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Immunologic Response after COVID-19 Vaccination in Heart Transplant Recipients

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Abstract

To date, all large-scale randomized controlled trials for COVID-19 vaccines have excluded solid organ transplant recipients; therefore, the effectiveness and safety of COVID-19 vaccines in preventing coronavirus infection using COVID-19 vaccines in patients with heart transplants have not been sufficiently studied. This paper presents the characteristics of humoral and cellular immunity in heart transplant recipients following vaccination against coronavirus infection. The study group consisted of 40 patients who underwent orthotopic heart transplantation between 2019 and 2014. They were vaccinated twice with the Vero Cell

vaccine (China) or received a 3-dose vaccination with Gam-COVID-Vac (Sputnik V, Russia) booster. 63% of vaccinated individuals with no previous COVID-19 history and 85% of patients with a history of COVID-19 were to develop humoral post-vaccination immunity. The humoral response in patients who seroconverted before vaccination showed high level of virus-specific IgG antibodies to SARS-CoV-2 S protein during the post-vaccination period, with a statistically increase observed 9-12 months after the booster. The specific cellular response to the SARS-CoV-2 S and N proteins remained low throughout the entire follow-up period, and was recorded in 5-40% of heart transplant recipients. A significantly increased number of S- and N-specific T cells was observed 4-6 months after the secondary immunization. Starting from 21-28 days after the primary vaccination and continuing for a year after the booster, increased plasmablasts ($CD27^{high}CD38^{high}$ B cells) were observed, correlating with neutralizing and spike-specific antibodies. In heart transplant recipients, vaccination against coronavirus infection does not result in increased serum autoantibodies (RF-IgG and IgA-RF, anti-SSR, cardiolipin IgG, β2-glycoprotein IgG, ANA, ANCA-Pro, anti-SLA/LP, anti-GD-IgA). In our study, vaccinated heart transplant recipients with a history of coronavirus infection showed an increased level of anti-IFN-α antibodies for 9-12 months after the basic vaccination. This finding, when associated with HLA alleles, must be taken into account for identifying patients at a potential risk of a severe disease.

Keywords
Heart transplant recipients; vaccination; SARS-CoV-2; COVID-19; humoral and cellular immune response; memory cells; autoantibodies

1. Introduction

Vaccination is one of the most effective tools to reduce severe morbidity and mortality from SARS-CoV-2 infection significantly. Heart transplant recipients (HTRs) are at an increased risk of poor outcomes from coronavirus disease 2019 (COVID-19) due to the mandatory immunosuppression and the presence of comorbidities. With the advent of COVID-19 vaccines and their widespread large-scale use, interest in studies on patients whose community is weakened due to organ transplantation has increased. To date, all large-scale randomized controlled trials of various COVID-19 vaccines have excluded solid organ transplant recipients (SOTRs). Therefore, the efficacy and safety of these vaccines for preventing the coronavirus infection in this cohort have not been sufficiently studied.

The SOTRs have been shown to have a less robust antibody response, to a lesser extent, a cellular response to vaccines [1, 2]. In prior studies, lower seroconversion rates were recorded in HTRs after COVID-19 vaccination. Overall, after two doses of mRNA vaccines, antibodies (Abs) to the spike protein receptor-binding domain (RBD) of SARS-CoV-2 were detected in 10-57% of patients, and a cellular response was recorded in 10-70% of HTRs [3, 4].

Increased intensity of immunosuppression, administration of antimetabolites, and agents inhibiting B-cell response are related to a decreased vaccine immunogenicity [5, 6]. Despite suboptimal seroconversion rates, vaccination is associated with a reduced risk of death from COVID-

19 [3, 4, 7] and an 80% reduced risk of symptomatic disease as compared to unvaccinated SOTRs [8]. Evaluation of post-vaccination immunity in primarily seronegative individuals and those who have recovered from the infection caused by the SARS-CoV-2 virus indicates that the preceding vaccination against COVID-19 contributes to the restructuring of the immune system and provides an opportunity for forming a secondary immune response to a subsequent vaccination against SARS-CoV-2, namely "hybrid immunity" [9-11]. Clinical efficacy, ongoing monitoring of rare serious adverse events, and vaccine immunogenicity data should be considered when developing a methodology for assessing the risks and benefits of vaccination for individual HTRs.

Autoimmune reactions are one of the factors influencing the successful prognosis in heart transplantation. Throughout the pandemic, cases of autoimmune diseases related to immunization against COVID-19 have been reported [12, 13]. Molecular mimicry, the production of certain autoantibodies (autoAbs) and the role of particular vaccine adjuvants appear contribute to significantly to autoimmune reactions. It has been shown that the SASR-CoV-2 virus can induce the production of more than 15 types of Abs (LAC – Lupus anticoagulant, ANA – Anti-nuclear Abs, Anti-β2 GPI – Anti-β2 glycoprotein I, Anti ACE-2 – Anti-angiotensin-converting enzyme 2, etc.) and can trigger 10 different autoimmune diseases (antiphospholipid syndrome, Guillain-Barré syndrome, Kawasaki disease, etc.) [12, 14]. The existing molecular mimicry between SARS-CoV-2 and human peptides/antigens does not exclude possible immune reactions in vaccinated individuals, including HTRs. This makes it necessary to develop vaccination strategies to reduce the risk of COVID-19 and the autoimmune dangers of a severe disease in the most vulnerable individuals. The spread of the infection requires all existing concepts and conventional approaches to be revised. The spread of infection requires all existing concepts and conventional approaches to be revised. As the understanding of pathophysiological mechanisms of COVID-19 accumulates, specific features of HTRs' management will become clearer.

Updated recommendations (May 21, 2021) from the International Society for Heart and Lung Transplantation (ISHLT) recommend that transplant candidates and transplant recipients should be vaccinated against coronavirus infection with an available vaccine. The International Society for Heart and Lung Transplantation (ISHLT) and the American Society for Transplantation (AST) have released guidelines for vaccination against COVID-19 in patients with chronic heart or lung failure, as well as in those who have undergone heart transplantation.

This article presents the characteristics of the cellular and humoral immune response and the results of the analysis of autoAbs production in HTRs after the basic 2-dose vaccination with inactivated whole virus Vero Cell vaccine (SARS-CoV-2, 19nCoV-CDC-TanHB02, China), followed by booster (3-dose full vaccination schedule) with the combined vector vaccine Gam-COVID-Vac (Sputnik V, Russia).

2. Materials and Methods

2.1 Patients, Design, and Ethics Statement

The study group consisted of 40 patients after orthotopic heart transplantation between 2009 and 2021 at the Republican Scientific and Practical Center "Cardiology" (Minsk, Republic of Belarus) who underwent 2- and 3-dose vaccination against SARS-CoV-2. To assess the antigen-specific cellular and humoral immune response following vaccination, HTRs were divided into two groups: group 1–19 patients with no history of coronavirus infection at the time of immunization

(seronegative patients) and group 2–21 patients who had recovered from COVID-19 (3-6 months before vaccination) or were asymptomatic (seropositive patients) (Table 1). All patients were administered dual immunosuppressive therapy (tacrolimus + mycophenolate or tacrolimus + everolimus) according to the protocol of the Ministry of Health of the Republic of Belarus.

Table 1 Clinical and demographic characteristics of investigated HTRs.

Groups	n	Average age, years *	Gender, m/f	COVID-19-infected HTRs within the vaccination period
Total	40	62.0 (56.0; 66.0)	38/2	9/40 (22.5%)
Group 1	19	63.5 (53.2; 68.8)	18/1	7/19 (36.8%)
Group 2	21	62.0 (57.0; 64.5)	20/1	2/21 (9.5%)

* Data are presented as median (lower quartile; upper quartile).

Based on international recommendations, and the vaccination protocol in the Republic of Belarus, HTRs were given a complete cycle of vaccination. The vaccination did not affect the function and survival of the allograft in HTRs.

Peripheral venous blood was sampled for research according to the following steps of study design (Figure 1):

(1) before the primary immunization (Vero Cell, inactivated, CoronaVac®);

(2) before the secondary immunization (Vero Cell, inactivated, CoronaVac®, 21-28 days after the primary immunization);

(3) before booster (4-6 months after the basic 2-dose vaccination, Sputnik V);

(4.1) 9-12 months after the basic 2-dose vaccination (unboosted);

(4.2) 9-12 months after booster (3-dose vaccination, full vaccination schedule, Vero Cell and Sputnik V).

