation between cellular and humoral immunity, but in 2 cases (4.16%) where the humoral response was absent, a positive cellular response was verified.

Keywords: COVID 19, SARS CoV-2, cellular and humoral immunity, vaccination

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Апстракт

Вовед. Пандемијата на COVID-19 го смени животот на целата планета, однесе приближно 7 милиони животи и повеќе од 776 милиони случаи пријавени на глобално ниво, а многу луѓе останаа со трајни секвели. Сеуште постои голем научен интерес како да ја спречиме, контролираме и излечиме оваа болест. Особено е важно разбирањето на одбрамбените одговори од страна на имунолошкиот систем против инфекцијата со SARS-CoV-2, со акцент на Т-лимфоцитните клетки кои го модулираат специфичниот имунолошки одговор, вклучувајќи го целуларниот и хуморалниот дел.

Цели. Да се одредат епидемиолошките и клиничките карактеристики, особено коморбидитетите кај пациентите кои прележале тежок и лесен COVID 19, да се одредат разликите во целуларниот и хуморалниот имунолошки одговор кај различните групи пациентите и да се корелираат со вакциналниот статус за SARS CoV- 2.

Методи. Проспективна кохортна студија во времетраење од 6 месеци реализирана на ЈЗУ Универзитетска клиника за пулмологија и алергологија и Институтот за имунобиологија и хумана генетика при Медицински факултет - Скопје. Во студијата беа вклучени 88 пациенти со наполнети 18 години

и постари, со прележан COVID 19 кои се поделени во две групи: лесна клиничка манифестација и пациенти со тешка клиничка манифестација и хоспитализација. Анализирани се параметрите од медицинската историја на болните, бројот и типот на коморбидитети, параметри за целуларен и хуморален имунолошки одговор, податок за вакцинација против SARS-CoV-2.

Заклучок. Тежината на клиничката слика корелира правопропорционално со бројот на коморбидитети, а обратнопропорционално со вакциналниот статус. Кај 87,7% од пациентите со тешка клиничка слика се застапени коморбидитети. Не се најде корелација со пушачкиот статус и тежината на клиничката слика. Студијата покажа дека 97,72% од сите пациенти имаа позитивни неутрализирачки антитела за SARS-CoV-2, со изразито висок титар над референтните вредности. Позитивен целуларен имунитет имаа 54,55% пациенти, со сигнификантно поголем опфат во групата со тежок COVID-19 и вакцинираните пациенти. Постои позитивна корелација помеѓу целуларниот и хуморалниот имунитет, но во 2 случаи (4,16%) каде изостана хуморалниот одговор верификуван е позитивен целуларен одговор.

Клучни зборови: COVID 19, SARS-CoV-2, целуларен и хуморален имунитет, вакцинација

Introduction

Coronavirus infectious disease 2019 (COVID-19) was declared a global pandemic by the World Health Organization in March 2020 and is still present in human pathology. While symptoms of COVID-19 in the majority of infected children and young adults are mild [1], severe illness occurs in approximately 13% of the population with mortality exceeding 7 million [2]. Serious risk factors for increased mortality rates in adults and immunocompromised individuals include: obesity, chronic lung disease, diabetes, organ transplantation, and cardiovascular comorbidities, as well as male sex and older age [3-8]. Despite being initially described as a respiratory disease, COVID-19 has been observed to affect other organs and systems over time, such as the cardiovascular system, central nervous system, kidneys, liver, thyroid, etc. [3-5].

