Polish Annals of Medicine

Journal homepage: https://www.paom.pl

Research paper

# In search of protective antibody for coronavirus disease 2019: A retrospective study

*Sayak Roy*[1], *Sandip Mukhopadhyay*[2], *Shatavisa Mukherjee*[3], *Shambo Samrat Samajdar*[3], *Santanu K Tripathi*[4]

[1] *Medica Superspeciality Hospital, Kolkata, India*
[2] *Department of Pharmacology, Burdwan Medical College, Burdwan, India*
[3] *Department of Clinical and Experimental Pharmacology, School of Tropical Medicine, Kolkata, India*
[4] *Department of Pharmacology, Netaji Subhash Medical College and Hospital, Bihta, Patna, India*

Article info

Article history
Received: November 22, 2021
Accepted: February 23, 2022
Available online: April 4, 2022

Keywords
COVID-19
SARS-CoV-2
Neutralizing antibody

Doi
https://doi.org/10.29089/paom/146894



Abstract

Introduction: The search for a protective antibody titer level to prevent coronavirus disease 2019 (COVID-19) infection, progression, and death is far from over. To date, no specific cut-off values have been established for these protective antibodies or neutralizing antibody (NAb) titers. NAb titers inhibit viral replication.

Aim: To find out the prevailing NAb titre which might give protection from COVID-19 infection, or complication arising out of it.

Material and methods: The data of COVID-19 patients with NAb titers who underwent reverse transcription polymerase chain reaction and presented with mild symptoms within 3 days after receiving the results were analyzed. The data were obtained from the clinic's electronic database. Of the recruited patients, 63 were included in the final analysis. All statistical analyses were performed using SPSS v. 21.

Results and discussion: A highly significant correlation (negative) existed between circulating NAb titer and duration of fever ($P < 0.001$); a positive significant correlation existed between the period from the 1st vaccine dose to the period of infection and the NAb titer level ($P < 0.001$). The NAb titer was significantly higher in the group that received both doses ($P = 0.00016$). Death and admission due to progression to moderate COVID-19 occurred in the group with a NAb titer of less than 10 U/L.

Conclusions: The risk of complications and death due to COVID-19 may increase if the protective antibody level remains less than 10 U/L. The sample size used in this study was relatively small; therefore, this finding cannot be generalized. Hence, more robust studies should be performed to determine the appropriate protective NAb titer levels.

*Corresponding author: Shambo Samrat Samajdar, Department of Clinical and Experimental Pharmacology, School of Tropical Medicine, 108, Chittaranjan Avenue, Kolkata – 700073, West Bengal, India.*
*E-mail address: shambo_sa2001@yahoo.co.in*

## 1. INTRODUCTION

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused a havoc worldwide for the past 20 months with significant morbidity and mortality.[1] Immunity against SARS-CoV-2 is acquired after developing an infection with this virus and obtaining external immunizations; however, the vaccine has demonstrated a significant reduction in the disease severity, but some patients still require hospitalization.[1] As of August 11, 2021, 203 295 170 confirmed COVID-19 cases and 4 303 515 deaths were reported worldwide.[2] The COVID-19 outbreak is one of the worst pandemics, yet the robust efficacy of the existing therapeutic options is not well defined. Primarily, the treatment is aimed at achieving symptomatic relief and targets patients with severe and high-risk cases with an anticipated worse prognosis, as recommended by the United States Food and Drug Administration (US FDA).[3,4] Furthermore, the risk of reinfection in the presence of neutralizing antibody (NAb) titers is ambiguous, as it might be due to a different strain. Besides the technical and methodological limitations, confirming the occurrence of reinfection caused by the same strain remains dubious. Lastly, the levels of NAb required to offer sufficient protection against new infections or reinfection are also unclear.[5] Various clinical studies investigating candidate vaccines have used NAb titers to quantify the seroconversion and indicate their efficacy. Several vaccines that have obtained approval for emergency use authorization or that are still in the late stages of trial are aimed at developing NAb titers against spike (S) protein or the receptor-binding domains (RBD).[6]

