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Evolution of neutralizing antibodies and cross-activity against different variants of SARS-CoV-2 in patients recovering from COVID-19

Wang-Da Liu a,b, Jann-Tay Wang a,c,*,1, Tai-Ling Chao d, Si-Man Ieong d, Ya-Min Tsai d, Po-Hsien Kuo e, Ming-Jui Tsai f, Yi-Jie Chen a, Guei-Chi Li a, Shu-Yuan Ho g, Hui-Hou Chen g, Yu-Shan Huang a, Chien-Ching Hung a,f,h, Yee-Chun Chen a,i, Sui-Yuan Chang d,g,**,1, Shan-Chwen Chang a,j

Original Article

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a Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan
b Department of Medicine, National Taiwan University Cancer Center, Taipei, Taiwan
c Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Zhunan, Taiwan
d Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University College of Medicine, Taipei, Taiwan
e Department of Internal Medicine, National Taiwan University Hospital Biomedical Park Hospital, Hsinchu, Taiwan
f Department of Internal Medicine, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin County, Taiwan
g Department of Laboratory Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan
h Department of Tropical Medicine and Parasitology, National Taiwan University College of Medicine, Taipei, Taiwan
i Center of Infection Control, National Taiwan University Hospital, Taipei, Taiwan
j School of Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

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* Corresponding author. Department of Internal Medicine, National Taiwan University Hospital, 7 Chung-Shan South Rd., Taipei City 10002, Taiwan.
** Corresponding author. Department of Laboratory Medicine, National Taiwan University Hospital, 7 Chung-Shan South Rd., Taipei City 10002, Taiwan.

E-mail addresses: b95401043@ntu.edu.tw (W.-D. Liu), wang jt1968@gmail.com (J.-T. Wang), d01424001@ntu.edu.tw (T.-L. Chao), ccccman86@gmail.com (S.-M. Ieong), kssn3981@gmail.com (Y.-M. Tsai), kphsien@gmail.com (P.-H. Kuo), flophoenix@gmail.com (M.-J. Tsai), jie7521@gmail.com (Y.-J. Chen), ligg2020n@gmail.com (G.-C. Li), 030144@ntu.gov.tw (S.-Y. Ho), 030182@ntu.gov.tw (H.-H. Chen), b101091021@gmail.com (Y.-S. Huang), hcc0401@ntu.edu.tw (C.-C. Hung), yeechunchen@gmail.com (Y.-C. Chen), sychang@ntu.edu.tw (S.-Y. Chang), changsc@ntu.edu.tw (S.-C. Chang).
1 Wang JT and Chang SY contributed equally to this work.

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Background: Patients recovering from COVID-19 may need vaccination against SARS-CoV-2 because acquired immunity from primary infection may wane, given the emergence of new SARS-CoV-2 variants. Understanding the trends of anti-spike IgG and neutralizing antibody titers in patients recovering from COVID-19 may inform the decision made on the appropriate interval between recovery and vaccination.

Methods: Participants aged 20 years or older and diagnosed with COVID-19 between January and December, 2020 were enrolled. Serum specimens were collected every three months from 10 days to 12 months after the onset of symptom for determinations of anti-spike IgG and neutralizing antibody titers against SARS-CoV-2 Wuhan strain with D614G mutation, alpha, gamma and delta variants.

Results: Of 19 participants, we found a decreasing trend of geometric mean titers of anti-spike IgG from 560.9 to 217 and 92 BAU/mL after a 4-month and a 7-month follow-up, respectively. The anti-spike IgG titers declined more quickly in the ten participants with severe or critical disease than the nine participants with only mild to moderate disease between one month and seven months after SARS-CoV-2 infection (−8.49 vs -2.34-fold, p < 0.001). The neutralizing activity of the convalescent serum specimens collected from participants recovering from wild-type SARS-CoV-2 infection against different variants was lower, especially against the delta variants (p < 0.01 for each variant with Wuhan strain as reference).