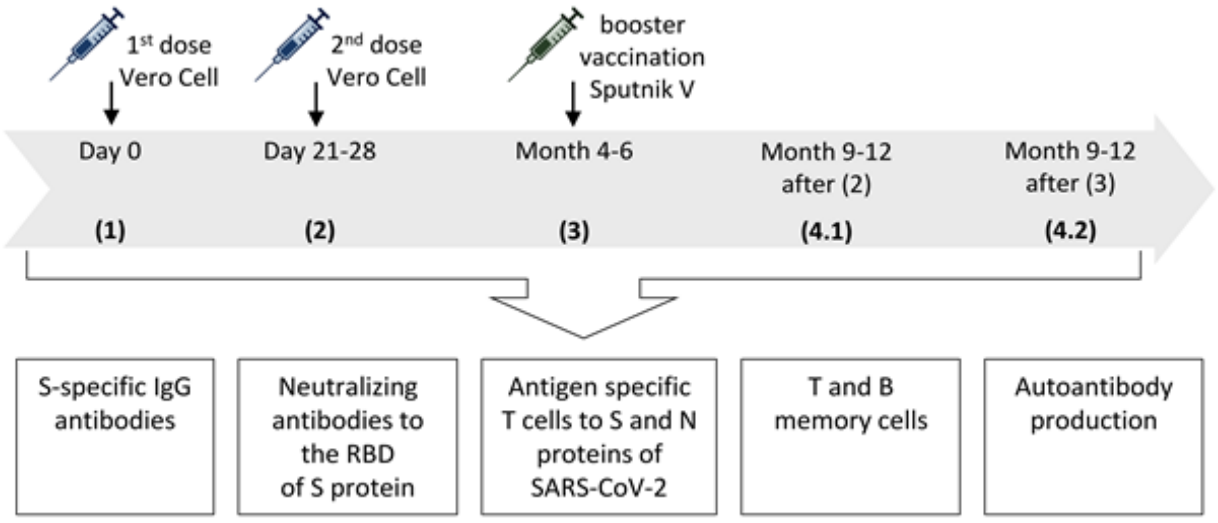

Figure 1 Study design.

The study was approved by the Ethics Committee of the Republican Scientific and Practical Center "Cardiology", and all patients provided informed written consent to participate in the study.

2.2 Peripheral Blood Mononuclear Cells Processing

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood by density gradient centrifugation for 30 min at 1500 rpm at 4°C according to the manufacturer's protocol (Roti-Sep, CarlRoth, Germany). PBMCs were washed twice in physiological saline for 10 min at 1500 rpm at 4°C. The concentration was calculated using a hematology analyzer Micros-60 (ABX, France).

Freshly isolated PBMCs were diluted in a cryopreservation medium based on RPMI-1640 (Gibco, Germany) supplemented with 20% DMSO (Fluka, Germany) and 20% fetal calf serum (Gibco, Germany) at a concentration of 1×10^7 cells/ml and cryopreserved in a programmed CryoMed Controlled Rate Freezer (Model 7451, Thermo Electron, Germany). PBMCs' samples were stored in a cryostorage system Cryo200 (Thermo Electron, Germany) at -180°C.

2.3 Cell Culture Method

To determine SARS-CoV-2-reactive T cells by assessing the expression of intracellular and extracellular activated markers and cytokines, the SARS-CoV-2 T Cell Analysis Kit (REAfinity™, Miltenyi Biotec, Germany) was used. The following antigens were chosen for *in vitro* stimulation of SARS-CoV-2-specific T cells:

- PepTivator SARS-CoV-2 Prot_S (Miltenyi Biotec, Germany) – a pool of lyophilized peptides covering the immunodominant domain of the sequence of surface spike glycoprotein ("S") of SARS-CoV-2 (GenBank MN908947.3, Protein QHD43416.1);
- PepTivator SARS-CoV-2 Prot_N (Miltenyi Biotec, Germany) – a pool of lyophilized peptides consisting of 15-dimensional sequences with an overlap of 11 amino acids, covering the complete sequence of the nucleocapsid phosphoprotein (N) of SARS-CoV-2 (GenBank MN908947.3, Protein QHD43423.2).

PBMCs were cultured at a concentration of 1×10^6 cells (100µL) per well in a 96-well flat-bottom plate in cell culture medium RPMI-1640 (Gibco, Germany) supplemented with 10% fetal calf serum (Capricorn, Germany), 1% L-Glutamine (Elabscience, China) and 1% antibiotic-antimycotic (Elabscience, China) in the presence of SARS-CoV-2 antigens S or N at a final concentration of 1 µg/ml, as well as positive (CytoStim, Miltenyi Biotec, Germany) and negative (sterile water/10% DMSO solution) controls for 6 h in a humidified atmosphere with 5% CO_2 at 37°C before subsequent staining with monoclonal antibodies. After 2 h of cultivation, Brefeldin A (Miltenyi Biotec, Germany) was added to each well, and the cultivation was continued for an additional 4 h.

2.4 Flow Cytometry

2.4.1 PBMCs Phenotyping

For staining, cocktails of monoclonal Abs CD3-FITC (Beckman Coulter, USA), CD19-FITC (Elabscience, China), CD38-PE (Elabscience, China), CD45RO-ECD (Beckman Coulter, USA), and CD27-PE/Cyanine5 (Elabscience, China) were added to 100 µL of the biological sample according to the manufacturer's instructions. Samples with monoclonal Abs were incubated in the dark at room

temperature for 15 min. Data were measured per 10000 T cells or B cells using a 10-channel CytoFlex flow cytometer (Beckman Coulter, USA) and analyzed with CytExpert software (version 2.3.0.84, Beckman Coulter, USA).

2.4.2 Intracellular Staining of Cytokines and Activated Surface Markers

To determine antigen-specific T cells after *in vitro* stimulation with SARS-CoV-2-specific peptides, PBMCs were washed and fixed with Inside Fix (Miltenyi Biotec, Germany) for 20 min at room temperature. After fixation, PBMCs were washed with Inside Perm (Miltenyi Biotec, Germany) and stained for lineage and activation markers as well as cytokines using monoclonal Abs CD3-APC, CD4-Vio Bright B515, CD8-VioGreenTM, IFN-γ-PE, TNF-α-PE-Vio770, CD14-VioBlue, CD20-VioBlue, and CD154-APC-Vio770 in accordance with the SARS-CoV-2 T Cell Analysis Kit, (REAfinity™, Miltenyi Biotec, Germany).

Data were measured per 30000 $CD3^+$ T lymphocytes using a 10-channel CytoFlex flow cytometer (Beckman Coulter, USA) and analyzed with CytExpert software (version 2.3.0.84, Beckman Coulter, USA). Doublets, debris, and dead cells as well as $CD14^+$ and $CD20^+$ cells were excluded. After pre-gating on CD3 as well as CD4 and CD8, respectively, activation marker and cytokine expression were assessed, e.g., CD154 for $CD3^+$ T cells (Figure 2A), CD154 and TNFα for $CD3^+$ T cells (Figure 2B), and TNFα and IFNγ for $CD3^+$ T cells (Figure 2C). Similar populations were analyzed among $CD3^+CD8^+$ T cells.

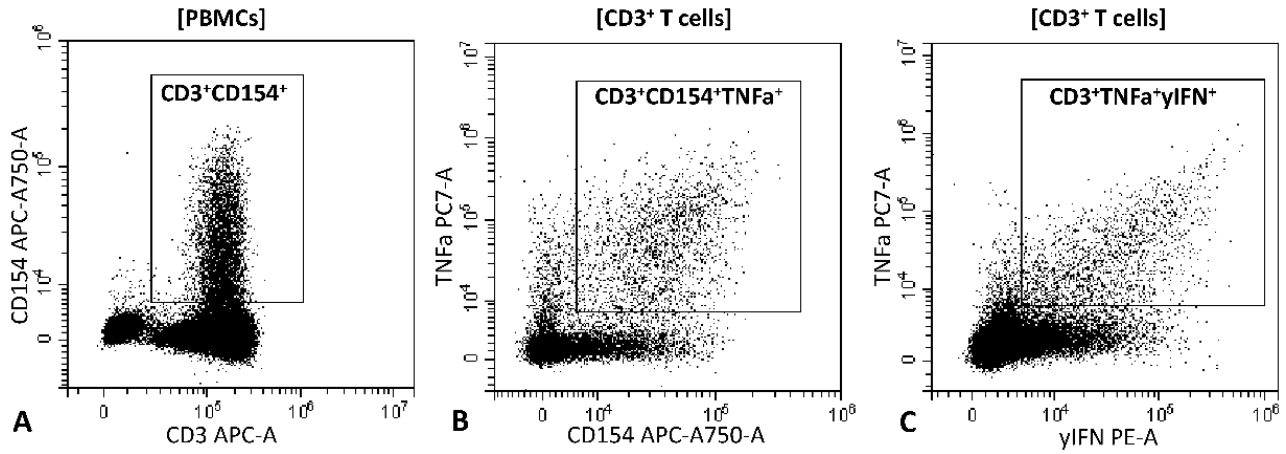

Figure 2 Identification of the main subpopulations of SARS-CoV-2 specific T lymphocytes by flow cytometry in HTRs: A – $CD3^+CD154^+$ T cells, B – $CD3^+CD154^+TNFα^+$ T cells, C – $CD3^+IFNγ^+TNFα^+$ T cells.

2.5 Chemiluminescence Immunoassay

Specific to the coronavirus, S protein IgG Abs were determined by the chemiluminescent microparticle immunoassay with the SARS-CoV-2 IgG II Quant Reagent Kit (Abbott, USA) using an Architect immunochemical analyzer (Abbott i1000 SR, USA). Values <7.1 BAU/ml were considered negative, values >7.1 BAU/ml were considered positive.