SARS-CoV-2 triggers a humoral immune response leading to the production of antibodies such as immunoglobulin (Ig) M, IgG, and IgA, which remain intact even when the levels of NAb decline.[7] These antibodies against the nucleocapsid (N) protein and S-protein, which target the S1 protein subunit and impede viral infection through several mechanisms, are formed within a few days to three weeks after infection.[8,9] The S1 subunit comprises the RBD that facilitates the binding of the virus to predisposed cells and is also the key target of NAb.[10,11] The levels of IgG, which act against RBD, are usually proportional to the levels of the neutralization titer. When the neutralization potency of Nab was quantified, the data revealed that higher levels were found to be a positive prognostic marker of survival.[12] In addition, patients with anti-SARS-CoV-2 antibodies are less susceptible to reinfection, as confirmed by a few outbreak investigation studies conducted in the United States.[13,14] NAb detection tests performed in vitro using serum or plasma for incubation assessed the capacity of these antibodies to prevent infection by inhibiting the multiplication of virus in cell culture.[8] It is critical to carefully understand the time that the antibodies take to appear and disappear to obtain the actual clinical status of the patient and avoid any false-positive or false-negative impression.[3]

Considering the ambiguity based on the available evidence regarding the NAb, we determined the correlation between NAb titer and fever duration, COVID-19 related complications or hospitalizations, or disease progression to moderate or severe stages using various hematological parameters. The retrospective patient data included were obtained from patients with mild COVID-19 infection, confirmed with a positive reverse transcription polymerase chain reaction (RT-PCR) report, who had received at least one shot of vaccine.

## 2. AIM

Primary, to determine the association between the prevailing NAb titer and the outcome of COVID-19 infection.
Secondary, to determine the association between the prevailing NAb titer and the following factors:
(1) Estimated glomerular filtration rate (eGFR),
(2) Glycated hemoglobin (HbA1c, in known diabetics),
(3) Systolic blood pressure and diastolic blood pressure,
(4) Hematological parameters C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), and D-dimer.

## 3. MATERIAL AND METHODS

A retrospective study was performed by searching the electronic records of patients with mild COVID-19. The current study included data from 63 patients with RT-PCR-confirmed COVID-19 infection of mild severity with a mean age of 57.38 years and a sex ratio of 1.33 : 1.00 (male to female). Patients who tested positive on the RT-PCR (performed within 3 days after the onset of symptoms); with available data on creatinine, NLR, CRP (normal <5.0 mg/L), D-dimer (high >500 ng FEU/mL), and NAb titer levels (positive >0.8 U/Ml, measured by double-antigen sandwich electrochemiluminescence immunoassay); and with complete records of vaccination history were included in the study. All hematological investigations were performed on the 6th to 7th day after symptom onset. With an estimated correlation coefficient of 0.35, an 80% power, and 5% probability of type 1 error, the estimated sample size was 62.

After performing an extensive database mining for the period of January 2021 to July 2021, we identified 73 patients who met the eligibility criteria. Of the 73 patients identified, 63 agreed to provide a written informed consent to share their clinical data for publication purposes. Each patient or the closest family member was followed-up over the phone, and information on the exact fever duration, need for hospitalization, and outcome at the hospital were obtained. The details of the study are presented in Figure 1.

Information on age, blood pressure levels, HbA1c level, and hematological parameters (CRP, NLR, and D-dimer) were obtained along with the prevailing NAb titer level. Data on the history of hypertension, type 2 diabetes mellitus (T2DM), dyslipidemia, atherosclerotic cardiovascular disease, chronic obstructive pulmonary disease, and malignancies were recorded. The dates of vaccine administration and duration of fever were obtained from all patients. Hospital records on the need

