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Immunological insights: assessing immune parameters in medical professionals exposed to SARS-CoV-2

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Abstract

Background The immunological background responsible for the severe course of COVID-19 and the immune factors that protect against SARS-CoV-2 infection are still unclear. The aim of this study was to investigate immune system status in persons with high exposure to SARS-CoV-2 infection.

Methods Seventy-one persons employed in the observation and infectious diseases unit were qualified for the study between November 2020 and October 2021. Symptomatic COVID-19 was diagnosed in 35 persons. Anti-SARS-CoV-2 antibodies were also found in 8 persons. Peripheral blood mononuclear cells subpopulations were analyzed by flow cytometry, and the concentrations of cytokines and anti-SARS-CoV-2 antibodies were determined by ELISA.

Results The percentages of cytotoxic T lymphocytes (CTLs), $CD28^+$ and T helper (Th) cells with invariant T-cell receptors were significantly higher in persons with symptomatic COVID-19 than in those who did not develop COVID-19' symptoms. Conversely, symptomatic COVID-19 persons had significantly lower percentages of: a) CTLs in the late stage of activation ($CD8^+/CD95^+$), b) NK cells, c) regulatory-like Th cells ($CD4^+/CTLA\text{-}4^+$), and d) Th17-like cells ($CD4^+/CD161^+$) compared to asymptomatic COVID-19' persons. Additionally, persons with anti-SARS-CoV-2 antibodies had a significantly higher lymphocyte count and IL-6 concentration than persons without these antibodies.

Conclusion Numerous lymphocyte populations are permanently altered by SARS-CoV-2 infection. High percentages of both populations: NK cells—as a part of the non-specific response, and T helper cells' as those regulating the immune response, could protect against the acute COVID-19 symptoms development. Understanding the immune background of COVID-19 may improve the prevention of this disease by identifying people at risk of a severe course of infection.

Trial registration This is a retrospective observational study without a trial registration number.

Keywords COVID-19, SARS-CoV-2 exposure, Anti-SARS-CoV-2 antibodies, Immunology system, Peripheral blood mononuclear cells

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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was declared a pandemic by the WHO on March 11, 2020 [1, 2]. The virus belongs to the same group of coronaviruses as SARS-CoV and MERS-CoV (Middle East respiratory syndrome coronavirus), which were responsible for deadly outbreaks in 2002 and 2012, respectively [3, 4]. The virus is spread by inhalation or contact with contaminated droplets, with an incubation period ranging from 2 to 14 days, depending on the mutations of the virus [5]. In principle, the first line of screening includes the detection of viral genomic material by reverse transcription polymerase chain reaction (RT–PCR), followed by complementary serological and radiological tests [6]. An infected person can be symptomatic or asymptomatic. SARS-CoV-2 invades epithelial cells, resulting in a diverse spectrum of symptoms. Before the reign of the Omicron variant, the severity of symptoms varies, but approximately 80% of patients have a mild infection [7]. In 15% of cases, the disease has a severe course with dyspnea, hypoxia, and lung lesions. Up to 5% are in critical condition with respiratory failure with ARDS (acute respiratory distress syndrome), shock, and/or multiorgan dysfunction [8–10].

The immune system is composed of two main branches: innate and adaptive immunity, both essential for defending the body against pathogens. Innate immunity is the first line of defense and provides a rapid, non-specific response to pathogens. It includes physical barriers like the skin and mucous membranes, as well as cellular components such as phagocytic cells (e.g., macrophages and neutrophils), natural killer cells, and the complement system. Innate immunity is always ready and responds within minutes to hours, recognizing common pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs). In contrast, adaptive immunity is highly specific, allowing for a stronger and faster response upon subsequent exposures to the same pathogen. It involves lymphocytes, namely B cells and T cells. B cells produce antibodies that neutralize pathogens, while T cells can directly kill infected cells or help coordinate the immune response. Adaptive immunity takes longer to develop, typically days to weeks, but provides long-lasting protection. Together, innate and adaptive immunity create a comprehensive defense mechanism, with the innate system providing immediate protection and the adaptive system offering tailored and lasting immunity [11, 12].

The primary gateway entry into immune cells for viruses is specific receptors [13]. For SARS-CoV infection, the ACE2 receptor is expressed, e.g., on type 2 alveolar cells in the lung. The spike (S) virus protein is merged with the ACE2 receptor followed by subsequent membrane fusion of alveolar cells and viruses [14]. After virus invasion, unrestrained virus replication evades innate immune cell activity and dampens antiviral interferon-based (IFN) responses [15, 16]. The site of virus entry is infiltrated by several adaptive immune cells (monocytes, macrophages, neutrophils), which leads to uncontrolled proinflammatory cytokine production, which is additionally aggravated by stimulation of Th1/Th17 cell subsets with viral epitopes [15–17]. Cytotoxic T cells are also recruited to the site of infection and serve as a contractor of virus-infected cell death in the lungs. Adaptive immunity, in terms of B cells and plasma cells, is also stimulated to produce antibodies specific to SARS-CoV-2, which may help to block viruses and provide systemic immunity in different organs.

Individuals with defects in innate or adaptive immunity demonstrate more severe viral infections [18]. T-cell immunity is more important for controlling many viral infections, while the activity of B cells and specific antibodies is crucial to minimize reinfection, particularly at mucosal sites. Finally, immune memory is often sufficient to prevent secondary disease, although not in all viral infections. This indicates a very important role of a properly functioning immune system in the effective management of viral infection [14, 18–20].

Constant exposure to viral infections can lead to an altered immune response characterized by a heightened state of immune vigilance and chronic activation. The immune system, particularly the adaptive immunity involving T cells and B cells, undergoes continual stimulation to recognize and combat the persistent threat of viruses. Over time, this can result in immune system fatigue or immunosenescence, where the efficacy of immune responses diminishes [14, 18–20]. Additionally, the chronic activation can lead to inflammation and tissue damage, contributing to the development of autoimmune disorders, where the immune system mistakenly targets the body's own cells. Regulatory mechanisms may become dysregulated, leading to either an exaggerated immune response or an impaired ability to control viral infections effectively. This altered immune landscape necessitates careful management to balance the need for protection against infections while preventing detrimental overactivity of the immune system [14, 18–20].

In the present study, we examined the activity and functioning of the immune system in healthcare professionals in a state of constant exposure to SARS-CoV-2. We tested the nonspecific response, the activity of individual T lymphocyte subpopulations, and the presence of virus-specific antibodies. The obtained immunological data were closely correlated with the clinical history of the subjects to define the immunological factors

responsible for the specific course of the disease, as well as for resistance to viral infection.

Materials and methods

Characteristics of the studied group

The study population included 71 persons with high exposure to SARS-CoV-2 infection (median age 47 with a standard deviation [SD] of 11 years). Physicians, nurses, and orderlies from the observation and infectious diseases unit at the Department of Pneumonology, Oncology, and Allergology were enrolled in the study from November 24, 2020, to October 25, 2021. During this period, blood samples were collected to perform all experiments. However, from persons with COVID-19, blood samples were obtained one month after infection (median: 36 ± 20 days) and when the acute symptoms of the disease had subsided. The study population was divided into two group: persons with symptomatic COVID-19 ($n=35$) and persons without COVID-19 symptoms ($n=36$). Characteristic of the study cohort is presented in Table 1. Characteristic of acute symptoms of COVID-19 observed in the individuals was presented in Table 2.

All persons employed in the observation and infectious diseases unit who gave their written informed consent to participate in the study were examined. All persons included in the study were properly secured in accordance with the procedures introduced in the hospital during the COVID-19 pandemic. The study was approved by the local Bioethics Committee at the Medical University of Lublin (approval number – KE-0254/244/2020).