2.6 Enzyme-Linked Immunosorbent Assay

Neutralizing Abs to the coronavirus spike-protein receptor-binding domain (RBD) were determined by enzyme immunoassay using the EIA COVID-19 RBD IgG diagnostic kit (TestLine, Czech Republic) according to the manufacturer's instructions. Values <18 BAU/ml were considered negative (no Abs or their level is below the limit of detection), values >22 BAU/ml were considered positive.

Serum Abs were detected using commercial kits presented in Table 2, according to the manufacturer's instructions.

Table 2 Spectrum of investigated autoantibodies.

Investigated autoantibodies	Commercial kit and manufacturer	Cut-off
Rheumatoid factor IgG (RF-IgG)	IgG Rheumatoid Factor ELISA (EUROIMMUN, Germany)	>20 RU/ml
Rheumatoid factor IgA (RF-IgA)	IgA Rheumatoid Factor ELISA (EUROIMMUN, Germany)	>20 RU/ml
Antibodies to cyclic citrulline-containing peptide (anti-CCP)	IMTEC-CCP-Antibodies (IgG) (HUMAN Diagnostics Worldwide, Germany)	>25 U/ml
IgG cardiolipin antibodies (anti-CL)	Anti-Cardiolipin IgG (NeoMedica, Serbia)	>18 GPLU/ml
Antibodies to IgG β2-glycoprotein (anti- β2GP)	Anti-beta-2-Glycoprotein I IgG (NeoMedica, Serbia)	>18 U/ml
Antinuclear antibodies (ANA)	ANA screen (NeoMedica, Serbia)	>1.2 U/ml
Antineutrophil cytoplasmic antibodies (ANCA-Pro)	ANCA-Pro (NeoMedica, Serbia)	>18 U/ml
Antibodies to soluble liver antigen/ hepatic-pancreatic antigen (anti-SLA/LP)	Anti-SLA/LP ELISA (IgG) (EUROIMMUN, Germany)	>20 RU/ml
Antibodies to galactose-deficient IgA (anti-GD-IgA)	Human Galactose-Deficient IgA1 Autoantibody (Anti-GD-IgA1) ELISA Kit (MyBioSource, USA)	-
Antibodies to interferon-alpha (anti-IFN-α)	ELISA Kit for Anti-Interferon Alpha Antibody (Anti-IFNa) (Cloud-Clone Corp., China)	>34 ng/ml

The optical density values were measured with a microplate reader Sunrise (Tecan, Austria) and a Naissa immunoanalyzer (NeoMedica, Serbia) set to 450 nm.

2.7 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8. Data were tested for normality with a Shapiro-Wilk test. The median (Me), lower (Q25), and upper (Q75) quartiles were used as descriptive statistics. Significant differences between the investigated groups were determined by the nonparametric Wilcoxon exact test (W) for dependent variables and the Mann-Whitney U test (M-U) for independent variables. Significance levels were set at $p < 0.05$. The correlation was estimated using Spearman's rank-based correlation coefficients (rho). RStudio version 2024.09.1 (Posit Software, USA) with the corrplot package version 0.95 was used to produce the correlation matrix. Positive correlations were displayed in blue, while negative correlations were shown in red. The color intensity and size of the circles were proportional to the correlation coefficients. To identify associations of HLA gene alleles with elevated autoAbs level, a contingency table and Fisher's exact test were used. Odds ratios (OR) and their 95% confidence intervals (CI) were adjusted in the GraphPad Prism by the Woolf logit method.

3. Results

3.1 Humoral Immune Response in HTRs Following Vaccination Against Coronavirus Infection

To characterize humoral post-vaccination immunity in HTRs, specific IgG Abs to the spike-protein (S-protein) of the SARS-CoV-2 were assessed in dynamics (Figure 3). The number of seronegative HTRs with no history of COVID-19 before vaccination was 44.4% (Figure 3) and seropositive 55.6%, which was confirmed by pre-vaccination Abs to the S-protein on average 464.6 BAU/ml. Therefore, all investigated HTRs were divided into 2 groups: group 1 consisted of HTRs who did not present with coronavirus infection before vaccination and did not have specific Abs to the S-protein; group 2 consisted of HTRs who had suffered from coronavirus infection 3-6 months before vaccination, or who had not had coronavirus infection but showed positive values of specific Abs to the S-protein (asymptomatic disease) (Figures 3B, C).

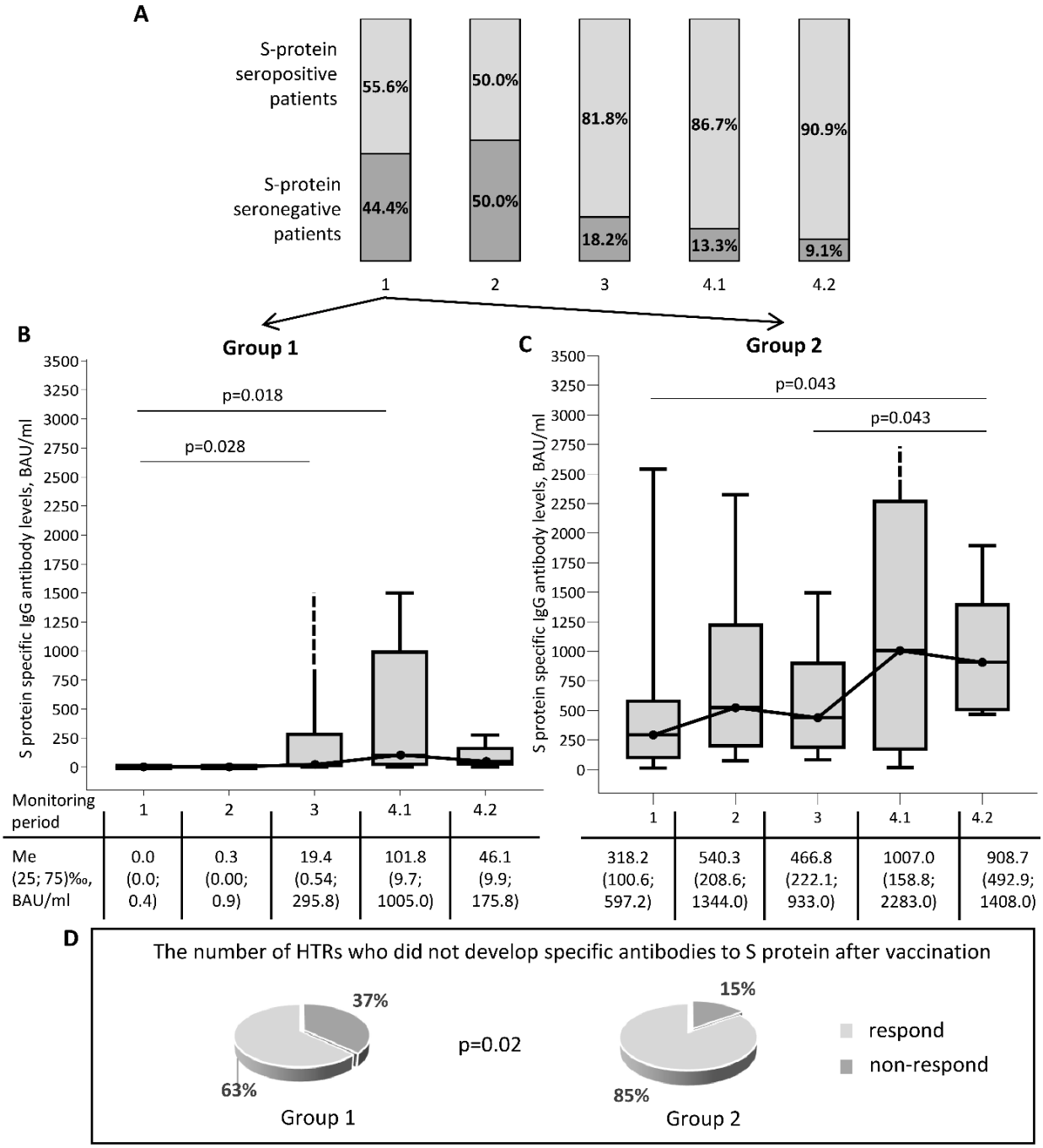

Figure 3 Dynamic Abs to the S-protein in HTRs after vaccination against coronavirus infection: A – percentage of seropositive and seronegative HTRs after vaccination; B – IgG to S protein in group 1; C – IgG to S protein in group 2; D – number of HTRs who did not develop specific antibodies to the S protein after vaccination.

In vaccinated individuals from group 1, increased Abs to the S-protein were observed starting from 4-6 months after the secondary immunization, with statistically significant differences seen for 9-12 months following basic 2-dose vaccination (0.0 (0.0; 0.04) and 101.8 (9.7; 1005.0) BAU/ml, p = 0.018, respectively, W). However, the antibody concentration remained relatively low, and 37% of HTRs from group 1 did not respond to vaccination, which was evidenced by the lack of virus-specific antibody production throughout the follow-up period (Figure 3D).

