A new set-up of vanishing antibodies: A biennial follow-up of five different clients' humoral responses against SARS-CoV-2 after systemic vaccination in an oncology hospital in Poland

Piotr Kosiorek[1,2], Samuel Stróż[2], Anna Hryniewicz[3], Robert Milewski[4], Klaudia Bartoszewicz[2] and Anna Stasiak-Barmuta[2]

1. Department of Emergency, Maria Sklodowska-Curie Bialystok Oncology Centre, Ogrodowa 12, 15-027, Białystok, Poland,

2. Department of Clinical Immunology, Medical University of Białystok, Jana Kilińskiego 1, 15-089, Białystok, Poland

3. Department of Rehabilitation, Medical University of Białystok, Jana Kilińskiego 1, 15-089, Białystok, Poland

4. Department of Biostatistics and Medical Informatics, Medical University of Białystok, Jana Kilińskiego 1, 15-089, Białystok, Poland

Correspondence to Piotr Kosiorek (pkosiorek@onkologia.bialystok.pl)

Abstract:

The humoral response of the COVID-19 vaccine varies from person to person. It largely depends on prior SARS-CoV-2 infection, obtaining an adequate immune response, and leaving a trace of changing antibody concentration over time. We retrospectively analyzed five clinical cases from selected patients and employees of the oncology hospital. All mild COVID-19 convalescents received the BNT162b2-Comirnaty mRNA vaccine three or four times. The levels of SARS-CoV-2 IgM- and IgG-specific antibodies, as well as S-RBD antibodies, were analyzed for two years. The concentration of antibodies was assessed in the laboratory using the chemiluminescent immunoassay CLIA, MAGLUMI. Results: (1) Active autoimmune disease stabilized the level of IgG-specific antibodies after systemic mRNA vaccination for at least six months. (2) Post-vaccination IgG and S-RBD levels decreased when vaccination was performed within three months of onset. (3) The booster dose administered only increased the S-RBD antibody levels. Declining IgG-specific antibodies were observed. (4) The S-RBD IgG levels were not correlated with the SARS-CoV-2 IgG levels in

the vaccinated convalescents. (5) Subsequent reinfection with SARS-CoV-2 after vaccination three times released a more significant specific antibody response. Based on the collected data, we suggest that monitoring S-RBD antibodies is sensitive but not equivalent to a specific humoral response for SARS-CoV-2 IgG. We suggested that administering at least three doses of the mRNA vaccine should serve as the basis for immunization. The three-month interval may be the best alternative to an immunization schedule for non-immunocompromised people.

KEYWORDS: COVID-19; BNT162b2; vaccination; S-RBD; SARS-CoV-2; seroconversion

1. Introduction

The common SARS-CoV-2 virus has infected approximately 600 million people across 228 countries worldwide, leaving behind natural immunity [1]. Acute COVID-19 infection can cause a cytokine storm that leads to acute respiratory failure (ARDS) and death [2], while severe inflammatory disease can result in chronic polymyositis syndrome in children (PIMS) [3]. Almost half (44.8%) of symptomatic infections in children and adults manifest as varied symptoms related to chronic infection, in the form of long-COVID/post-COVID clinical syndrome, cognitive and physical deficits, pulmonary fibrosis, myocarditis, or neurological deficits [4, 5]. The findings so far show that to acquire antiviral immunity to SARS-CoV-2, one does not need to come into contact with the virus and become infected [6]. Being around immunized people can provide a cellular and humoral response. To what extent is it active and provides self-immunity? How to check if we have acquired the appropriate anti-virus response, or only had contact with it? When should a booster be given?

The situation applies to everyone, including the healthy, those sick with a viral disease in any stage, those with impaired immunity, and young people. The data we collected show that it is necessary to measure the concentration of IgG-specific antibodies after the onset of SARS-CoV-2 and to measure the concentration of post-vaccine IgG (anti-S, anti-RBD, and S-RBD) [6]. IgM and IgG antibody levels may indicate early- or late-phase infection [7]. Administration of antibody response acquisition during the early phase has only demonstrated therapeutic implications for symptomatic COVID-19 cases [8], and vaccination of people with a history of SARS-CoV-2 without knowledge of their humoral response is considered safe [9]. In our

hospital, most medical staff and patients underwent vaccinations without knowing that they had acquired a humoral response beforehand [10].

The decline in humoral immunity over time following coronavirus infection, including SARS-CoV-2, is typical in mild cases [11, 12, 13, 14, 15, 16]. Then, do all infections require antibody monitoring? Barrière et al. [17] found that after administering a second dose of the vaccine (complete vaccination) in cancer patients, the individual humoral anti-S antibody response level could be determined after three to four weeks. It has been proven that the level of S-RBD antibodies is correlated with protection against symptomatic SARS-CoV-2 infection [18, 19], and not asymptomatic infection [20]. Thus, after the third dose and subsequent boosters, does the level of only anti-S antibodies determine immunity? In a selected population of healthy people, the level of the humoral response can last up to 13 months [19]. During our study, the Delta strain of SARS-CoV-2 accounted for over 95% of infections in Poland. At that time, rapid neutralization by current vaccines of the new Delta virus [20, 21] was reported after complete inoculation with BNT162b2. We asked whether, during the next wave of infections in Europe with the different Omicron virus types, the administration of a booster in healthy people would stimulate the immune system again [22, 23], or if it would only cause an increase in the S-RBD in response to the vaccine? The description of five different courses of SARS-CoV-2 infection until the administration of subsequent doses of the Comirnaty vaccine shows that a specific immune response expires approximately one year after the onset of the disease and is not induced after subsequent artificial immunization [10]. We call this effect vanishing antibodies. Another illness with a new variant of the SARS-CoV-2 virus can stimulate a new response to a different level [24-26]. We call this a new set-up. In other data, currently unpublished, article focused on a year-long case observation of the COVID-19 humoral response, we saw that an early double infection (without or after vaccination), rather than a third booster, raises antibodies to a higher "protective" IgG level.

2. Material and methods

Before vaccines were introduced in 2021, the natural immunity in the SARS-CoV-2 pandemic was investigated by analyzing antibody concentrations. Antibody measurements were analyzed routinely for oncology patients and hospital staff for medical purposes during the COVID-19 pandemic. Retrospectively,

Table 1. Characteristics of the five selected person measurements. Two-year monthly schedule with antibody measurements during hospital visits or outpatient appointments. SARS-time of infection; Com1, Com2, Com3, Com4-time of Comirnaty vaccination.

Case	Months	VIII	X	XI	XII			I			II		III	IV
#1	Timeline (samples 1-8)	1	2	3	4	5	6	7	8	9	10	11	12	13
	Event		SARS					Com1			Com2			
	Data	2020-08-07	2020-10-06	2020-11-10			2020-12-22	2021-01-04	2021-01-13	2021-01-21	2021-02-01	2021-02-23		2021-04-22
	SARS-CoV-2 IgG	0.001	0.027	0.004			1.049		0.923	0.922		0.456		0.193
	SARS-CoV-2 IgM	0.140	0.243	0.884			0.434		0.674	0.551		0.392		0.097
	S-RBD IgG	0.000	0.000	0.000			0.000		100.000	1050.000		600.000		401.4
#2	Timeline (samples 1-3)	1	2	3	4	5	6	7	8	9	10	11	12	13
	Event												SARS	
	Data										2021-02-02		2021-03-19	2021-04-13
	SARS-CoV-2 IgG										0.422		0.831	2000.000
	SARS-CoV-2 IgM										0.140		2.140	26.590
	S-RBD IgG										0.000		0.000	310.9
#3	Timeline (samples 1-10)	1	2	3	4	5	6	7	8	9	10	11	12	13
	Event				SARS			Com1						
	Data		2020-10-06	2020-11-17	2020-12-05	2020-12-22	2020-12-30	2021-01-04	2021-01-13	2021-01-21	2021-01-26	2021-02-23		2021-04-15
	SARS-CoV-2 IgG		0.018	0.029	0.031	0.017	0.858		1.272	1.151	0.900	0.619		0.323
	SARS-CoV-2 IgM		0.335	0.847	0.840	0.850	0.779		6.181	0.028	1.000	0.986		0.131
	S-RBD IgG		0.000	0.000	0.000	0.000	13.070		500.000	400.000	300.00	250.000		153.4
#4	Timeline (samples 1-4)	1	2	3	4	5	6	7	8	9	10	11	12	13
	Event							Com1				Com2		
	Data		2020-10-30	2020-11-17				2021-01-04				2021-02-12	2021-03-24	2021-03-29
	SARS-CoV-2 IgG		0.042	0.042									0.035	0.035
	SARS-CoV-2 IgM		0.179	0.179									0.217	0.217
	S-RBD IgG		0.001	0.001									128.000	128.000
#5	Timeline (samples 1-6)	1	2	3	4	5	6	7	8	9	10	11	12	13
	Event										SARS			
	Data	2020-10-05	2020-10-12	2020-10-19	2020-12-01	2020-12-10					2021-01-12	2021-02-15	2021-03-06	
	SARS-CoV-2 IgG	11.680	11.680	9.450	7.426							4.720	4.720	
	SARS-CoV-2 IgM	6.664	6.664	5.240	3.050							0.185	0.185	
	S-RBD IgG	0.001	0.001	0.001	100.000							100.000	100.000	

