New Microbiologica, 48, 4, 314-321, 2025, ISSN 1121-7138

FULL PAPER

# Anti-SARS-CoV-2 IgG Cut-off Value for Predicting Neutralizing Antibodies and Reinfection Risk in Natural and Hybrid Immunity: A Longitudinal Observational Study

Arzu Nazlı[1], Özgür Appak[2], Ahmet Naci Emecen[3], Ahmad Nejat Ghaffari[1], Gökçen Ömeroğlu Şimsek[4], Arzu Sayıner[2], Vildan Avkan-Oguz[1]

[1] *Infectious Diseases and Clinical Microbiology Department, Dokuz Eylul University Faculty of Medicine, Izmir, Türkiye;*
[2] *Division of Medical Virology, Department of Medical Microbiology, Dokuz Eylul University Faculty of Medicine, Izmir, Türkiye;*
[3] *Division of Epidemiology, Public Health Department, Dokuz Eylul University Faculty of Medicine, Izmir, Türkiye;*
[4] *Pulmonary Diseases Department, Faculty of Medicine, Dokuz Eylul University, Izmir, Türkiye.*

SUMMARY

Understanding immune responses to SARS-CoV-2, particularly the dynamics of natural versus hybrid immunity (infection plus vaccination), is essential for shaping public health strategies. A prospective cohort of 171 adults with mild to moderate COVID-19 was followed from February to July 2021. Participants were grouped as having natural (without COVID-19 vaccination, n=129) or hybrid (vaccinated before and/or after infection, n=42) immunity. Anti-RBD IgG and neutralizing antibodies were measured at the 1st, 3rd, and 6th months. ROC analysis identified an IgG cut-off to predict neutralizing antibody positivity. Reinfections were monitored for 12 months. Hybrid immunity was associated with significantly higher IgG levels across all time points. IgG and NAb levels were strongly correlated (r=0.86 at 1st, r=0.80 at 3rd month). An IgG threshold of 303.8 AU/mL predicted NAb positivity with 91.9% sensitivity and 90% specificity. Reinfection occurred in 4.6% of participants during the Omicron wave, often despite high antibody levels. An IgG level ≥303.8 AU/mL is a strong predictor of NAb positivity and can serve as a practical surrogate marker of functional immunity, especially where neutralization testing is unavailable. Reinfections despite high IgG/NAb levels highlight the limitations of static thresholds, particularly against different variants.

*Received* September 24, 2025 *Accepted* November 02, 2025

## INTRODUCTION

Since its emergence in late 2019, SARS-CoV-2 has presented substantial challenges to public health worldwide (Zaçe *et al.*, 2025; Gomes *et al.*, 2024). Seroprevalence studies, which estimate the proportion of a population with detectable antibodies, have been instrumental in tracking the pandemic's progress and reinfection rates (Bobrovitz *et al.*, 2023; Rostami *et al.*, 2021; Wei *et al.*, 2022; Alp-Çavuş *et al.*, 2023). Neutralizing antibodies targeting the receptor-binding domain of the SARS-CoV-2 spike protein have been identified as correlates of protection, associated with reduced risk of reinfection, severe disease, and mortality (Khoury *et al.*, 2023; Garcia-Beltran *et al.*, 2021). To detect neutralizing antibody levels, the classical live virus neutralization test (VNT) is the gold standard, which requires a biosafety level 3 (BSL-3) laboratory (Manenti *et al.*, 2020). Recognizing the cost and time challenges of VNT, researchers have turned to the practicality of enzyme-linked immunosorbent assay (ELISA)-based surrogate virus neutralization tests (sVNTs) (Valcourt *et al.*, 2021; Zaballa *et al.*, 2023; Gillot *et al.*, 2024). The correlation between sVNTs and commercial kits that detect SARS-CoV-2-specific anti-Receptor binding domain (RBD), as evaluated in various studies, has shown a strong correlation between anti-IgG ELISA tests and neutralization. However, the need for more research with a clear cut-off is evident, highlighting the current gaps in knowledge and the need for further investigation (Kitagawa *et al.*, 2022; Gütlin *et al.*, 2024, Güzel *et al.*, 2025; Lee *et al.*, 2022).

*Key words:*
SARS-CoV-2, Hybrid immunity, Neutralizing antibody, Anti-RBD IgG, Reinfection.

*Corresponding author:*
Arzu Nazli
E-mail: arzu.nazli@deu.edu.tr

©2025 by EDIMES - Edizioni Internazionali Srl. All rights reserved

The hybrid immunity, derived from a combination of SARS-CoV-2 infection and vaccination, has been shown to provide strong and sustained protection compared to infection or vaccination alone (Bratcher *et al.*, 2021; Zhang *et al.*, 2025). Despite the growing body of evidence, the dynamics of natural and hybrid immunity and reinfection, especially in settings with heterogeneous vaccination coverage, remain insufficiently understood and require further investigation.

In this study, we prospectively collected longitudinal serum samples from infected people from the public (not healthcare workers) before or after vaccination. We examined the correlation between NAb and anti-RBD IgG levels to estimate protective immunity based on anti-RBD IgG results.

## METHODS

### *Participants*

From February 17, 2021 to July 17, 2021, participants aged 18 and older who had positive RT-PCR tests and mild to moderate COVID-19 disease presentation as defined by international COVID-19 management guidelines were recruited from a pandemic outpatient clinic of a university hospital in western Turkey. All patients were cared for as outpatients at their first attendance. Some of the patients were hospitalized at their second attendance for closer follow-up; they were also included if they did not need high oxygen support. Exclusion criteria were as follows:

1) participants with severe disease presentation who needed high oxygen support;
2) those younger than 18 years old;
3) those who declined consent.

