

COMPARISON OF ANTI-RECEPTOR BINDING DOMAIN
OF SARS-COV-2 SPIKE PROTEIN HUMORAL RESPONSE
BETWEEN INFECTED, VACCINATED AND VACCINATED
AFTER NATURAL EXPOSURE SUBJECTS OVER
12-MONTHS FOLLOW-UP PERIOD

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Abstract

The duration and dynamics of the humoral immune response against SARS-CoV-2 in different groups of individuals are important for determining the risk of reinfections and breakthrough infections. The aim of this study was to monitor and compare the humoral immune response against SARS-CoV-2 over a 12-month period by testing anti-RBD IgG, anti-S IgA, and virus neutralizing antibodies (VNAb). The study included 107 participants belonging to the following groups: COVID-19 convalescent individuals (CI), vaccinated seronaive (VS) and vaccinated convalescent participants (VC). The mean values of anti-RBD IgG and VNAb antibodies in period I were statistically significantly higher in the two groups of vaccinated participants compared to CI. In period II and period III, the mean IgG and IgA levels were statistically significantly higher in VC individuals compared to CI or VS. The mean values of anti-S IgA in all three periods were statistically higher in the group of participants with hybrid humoral immunity compared with CI and VS. The results showed that after the third month and until the end of the study, the mean anti-RBD IgG, anti-S IgA, and VNAb antibody values remained highest in the vaccinated convalescents. In vaccinated seronaive the mean values for the three parameters in the first three months were higher than those in CI. A greater decline was

observed in the group of VS and after 7 months the mean anti-RBD IgG, anti-S IgA, and VNAb values in this group were similar to those of the convalescents-only group.

Key words: antibodies, immunity, RBD, SARS-CoV-2, vaccinated

Introduction. Since the onset of the SARS-CoV-2 pandemic, a number of studies have been initiated on the dynamics and duration of naturally acquired humoral immunity. After most of them confirmed kinetics similar to those observed in other viral infections during COVID-19 illness and in the early reconvalescent phase [1], it remained to clarify the long-term duration and dynamics of humoral immunity. Although they are fewer and limited in the number of participants, some studies on the other two coronaviruses that can present with a severe clinical course (SARS-CoV and MERS-CoV) have shown the presence of viral neutralizing activity even after 2–3 years of infection [2, 3]. Some SARS-CoV-2 studies also showed the presence of antibodies and virus neutralizing activity after 6–12 months of disease onset [4–6]. The severe clinical course in some patients and the significant social and health consequences have necessitated the use of vaccines designed to initiate an immune response against certain SARS-CoV-2 epitopes. For the first time, vaccine preparations were administered using mRNA that encodes particular recombinant spike proteins of the virus [7]. The studies about the immune response in the first months after completion of vaccination course have shown seropositivity to the receptor-binding domain (RBD) of S1 in over 90% of vaccinated individuals and a high rate of virus-neutralizing potential [8, 9]. Some authors have studied the long-term immune response to SARS-CoV-2 after vaccination and all of them have shown a decline of anti-RBD IgG and VNAb after the sixth month [10, 11]. However, further studies are needed on the long-term dynamics of the humoral response depending on the mode of acquisition.

The present study aims at following the extent, dynamics, and duration of the antibody immune response to the receptor-binding domain (RBD) in convalescent patients, vaccinated seronaive after completion of the vaccination course individuals, and in vaccinated convalescents and to compare its parameters between the different groups.

Materials and methods. Participants. In this study, the concentrations of IgG, IgA, and virus neutralizing antibodies (VNAb) against SARS-CoV-2 RBD were examined in a total of 107 health care workers in the period from November 2020 to March 2022. For detection of asymptomatic infections and epidemiological control of the spread of SARS-CoV-2, health care workers were tested weekly with RT-PCR or rapid antigen test during this period. In addition, at the start of the vaccination campaign in the end of 2020, health care workers were tested for anti-RBD IgG to optimize the application of the limited then number of vaccines. The required information regarding participants (type of vaccine, date of vaccination and/or onset of the disease) was collected using available medical records and a

questionnaire completed personally by each patient. All participants signed an informed consent according to the principles of the Declaration of Helsinki.

