Systemic COVID-19 vaccination also enhances the humoral immune response after SARS CoV-2 infection in the population of an oncology hospital in Poland. Criteria for COVID-19 re-immunization are needed.

Piotr Kosiorek (pkosiorek@onkologia.bialystok.pl)
Oncology Center Bialystok
Dorota Kazberuk
Oncology Center Bialystok
Anna Hryniewicz
Medical University of Białystok
Robert Milewski
Medical University of Białystok
Samuel Stróż
Medical University of Białystok
Anna Stasiak-Barmuta
Medical University of Białystok

Research Article

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Abstract

Systemic vaccination of the BNT162b2 mRNA stimulates humoral response. Our study aimed to compare the intensity of humoral immune response, measured by SARS CoV-2 IgG, SARS CoV-2 IgM, and neutralization S-RBD IgG antibodies level, post COVID-19 vaccination versus post-SARS CoV-2 infection. We analyzed 1060 people in the following groups: convalescents, healthy vaccinated, vaccinated with Comirnaty, AstraZeneca, Moderna, Johnson & Johnson, and vaccinated SARS CoV-2 convalescents. A concentration of SARS CoV-2 IgG, SARS CoV-2 IgM, and neutralizing S-RBD IgG was estimated in the oncology hospital laboratory by chemiluminescent immunoassay - CLIA, MAGLUMI. Results: 1. We observed a rise of antibodies response in both convalescent SARS CoV-2 and COVID-19 vaccinated groups 2. The level of all antibodies' concentrations in vaccinated COVID-19 convalescents was significantly higher. 3. We differentiated asymptomatic SARS CoV-2 convalescents from the control group. Our analysis suggested that it is essential to monitor SARS CoV-2 IgG antibodies concentrations as an indicator of asymptomatic COVID-19 and equivalent to the effectiveness of humoral response in convalescents and vaccinated people. Considering the time-limited effects of post-infection SARS CoV-2 recovery or vaccination, among others physiological half-life, we suggested monitoring IgG antibodies level as a criterium for the next vaccination.

Introduction

Vaccination is the best form of prevention of infectious diseases. The Pfizer-BioNTech vaccine BNT162b2 (Comirnaty) [1] is recognized so far as among the most effective vaccine in preventing SARS Co-V-2 infection and severe COVID-19 [2,3,4]. A rising level of specific antibodies – IgM and IgG – and neutralizing S-RBD IgG as a humoral immune response is an effect of vaccination. A similar effect is observed post SARS CoV-2 exposure (COVID-19; Coronavirus disease 2019) [5]. They have been proving that in symptomatic SARS CoV-2 infections, the level of neutralizing S-RBD antibodies correlates with the severity of COVID-19 and with hospitalization [5,6]. This correlation was observed neither in asymptomatic convalescent COVID-19 patients nor in healthy vaccinated individuals after the first dose of systemic double vaccination [7,8]. There was evidence that after a second dose of systemic vaccinations with mRNA Pfizer-BioNTech, the neutralizing antibody level was lower than that in vaccinated convalescent COVID-19 patients [8]. Higher levels of neutralizing antibodies after SARS CoV-2 exposure and COVID-19 vaccinations are seen in autoimmune disease [9,10]. Seroconversion to neutralizing antibodies is gradual and depends on a patient's clinical state resulting from immune defense mechanisms, such as neutralization, complement activation, and cell cytotoxicity (ADCC; Antibody-dependent cellular cytotoxicity) [7,11,12]. In rare situations, SARS CoV-2 infection gives rise to systemic inflammatory response syndrome (SIRS) with systemic inflammatory multiorgan dysfunction (MODS) [13]. Depending on the phase and intensity of the inflammatory response, giving convalescents serum plasma antibodies for COVID-19 treatment may reduce or strengthen the inflammation [14]. There is much controversy involving convalescent-derived serum plasma for COVID-19 therapy [26]. Serum

plasma antibodies are very effective at an early stage of COVID-19 when the inflammation process has not yet involved elements of the humoral response [12,15,26].

The evaluation of systemic vaccinations against SARS CoV-2 invariably raises questions about seroprotective antibody concentration levels and their half-life [11]. Aging of the immune system is, on the one hand, a risk factor for contracting COVID-19 and, on the other hand, determines poor postvaccination response [16]. The question then arises whether elderly individuals require an additional dose of the vaccine [16,17]. Should be asked the same question about immunosuppression, people with immunodeficiency syndromes, post-transplantation patients, or oncologic or dialysis patients? Many published papers prove the safety of BNT162b2 vaccination in dialysis patients [18], patients after lymphoma therapy [19], and the safety of the third dose in kidney transplant recipients [20]. In healthy individuals, partial seroprotection, a mean of approximately 53% (32-68%, confidence interval 95%), is reached 14 days after the first dose of the BNT162b2 vaccine. Seven days after the second dose, seroprotection reached 95% [16]. Seroprotection is even higher for SARS CoV-2 variants B.1.1.7 and B.1.351, 75% and 97% after the first and second doses, respectively [21].