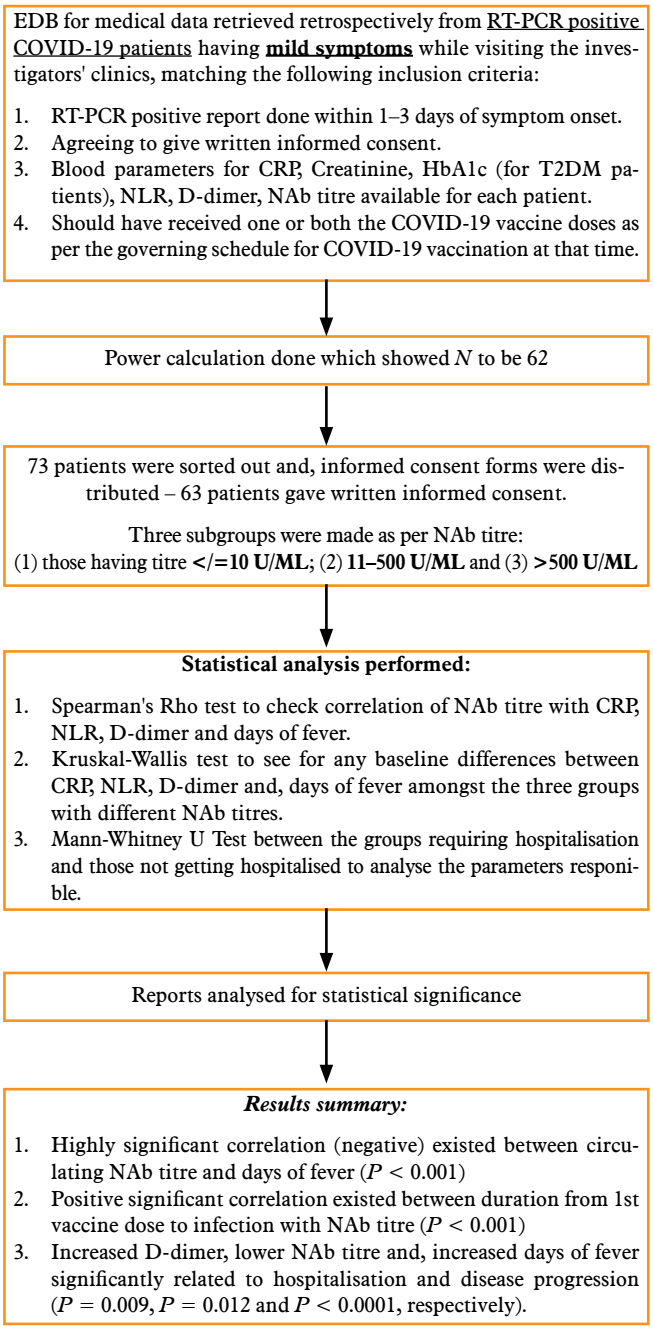


**Figure 1. Study flowchart. Comments: RT-PCR – reverse transcription polymerase chain reaction, COVID-19 – coronavirus disease 2019, NAb – neutralizing antibodies, CRP – C-reactive protein, NLR – neutrophil-lymphocyte ratio, eGFR – estimated glomerular filtration rate, T2DM – type 2 diabetes mellitus, HbA1c – glycated hemoglobin.**

for hospitalization and outcomes were also assessed by asking the patient or the next of kin.

All the above mentioned parameters were specifically examined in a cohort of hospitalized patients and compared with the data from the non-hospitalized group. Furthermore, the patients were divided into three subgroups based on the NAb titer levels: (1) those with a titer level of ≤10 U/mL, (2) those with a titer level of 11–500 U/mL, and (3) those with a titer level of >500 U/mL; the association of NAb titer levels with blood parameters (CRP, NLR, and D-dimer) and duration of fever was also analyzed.

The data were evaluated for completeness and then included in the statistical analysis. The descriptive data were expressed as mean or percentages, along with its standard deviation, wherever applicable. Different levels were expressed as 95% confidence intervals (CIs). The mean values were compared for hypothesis testing, and a correlation analysis was performed. Non-parametric tests, such as the Mann–Whitney U test, Kruskal–Wallis test, and Spearman's rank correlation coefficient were performed using an online calculator.[15] A $P$ value of less than 0.05 was considered significant. A statistical analysis of various parameters was performed using appropriate statistical software packages, such as Statistical Package for the Social Sciences (Windows v. 21.0; SPSS Inc., Chicago, IL, USA), GraphPad Prism, Social Science Statistics, and Microsoft Excel.

## 4. RESULTS

The patients' baseline characteristics are shown in Tables 1 and 2.

Of the 63 patients, 6 were hospitalized, and the relevant characteristics of this cohort are presented in Table 3.

In the hospitalized cohort, 83.33% ($n$ = 5/6) of the patients had a history of T2DM. Of the total patients with

