Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2022;11(4):1534-40

A trend analysis of Sars-Cov-2 anti-nucleocapsid IgG antibodies with a recommendation of the cut-off classification to identify naïve, vaccinated, and infected cases

Serhat Uysal, Kutbeddin Demirdag, Mesut Batur, Safak Ozer Balin, Mehmet Ali Asan, Ayse Sagmak Tartar, Ayhan Akbulut

Fırat University School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Elazıg, Türkiye

Received 05 July 2022; Accepted 23 September 2022
Available online 27.11.2022 with doi: 10.5455/medscience.2022.07.153

Abstract

Determination of the vaccination's effect on immune responses with the emergence of the Sars-Cov-2 pandemic become an important issue. This study aimed to evaluate the response of the anti-nucleocapsid IgG (anti-N IgG) index in naïve, vaccinated, or infected cases, in addition to suggesting 'How to distinguish between vaccinated versus infected cases via anti-N IgG?'. Anti-N levels of the naïve [0.03 (0.02–0.06)], vaccinated [0.7 (0.2–1.96)], and infected [3.07 (1.44–5.2)] groups were statistically different ($p<0.0001$). Anti-N levels were statistically different in all periods of the infected and vaccinated sub-groups ($p<0.0001$). An inter-group analysis of the cases with repeated tests established that there was no difference between the not-vaccinated (-1.44±1.27) and after-infection early-vaccinated (-1.62±0.93) groups ($p=0.717$). A significant difference was observed between the after-infection early-vaccinated (-1.62±0.93) and late-vaccinated groups (1.5±1.61) ($p<0.0001$). Three-category cut-off levels were calculated for the naive, vaccinated, and infected cases as ≤0.32, 0.32–1.40, and ≥1.40 respectively. The area under the receiver operating curve (AUROC) of this classification was 0.907 (95% CI, 0.879–0.930) and the AUROC of the manufacturer's cut-off (≥1.40) was 0.790 (95% CI, 0.754–0.823) ($p<0.0001$). This study demonstrates that early vaccination after the infection does not affect the trend of the anti-N level. The manufacturers should set out to update their tests dating back to the pre-vaccine period for the post-vaccine period.

Keywords: COVID-19, anti-nucleocapsid IgG, ınactive vaccine, naïve, SARS-Cov-2, vaccination, serology

Introduction

Following the coronavirus infection 2019(COVID-19), antibodies and cellular responses specific to severe acute respiratory syndrome coronavirus-2(SARS-Cov-2) are induced. In particular, the presence of antibodies is one of the most important signs that a person has contracted SARS-Cov-2 [1]. After the COVID-19 pandemic, numerous commercial products functioning with serological methods swiftly came into use [Emergency Use Authorization(EUA)] to identify the immune memory generated against SARS-Cov-2, and the experience regarding the clinical use of these products began to be collected [1-4]. Considering that vaccines targeting the spike antigen are used more frequently and the availability of anti-nucleocapsid-IgG (anti-N IgG) antibodies to distinguish between the status of being vaccinated and infected, anti-N IgG represents a significant advantage [5,6].

***Corresponding Author:** Serhat Uysal, Fırat University School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Elazıg, Türkiye
E-mail: serhatuysalmd@gmail.com

On the other hand, the introduction of vaccine use around the world has caused, many commercial serological products that were only calibrated for a previous COVID-19 to become partially out of date. Especially, inactivated whole virus vaccines are used more extensively in some countries. Therefore, the issue of how the ability to distinguish between the status of having been vaccinated with an inactivated vaccine and the status of having been previously infected with SARS-Cov-2 will be affected constitutes a serious point of consideration for clinicians [7]. Because antibody tests do not provide much benefit for early diagnosis but are more suitable for screening populations that have formed an active immune response, mainly for establishing the vaccine efficacy, the issue of how the tests that function with antibodies generated against nucleocapsid protein are affected in the cases of having been vaccinated, becomes all the more critical. Although some studies demonstrate that the post-vaccine antibody response is similar to the post-COVID antibody response, there is more substantial evidence suggesting that antibody levels measured after COVID-19 are much higher than antibody levels measured after full-dose vaccination [8-10]. On the other hand, as of the time when the manufacturers introduce the relevant

serological test into the market, the cut-off level specified in the user manual may become invalid due to clinical conditions such as vaccination[11]. Consequently, there is still a need for studies that examine the responses of anti-N IgG after vaccination with an inactivated vaccine and contribute to the knowledge of how to use the anti-N IgG test in individuals after vaccination.

This study aims to investigate the characteristics of the IgG-type antibody response against the nucleocapsid protein in clinical practice such as previous-COVID-19 or vaccination with inactivated vaccines and the changes in these responses that can occur over time. Also, this study aimed to investigate 'How to distinguish between vaccinated versus infected patients via anti-N IgG?'.

Materials and Methods

Ethics

This retrospective study was approved by Republic of Turkey Ministry of Health (approval number-date:2021-11-10T19_11_15/12 November 2021). Ethics approval was obtained from Institutional Clinical Research Ethics Committee (Code-date:2021/13-10/12.16.2021) and conducted in Fırat University School of Medicine Hospital. All procedures were carried out to comply with regulatory laws and the ethical standards of the Declaration of Helsinki.