Materials And Methods

Our study observed a group of 1063 people, 786 females (74%) and 277 males (26%), in the age bracket between 18 and 89 years old. People over 50 years old constituted 45% (N=479) of the group. Because of different postvaccination reactions, three subjects we considered early hyper responders IgM and IgG, and we excluded them from further evaluation. The final group of 1060 subjects (Table 1.) we divided into five subgroups: Control group G0 (N=154) was unvaccinated persons with no clinical signs of COVID-19 who tested negative for SARS CoV-2 by RT-PCR. Group G1 (N=76) included entirely symptomatic COVID-19 patients who tested positive for SARS CoV-2 by RT-PCR. Group G2 included 472 healthy individuals vaccinated with both doses of Comirnaty. Group G3 included 42 persons vaccinated with AstraZeneca (N=21), Moderna (N=19), and Johnson & Johnson (N=2) according to the vaccination scheme. Group G4 included 312 COVID-19 convalescents vaccinated with Comirnaty. Group G5 consisted of 4 individuals with COVID-19 convalescents infected with SARS CoV-2 after a complete systemic vaccination cycle. Two positive RT-PCR tests confirmed the infection.

After a retrospective analysis of concentrations of SARS CoV-2 IgG antibodies in group G0 (N=154), we identified subgroup G01 of 43 persons who had higher than cut-off value (0.2 AU/ml) concentrations of specific IgG antibodies. The existence of specific SARS CoV-2 antibodies we recognized as evidence of viral contact, and the patients were qualified as SARS CoV-2 convalescents with no symptomatic COVID-19 history. Finally G0=111. Among 43 analyzed cases (G01), 13 persons were in the seroconversion phase (both positive IgM>1.0 AU/ml and IgG>0.2 AU/ml), 30 persons were in a late phase producing secondary antibodies (positive IgG, negative IgM), but eight persons were in an early phase of the humoral viral response (positive IgM, negative IgG).

2.1. Materials

Our study materials were blood specimens taken through venepuncture sampling. The concentration of antibodies was evaluated 4 hours after blood collection. If the immediate assessment was not possible, the serum was collected and stored at -80 °C.

2.2. Methods

All 1063 participants had elevated levels of IgM and IgG antibodies oriented specifically towards SARS CoV-2; in 546 subjects, IgG anti-S (S-RBD) antibodies we detected by chemiluminescent immunoassay-CLIA (MAGLUMI, Snibe Diagnostic, Shenzhen China).

The results were greater or equal to 1.0 AU/ml SARS CoV-2 IgG, IgM, and S-RBD we considered reactive and recognized as positive according to the manufacturer's protocol.

Of the study participants, 827 were vaccinated, including Comirnaty 787 persons. BNT162b2 was 95% effective in preventing COVID-19, and similar vaccine efficacy observes across subgroups defined by age, sex, race, ethnicity[1]. Table 2 is data on gender and age supplement to Table 1.

The Bioethics Commission of Medical University gave consent for our research.

3. Statistical analysis

Nonparametric statistical methods we applied for the case-control analyses because of a lack of considered variables with a normal distribution. Comparing two groups to each other, the Mann-Whitney test was adopted, whereas the Kruskal-Wallis test was applied to assess more than two groups. The statistically actual results were assumed to be $p<0,05$: We used PQStat Software 2021 and Tibco statistic 13.3.

Results

After evaluating the concentrations of specific antibodies in the control group of healthy unvaccinated subjects, we identified a subgroup of SARS CoV-2 convalescents (G01) after an asymptomatic COVID-19. The concentration of antibodies from IgG class in that subgroup was comparable to the concentration observed in COVID-19 convalescents with entirely symptomatic disease confirmed by a positive RT PCR test.

Our research showed the presence of SARS CoV-2 antibodies in both the convalescent and COVID-19 vaccinated groups, wherein the concentration of antibodies decreased with time in both groups. Figure 1.

Significantly higher IgG- and IgM-specific antibody concentrations in vaccinated COVID-19 convalescents (G4). Figure 2.