Measurements of anti-RBD IgG, anti-S IgA and VNAb were conducted in three periods: period I – 30–90 days from the onset of infection or completion of vaccination course, period II – 91–210 days, and period III – 211–365 days. A total of 47 individuals were included in the group of convalescent individuals (CI), 46 in the group of vaccinated seronaive (VS), and 14 were vaccinated 6–8 months after infection (VC). Summarised information on the number of participants and measurements in the three periods for each group is presented in Table 1. The infections occurred between November 2020 and November 2021, before the appearance of Omicron variant. In both groups of vaccinated participants – seronaive and vaccinated after illness, the mRNA vaccine, produced by Pfizer/BioNTech was administered in a two-dose regimen according to the manufacturer’s conditions. In the group of VC, the vaccination was conducted 6–8 months after infection.

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Numbers of convalescents, vaccinated seronaive, and vaccinated convalescent participants tested for anti-RBD IgG, anti-S IgA and VNAb

Group	Individuals tested for anti-RBD IgG			Individuals tested for Anti-S IgA			Individuals tested for VNAb		
	Period I	Period II	Period III	Period I	Period II	Period III	Period I	Period II	Period III
Convalescent individuals	36	37	32	20	27	24	20	27	24
Vaccinated seronaive individuals	46	33	30	27	29	22	31	29	27
Vaccinated convalescent individuals	12	14	7	11	13	7	12	14	7

Methods. *Anti-RBD IgG antibody testing.* The method used was an automated system VIDAS[®] SARS-COV-2 IgG/IgM(9COG/9COM)BIOMÉRIEUX, France. The test principle combines a two-step “sandwich” enzymatic method with a final fluorescence detection – ELFA. The cut-off value for positivity was 1.00. According to the manufacturer’s instructions, the value obtained can be converted into BAU/ml by the following formula, where the k is a pre-set factor equal to 20.33: $\text{BAU/ml} = k \cdot \text{TV}$.

Anti-S1 IgA antibody testing. Anti-SARS-CoV-2 IgA ELISA (EUROIMMUN, Germany) was used. The principle of the assay is based on the two-step “sandwich” method. The test results were given in Ratio. The ratio was calculated using the value of optical density (OD) of the sample/control and OD of the calibrator,

measured on wave length of 450 nm. A Ratio greater than 1.1 was accepted as a positive result.

sVNAT study. For this purpose, we used the Surrogate virus neutralization test (sVNT) (GenScript, China). The assay is performed in a similar manner to ELISA and quantifies the percentage of virus-neutralizing activity to the receptor binding domain (RBD) of the Spike protein. The results were presented in percentage virus-neutralizing activity. Cut-off value was 30% and results greater than this percentage were considered positive.

Statistical analyses. The programming language used was Python 3.9. When testing for a statistically significant difference between two populations, a significance level of $p \leq 0.05$ was chosen at which we could reject the null hypothesis. The student's t -test was applied to assess the presence of a statistically significant difference between means. Such a test was conducted for IgG, IgA, and VNAb between the group of CI, the group of VS, and VC for the three distinct periods.

Results. In the group of CI statistically significant difference was observed for anti-RBD IgG between periods I and II ($p = 0.018$) and no statistically significant difference between the three periods for anti-S IgA and VNAb was observed. In the group of VS significant difference was observed between periods I and II ($p = 0.0001$) and period II and III ($p = 0.018$) for anti-RBD IgG and between period I and II for anti-S IgA ($p = 0.004$) and VNAb ($p = 0.0001$). In the group of VC significant difference was observed between period I and II for anti-RBD IgG ($p = 0.04$) and no difference was observed between the three periods for anti-S IgA and VNAb.

The data regarding maximum, minimum and mean values of viral-specific IgG, IgA, and VNAb in the three periods for CI, VS and VC are presented in Table 2. Figure 1 shows the dynamics of mean anti-RBD IgG, anti-S IgA and VNAb values correspondingly in the three periods in convalescents, vaccinated seronaive, and vaccinated convalescent individuals. Statistical analysis was performed to assess the presence of a significant difference in IgG, IgA, and VNAb values between the three groups for the three periods of investigation. The analysis showed the following correlations: the mean values of anti-RBD IgG in period I in the two groups of vaccinated participants were statistically higher compared to the CI ($p = 0.0001$ for both groups). In this period, no significant difference was found between VS and VC ($p = 0.39$). In period II and period III, the mean IgG levels were statistically significantly higher in vaccinated, previously infected compared to either CI or VS ($p = 0.0001$ for period II and $p = 0.001$ for period III for both groups). No statistically significant difference was observed between CI and VS for period II ($p = 0.129$) and III ($p = 0.745$). The mean anti-S IgA values in all three periods were statistically higher in VC group compared to CI ($p = 0.0001$ for period I and period III, $p = 0.001$ for period III) and compared to VS ($p = 0.002$ for period I and $p = 0.0001$ for period II and period III). There