In scientific aspect, the enigma of diversity in the course of disease still remains. Understanding the immune system's defensive responses against SARS-CoV-2 infection is of particular importance. The immune response involves the pro- and anti-inflammatory cells of the immune system, particularly T lymphocyte cells that modulate the specific immune response, including cellular and humoral immunity, the activity of natural killer cells, the antiviral action of interferon, and the proliferation of T and B lymphocytes [9,10]. In healthy subjects, there is a correlation and coordination in the cascading immune response that occurs with viral infection. Antiviral immunity generally consists of neutralizing antibodies that block viral infection and cytotoxic CD8+ T cells, which eliminate cells infected with the virus. There is compelling evidence regarding the role of neutralizing antibodies in the protective immune response to SARS-CoV-2 infection [11]. However, the role of CD4+ and CD8+ T cells after viral entry is complex and requires comprehensive analysis. SARS-CoV-2 induces unrestricted generation and release of various mediators and cytokines in the bloodstream. A consequence of the action of inflammatory mediators is systemic inflammation, dysregulation of innate and acquired immunity responses, and further infiltration of various immune effector cells into various tissues. Numerous scientific publications have shown that the overexpression of cytokines and an exaggerated immune response ultimately cause organ dysfunction and cytotoxicity [9,12]. Jouan and Wang, and numerous other authors have suggested that an inadequate immune response lies behind the pathogenesis of ARDS [13,14]. According to Zhang *et al*., both genetic and acquired factors clearly demonstrate the critical role of effective interferon signaling during acute infection. Severe clinical outcomes are characterized by a slow decline in viral load and early and persistent inflammation with elevated interferon (IFN)-α, TNF, and IFN-γ [15]. T cell responses develop early and are correlated with protection, but in severe disease, they are relatively impaired and are associated with intense activation and lymphopenia [16]. Current evidence suggests that SARS-CoV-2-specific T cell responses are essential for viral clearance, can prevent infection without seroconversion, provide strong memory, and mediate the identification of viral variants. They also increase after vaccination, providing excellent protection against severe infection and death [17]. Antibody responses are highly effective in clinical care, and their analysis is facilitated by relatively easy detection and evaluation [18,19]. The immune response of an organism exposed to SARS-CoV-2 infection remains a topic of considerable scientific and professional interest.

Aims of the study

1. To determine the epidemiological, clinical, and pathological features of COVID-19 patients with severe clinical manifestations requiring hospitalization.
2. To determine epidemiological, clinical, and pathological features in patients who have had COVID-19 with mild clinical manifestations, or asymptomatic patients who have tested positive for SARS-CoV-2.

3. To determine differences in cellular immune response in patients with severe COVID-19 and those with mild or asymptomatic clinical manifestations.
4. To determine differences in humoral immune response in patients with severe COVID-19 and those with mild or asymptomatic clinical manifestations.
5. To correlate clinical, pathological and immunological characteristics with the vaccination status of patients for SARS CoV-2 virus.

Material and methods

Material

The study was performed at the University Clinic for Pulmonology and Allergology and the Institute of Immunobiology and Human Genetics at the Faculty of Medicine-Skopje. Eighty-eight patients were enrolled, aged 18 years or older, with previous COVID-19, examined and/or treated at the University Clinic for Pulmonology and Allergology-Skopje. All patients provided voluntarily and handwritten informed consent to participate in the study. Patients were divided into groups according to the following criteria: Group A: COVID-19 patients with severe clinical manifestations for which hospitalization was required; Group B: Patients who have had COVID-19 with mild clinical manifestations, or asymptomatic patients who have tested positive for SARS-CoV-2. Exclusion criteria in the study were: patients with malignant diseases, psychiatric patients, prisoners, pregnant women, individuals under 18 years of age, patients who are not legally capable of giving informed consent.

Methods

This was a prospective cohort study conducted over a period of 6 months. The parameters of patients' medical history, epidemiological data - gender, age, smoking status, data on COVID-19 recoveries and severity of illness, number and type of comorbidities, data on SARS-CoV-2 vaccination - number of doses and type of vaccine, parameters of cellular and humoral immune response to SARS-CoV-2 were analyzed.

Each patient's blood was taken for standard biochemical and immunological analyses, as follows:

- For cellular immunity using the *QuantiFERON* SARS-CoV-2 method - enzyme immune method ELISA (Enzyme-linked immunosorbent assay), which allows measuring the generated IFN-γ of CD4+ and CD8+ T cells in plasma samples stimulated by SARS-CoV-2 antigens - antigen 1 (CD4+ T cell epitopes from the S1 subunit RBD of the S protein from SARS-CoV-2), antigen 2 (CD4+ and CD8+ T cell epitopes from the S1 and S2 units of the S protein from SARS-CoV-2).
- For humoral immunity (IgG for SARS CoV-2) using an automatic chemiluminescent analytical method (CLIA).