Written informed consent was obtained for serial blood sample collection. At the baseline visit, demographic data, date of positive PCR test, symptom onset, symptom type, hospitalization or home isolation details, comorbidities, and laboratory and radiologic findings were recorded. Serum samples were collected at baseline (1st visit; ~4 weeks after PCR test), 2nd visit (~12 weeks), and 3rd visit (~24 weeks). Missing follow-up data were excluded from longitudinal analyses. SARS-CoV-2 IgG antibodies were measured in 331 serum samples, while neutralizing assays were performed in 254 serum samples of participants completing at least two visits.

The natural immunity group participants had never received any SARS-CoV-2 vaccine before enrolment or during the six-month follow-up. Participants in the hybrid immunity group received at least one dose of the SARS-CoV-2 vaccine before infection and/or within the first six months post-infection.

Reinfections were identified as symptomatic patients confirmed with RT-PCR testing ≥90 days after the initial diagnosis during the 6-month study period, and the positive SARS-CoV-2 RT-PCR test was confirmed by retrospective review of national electronic health records within 6 months after the study finished.

### *Serological Assay*

Anti-RBD antibody levels were measured using the Architect analyzer's SARS-CoV-2 IgG II Quant (Abbott Diagnostics) chemiluminescent microparticle immunoassay. Values ≥50 AU/ml were considered positive, as recommended by the manufacturer. The quantitation range was 21–40,000 AU/ml. Median values and ranges were calculated to assess the variation in antibody responses across visits.

### *Neutralization Assay (NA) Tests*

The SARS-CoV-2 class surrogate virus-neutralizing test (GenScript, Piscataway, NJ, USA) was used to determine neutralizing antibodies. The assay detects antibodies that block the RBD from binding to the ACE2 receptor. The percentage of inhibition was calculated according to the manufacturer's instructions using the following formula:

$$\% \text{ Inhibition} = [1 - (\text{OD_sample} / \text{OD_negative control})] \times 100$$

A cutoff of ≥30% was used to define positivity, with calibration against the gold-standard PRNT90. This assay provides a quantitative evaluation of neutralizing antibody efficacy over time.

### *Statistical Analysis*

Descriptive statistics are presented as numbers (%), mean±standard deviation (SD), or median (minimum-maximum). Categorical variables were compared using the chi-squared test or Fisher's exact test, as appropriate. Continuous variables were compared using the Student's t-test, Mann-Whitney U test, or Kruskal-Wallis test, depending on the normality of the data. Pairwise comparisons were performed using the post-hoc Dunn test. Correlation analyses examined the relationship between IgG and NA tests at the 1st, 3rd, and 6th months. Generalized estimating equations (GEE) assessed the association between ln-IgG titers (outcome variable) and various predictors, accounting for the exchangeable correlation structure in repeated measures data. To address multicollinearity, hospitalization was included as a predictor in place of symptoms or disease severity. Two-sided p-values less than 0.05 were considered statistically significant. All analyses and visualizations were performed using R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

### *Demographic features of participants*

This study recruited 171 participants (female, n=109, 63.7%). The mean age was 46.7±13.8 years. Seventy-six (44.4%) participants had at least one comorbidity. The most common were hypertension (n=33, 19.3%), diabetes (n=17, 9.9%), and hypothyroidism (n=17, 9.9%). The mean time from symptom onset to

**Table 1** - *General characteristics of the participants and comparison by vaccination status before infection.*

| | *Total (n=171)* | *Immunity group at 1 month* | | *p* |
|---|---|---|---|---|
| | | *Natural n=129(%)* | *Hybrid n=42(%)* | |
| Age, mean±SD | 46.7±13.8 | 43.4±11.3 | 57.0±15.6 | <0.001 |
| Gender | | | | 0.23 |
| Male | 62 (36.3) | 43 (33.3) | 19 (45.2) | |
| Female | 109 (63.7) | 86 (66.7) | 23 (54.8) | |
| BMI, mean±SD, n=167 | 26.4±4.26 | 26.4±4.42 | 26.5±3.79 | 0.84 |
| Comorbidity | | | | 0.17 |
| No | 95 (55.6) | 76 (58.9) | 19 (45.2) | |
| Yes | 76 (44.4) | 53 (41.1) | 23 (54.8) | |
| Symptoms | | | | 0.31 |
| No | 13 (7.60) | 8 (6.2) | 5 (11.9) | |
| Yes | 158 (92.4) | 121 (93.8) | 37 (88.1) | |
| Hospitalization | | | | 0.58 |
| No | 151 (88.3) | 115 (89.1) | 36 (85.7) | |
| Yes | 20 (11.7) | 14 (10.9) | 6 (14.3) | |

*SD: Standard Deviation, BMI: Body Mass Index.

PCR positivity was 2 days (-3; 27), and there was no difference between the natural and hybrid immunity groups (p=0.67). Twenty of 171 patients (11.7%) were hospitalized, but none needed intensive care (*Table 1*).