Patients

Cases over the age of 18 years, who have been subjected to the Abbott SARS-Cov-2 anti-N IgG test between 01 March 2020 and 31 December 2021 were included in the study [12]. Age, gender, the time elapsed since the onset of symptoms (days) where patients have a history of COVID-19 or (if asymptomatic) the time elapsed since PCR positivity (days), and the time elapsed after the dose of vaccination(days) in vaccinated cases, as well as SARS-Cov-2 anti-N IgG test results, were recorded. The semi-quantitative results of the SARS-Cov-2 anti-N IgG index were defined by comparing the signal of the chemiluminescent relative light unit in the reaction tube of the sample to the calibrator (S/C-index) stored in the system (ARCHITECT-*i*1000SR, Abbott, USA) [12].

Within the examination of the data of the cases participating in the study, patients with missing data, patients who are likely to have humoral immunity disorder (such as hematological malignancy), and patients aged<18 years were excluded. Patients included in the study are assigned to four main groups. These groups are defined as only vaccinated cases[Vaccinated=Cov(-)Vac(+)], unvaccinated patients with COVID-19 [Infected=Cov(+)Vac(-)], not infected and not vaccinated cases[Naïve=Cov(-)Vac(-)], and both infected and vaccinated patients[Both=Cov(+)Vac(+)]. Among patients with a single test result, one-month periods were established by taking as starting point the day of the first symptom (or positive PCR) in Cov(+)Vac(-) patients and the day of the first vaccination in Cov(-)Vac(+) cases.

The anti-N antibody responses of patients who received two doses of the inactivated COVID-19 vaccine [Sinovac Life Sciences (Beijing, China)] was evaluated in cases with repeated anti-N IgG tests. These cases were divided into four groups. The first group was defined as 'cases not vaccinated between the first and last test' (Not-vaccinated). The second group was defined as 'cases vaccinated in the early period of the post-COVID-19 infection (within the first four months after the symptom onset and/or after the first positive PCR) and vaccinated between the first and last test' (Post-Covid-Early-vaccinated). The third group was defined as 'lately vaccinated cases (>4 months after the symptom onset or positive PCR) and vaccinated between the first and last test' (Post-Covid-Late-vaccinated). The fourth group was defined as 'not-infected and only vaccinated cases between the first and last test' (Only-vaccinated).

Statistics

Numbers and percentages were used for categorical data in descriptive analyses. Mean±standard deviation (mean±SD), One-way ANOVA, and Student's T-tests were used for parametric and median (25percentile–75percentile), Kruskal-Wallis and Mann-Whitney-U tests were used for the non-parametric continuous variables. Categorical data were compared using the chi-square (or Fisher's) tests and the Mc-Nemar test. Wilcoxon (or paired-T) test was applied for the analysis of the repeated anti-N IgG measures (intra-group comparisons). The difference of repeated measures was compared with One-way ANOVA and Student t-test (inter-group comparisons). The Spearman correlation and linear regression analysis with the adjusted coefficients of determination (adj-R^2) were applied to estimate the effect of $Time_{cov\text{-}19}$ on anti-N antibody response. The area under the receiver operating curve (AUROC) was used to estimate discrimination ability and novel cut-off points of anti-N IgG in Naïve, vaccinated, and infected cases. P<0.05 was selected for the statistical significance of the alpha. Adjusted p-values'(F) were used for the pairwise comparisons. Power>80% was selected as a probability of avoiding a type-II error. Statistical analyses were conducted with IBM-SPSS-V22 and G*power-V3.1[13].

Results

A total of 729 cases, of which 297(36.5%) were female, were included in the study. The median age of the cases was 36(18-81) years. Demographics and distribution of the cases were given in Table 1. There was a significant difference in Anti-N IgG levels between the groups (p<0.0001) and a significant difference was observed between all pairwise comparisons *(for all; p<0.001<F=0.00833)*. (Table 1).

The anti-N IgG level was positively correlated with age *(r=0.172, p=0.00077)* but negatively correlated with $Time_{cov\text{-}19}$ *(r=-0.356, p<0.0001)* in Cov(+)Vac(-) cases. The negative effect of $Time_{cov\text{-}19}$ on anti-N IgG levels was confirmed with linear regression (Figure 1). A pairwise comparison of the periods in Cov(+)Vac(-) cases confirmed the decrease of the anti-N IgG is statistically significant between the 1st–7th, 2nd–7th, 3rd–7th, and 4th–7th months *(Kruskal-Wallis, p<0.0001) (for all; p<0.0001< F=0.00283)* (Figure 2). The anti-N IgG levels of Cov(+)Vac(-) and Cov(-)Vac(+) groups in the 1st, 2nd and 3rd month periods were significantly different *(for all; p<0.0001<F=0.005)* (Figure 2). The highest anti-N IgG positivity ratio was detected in the second period among Cov(+)Vac(-) cases and significantly decreased in the 6th period (46.7%) according to the previous periods (chi-square trend; $X^2=41.36$, *p<0.0001)* (Figure 2). Anti-N IgG levels were significantly different among the periods of the Cov(-)Vac(+) cases *(p<0.0001)*. Pairwise comparison of the periods states that; the difference between 1st–2nd, 1st–3rd, and 3rd–(>6th) months were statistically significant (Figure 2). Anti-N IgG level was negatively correlated with age in Cov(-)Vac(+) cases (r=-0.282,p<0.0001).