**Table 1. Baseline patient pharacteristics ($n$ = 63).**

| Parameters | Observed values as mean ± SD (95% CI) |
|---|---|
| Mean age, years | 57.38 ± 10.657 (54.7–60.06) |
| Mean HbA1c, % | 2.84 ± 3.58 (1.93–3.74) |
| Mean SBP, mm Hg | 118.70 ± 17.38 (114.32–123.08) |
| Mean DBP, mm Hg | 71.11 ± 8.14 (69.06–73.16) |
| Mean days of fever | 3.73 ± 3.87 (2.75–4.70) |
| Comorbidities* | |
| Hypertension | 27 (42.86%) |
| Type 2 diabetes mellitus | 25 (39.68%) |
| Dyslipidemia | 15 (23.81%) |
| History of ASCVD | 3 (4.76%) |
| COPD | 2 (3.17%) |
| Malignancy | 1 (1.58%) |
| Mean measures of blood parameters | |
| eGFR, mL/min/1.73 m$^2$ | 92.21 ± 15.75 (88.24 – 96.17) |
| CRP, mg/dL | 12.79 ± 21.40 (7.40 – 18.17) |
| NLR | 2.98 ± 1.46 (2.61 – 3.35) |
| D-dimer, ng FEU/mL | 460.13 ± 311.18 (381.76 – 538.50) |
| NAb titre, U/mL | 868.59 ± 1012.85 (613.51 – 1123.68) |

Comments: * Values represented as $n$(%). SBP – systolic blood pressure; DBP – diastolic blood pressure; T2DM – type 2 diabetes mellitus; ASCVD – atherosclerotic cardiovascular disease; COPD – chronic obstructive pulmonary disease; HbA1c – glycated haemoglobin; NAb – neutralizing antibodies; CRP – C-reactive protein; NLR – neutrophil and lymphocyte ratio; eGFR – estimated glomerular filtration rate.

T2DM, 69.56% ($n$ = 16/25) received the first dose of vaccine, while 39.13% ($n$ = 9/25) received both doses. The mean NAb titer level in the T2DM cohort was 872.89 ± 979.14 U/mL (95% CI: 551.06–1194.73).

In the NAb titer group, the age, eGFR, duration of fever, and hematological parameters were obtained (Table 4).

The association of various parameters with NAb titers was assessed using Spearman's rank correlation (Table 5). The Mann–Whitney U test was performed to determine the differences in parameters between the hospitalized and non-hospitalized groups (Table 6). The Kruskal–Wallis test was performed to compare the three Nab titer subgroup categories to determine any baseline differences between various parameters (Table 7).

**Table 2. Baseline vaccination and hospitalization status.**

| Parameters | Values as mean ± SD (95% CI) |
|---|---|
| Vaccination details | |
| Mean duration from 1st vaccine dose to RT-PCR positivity, days | 40.51 ± 26.64 (33.80–47.22) |
| Patients getting single vaccine dose | 35 (55.55%) |
| Patients getting two doses of vaccine | 28 (44.44%) |
| Mean NAb titre after single vaccine dose | 468.814 ± 891.58 (162.54–775.08) |
| Mean NAb titre after both doses of vaccine | 1368.31 ± 942.629 (1002.81–1733.83) |
| Hospitalization* | |
| Patient getting admitted with Moderate COVID-19 | 4 (6.34%) |
| Patients expired after 1st dose of vaccine in the whole population | 1 (1.58%) |
| Patients expired after 2nd dose of vaccine in the whole population | 1 (1.58%) |

Comments:* Values represented as $n$ (%).

**Table 3. Characteristics in hospitalised patients ($n$ = 6)**

| Parameters | Observed means mean ± SD (95% CI) |
|---|---|
| Age, years | 62.83 ± 10.45 (51.86–73.81) |
| HbA1c ($n$ = 5 out of 6) | 7.46 ± 0.378 (±5.07%) |
| Duration of infection date from day of 1st vaccine | 30.17 ± 21.82 (7.27–53.07) |
| eGFR, mL/min/1.73 m$^2$ | 96.33 ± 17.78 (77.67–115.00) |
| CRP, mg/dL | 27.53 ± 29.47 (–3.4–58.46) |
| NLR | 4.63 ± 2.47 (2.05–7.23) |
| D-dimer, ng FEU/mL | 935.17 ± 657.70 (244.95–1625.39) |
| NAb titre, U/mL | 6.23 ± 3.51 (2.54–9.91) |

Comments: HbA1c – glycated haemoglobin, NAb – neutralizing antibodies; CRP – C-reactive protein; NLR – neutrophil to lymphocyte ratio; eGFR – estimated glomerular filtration rate.