### *Antibody assay results, correlation between assays*

Serum samples were taken from 171 (100%) participants on the first, 94 (55%) participants on the second, and 66 (38.6%) participants on the third visit (*Table 2*). SARS-CoV-2 IgG antibody testing was performed on 331serum samples, and neutralizing antibody testing was performed on 254 serum samples 94 (55%) in the first, 94 (55%) in the third, and 66 (38.6%) in the sixth month (*Table 2*). Anti-RBD IgG titers on the days after SARS-CoV-2 RNA positivity stratified by NA positivity are shown in *Figure 1*.

In the first month, the antibody titers of two patients (1.2%) were found to be below 50 AU/ml, the threshold set by the manufacturer for protective immunity, and thus considered negative (*Table 2*). Both were in the unvaccinated group (2/129; 1.5%). One of them was a 34-year-old woman with Behcet's disease who was using colchicine. She did not have a neutralization assay test. This patient did not have samples for other months. The other patient (ZBD) was a 20-year-old woman without comorbidity. She had negative IgG titers also in the third month. Her neutralization assay tests in the first and third months were also negative. She doesn't have samples for the sixth month.

In the third month, the other IgG-negative participant was a 48-year-old man with DM and HT. He had a positive IgG level (87.3 AU/ml) in the first month and a negative IgG level (49.5) in the third month, with negative NA tests on both time points. He was the only seroreverted patient in the study. After one dose of mRNA vaccine, he had a high IgG level (4682 AU/ml) and a positive (93.1%) NA test. In the sixth month, all the samples were positive for IgG tests.

In neutralization assays, in the first month, 10 (10.6%) patients (nine female and one male with a median age of 41 (20-49) years) had negative results. All were in the naturally immunized group. Nine had positive IgG titers with a median of 97.2 AU/ml (77.7-432). Two of them were vaccinated and had positive NA in the third month.

**Table 2** - *Number of positives, median antibody titers of the cohort at 1st, 3rd, and 6th months, and correlation results.*

| *Month* | *Anti-RBD IgG antibody AU/ml* | | *NA %* | | *Correlation r* |
|---|---|---|---|---|---|
| | *Positive n (%)* | *Median (min-max)** | *Positive n (%)* | *Median (min-max)** | |
| 1st | 169/171 (98,8) | 1300 (50.3-40000) | 84/94 (89,4) | 81 (31.7-100) | 0.86 |
| 3rd | 92/94 (97,9) | 1272 (50.5-40000) | 78/ 94 (83,0) | 85.4 (30.6-96.0) | 0.80 |
| 6th | 66/66 (100) | 11452 (236-40000) | 65/66 (98,5) | 95.2 (48.9-96.0) | 0.36 |

*For IgG or Neutralizing Antibody (NA), positive results only.

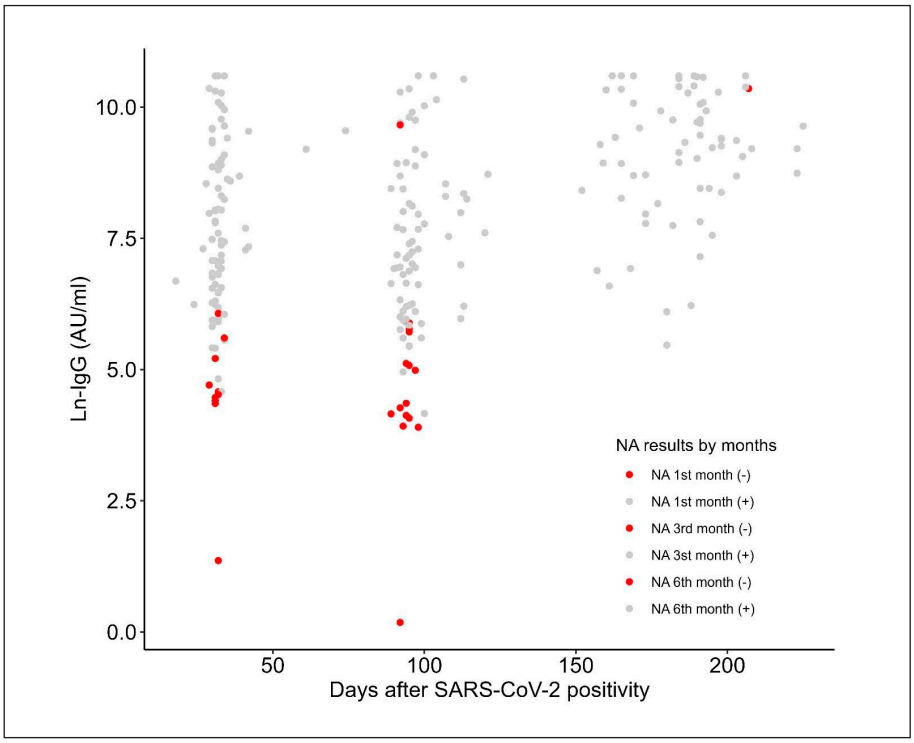



**Figure 1**
*Anti-RBD IgG titers at the days after SARS-CoV-2 RNA positivity stratified by Neutralization Assay (NA) positivity.*

In the 3rd month, 16 (17%) participants had negative NA, and eight were also negative in the first month. They had not been vaccinated from the first to third months. The other eight were positive and decreased their NA percent over time without vaccination.
One (1.5%) participant had negative neutralizing antibodies in the sixth month. She was a 54-year-old woman with Sjögren's Syndrome who was taking hydroxychloroquine. She was unvaccinated and had high IgG levels in the first and third months, with a positive neutralizing antibody titer. After she had one dose MRNA vaccine, even if she had high IgG levels (31369 AU/ml), she had a negative neutralizing antibody.
*Table 2* shows the correlation analysis between specific IgG and neutralizing antibody values at 1, 3, and 6 months. Strong positive correlations existed between the assays in the first month (r=0.86) and the third month (r=0.80). However, in the sixth month, the correlation was weak (r=0.36).
In ROC analysis, when the Anti-RBD IgG antibody threshold for neutralization antibody positivity was 303.8 AU/ml, sensitivity was 91.9%, specificity was 90%, positive predictive value was 98.3%, and negative predictive value was 64% (*Figure 2*).