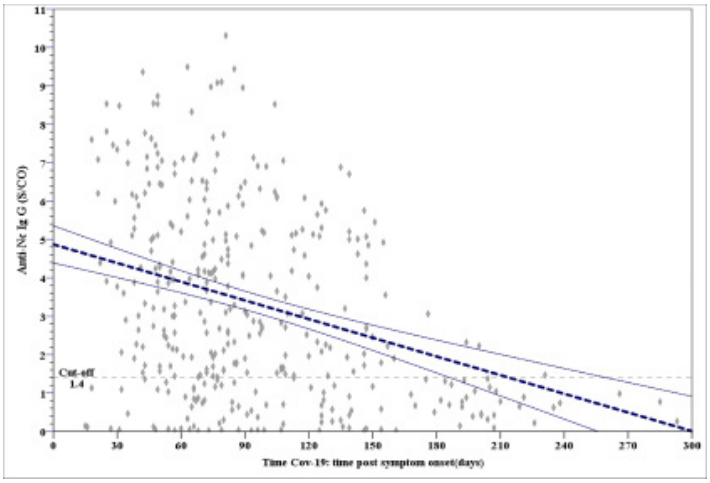


Figure 1. The plot with curve estimation of the regression equation between $Time_{cov-19}$ and anti-N IgG. The linear curve fitting equation of **'Y=aX+b'** was estimated via the adjusted coefficients of determination **(adj-R^2)** in simple linear regression analysis. The curve of **"Anti-N IgG = (-0.016*$Time_{cov-19}$)+4.87"** was illustrated with the lines of %95 CI (adj-R^2=0.124; F=54.45, p<0.00001). Anti-N IgG prediction lines of ''$Time_{Cov-19}$: Time post symptom onset (days) in cases with Anti-N IgG in Cov(+)Vac(-) cases''

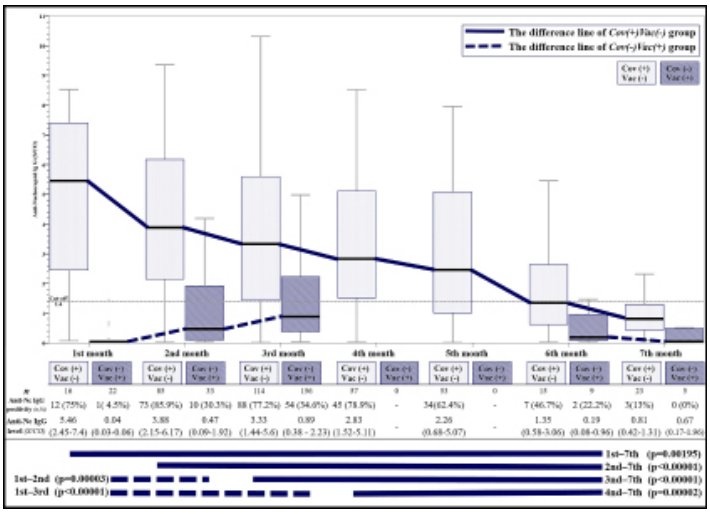


Figure 2. Periodic changes of SARS-CoV-2 anti-nucleocapsid IgG antibodies over seven months post-infection and post-vaccination. Periods were classified as monthly intervals. Cov(+)Vac(-)=Only Covid-19 infected cases; Cov(-)Vac(+)=Only vaccinated cases; Anti-Nc IgG (S/C) level =Levels of Anti-Nucleocapsid Ig G sample/calibrator index. In patients with Cov(+)Vac(-), anti-Nc IgG level decreases as the period increases (Kruskal-Wallis; H=42.5; df=6; p=0.00000015). Anti-Nc levels were significantly different among the periods of the covid-19 infected - Cov(+)Vac(-) cases (Kruskal-Wallis; H=42.5; df=6; p=0.00000015). Bonferroni adjusted p-value (F) was selected as <0.00283 for the pairwise comparisons of the periods of Cov(+)Vac(-) cases. Pairwise comparisons of the periods of Cov(-)Vac(+) cases; 1st–2nd(p=0.498), 1st–3rd(p=0.338), 1st–4th(p=0.133), 1st–5th(p=0.034), 1st–6th(p=0.059), 1st–7th(p=0.00195< F=0.00283 -sig), 2nd–3rd(p=0.155), 2nd–4th(p=0.036), 2nd–5th(p=0.0075), 2nd–6th(p=0.02), 2nd–7th(p<0.00001<F=0.00283 -sig), 3rd–4th(p=0.408), 3rd–5th(p=0.101), 3rd–6th(p=0.119), 3rd–7th(p<0.00001<F=0.00283 -sig), 4th–5th(p=0.348), 4th–6th(p=0.244), 4th–7th(p=0.00002< F=0.00283 -sig), 5th–6th(p=0.191), 5th–7th(p=0.005), 6th–7th(p=0.162)

The number of patients with two anti-N IgG tests at two different times is 80 (test count = 160). Characteristics of the cases with repeated anti-nucleocapsid IgG tests were given in Table 2. In the Post-Covid-Early-vaccinated group, the first anti-N IgG levels were statistically higher than the last anti-N IgG (p=0.0117). The differences between the first and last measures of the anti-N IgG within the sub-groups were given in figure 3.

Pairwise comparisons concerning the variability of the difference of the repeated tests between the groups showed that there was no difference between the not-vaccinated and Post-Covid Early-vaccinated groups *(p=0.717)*. There was a significant difference between the not-vaccinated (-1.44±1.27) and Post-Covid Late-vaccinated group (1.5±1.61) in terms of the difference between the first and last test *(t(60)=-7.262, p<0.000001<F=0.00833)*. There was a significant difference between not-vaccinated (-1.44±1.27) and the Only-vaccinated group (0.48±1.56) *(p=0.00103<F)*. A significant difference was observed between Post-Covid Early-vaccinated (-1.62±0.93) and Only-vaccinated group (0.48±1.56) *(t(16)=-3.355,p=0.00402<F)*. There was also a significant difference between Post-Covid Early-vaccinated (-1.62±0.93) and Post-Covid Late-vaccinated group (1.5±1.61) *(t(47)=-5.279, p<0.0001<F)*. There was no difference between Post-Covid Late-vaccinated (1.5±1.61) and the Only-vaccinated group (0.48±1.56) *(p=0.078>F)*.