**Table 4. Patient Characteristics as per NAb titre categories**

| Parameters | Groups as per NAb titre ≤10 | 11–500 | >500 |
|---|---|---|---|
| Age, years | 58.42 ± 8.88 (54.14–62.70) | 57.53 ± 9.56 (52.24–62.83) | 56.62 ± 12.39 (51.91–61.34) |
| eGFR, mL/min/1.73 m$^2$ | 94.42 ± 15.82 (86.80–102.04) | 92.20 ± 18.28 (82.07–102.33) | 90.76 ± 14.69 (85.17–96.35) |
| NLR | 3.25 ± 1.78 (2.39–4.11) | 3.38 ±1.75 (2.42–4.35) | 2.59 ± 0.93 (1.2–4.8) |
| CRP, mg/dL | 12.35 ± 19.18 (3.11–21.60) | 7.44 ± 9.30 (2.28–12.59) | 15.83 ± 26.63 (5.7–25.96) |
| D-dimer | 552.53 ± 461.26 (330.20–774.85) | 463.33 ± 173.54 (367.23–559.44) | 397.93 ± 229.73 (310.55–485.32) |
| Days of fever | 6.89 ± 5.15 (4.41–9.37) | 3.07 ± 2.40 (1.73–4.39) | 2.00 ± 1.74 (1.33–2.66) |
| NAb titre, U/Ml | 3.15 ± 3.57 (1.43–4.87) | 118.91 ± 151.03 (35.27–202.55) | 1823.37 ± 711.82 (1552.61–2094.13) |

Comments: Values expressed as mean ± SD (95% CI).

**Table 5. Association of various parameters with NAb titres**

| Parameters | Spearman's correlation coefficient | $P$ value | Type of correlation |
|---|---|---|---|
| Days of fever | –0.469 | 0.000* | Negative |
| CRP | –0.200 | 0.116 | |
| D-dimer | –0.191 | 0.134 | |
| NLR | –0.105 | 0.412 | |
| eGFR | –0.110 | 0.391 | |
| SBP | –0.022 | 0.875 | |
| DBP | 0.058 | 0.654 | |
| Age | 0.010 | 0.940 | Positive |
| Duration of 1st dose of vaccine to infection, days | 0.508 | 0.000* | |
| HbA1c | 0.028 | 0.895 | |

Comments: *Statistical significance at $P < 0.001$.

**Table 6. Mann-Whitney $U$ test between hospitalised group and non-hospitalised group for various parameters**

| Test | CRP | NLR | D-dimer | NAb | AGE | eGFR | Days of fever |
|---|---|---|---|---|---|---|---|
| Mann–Whitney $U$ | 105.00 | 91.00 | 59.50 | 64.00 | 120.50 | 128.00 | 0.000 |
| $Z$ | –1.548 | –1.873 | –2.611 | –2.516 | –1.184 | –1.008 | –4.038 |
| $P$ Value | 0.122 | 0.061 | 0.009* | 0.012* | 0.237 | 0.313 | 0.000* |

Comments: *Statistical significance at $P < 0.05$.

**Table 7. Kruskal–Wallis test.**

| | CRP | NLR | D-dimer | days of fever |
|---|---|---|---|---|
| Chi-Square | 0.551 | 2.950 | 2.611 | 15.617 |
| df | 2 | 2 | 2 | 2 |
| $P$ value | 0.759 | 0.229 | 0.271 | 0.000* |

Comments: *Statistical significance at $P$ <0.05.

Clearly, compared with those who received the first dose of vaccine, patients who received both doses showed a significantly ($P = 0.00008$) high titer level according to the Mann–Whitney $U$ test. The observed value of $U$ was 203.5, while the $Z$ statistic was $-3.955$.

## 5. DISCUSSION

The present study demonstrated that the NAb in response to COVID-19 vaccination are not similar in all patients. The patients may develop infection even after receiving vaccination. Hospitalization and severe disease are more common in patients with lower NAb titer levels (<10 U/L). To precisely estimate the period to offer a booster dose of vaccine, several researchers have attempted to investigate the NAb titer levels. A database evaluation performed in Taiwan reported that the Nab titer levels peaked between week 5 and week 8 and progressively decreased thereafter in a patient with SARS infection with an estimated half-life of 6.4 weeks.[16] The study also noted that, compared with those with a shorter duration of illness, patients with a longer illness duration had potentially lower NAb titer levels ($P = 0.008$). When the study compared patients who had a seroconversion within 2 weeks with those who had a seroconversion beyond 3 weeks, the death rate ($P = 0.004$) was nearly four-fold higher and the duration of survival was shorter ($P = 0.013$); these patients were predominantly older (>60 years old, $P = 0.01$) and belonged in the early antibody detectable group. This finding could be possibly due to the priming effect of a preceding non-SARS-CoV infection, which also aligns with the higher age, since the infection rates increase with advancing age.[16]