### *Comparison of antibody levels at the 1st, 3rd, and 6th months according to vaccination status*

The vaccination status and follow-up of the participants are shown in *Figure 3*.
Among 171 participants, 129 (75.4%) were unvaccinated at the time of infection. In addition, 24 (14.0%) had received one dose of the vaccine, while 18 (10.5%) had received two doses prior to infection. During the follow-up period, additional vaccinations were administered as follows: within 1–3 months after infection, 13 (10.1%) of the initially unvaccinated participants received an additional vaccine dose, along with 3 (12.5%) of those who had one dose and 6 (33.3%) of those who had two doses. Within 3–6 months post-infection, 44 (34.1%) of the initially unvaccinated participants, 3 (12.5%) of those with one dose, and 3 (16.7%) of those with two doses received further vaccinations. By the end of the six-month follow-up,

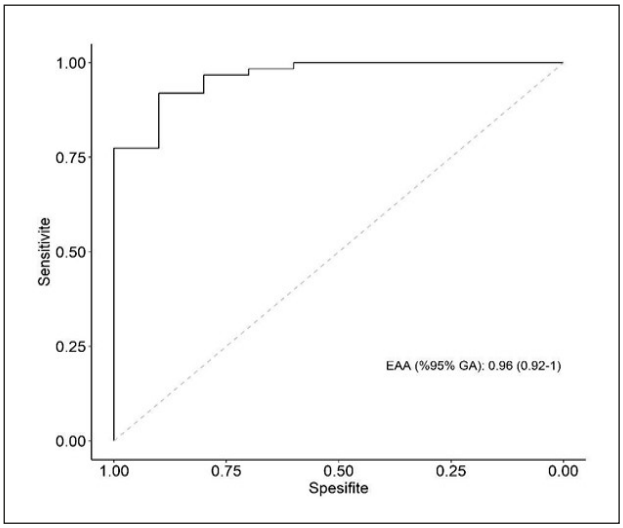
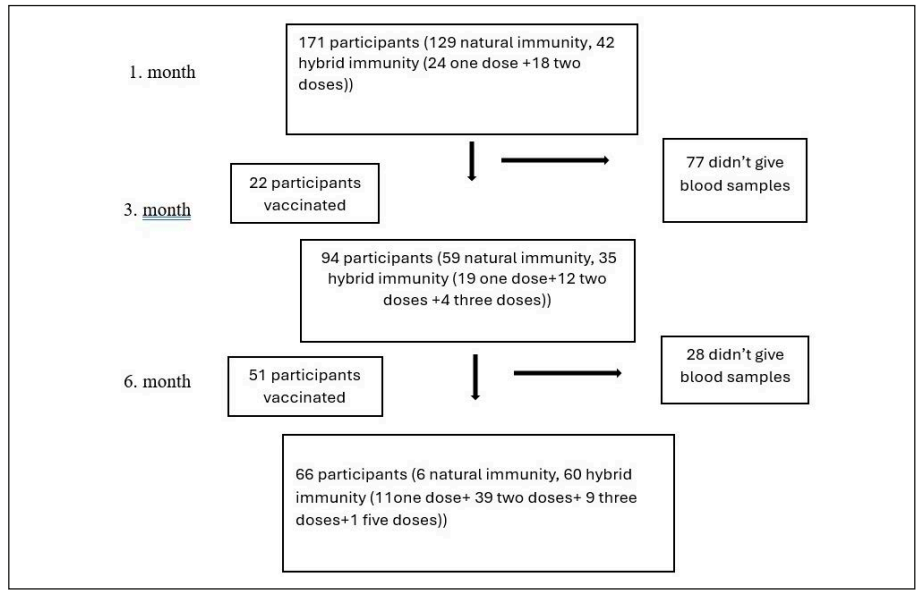