Case	Months	V		VI		VII				VIII	IX		X	XI
#1	Timeline (samples 9-16)	14	15	16	17	18	19	20	21	22	23	24	25	26
	Event											Com3		
	Data	2021-05-11	2021-05-27	2021-06-07	2021-06-30				2021-07-30	2021-08-18	2021-09-17	2021-09-28	2021-10-15	
	SARS-CoV-2 IgG	0.7	0.489	0.399	0.236				0.171	0.095	0.078		0.074	
	SARS-CoV-2 Ig M	0.077	0.127	0.170	0.010				0.010	0.026	0.010		0.026	
	S-RBD IgG	275.000	202.000	158.000	119.000				104.000	84.000	50.920		1000.000	
#2	Timeline (samples 4-11)	14	15	16	17	18	19	20	21	22	23	24	25	26
	Event				Com1		Com2							
	Data	2021-05-21			2021-06-01	2021-07-08	2021-07-14	2021-07-16	2021-07-30	2021-08-27			2021-10-29	
	SARS-CoV-2 IgG	43.14			40.000	35.94	31.000	30.62	30.76	13.97			13.550	
	SARS-CoV-2 IgM	2.277			1.500	1.256	1.000	0.997	0.519	0.330			0.043	
	S-RBD IgG	132.000			145.000	1997.000	2000.000	2000.000	2000.000	895.300			533.100	
#3	Timeline (samples 11-18)	14	15	16	17	18	19	20	21	22	23	24	25	26
	Event	Com2										Com3		
	Data	2021-05-11	2021-05-27	2021-06-07	2021-06-30				2021-07-30	2021-08-18	2021-09-17	2021-09-28	2021-10-15	
	SARS-CoV-2 IgG	0.222	28126.000	1.542	1.015				0.633	0.575	0.367	0.260	0.230	
	SARS-CoV-2 IgM	0.048	0.151	0.158	0.010				0.010	0.018	0.100	0.010	0.10	
	S-RBD IgG	89.43	63.56	626.2	425.800				181.700	127.500	66.500	32.400	602.300	
#4	Timeline (samples 5-9)	14	15	16	17	18	19	20	21	22	23	24	25	26
	Event			SARS								Com3		
	Data	2021-04-28		2021-06-01		2021-07-02			2021-07-30			2021-09-16	2021-10-29	
	SARS-CoV-2 IgG	0.035				0.010			0.014				0.061	
	SARS-CoV-2 IgM	0.217				0.001			0.001				0.001	
	S-RBD IgG	128.000				34.190			16.240				16.240	
#5	Timeline (samples 7-10)	14	15	16	17	18	19	20	21	22	23	24	25	26
	Event	Com1		Com2										Com3
	Data	2021-04-21	2021-05-19	2021-06-02					2021-07-29					2021-11-20
	SARS-CoV-2 IgG	1.970	6.031	5.063					3.019					
	SARS-CoV-2 IgM	0.069	1.051	1.051					0.198					
	S-RBD IgG	94.61	2000.000	2000.000					2000.000					

Case	Months	XII		I	II		III	IV	V	VI	VII	VIII	IX	X
#1	Timeline (samples 17-24)	27	28	29	30	31	32	33	34	35	36	37	38	39
	Event			SARS										
	Data	2021-12-03		2022-01-04	2022-02-03	2022-02-18	2022-03-28			2022-06-24		2022-08-19		2022-10-20
	SARS-CoV-2 IgG	0.049		0.029	36.390	98.590	9.400			1.300		18.900		48.100
	SARS-CoV-2 IgM	0.020		0.000	0.130	0.090	0.080			0.090		0.001		0.010
	S-RBD IgG	214.800		123.700	396.690	234.200	136.440			71.500		600.138		312.748
#2	Timeline (samples 12-15)	27	28	29	30	31	32	33	34	35	36	37	38	39
	Event	Com3												
	Data	2021-12-06	2021-12-31				2022-03-05				2022-07-01			2022-10-26
	SARS-CoV-2 IgG		10.760				11.100				4.900			46.200
	SARS-CoV-2 Ig M		0.193				0.540				0.160			0.010
	S-RBD IgG		1362.900				739.580				138.100			198.706
#3	Timeline (samples 19-26)	27	28	29	30	31	32	33	34	35	36	37	38	39
	Event				SARS							Com4		
	Data	2021-12-03	2021-12-31		2022-02-03	2022-02-18	2022-03-28			2022-06-24		2022-08-19	2022-09-14	2022-10-22
	SARS-CoV-2 IgG	0.214	0.237		36.390	245.000	43.400			16.700		8.100	6.900	36.700
	SARS-CoV-2 IgM	0.10	0.026		0.130	0.370	0.050			0.020		0.001	0.001	0.010
	S-RBD IgG	226.500	102.700		396.690	108.060	89.830			48.720		35.035	87.505	63.995
#4	Timeline (samples 10-15)	27	28	29	30	31	32	33	34	35	36	37	38	39
	Event		SARS											
	Data	2021-12-03	2021-12-31		2022-02-09				2022-05-12	2022-06-03		2022-08-17		2022-10-20
	SARS-CoV-2 IgG	0.001			1.700				1.100	1.100		0.600		50.6
	SARS-CoV-2 IgM	0.010			0.200				0.220	0.220		0.160		0.010
	S-RBD IgG	535.500			1317.000				4239.000	4239.000		2824		2484.57
#5	Timeline (samples 11-12)	27	28	29	30	31	32	33	34	35	36	37	38	39
	Event									Com4				
	Data							2022-04-12		2022-07-29				2022-10-20
	SARS-CoV-2 IgG							41.274						52.100
	SARS-CoV-2 IgM							2.268						1.200
	S-RBD IgG							2000.000						10000.000

we decided to follow five selected people for approximately 24 months—with most measurements taken monthly (timeline).

Table 1 presents a monthly timeline with antibody measurements.

Table 1. The complete characteristics of the antibody collection in five subjects (Case #1,2,3,4,5) in months (samples timeline). Event: SARS – SARS-CoV-2 infection time (positive PCR or antigen test); Com1, Com2, Com3, Com 4 – vaccination time (Comirnaty).

Three patients (#2, #4, #5) and two hospital staff persons (#1,#3) were chosen. Case #2 was chosen because of their rapid response to SARS-CoV-2 infection (hyper-responder with SARS-CoV-2 IgG >2000 AU/mL in less than 14 days), providing an excellent case of early seroconversion relevant to acute viral infection. The other two persons were selected as the controls, one being COVID-19 convalescent (#3) and one lacking a SARS-CoV-2 history (#1). The other two patients passed COVID-19 twice with mild (#4) and severe (#5) respiratory problems. Case #3 stayed at the hospital and was cured against COVID-19 using remdesivir (Veklury) for five days. Case #1 patient was chosen because of a lack of a history of COVID-19, but analyses of his seroconversion proved past SARS-CoV-2 infection (IgG cut-off >0.2 AU/mL). Case #4 stayed and cured COVID-19 at home. Case #5 remained at the hospital because of bilateral pneumonia and was given steroids and early plasma antibodies. All five were vaccinated with BNT162b2 mRNA (Comirnaty) according to the manufacturer's schedules and took booster vaccines at different times (gray arrows in figures; Com1, Com2, Com3, Com4).