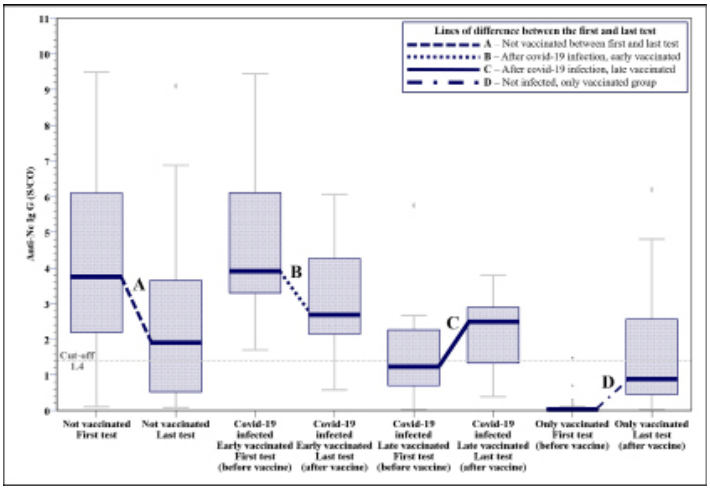


Figure 3. Intra-group analysis with pairwise comparisons of the first and last anti-nucleocapsid IgG tests. The intragroup (within-subject) effect was analyzed via Wilcoxon signed-rank test. The first test [3.75 (2.19-6.10)] and last test [1.9 (0.52-3.65)] in cases with a not-vaccinated group (**A**) was significantly different (Wilcoxon; Z=-3.841; r=-0.838; p=0.00012). First test [3.91 (3.3-6.11)] and last test [2.69 (2.15-4.27)] in Post-Covid Early-vaccinated (within the first four months after the symptom onset and/or after the first positive PCR) group the difference between the first and last test (**B**) was significantly different (Wilcoxon; Z=-2.521; r=-0.891; p=0.0117). The first test [1.23 (0.7-2.26)] and last test [2.49 (1.33-2.89)] in post-Covid Late-vaccinated (four or more months after the symptom onset and/or after the first positive PCR) cases the difference between the first and last test (C) was not significantly different (Wilcoxon; Z=-1.172; p=0.241). First test [0.03 (0.02-0.06)] and last test [0.88 (0.45-2.56)] in only-vaccinated (not-infected) cases the difference between the first and last test (**D**) was significantly different (Wilcoxon; Z=-5.484; r=-856.5; p<0.000001)

The discrimination ability of the anti-N IgG was assessed with the AUROC=0.952(95%CI 0.931–0.969). The cut-off level of the naïve versus infected cases was calculated as S/C≤0.32 with a sensitivity of 94.5% and specificity of 80.74%. The cut-off level of the vaccinated versus infected cases was calculated as S/C>1.37. This level was similar to the diagnostic susceptibility tests as a manufacturer's cut-off (S/C≥1.40). The AUROC= 0.815(95%CI,0.780–0.847), sensitivity (76%), and specificity (82.8%) were identified for both S/C≥1.40 and S/C>1.37. The modified three-category cut-off classification was calculated for Naive, vaccinated, and infected cases as (S/C≤0.32), (0.32<S/C<1.40), and (S/C≥1.40) respectively. The AUROC difference between the modified three-category cut-off and the manufacturer's cut-off was statistically significant (Figure 4).

Table 1. Demographics and distribution of the cases

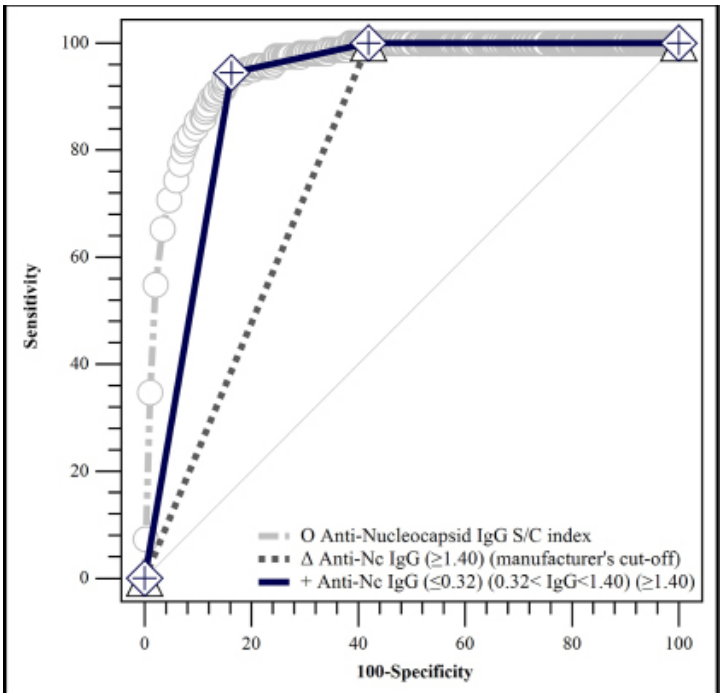
		Naïve Cov(-)Vac(-)	Infected Cov(+)Vac(-)	Vaccinated Cov(-)Vac(+)	Both Infected and vaccinated Cov(+)Vac(+)	Total
		164 (20.2%)	346 (42.6%)	225 (27.7%)	78 (9.6%)	813 (100%)
	Anti-Nc Ig G level (S/C)	0.03 (0.02-0.07)	3.07 (1.44-5.20)	0.67 (0.17-1.96)	1.84 (0.91-2.85)	1.16 (0.11-3.34)
	$Gender_{(Female)}$	80 (48.8%)	94 (27.2%)	120 (53.3%)	39 (50.0%)	333 (41%)
	$Age_{(Years)}$	37 (29-45)	35 (28-44)	38 (29-45)	36 (28-45)	36 (28-45)
	Anti-Nc $IgG_{(Positive)}$	-	262 (75.7%)	67 (29.8%)	50 (64.1%)	379 (46.6%)
Vaccination status N (%)	**Not-vaccinated**	164 (100%)	343 (100%)	-	-	507 (62.6%)
	Only first dose	-	-	23 (10.2%)	14 (17.9%)	37 (4.6 %)
	Second dose	-	-	202 (89.8%)	64 (82.1%)	266 (32.8%)
	$Time_{Vac-1}$ (days)	-	-	64 (62-76)	63 (59-70)	64 (60-75)
	$Time_{Vac-2}$ (days)	-	-	36 (34-48)	35 (32.5-43.5)	36 (34-47)
	$Time_{Cov-19}$ (days)	-	80 (56-113)	-	191 (147-210)	91 (64-146)