A data collection instrument is a specially designed database, prepared according to the needs of the study, to collect sufficient data for verification of the assumeptions made. A standard descriptive statistical analysis, with determination of frequencies, was made with Microsoft Excel, 2016 and IBM SPSS Statistics, 2022.

Results

Our study included 88 patients with an equal gender distribution, 44(50%) men and 44(50%) women. Patients' age ranged from 31 to 83 years, with a mean age of 53.43 years, predominantly between 44 and 61.75 years. According to the average age, the majority belonged to the group of working population. Predominantly, or half, were non-smokers, a total of 44(50%), while 29 (32.95%) were smokers, and 15(17.05%) were former smokers. According to the severity of clinical presentation, more patients had a severe clinical course with hospitalization (group A), 49 patients (55.68%), while 39 patients (44.32%) had a mild illness or were asymptomatic (group B). Among

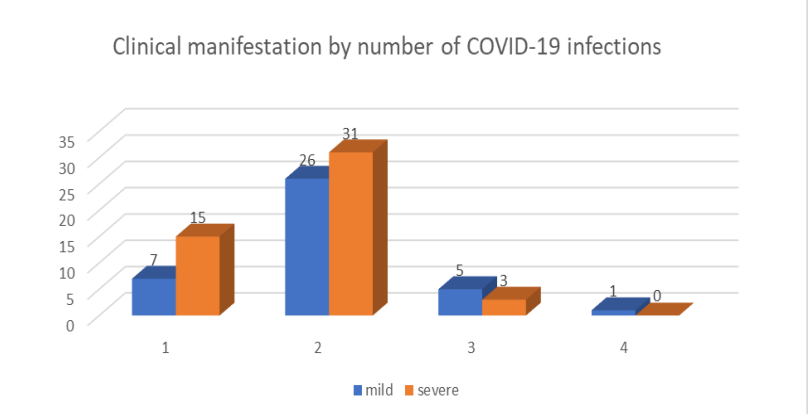

Fig. 1. Severity of clinical presentation, according to number of confirmed COVID-19 infections

those with one COVID-19 infection, 7(7.95%) pts had a mild clinical course, while 14(17.05%) had a severe illness; with two COVID-19 infections, 26(29.55%) pts had a mild, while 31(35.23%) pts had a severe clinical presentation; with three COVID-19 infections, 5 (5.68%) pts had a mild, and 3(3.41%) had a severe illness; and with four COVID-19 infections, 1(1.14%) patient had a mild illness, and there were no patients requiring hospitalization (Figure 1).

Out of the total 88 pts, 71(80.68%) were vaccinated, while 17(19.32%) were unvaccinated. Patients with mild clinical presentation had a slightly lower vaccination coverage 33(37.5%), compared to 38(43.18%) unvaccinated pts with severe clinical presentation, while 6(6.82%) unvaccinated pts had mild clinical presentation, compared to 11(12.5%) unvaccinated pts with severe clinical presentation. Of the 29 smokers, 15(51.72%) pts had a severe illness, compared to 14(48.28%) with a mild course. Of the 44 non-smokers, 26(59.09%) had a severe illness, while 18(40.91%) had a mild presentation or were asymptomatic. Of the 15 former smokers, 8(53.33%) had a severe illness with hospitalization, while 7(46.67%) had a mild clinical course.

There were 26 (29.55%) pts without comorbidities, while a total of 62(71.45%) had comorbidities, of which 14(15.91%) pts had 1 comorbidity, 20(22.73%) had 2, and 28(31.81%) had 3 or more comorbidities. Correlation of comorbidities with smoking status: among those with comorbidities, 16 (18.18%) patients were smokers, 32(36.36%) were non-smokers, and 14(15.91%) were former smokers, while among those without comorbidities, 13(14.77%) pts were smokers, 12(13.64%) were non-smokers, and 1(1.14%) was former smoker. Of the total of 26 pts without comorbidities, 19(73.07%) were in group B (mild illness), while 7(26.92%) pts had a severe clinical picture (group A). Of the total of 14 pts with 1 comorbidity, 9(64.28%) were in group A, while 5(35.71%) were in group B. Of the total of 20 pts with 2 comorbidities, 14(70%) were in group A, and 6(30%) were in group B. Of the total of 28 pts with 3 or more comorbidities, 20(71.43%) had a severe illness while 8 (28.57%) had a mild clinical course. Comorbidities were present in 4 out of the total of 49(87.7%) pts in group A (Figure 2).