2.1. Characteristics of the people

Case #1. Thirty-four-year-old Caucasian male, no chronic diseases, non-smoker, undergoing hypertension treatment.

Case #2. Sixty-eight-year-old Caucasian women, with chronic diseases (rheumatoid arthritis and degeneration of the spine), non-smoker, hypertension treatment, taking NSAIDs and treated with NSAIDs for COVID-19 infections at home.

Case #3. Forty-nine-year-old Caucasian male, hospitalized early for COVID-19 because of headaches and a cough, shortness of breath (saturation 88%), with twice COVID-19 drug therapy: remdesivir (Veklury) and molnupiravir (Lagevrio), respectively.

Case #4. Fifty-nine-year-old Caucasian woman, non-hospitalized for COVID-19. First time SARS-CoV-2 infection with flu, second with mild gastrointestinal problems.

Case #5. Sixty-eight-year-old Caucasian male postponed the urgent urology operation because of a mild second SARS-CoV-2 infection in 2021. Now is under prostate cancer hormonotherapy.

Chest CTs for Cases #2, #3, and #5 showed no post-COVID changes three months after treatment.

2.2. Materials

Our study materials were blood specimens taken through venipuncture sampling. The concentration of antibodies was evaluated four hours after blood collection. If an immediate assessment was impossible, the serum was collected and stored at –80 °C. Due to the fact that most of the data (2021) are in AU/mL, the results of measurements in BAU/mL in 2022 were converted into AU/mL (1.0 BAU/mL = 4.33 AU/mL).

2.3. Methods

The antibody concentrations were detected by the chemiluminescent immunoassay CLIA (MAGLUMI, Snibe Diagnostic, Shenzhen, China). Per the manufacturer's protocol, results greater or equal to 1.0 AU/mL of SARS-CoV-2 IgG, IgM, and S-RBD were considered reactive and positive. The maximum limits of the antibody measurements were assumed: 2000 AU/mL of S-RBD (initially, it was 100 AU/mL), with the upper limit of the SARS-CoV-2 antibody measurements being 2000 AU/mL. According to our earlier observations in this population, the cut-off value for SARS-CoV-2 IgG should be >0.2 AU/mL for a positive test result. Antibody tests were ordered by a hospital doctor at different times, from vaccination to illness. The results are presented in a table depending on the time of the test (timeline). The patients provided their written consent for performing humoral immunity tests and for participating in this study.

The Bioethics Commission of the Medical University provided consent for our research.

3. Results

The immunization (infection and vaccination) results for each person are shown as a timeline (measurements 1-39), presented in five diagrams for each figure (1,2,3,4,5). The diagrams are presented as follows:

   (a) SARS-CoV-2 IgM with IgG;

   (b) SARS-CoV-2 IgG with S-RBD IgG;

(a`) and (b`) is a part of the diagram (a) or (b), respectively, to explain the dynamic changes of humoral response;

(c) All three antibody concentrations are time-related in the logarithmic chart.

Figures 1–5 present the humoral response of each person after infection and vaccination. The legend in the figures shows arrows indicating systemic vaccinations (Com1, Com2, Com3, Com4) and the symbol of the virus pointing to SARS time infections.

Case #1: Figure 1. (a) All IgG and IgM antibodies (samples 1–24). A retrospective case study showed that the person had had SARS-CoV-2 infection in October 2020 without clinical symptoms, so we present (a`) an additional diagram with only 1–26 measurements (1–16 samples) separately to point out this phenomenon. There is a typical seroconversion, seen as an increase in IgM and IgG, assumed as positive results (cut-offs: IgM >1.0 AU/mL and IgG >0.2 AU/mL). The results should be considered clinically asymptomatic of previous SARS-CoV-2 infection. As hospital staff, he was routinely vaccinated in January 2021 with two doses of BNT162b2 (Comirnaty) on a schedule over 28 days (see arrows). (b) Post-vaccination effects are seen as S-RBD gains and triple antibody re-synthesis in SARS-CoV-2 infection and vaccination ((a`) The three peaks of IgM and IgG mean three seroconversions in a row). (c) Logarithmic representation of the three antibody concentrations. Notable here is a conversion to higher SARS-CoV-2 IgG concentrations than baseline before SARS-CoV-2 infection at the beginning of the diagram. A booster was given eight months after the second dose of Comirnaty (see arrow) due to another wave of illness (autumn) for medical personnel (only increased S-RBD). On the contrary, at that time, the second vaccination did not work—(b) only increased S-RBD after the booster, but together with COVID-19 (raised S-RBD with SARS-CoV-2 IgG). This phenomenon is called enhanced humoral response without classical seroconversion (fast release IgG-specific antibodies). (c) After a few months, he passed SARS-CoV-2 asymptomatically, which provided a new set-up of humoral response (in the red frame). As a hospital person, he is still working with COVID-19 patients.
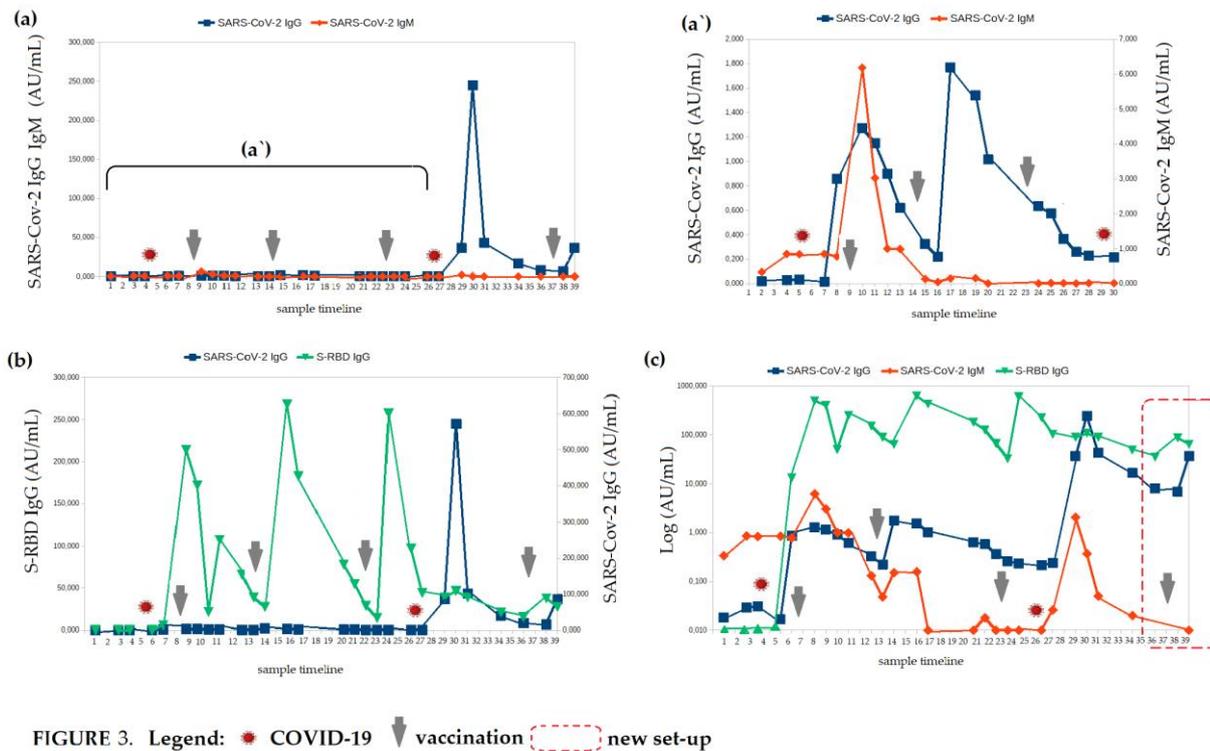
FIGURE 1. Legend: ● COVID-19   ⬇ vaccination   ⬚ new set-up
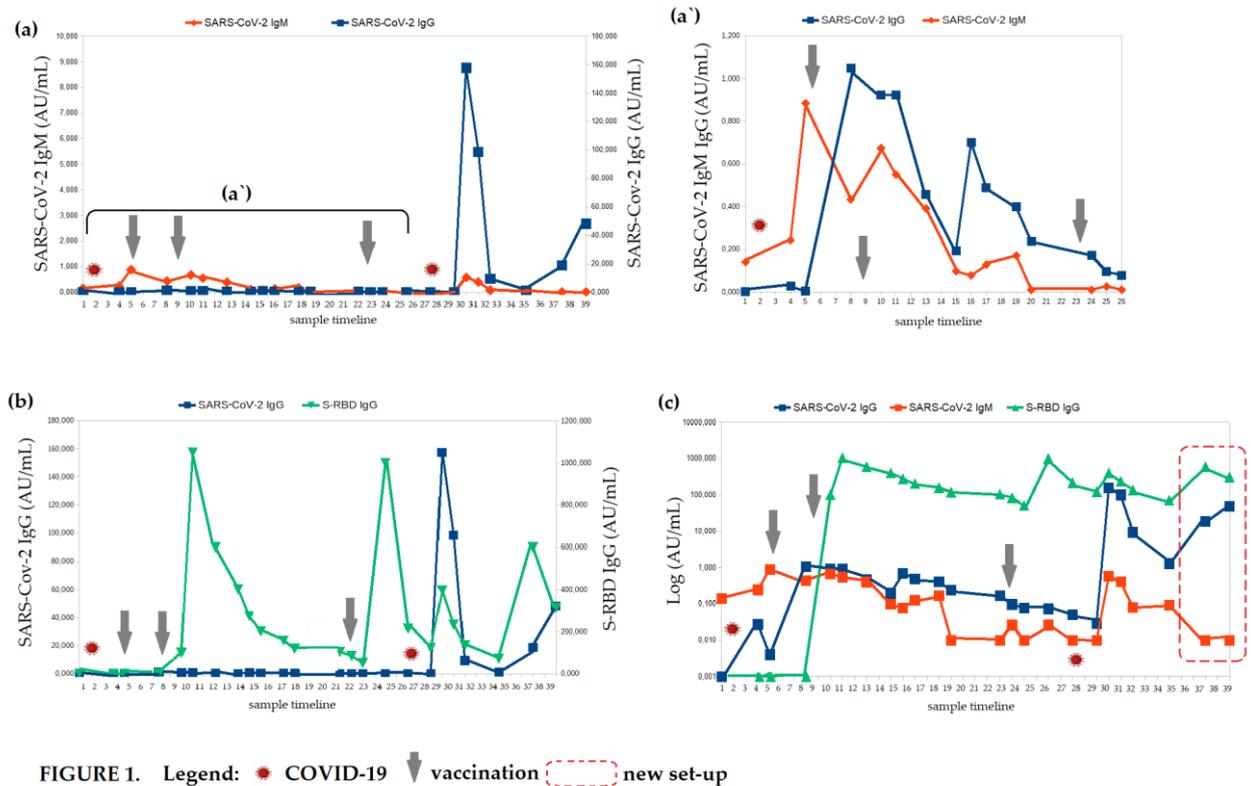
Figure 1. (a, a`, b, c) – presents all three antibodies in time (samples 1–24). Figure legend points to the time of COVID-19 passed, vaccinations were given (arrows) and new set-up, a time to stabilize antibodies after six months (box with dotted red line)