All variables were given as N (%): count (percent) or Med (25p–75p): median (25percentile–75percentile) in the table. Anti-Nc IgG: Anti-nucleocapsid immunoglobulin G, $Time_{Cov-19}$: Time to anti-Nc IgG test after the first symptom onset (days), $Time_{Vac-1}$: Time to anti-Nc IgG test after the first dose of vaccine (days), $Time_{Vac-2}$: Time to anti-Nc IgG test after the second dose of vaccine (days). Anti-Nc IgG level: Anti-nucleocapsid Ig G S/C index level, Anti-Nc $IgG_{positive}$: Anti-Nc IgG level (S/C) ≥1.40 (cut-off)

Table 2. Characteristics of the cases with repeated anti-nucleocapsid IgG test

	Not-vaccinated 21 (26.3 %)		Post-Covid Early-vaccinated 8 (10 %)		Post-Covid Late-vaccinated 41 (51.3 %)		Only vaccinated 10 (12.5 %)	
	First test	**Second test**	**First test**	**Second test**	**First test**	**Second test**	**First test**	**Second test**
Age (years)	34 (29–42)		41 (33–52)		35 (29 – 42)		37 (29 – 43)	
Gender (female)	9 (42.9%)		2 (25 %)		19 (46.3%)		4 (40%)	
$Time_{Vac-1}$ (days)	57.5 (49–62)	91 (77–160)	–	63.5 (52–66)	7 (4–19)	65 (64–69)	–	63 (63 – 64)
$Time_{Cov-19}$ (days)	73 (40–98)	126 (105–168)	68 (47–83)	142 (120–155)	129 (127–146)	196 (193–210)	–	–
Not-vaccinated	17 (81%)	17 (81%)	8 (100%)	–	41 (100%)	–	10 (100%)	–
Two doses vaccinated	4 (19%)	4 (19%)	–	8 (100%)	–	41 (100%)	–	10 (100%)
Anti-Nc IgG level (S/C)	3.8 (2.19–6.1)	1.9 (0.52–3.65)	3.9(3.3 – 6.11)	2.7(2.15–4.27)	0.03(0.02–0.06)	0.88(0.45–2.56)	1.23(0.7–2.26)	2.5(1.33–2.89)
Anti-Nc Ig G (positive)	16 (76.2 %)	13 (61.9%)	8 (100%)	7 (87.5%)	1 (2.4%)	15 (36.6%)	5 (50%)	7 (70%)
The time between tests (days)	64 (44–74)		70 (57–75)		69 (63–74)		65 (64–69)	
Difference between tests	-1.44 (-1.99–-0.61)		-1.265 (-2.2– -1.05)		0.70 (0.38–2.54)		0.975 (-0.57–1.61)	

All variables were given as N (%); count (percent) or Med (25p–75p); median (25percentile–75percentile) in the table. Not-vaccinated: Cases not vaccinated between the first and last test, Post-Covid Early-vaccinated: Cases vaccinated between the first and last test and vaccinated within the first four months after the symptom onset or/and after the first positive PCR, Post-Covid Late-vaccinated: Cases vaccinated between the first and last test and vaccinated after the four-month post symptom onset or/and after the first positive PCR, Only vaccinated: Cases were not-infected with Covid-19 and only vaccinated between the first and last test, $Time_{Cov-19}$: Time to anti-Nc IgG test after the first symptom onset (days), $Time_{Vac-1}$: Time to anti-Nc IgG test after the first dose of vaccine (days), $Time_{Vac-2}$: Time to anti-Nc IgG test after the second dose of vaccine (days), Anti-Nc IgG level: anti-nucleocapsid IgG S/C index. Anti-Nc $IgG_{positive}$: anti-Nc IgG level (S/C) ≥1.4 (cut-off), the difference between tests was calculated as ''Last anti-Nc IgG S/C–First anti-Nc IgG S/C''