Conclusion: Acquired immunity from primary infection with SARS-CoV-2 waned within 4–7 months in COVID-19 patients, and neutralizing cross-activities against different SARS-CoV-2 variants were lower compared with those against wild-type strain.

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has become pandemic and caused a high number of morbidities and mortalities since 2020. The occurrences of SARS-CoV-2 reinfection following primary infection in this pandemic have raised public health concerns.\(^2\)–\(^4\)

A longitudinal study for antibody kinetics after primary SARS-CoV-2 infection showed that neutralizing antibodies could be detected even 300 days after primary infection with only a slight decrease.\(^2\) Another study also revealed that the protection effectiveness against reinfection with SARS-CoV-2 was 85% or greater, which could last for seven months.\(^6\) However, breakthrough infections occurred in individuals with waning immunity that was acquired from either infection or vaccination; moreover, reinfections with different SARS-CoV-2 variants were also reported.\(^7\)

Until now, vaccination of patients recovering from SARS-CoV-2 infection has been recommended. However, the appropriate timing of vaccination for these populations remains debating.\(^8\) Given the emergence of new SARS-CoV-2 variants, a shorter interval between recovery and vaccination might be necessary. In this study, we aimed to evaluate the trends of anti-spike protein IgG and neutralizing antibody levels of patients who had recovered from coronavirus disease 2019 (COVID-19) and to assess the cross-neutralizing activities of the convalescent sera against different variants of SARS-CoV-2.
NTUH (NTUH 202002002RIND) and written informed consent was obtained from the participants.

Neutralization assays

Plaque reduction neutralization test (PRNT) was performed on the sequentially collected serum specimens to determine the neutralizing antibody titers against SARS-CoV-2. The serum specimens used in these assays were heat-inactivated at 56°C for 30 min, and then 2-fold serially diluted in serum-free DMEM media, from 1:80 to 1:1280. PRNT was performed in triplicate in 24-well tissue culture plates. The clinical isolates of SARS-CoV-2 used in the assay included SARS-CoV-2/NTU03/TWN/human/2020 (EPI_ISL 413592), which exhibits the D614G mutation, SARS-CoV-2/NTU49/TWN/human/2020 (EPI_ISL 1010728) as alpha variant, SARS-CoV-2/CGU56/TWN/human/2021 (EPI_ISL 2249615) as gamma variant, and SARS-CoV-2/NTU92/TWN/human/2021 (EPI_ISL 3979387) as delta variant. SARS-CoV-2 (50–100 plaque-forming units, pfu) was incubated with diluted test sera for 1 h at 37°C before adding to the Vero E6 cell monolayer for another 1 h. Subsequently, virus-serum mixtures were removed and the cell monolayer was washed once with phosphate buffered saline before covering with DMEM media containing 2% fetal bovine serum (FBS) and 1% methylcellulose for 5–7 days. The cells were fixed with 10% formaldehyde overnight. After removal of overlay media, the cells were stained with 0.7% crystal violet and the plaques were counted.

To facilitate conversion of 50% PRNT (PRNT50) to International Unit (IU/mL) the WHO international standard reference panel (20/268 [including reference samples 20/150, 20/148, 20/144, and 20/140]) from the National Institute for Biological Standards and Control (NIBSC; Potter’s Bar, UK) was used to generate an equation for converting PRNT50 to IU/mL: $y = (x-9.3313)/1.453$, where $y$ is the value of IU/mL and $x$ is the value of the PRNT50). In this study, a PRNT50 titer lower than 80 will be labeled as 40 (21.1 IU/mL) for further statistical analysis.

Detection of IgG against SARS-CoV-2 spike protein

SARS-CoV-2 spike (S) protein-specific IgG in serum samples was determined using SARS-CoV-2 IgG II Quant assay (Abbott, Abbott Park, Illinois, U.S.A.) according to the manufacturer’s instructions. An IgG level higher than 50 AU/mL (7.1 BAU/mL) was considered positive.