In HTRs with a history of coronavirus infection (group 2), high Abs to the S-protein were determined before vaccination as compared to individuals with no history of COVID-19 (318.2 (100.6; 597.2) and 0.0 (0.0; 0.04) BAU/ml, p = 0.000…, M-U). After the subsequent 2-dose basic vaccination, the Abs level remained stable for 6 months (p = 0.98, W). In the long-term period, a trend towards increased IgG to the S-protein was seen 9-12 months following the second Vero Cell vaccine, with significantly higher Abs to the S-protein recorded by 9-12 months after booster (318.2 (100.6; 597.2)) *vs* (908.7 (492.9; 1408.0) BAU/ml, p = 0.043, respectively, W). Herewith, the number of patients who did not respond to vaccination (decreased initial Abs during the follow-up) was 15%, which is significantly lower compared to group 1 (p = 0.02) (Figure 3D).

Figure 4 presents the results of the correlation analysis between S-reactive IgG Abs and neutralizing IgG Abs to the RBD of the SARS-CoV-2 S protein at different points of the follow-up after 2- and 3-dose vaccination. In group 1, a strong positive statistically significant correlation between the studied parameters appeared 4-6 months after the secondary immunization and persisted during a year after 2-dose and 3-dose vaccination schedule. In group 2, a strong positive statistically significant correlation between neutralizing and virus-specific IgG to the S-protein was recorded at the initial point before vaccination and persisted up to 4-6 months after the secondary immunization.

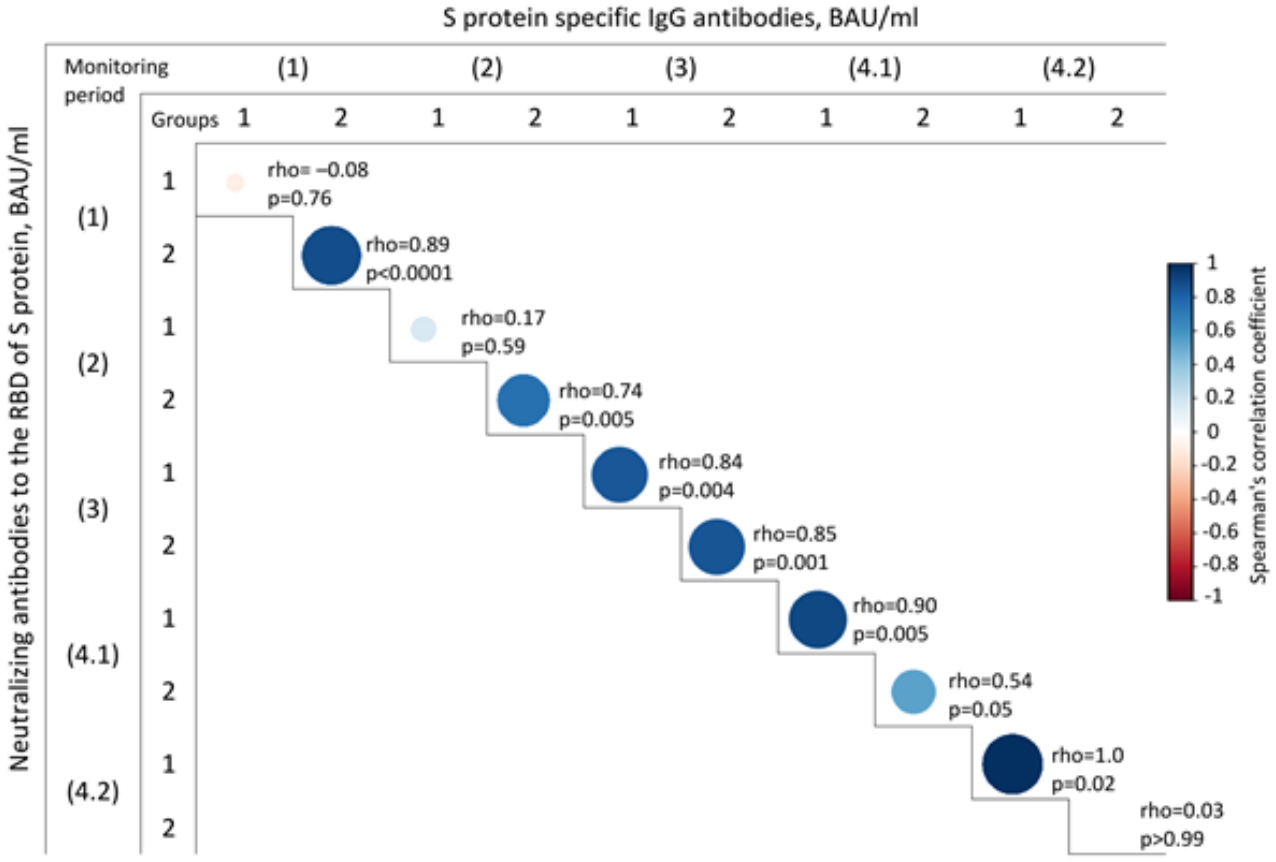

Figure 4 Correlation of Abs to the S-protein with neutralizing Abs to the RBD.

3.2 Dynamic in Vitro Assessment of SARS-CoV-2-Reactive T Cells in HTRs Following Vaccination

Table 3 shows the number of virus-specific T lymphocytes reacting *in vitro* with SARS-CoV-2 S- and N-peptides.

Table 3 The number of S- and N-reactive T cells in HTRs in dynamics following vaccination against coronavirus infection, *% (Me (25; 75)‰).*

T cell phenotype	*in vitro* stimulator	Group	No	Monitoring period					p(W)
				before vaccination	21-28 days after dose 1	4-6 months after dose 2	9-12 months after dose 2	9-12 months after booster	
				1	**2**	**3**	**4.1**	**4.2**	
$CD3^+154^+$	S	1	a	0.96 (0.43; 1.42)	1.11 (0.82; 1.56)	0.54 (0.52; 1.16)	0.72 (0.19; 1.44)	1.35 (0.29; 1.65)	
		2	b	0.93 (0.63; 1.49)	1.00 (0.52; 2.12)	1.81 (1.25; 2.93)	1.19 (1.10; 2.48)	0.90 (0.64; 0.93)	$p_{1-3} = 0.043$
	N	1	c	0.39 (0.23; 0.60)	1.43 (0.94; 2.22)	0.42 (0.32; 1.14)	1.17 (0.84; 1.31)	0.38 (0.23; 1.76)	$p_{1-2} = 0.028$
		2	d	0.71 (0.40; 1.28)	0.64 (0.46; 0.87)	1.71 (0.95; 2.69)	1.04 (0.79; 2.24)	0.33 (0.22; 0.64)	$p_{1-3} = 0.043$
$CD3^+IFN\gamma^+TNF\alpha^+$	S	1	e	0.19 (0.09; 0.30)	0.07 (0.05; 0.12)	0.04 (0.03; 0.13)	0.05 (0.03; 0.12)	0.03 (0.02; 0.04)	
		2	f	0.18 (0.06; 0.44)	0.23 (0.09; 0.32)	0.26 (0.12; 0.53)	0.26 (0.13; 0.40)	0.13 (0.11; 0.56)	$p_{1-2} = 0.08$ $p_{1-3} = 0.07$
	N	1	g	0.15 (0.08; 0.25)	0.03 (0.01; 0.08)	0.09 (0.06; 0.14)	0.10 (0.06; 0.14)	0.05 (0.03; 0.08)	
		2	h	0.07 (0.05; 0.45)	0.14 (0.11; 0.41)	0.43 (0.09; 1.47)	0.07 (0.03; 0.16)	0.09 (0.09; 0.10)	$p_{1-2} = 0.014$ $p_{1-3} = 0.040$
$CD8^+IFN\gamma^+TNF\alpha^+$	S	1	i	0.19 (0.00; 0.35)	0.07 (0.02; 0.12)	0.20 (0.03; 0.34)	0.05 (0.03; 0.16)	0.06 (0.02; 0.08)	
		2	j	0.30 (0.08; 0.50)	0.25 (0.08; 0.41)	0.50 (0.39; 1.67)	0.47 (0.31; 0.60)	0.23 (0.09; 0.52)	$p_{1-3} = 0.012$
	N	1	k	0.13 (0.05; 0.31)	0.04 (0.01; 0.16)	0.06 (0.03; 0.12)	0.15 (0.06; 0.24)	0.04 (0.03; 0.06)	
		2	l	0.08 (0.01; 0.20)	0.24 (0.18; 0.38)	0.49 (0.34; 1.40)	0.07 (0.02; 0.26)	0.07 (0.04; 0.10)	$p_{1-3} = 0.043$
p(W)				$p_{a-c} = 0.028$ $p_{b-d} = 0.010$ $p_{f-h} = 0.006$ $p_{j-l} = 0.021$			$p_{f-h} = 0.07$	$p_{b-d} = 0.043$ $p_{f-h} = 0.07$	
p(M-U)					$p_{g-h} = 0.004$	$p_{a-b} = 0.042$ $p_{e-f} = 0.051$ $p_{i-j} = 0.017$		$p_{i-j} = 0.019$	

Previously provided findings demonstrate an insignificant number of antigen-specific S-reactive T lymphocytes [15-17]. It differs in individuals who have not had a coronavirus infection before vaccination compared to patients with a history of COVID-19. In patients from group 2, a significantly increased number of S-specific $CD3^+154^+$ cells were observed 4-6 months after the secondary immunization (before booster), along with a tendency for increased T lymphocytes with intracellular IFNγ and TNFα production ($p = 0.07$, W), followed by a decrease over the year. A similar analysis of the number of virus-specific T lymphocytes was carried out for the main subpopulations of lymphocytes expressing $CD4^+$ and $CD8^+$ co-receptors. Differences in the number of S-reactive $CD8^+IFN\gamma^+TNF\alpha^+$ T lymphocytes were also observed in dynamics 4-6 months after the secondary immunization, indicating a trend towards an increase in patients with a history of coronavirus infection.