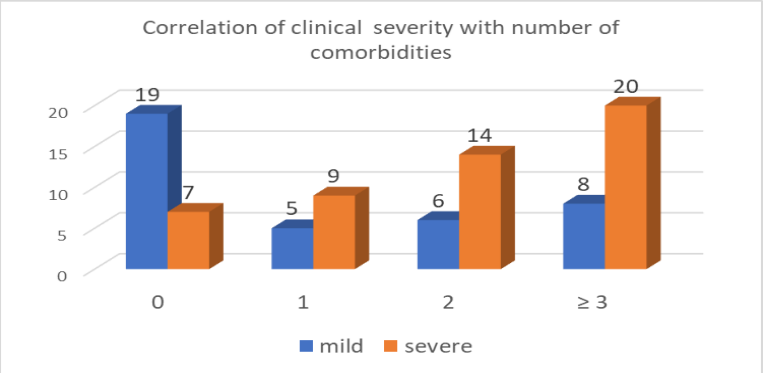

Fig. 2. Comorbidities correlated with severity of clinical presentation

Cellular immunity was determined by measuring IFN-γ produced by CD4+ and CD8+ T cells in plasma samples stimulated with SARS-CoV-2 antigens-antigen 1 (CD4+ T cell epitopes from the S1 subunit RBD of the S protein of SARS-CoV-2), antigen 2 (CD4+ and CD8+ T cell epitopes from the S1 and S2 subunits of the S protein of SARS-CoV-2) using the QuantiFERON SARS-CoV-2 method. In our study, 38(43.18%) pts

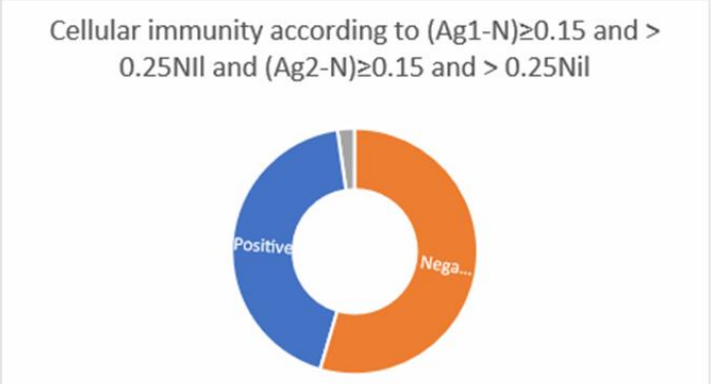

Fig. 3. Cellular immunity according to (Ag1-N) ≥0.15 and > 0.25NIl and (Ag2-N) ≥0.15 and > 0.25Nil

had negative cellular immunity, 48(54.55%) had positive cellular immunity, and 2(2.27%) had undetermined cellular immunity (Figure 3).
Correlation of cellular immunity with severity of clinical course: out of a total of 49 pts in group A (severe illness), 30(61.22%) had positive cellular immunity, 18(36.73%) had negative cellular immunity, and 1(2.04%) patient had undetermined cellular immunity. Out of a total of 39 pts in group B (mild illness), 18(46.15%) had positive cellular immunity, 20(51.28%) had negative cellular immunity, and 1(2.56%) patient had undetermined cellular immunity (Figure 4).