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Minimum positive, maximum, and mean values for anti-RBD IgG (BAU/ml), anti-S IgA (Ratio), and VNAb (%)

Group	Period	IgG – BAU/ml			IgA – Ratio			VNAb – %		
		Min.Positive Value	Max. Value	Mean Value	Min.Positive Value	Max. Value	Mean Value	Min.Positive Value	Max. Value	Mean Value
Convalescent individuals	I	20.33	504.387	195.6	1.10	9.58	3.15	32.3	96.8	70.45
	II	20.33	483.854	119.25	1.25	9.59	2.92	33.6	97	63.24
	III	20.33	358.215	89.35	1.20	8.50	3.21	32.7	96.7	61.44
Vaccinated seronaive individuals	I	105.11	784.535	482.92	1.38	10.53	4.91	58.2	97.8	89.03
	II	30.495	507.03	164.2	1.14	9.56	2.71	36.8	98	60.49
	III	21.753	315.115	95.21	1.1	9.8	1.99	35.1	94.5	50.6
Vaccinated Convalescents individuals	I	134.18	766.237	532.5	2.48	14.5	8.53	79.5	98	95.08
	II	126.05	778.638	375.28	3.22	12.09	6.48	49.6	97.7	90.9
	III	115.28	300.884	221.59	3.29	9.2	6.12	66.8	96.6	88.43

was a statistically significant difference between the IgA mean values of the vaccinated seronaive group and the group of CI in the first period ($p = 0.043$), with higher IgA mean values in the VS group. In period II, no significant difference was found for anti-S IgA between CI and VS ($p = 0.736$). In period III, a statistically significant difference appeared between the two groups ($p = 0.05$), although in this period the mean IgA values were higher in the group of CI. The mean VNAb values in period I were statistically higher in the group of VC compared to CI ($p = 0.003$) and in the group of VS compared to CI ($p = 0.001$). In this period, no significant difference was found between the mean VNAb values of VS and VC ($p = 0.071$). In period II and period III the mean values were statistically significantly higher in the group of VC compared to VS ($p = 0.0001$ for both periods) and CI ($p = 0.0001$ for period II and $p = 0.005$ for period III). In period II and period III, there was no significant difference in the mean VNAb values between VS and CI ($p = 0.65$ for period II and $p = 0.089$ for period III).

Discussion. The differences on the dynamics and duration of humoral immunity against SARS-CoV-2 acquired in different ways is important for optimizing local guidelines for vaccine prophylaxis of this disease depending on the individual's