Table 1 Characteristic of the study cohort

Characteristic	Persons without COVID-19 symptoms	Persons with COVID-19 symptoms	X^2	*p* value
Age (median ± standard deviation)	48.5 ± 8.89 years	45 ± 17.41 years	-	0.0255
Age, n (%)				
< 47 years ($n=33$)	15 (45.45)	18 (54.55)	0.68	0.4096
≥ 47 years ($n=38$)	21 (55.3)	17 (44.7)		
Gender, n (%)				
Male ($n=14$)	5 (35.7)	9 (64.3)	1.568	0.2104
Female ($n=57$)	31 (54.4)	26 (45.6)		
COVID-19 assays, n				
Molecular ($n=29$)		29	Not applicable	
Antigenic ($n=2$)		2		
Not performed ($n=40$)	36	4		
Comorbidities influencing immunologic system, n (%)				
Yes ($n=32$)	13 (40.6)	19 (59.4)	2.368	0.1238
No ($n=39$)	23 (59.0)	16 (41.0)		
Drugs influencing immunologic system, n (%)				
Yes ($n=24$)	9 (37.5)	15 (62.5)	2.529	0.1117
No ($n=47$)	27 (57.4)	20 (42.6)		
Inhaled corticosteroids, n (%)				
Yes ($n=10$)	5 (50.0)	5 (50.0)	0.002	0.9643
No ($n=61$)	31 (50.8)	30 (49.2)		
Vitamin D, n (%)				
Yes ($n=39$)	19 (48.7)	20 (51.3)	0.137	0.7113
No ($n=32$)	17 (53.1)	15 (46.9)		
Smoking status, n (%)				
Yes ($n=32$)	17 (53.1)	15 (46.9)	0.137	0.7113
No ($n=39$)	19 (48.7)	20 (51.3)		
Current smokers, n (%)				
Yes ($n=14$)	8 (57.1)	6 (42.9)	0.289	0.5909
No ($n=57$)	28 (49.1)	29 (50.9)		

Table 2 Characteristic of acute symptoms of COVID-19

Symptoms	Persons ($n=35$) with symptomatic COVID-19, n, (%)
Disruption of smell and taste (in one case – hypersensitivity)	22, (62.9%)
Weakness	20, (57.0%)
Bone and muscle pain	17, (48.6%)
Fever	16, (45.7%)
Dyspnea	12, (31.6%)
Headache	10, (28.6%)
Cough	8, (22.9%)
Rhinitis and sinusitis	8, (22.9%)
Nausea and vomiting	4, (11.4%)
Cutaneous hyperalgesia	4, (11.4%)
Pharyngitis and throatache	3, (8.6%)
Diarrhea and abdominal pain	2, (5.7%)
Chest pain	2, (5.7%)
Problems with concentrations and sleep disturbance	1, (2.9%)

Diagnostic laboratory tests

Routine blood counts and chemistry tests were performed in the whole studied population. We assessed the number of white blood cells, red blood cells, and platelets, as well as the percentage and number of lymphocytes, neutrophils, monocytes, eosinophils, and basophils. In biochemical analyses, the levels of hemoglobin, activated partial thromboplastin time (APTT), prothrombin time (PT), INR (international normalized ratio), D-Dimers, ferritin, ASP (aspartate transaminase), ALT (alanine transaminase), CRP (C-reactive protein), bilirubin, creatinine, eGFR (estimated glomerular filtration rate), LDH (lactate dehydrogenase) and albumin were examined.

Flow cytometry technique

We collected peripheral blood for plasma and for isolation of peripheral blood mononuclear cells (PBMCs) from the antecubital vein into an EDTA- (ethylenediaminetetraacetic acid) or heparin-containing tube from all studied persons. The flow cytometry technique and fluorochrome-labeled monoclonal antibodies were used to define different PBMC populations. We used a FACS Calibur flow cytometer (Beckton Dickinson, USA) equipped with an argon-ion laser (emitted wavelength – 488 nm) and a helion-neon laser (emitted wavelength—633 nm). Monoclonal antibodies were labeled with the following fluorochromes: FITC (fluorescein isothiocyanate): anti-CD3, anti-CD4, anti-CD14, anti-CD19; PE (phycoerythrin): anti-CD1d, anti-CD8, anti-CD19, anti-CD56 + CD16, anti-CTLA-4, anti-TGF-βRII, anti-CXCR3, anti-HLA-DR; APC (allophycocyanin): anti-CD25, anti-CD28, anti-CD95, anti-PD-1, anti-CD161, anti-TCRγδ, anti-INF-γ, anti-IL-10, anti-ROR-γT, anti-CTLA-4, anti-CD38; PerCP-Cy5.5 (peridinin chlorophyll protein-Cyanine5.5): anti-T-bet, anti-CD25;, BB700 (Brilliant Blue 700): anti-invNKT, anti-CD24, anti-CD8, anti-CD27;, and AF647 (Alexa Fluor): anti-STING, anti-FoxP3, anti-STAT-6, anti-ROR-γT. Antibodies were purchased from Becton Dickinson (USA).

Mononuclear cells were isolated from whole blood (collected into heparin-containing tubes) by 20 min. centrifugation at 2,800 rpm (revolutions per minute) with a density gradient on Lymphoprep (Stem Cell Technologies, Canada). After harvesting, the cells were washed twice in phosphate-buffered saline (PBS) by centrifugation for 6 min at 2,200 rpm. Then, the cells were counted and incubated for 20 min with antibodies against extracellular antigens. An excess of antibodies was removed by washing in PBS and centrifugation for 6 min at 2,200 rpm. To determine the expression of intracellular antigens, the cells were permeabilized by incubation with a transcription factor buffer set (Beckton Dickinson, USA) according to the manufacturer's instructions. After the process of permeabilization and neutralization of the Fix/Perm solution with the Perm/Wash solution, the cells were incubated with the appropriate antibodies for 40 min at 5 °C. Excess antibodies were removed by washing in Perm/Wash solution and centrifugation for 6 min at 2,200 rpm. The labeled cells were immediately acquired. Cytometric analysis was performed with CellQuest Pro software (Beckton Dickinson, USA). The percentage of individual PBMC subpopulations as well as the mean fluorescence intensity (MFI) of the various antibodies were analyzed. A short characterization of peripheral blood mononuclear cell subpopulations and the markers that were used for specific cell definitions are presented in Table 3.

Enzyme-linked immunosorbent assay (ELISA) assays

We analyzed the concentrations of four cytokines, IFN-γ, TNF-α (tumor necrosis factor α), IL-6, and IL-10, as well as anti-COVID-19 IgM and IgG antibodies, in plasma obtained after centrifugation of whole blood collected into EDTA-containing tubes. The plasma was pipetted in equal amounts into Eppendorf tubes to avoid freeze–thaw cycles and stored at -80 °C until analyses were performed. We used thawed, room-temperature plasma samples for analysis.

We used commercially available ELISA kits (Diaclone, France): Human IFN-γ ELISA KIT (limit of detection: 5 pg/ml), Human TNF-α ELISA KIT (limit of detection:

Table 3 Definition of the peripheral blood mononuclear cells' subpopulations tested in the study

Short characterization of peripheral blood mononuclear cells subpopulations	Immunophenotype' definition of each cells subpopulation
Subpopulation of T lymphocytes; subpopulation of B lymphocytes	CD3+; CD19+
Subpopulation of T helper (Th) lymphocytes with coexpression of PD-1 (programmed death 1) receptor ("exhausted"T cells)	CD4+/PD-1+
Subpopulation of cytotoxic T lymphocytes (CTLs) with coexpression of PD-1 (programmed death 1) receptor ("exhausted"T cells)	CD8+/PD-1+
Subpopulation of Th cells with coexpression of costimulatory marker essential for immunological synapse formation	CD4+/CD28+
Subpopulation of CTLs with coexpression of costimulatory marker essential for immunological synapse formation	CD8+/CD28+
Subpopulation of Th cells with coexpression of late activation marker	CD4+/CD95+
Subpopulation of CTLs with coexpression of late activation marker	CD8+/CD95+
Subpopulation of natural killer (NK) cells	CD3-/CD16+/CD56+
Subpopulation of activated and stimulated T lymphocytes	CD3+/HLA-DR+
Subpopulation of Th lymphocytes with intracellular expression of TGF-β (tumor growth factor β) and extracellular IL-2 (interleukin 2) receptor (CD25), as well as CTLA-4 (cytotoxic T-lymphocyte associated protein 4) molecule, defined as regulatory T cells (Tregs)	CD4+/TGF-β+/CTLA-4+/CD25+
Subpopulation of B lymphocytes capable of lipid antigen presentation, which stimulate other immune cells to cytokine production and adhere to endothelium during inflammation	CD19+/CD1d+/CD38+/CD24+
Subpopulation of Th cells with NK cells-like features of invariant nature	CD4+/CD161+/Inv NK V24+
Subpopulation of CTLs with NK cells-like features of invariant nature	CD8+/CD161+/Inv NK V24+
Subpopulation of Th cells with intraepithelial lymphocytes (IELs) features of invariant nature	CD4+/TCR γδ+/Inv NK V24+
Subpopulation of CTLs with intraepithelial lymphocytes (IELs) features of invariant nature	CD8+/TCR γδ+/Inv NK V24+
Subpopulation of peripheral blood monocytes with the ability to induce type I interferon (IFN) production	CD14+/STING+
Subpopulation of peripheral blood monocytes with the ability to produce IL-10 (interleukin 10) cytokine	CD14+/IL-10+
Subpopulation of CTLs with chemotactic migration marker and able to produce IFN-γ	CD3+/CD8+/CXCR3+/INF-γ+
Subpopulation of Th lymphocytes with chemotactic migration marker, which are able to differentiate into Th1 subset	CD4+/CXCR3+/T-bet+
Subpopulation of Th lymphocytes with chemotactic migration marker, which are able to differentiate into Th2 subset	CD4+/CXCR3+/STAT-6+
Subpopulation of Th lymphocytes with chemotactic migration marker, which are able to differentiate into Th17 subset	CD4+/CXCR3+/ROR-γT+
Subpopulation of Th lymphocytes with intracellular expression of FoxP3 (forkhead box P3) and extracellular IL-2 receptor (CD25), as well as CTLA-4 molecule, defined as Treg cells	CD4+/CTLA-4+/CD25+/FoxP3+
Subpopulation of B lymphocytes capable of immunoglobulin synthesis and IL-10 cytokine production	CD19+/CD27+/IL-10+
Subpopulation of T helper lymphocytes able to produce INF-γ and of invariant nature	CD4+/Inv NK V24+/INF-γ+
Subpopulation of T cytotoxic lymphocytes able to produce INF-γ and of invariant nature	CD8+/Inv NK V24+/INF-γ+