A recent study reported that to reach a 50% protective effect against noticeable SARS-CoV-2 infection, the neutralization level should be 20.2% of the mean convalescent level (95% CI: 14.4%–28.4%); furthermore, to protect against severe infection, the neutralization level should be 3% of the mean convalescent level (95% CI: 0.7%–13%, $P = 0.0004$).[1] Thus, the study suggested that the level of neutralization can adequately predict the magnitude of immune protection, which can serve as a model for devising vaccine strategies in the current uncertain scenario of the pandemic.[1] Analysis from a previous study in Germany showed that the probability of NAb seropositivity significantly increased in patients with greater loss of appetite, muscle weakness, chills or hot flashes, and a reduced sense of taste.[17] Additionally, further studies found that an 80% vaccine efficacy against symptomatic primary infection could be achieved with the NAb levels are 40 923 and 63 383 for anti-spike and anti-RBD, respectively. However, the NAb levels are not proportionate to the levels required to protect asymptomatic patients.[18]

A study conducted in 162 COVID-19 patients from the COVID-BioB study cohort showed that more than 43% of patients developed NAbs within the first week of infection, while nearly 79% developed NAbs on the second week. Thus, the remaining 28% of patients failed to develop NAbs. The status of IgG development against spike proteins was similar to that of NAbs. The univariate analysis of these data showed that faster development of NAbs from symptom onset was associated with significantly less time to return a negative RT-PCR result ($P = 0.002$), which indicates elimination of the virus. Furthermore, the study found that although the initial NAb titer did not influence the duration of hospitalization, it did correlate with the severity of infection. The absence of NAb response in immediately after the onset of infection was a significant factor of worsening prognosis and subsequent mortality, regardless of sex and age (HR: 2.918, 95% CI: 1.321–6.449; $P = 0.008$), pre-existing comorbidities, levels of other immune-inflammatory markers, and lack of IgA (HR: 2.67, 95% CI: 1.2–5.9; $P = 0.015$).[19] Another study conducted in 49 patients from China who had recently recovered from COVID-19 pneumonia found that the NAb titers were significantly higher in older patients ($P = 0.020$), those with symptomatic infection ($P = 0.044$), more extensive pulmonary involvement ($P < 0.001$), abnormal CRP level ($P < 0.01$), and increased lactate dehydrogenase level ($P = 0.019$). Results of the multivariate analysis showed that the severity of pneumonia and comorbidities were positively associated with the level of NAb titers ($P = 0.02$), as well as the corticosteroid treatment ($P = 0.01$).[20] The current study inferred that an Nab titer of <10 U/mL was significantly associated with increased hospitalization, disease progression, and mortality ($P = 0.012$), which is in agreement with the published evidence.

Nevertheless, there are several challenges in the detection of antibody titers. In anticipation of a neglectful behavior in those with higher antibody levels, the US FDA has recommended against the detection of antibody titers.[21] However, whether antibodies are produced after spontaneous infection and after receiving mRNA vaccines needs to be clarified further. mRNA vaccines induce antibodies directed against spike proteins, unlike natural infections, which induce nucleocapsid proteins. This might lead to negative antibody test results in patients who were vaccinated but were not naturally infected with SARS-COV-2.[21]

A retrospective cohort study from NHS England showed that advanced age, male sex, obesity, and some comorbidities were strongly related to COVID-19 deaths, while smoking, history of malignancy, and chronic hepatic disease had more robust links with non-COVID deaths. When comparing the association with ethnicity, non-white ethnic groups had twice the likelihood of dying due to COVID-19, while the white ethnic groups had greater odds of dying due to non-COVID-19-related illness.[22] Other individual studies and meta-analyses of such studies have found a significant association of elevated neutrophil counts, D-dimer level, CRP level, interleukin-6 level, and diminished lymphocyte counts with poor prognosis in patients with COVID-19, after adjusting for confounders (comorbidities and advanced age).[23–25] An NLR of more than 4 has been associated with intensive care unit admission, while an NLR of less than 3 was associated with clinical improvement in 74 hospitalized patients.[26]