Case #2: Figure 2 (a) Perspective case analysis (samples started at 10 till 39 measurements); the person, had symptomatic COVID-19 infection in March 2021, with a sore throat and muscles, a fever, and the chills. (a) Antibody collection for assays was routinely scheduled (considered a hyper-responder) as a hospital-monitored oncology patient, vaccinated in July 2021 with two doses of BNT162b2 (Comirnaty) on a schedule over 28 days (see arrows). A booster was given after half a year delay, as an auto-immunologic person. (c) A logarithmic representation of the three antibody concentrations. (b) Post-vaccination effects, shown as an increase in S-RBD extended to six months and no re-synthesis of antibodies in the vaccine prompt. (b, c) No fluctuations in the IgM and IgG concentrations. The conversion to higher IgG concentrations before SARS-CoV-2 infection and the exceptional smooth/stable course of the immune antibody curves are noteworthy. (c) A new set-up of antibodies can be seen in the red frame. She hasn't given any data on infection lately.
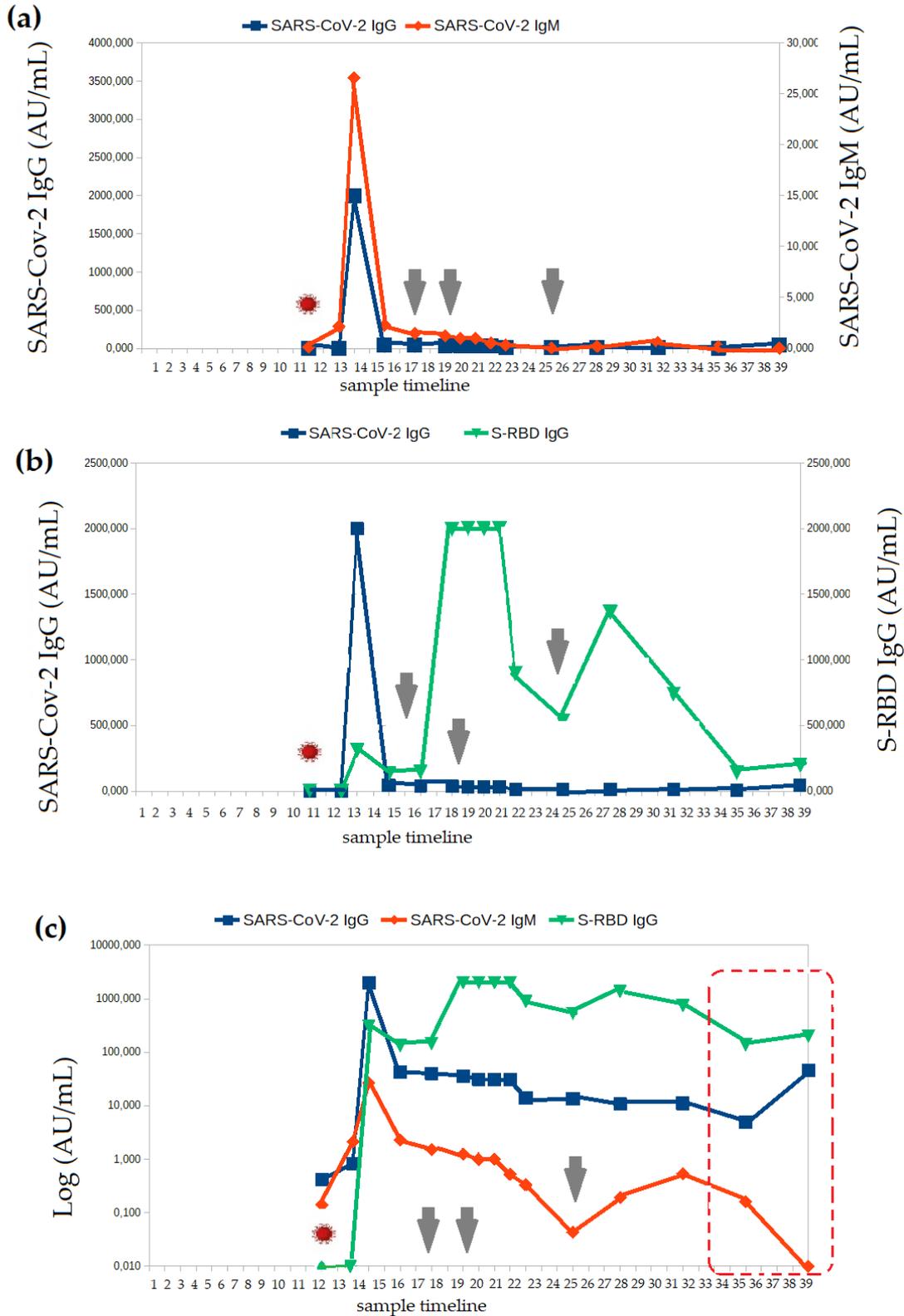
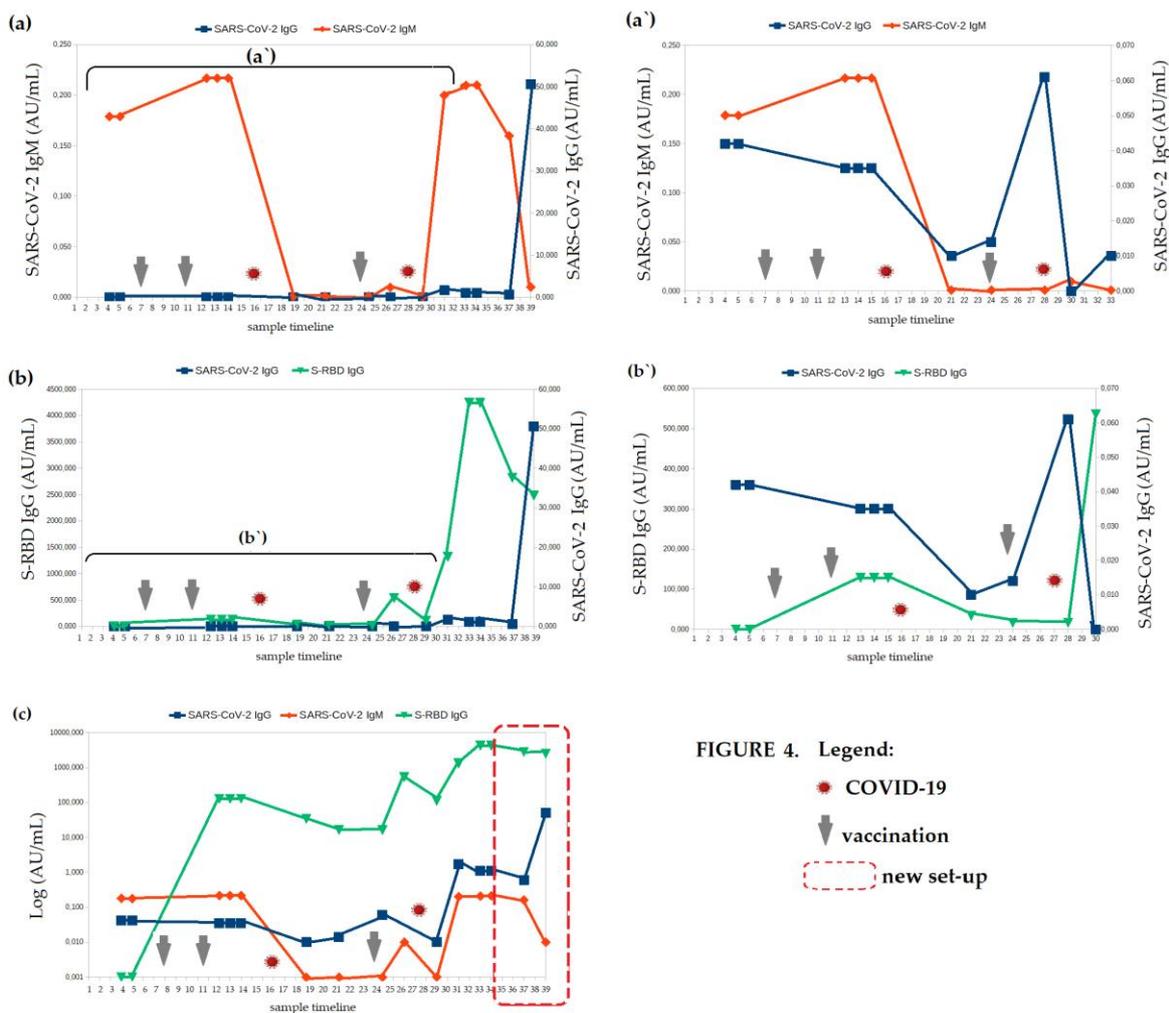FIGURE 2.   Legend: 🦠 COVID-19   🡇 vaccination   ⌐ ⌐ new set-up