**Figure 2** - *Relation between Anti-RBD IgG antibody and neutralizing antibody positivity in ROC analysis.*

**Figure 3**
*Study follow-up chart.*



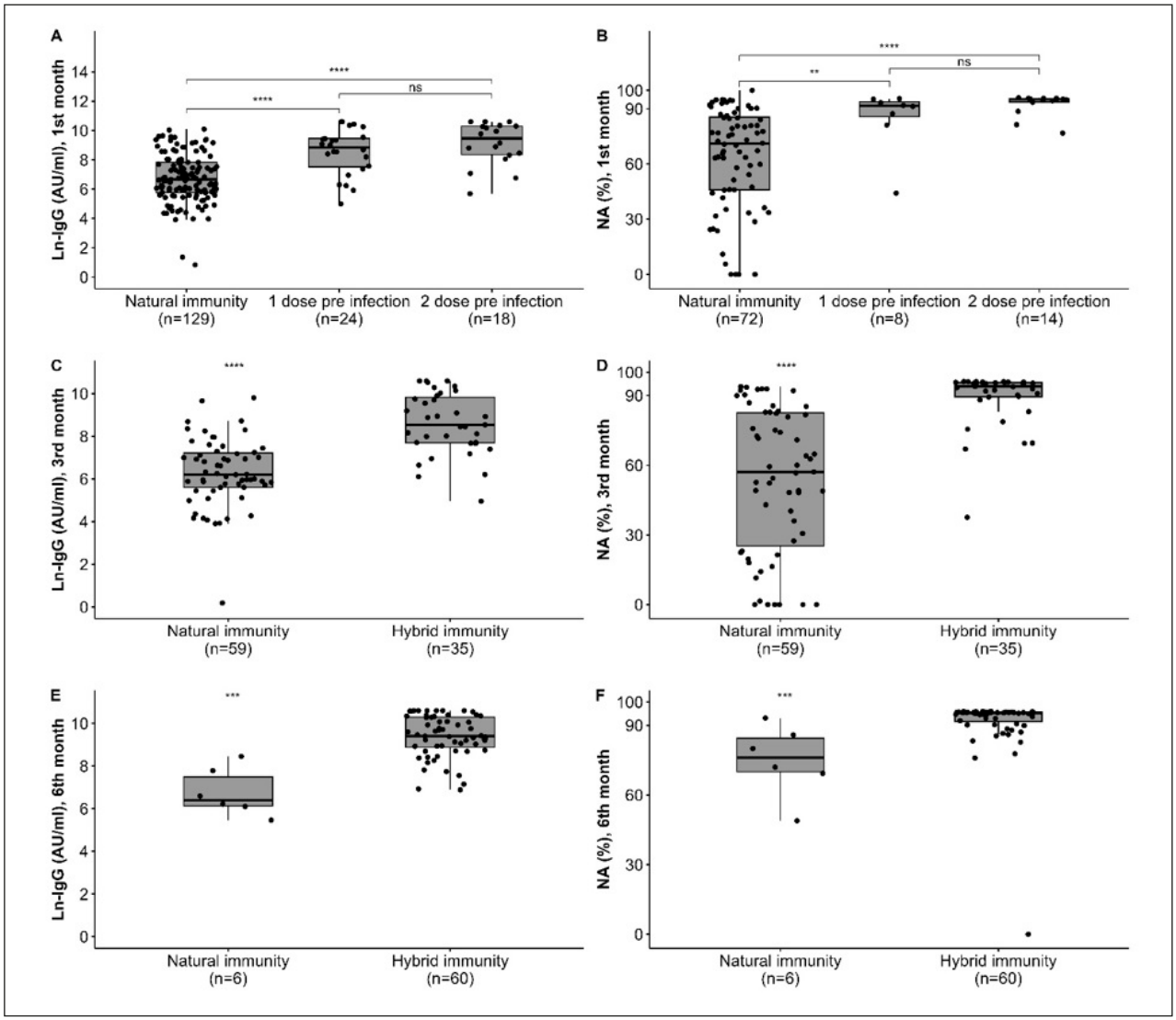




**Figure 4** - *Comparison of IgG and NA titers at the 1st, 3rd, and 6th months between natural and hybrid immunity groups.*

most participants in the hybrid group had completed their booster vaccinations, while 9 (7.0%) remained unvaccinated throughout the study period.

In the first month, the mean duration was 27±23.1 days from the last vaccination dose until the infection. There was a statistically significant difference between the first-month IgG values of the natural immunity group (median: 780, min-max: 2.30-24137), the group that received one dose of vaccine (median: 7034, min-max: 147-40000), and the group that received two doses of vaccine (median: 13468, min-max: 296-40000) before infection (p<0.001) (*Figure 4A*). There was no difference in IgG and neutralizing antibody titers between those who received a single dose of vaccine and those who received two doses of vaccine before infection (p=0.29).

In post hoc analysis, both IgG and neutralizing antibodies were higher in participants with hybrid immunity than with natural immunity at all time points. The median titer increased by booster doses (*Figure 4*).

### *Factors Affecting Antibody Levels*

We investigated the predictors affecting log-antibody titers measured in the first, third, and sixth months by the Generalized Estimating Equations (GEE) model. Age (β=0.027, z=3.14) was positively associated with log-antibody titers. The presence of comorbidities (β=−0.658, z=−3.55 was negatively related to log-antibody titers. Individuals with mild-to-moderate COVID-19 clinical status exhibited significantly lower antibody levels than those with severe status (β=-1.426, z=−5.16). Vaccination status was a strong predictor of log-antibody titers. Non-significant variables included gender, BMI, and time (*Table 3*).

No cases of re-infection were identified during the six-month follow-up period. When retrospectively evaluated for an additional six months, 56 patients (56/171) underwent PCR testing, and eight (4.6%) tested positive for COVID-19. They did not have hospitalization or mortality. All patients diagnosed with re-infection had received two doses of mRNA vaccine, with an average of 137.6 days (109–169) since their last vaccination at the time of re-infection.

Only one patient had a negative IgG antibody titer; seven had high IgG antibody levels with a median of 15352 (AU/ml (7686–24441 AU/ml), and measured median of 34.6 days (range: 10–61 days) after their mRNA vaccination. The median time for re-infection after a positive IgG test was 107 days (79-278). Six patients had positive neutralization assay tests (*Table 4*).