The current study showed that lower NAb titers, elevated D-dimer levels, and increased duration of fever were significantly associated with hospitalization and disease progression ($P = 0.012$, $P = 0.009$, and $P < 0.0001$, respectively). Our study showed a significant correlation (negative) for febrile days, depending on the prevailing NAb titer ($P < 0.001$). Similarly, a significant correlation was also found between the duration of infection from the date of the first vaccine dose and NAb titer formation ($P < 0.001$). All patients who progressed to either moderate or severe stage or died had a lower mean NAb titer level (3.15 ± 3.57 U/M1; CI: 1.43–4.87), prolonged duration of fever, and increased D-dimer, CRP, and NLR levels. All hospitalized patients with T2DM had a high mean HbA1c level of 7.46 ± 0.378 (±5.07%). The association of higher morbidity and mortality in patients with diabetes is well established; it is the second most common comorbidity in SARS-CoV-2-infected patients after hypertension.[27] Studies have shown poorer outcomes with hypertensive elderly patients.[28]

However, that study failed to show a significant association with other inflammatory markers between hospitalized and non-hospitalized patients.

### Limitations of the study

The study has certain limitations: (1) the retrospective nature of the study can cause a small bias; (2) the small sample size limited the applicability of our the findings in the general population; (3) due to the small number of patients in subgroups with NAb titers between 11 and 500, both groups were merged to perform the Kruskal-Wallis test to identify the baseline significant difference; and (4) the subgroups were formed on the basis of the prevailing NAb titer arbitrarily, since the relevant literature on classification of NAb titers is sparse.

## 6. CONCLUSIONS

Even post-vaccinated people may still develop COVID-19. However, a higher NAb titer is associated with better protection against severe form of COVID-19. A titer of less than 10 U/mL may not be able to prevent hospitalization or severe disease. However, larger studies are recommended to generalize and validate the findings of the present study.

Although this is a study with a small sample size, it definitely suggests some future directions:

(1) The protective antibody titer must be determined before a booster dose is planned.
(2) The specific clinical parameters that determine the rate of production of antibodies must be evaluated.
(3) The effects of different COVID-19 mutant variants must be analyzed in light of the variable immunogenicity.
(4) A yearly vaccination is needed since the titer expires after a few months.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Funding

None.

## Acknowledgments

The authors would like to acknowledge the help of Editage in helping perform editing.

## Ethics

Institutional EC clearance was obtained (Ref No: HREC-AAC/02). Consent was obtained from each study participant.

## References

1 Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med*. 2021;27(7):1205–1211. https://doi.org/10.1038/s41591-021-01377-8.
2 WHO. *WHO COVID-19 Dashboard*. https://covid19.who.int/. Published 2021. Accessed: August 11, 2021.
3 Roy S. Journey so far with COVID 19 – a comprehensive review. *Eur J Clin Exp Med*. 2020;18(4):303–317. https://doi.org/10.15584/ejcem.2020.4.7.
4 NIH. *Coronavirus disease 2019 (COVID-19) treatment guidelines covid-19 treatment guidelines*. https://files.covid19treatmentguidelines.nih.gov/guidelines/covid19treatmentguidelines.pdf. Accessed: August 11, 2021.
5 Roy S. COVID-19 reinfection in the face of a detectable antibody titer. *Cureus*. 2021;13(3):e14033. https://doi.org/10.7759/cureus.14033.
6 Poland GA, Ovsyannikova IG, Kennedy RB. SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates. *Lancet*. 2020;396(10262):1595–1606. https://doi.org/10.1016/S0140-6736(20)32137-1.
7 Ogega CO, Skinner NE, Blair PW, et al. Durable SARS-CoV-2 B cell immunity after mild or severe disease. *J Clin Invest*. 2021;131(7):e145516. https://doi.org/10.1172/JCI145516.
8 CDC. *Information for laboratories about coronavirus (COVID-19). 2020 Centers for Disease Control and Prevention*. https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html. Accessed: August 11, 2021.
9 Gharbharan A, Jordans CCE, GeurtsvanKessel C, et al. Effects of potent neutralizing antibodies from convalescent plasma in patients hospitalized for severe SARS-CoV-2 infection. *Nat Commun*. 2021;12(1):3189. https://doi.org/10.1038/s41467-021-23469-2.
10 Qu J, Wu C, Li X, et al. Profile of immunoglobulin G and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2020;71(16):2255–2258. https://doi.org/10.1093/cid/ciaa489.
11 Wolfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020;581(7809):465–469. https://doi.org/10.1038/s41586-020-2196-x.