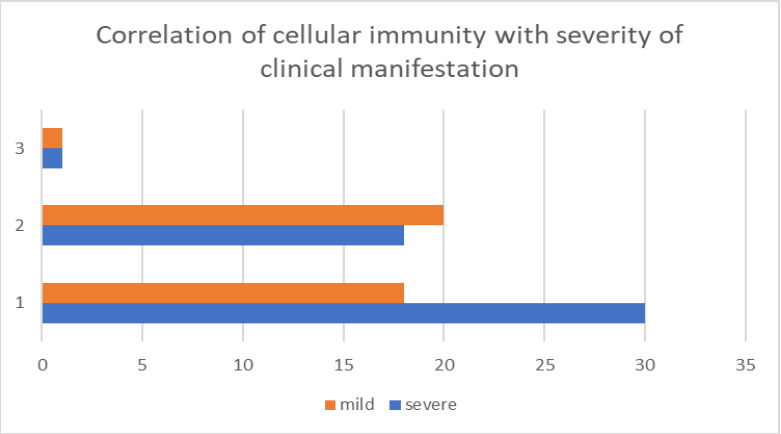

Fig. 4. Correlation of cellular immunity with severity of clinical course

Out of a total of 71 vaccinated pts, 37(52.11%) had positive cellular immunity, 33(46.48%) had negative cellular immunity, and 1(1.41%) vaccinated patient had undetermined cellular immunity. Out of 17 unvaccinated pts, 11 (64.71%) had positive cellular immunity, 5(29.41%) had negative cellular immunity, and 1(5.88%) patient had undetermined cellular immunity.
According to the titer of IgG antibodies (IU/mL) using the CLIA chemiluminescent method- reference value >1 IU/ml, antibodies were absent in only 2 pts, while the remaining 86(97.72%) pts had a positive titer. The distribution ranged from 2.28 to 2756 IU/mL, with a mean antibody value of 307.92 IU/mL. Patients with severe COVID-19 had a higher IgG antibody titer, an average of 384.06 IU/mL, while pts with mild COVID-19 had a lower average IgG antibody titer, of 228.9 IU/mL. Out of 71 vaccinated patients, 68(95.77%) had a positive IgG titer, 1(1.41%) patient had a borderline IgG titer, and 2(2.82%) pts had a negative IgG titer, while out of 17 unvaccinated patients, 14(82.35%) had a positive IgG titer, 1(5.88%) had a borderline IgG titer, and 2(2.27%) had a negative IgG titer.
Among patients with positive cellular immunity, 46(95.83%) had a positive IgG titer, 2(4.17%) had a negative IgG titer, and there were no patients with a borderline IgG titer. Among patients with negative cellular immunity, 34(89.47%) had a positive IgG titer, 2(5.26%) had a negative IgG titer, and 2(5.26%) had a borderline IgG titer. Among patients with indeterminate cellular immunity, 2(100%) had a positive IgG titer, while there were no patients with a negative and borderline IgG titer (Figure 5).

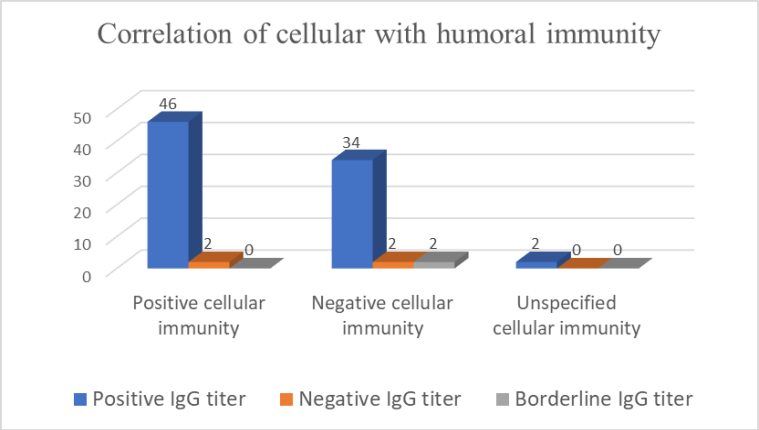

Fig. 5. Correlation of cellular with humoral immunity

The average IgG titer in pts with positive cellular immunity was 389.47 IU/mL, in pts with negative cellular immunity, it was 218.98 IU/mL, and in pts with indeterminate cellular immunity, it was 40.75 IU/mL.