Figure 2. (a, b, c) – presents all three antibodies in time (samples 1-15). Figure legend points to the time of COVID-19 passed, vaccinations were given (arrows) and new set-up, a time to stabilize antibodies after six months (box with dotted red line)
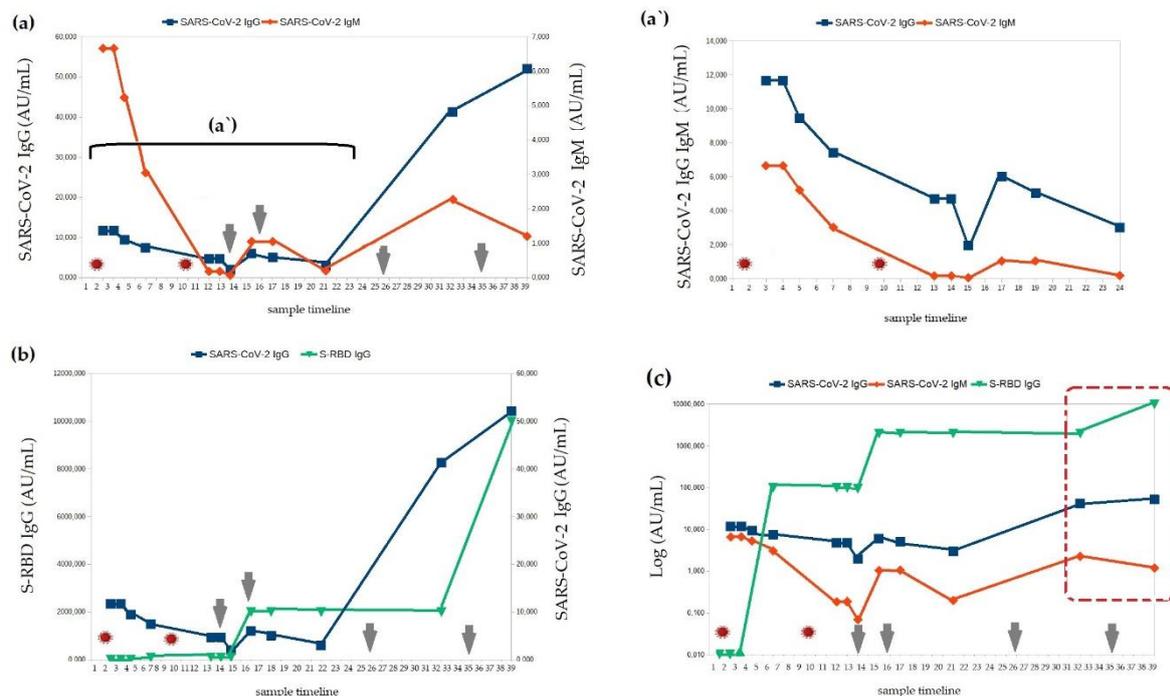

FIGURE 3. Legend: ☀ COVID-19   ▼ vaccination   ⌞ ⌝ new set-up

Figure 3. (a, a`, b, c) – presents all three antibodies in time (samples 1-26). Figure legend points to the time of COVID-19 passed, vaccinations were given (arrows) and new set-up, a time to stabilize antibodies after six months (box with dotted red line)