The classification methods are given in the figure	AUROC	SE	95% CI
(○)Anti-nucleocapsid IgG S/C index	0.952	0.0083	0.931 – 0.969
(Δ)Anti-nucleocapsid IgG (*cut-off ≥ 1.40 = cut-off >1.37*)***	0.790	0.0126	0.754 – 0.823
(+)Anti-nucleocapsid IgG(*S/C ≤ 0.32) (0.32 < S/C < 1.40) (S/C ≥ 1.40)*)****	0.907	0.0105	0.879 – 0.930

A single category of the cut-off level	AUROC	95% CI for AUROC		Sensitivity (%)	95% CI for Sensitivity	Specificity (%)	95% CI for Specificity	+LR	95% CI	-LR	95% CI
S/C ≤ 0.32 *	0.876	0.850	0.899	94.51	89.8 - 97.5	80.74	77.3 - 83.9	4.91	4.1 - 5.8	0.068	0.04 - 0.1
0.32<S/C<1.40**	0.670	0.635	0.704	58.2	49.6-66.4	75.93	72.3-79.3	2.42	2.0-3.0	0.55	0.5-0.7
S/C ≥ 1.40***	0.815	0.780	0.847	76.01	71.2 - 80.4	82.78	78.6 - 86.4	4.41	3.5 - 5.5	0.29	0.2 - 0.4

Figure 4. Multiple comparisons of the area under the receiver operating curves. *Cut-off level of the Naive versus other cases was calculated as S/C ≤0.32. **Cut-off category of the vaccinated versus other (infected + naive) cases ***Cut-off level of the infected versus other cases was calculated as S/C>1.37. S/C>1.37 had a similar AUROC, specificity, and sensitivity as the manufacturers' cut-off (S/C≥1.40). Therefore, a cut-off level between vaccinated versus infected cases was selected as the manufacturers' Cut-off(S/C≥1.40). ***Modified three categorical anti-Nc IgG cut-off levels were calculated via the Youden index for Naive, vaccinated, and infected cases as (S/C≤0.32), (0.32<S/C<1.40) and (S/C≥1.40) respectively. The AUROC of this modified anti-Nc IgG was 0.907 (95%CI: 0.879-0.930) and the AUROC of the manufacturer's cut-off (S/C ≥1.40) was 0.790 (0.754-0.823). The AUROC difference between the modified-tree categorical cut-off and the manufacturer's cut-off was statistically significant (Z=10.3; Difference between areas=0.117 (95%CI: 0.0946-0.139); p<0.0001)

Discussion

It is also well known that neutralizing antibodies are seen as the major marker of protection from COVID-19, and there exists a strict correlation between neutralizing antibodies and some of the binding antibodies [14-17]. Among the binding antibodies, SARS-Cov-2 Anti-N IgG also exhibits a positive correlation with neutralizing antibodies, which is much higher than that some other binding antibodies exhibit with neutralizing antibodies [18-20]. Therefore, it is obvious that anti-N IgG is undeniably closely associated with protection against possible new infection emergence, and the ability of Anti-N IgG to detect the condition of individuals vaccinated with whole virus vaccines or infected with COVID-19 stands out as a topic of interest.

It is well known that antibody levels increase markedly with vaccination against SARS-Cov-2 [10,21,22]. The consensus is that the anti-N IgG antibody test might be beneficial in late diagnosis or epidemiological studies in patients who are thought to be infected with COVID-19. Therefore, anti-N IgG has no diagnostic role in spike-based vaccination [5,6]. Yet the elucidation of the difference between the condition of having been vaccinated and the condition of having been infected with COVID-19 merely based on anti-N IgG results is much more complicated since there is an increase in anti-N IgG after vaccination with an inactive vaccine [10,21,22]. Although some studies demonstrate that the post-vaccine antibody response is similar to the post-COVID antibody response, there is more substantial evidence suggesting that antibody levels measured after COVID-19 are much higher than antibody levels measured after full-dose vaccination [8-10]. Therefore, having been infected with COVID-19 is the major trigger of the antibody levels developed against SARS-Cov-2 as well as anti-N IgG. Anti-N IgG levels in vaccinated individuals are significantly lower than in post-COVID-19 patients [24]. Thus, anti-N IgG levels would be higher in post-COVID-19 than the vaccinated cases, and anti-N IgG levels would be increased as per **"naive<vaccinated<infected"**. In this way, an index range that can distinguish the condition of having been vaccinated from being naive or infected from among the anti-N IgG levels can be calculated and used as a diagnostic tool.

Although a long time has elapsed since the EUA, the conditions indicating how we are to use these serological tests in terms of vaccination remain unclear. This is because no study or guideline is showing how a successful vaccination is confirmed or how the effect of having been vaccinated can be distinguished from COVID-19, although there are many serological tests with EUA[2]. In naïve patients included in our study, the ratio of positivity (S/C≥1.40) did not exceed 35% even between 60-90 days (3rd month period) when the highest anti-N IgG positivity levels were reached after the first dose of vaccine. It has been found that the anti-N IgG was higher in vaccinated individuals who received inactive vaccination compared to naive individuals. However, it was significantly lower than those who had COVID-19. It is stated that the cut-off value determined by the manufacturer may lose its validity in various special cases and separate cut-off levels may need to be calculated [11]. In this study, a three-category cut-off scale was found to provide a higher benefit than the manufacturers' cut-off used alone. Therefore, manufacturers can address this issue and set out to update these tests dating back to the pre-vaccine period for the post-vaccine period. In this regard, it would be beneficial to revise the anti-N IgG cut-off levels via prospective and controlled studies for naive, vaccinated, and infected patients. It has been found that the anti-N levels recorded after COVID-19 were higher in elder people than in younger ones [5,25]. Also in our study, it was found that the elderly COVID-19 patients had higher anti-N levels compared to the younger age group. In contrast, various types of antibody responses after vaccination were found to be lower in elder individuals than in younger individuals [21,22]. One of the main factors leading to higher antibody levels in elder patients' period of post-infection may be the prevalence of severe COVID-19 infection in older patients [25]. This suggests that high anti-N levels in elderly individuals infected with COVID-19 may result from a severe infection that usually occurs in elderly patients [5,8,18,25]. While there is a positive correlation between age and anti-N levels among individuals who have been infected with COVID-19, this correlation is negative in vaccinated people. In other words, elderly patients cannot produce an antibody response to the vaccine which is as high as in young individuals [21,22].