After we analyzed the strength of specific IgG and IgM generation after vaccination, we did not find any significant difference between the studied vaccines (Pfizer, AstraZeneca, Moderna). The sample size of

the Johnson & Johnson vaccine recipients (N=2) was too small to render a meaningful result; hence, we excluded it from further analysis.

Figure 3 shows the levels of antibodies in the following groups: (a) evaluated in individual groups at time intervals after full vaccination and (b) after a positive RT PCR test result for SARS CoV-2 (COVID-19). The completed analysis showed a linear dependence over time for both vaccination response and response naturally taken from viral infection after clinically mild or moderate COVID-19. We observed the effect mentioned above in each studied group up to 180 days post-exposure and up to 90 days post-vaccination.

Discussion

The evaluation of concentrations of IgG and IgM antibodies specific to SARS CoV-2 that we conducted for this study allowed us to identify a new subgroup among the healthy, unvaccinated controls: subjects who had a previous asymptomatic SARS CoV-2 infection. Every patient in this subgroup presented lower concentrations of specific SARS CoV-2 IgG antibodies than the cut-off assumed for a positive result by laboratory tests, between 0.2 and 1.0 AU/ml.

Based on the concentration of neutralizing antibodies, we propose the following hierarchy of activation of the humoral response: unvaccinated (G0) < convalescents (G1) < vaccinated (G2, G3) < vaccinated convalescents (G4) < patients with double SARS CoV-2 infection after being fully vaccinated (G5).

Group G4 was exposed to the virus three times: the first time they were infected with SARS CoV-2 virus and the following two times when they were injected through immunization. Every contact with antigen altered the immune system, causing a burst of antibody production lasting up to 14 days [22,23]. The triple exposure of this group caused the most significant rise in neutralizing antibodies among all groups observed.

Although group G5 (N=4), vaccinated people who developed entirely symptomatic COVID-19, was excluded from further statistical analysis due to the group's small size, the results we obtained for that group correlate with literature data, which shows the most significant growth in antibody concentration in vaccinated and infected people [8,21].

5.1. Who needs the third dose of the vaccine?

Synthesis of antibodies from the IgM class is the first phase of the humoral response after initial contact with the antigen. IgG antibody synthesis occurs after the second or repeated contact with the same antigen. Conducted analysis of concentrations of specific SARS CoV-2 antibodies IgG and IgM and their mutual dependence let us discover early and late phases of humoral response to SARS CoV-2 infection and COVID-19 vaccine [17,21]. Indirectly, it allowed us to identify individuals who had asymptomatic COVID-19. One should remember that the timing of blood sample collection is fundamental for the correct evaluation of the concentrations of IgM and IgG antibodies. This timing depends on the patient's clinical

status and dynamic changes in the immunologic system, e.g., after SARS CoV-2 infection, and can effectively optimize the planning of the next vaccine dose against the Delta variant [22]. Of course, such an evaluation needs to compare on clinical examinations, identify infection symptoms of lack of thereof, serological investigation – the presence or lack of virus antigens.

In Israel, we observed an increase in SARS infection among people vaccinated with the BNT1262b vaccine 146 days post-vaccination [23]. This increase was the most pronounced in people over 60 [23]. In our opinion, it is reasonable to start measuring specific antibodies IgG (late effect) and IgM (early effect) for vaccination evaluation at serological window neutralizing antibodies. As we know so far, a humoral response to neutralizing antibodies post-vaccination is rapid and decreasing. In addition, in some people without exposure to SARS CoV-2, humoral and cellular responses are detected late, indicating widespread immunity to SARS in the population [24]. In summary, our results indicate the importance of evaluating and monitoring immunological response parameters in vaccinated healthy people and convalescents, which will help establish the necessity for the third and the next dose of vaccine against SARS CoV-2. The manuscript we sent was a preprint [25].

Declarations

Author Contributions: Piotr Kosiorek, Anna Hryniewicz, and Anna Stasiak-Barmuta analyzed the data and drafted the manuscript. Robert Milewski participated in data analysis and extensively reviewed the manuscript. Other authors contributed to clinical and laboratory data acquisition and reviewed the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest

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25. The manuscript has been submitted as a preprint in the below link: https://www.researchsquare.com/article/rs-858160/v4.

Tables

Table 1. Population characteristics and outcomes in the case-control study.