Case #3: Figure 3 (a) Retrospective case analysis. The person had a symptomatic COVID-19 infection in December 2020, including a sore throat, a cough, a fever, and dyspnea. Routinely, antibodies were collected for assay; (a`) during inpatient treatment, remdesivir (Veklury) was administered for five days while being monitored by medical personnel in the hospital, and vaccinations occurred in January and in May 2021 with two doses of BNT162b2 (Comirnaty) because this patient underwent an urgent spine operation in March. A booster was given five months after the second dose of Comirnaty because of lost antibodies—SARS-CoV-2 IgG of 0.26 AU/mL and S-RBD IgG of 32.40 AU/mL (see arrow Figure 3 (a, b, c) timeline 23-24). (b) Here, we can see the post-vaccination effects, shown as an increase in both S-RBD and SARS-CoV-2 IgG extended to

over three months (SARS-CoV-2 IgG = 0.237 AU/mL, S-RBD IgG = 102.700 AU/mL and SARS-CoV-2 IgM =

0.026 AU/mL; timeline 28), as well as a decrease in IgG antibodies in the prompt for SARS-CoV-2 (IgG =

16.700 AU/mL and IgM = 0.020 AU/mL; timeline 35) and vaccination (S-RBD IgG = 48.720

AU/mL) after five months. Fortunately, the Polish government and the minister of health made it possible

for medical workers to receive the 4th dose (booster) in only 2 months (timeline 37). After three months, he

passed into mild symptomatic COVID-19, cured after five days by molnupiravir (Lagevrio), which provided

a new set-up of humoral responses (Figure 3c in the red frame). In summary, a third dose of the vaccine was

used due to the significant decrease in immunity, the antibodies reduction, and the patient's clinical

condition (Figure 3a`,b).

FIGURE 4. Legend:
- COVID-19
- vaccination
- new set-up

Figure 4. (a, a`, b, b`, c) – presents all three antibodies in time (samples 1-15). Figure legend points to the time of COVID-19 passed, vaccinations were given (arrows) and new set-up, a time to stabilize antibodies after six months (box with dotted red line)

Case #4: Figure 4 (a) Retrospective case analysis. Delayed humoral response. She past twice SARS-CoV-2 with mild symptoms (COVID-19). She is an out-patient at the breast unit. In Figure 4 (a) and (a'), we see a crossing-line dynamic of all antibodies with little correlation with vaccination (Com3; timeline 24). Fig 4. (b), (b`), and (c) present delayed effects in time SARS-CoV-2 infection (ending "old" SARS-CoV-2 IgG and replacing them with "new." Now all three antibodies are in response. Building a new humoral strategy: seroconversion (IgM), humoral response (S-RBD), immunity (SARS-CoV-2 IgG). That is also an example of a new set-up raise of IgG-specific antibodies at the end of observation. That person is still a riddle. That is why she did not yet decide on vaccination (Com4).

FIGURE 5.   Legend: ☀ COVID-19   ▼ vaccination   ⌐ ¬ new set-up

Figure 5. (a, a`, b, c) – presents all three antibodies in time (samples 1-12). Figure legend points to the time of COVID-19 passed, vaccinations were given (arrows) and new set-up, a time to stabilize antibodies after six months (box with dotted red line)

Case #5: Figure 5 (a) Retrospective case analysis (measurement 2-39). The selected patient underwent COVID-19 with pneumonia and was administered steroids for respiratory failure and convalescent plasma with antibodies. Another "reinfection" of SARS-CoV-2 may have been due to the long-standing genetic material of the virus (RT-PCR positive) before urological surgery (no clinical correlation). Figure 5 (a), (a'), (b), and (c) are the only evidence of correlation with vaccinations (Com1, Com2). As you can see, stable levels of the three classes of antibodies are maintained in cancer patients after surgery and prostate cancer hormone therapy (Com3 and Com4 vaccinations). This is evidence that COVID-19 in some immunosuppressed patients is well tolerated.

A logarithmic representation of all three antibody concentrations can be seen in Figures 1c, 2c, 3c, 4c, and 5c. The effect of the new increasing level of IgG antibodies was obtained. Conversion to higher IgG concentrations was noticeable (after disease and after complete vaccination) initially after infection with SARS-CoV-2 and a symmetrical course of S-RBD IgG curves of immune antibodies in the time immediately after the third dose of Comirnaty. There was a difference in antibody surge after COVID-19 getting sick with the boosted vaccine (third dose) between a symptomatic patient (Figure 3b) and SARS-CoV-2 asymptomatic patient (Figure 1b). The symptom convalescent patient had a higher IgG-specific level.

Following autoimmunity (Case #2), the antibody response after disease and inoculation was stable. Significantly, it was strengthened by administering the first mRNA vaccine, respectively, after three months. The results indicate that the active autoimmune disease stabilized the level of specific IgG after systemic mRNA vaccination for at least half a year.

Looking at all cases of vaccinated convalescents, we believe a booster dose should be given later than 28 days after SARS-CoV-2 infection. Then, we can observe fluctuations in antibodies, suggesting a change in response to the subsequent immunization, depending on the patient's condition.

We expected a longer duration of neutralizing antibodies after vaccination. The level of post-vaccine IgG antibodies decreased when vaccination was performed within three months after disease onset, depending on the patient's condition. We can change this by appropriately tracking S-RBD IgG and SARS-CoV-2 IgG

antibodies one month after the disease [17]. According to observations, raising IgG-specific antibodies will better occur by administering the vaccine's second (next) dose three months after the first.

The administered booster dose (third dose) only increased the concentration of S-RBD antibodies. Despite the decrease in specific IgG, their level was higher than before disease and vaccination. Declining IgG-specific antibodies were observed. S-RBD IgG levels are not correlated with SARS-CoV-2 IgG levels in vaccinated convalescents [10], but they are related to symptomatic ones [16]. Surprisingly, there was no apparent spike in IgG antibody levels after the third dose of the vaccine after six months, despite a persistently elevated level. Based on the collected data, we suggest that monitoring S-RBD antibodies is sensitive, but not equivalent to a specific humoral response for SARS-CoV-2 IgG. The observations of the five presented cases show that the measurement of S-RBD correlates with specific IgG only in the period from one to three months following disease and inoculation, but not for the booster doses given in the six months after the initiation of vaccination.

4. Discussion

Many clinical data indicate that vaccination with a third dose of Comirnaty is useful in immunocompromised people [10, 11], the chronically ill [12], or people undergoing oncological treatment [13, 14]. An analysis of specific antibody concentration charts at a cut-off for SARS-CoV-2 of >0.2 AU/mL was used [10], allowing us to observe asymptomatic COVID-19 and the natural course of the disease [15].

Patterns of antibody seroconversion in response to SARS-CoV-2 infection and the BNT162b2 mRNA vaccine were apparent in the five separate cases. In the case of Case #3 and Case#4, it turned out to be effective. Currently, a wave of infection with the SARS-CoV-2 virus strain Omicron is going through Europe and the USA, as the BNT162b2 vaccine is effective against this strain.

The ideal past seroconversion is when IgG antibodies are formed after stimulation and are maintained for longer (Case#4, Case #5). For Case #1, Comirnaty vaccination needed to be postponed one more month after getting sick; for Case #2 and Case #4, double vaccination did not show/ change anything (excess antibodies); for Case #3, in terms of the vaccination, the third and the next dose significantly improved the initial level of antibodies, indicating that it is safe and advisable, and similarly to Case #1, the first dose of the Comirnaty

vaccine was taken too early. Analyzing a new set-up in these five cases shows that in Case #2, subsequent infection was not needed to reach a stable level of IgG antibodies.

5. Conclusion

To sum up, administration of the vaccine too early in the case of people with excess antibodies (e.g., Case #2) does not produce the expected effect, as too early an administration (Cases #1 and #3) does not allow to hit the serological window after falling ill. Following re-administration (Case #3 and Case #4) after five months from the administration of the first dose, based on individual indications for vaccination, the level of antibodies responds correctly.

The analysis of these five cases of antibody changes over time during the COVID-19 pandemic provides students training material and allows them to observe and control the effects of treatment—including when to administer the third or subsequent dose of the vaccine at the appropriate time for the natural course of the disease [25, 26].