The most significant changes of virus-specific T lymphocytes reacting *in vitro* with the SARS-CoV-2 Prot_N nucleocapsid phosphoprotein (N-protein) between the two study groups were observed 4-6 months after the secondary immunization, with statistical differences in the number of N-reactive $CD3^+154^+$, $CD3^+IFN\gamma^+TNF\alpha^+$, $CD8^+IFN\gamma^+TNF\alpha^+$ T lymphocytes, mostly in HTRs with a history of COVID-19. It should be noted that when analyzing the dynamic changes in the number of antigen-specific T cells to N-protein in group 1, increased reactive $CD3^+154^+$ were observed 21-28 days after the primary immunization. Booster vaccination did not result in statistically significant changes in the number of specific T lymphocyte subpopulations during the 9-12 months follow-up. In general, the cellular immune response to the S-protein prevailed over the reaction of T cells to the N-protein. At the same time, HTRs with a previous coronavirus infection, in contrast with seronegative HTRs, showed a greater number of specific N-reactive cells after vaccination ($CD3^+IFN\gamma^+TNF\alpha^+$ 21-28 days after the primary immunization) and S-reactive cells ($CD3^+154^+$, $CD3^+IFN\gamma^+TNF\alpha^+$, $CD8^+IFN\gamma^+TNF\alpha^+$ 4-6 months after the secondary immunization and $CD8^+IFN\gamma^+TNF\alpha^+$ a year after booster). In our study, S- and N-reactive T cells were detected in 5-40% of HTRs at different follow-up periods after vaccination, mainly in those with a history of COVID-19.

3.3 Memory Cell Dynamics in HTRs Following SARS-CoV-2 Vaccination

Table 4 presents the results of statistical processing of the memory cells (memory B cells $CD19^+CD27^+$, $CD27^{high}CD38^{high}$ plasmablasts, naïve T $CD45RO^-CD27^+$ lymphocytes, terminally-differentiated memory T cells $CD45RO^-CD27^-$) over time following vaccination.

Table 4 Dynamic memory cells in HTRs after vaccination against coronavirus infection, %, *(Me (25; 75)‰).*

Monitoring period	Subpopulations of T and B cells			
	B cells		T cells	
	$CD19^{+}27^{+}$	$CD27^{high}38^{high}$	$CD45RO^{-}27^{+}$	$CD45RO^{-}27^{-}$
1 before vaccination	32.35 (29.08; 39.75)	0.07 (0.03; 0.16)	36.30 (27.40; 44.90)	19.50 (11.40; 27.20)
2 21-28 days after dose 1	36.70 (32.25; 51.90)	0.28 (0.09; 0.67)	30.40 (16.90; 49.00)	28.90 (8.14; 36.70)
3 4-6 months after dose 2	39.40 (36.10; 48.20)	0.30 (0.11; 0.35)	20.70 (11.50; 49.80)	15.10 (10.80; 30.70)
4.1 9-12 months after dose 2	56.70 (54.90; 59.90)	0.48 (0.36; 0.61)	22.50 (20.40; 37.50)	22.20 (16.80; 33.80)
4.2 9-12 months after booster	31.20 (23.90; 38.90)	0.42 (0.14; 1.30)	22.70 (21.90; 34.30)	24.60 (16.00; 28.90)
p(W)	$p_{1-3} = 0.062$ $p_{1-4.1} = 0.043$	$p_{1-2} = 0.054$ $p_{1-3} = 0.032$ $p_{1-4.1} = 0.034$ $p_{1-4.2} = 0.036$	$p_{1-3} = 0.043$	$p_{1-2} = 0.097$ $p_{1-4.2} = 0.061$

Three weeks after the primary immunization, a trend towards increased $CD27^{high}CD38^{high}$ cells and $CD45RO^{-}27^{-}$ T cells was observed, combined with decreased naïve $CD45RO^{-}27^{+}$ T lymphocytes.

When analyzing the number of memory cell subpopulations 4-6 months after the secondary immunization, increased $CD27^{high}CD38^{high}$ plasmablasts were found ($p = 0.032$, W). A tendency towards an increase in naïve $CD19^{+}CD27^{+}$ B cells was also seen. In patients after the secondary immunization, the number of memory B cells significantly exceeded similar values before vaccination ($p = 0.043$ for $CD19^{+}27^{+}$ and $p = 0.034$ for $CD27^{high}CD38^{high}$, W) 9-12 months later.

Moderate positive correlations were found between the number of plasmablasts and neutralizing and S-specific antibody levels in different periods of monitoring the post-vaccination immunity (from rho = 0.35 to rho = 0.44, $p = 0.046$ and $p = 0.003$, respectively). Therefore, the number of $CD27^{high}CD38^{high}$ B lymphocytes may be included in the list of immunological criteria for assessing the efficacy of vaccination against COVID-19.

3.4 Analysis of Post-Vaccination Autoantibody Production in HTRs Following SARS-CoV-2 Vaccination

Table 5 presents the serum autoAbs characteristics of HTRs following vaccination. The median concentrations of all investigated parameters were recorded at levels below the reference positive values of the diagnostic test system used, with no statistically significant changes observed in different follow-up periods after vaccination.

Table 5 Dynamic serum autoAb levels in HTRs after vaccination against coronavirus infection, % *(Me (25; 75)‰).*

Parameter	Monitoring period				
	before vaccination **1**	21-28 days after dose 1 **2**	4-6 months after dose 2 **3**	9-12 months after dose 2 **4.1**	9-12 months after booster **4.2**
RF-IgG, *RU/ml*	38.7 (26.8; 48.4)	36.1 (25.4; 46.5)	34.8 (28.6; 44.1)	36.4 (26.5; 62.9)	34.3 (28.7; 43.1)
RF-IgA, *RU/ml*	48.1 (24.6; 92.3)	29.3 (13.6; 65.5)	55.8 (19.3; 68.9)	41.8 (13.3; 69.1)	20.0 (17.2; 69.4)
Anti-CCP, *U/ml*	1.90 (1.50; 3.00)	2.45 (1.78; 6.05)	1.30 (0.92; 2.65)	1.58 (1.27; 2.29)	1.03 (0.75; 1.78)
Anti-cardiolipin IgG, *U/ml*	1.50 (0.59; 2.47)	2.03 (0.58; 2.73)	1.35 (0.50; 1.96)	0.70 (0.50; 1.09)	2.36 (1.67; 6.95)
Anti-β2 GPI IgG, *U/ml*	2.84 (2.36; 4.78)	4.02 (2.37; 5.09)	2.25 (1.51; 4.31)	4.01 (3.78; 5.47)	5.74 (5.07; 6.81)
ANA (IgG), *U/ml*	0.14 (0.14; 0.19)	0.15 (0.13; 0.23)	0.16 (0.13; 0.22)	0.14 (0.13; 0.23)	0.16 (0.14; 0.20)
ANCA-Pro, *U/ml*	0.67 (0.56; 0.89)	0.76 (0.50; 1.35)	0.50 (0.50; 0.69)	0.57 (0.50; 1.29)	0.86 (0.50; 1.01)
Anti-SLA/LP, *RU/ml*	3.02 (0.00; 6.08)	3.36 (2.34; 7.34)	2.34 (0.00; 5.24)	3.02 (2.00; 4.64)	2.34 (0.00; 5.57)
Anti-GD-IgA1, *ng/ml*	0.00 (0.00; 0.00)	0.00 (0.00; 0.81)	1.13 (0.86; 1.42)	0.00 (0.00; 0.00)	0.03 (0.02; 0.71)

An individual analysis revealed increased IgA rheumatoid factor (RF-IgA) in 6.5% of HTRs by 4-6 months after the secondary immunization (43.5 (41.2; 45.8) vs 104.7 (84.1; 125.2) RU/ml) and in 16.1% of HTRs during the 9-12 months follow-up after the secondary immunization (30.0 (21.9; 60.5) *vs* 72.8 (57.8; 126.0) RU/ml), which was transient. Herewith, no correlation was found between the RF-IgA and S-specific Abs (rho = -0.19, p = 0.12) (Figure 5).