Discussion

Although 5 years have passed since the first known case of COVID-19 was detected in China in November 2019, and in our country the first case was reported on February 26, 2020, the disease is still present in everyday clinical practice. Epidemiological predictions are that the disease will remain among us as a predominantly seasonal infection, with a relatively lower risk of severe clinical picture globally, but with an individually variable risk, particularly in immunocompromised individuals [20,21]. The virus has mutated over time, but the human immune system, including both humoral and cellular immunity, has enabled a more appropriate defensive response, hence, the severe clinical pictures of the past that were due to an inadequate immune response, including cytokine storms, fluid extravasation, interstitial pneumonias and ARDS [6], are now rare. According to our results and according to the available clinical studies, there is a strong relationship between comorbidities and severity of the illness; patients who were not vaccinated had a more severe clinical outcome [21,22]. Our data coincide with data from the world literature, confirming the relationship between the levels of neutralizing IgG antibodies after a severe infection, but also their protective role in mild forms of the disease [23,24]. Infection and vaccination with SARS-CoV-2 induce immune responses of both T-cells and B-cells in immunocompetent individuals. However, the mechanisms of antiviral effects mediated by CD 4+ T cells are not fully elucidated. The study by Shimizu J *et al*. showed that the inhibitory effect on viral replication was mostly attributed to interferon-γ (IFN-γ) present in the supernatant of polyclonally stimulated human CD4+ T cells. These results highlight the potential role of IFN-γ as a mediator against SARS-CoV-2 derived from CD 4+ T cells and suggest that understanding the IFN-γ-dependent susceptibility of SARS-CoV-2 is necessary in controlling clinical outcomes [25]. In addition, the characterization of new SARS-CoV-2 variants in terms of IFN-γ susceptibility will have important implications on the selection of therapeutic strategies. In our study, more than half of the patients (54.55%) had positive cellular immunity, with evidence of IFN-γ from CD4+ and CD8+ T cells in plasma samples stimulated with SARS-CoV-2 antigens. Most of the positive subjects had previously had a severe clinical picture (30 out of 48 patients, or 62.5%), significantly more than patients with a mild clinical picture where a higher percentage had negative cellular immunity (20 out of 38, or 52.63%). In two patients from the group with positive cellular immunity (4.16%), a negative IgG titer was determined, which confirms that even in the absence of a humoral response, cellular immunity has its own independent role in the human defense system. T-cell memory encompasses a broad recognition of viral proteins, estimated at about 30 epitopes in each individual. This may limit the impact of individual viral mutations and will probably underpin protection against severe disease from viral variants [26]. We were surprised that almost all subjects had exceptionally high titers of IgG neutralizing antibodies, which can be due to an immune response to the disease, to vaccination, but probably also to numerous reinfections with SARS CoV2, which were with milder and/or asymptomatic clinical manifestations, leading to a booster immune response.

The study by Simard *et al.* as well as numerous other studies support the importance of promoting vaccination in all individuals, particularly in those with pre-existing medical conditions, to reduce severe complications, even during the Omicron wave [27].

Conclusion

The severity of the clinical presentation correlates directly with the number of comorbidities, and inversely with the vaccination status. The majority of severe COVID-19 pts (87.7%) had comorbidities, 40.8% had 3 or more comorbidities, the most common being hypertension, obesity, cardiomyopathy, COPD, and diabetes mellitus. There was no significant difference in the severity of the clinical course in the vaccinated cohort, but unvaccinated patients had a greater number of severe clinical manifestations that required hospitalization. No correlation was found between smoking status and severity of the clinical picture. Patients with severe COVID-19 had a significantly higher IgG antibody titer, averaging 384.06 IU/mL, compared to 228.9 IU/mL in patients with mild COVID-19. The study showed that 97.72% of all patients had positive neutralizing antibodies for SARS-CoV-2, with a significantly high titer above the reference values. Positive cellular immunity was found in 54.55% of patients, with a significantly higher presence in the group with severe COVID-19. No significant difference was found in the response to cellular immunity in vaccinated patients with different clinical manifestations, while unvaccinated patients had a higher percentage of positive findings (11 out of 17 patients - 64.71%). There was a positive correlation between cellular and humoral immunity, but in 2 cases (4.16%) where the humoral response was absent, a positive cellular response was verified.

Conflict of interests: None declared.

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