Groups	Participants	G0	G01	G1	G2	G3	G4	G5
All	1060	111	43	76	472	42	312	4
%	100	10.69	4.05	7.17	44.52	3.96	29.43	0.38
2020	499 (47.07%)	51	14	30	215	7	179	3
2021	561 (52.92%)	60	29	46	257	35	133	1
COVID-19 test								
Negative	671 (63.3%)	111	43	0	471	35	11	0
Positive	389 (36.69%)	0	0	76	1	7	301	4
COVID-19 response								
no	662 (62.45%)	111	43	0	470	35	2	0
30-50 days	18 (1.69%)	0	0	13	1	2	1	2
60-90 days	81 (7.64%)	0	0	18	0	3	58	2
>180 days	299 (28.2%)	0	0	45	1	0	251	0
Vaccinated								
no	233(21.79%)	111	43	76	0	0	0	0
yes	827 (78.01%)	0	0	0	472	24	312	4
Comirnaty	787 (74.,2%)	0	0	0	472	0	312	4
Astra Zeneca	21 (1.98%)	0	0	0	0	21	0	0
Moderna	19 (1.79%)	0	0	0	0	19	0	0
J&J	2 (0.19%)	0	0	0	0	2	0	0
Vaccinated response								
no	749 (70.66%)	111	43	76	288	31	197	2
<14 days	58 (5.47%)	0	0	0	28	4	26	0
14-90 days	183 (17.3%)	0	0	0	111	0	64	1
>90 days	70 (6.6%)	0	0	0	45	0	25	1
IgG (AU/ml)	1060							
<1.0	685 (64.62%)	111	19	41	420	27	192	3
>0.2	375 (35.37%)	0	43	42	103	19	167	1
>1.0	247 (23,30%)	0	24	35	52	15	120	1
IgM (AU/ml)	1060							
<1.0	879 (82.92%)	103	33	65	397	36	242	3
>1.0	181 (17.07%)	18		11	75	6	70	1
S-RBD IgG (AU/ml)	546							
<1.0	81 (7.64%)	31	2	6	31	4	7	0
>1.0	465 (43.86%)	20	22	33	220	21	147	2
> 50	295 (27.83%)	0	3	11	154	8	117	2
> 100	246 (23.20%)	0	3	8	126	6	101	2
> 500	57 (5.38%)	0	0	1	14	4	36	2
> 1000	38 (3.58%)	0	0	0	10	3	23	2

The average age of 1060 participants is 47.5 years. There is normal population distribution. All antibodies concentration results are in AU/ml. The COVID-19 test represents population groups tested positive or negative RT-PCR. COVID-19 response represents the population groups where antibodies with a temporal correlation with infection tested, as measured by a positive test. Vaccinated COVID-19 represents the vaccine population groups. Vaccination response refers to the population groups with a temporal correlation with the performance of the vaccination. We rejected from 1063 participants three persons assumed as hyper responders IgM and IgG because of spectacular higher antibodies concentrations measured (>2000 AU/ml IgG and >700 and >300 AU/ml IgM). The person with an over-response to IgG was also an early responder (<14 days) due to an autoimmune disease. In the G5 group too few people to conclude, but four people had high levels of S-RBD IgG and low levels of SARS CoV-2 IgM and IgG. This phenomenon seems to be related to the loss of antibodies following a previous complete vaccination and the duplication of SARS CoV-2 infection.

Table.2 Data on gender and age supplement to Table 1.

Groups	Participants	G0	G01	G1	G2	G3	G4	G5
All	1060	111	43	76	472	42	312	4
%	100	14.62	4.05	7.17	45.66	3.96	29.43	0.38
Sex								
Female	783(73.87%)	94	10	33	63	346	23	3
Male	277(26.13%)	18	33	10	125	19	91	1
Age range								
< 35	220(20.75%)	39	8	20	102	4	47	0
36-49	361(34.06%)	39	12	23	163	15	109	0
>50	479(45.18%)	34	23	33	206	23	156	4

Extending preferential vaccinations to only some age groups in Poland prevented us from correctly interpreting these data. No relationship with gender is in the analyzed groups.

Figures

Figure 1

Figure shows the absolute concentrations of SARS CoV-2 IgG **(a)** IgM **(c)** a neutralizing S-RBD IgG **(b)** antibodies in each study group G0-G5. (*p, Kruskal-Wallis ANOVA).

Figure 2

Figure shows the absolute concentrations of SARS CoV-2 IgG and IgM antibodies **(a, c)** and neutralizing S-RBD IgG **(b, d)** for vaccinated and COVID-19 individuals (*p, Kruskal-Wallis ANOVA).

Figure 3

Figure 3 shows the determination of antibodies in the subsequent study groups (a) performed in each group at intervals after full vaccination (b) and after obtaining a positive result for SARS CoV-2 (COVID-19) (* p, Kruskal-Wallis).

Supplementary Files

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