8 pg/ml), Human IL-6 ELISA KIT (limit of detection: 2 pg/ml) and Human IL-10 ELISA KIT (limit of detection: 4.9 pg/ml). All procedures were performed according to the manufacturer's instructions. The absorbance was read at 450 nm for all cytokines on a BioTek ELx800 Absorbance Microplate Reader (BioTek, USA). Standard curves were generated for each cytokine, from which concentration results in pg/mL were obtained. Analysis was performed with a Gen5 3.03 Microplate Reader and Imager Software (BioTek, USA).

For identification of anti-COVID-19 IgM and IgG antibodies, the EDI™ Novel Coronavirus COVID-19 IgG Elisa Kit (limit of detection: 0.0666) and EDI™ Novel Coronavirus COVID-19 IgM Elisa Kit (limit of detection: 0.0669) (Epitope Diagnostics, USA) were used. The assays allow for the qualitative identification of human IgG and IgM reacting to multiple epitopes of the SARS-CoV-2 full-length nucleocapsid protein. The procedures were performed according to the manufacturer's instructions. Absorbance was read at 450 nm on a BioTek ELx800 Absorbance Microplate Reader (BioTek, USA) and visualized with a Gen5 3.03 Microplate Reader and Imager Software (BioTek, USA). Interpretation of the results was performed according to the manufacturer's guidelines. For IgM antibodies, the mean absorbance of 3 negative controls (xNC) was calculated, and then the OD (optical density) of the samples was compared against the calculated manufacturer's thresholds as follows: positive

cutoff = 1.1 X (xNC + 0.1) and negative cutoff = 0.9 x (xNC + 0.1). For IgG antibodies, the mean absorbance of 3 negative controls (xNC) was calculated, and then the OD of the samples was compared against the calculated manufacturer's thresholds as follows: positive cutoff = 1.1 X (xNC + 0.18) and negative cutoff = 0.9 x (xNC + 0.18). The results were considered positive if the OD value was above the positive threshold.

Statistical analysis

We expressed the data as numbers and percentages (for categorized variables), as well as medians and interquartile ranges (IQRs) (for continuous variables). We used Pearson's chi-square test to compare the characteristics of the groups divided according to the development of SARS-CoV-2 infections and the presence of anti-SARS-CoV-2 antibodies. We used the U-Mann–Whitney test to test the equality of population medians among groups with and without SARS-CoV-2 infection. For multiparameter variation analysis, a nonparametric Kruskal–Wallis ANOVA test was used. The Shapiro–Wilk test was used for normality assumption. These tests were performed with Statistica v. 13.1 (Tibco Software, USA).

Results

Comorbidities of the study cohort and demographic factors

Forty-three persons enrolled in the study had comorbidities, and 32 had comorbidities that could affect the immune system. Allergies were described in 20 persons. Twenty-four persons constantly took immunosuppressive medication (such as L-thyroxin, non-steroid anti-inflammatory drugs, anti-histaminic drugs) and 10 used inhaled glucocorticosteroids. Inhaled glucocorticosteroids were administered once or twice daily in accordance with the summary of product characteristics for each drug. Vitamin D in prophylactic doses was used by 39 persons. Thirty-two persons declared smoking cigarettes, including 14 persons who were current smokers at the time of inclusion in the study. The median number of pack years was 8 ± 11.2. Of note, only persons not vaccinated against COVID-19 were enrolled in the study. Comorbidities in the entire study group and in two subgroups with and without symptoms of COVID-19 are presented in Table 4.

The most common symptoms of COVID-19 were loss of smell and taste, dyspnea, weakness, bone and

Table 4 Comorbidities in the study cohort

	Comorbidities in whole studied group n (%)	Comorbidities in persons with COVID-19 symptoms n (%)	Comorbidities in persons without COVID-19 symptoms n (%)
Comorbidities	**75 (100)**	**41 (100)**	**34 (100)**
Hypertension	16 (21.3)	8 (19.5)	8 (23.5)
Hypothyroidism	10 (13.3)	7 (17.1)	3 (8.8)
Asthma	8 (10.7)	4 (9.75)	4 (11.8)
Type 2 diabetes	7 (9.3)	5 (12.2)	2 (5.9)
Joint degeneration	5 (6.7)	3 (7.3)	2 (5.9)
Cardiac arrhythmias and other heart conditions	5 (6.7)	2 (4.9)	3 (8.8)
Psoriatic arthritis, rheumatoid arthritis or ankylosing spondylitis	3 (4)	2 (4.9)	1 (2.95)
Stomach ulcer disease	3 (4)	1 (2.4)	2 (5.9)
Ischemic heart disease	2 (2.7)	-	2 (5.9)
Crohn's disease	2 (2.7)	1 (2.4)	1 (2.95)
Lung cancer or breast and ovarian cancer	2 (2.7)	1 (2.4)	1 (2.95)
Chronic obstructive pulmonary disease	2 (2.7)	1 (2.4)	1 (2.95)
Liver damage (giardiasis, Gilbert's syndrome)	2 (2.7)	1 (2.4)	1 (2.95)
Chronic sinusitis	1 (1.3)	1 (2.4)	-
Hypercholesterolemia and obesity	1 (1.3)	1 (2.4)	-
Type 1 diabetes	1 (1.3)	1 (2.4)	-
Hashimoto's disease	1 (1.3)	-	1 (2.95)
Pulmonary embolism	1 (1.3)	1 (2.4)	-
Irritable bowel syndrome	1 (1.3)	1 (2.4)	-
Migraine	1 (1.3)	-	1 (2.95)
Varicose veins	1 (1.3)	-	1 (2.95)

muscle pain, and fever (Table 2). The age of COVID-19 persons was significantly lower than that of those who did not develop COVID-19 ($p=0.0225$). However, this could be related to greater exposure to SARS-CoV-2 in younger people (e.g., more frequent shifts). COVID-19 was slightly more common in people with comorbidities ($p=0.1238$) and persons who used immunosuppressive medication ($p=0.1117$). Other demographic factors did not affect the risk of symptomatic COVID-19 (Table 1). Moreover, the results of all basic laboratory tests did not differ in the group of people with and without SARS-CoV-2 infections.

Seroprevalence analysis: detecting SARS-CoV-2 antibodies and viral mRNA in our study group

Symptomatic COVID-19 was diagnosed in 35 persons (49.3% of the study group), who have also been found to have anti-SARS-CoV-2 antibodies (in the IgM or IgG class). Moreover, anti-SARS-CoV-2 antibodies were also found in 8 persons who did not develop symptoms of COVID-19. In summary, 43 persons (60.6% of the study population) had anti-SARS-CoV-2 antibodies.