**Table 3** - *The results of the GEE model to investigate the predictors affecting log-antibody titers measured at 1st, 3rd, and 6th months.*

| | *Ln-IgG* | | | *NA* | | |
|---|---|---|---|---|---|---|
| | *Estimate* | *Robust SE* | *Robust z* | *Estimate* | *Robust SE* | *Robust z* |
| Age | 0.03 | 0.01 | 3.19* | 0.45 | 0.16 | 3.10* |
| Female gender | -0.03 | 0.19 | -0.18 | -4.29 | 3.07 | -1.40 |
| BMI | 0.02 | 0.02 | 0.87 | 0.54 | 0.35 | 1.55 |
| Comorbidity (no) | -0.65 | 0.18 | -3.70* | -9.02 | 3.13 | -2.88* |
| Hospitalization (ref) | 1.17 | 0.26 | 4.47* | 14.00 | 4.09 | 3.43* |
| Vaccination before infection (ref) | 1.12 | 0.25 | 4.42* | 14.58 | 3.21 | 4.54* |
| Vaccination between 1st and 3rd month postinfection (ref) | 1.74 | 0.36 | 4.79* | 23.77 | 4.42 | 5.39* |
| Vaccination between 3rd and 6th month postinfection (ref) | 2.88 | 0.36 | 7.97* | 26.93 | 5.31 | 5.07* |
| Time | -0.14 | 0.07 | -2.05* | -0.79 | 1.31 | -0.70 |

Ref: reference group, *: p<0.05.

**Table 4** - *Vaccination status, IgG antibody and neutralization antibody results of the infected participants.*

| | *Age, sex* | *First infection date* | *Re-infection date* | *Last vaccination date* | *Vaccination dose* | *Antibody test date* | *Antibody level (AU/ml)* | *Neutralisation assay (%)* |
|---|---|---|---|---|---|---|---|---|
| 1 | 42 M | 06.03.21 | 09.02.22 | 24.08.21 | 2mRNA | 13.09.21 | 16218 | Positive (95,7) |
| 2 | 46 F | 16.03.21 | 13.01.22 | 12.08.21 | 2mRNA | 30.09.21 | 10490 | Positive (95,5) |
| 3 | 44 M | 15.03.21 | 13.01.22 | 26.08.21 | 2mRNA | 26.10.21 | 15352 | Positive (95,7) |
| 4 | 47 F | 16.03.21 | 15.01.22 | 15.09.21 | 2mRNA | 30.09.21 | 11877 | Positive (95,5) |
| 5 | 44 F | 14.03.21 | 27.02.22 | 15.11.21 | 2mRNA | 23.09.21 | 20520 | Positive (95,7) |
| 6 | 42F | 24.03.21 | 04.01.22 | 02.08.21 | 2mRNA | 24.09.21 | 7686 | Positive (95,7) |
| 7 | 34 F | 18.03.21 | 02.01.22 | 15.09.21 | 2mRNA | 26.04.21 | 2,3 | – |
| 8 | 55 M | 17.02.21 | 27.01.22 | 01.09.21 | 2SIN+2 mRNA | 05.04.21 | 24441 | – |

*M: male, F: Female, mRNA: mRNA vaccine (Biontech) against SARS-CoV-2, SIN: inactivated virus vaccine(Sinovac) against SARS-CoV-2.

## DISCUSSION

In this study, we evaluated the longitudinal course of SARS-CoV-2 IgG and neutralizing antibody levels, the factors influencing them, and the frequency of reinfection in patients with mild to moderate COVID-19. Our findings provide valuable insight into post-infection immune dynamics, with potential implications for both clinical practice and future research.

We found that median SARS-CoV-2 IgG and NAb titers were significantly higher in individuals with hybrid immunity compared to those with natural immunity. In the first month after infection, no significant difference was observed between one and two pre-infection vaccine doses; however, booster doses at the third and sixth months led to higher antibody levels. These results are consistent with previous studies demonstrating that hybrid immunity elicits stronger and more durable antibody responses than natural infection alone (Morgan *et al.*, 2024; Bobrovitz *et al.*, 2023; Kirwan *et al.*, 2023; Feldstein *et al.*, 2025).

Regarding predictors of antibody levels, comorbidities were associated with lower responses, while older age, greater disease severity/hospitalization, and hybrid immunity – regardless of dose number – were linked to higher titers, in agreement with earlier reports (Güzel *et al.*, 2025; Bratcher *et al.*, 2021). The positive correlation between IgG and NAb was strong at months 1 and 3, but weaker at month 6, likely reflecting the low number of negative results at that time. Similar strong correlations have been documented for anti-S-RBD Ig measurements and NAb detection (Kitagawa *et al.*, 2022; Gütlin *et al.*, 2024; Güzel *et al.*, 2025).

We identified an IgG threshold of 303.8 AU/mL for predicting NAb positivity (>30%) with >90% sensitivity and specificity. This is comparable to the 302.8 AU/mL cut-off reported for >60% neutralization (Güzel *et al.*, 2025), while Lee *et al.* (2022) suggested 120.1 AU/mL and 449.7 AU/mL for >20% and >50% NAb, respectively. Differences in cut-off values may stem from variations in assay type, inhibition thresholds, study populations, and statistical methodology (Gütlin *et al.*, 2024; Güzel *et al.*, 2025; Lee *et al.*, 2022).