Statistical analysis

The geometric mean titers (GMTs) of SARS-CoV-2 anti-spike IgG and neutralizing antibodies were calculated in log-transformed data for statistics. The GMTs of IgG against SARS-CoV-2 spike protein between participants of different severity categories were analyzed using Student’s t-test. Neutralizing antibody titers against different variants were analyzed using paired t-tests, with the titer against Wuhan strain as reference. Pearson’s product–moment correlation was performed to evaluate the relationship between anti-spike IgG and neutralizing titer. A two-tailed P value less than 0.05 was considered statistically significant. All analyses were performed using Stata/SE software, Version 11.0 (https://www.stata.com).

Results

A total of 19 participants were enrolled, including nine with mild to moderate disease (Group 1), five with severe disease (Group 2) and five with critical disease who developed respiratory failure that led to intubation and mechanical ventilator support (Group 3). The demographic and clinical features of the participants are shown in Table 1. Participants with severe or critical disease tended to be older and have more comorbidities. In Group 1, one (11.1%) participant received treatment with lopinavir/ritonavir while the other eight (88.9%) participants received hydroxychloroquine. All of the five participants of Group 2 received a 5-day course of remdesivir. For participants with critical disease, four (80%) received high-dose corticosteroids while two (40%) received tocilizumab. None of the participants died of COVID-19, and there were no cases of reinfection during the observation period. Of the 19 participants, full-length virus genome sequences were determined for 12 participants using the virus isolated from their respiratory specimens. The viral sequences were deposited in the GISAID database (Supplementary Table 1). The spike sequences of these strains were very similar to those of the original SARS-CoV-2 strain, with seven having additional D614G mutation and one S254F mutation and none having deletions at V69, H70, and Y144.

The GMT of anti-spike IgG of the participants in Groups 1 and 2 reached the peak one month after the onset of symptoms, with the level of 125.9 BAU/mL (95% confidence interval [CI], 73.8–214.8) and 869.5 (95% CI, 472.9–1598.6), respectively, while the antibody level reached the peak (1032 BAU/mL) during the first 10 days after symptoms onset of Group 3 (95% confidence interval [CI], 18.4–57971.9). Waning of anti-spike IgG titers from all participants was noted, with GMT decreasing from 560.9 to 217 and 92 BAU/mL after a 4-month and a 7-month follow-up, respectively. Of note, the anti-spike IgG titers declined more quickly in the participants with severe or critical disease than those with only mild to moderate disease between one month and seven months after SARS-CoV-2 infection ($-8.49 \times -2.34$-fold, $p < 0.001$) (Fig. 1).

The GMT of neutralizing antibodies against SARS-CoV-2 with D614G mutation showed a similar trend, which decreased from 382.8 (95% CI, 199.6–734.3) at the first month to 80.9 (95% CI, 32.7–200.1) IU/mL at the seventh month after symptom onset. The neutralizing activity against different variants of the serum specimens, especially delta variants, obtained from participants recovering from wild-type SARS-CoV-2 infection regardless of the presence of D614G mutation was lower than that against wild-type virus. The GMTs of neutralizing antibodies against alpha, gamma and delta variants of the serum specimens collected at the first month after primary infection were much lower than that against wild-type virus ($p < 0.01$ for each variant) (Table 2).
Throughout the follow-up period, the neutralizing activity against delta variant was lowest among the four types of SARS-CoV-2 tested in this study of participants of three different severity categories (Fig. 2). Of all samples with a neutralizing antibody titer higher than 48.63 IU/mL, which was the lower limit of detection of neutralizing antibody titer in our study, only one was tested negative for anti-spike IgG. Pearson’s product–moment
correlation analysis revealed a statistically significantly positive correlation between the quantitative levels of anti-spike IgG and the titers of neutralizing antibodies against the virus strain with D614G mutation ($r = 0.78$, $p < 0.0001$). Such correlations remained even though the neutralizing activities against other SARS-CoV-2 variants remained lower.