The presence of viral mRNA was tested by RT–PCR in 29 symptomatic persons. Two persons were confirmed to have COVID-19 by an antigen test. Both tests (molecular or antigen) were performed at the time of COVID-19 symptom occurrence. In asymptomatic persons, no molecular or antigen tests were performed. However, in 4 persons with COVID-19 symptoms, the test for SARS-CoV-2 infection determination was not performed. Hospitalization was necessary in 3 persons, who were treated with standard anti-inflammatory, antipyretic and antitussive drugs (nonsteroidal anti-inflammatory drugs and thiocodin or levodropropizinum) and additionally received antiviral therapy (remdesivir), antithrombotic therapy (enoxaparin in a prophylactic dose), steroid therapy (dexamethasoni) and oxygen therapy. The remaining persons were treated at home with standard anti-inflammatory, antipyretic and antitussive drugs. No deaths due to SARS-CoV-2 infection were recorded.

According to the COVID-19 symptoms, the presence of anti-SARS-CoV-2 antibodies in the serum, during the statistical analysis, the following groups were compared:

1. Persons with symptomatic COVID-19 *vs.* persons without COVID-19 symptoms;
2. anti-SARS-CoV-2 serum-positive persons (both symptomatic and asymptomatic for COVID-19) *vs.* anti-SARS-CoV-2 serum-negative persons;
3. anti-SARS-CoV-2 serum-positive asymptomatic COVID-19 persons *vs.* anti-SARS-CoV-2 serum-positive symptomatic COVID-19 persons.

Immunological status in persons with symptomatic COVID-19

In persons who developed symptomatic COVID-19 compared to those who did not develop symptoms of SARS-CoV-2 infection, the percentages of the following PBMC populations were significantly higher: cytotoxic T-cell lymphocytes with the expression of CD28 costimulatory molecules ($CD8^+/CD28^+$) and CD4-positive T helper cells with the expression of invariant T-cell receptors (TCRs) corresponding to natural killer T (NKT) cells ($CD4^+/TCR\gamma\delta^+$/Inv NK $V24^+$). The following percentages of the PBMC populations were significantly lower in symptomatic COVID-19 persons than in asymptomatic persons: CTLs in the late stage of activation ($CD8^+/CD95^+$), NK cells, regulatory-like T helper cells ($CD4^+/CTLA\text{-}4^+$), and Th17-like cells expressing CD4 and CD161 molecules. The expression of the coinhibitory molecule CTLA-4 on CD4-positive cells, the expression of TCRγδ on CD4-positive cells, and the expression of invariant NK V24 on CD4-positive cells were significantly higher in symptomatic COVID-19 persons than in persons without COVID-19 symptoms. However, the expression of ROR-γT (transcription factor indicative of a differentiation of T helper lymphocytes toward Th17 cells) on CD4-positive cells was significantly lower in persons with symptomatic COVID-19 than in persons without COVID-19 symptoms (Table 5). The graphical representation of the most significant relation in the percentages of study lymphocyte subpopulations between asymptomatic and symptomatic COVID-19 is shown in Fig. 1. Exemplary cytometric analysis of selected markers is shown in Figs. 2 and 3.

Additionally, we checked the impact of comorbidities on the state of the immune system, in terms that it plays a leading role in viral infection predispositions, including SARS-CoV-2. However, our study group was too small and too heterogeneous to develop a multivariate analysis to demonstrate it (data shown in Table 4). We conducted a comparison of the percentage of lymphocyte subpopulations examined by flow cytometry in the largest groups with comorbidities (hypertension, hypothyroidism, asthma, type 2 diabetes). We used the nonparametric Mann–Whitney U test for this purpose. We found no significant differences in the percentage of lymphocyte subpopulations between these groups. Only in the group of persons with bronchial asthma was a slightly increased percentage of Th2 lymphocytes (CD4 + , CXCR3 + , STAT-6 +) found.

Analysis of antibodies against SARS-CoV-2 in persons with symptomatic and asymptomatic COVID-19

Forty-three persons had symptomatic COVID-19 and/or IgM or IgG class antibodies against SARS-CoV-2

Table 5 Significant differences in PBMC subpopulations (percentage (%) or molecule expression defined as the mean fluorescence intensity (MFI)) in persons with COVID-19 symptoms and in persons without COVID-19 symptoms. * *p* value for U-Mann–Whitney unifactorial test; ** *p* value for Kruskal–Wallis ANOVA multifactorial test

Subpopulations of PBMCs (% or mean fluorescence intensity)	Persons without symptoms of COVID-19 (median ± IQR)	Persons with symptoms of COVID-19 (median ± IQR)	*p* value (*)	*p* value (**)
$CD8^+/CD28^+$ cells (%)	46.51 ± 8.56	54.57 ± 18.97	0.0122	0.0066
$CD8^+/CD95^+$ cells (%)	70.15 ± 13.3	57.68 ± 26.54	0.0246	0.0226
NK cells (%)	13.71 ± 10.73	10.78 ± 6.73	0.039	0.0202
$CD4^+/CTLA\text{-}4^+$ cells (%)	4.49 ± 4.52	1.435 ± 1.97	0.000052	0.0004
CTLA-4 expression on $CD4^+$ cells (MFI)	15.59 ± 7.16	22.4 ± 20.83	0.000175	0.0007
$CD4^+/CD161^+$ cells (%)	15.62 ± 10.215	12.03 ± 9.09	0.0518	0.097
TCRγδ expression on $CD4^+$ cells (MFI)	78.36 ± 69.905	133.085 ± 82.12	0.00647	0.0104
$CD4^+/TCR\gamma\delta^+/$Invariant TCR $V24^+$ cells (%)	49.61 ± 13.1	55.13 ± 16.17	0.0491	0.0279
Invariant TCR V24 expression on $CD4^+$ cells (MFI)	61.1 ± 34.93	72.61 ± 40.84	0.0307	0.0139
RORγT expression on $CD4^+$ cells (MFI)	73.825 ± 30.78	60.18 ± 28.63	0.0125	0.0126

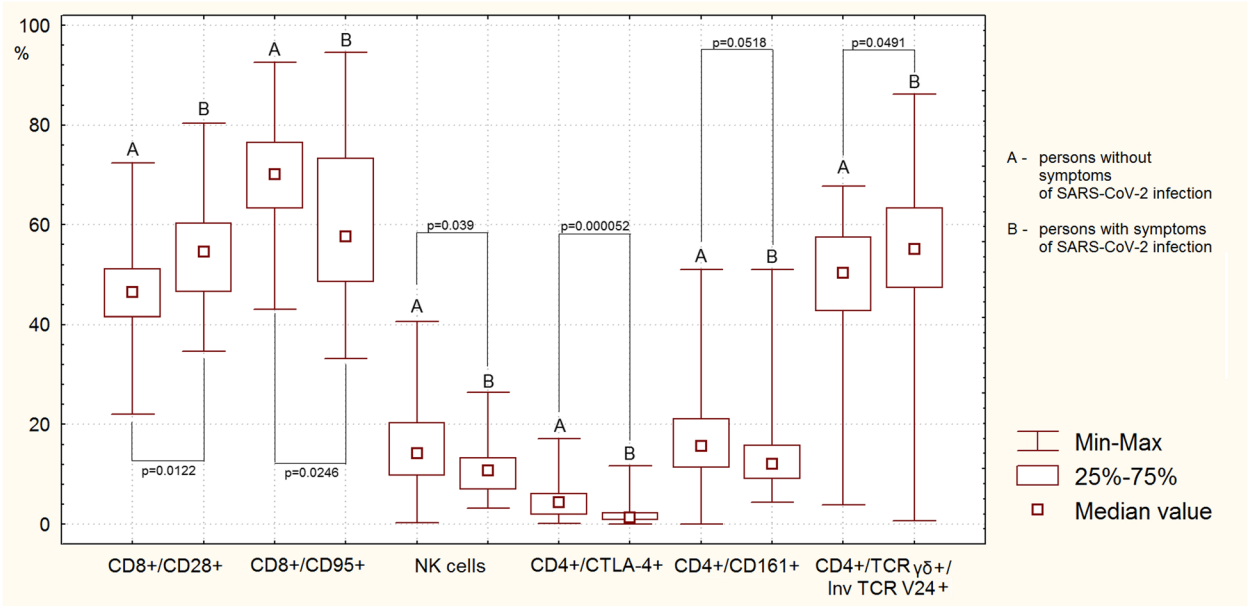

Fig. 1 The graphical representation of the most significant relation in the percentages of study lymphocyte subpopulations between asymptomatic and symptomatic COVID-19

(60.6% of the studied group). Demographic and clinical factors did not influence the risk of developing anti-SARS-CoV-2 antibodies. Persons with anti-SARS-CoV-2 antibodies had a significantly higher lymphocyte count (2,030 *vs.* 1,690 per mm^3, $p = 0.05$) and IL-6 concentration (1.16 *vs.* 0.9 pg/mL, $p = 0.0378$) but a lower albumin concentration (4.58 *vs.* 4.73 g/dL, $p = 0.0449$) than persons without these antibodies.