Reinfections were not observed in the first six months, but occurred in 4.6% of participants during the following six months, all during the Omicron wave in Turkey (Sayan *et al.*, 2021). Most cases occurred within ~3 months of high antibody levels. This aligns with reports of waning protection despite hybrid immunity (Dépéry *et al.*, 2025; Chu *et al.*, 2024; Tan *et al.*, 2025; Sheehan *et al.*, 2025; Chen *et al.*, 2024; Alp-Çavuş *et al.*, 2023; Moore *et al.*, 2025).

The study has several limitations. COVID-19 vaccines became available during follow-up, and vaccination status changed over time; by the sixth month, only 10.6% remained unvaccinated, limiting separate analysis of natural immunity at later time points. Reinfections were assessed prospectively for the first six months and retrospectively thereafter, possibly underestimating rates by missing asymptomatic cases. Although no reinfections occurred during the first six months, eight reinfections were retrospectively identified during the subsequent Omicron wave. All reinfections identified during follow-up were symptomatic and confirmed by RT-PCR. Asymptomatic infections may have been missed, as testing was performed only upon symptom onset. Lack of variant data precluded analysis of variant-specific neutralization. Finally, the study cohort consisted of adult outpatients with mild to moderate disease, so findings may not generalize to severe or immunocompromised populations.

## CONCLUSIONS

Hybrid immunity, resulting from SARS-CoV-2 infection combined with vaccination, induces stronger and more sustained IgG and neutralizing antibody responses than natural infection alone. An IgG level ≥303.8 AU/mL is a reliable surrogate marker for predicting NAb positivity with high sensitivity and specificity, offering a practical tool in settings where neutralization assays are unavailable.

However, the occurrence of reinfections despite high IgG and NAb levels – particularly during the Omicron wave – highlights the limitations of static serological thresholds in predicting protection against evolving variants. These findings emphasize the need for continued surveillance, booster immunization strategies, and further prospective studies incorporating variant analysis to refine antibody-based correlates of protection.

### *Ethical Approval and consent to participate*

The Dokuz Eylul University Research Ethics Committee (Date: June 15, 2020; Number: 2020/13-30) and the Scientific Research Platform of the Turkish Ministry of Health granted ethical approval. All the participants included in the study signed an informed consent form.

### Disclosure

The authors declare no disclosure issues.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Author contribution

AN, ANE, VAO, and AS participated in the study protocol design, data collection, and manuscript drafting. AN, ANG, OA, and AAS participated in the laboratory analysis and laboratory protocol design. AN, ANE, AS, and VAO participated in the data analysis, manuscript drafting, and revision.AN, ANG, ANE, and GOS participated in the data collection, manu-

script revision, and approval. All the authors read and approved the final manuscript.

**Funding**
The study was supported under Dokuz Eylul University Scientific Research Projects (project number: 2456).

**Availability of data and materials**
The datasets used and analyzed during the study are available from the corresponding author upon request.

**Declaration of generative AI in scientific writing**
During the preparation of this work the author(s) used AI-assisted tool in the writing process to improve the readability and language of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

## References

Alp- Çavuş S., Çelik M., Süner A.F., Güzel I., Irmak Ç., et al. (2023). Pre-infection antibody levels of vaccinated healthcare workers with SARS-CoV-2 breakthrough infection: A nested case-control study. *Immunol Lett*. **262**, 1-6.

Bobrovitz N., Ware H., Ma X., Li Z., Hosseini R., et al. (2023). Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the omicron variant and severe disease: a systematic review and meta-regression. *Lancet Infect Dis*. **23** (5), 556-567.

Bratcher A., Kao S.Y., Chun K., Petropoulos C.J., Gundlapalli A.V., et al. (2024). Quantitative SARS-CoV-2 Spike Receptor-Binding Domain and Neutralizing Antibody Titres in Previously Infected Persons, United States, January 2021-February 2022. *Emerg Infect Dis*. **30** (11), 2352-2361.

Chen D., Zhang W., Xiao B., Xu B., Yang X., et al. (2024). Effect of wild-type vaccine doses on BA.5 hybrid immunity, disease severity, and XBB reinfection risk. *J Virol*. **98** (12), e0128524.

Chu J., Dai Q., Dong C., Kong X., Tian H., et al. (2024). The serological IgG and neutralizing antibody of SARS-CoV-2 omicron variant reinfection in Jiangsu Province, China. *Front Public Health*. **12**, 1364048.

Dépéry L., Bally I., Amen A., Némoz B., Buisson M., et al. (2025). Anti-SARS-CoV-2 serology based on ancestral RBD antigens does not correlate with the presence of neutralizing antibodies against Omicron variants. *Microbiol Spectr*. **13** (1), e0156824.

Feldstein L.R., Ruffin J., Wiegand R.E., Borkowf C.B., James-Gist J., et al. (2025). Effectiveness of mRNA COVID-19 vaccines and hybrid immunity in preventing SARS-CoV-2 infection and symptomatic COVID-19 among adults in the United States. *J Infect Dis*. jiad007.

Garcia-Beltran W.F., Lam E.C., Astudillo M.G., Yang D., Miller T.E., et al. (2021). COVID-19-neutralizing antibodies predict disease severity and survival. *Cell*. **184** (2), 476-488.e11.

Gillot C., Favresse J., David C., Maloteau V., Dogne J.M., et al. (2024). An Evaluation of a SARS-CoV-2 Pseudovirus Neutralization Test and A Comparison to a SARS-CoV-2 Surrogate Virus Neutralization Test in a COVID-19 Long-Term Follow-Up Cohort. *Microbiol Res*. **15**, 422-430.