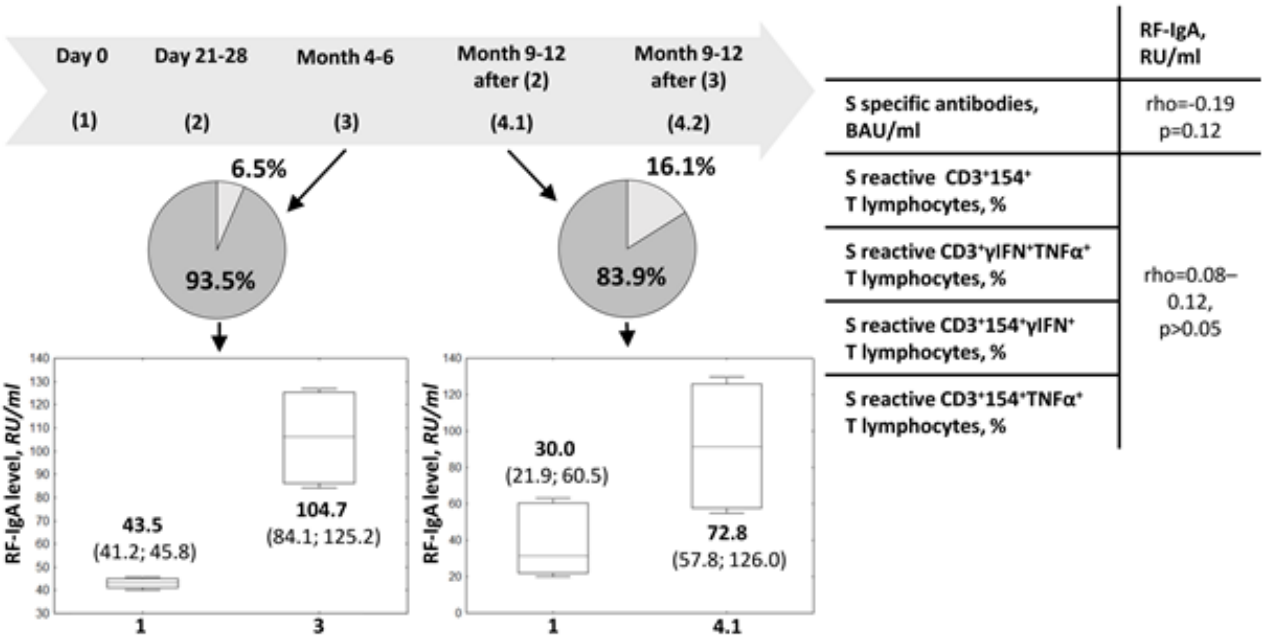

Figure 5 Individual analysis of serum RF-IgA in HTRs in dynamics after vaccination against coronavirus infection.

During the pandemic, the presence of autoAbs to IFN-I was reported in a proportion of patients (25%, autoAbs concentration >34 ng/ml) with a severe coronavirus infection [18]. In this regard, in HTRs vaccinated against coronavirus infection, the production of serum anti-IFN-α Abs was assessed. In HTRs from group 1, the median level of anti-IFN-α Abs remained below the accepted threshold (≤34 ng/ml) throughout the entire follow-up after vaccination (Table 6).

Table 6 Dynamic anti-IFN-α Abs in HTRs after vaccination against coronavirus infection, *ng/ml*, *(Me (25; 75)‰).*

Groups	Monitoring period					p(W)
	before vaccination **1**	21-28 days after dose 1 **2**	4-6 months after dose 2 **3**	9-12 months after dose 2 **4.1**	9-12 months after booster **4.2**	
1	15.5 (5.5; 35.2)	19.5 (3.1; 27,1)	16.3 (5.8; 36.2)	15.1 (6.1; 36.0)	5.6 (2.5; 28.3)	$p_{1\text{-}4.2} = 0.068$
2	22.4 (10.7; 36.3)	19.2 (4.0; 36.1)	20.9 (8.5; 37.0)	51.8 (40.7; 68.5)	29.8 (15.2; 43.2)	$p_{1\text{-}4.1} = 0.043$ $p_{1\text{-}4.2} = 0.08$
p(M-U)				0.07	0.015	

The number of HTRs with anti-IFN-α Abs ranged from 30% (4-6 months after the basic 2-dose vaccination) to 0% at 9-12 months following booster (Figure 6). In HTRs from group 2, a statistically significant increase in the level of anti-IFN-α Abs was seen for a period of 9-12 months after the basic vaccination, with a continuing rise by 9-12 months after the booster. A growth in the total number of anti-IFN-α Abs in subjects from group 2 was also confirmed by an increased percentage of HTRs presenting with increased anti-IFN-α Abs from 28.6% at 21-28 days after the primary

immunization to 83.3% by 9-12 months after the secondary immunization (Figure 6). There was no correlation between the level of anti-IFN-α Abs and the concentration of S-specific Abs, along with the established relationship between the investigated indicator and the antigen-specific post-vaccination immune response (anti-IFN-α Abs *vs* S-reactive $CD3^+IFN\gamma^+TNF\alpha^+$: rho = 0.83, p = 0.05).

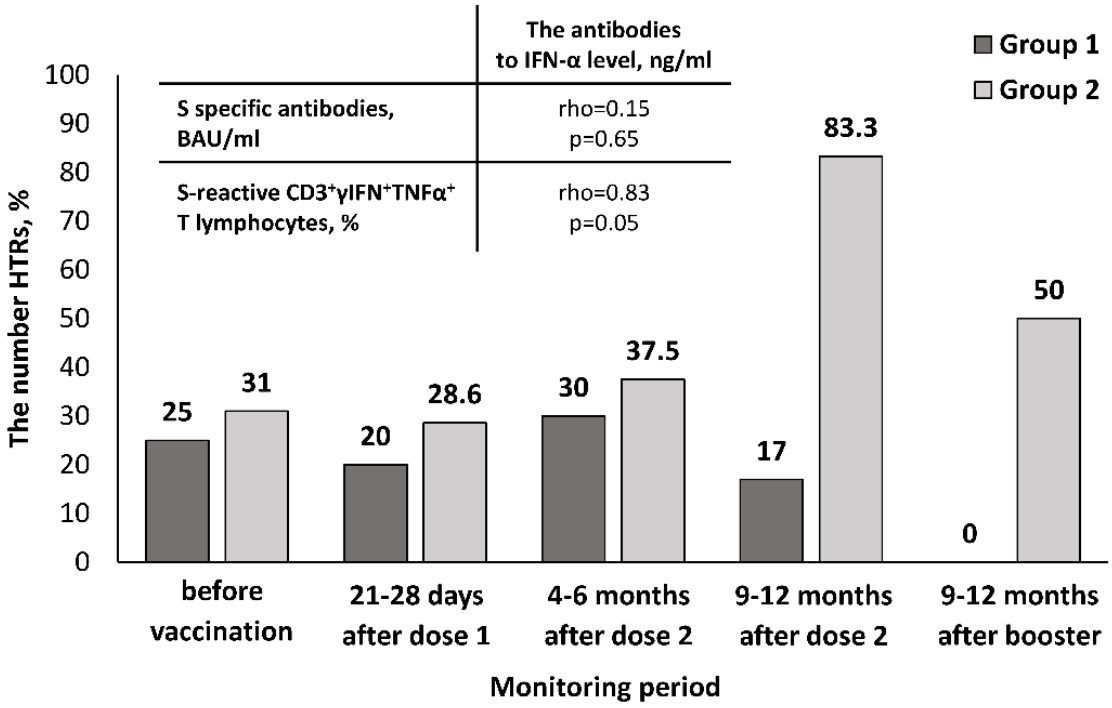

Figure 6 The number of HTRs with increased anti-IFN-α Abs in dynamics after vaccination against coronavirus infection.

Taking into account the available information on HTR' HLA typing, we assessed the increased level of anti-IFN-α Abs in association with specific genetic variants of HLA (Table 7). HTRs with the HLA-B27 allele had a lower adds of increased anti-IFN-α Abs. In comparison, carriers of HTRs with HLA-DRB1*03 and HLA-DQB1*02 alleles had higher odds of increased anti-IFN-α Abs following a viral infection or vaccination.

Table 7 HLA alleles association with increased anti-IFN-α antibodies in HTRs after vaccination against coronavirus infection.