The following percentages of the PBMC populations were significantly higher in persons with anti-SARS-CoV-2 antibodies than in the rest of the study group: cytotoxic T-cell lymphocytes with the expression of CD28 costimulatory molecules ($CD8^+/CD28^+$), activated T cells ($CD3^+/HLA\text{-}DR^+$), activated CD4-positive cells with the ability to produce IFN-γ, and T cells with the expression of CD4 molecules and the expression of T-bet transcription factors (prone to differentiation in Th1 cells). However, the percentages of regulatory-like T cells ($CD4^+/CTLA\text{-}4^+$) and regulatory T cells ($CD4^+/FoxP3^+/CTLA\text{-}4^+/CD25^+$) were significantly lower in serum-positive

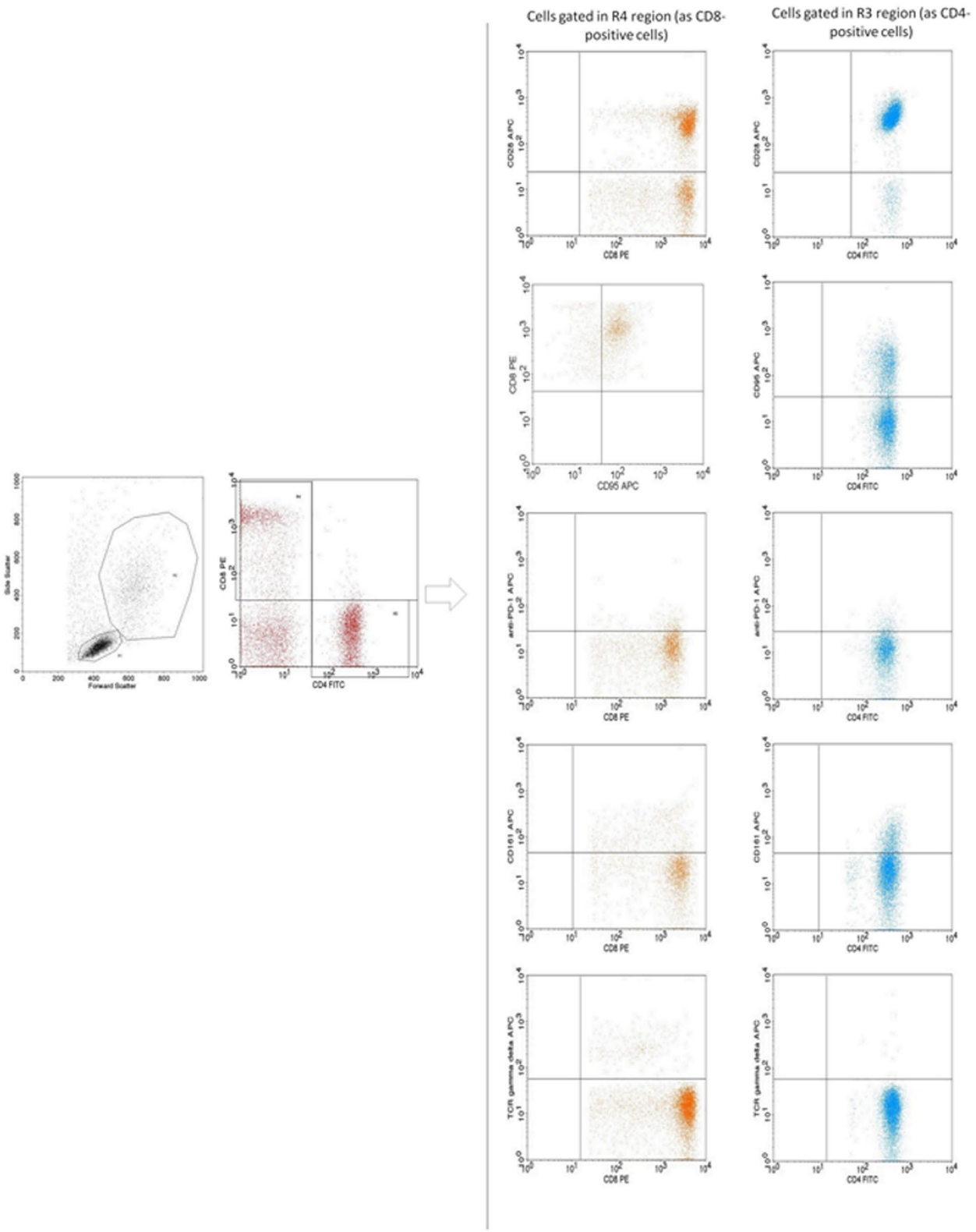

Fig. 2 An exemplary cytometric analysis of the peripheral blood mononuclear cell immunophenotype. In region R1, lymphocyte populations were gated and then scored for CD4 (gate R3) and CD8 (gate R4). The expression of individual analyzed markers was assessed on the surface of CD4-positive and CD8-positive lymphocytes