This may be the result of the weakness that occurs over time in the immune system in elders [26]. Therefore, decreased immune function in vaccinated elders' may result in a lower antibody response[26]. On the other hand, immune weakness in elderly patients can result in severe COVID-19 infection, resulting in a higher antibody response [5,8,18,25].

There are in part deviating results regarding the persistence of antibodies developed after vaccination with both inactivated and spike-based mRNA vaccines and the persistence of antibodies produced after COVID-19 [27]. Although anti-N IgG persists longer in those with severe infection than those with mild, and possibly in those with high initial antibody response than those without; the anti-N IgG levels significantly decrease in the late periods after infection compared to early periods [28-31]. In particular, it is stated that anti-N IgG levels show a significant decrease between the 4th and 8th months as compared to the initial months [25,30,32]. There is a clear negative correlation between the time from COVID-19 to the test and the anti-N level [5,8,18,25,33]. In this study, the fact that patients with not-vaccinated and/or not-infected between the repeated tests had a significantly lower second test result than their first anti-N IgG test. Additionally, in patients after COVID-19 a negative correlation was established between the time from the first symptom until the test and the anti-N IgG level. In contrast, some studies suggest that post-COVID-19 patients present with persistent and/or detectable IgG levels for eight months or more [16,17,34,35], it was thought that this deviation in related studies could be due to some unnoticed special factors such as symptom severity or ethnicity [36].

One of the findings regarding the effect of vaccination on anti-N IgG and noticed within the scope of our study is that the course of decrease in anti-N IgG in individuals vaccinated with the inactivated vaccine in the first four months after COVID-19 exhibits a similar downfall as in those who were not vaccinated in the first four months after COVID-19. In those vaccinated after four months of COVID-19, however, the decrease in anti-N IgG disappears in most patients, and anti-N IgG increases again in some patients.

Conclusion

This study points out that the antibody responses could be decreased over time after COVID-19 infection the anti-N IgG increases mostly with COVID-19 infection and secondly with vaccination. The factors affecting the course of an existing anti-N IgG were found to be age, the time elapsed after the disease (or vaccine), and vaccination after a period longer than four months after the disease. There is a difference that can be detected by currently available tests between naive, vaccinated, and infected individuals. Therefore, the manufacturers of the assays should make a new cut-off classification by considering the vaccination effects.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

This retrospective study was approved by Republic of Turkey Ministry of Health (approval number-date:2021-11-10T19_11_15/12 November 2021). Ethics approval was obtained from Institutional Clinical Research Ethics Committee (Code-date:2021/13-10/12.16.2021) and conducted in Fırat University School of Medicine Hospital. All procedures were carried out to comply with regulatory laws and the ethical standards of the Declaration of Helsinki.

References

1. Coronavirus disease (COVID-19): Serology, antibodies and immunity. https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease-covid-19-serology access date 21 December 2021
2. In Vitro Diagnostics EUAs - Serology and Other Adaptive Immune Response Tests for SARS-CoV-2. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-serology-and-other-adaptive-immune-response-tests-sars-cov-2 access date 21 December 2021
3. Burbelo PD, Riedo FX, Morishima C, et al. Sensitivity in detection of antibodies to nucleocapsid and spike proteins of severe acute respiratory syndrome coronavirus 2 in patients with coronavirus disease 2019. J Infect Dis. 2020;222:206-13.
4. Caini S, Bellerba F, Corso F, et al. Meta-analysis of diagnostic performance of serological tests for SARS-CoV-2 antibodies up to 25 April 2020 and public health implications. Euro Surveill. 2020;25:pii=2000980.
5. Chansaenroj J, Yorsaeng R, Posuwan N, et al. Long-term specific IgG response to SARS-CoV-2 nucleocapsid protein in recovered COVID-19 patients. Sci Rep. 2021;11:23216.
6. Bradley T, Grundberg E, Selvarangan R, et al. Antibody Responses after a Single Dose of SARS-CoV-2 mRNA Vaccine. N Engl J Med. 2021;384:1959-61.
7. Dinç H, Özdemir YE, Alkan S, et al. Evaluation of the Diagnostic Performance of Different Principles of SARS-CoV-2 Commercial Antibody Tests in COVID-19 Patients. Mikrobiyol Bul. 2021;55:207-22.
8. Buonfrate D, Piubelli C, Gobbi F, et al. Antibody response induced by the BNT162b2 mRNA COVID-19 vaccine in a cohort of health-care workers, with or without prior SARS-CoV-2 infection: a prospective study. Clin Microbiol Infect. 2021;27:1845-50.
9. Bradley T, Grundberg E, Selvarangan R. Antibody responses boosted in seropositive healthcare workers after single dose of SARS-CoV-2 mRNA vaccine. medRxiv. 2021;2021.02.03.21251078.
10. Benjamanukul S, Traiyan S, Yorsaeng R, et al. Safety and immunogenicity of inactivated COVID-19 vaccine in health care workers. J Med Virol. 2022;94:1442-9.
11. Ota S, Sugawa S, Suematsu E, et al. Possibility of underestimation of COVID-19 prevalence by PCR and serological tests. J Microbiol Immunol Infect. 2021;S1684-1182(21)00192-4.
12. Manual of the Abbott SARS-CoV-2 IgG (CoV-2 IgG) for use with ARCHITECT 6R86-20/6R86-30-H14806R05 (For use under an Emergency Use Authorization (EUA) Only) in: https://www.fda.gov/media/137383/download. https://www.fda.gov/media/137383/download access date 21 December 2021
13. Faul F, Erdfelder E, Lang AG, et al. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39:175-91.
14. WHO Guidelines on clinical evaluation of vaccines: regulatoryexpectations. https://www.who.int/biologicals/expert_committee/WHO_TRS_1004_web_Annex_9.pdf access date 27 December 2021
15. Knezevic I, Mattiuzzo G, Page M, et al. WHO International Standard for evaluation of the antibody response to COVID-19 vaccines: call for urgent action by the scientific community. Lancet Microbe. 2022;3:e235-e40.
16. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science. 2020;370:1227-30.
17. Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. N Engl J Med. 2021;384:533-40.
18. Rockstroh A, Wolf J, Fertey J, et al. Correlation of humoral immune responses to different SARS-CoV-2 antigens with virus neutralizing antibodies and symptomatic severity in a German COVID-19 cohort. Emerg Microbes Infect. 2021;10:774-81.