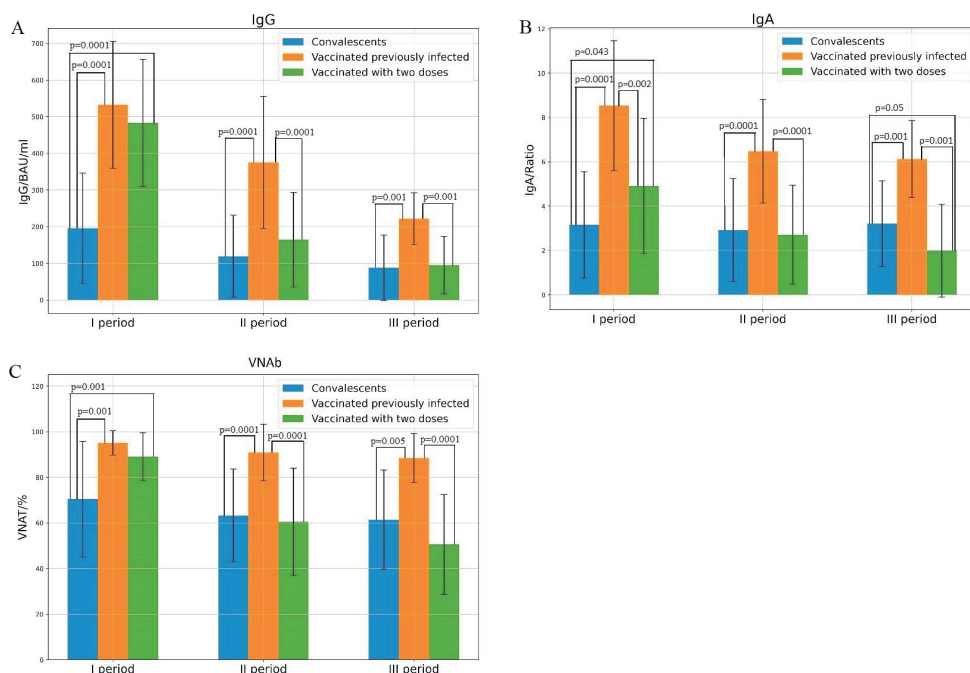


Fig. 1. **A:** Dynamics of mean anti-RBD IgG positive values for the three periods in convalescents, vaccinated seronaive and vaccinated convalescent patients. **B:** Dynamics of mean IgA values for the three periods in convalescents, vaccinated seronaive and vaccinated convalescents. **C:** Dynamics of mean VNAb values for the three periods in convalescents, vaccinated seronaive and vaccinated convalescents. The statistically significant differences are designated in the figure

status. In this study as well as in others, a high percentage of virus neutralizing activity was observed over a one-year period after illness [4, 5]. For anti-S IgA, some authors reported a rapid decline in the first 3 months and less than 20% of IgA-positive individuals 12 months after illness [10, 12]. Although these data are consistent with what has been studied regarding antibody kinetics during and after most viral infections, results from several other studies correlated with the stability in IgA levels to SARS-CoV-2 which this study also observed [13, 14]. The mechanisms behind this phenomenon are not yet fully understood and further studies would be necessary to explain it. One of the reasons for the stability of anti-S IgA might be the longer persistence of viral particles after infection (mRNA and proteins) in the epithelium cells, which some authors had observed [15].

This study observes a higher rate of viral inhibition and higher mean values of anti-RBD IgG and anti-S IgA in the first three months in the group of VS compared to CI. After the third month, significant decline was found, resulting in mean values for the three parameters similar (for anti-RBD IgG and VNAb), or significantly lower (for anti-S IgA) to those in the group of CI in the third

period. In this study, higher peak values were found of anti-S-RBD antibodies in vaccinated compared to CI, but COPPETA et al. [10] observed no difference in results between vaccinated seronaive and convalescent individuals after 6 months.

The immune response acquired through disease and vaccination is termed hybrid [16]. In the group of participants with hybrid humoral immunity (VC) this study observed the highest mean anti-RBD IgG, anti-S IgA levels and VNAb over the entire follow-up period in comparison to CI and VS. Although this study included a smaller number of participants in the VC group ($n = 14$), the differences we observed in the duration and dynamics of the humoral immune response between CI, VS and VC correlated with the results obtained by other larger studies. PEREZ-ALOS et al. [17] reported significantly higher concentrations of viral – specific IgA, IgG, and VNAb in participants with a hybrid immune response compared to vaccinated participants and a smaller decline over the follow-up period of 230 days. In another large study that included a total of 1081 participants similar to the results of this study were obtained about the different kinetics of anti-RBD IgG, anti-S IgA and VNAb [18].

The higher mean antibody values and the greater stability observed within this study during the follow-up period in vaccinated convalescent individuals is probably a result of the greater number of antigen encounters that activated the mechanisms of secondary immune response [19]. Most studies in recovered COVID-19 patients who were subsequently vaccinated were done in a single-dose regimen for both mRNA vaccines and showed an immune response beginning as early as day seven after administration, even in individuals with negative antibody values before vaccination. This suggests the presence of immune memory and the possibility of rapid immune response with high potential more than 10 months after infection [20]. The participants in this study were vaccinated with two doses of mRNA vaccine and the results are similar to those obtained after one-dose regimen [17]. Although there was a decrease in the mean anti-RBD and anti-S IgA values, the mean viral inhibition in all three periods in VC group was about 90%. This is probably a result of combined virus neutralizing activity of IgG and IgA class antibodies. Although there was a decrease in the mean IgG and IgA values in VC, they remained higher throughout the follow-up period. The higher activity of the antibodies may also be due to the higher affinity normally observed for antibodies secreted by memory B cells in the secondary immune response [19]. These reasons also explain the higher potential of hybrid immunity to the different variants of SARS-CoV-2, including the Omicron variant and the lower risk of breakthrough infections in vaccinated convalescent individuals, reported by some authors [19,21].

Conclusion. The current study demonstrates the importance of vaccinating COVID-19-recovered individuals and the need for administering booster doses to vaccinated seronaive 6 months after completion of the vaccination course. As a limitation of our study, we can point to the relatively small number of participants.

Nevertheless, the results were sufficient to define the main trends in antibody kinetics in the different groups.

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