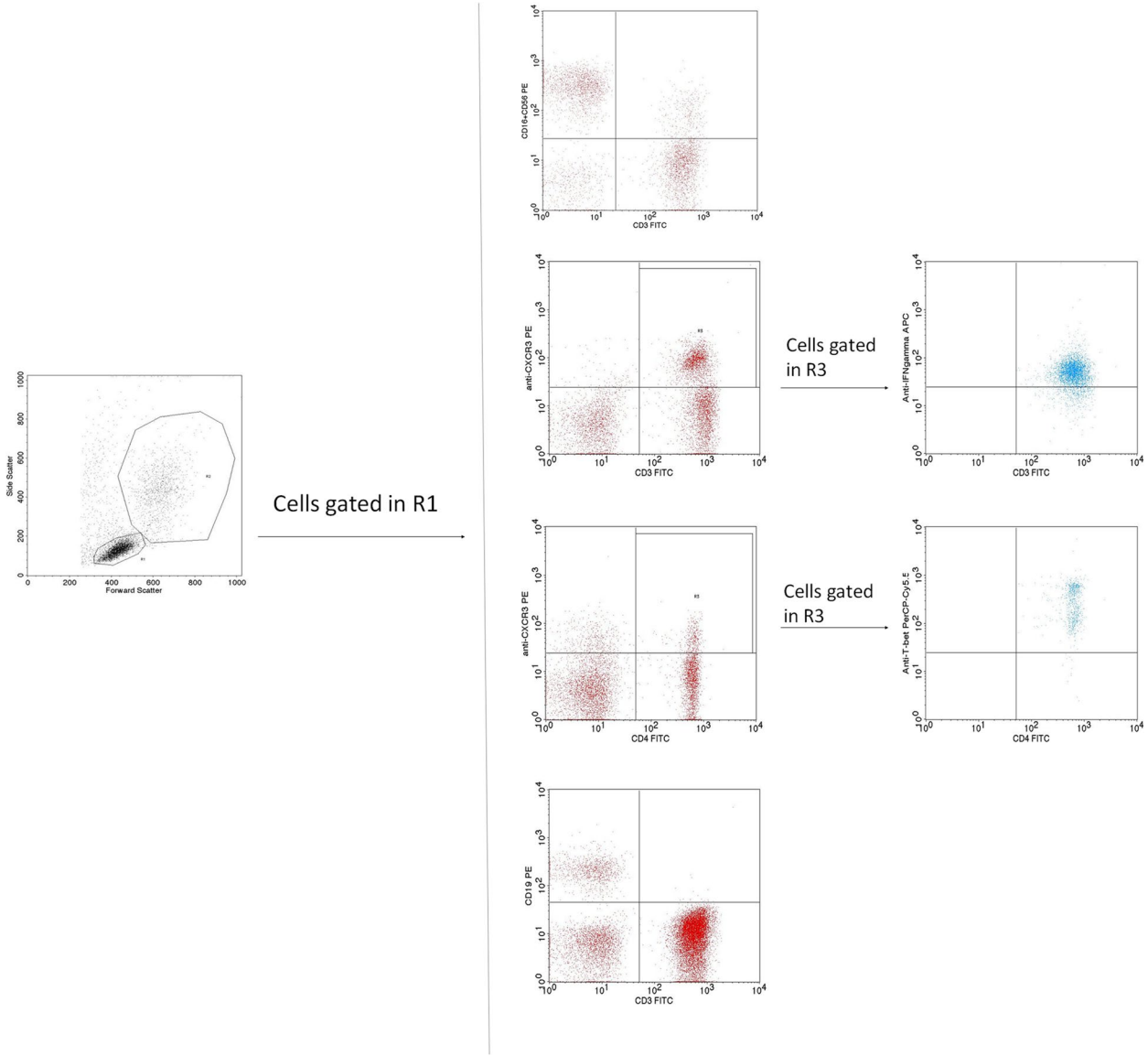

Fig. 3 An exemplary cytometric analysis of the peripheral blood mononuclear cell immunophenotype. In region R1, lymphocyte populations were gated and then scored for individual marker expression. Cells gated in regions R3 (CD3-positive/CXCR3-positive and CD4-positive/CXCR3-positive) were analyzed for their ability to produce INF-γ and intracellular T-bet expression, respectively

persons than in persons without anti-SARS-CoV-2 antibodies. Expression of the coinhibitory molecule CTLA-4 on CD4-positive cells, expression of invariant TCR V24 on CD4-positive cells, expression of receptor for C-X-C chemokines (CXCR3) on CD3-positive, CD4-positive, CD8-positive, and T-bet-positive T cells, and the expression of CD27 on B lymphocytes (mature B cells capable of producing antibodies) were significantly higher in persons with anti-SARS-CoV-2 antibodies than in the rest of the study group. However, the expression of ROR-γT on CD4-positive cells was significantly lower in persons with anti-SARS-CoV-2 antibodies than in the rest of the study group (Table 6).

Comparison of the immune system status between symptomatic COVID-19 anti-SARS-CoV-2 serum-positive persons and persons without infection symptoms but with anti-SARS-CoV-2 antibodies

The following percentages of the PBMC populations were significantly higher in persons with anti-SARS-CoV-2 antibodies but without COVID-19 symptoms than in persons with symptomatic SARS-CoV-2 infection and with

Table 6 Significant differences in PBMC subpopulations (percentage (%) or molecule expression defined as the mean florescence intensity (MFI)) in persons without anti-SARS-CoV-2 antibodies and in persons with anti-SARS-CoV-2 antibodies. * *p* value for U-Mann–Whitney unifactorial test; ** *p* value for Kruskal–Wallis ANOVA multifactorial test

Subpopulations of PBMCs (% or mean fluorescence intensity)	Persons without anti-SARS-CoV-2 antibodies (median ± IQR)	Persons with anti-SARS-CoV-2 antibodies (median ± IQR)	*p* value (*)	*p* value (**)
$CD8^+/CD28^+$ cells (%)	46.43 ± 9.105	52.77 ± 14.24	0.00524	0.0052
$CD3^+/HLA\text{-}DR^+$ cells (%)	11.955 ± 6.56	15.56 ± 6.12	0.0291	0.0291
$CD4^+/CTLA4^+$ cells (%)	4.435 ± 4.81	2.05 ± 3.25	0.0419	0.042
$CD4^+/IFN\text{-}\gamma^+$ (%)	85.44 ± 9.35	88.15 ± 7,85	0.0279	0.0279
CTLA-4 expression on $CD4^+$ cells (MFI)	15.68 ± 10.12	20.6 ± 14.23	0.0228	0.0229
Invariant TCR V24 expression on $CD4^+$ cells (MFI)	61.1 ± 34.93	69.21 ± 43.00	0.05	0.0508
CXCR3 expression on $CD3^+$ cells (MFI)	73.05 ± 20.46	88.745 ± 36.36	0.0165	0.0165
CXCR3 expression on $CD8^+$ cells (MFI)	78.18 ± 24.97	92.54 ± 36.18	0.0218	0.0218
CXCR3 expression on $CD4^+$ cells (MFI)	60.86 ± 28.42	76.915 ± 44.45	0.05	0.0536
$CD4^+/T\text{-}bet^+$ (%)	59.77 ± 16.37	70.765 ± 17.04	0.0368	0.0368
CXCR3 expression on $CD4^+/T\text{-}bet^+$ cells (MFI)	62.26 ± 25.25	75.635 ± 42.45	0.05	0.0581
ROR-γT expression on $CD4^+$ cells (MFI)	73.92 ± 27.68	61.105 ± 30.79	0.0193	0.0193
CXCR3 expression on $CD4^+/ROR\text{-}\gamma T^+$ cells (MFI)	63.7 ± 26.58	78.405 ± 43.35	0.0232	0.0233
$CD4^+/FoxP3^+/CTLA\text{-}4^+/CD25^+$ cells (%)	79.91 ± 17.46	66.67 ± 22.86	0.0489	0.0489
CD27 expression on $CD19^+$ cells (%)	366.665 ± 205.62	456.45 ± 203.14	0.0335	0.0335

anti-SARS-CoV-2 antibodies: CTLs in the late stage of activation ($CD8^+/CD95^+$), regulatory-like T cells ($CD4^+/CTLA\text{-}4^+$), T helper cells (both $CD4^+$ and $CD4^+/T\text{-}bet^+$) with the expression of a receptor for C-X-C chemokines (CXCR3), T helper cells with the STAT-3 transcription factor (which promotes Th cell differentiation into the Th2 subtype) and with the ROR-γT transcription factor (which promotes Th cell differentiation into Th17 lymphocytes). The percentage of T helper cells with CD161 and invariant TCR V24 expression, the percentage of T helper cells with the ability to produce IFN-γ and the expression of IFN-γ in these cells were significantly lower in asymptomatic, antibody-positive persons than in persons with COVID-19 symptoms (Table 7).

Discussion

Influence of clinical factors on the risk of COVID-19 development

COVID-19 has a very heterogeneous presentation. In most patients, the infection is mild, with upper respiratory tract symptoms, fever, and loss of taste and/or smell. In contrast, some patients may develop a severe

Table 7 Significant differences in PBMC subpopulations (percentage (%) or molecules expression defined as mean fluorescence intensity (MFI) in asymptomatic COVID-19 antibodies-positive persons and in symptomatic COVID-19 antibodies-positive persons. * - *p* value for U-Mann Whitney unifactorial test; ** - *p* value for Kruscal-Wallis ANOVA multifactorial test

Subpopulations of PBMCs (% or mean fluorescence intensity)	Persons without COVID-19 symptoms and with anti-SARS-Cov-2 antibodies (median ± IQR)	Persons with COVID-19 symptoms and with anti-SARS-CoV-2 antibodies (median ± IQR)	*p* value (*)	*p* value (**)
$CD4^+/CD95^+$ cells (%)	65.465 ± 24.395	50.15 ± 21.92	0.0409	0.1495
$CD4^+/CTLA\text{-}4^+$ cells (%)	3.83 ± 2.775	1.61 ± 2.342	0.0297	0.0297
$CD4^+/CD161^+$/Invariant TCR $V24^+$ cells (%)	46.855 ± 3.79	57.08 ± 17.45	0.0261	0.0261
$CD4^+/CXCR3^+$ cells (%)	33.67 ± 8.31	21.605 ± 20.08	0.0249	0.0249
$CD4^+/CXCR3^+/T\text{-}bet^+$ cells (%)	33.16 ± 9.65	21.845 ± 17.74	0.0373	0.0373
$CD4^+/STAT3^+$ cells (%)	1.445 ± 0.625	0.775 ± 0.85	0.022	0.022
$CD4^+/ROR\text{-}\gamma T^+$ cells (%)	79.25 ± 51.38	24.99 ± 58.98	0.027	0.0271
IFN-γ expression in $CD4^+$ cells (MFI)	138.625 ± 58.385	181.85 ± 98.87	0.05	0.0507
$CD4^+/IFN\text{-}\gamma^+$ cells (%)	3.185 ± 3.61	5.185 ± 6.61	0.047	0.1024

infection, with respiratory failure and serious organ damage. The patient's clinical factors are crucial in determining the risk of infection severity [8, 21]. Many studies have reported that the main risk factors for severe COVID-19 are age, male sex, smoking, obesity, and comorbid chronic diseases such as hypertension or type 2 diabetes [8].