Gomes B.B.M., Ferreira N.N., Garibaldi P.M.M., Dias C.F.S.L., Silva L.N., et al. (2024). Impact of SARS-CoV-2 variants on COVID-19 symptomatology and severity during five waves. *Heliyon*. **10** (22), e40113.

Gütlin Y., Albertos Torres D., Gensch A., Schlotterbeck A.K., Stöger L., et al. (2024). Anti-SARS-CoV-2 total immunoglobulin and neutralising antibody responses in healthy blood donors throughout the COVID-19 pandemic: a longitudinal observational study. *Swiss Med Wkly*. **154**, 3408.

Güzel I., Öztürk G., Appak Ö., Çağlayan D., Süner A.F., et al. (2025). Neutralizing and binding antibody dynamics following primary and booster COVID-19 vaccination among healthcare workers. *BMC Infect Dis*. **25** (1), 218.

Khoury D.S., Schlub T.E., Cromer D., Steain M., Fong Y., et al. (2023). Correlates of Protection, Thresholds of Protection, and Immunobridging among Persons with SARS-CoV-2 Infection. *Emerg Infect Dis*. **29** (2), 381-388.

Kirwan PD, Hall VJ, Foulkes S, Otter AD, Munro K, et al. (2023). Effect of second booster vaccinations and prior infection against SARS-CoV-2 in the UK SIREN healthcare worker cohort. *Lancet Reg Health Eur*. **36**, 100809.

Kitagawa Y., Imai K., Matsuoka M., Fukada A., Kubota K., et al. (2022). Evaluation of the correlation between the access SARS-CoV-2 IgM and IgG II antibody tests with the SARS-CoV-2 surrogate virus neutralization test. *J Med Virol*. **94** (1), 335-341.

Lee B., Ko J.H., Park J., Moon H.W., Baek J.Y., et al. (2022). Estimating the Neutralizing Effect and Titer Correlation of Semi-Quantitative Anti-SARS-CoV-2 Antibody Immunoassays. *Front Cell Infect Microbiol*. **12**, 82259

Manenti A., Maggetti M., Casa E., Martinuzzi D., Torelli A., et al. (2020). Evaluation of SARS-CoV-2 neutralizing antibodies using a CPE-based colorimetric live virus micro-neutralization assay in human serum samples. *J Med Virol*. **92** (10), 2096-2104.

Moore M., Anderson L., Schiffer J.T., Matrajt L., Dimitrov D. (2025). Durability of COVID-19 vaccine and infection induced immunity: A systematic review and meta-regression analysis. *Vaccine*. **30** (54), 126966.

Morgan G., Fung C.Y.J., Gingras A.C., Colwill K., Briollais L., et al. (2024). Characterizing the SARS-CoV-2 antibody response and associations with patient factors: Serological profiling of participants enrolled in the GENCOV study. *Clin Biochem*. **110**, 110859.

Rostami A., Sepidarkish M., Leeflang M.M.G., Riahi S.M., Nourollahpour Shiadeh M., et al. (2021). SARS-CoV-2 seroprevalence worldwide: a systematic review and meta-analysis. *Clin Microbiol Infect*. **27** (3), 331-340.

Sayan M., Arikan A., Isbilen M. (2022). Circulating dynamics of SARS-CoV-2 variants between April 2021 and February 2022 in Turkey. Can. *J. Infect. Dis. Med. Microbiol*. 1-7.

Sheehan J., Trauth A.J., Hagensee M.E., Ramsay A.J. (2025). Characterization of Vaccine-Enhanced Humoral Immune Responses Against Emergent SARS-CoV-2 Variants in a Convalescent Cohort. *Pathogens*. **14** (1), 44.

Tan S.T., Rodríguez-Barraquer I., Kwan A.T., Blumberg S., Park HJ, et al. (2025). Strength and durability of indirect protection against SARS-CoV-2 infection through vaccine and infection-acquired immunity. *Nat Commun*. **16** (1), 1090.

Valcourt E.J., Manguiat K., Robinson A., Lin Y., Abe K.T., et al. (2021). Evaluating humoral immunity against SARS-CoV-2: validation of a plaque-reduction neutralization test and a multilaboratory comparison of conventional and surrogate neutralization assays. *Microbiol Spectr*. **9**, e00886-e00821.

Wei J., Pouwels K.B., Stoesser N., Matthews P.C., Diamond I., et al. (2022). Antibody responses and correlates of protection in the general population after two doses of the ChAdOx1 or BNT162b2 vaccines. Antibody responses and correlates of protection in the general population after two doses of the ChAdOx1 or BNT162b2 vaccines. *Nat Med*. **28** (5), 1072-1082.

Zaballa M.E., Perez-Saez J., de Mestral C., Pullen N., Lamour J., et al. (2023). *Lancet Reg Health Eur*. **24**, 100547.

Zaçe D., Çekrezi A., Jones C., Ferrari L., De Simone G., et al. (2025). The evolving landscape of COVID-19: factors associated with in-hospital COVID-19 related mortality during the 2023-24 phase of JN.1 subvariant dominance. *BMC Infect Dis*. **25** (1), 130.

Zhang S., Kong X., Zhen Q., Wei Y., Shi C., et al. (2025). Dynamic Changes and Trends of SARS-CoV-2 Antibodies Induced by Infection and Vaccination Across Multiple Time Points. *J Med Virol*. **97** (1), e70161.