		Allele	Occurrence, %	Odds ratio [95% confidence interval]	p	HLA association
HLA loci (phenotype)	A	1	11.8	4.75 [0.44-51.11]	0.29	predisposition
		2	41.2	0.36 [0.09-1.54]	0.30	
		3	29.4	2.67 [0.59-12.02]	0.27	
		11	17.6	1.42 [0.24-8.26]	>0.99	
		23	2.9	4.24 [0.16-111.7]	0.43	
		24	20.6	0.28 [0.05-1.64]	0.24	
		25	8.8	11.48 [0.54-241.3]	0.07	
		26	14.7	0.29 [0.03-2.87]	0.37	

	28	8.8	0.64 [0.05-7.83]	>0.99	
	29	2.9	4.24 [0.16-111.7]	0.43	
	30	5.9	1.36 [0.08-23.61]	>0.99	
	31	5.9	1.36 [0.08-23.61]	>0.99	
	32	2.9	0.42 [0.02-11.03]	>0.99	
	7	26.5	0.58 [0.12-2.85]	0.7	
	8	8.8	2.92 [0.24-35.68]	0.56	
	13	2.9	4.24 [0.16-111.70]	0.43	
	14	2.9	0.42 [0.02-11.03]	>0.99	
	18	20.6	2.06 [0.38-11.04]	0.43	
	27	17.6	0.07 [0.003-1.40]	0.03	resistance
	35	17.6	0.62 [0.10-3.91]	0.68	
	37	5.9	7.59 [0.34-170.80]	0.18	
	38	14.7	2.25 [0.33-15.54]	0.63	
B	41	5.9	1.36 [0.08-23.61]	>0.99	
	44	14.7	2.25 [0.33-15.54]	0.63	
	47	2.9	0.42 [0.02-11.03]	>0.99	
	51	8.8	0.64 [0.05-7.83]	>0.99	
	52	2.9	0.42 [0.02-11.03]	>0.99	
	56	2.9	0.42 [0.02-11.03]	>0.99	
	57	14.7	2.25 [0.33-15.54]	0.63	
	58	2.9	4.24 [0.16-111.70]	0.43	
	60	5.9	1.36 [0.08-23.61]	>0.99	
	61	5.9	1.36 [0.08-23.61]	>0.99	
	62	11.8	0.41 [0.04-4.34]	0.62	
	1	16.7	0.65 [0.05-8.02]	>0.99	
	2	27.8	0.29 [0.03-2.92]	0.37	
	4	44.4	0.76 [0.15-3.92]	>0.99	
C	5	16.7	11.87 [0.56-251.00]	0.07	predisposition
	6	44.4	2.96 [0.57-15.40]	0.24	
	7	88.9	1.11 [0.28-4.42]	>0.99	
	9	22.2	0.41 [0.03-4.43]	0.62	
	10	16.7	3.00 [0.24-36.88]	0.56	
	*01	32.3	0.51 [0.10-2.53]	0.47	
	*03	16.1	25.24 [1.25-509.40]	0.006	predisposition
	*04	16.1	2.55 [0.36-17.96]	0.37	
	*07	12.9	1.55 [0.19-12.64]	>0.99	
	*08	9.7	0.17 [0.01-3.68]	0.25	
DRB1	*09	3.2	4.68 [0.18-124.2]	0.41	
	*11	22.6	0.51 [0.08-3.14]	0.67	
	*13	16.1	2.55 [0.36-17.96]	0.37	
	*14	3.2	0.46 [0.02-12.10]	>0.99	
	*15	35.5	0.76 [0.17-3.42]	>0.99	
	*16	19.4	0.23 [0.02-2.28]	0.36	

	*02	19.4	11.25 [1.13-112.5]	0.03	predisposition
	*03	41.9	0.86 [0.20-3.63]	>0.99	
	*04	6.5	0.26 [0.01-5.86]	0.50	
DQB1	*05	48.4	0.32 [0.07-1.43]	0.17	
	*06	45.2	1.18 [0.28-4.88]	>0.99	
	*08	3.2	4.68 [0.18-124.2]	0.41	
	*15	3.2	4.68 [0.18-124.2]	0.41	

4. Discussion

The epidemiological situation changed due to the spread of COVID-19, necessitating the revision of all usual norms, including schedules and protocols for managing patients with various pathologies. As a respiratory virus infection, COVID-19 is highly dangerous for patients with comorbidities, reduced or suppressed immunity [19]. HTRs are at a particular risk [20]. Vaccines against symptomatic COVID-19 demonstrate a marked efficacy within clinical trials of immunocompetent individuals with significantly reduced COVID-19 severity, hospital admission, and death rates [21]. Most vaccines target the virus spike protein and RBD, which facilitate viral entry. They are designed to stimulate both cellular (T-regulatory and T helper cells) and humoral (IgG anti-spike and/or anti-RBD antibody) immune response [22]. Since immunosuppressed SOTRs are at an increased risk of poor COVID-19 outcomes, most organizations, including the ISHLT, have promoted vaccination for this population despite existing uncertainty about vaccine responses and their clinical efficacy [23]. However, throughout the pandemic, cases of autoimmune disease associated with the COVID-19 immunization have been reported [12, 13]. Accumulated evidence suggests that SARS-CoV-2 possesses the ability to cause hyperstimulation of the immune system, resulting in the synthesis of multiple autoAbs with a trigger effect on pre-existing autoimmune diseases [14]. Thus, with the advent of COVID-19 vaccines and their widespread large-scale use, there appeared an increased interest in studying their effects in immunocompromised patients, in particular those after SORTs.

In our study, post-vaccination immunity (production of virus-specific IgG to the S-protein, identification of SARS-CoV-2-reactive T lymphocytes, T and B memory cells) was analyzed. The possible development of autoimmune reactions in HTRs, followed either a 2-dose vaccination with the whole inactivated Vero Cell vaccine (China), or a 3-dose vaccination (full vaccination schedule) with the combined vector vaccine Gam-COVID-Vac (Sputnik V, Russia) as a booster, was assessed. Available data suggest that COVID-19 vaccines may be less effective in the immunocompromised population, who are at an increased risk of severe coronavirus infection [2, 24]. The study of the immunological efficacy of vaccines is classically carried out by comparing the titers of specific Abs in the blood serum of individuals vaccinated before and at different periods after immunization, as well as by comparing these results with the antibody levels obtained at the same time during examination of those who were administered a placebo or a comparison drug. The presented data show that before vaccination, 55.6% of the investigated HTRs with no history of coronavirus infection had Abs to the SARS-CoV-2 S protein, which is indicative of an asymptomatic disease in this group (Figure 3). Therefore, to correctly assess the post-vaccination immunity, the studied HTRs were divided into 2 groups: seronegative before vaccination (group 1) and seropositive before vaccination (group 2). In seronegative recipients, despite significantly increased IgG to S-protein recorded both 9-12 months following the basic 2-dose vaccination and 9-12 months after booster,

the Abs concentration remained relatively low. In seropositive patients, a high Abs level to S-protein (318.2 (100.6; 597.2) BAU/ml) was determined before vaccination, which was then recorded throughout the entire follow-up period with a statistically significant increase by 9-12 months after booster (908.7 (492.9; 1408.0) BAU/ml, p = 0.043). According to our data, more effective humoral post-vaccination immunity in individuals who have recovered from COVID-19 is confirmed by both the number of recipients who responded to vaccination ((85%, group 2) *vs* 67% (group 1), p = 0.02) (Figure 3) and the number of individuals who have fallen ill with COVID-19 during the vaccination period (9.5% (group 2) *vs* 36.8% (group 1), p = 0.015) (Table 1). Boyarsky et al assessed the immune response to the second dose of mRNA vaccines (Pfizer-BioNTech and Moderna) in SORTs. Anti-spike protein Abs were detected in 54% of individuals at a median of 29 days from the second vaccine dose, in stark contrast to higher rates noted in the general population. 57% of HTRs had detectable IgG, while the lung transplant recipients showed IgG Abs in 39% only [3]. Ran Zhuo et al compared the amount of SARS-CoV-2 S-protein Abs using the WHO International Standard Units and demonstrated that natural SARS-CoV-2 infection induced a greater antibody response as compared to vaccines. This is evidenced by a significantly higher neutralizing antibody titer in seroconverted unvaccinated individuals [4, 10]. Our results are consistent with the numerous data indicating that in vaccinated patients with previous COVID-19, hybrid immunity is characterized by a more pronounced regulatory and functional potential [4, 11, 25] and is accompanied by a persisting high level of virus-specific Abs to the S-protein in the post-vaccination follow-up in dynamics.

Coronavirus-specific T cells were expected to be present only in certain individuals, with their frequency being low compared to other specific T cells. The intensity of post-vaccination virus-specific immunity in HTRs was assessed by *in vitro* stimulation of antigen-specific T cells with SARS-CoV-2 S- and N-proteins, which, in turn, causes the secretion of effector cytokines and up-regulation of activation markers, followed by the detection and isolation of antigen-specific T cells. As can be seen from the data provided (Table 3), antigen-specific S- and N-reactive T lymphocytes were recorded in HTRs from both study groups before vaccination. This was followed by an increase in $CD3^+154^+$, $CD3^+IFN\gamma^+TNF\alpha^+$, and $CD8^+IFN\gamma^+TNF\alpha^+$ by 4-6 months after the secondary immunization. During a subsequent monitoring of post-vaccination cellular immunity, the number of S- and N-reactive T cells decreased regardless of whether the vaccine was boosted. Dynamic differences in the number of spike-reactive $CD8^+IFN\gamma^+TNF\alpha^+$ T lymphocytes were also seen 4-6 months after the secondary immunization. Braun J. et al detected spike-reactive $CD4^+$ T cells not only in 83% of patients with COVID-19 but also in 35% of healthy donors and indicated that spike protein cross-reactive T cells were present, having probably been generated during previous encounters with endemic coronaviruses [26]. According to our data, S- and N-reactive T cells were detected in 5-40% of HTRs at different follow-up periods after vaccination. According to Sizyakina et al, mild and moderate coronavirus infection results in changed parameters of the immune system functioning, recorded one year after infection. The maximum range of changes affects the humoral adaptive immunity. The authors have shown that the restructuring of the immune response after COVID-19 ensures high immunogenicity with even a single dose of the vaccine based on SARS-CoV-2 peptide antigens (EpiVacCorona). The secondary response was accompanied by decreased circulating $CD4^+$T lymphocytes, which interacted with antigen-presenting cells. There was an increased $CD40^+$ B lymphocytes responsible for T-B cooperation and a reduced proportion of circulating unswitched memory B cells [10].