| Disease severity | Wuhan | Alpha | Gamma | Delta |
|------------------|-------|-------|-------|-------|
| Severe (N = 20)  | 2.47  | Ref 2.1 | p = 0.01 (0.12–0.79) | 2.17 | p = 0.002 (0.15–0.6) | 2.01 | p < 0.001 (0.32–0.71) |
| Moderate (N = 22) | 2.23  | Ref 2.02 | p = 0.01 (0.06–0.43) | 1.73 | $p < 0.001$ (0.25–0.67) | 1.7 | $p < 0.001$ (0.36–0.74) |
| Mild (N = 33)     | 1.7   | Ref 1.52 | $p = 0.008$ (0.04–0.25) | 1.45 | $p = 0.01$ (0.06–0.36) | 1.48 | $p = 0.007$ (0.05–0.29) |

Note. Neutralization titer was recorded in log IU/mL.

Table 2 Neutralizing activity of different SARS-CoV-2 variants to convalescent serum collected from patients recovered from Wuhan strain.

Figure 1 Evolution of anti-spike IgG of patients with different severity.
Discussion

In this longitudinal follow-up study of the anti-spike IgG and neutralizing antibody titers in patients recovering from COVID-19, we found that both titers waned over time and the neutralizing activities against different SARS-CoV-2 variants were much lower than those against the original strain causing the first wave of SARS-CoV-2 infection.

Until now, our understanding of the durability of antibodies against SARS-CoV-2 acquired from primary infection with SARS-CoV-2 remains relatively limited. Our study demonstrated that sustained antibody responses could be detected more than six months after primary infection, which is in line with the findings of other studies. \(^{10,11}\) We also found that a stronger antibody response was associated with a more severe disease status, which echoed the study by den Hartog et al. \(^{12}\) However, those with more severe disease had a more rapid decline of antibody titers, as previous studies have described. \(^{13}\) An increasing age was shown to be associated with a stronger neutralizing antibody response. \(^{14,15}\) The patients with milder disease in our study was much younger than the patients enrolled in the study by den Hartog et al., which might explain this difference. Moreover, the patients with severe or critical disease in our cohort tended to have more comorbidities such as malignancies, which might interfere with the antibody response.

Our study revealed that serum specimens obtained from patients who recovered from infection with wild-type strain showed lower neutralizing activities against different variants, especially against the delta variant. Previous studies have demonstrated that the delta variant was resistant to neutralization by certain monoclonal antibodies, including bamlanivimab, and the convalescent serum specimens obtained from affected individuals were fourfold less potent against the delta variant than against the alpha variant. \(^{16}\) A recent study has shown that the level of neutralizing activity from either vaccination or convalescent cohorts was predictive of seroprotection against severe COVID-19, and a sustained protective effect might persist despite the decrease of neutralizing activity. \(^{17}\) Rosati et al. demonstrated that SARS-CoV-2 antibodies induced by wild-type strain potently recognized alpha-spike-receptor-binding-domain (RBD) but only showed slightly lower affinity to delta-spike-RBD in pseudovirus tests. \(^{18}\) In addition, Betton et al. demonstrated that the neutralizing activity of serum specimens from patients recovering from infection with wild-type strain lasted at least for six months, regardless of a decreased IgG level, which could even confer a cross-protection against the D614G, alpha, beta, and gamma variants. \(^{19}\)