12 Garcia-Beltran WF, Lam EC, Astudillo MG, et al. COVID-19-neutralizing antibodies predict disease severity and survival. *Cell*. 2021;184(2):476–488.e11. https://doi.org/0.1016/j.cell.2020.12.015.

13 Addetia A, Crawford KHD, Dingens A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J Clin Microbiol*. 2020;58(11):e02107–02120. https://doi.org/10.1128/JCM.02107-20.

14 Pray IW, Gibbons-Burgener SN, Rosenberg AZ, et al. COVID-19 outbreak at an overnight summer school retreat – Wisconsin, July-August 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(43):1600–1604. https://doi.org/10.15585/mmwr.mm6943a4.

15 Stangroom J. *Mann-Whitney U Test Calculator*. https://www.socscistatistics.com/tests/mannwhitney/default2.aspx. Accessed: February 15, 2022.

16 Ho MS, Chen WJ, Chen HY, et al. Neutralizing antibody response and SARS severity. *Emerg Infect Dis*. 2005;11(11):1730–1737. https://doi.org/10.3201/eid1111.040659.

17 Aziz NA, Corman VM, Echterhoff AKC, et al. Seroprevalence and correlates of SARS-CoV-2 neutralizing antibodies from a population-based study in Bonn, Germany. *Nat Commun*. 2021;12:2117. https://doi.org/10.1038/s41467-021-22351-5.

18 Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *medRxiv*. 2021.06.21.21258528. https://doi.org/10.1101/2021.06.21.21258528.

19 Dispinseri S, Secchi M, Pirillo MF, et al. Neutralizing antibody responses to SARS-CoV-2 in symptomatic COVID-19 is persistent and critical for survival. *Nat Commun*. 2021;12(1):2670. https://doi.org/10.1038/s41467-021-22958-8.

20 Chen W, Zhang J, Qin X, et al. SARS-CoV-2 neutralizing antibody levels are correlated with severity of COVID-19 pneumonia. *Biomed Pharmacother*. 2020;130:110629. https://doi.org/10.1016/j.biopha.2020.110629.

21 FDA. *Antibody testing is not currently recommended to assess immunity after COVID-19 vaccination: FDA safety communication*. https://www.fda.gov/medical-devices/safety-communications/antibody-testing-not-currently-recommended-assess-immunity-after-covid-19-vaccination-fda-safety. Published May 19, 2021.

22 Bhaskaran K, Bacon S, Evans SJ, et al. Factors associated with deaths due to COVID-19 versus other causes: population-based cohort analysis of UK primary care data and linked national death registrations within the OpenSAFELY platform. *Lancet Reg Health Eur*. 2021;6:100109. https://doi.org/10.1016/j.lanepe.2021.100109.

23 Lanini S, Montaldo C, Nicastri E, et al. COVID-19 disease-temporal analyses of complete blood count parameters over course of illness, and relationship to patient demographics and management outcomes in survivors and non-survivors: A longitudinal descriptive cohort study. *PLoS One*. 2020;15(12):e0244129. https://doi.org/10.1371/journal.pone.0244129.

24 Zeng ZY, Feng SD, Chen GP, et al. Predictive value of the neutrophil to lymphocyte ratio for disease deterioration and serious adverse outcomes in patients with COVID-19: a prospective cohort study. *BMC Infect Dis*. 2021;21:80. https://doi.org/10.1186/s12879-021-05796-3.

25 Moutchia J, Pokharel P, Kerri A, et al. Clinical laboratory parameters associated with severe or critical novel coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis. *PLoS One*. 2020;15(10);e0239802. https://doi.org/10.1371/journal.pone.0239802.

26 Roy S, Samajdar SS, Tripathi SK, Mukherjee S, Bhattacharjee K. Outcome of different therapeutic interventions in mild COVID19 infection. *J Clin Immunol Microbiol*. 2021;2(2):1–10. http://doi.org/10.46889/JCIM.2021.2203.

27 Yang J, Zheng Y, Gou X, et al. Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: a systematic review and meta-analysis. *Int J Infect Dis*. 2020;94:91–95. https://doi.org/10.1016/j.ijid.2020.03.017.

28 Cioni G. The role of angiotensin-converting-enzyme 2 in the age- and sex related poor prognosis of COVID-19. A comment on recent findings on novel coronavirus infection by SARS-CoV-2. *Pol Ann Med*. 2020;27(1):85–87. https://doi.org/10.29089/2020.20.00100.