When monitoring immunological parameters in HTRs, starting from 3 weeks after the primary immunization, the most pronounced changes were characterized by a statistically significant increase in the number of $CD27^{high}CD38^{high}$ plasmablasts, which persisted for 9-12 months after both the basic and full (booster including) vaccination with positive correlations between the number of plasmablasts and the level of neutralizing and S-specific Abs. Moreover, following 2-dose vaccination 9-12 months later, the total number of $CD19^{+}CD27^{+}$ memory B cells exceeded similar values before immunization. Schulz E. et al., 2021 showed, that absolute numbers of $CD19^{+}IgD^{+}CD27^{-}$ naïve B cells, $CD19^{+}IgD^{+}CD27^{+}$ pre-switched memory B cells, and $CD19^{+}IgM^{-}CD38^{++}$ plasmablasts were significantly associated with the anti-SARS-CoV-2 response in univariate analysis. However, only the number of $CD19^{+}IgD^{+}CD27^{-}$ naïve cells was considered to be an independent predictor of a strong vaccination response in multivariate analysis, indicating their functional importance for inducing a humoral immune response [27]. Indeed, the production of specific Abs to a new antigen depends on antigen-specific B cell clones in the population of $CD19^{+}IgD^{+}CD27^{-}$ naïve B cells. Thus, a sharply reduced pool of $CD19^{+}IgD^{+}CD27^{-}$ naïve cells diminishes the likelihood of the emergence of B cells with a sufficiently avid B cell receptor that can successfully interact with follicular T helper cells and subsequently undergo somatic hypermutation with an optimal antibody response. Therefore, the association between the magnitude of the humoral post-vaccination response and the increase in $CD19^{+}IgD^{+}CD27^{-}$ naïve B cells and $CD27^{high}CD38^{high}$ plasmablasts is most likely a causal relationship [21]. Herewith, decreased $CD45RO^{-}CD27^{+}$ naïve T cells were observed 4-6 months after basic vaccination, persisting for a year after booster, combined with a tendency to increased $CD45RO^{-}CD27^{-}$ cells. This indicates the immunogenesis of T and B lymphocytes in peripheral lymphoid organs following antigen stimulation, which leads to their differentiation into activated and terminally differentiated memory cells.

A possible link between COVID-19 vaccination and autoimmune/inflammatory disease has been demonstrated by numerous researchers. Like many viruses, such as Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus, and human T-lymphotropic virus-1, SARS-CoV-2 may possess the ability to promote autoimmune diseases. The mechanisms of their induction by COVID-19 vaccination and SARS-CoV-2 infection may be similar. The most likely contributing factors to autoimmune diseases in COVID-19 are the ability of SARS-CoV-2 to hyperstimulate the immune system, induce excessive netosis and neutrophil-associated cytokine reactions, and molecular similarity between the host's and virus antigens [13, 14, 18].

To exclude adverse effects due to vaccination against SARS-CoV-2, a study of the blood serum of HTRs was performed to detect the following autoAbs in the dynamics of the post-vaccination period: (RF-IgG and RF-IgA), anti-CCP, anti-CL, anti-β2GP, ANA, ANCA-Pro, anti-SLA/LP, anti-GD-IgA, and anti-IFN-α. In HTRs, vaccination against coronavirus infection did not result in increased serum autoAbs. Some HTRs (6.5-16.1%) showed an increased level of RF-IgA by 4-6 and 9-12 months after 2-dose vaccination, which was transient and did not correlate with the concentration of S-specific and virus-neutralizing (RBD) Abs. Rheumatoid factors (RFs) are autoAbs that target the Fc-region of immunoglobulins and are found both in patients with rheumatic disease and healthy individuals. Many studies show that an immune/viral trigger can transiently induce an RF-response [28]. Since SARS-CoV-2 primarily affects the mucosa, which may influence the production of IgA-RF, further investigation of the effect of vaccination on autoimmune reactions and monitoring of HTRs with elevated RF in the post-vaccination period is needed.

The immune response against SARS-CoV-2 has been extensively studied, and defects in antiviral mechanisms are associated with disease severity. In particular, decreased type I interferon (IFN-I) has been reported in critically ill COVID-19 patients. This defect may be caused either by inherited genetic deficiencies in IFN-I signal transduction or by the emerging circulating Abs to IFN-I. In HTRs from group 1, the median autoantibody level to IFN-α remained below the accepted threshold throughout the entire follow-up after vaccination. Herewith, the number of HTRs with anti-IFN-α Abs ranged from 30% (4-6 months after the basic 2-dose vaccination) to 0% at 9-12 months following booster (Figure 6). In HTRs from group 2, a statistically significant increase in the level of anti-IFN-α Abs rise by 9-12 months after the booster. A growth in the total number of anti-IFN-α Abs in subjects from group 2 is also confirmed by the increased percentage of HTRs presenting with increased anti-IFN-α Abs from 28.6% at 21-28 days after the primary immunization to 83.3% by 9-12 months after the secondary immunization. At the same time, there was no correlation between the level of anti-IFN-α Abs and the concentration of S-specific immunoglobulins, along with the established relationship between the investigated indicator and the antigen-specific post-vaccination immune response (anti-IFN-α Abs *vs* S-reactive $CD3^+IFN\gamma^+TNF\alpha^+$: rho = 0.83, p = 0.05). Impaired type I IFN activity in COVID-19 patients is associated with poor control of viral replication and excessive inflammation. Genetic defects in Toll-like receptor 3 and IFN-α receptor have been identified as underlying the deficient IFN response in some cases of severe COVID-19. Anti-IFN-α Abs have been detected in approximately 10% of patients with life-threatening COVID-19, resulting in an autoimmune phenocopy of inborn errors of type I IFN signaling [29].

Moreover, in HTRs, a protective effect of the HLA-B27 allele was established in relation to increased levels of anti-IFN-α Abs. Along with this, carriers of the HLA-DRB1*03 and HLA-DQB1*02 alleles are predisposed to increased anti-IFN-α Abs following a viral infection or vaccination. It should be noted that in this case, based on the OR, one can discuss the identified association. However, the extensive range of the CI indicated insufficient sampling, necessitating further studies foe a more accurate interpretation of the data and confirmation of the revealed correlation. Nevertheless, the revealed trend towards increased anti-IFN-α Abs in HTRs with hybrid immunity after vaccination against SARS-CoV-2, linked to genetic markers, may be helpful in clinical practice to identify patients at potential risk of severe disease.

5. Conclusions

In vaccinated HTRs, humoral post-vaccination immunity is formed in 63% of subjects with no history of previous COVID-19 and in 85% of those with a history of COVID-19. The humoral response in patients who seroconverted before vaccination is characterized by consistently high levels of virus-specific antibodies to the SARS-CoV-2 S protein during the post-vaccination period, with a statistically significant increase observed 9-12 months after the booster. The specific cellular response to the SARS-CoV-2 S and N proteins remained low throughout the follow-up period and was recorded in 5-40% of HTRs. Statistically significant changes in the number of S- and N-reactive lymphocytes were observed in patients with a history of COVID-19, 4-6 months after the secondary immunization.

Vaccination against COVID-19 does not result in an increased number of autoAbs in HTRs. In some HTRs (6.5-16.1%), increased RF-IgA was found by 4-6 months during the 9-12 months follow-up after 2-dose vaccination with Vero Cell, which was transient. In HTRs with a history of coronavirus

infection, a statistically significant increase in the level of anti-IFN-α Abs was observed for a period of 9-12 months after the basic vaccination. This should be taken into account, as it is associated with HLA alleles, to identify patients at a potential risk of developing a severe disease.

Taken together, the presented data show that previous COVID-19 promotes the formation of immunological memory and testify to a more pronounced regulatory and functional potential of post-vaccination immunity in HTRs with hybrid immunity. To develop a methodology for risk-benefit analysis for individual HTRs vaccination strategy, clinical efficacy, ongoing monitoring of rare serious adverse events, and vaccine immunogenicity data should be taken into account.

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Author Contributions

M.Z. and A.K. designed the study; M.Z., D.N., E.N. drafted the manuscript; O.Sh., M.K. and A.K. patients enrolment and samples proceeding, D.N., E.N., G.I., I.R., A.V., S.K and M.Z. carried out samples proceeding and immunological experiments; M.Z., D.N., T.D. and E.N. analyzed and discussed the data and generated the Figures and Tables. All authors have read and approved the published version of the manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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