19. Burbelo PD, Riedo FX, Morishima C, et al. Detection of nucleocapsid antibody to SARS-CoV-2 is more sensitive than antibody to spike protein in COVID-19 patients. medRxiv. 2020;2020.04.20.20071423.

20. Marklund E, Leach S, Nyström K, et al. Longitudinal Follow Up of Immune Responses to SARS-CoV-2 in Health Care Workers in Sweden With Several Different Commercial IgG-Assays, Measurement of Neutralizing Antibodies and CD4(+) T-Cell Responses. Front Immunol. 2021;12:750448.21.

21. Yigit M, Ozkaya-Parlakay A, Cosgun Y, et al. Should a third booster dose be scheduled after two doses of CoronaVac? A single-center experience. J Med Virol. 2022;94:287-90.

22. Bayram A, Demirbakan H, Günel Karadeniz P, et al. Quantitation of antibodies against SARS-CoV-2 spike protein after two doses of CoronaVac in healthcare workers. J Med Virol. 2021;93:5560-5567.

23. Dobaño C, Jiménez A, Rubio R, et al. Spike-based COVID-19 immunization increases antibodies to nucleocapsid antigen. Transl Res. 2021;240:26-32.

24. Azak E, Karadenizli A, Uzuner H, et al. Comparison of an inactivated Covid19 vaccine-induced antibody response with concurrent natural Covid19 infection. Int J Infect Dis. 2021;113:58-64.

25. Deshpande GR, Kaduskar O, Deshpande K, et al. Longitudinal clinico-serological analysis of anti-nucleocapsid and anti-receptor binding domain of spike protein antibodies against SARS-CoV-2. Int J Infect Dis. 2021;112:103-10.

26. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. J Clin Invest. 2013;123:958-65.

27. Favresse J, Bayart JL, Mullier F, et al. Antibody titres decline 3-month post-vaccination with BNT162b2. Emerg Microbes Infect. 2021;10:1495-8.

28. Rijkers G, Murk JL, Wintermans B, et al. Differences in antibody kinetics and functionality between severe and mild severe acute respiratory syndrome Corona virus 2 infections. J Infect Dis. 2020;222:1265-9.

29. Lynch KL, Whitman JD, Lacanienta NP, et al. Magnitude and kinetics of anti-severe acute respiratory syndrome coronavirus 2 antibody responses and their relationship to disease severity. Clin Infect Dis. 2021;72:301-8.

30. Van Elslande J, Oyaert M, Ailliet S, et al. Longitudinal follow-up of IgG anti-nucleocapsid antibodies in SARS-CoV-2 infected patients up to eight months after infection. J Clin Virol. 2021;136:104765.

31. Self W H, Tenforde MW, Stubblefield WB, et al. Decline in SARS-CoV-2 antibodies after mild infection among frontline health care personnel in a multistate hospital Network - 12 States, April-August 2020. MMWR Morb Mortal Wkly Rep. 2020;69:1762-6.

32. Xia W, Li M, Wang Y, et al. Longitudinal analysis of antibody decay in convalescent COVID-19 patients. Sci Rep. 2021;11:16796.

33. Choudhary HR, Parai D, Dash GC, et al. IgG antibody response against nucleocapsid and spike protein post-SARS-CoV-2 infection. Infection. 2021;49:1045-8.

34. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science. 2021;371:eabf4063.

35. Gudbjartsson D F, Norddahl G L, Melsted P, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. N Engl J Med. 2020;383:1724-34.

36. Allen N, Brady M, Carrion Martin AI, et al. SARS-CoV-2 Antibody Testing in Health Care Workers: A Comparison of the Clinical Performance of Three Commercially Available Antibody Assays. Microbiol Spectr. 2021;9:e0039121.