Following Chen's analysis, age was the most significant risk factor for severe COVID-19 [22]. According to McIntosh, the median age of patients affected by severe complications during COVID-19 ranges from 49 to 56 years [23]. Moreover, the mortality rate due to SARS-CoV-2 infection increases with age and significantly rises above 80 years old [8]. Our study showed that the age of persons who developed COVID-19 was significantly lower than that of those without COVID-19. These results do not correspond to results obtained thus far in other studies [8]. This is likely related to the higher rate of exposure to SARS-CoV-2 in younger people than in older people working in health care. Moreover, the median age of our population was 47 years, which was lower than the age at which severe COVID-19 is more frequently diagnosed.

According to Jin et al., both men and women have the same susceptibility to infection, while male patients are more likely to experience poorer treatment outcomes and death [24]. One hypothesis explaining gender as a determinant of risk is the protective effect of hormonal factors in women [25]. However, our study found no effect of gender on the symptomatology of COVID-19. Similarly, our study did not show a significant effect of cigarette smoking and vitamin D supplementation on the risk of COVID-19 development. Again, our study is not representative in this respect, as many more women than men are employed in health care, many people use vitamin D supplementation, and few people smoke cigarettes (especially in pulmonary departments).

Barek et al., in their meta-analysis including 10,014 patients, showed that male and elderly patients were severely affected by SARS-CoV-2 infection and that comorbidities could significantly affect the prognosis and severity of COVID-19 [26]. Similar results were obtained by Sanyaolu et al. In their analysis, they showed that patients with COVID-19 who had comorbidities, such as hypertension or diabetes, were more likely to have a more severe course and progression of the disease [27]. In addition, older patients, especially those aged 65 and older, who have comorbidities and are infected with SARS-CoV-2 had increased rates of intensive care unit (ICU) admission and mortality [27]. Moreover, COVID-19 had a milder course in children. It was shown that in this group of patients, where age is not an aggravating factor, comorbidities such as obesity, diabetes, heart disease, and chronic lung diseases influenced the higher incidence of severe COVID-19 [28]. Another study showed that older age, the severity of respiratory failure, and renal impairment at presentation, but not other comorbidities, were predictors of 28-day mortality from COVID-19 [29]. In comparison, the study by Xue et al. showed that neither comorbidities nor chronological age were strong predictors of prognosis and disease outcome [30]. Our study confirmed previous assumptions that symptomatic COVID-19 was more common in patients with comorbidities and in those taking immunosuppressive drugs. At the same time, we did not show a significant effect of comorbidities or the use of immunosuppressants on COVID-19 development due to the insufficient size of the study group.

Pott and Cominetti noted in their study that the heterogeneity of the results of prognostic factors could be due to different study designs and different populations and the influence of confounding factors. In their results on a group of geriatric patients, the authors showed that comorbidities were the main determinants in the severity and mortality of COVID-19 [31].

The immune system status and high-risk exposure to SARS-CoV-2

During SARS-CoV-2 infection, immune dysregulation is observed; however, there is still insufficient knowledge on the immunological signals that could be used as predictors of enhanced resistance to viral infections [17, 19]. Retrospective reports regarding the information included in simple blood tests of COVID-19 survivors have shown that a decreasing trend in the percentage of white blood cells in patients with severe symptoms in comparison to patients with moderate symptoms of COVID-19 was frequently observed [32–34]. Chen et al. showed that the absolute numbers of T lymphocytes, CD4-positive T cells and CD8-positive T cells were decreased in almost all patients regardless of COVID-19 severity; however, their changes were markedly lower in those with severe disease manifestations than in those with moderate disease manifestations [35].

In our study, we did not observe any significant differences in the percentages of essential T lymphocyte populations, neutrophils, or monocytes in people with symptomatic viral infection and in those with asymptomatic COVID-19. This was in contrast with the study of Zahran AM et al., where they found that lymphopenia, alterations in almost all white cell populations and the depletion of CD4-positive and CD8-positive cells were observed in COVID-19 patients [36]. In our study, one of the most important observations was that the percentage of NK cells was significantly higher in the group without COVID-19 symptoms than in those with COVID-19. Wang F et al. measured the levels of peripheral

lymphocyte subsets in 60 hospitalized COVID-19 patients before and after treatment by flow cytometry and their association with clinical characteristics. Total lymphocytes, CD4-positive T cells, CD8-positive T cells, B cells, and natural killer cells decreased in COVID-19 patients, and severe cases had a lower level than mild cases [37]. Of note, natural killer cells are innate lymphocytes representing the first and invaluable line of defense against tumor cells and viral infections [17, 38]. It was shown that increasing NK cell activity promotes the alleviation of even severe infections [38]. Mazzoni A et al. showed high expression of cytotoxic granzyme A in NK cells in patients who needed intensive care due to COVID-19 [39]. However, in our research, it seems that these cells may have a protective role against the acute clinical manifestation of COVID-19. In further observations (data not shown), we found that people with a very high percentage of NK cells among PBMCs did not develop symptomatic COVID-19 until the appearance of the Omicron variant. Consistent with our and previous reports, Giamarellos-Bourboulis E et al. also showed depletion of CD4 lymphocytes, CD19 lymphocytes, and natural killer cells in all patients with severe respiratory failure during COVID-19 [15].

Moreover, in our study, within the group with symptoms of SARS-CoV-2 infection compared to people without symptoms of COVID-19, a significantly higher percentage of CD8-positive T cells with CD28 expression was observed. More interestingly, these cells did not express the CD95 molecule, which is defined as a late activation marker. It seems that in people with symptoms of infection, the described cell population could be defined as not completely activated lymphocytes that do not fulfill their task of eliminating cells infected with the virus. A study by Bobcakova A et al. tested the expression of various markers on immune cells in adults suffering from COVID-19 infection [40]. At the time of patient admission to the hospital, they found a significantly higher percentage of activated T $CD8^+$ lymphocytes, defined as CD38-positive cells, as well as significantly lower expression of CD159/NKG2A on T cytotoxic lymphocytes and NK cells in people who did not survive after COVID-19 infection compared with survivors [40]. In our study, a higher percentage of CD4-positive cells with TCRγδ molecules, as well as higher expression of Inv NK T markers and TCRγδ molecules on CD4-positive cells in people with SARS-CoV-2 infection, were observed. The CD28 marker is one of the most crucial proteins expressed on T cells that supplies the costimulatory signals needed for T-cell activation and survival [41, 42]. Stimulation of T cells through CD28, in addition to the interaction of TCR with viral antigens presented by MHC class I molecules, could provide a potent signal for the production of various interleukins, including IL-6. Invariant T cells after the presentation of foreign antigens also secrete proinflammatory cytokines and are capable of lysing infected cells [41–43]. Therefore, these changes could be responsible for the acute symptoms of the disease and hyperinflammation. We can conclude that targeting highly inflammatory parameters could diminish hyperinflammation and restore the proper functioning of T cells.

In people who are constantly exposed to the virus, humoral immunity seems to be of great importance [44, 45]. In our study, the examined population of healthcare workers constantly exposed to the SARS-CoV-2 virus was divided into two groups: persons who presented anti-SARS-CoV-2 antibodies and those without antibodies in serum. High immune system stimulation in the form of increased percentages of activated CD3-positive lymphocytes (cells with HLA DR expression), $CD8^+$/$CD28^+$ lymphocytes, T helper cells able to produce INF-γ, and CD4-positive cells with intracellular expression of the T-bet marker was observed in persons with anti-SARS-CoV-2 antibodies when compared with the group without such antibodies. Of note, high expression of the CXCR3 molecule was found on all CD3-positive, CD4-positive, CD8-positive and CD4-positive cells with ROR-γT expression in persons with anti-SARS-CoV-2 antibodies. The CXCR3 molecule is a chemokine receptor that is highly expressed on all effector T cells and plays a substantial role in T-cell trafficking and functioning [46, 47]. CXCR3 acts in driving T helper cells and CD8-positive T cells to peripheral sites of inflammation and facilitating their interaction with antigen presenting cells, which could generate effector and memory cells [46, 47]. These results indicate that in people with anti-SARS-CoV-2 antibodies, the immune system is in a steady-state position, ready for a confrontation with COVID-19 to fight the infection.