In the real-world setting, previous SARS-CoV-2 infection within 13 months was shown to be an independent protective factor against reinfection of delta strain, and patients with reinfection tended to have milder disease. \(^{20-22}\) However, even though patients infected with wild-type SARS-CoV-2 had more sustained and higher neutralizing antibody titers than those in vaccinated people, neutralizing antibodies from convalescent sera of patients infected with wild type, alpha, beta or delta variant had low cross-reactivity to omicron variants. \(^{23-25}\) Therefore, given the high transmissibility of the circulating delta/omicron variants in the community,
there should be no delay in vaccination among patients recovering from COVID-19 since a waning immunity has been observed. In a retrospective cohort study from Israel, in which 149,032 patients recovering from SARS-CoV-2 infection were followed for 270 days, the reinfection rate among unvaccinated patients were significantly higher than those with vaccination after primary infection.26 Moreover, Hall et al. also demonstrated that a booster vaccination for previously infected individuals provided a sustained protection, which provides supportive evidence for the recommendation of a booster dose of SARS-CoV-2 vaccination.27

Our study demonstrated a strong correlation between anti-spike IgG level and neutralizing activity. However, the correlation between anti-spike IgG level and neutralizing activity with the real-world protection remains debating. A predictive model by Khoury DS et al. showed 20% of convalescent serum specimens (54 IU/mL) can approximate 50% vaccine efficacy.17 In our study, the mean neutralizing activity of the serum specimens obtained 12 months after primary infection fell below this standard. However, the mean neutralizing activity against other variants such as the alpha or delta strain showed a faster waning in those obtained at month seven or even earlier. Another method to predict clinical efficacy is the BAU conversion model by Feng et al., which suggested that anti-spike IgG titers higher than 264 and 899 BAU/mL were correlated with 80% and 90% vaccine efficacy against the alpha variants, respectively.28 However, in our cohort, the serum specimens obtained seven months after primary infection also failed to meet this standard. Hence, a shorter interval between recovery from COVID-19 and a booster vaccination should be considered.

This is the first study that demonstrates the longitudinal follow-up of anti-spike IgG among patients with different severity in the early stage of the pandemics in Taiwan, which is previously less discussed. However, there are still several limitations in our study. First, the case number in this study was small and some participants missed blood sampling, which might dampen the power of statistical analyses. Secondary, not all the virus strains from the participants were available though there were no documented cases of infection with alpha strain in Taiwan before April, 2021. Therefore, there was a strong epidemic evidence of wild-type strain infection in our cohort even though the presence of D614G mutation was not completely clarified. Nevertheless, a recent study by Weissman et al. reported that even though D614G mutation increased SARS-CoV-2 transmission efficiency, such mutation increased the susceptibility of the virus strain to neutralization by receptor-binding domain (RBD) monoclonal antibodies and convalescent sera.29 Third, there were no domestic cases of reinfection in Taiwan during the study period, which might preclude us from evaluating the association between

Figure 3  Correlation between anti-spike IgG titer and neutralizing activity against different SARS-CoV-2 variants. (A. Wuhan strain with D614 mutation, r = 0.78, p < 0.001; B. alpha variant, r = 0.64, p < 0.001; C. gamma variant, r = 0.58, p < 0.001; D. delta variant, r = 0.67, p < 0.001).
waning antibody titers and protection. Last, we used the cut-off level of 21 IU/mL as the lower limit of detection for neutralizing activity in the estimation of Pearson’s correlation model for all neutralizing titers below 42 IU/mL, which might interfere the identification of correlation between IgG and neutralizing activity. Nevertheless, a strong correlation was still observed as previous study.\(^{30}\)

**Conclusions**

Our study found waning antibody titers within 4–7 months after primary infection with SARS-CoV-2. Moreover, the neutralizing cross-activities against different SARS-CoV-2 variants were lower compared with that against wild-type strain virus. Our findings imply that vaccination of individuals who have recovered from COVID-19 should be considered in the ongoing pandemic with emergence of new SARS-CoV-2 variants. An interval with no longer than seven months could be considered, though the optimal timing for vaccination warrants further evaluations.

**Declaration of competing interest**

None to declare.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jjfma.2022.11.015.

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