Persistently elevated levels of specific IgG after vaccination, even at low levels, after COVID-19 infection suggests that SARS-CoV-2 IgG-specific antibodies help in monitoring humoral immunity for a long time [26]. Crucially, however, they provide a new humoral response re-build up, observed twice following infection.

Moreover, we have to add that in the five cases, we primarily saw good SARS-CoV-2 response seroconversion (IgM and IgG) after mild infection and some different responses after vaccination. A lack of a humoral-specific response for complete BNT162b2 vaccination and a booster (Case #2, Case #4) does not mean seroconversion (S-RBD response). The mechanism of poor, late seroconversion is supposed to be involved in a substantial primary SARS-CoV-2 response (early responder). Moreover, a poor humoral response (seroconversion of IgG and IgM) was observed for the second and subsequent booster vaccinations in healthy SARS-CoV-2 asymptomatic cases (Case #1 and #3), not only for immunocompromised people [27, 28].

Author Contributions: P.K., S.S., and A.S.-B. analyzed the data and drafted the manuscript. R.M., A.H., and K.B. participated in data analysis and extensively reviewed the manuscript. P.K., S.S., and A.S.-B.

contributed to the clinical and laboratory data acquisition and reviewed the manuscript. All authors read and agreed to the published version of the manuscript.

Institutional Review Board Statement: The Bioethics Commission of the Medical University approved this research. Approval code: APK.002.267.2021; approval date: 29 April 2021.

Informed Consent Statement: Written informed consent was obtained from the patient(s) to publish this paper.

Data Availability Statement: Data are available on request.

Conflicts of Interest: The authors declare no conflicts of interest.

References:

1. Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, Agudelo M, Barnes CO, Gazumyan A, Finkin S, Hagglof T, Oliveira TY, Viant C, Hurley A, Hoffmann HH, Millard KG, Kost RG, Cipolla M, Gordon K, Bianchini F, Chen ST, Ramos V, Patel R, Dizon J, Shimeliovich I, Mendoza P, Hartweger H, Nogueira L, Pack M, Horowitz J, Schmidt F, Weisblum Y, Michailidis E, Ashbrook AW, Waltari E, Pak JE, Huey-Tubman KE, Koranda N, Hoffman PR, West AP, Jr., Rice CM, Hatziioannou T, Bjorkman PJ, Bieniasz PD, Caskey M, Nussenzweig MC. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature. 2020;584(7821):437-42. Epub 2020/06/20. DOI: 10.1038/s41586-020-2456-9. PubMed PMID: 32555388; PMCID: PMC7442695.

2. Hsu CY, Lai CC, Yeh YP, Chang-Chuan C, Chen HH. Progression from Pneumonia to ARDS as a Predictor for Fatal COVID-19. J Infect Public Health. 2021;14(4):504-7. Epub 2021/03/21. DOI: 10.1016/j.jiph.2020.12.026. PubMed PMID: 33743372; PMCID: PMC7834113.

3. Okarska-Napierala M, Ludwikowska KM, Szenborn L, Dudek N, Mania A, Buda P, Ksiazyk J, Mazur-Malewska K, Figlerowicz M, Szczukocki M, Kucinska B, Werner B, Stopyra L, Czech A, Berdej-Szczot E, Gawlik A, Opalinska P, Mazur A, Januszkiewicz-Lewandowska D, Niszczota C, Jackowska T, Wysocki J, Mois Co RSG, Kuchar E. Pediatric Inflammatory Multisystem Syndrome (PIMS) Did Occur in Poland during Months with Low COVID-19 Prevalence, Preliminary Results of a Nationwide Register. J Clin Med. 2020;9(11). Epub 2020/10/28. doi: 10.3390/jcm9113386. PubMed PMID: 33105634; PMCID: PMC7690437.

4. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, Hu JL, Xu W, Zhang Y, Lv FJ, Su K, Zhang F, Gong J, Wu B, Liu XM, Li JJ, Qiu JF, Chen J, Huang AL. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020;26(8):1200-4. Epub 2020/06/20. DOI: 10.1038/s41591-020-0965-6. PubMed PMID: 32555424.

5. Asadi-Pooya AA, Nemati H, Shahisavandi M, Akbari A, Emami A, Lotfi M, Rostamihosseinkhani M, Barzegar Z, Kabiri M, Zeraatpisheh Z, Farjoud-Kouhanjani M, Jafari A, Sasannia F, Ashrafi S, Nazeri M, Nasiri S. Long COVID in children and adolescents. World J Pediatr. 2021. Epub 2021/09/04. DOI: 10.1007/s12519-021-00457-6. PubMed PMID: 34478045; PMCID: PMC8414448.

6. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Perez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW, Jr., Hammitt LL, Tureci O, Nell H, Schaefer A, Unal S, Tresnan DB, Mather S, Dormitzer PR, Sahin U, Jansen KU, Gruber WC, Group CCT. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603-15. Epub 2020/12/11. DOI: 10.1056/NEJMoa2034577. PubMed PMID: 33301246; PMCID: PMC7745181.

7. Mazzini L, Martinuzzi D, Hyseni I, Benincasa L, Molesti E, Casa E, Lapini G, Piu P, Trombetta CM, Marchi S, Razzano I, Manenti A, Montomoli E. Comparative analyses of SARS-CoV-2 binding (IgG, IgM, IgA) and neutralizing antibodies from human serum samples. J Immunol Methods.

2021;489:112937. Epub 2020/12/01. DOI: 10.1016/j.jim.2020.112937. PubMed PMID: 33253698; PMCID: PMC7695554.

8. Arunachalam PS, Scott MKD, Hagan T, Li C, Feng Y, Wimmers F, Grigoryan L, Trial M, Edara VV, Lai L, Chang SE, Feng A, Dhingra S, Shah M, Lee AS, Chinthrajah S, Sindher SB, Mallajosyula V, Gao F, Sigal N, Kowli S, Gupta S, Pellegrini K, Tharp G, Maysel-Auslender S, Hamilton S, Aoued H, Hrusovsky K, Roskey M, Bosinger SE, Maecker HT, Boyd SD, Davis MM, Utz PJ, Suthar MS, Khatri P, Nadeau KC, Pulendran B. Systems vaccinology of the BNT162b2 mRNA vaccine in humans. Nature. 2021. Epub 2021/07/13. DOI: 10.1038/s41586-021-03791-x. PubMed PMID: 34252919.

9. Koch T, Mellinghoff SC, Shamsrizi P, Addo MM, Dahlke C. Correlates of Vaccine-Induced Protection against SARS-CoV-2. Vaccines (Basel). 2021;9(3). Epub 2021/04/04. DOI: 10.3390/vaccines9030238. PubMed PMID: 33801831; PMCID: PMC8035658.

10. Kosiorek P, Kazberuk DE, Hryniewicz A, Milewski R, Stroz S, Stasiak-Barmuta A. Systemic COVID-19 Vaccination Enhances the Humoral Immune Response after SARS-CoV-2 Infection: A Population Study from a Hospital in Poland Criteria for COVID-19 Reimmunization Are Needed. Vaccines (Basel). 2022;10(2). Epub 2022/02/27. DOI: 10.3390/vaccines10020334. PubMed PMID: 35214792; PMCID: PMC8875391.

11. Di Noia V, Pimpinelli F, Renna D, Barberi V, Pellini R, Morrone A, Giannarelli D, Cognetti F. Clinical characteristics are limiting the durability of humoral response to BNT162b2 in patients with solid cancer. Ann Oncol. 2022;33(3):350-2. Epub 2021/12/04. DOI: 10.1016/j.annonc.2021.11.015. PubMed PMID: 34861377.

12. Pitzalis M, Idda ML, Lodde V, Loizedda A, Lobina M, Zoledziewska M, Virdis F, Delogu G, Pirinu F, Marini MG, Mingoia M, Frau J, Lorefice L, Fronza M, Carmagnini D, Carta E, Orru V, Uzzau S, Solla P, Loi F, Devoto M, Steri M, Fiorillo E, Floris M, Zarbo IR, Cocco E, Cucca F. Effect of Different Disease-Modifying Therapies on Humoral Response to BNT162b2 Vaccine in Sardinian Multiple Sclerosis Patients. Front Immunol. 2021;12:781843. Epub 2021/12/28. DOI: 10.3389/fimmu.2021.781843. PubMed PMID: 34956211; PMCID: PMC8697018.