In prolonged inflammatory stimulation, the immune system could activate the specific mechanism that regulates its excessive action, e.g., the expression of negative immunological check-points on effector T cells or activation of regulatory T lymphocytes [48]. However, in the context of stimulating immune system activity during viral infections, excessive activity of regulatory T lymphocytes does not seem to be a desirable action. Current evidence suggests that the level of peripheral T regulatory cells is prominently reduced in patients with severe COVID-19 compared to patients with mild disease [49, 50]. Zahran AM et al. analyzed cytotoxic T-lymphocyte antigen-4 (CTLA-4)-expressing cells among CD4-positive and CD8-positive cells in 24 COVID-19 patients. The percentage and absolute count of CD4 + /CD8 + cells were significantly reduced in COVID-19 cases compared

to healthy controls, and the proportions of apoptotic and CTLA-4-expressing CD4+/CD8+cells were significantly upregulated in COVID-19 patients [36]. This is inconsistent with our results; however, Zahran's research included a fairly small group of patients. In contrast, in our study, in persons without anti-SARS-CoV-2 antibodies in the serum, the percentages of $CD4^+$/CTLA-4^+ cells as well as $CD4^+$/FoxP3^+/$CD25^+$ (defined as regulatory T lymphocytes) cells were significantly higher than those in antibody-positive persons. However, the reasons for the reduced level of T regulatory cells in the peripheral blood of persons with symptomatic COVID-19 and anti-SARS-CoV-2 antibodies are not entirely understood. One of the possibilities is that those cells might have migrated to the lungs to prevent tissue damage.

Moreover, in our study, the percentages of CD4-positive T lymphocytes with CD161 expression as well as intracellular retinoic acid-related orphan receptor gamma T (ROR-γT) intracellular expression were significantly higher in persons without symptoms of infection than in persons with symptomatic disease. The CD161 molecule is also found on NK cells; however, there have been various reports of modulated expression of CD161 on NK cells during viral infections [51]. For instance, reduced CD161 expression in acute hepatitis C virus (HCV) infection predicted viral clearance and correlated with increased liver inflammation in chronic HCV infection [52, 53]. Patients with chronic human immunodeficiency virus (HIV) infection have depleted CD161+NK cells compared to healthy donors [54, 55]. Recently, a population of NK cells with memory-like properties has been described in the context of cytomegalovirus (CMV), referred to as "adaptive" NK cells [38, 56]. Moreover, Th17 cells could inhibit Treg differentiation and play an important role in maintaining mucosal barriers and contributing to pathogen clearance at mucosal surfaces [38, 41]. In our study, we conclude that the high percentage of CD4-positive T lymphocytes with high CD161 expression, together with the high percentage of NK cells, could be defined as the first line of defense against COVID-19. Moreover, T helper lymphocytes, which are able to differentiate into the Th17 subset (ROR-γT-positive cells), seem to play an important role in the immune response against SARS-CoV-2.

In our study, the analysis of immune status in persons with and without SARS-CoV-2 infections has some weaknesses. First, it was a preliminary study conducted on a small group of healthcare professionals who were constantly exposed to the virus. No randomization of the study subjects was carried out; therefore, the subgroups are not equivalent to the population at large in terms of their key characteristics. Moreover, the size of the group should be equalized in terms of the number of men and women included in the study. This may have contributed to the lack of association between gender and symptomatology in this study. Moreover, blood sampling in persons with symptomatic COVID-19 is usually performed more than one month after the onset of the disease. However, in a few people, it was done later due to prolonged symptoms of the disease or earlier if the symptoms were mild, the virus tests were negative, and the employees returned work. These differences may have influenced the activity of the immune system. Moreover, it is not clear whether the status of the immune system affects the risk of SARS-CoV-2 infection. In persons with symptomatic COVID-19, blood for immunological analyses was collected after infection. Therefore, the differences in PBMC subpopulations in people with symptomatic COVID-19 and in persons without this infection could be the consequence of SARS-CoV-2 infection. We should also keep in mind that some of the studied subjects could have a history of asymptomatic COVID-19 infection without positive test results and with negative antibodies. As shown, up to 20% of the study group did not produce specific antibodies after infection. This could indicate an asymptomatic course of the disease without antibody testing. Indeed, this situation could also affect immune system activity in those persons. The highlights of our study are presented graphically in Fig. 4.

By 2020, the probability of SARS-CoV-2 infection is relatively high and could affect most of the world's population. In everyday life, we are constantly exposed to contact with infected asymptomatic persons. Therefore, it seems imperative to gain knowledge of immune mechanisms that defend us effectively against symptomatic COVID-19 development. Knowing the immunological background responsible for the severe course of COVID-19, it is possible to better plan the prophylactic actions of this disease. First, it would be more rational to use drugs for preexposure prophylaxis, such as antibodies against the viral spike receptor binding domain (e.g., tixagevimab and cilgavimab). Secondarily, more stringent isolation or vaccination rules can be implemented in people immunologically predisposed to the severe course of this disease. However, additional studies will still be necessary to fully understand the immunological changes during constant and high exposure to SARS-CoV-2 infection.

Highlights (graphical highlights presented in Fig. 4)

1. *NK cells* play an important role in protection against COVID-19.
2. CD28+*T cells (CTLs) and T helper (Th) cells* are elevated in symptomatic COVID-19 patients.

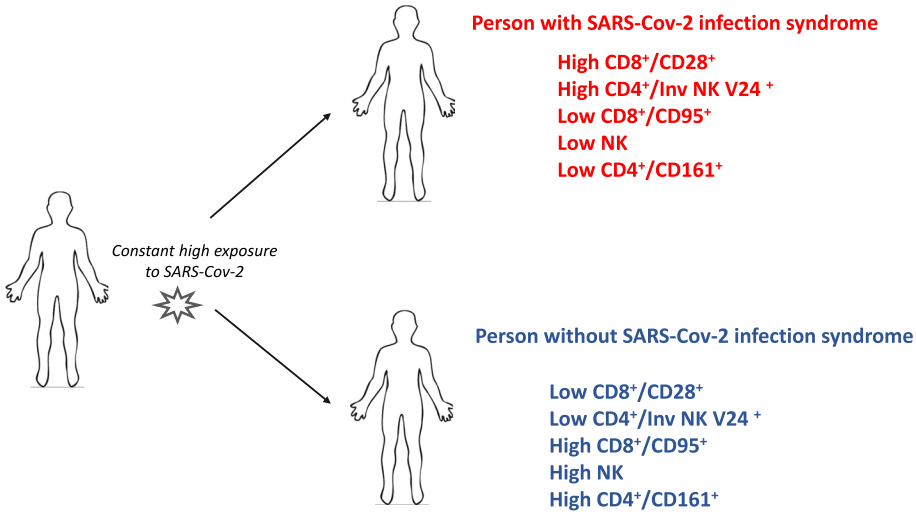

Fig. 4 Graphical abstract highlights the most important conclusions from the work

3. *Higher lymphocyte count and IL-6* positivity correlate with anti-SARS-CoV-2 antibodies.
4. Numerous lymphocyte populations contribute to the severity of COVID-19.
5. Understanding the immune background of COVID-19 could improve its prevention.

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Authors' contributions
KWK: designing research studies, conducting experiments, acquiring data, writing the manuscript; PK: designing research studies, analyzing data, writing the manuscript; JB: designing research studies, conducting experiments, writing the manuscript; TK: conducting experiments, acquiring data, writing the manuscript; AG: conducting experiments, acquiring data, writing the manuscript; NK: acquiring data, analyzing data; KS: acquiring data, analyzing data; AHW: acquiring data, analyzing data; MWS: acquiring data, analyzing data; IC: acquiring data, analyzing data; JM: designing research studies, supervising the research.

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Availability of data and materials
The datasets generated and/or analysed during the current study are not publicly available due to the fact that this data is sensitive data but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Bioethics Committee at the Medical University of Lublin (approval number – KE-0254/244/2020).

Consent for publication
Yes.

Competing interests
The authors declare no competing interests.

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