13. Polewska K, Tylicki P, Biedunkiewicz B, Rucinska A, Szydlowska A, Kubanek A, Rosenberg I, Rodak S, Slizien W, Renke M, Debska-Slizien A, Tylicki L. Safety and Tolerability of the BNT162b2 mRNA COVID-19 Vaccine in Dialyzed Patients. COViNEPH Project. Medicina (Kaunas). 2021;57(7). Epub 2021/08/07. DOI: 10.3390/medicina57070732. PubMed PMID: 34357013; PMCID: PMC8307559.

14. Havlin J, Skotnicova A, Dvorackova E, Hubacek P, Svorcova M, Lastovicka J, Sediva A, Kalina T, Lischke R. Impaired Humoral Response to Third Dose of BNT162b2 mRNA COVID-19 Vaccine Despite Detectable Spike Protein-specific T cells in Lung Transplant Recipients. Transplantation. 2022;106(3):e183-e4. Epub 2021/12/03. DOI: 10.1097/TP.0000000000004021. PubMed PMID: 34856599; PMCID: PMC8862668.

15. Oliveira-Silva J, Reis T, Lopes C, Batista-Silva R, Ribeiro R, Marques G, Pacheco V, Rodrigues T, Afonso A, Pinheiro V, Araujo L, Rodrigues F, Antunes I. Humoral response to the SARS-CoV-2 BNT162b2 mRNA vaccine: Real-world data from a large cohort of healthcare workers. Vaccine. 2022;40(4):650-5. Epub 2021/12/26. DOI: 10.1016/j.vaccine.2021.12.014. PubMed PMID: 34952755; PMCID: PMC8664659.

16. Adams O, Andree M, Rabl D, Ostermann PN, Schaal H, Lehnert E, Ackerstaff S, Muller L, Fischer JC. Humoral response to SARS-CoV-2 and seasonal coronaviruses in COVID-19 patients. J Med Virol. 2022;94(3):1096-103. Epub 2021/10/31. DOI: 10.1002/jmv.27427. PubMed PMID: 34716706; PMCID: PMC8662174.

17. Barriere J, Carles M, Audigier-Valette C, Re D, Adjtoutah Z, Seitz-Polski B, Gounant V, Descamps D, Zalcman G. Third dose of anti-SARS-CoV-2 vaccine for patients with cancer: Should humoral responses be monitored? A position article. Eur J Cancer. 2022;162:182-93. Epub 2022/01/12. DOI: 10.1016/j.ejca.2021.12.011. PubMed PMID: 35016032; PMCID: PMC8674546.

18. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, Subbarao K, Kent SJ, Tricks JA, Davenport MP. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med. 2021;27(7):1205-11. Epub 2021/05/19. DOI: 10.1038/s41591-021-01377-8. PubMed PMID: 34002089.

19. Krammer F. A correlate of protection for SARS-CoV-2 vaccines is urgently needed. Nat Med. 2021;27(7):1147-8. Epub 2021/07/10. DOI: 10.1038/s41591-021-01432-4. PubMed PMID: 34239135.

20. Gallais F, Gantner P, Bruel T, Velay A, Planas D, Wendling MJ, Bayer S, Solis M, Laugel E, Reix N, Schneider A, Glady L, Panaget B, Collongues N, Partisani M, Lessinger JM, Fontanet A, Rey D, Hansmann Y, Kling-Pillitteri L, Schwartz O, De Seze J, Meyer N, Gonzalez M, Schmidt-Mutter C, Fafi-Kremer S. Evolution of antibody responses up to 13 months after SARS-CoV-2 infection and risk of reinfection. EBioMedicine. 2021;71:103561. Epub 2021/08/30. DOI: 10.1016/j.ebiom.2021.103561. PubMed PMID: 34455390; PMCID: PMC8390300.

21. Planas D, Voyeur D, Baidaliuk A, Staropoli I, Grivel-Benhassine F, Rajah MM, Planchais C, Parrot F, Robillard N, Puech J, Prot M, Gallais F, Gantner P, Velay A, Le Guen J, Kassis-Chikhani N, Edriss D, Belec L, Seve A, Courtellemont L, Pere H, Hocqueloux L, Fafi-Kremer S, Prazuck T, Mouquet H, Bruel T, Simon-Loriere E, Rey FA, Schwartz O. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature. 2021;596(7871):276-80. Epub 2021/07/09. DOI: 10.1038/s41586-021-03777-9. PubMed PMID: 34237773.

22. Kurhade C, Zou J, Xia H, Liu M, Yang Q, Cutler M, Cooper D, Muik A, Sahin U, Jansen KU, Ren P, Xie X, Swanson KA, Shi PY. Neutralization of Omicron sublineages and Deltacron SARS-CoV-2 by three doses of BNT162b2 vaccine or BA.1 infection. Emerg Microbes Infect. 2022;11(1):1828-32. Epub 2022/07/07. DOI: 10.1080/22221751.2022.2099305. PubMed PMID: 35792746; PMCID: PMC9331225.

23. Moy JN, Anderson M, Shen X, Fu J, Stec M, Gosha A, Naquiallah D, Kinslow J, Montefiori DC, Cloherty G, Landay A. Neutralizing Antibody Activity to SARS-CoV-2 Delta (B.1.617.2) and Omicron (B.1.1.529) After One and Two Doses of BNT162b2 Vaccine in Infection-Naive and Previously-Infected Individuals. J Infect Dis. 2022. Epub 2022/06/28. DOI: 10.1093/infdis/jiac261. PubMed PMID: 35759252; PMCID: PMC9278194.

24. Buchan SA, Nguyen L, Wilson SE, Kitchen SA, Kwong JC. Vaccine Effectiveness of BNT162b2 Against Delta and Omicron Variants in Adolescents. Pediatrics. 2022. Epub 2022/06/17. DOI: 10.1542/peds.2022-057634. PubMed PMID: 35706105.

25. Kuechler AS, Weinhold S, Boege F, Adams O, Muller L, Babor F, Bennstein SB, Pham TU, Hejazi M, Reusing SB, Hermsen D, Uhrberg M, Schulze-Bosse K. A Diagnostic Strategy for Gauging Individual Humoral Ex Vivo Immune Responsiveness Following COVID-19 Vaccination. Vaccines (Basel). 2022;10(7). Epub 2022/07/28. DOI: 10.3390/vaccines10071044. PubMed PMID: 35891208; PMCID: PMC9322304.

26. Lo Sasso B, Agnello L, Giglio RV, Gambino CM, Ciaccio AM, Vidali M, Ciaccio M. Longitudinal analysis of anti-SARS-CoV-2 S-RBD IgG antibodies before and after the third dose of the BNT162b2 vaccine. Sci Rep. 2022;12(1):8679. Epub 2022/05/24. DOI: 10.1038/s41598-022-12750-z. PubMed PMID: 35606426; PMCID: PMC9126106.

27. Re D, Seitz-Polski B, Brglez V, Carles M, Graca D, Benzaken S, Liguori S, Zahreddine K, Delforge M, Bailly-Maitre B, Verriere B, Chamorey E, Barriere J. Humoral and cellular responses after a third dose of SARS-CoV-2 BNT162b2 vaccine in patients with lymphoid malignancies. Nat Commun. 2022;13(1):864. Epub 2022/02/16. DOI: 10.1038/s41467-022-28578-0. PubMed PMID: 35165284; PMCID: PMC8844396.

28. Zheng J, Deng Y, Zhao Z, Mao B, Lu M, Lin Y, Huang A. Characterization of SARS-CoV-2-specific humoral immunity and its potential applications and therapeutic prospects. Cell Mol Immunol. 2022;19(2):150-7. Epub 2021/10/15. DOI: 10.1038/s41423-021-00774-w. PubMed PMID: 34645940; PMCID: PMC8513558.