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BRIEF REPORT

Duration of Humoral Immunity and Cross-Neutralizing Activity Against the Alpha, Beta, and Delta Variants After Wild-Type Severe Acute Respiratory Syndrome Coronavirus 2 Infection: A Prospective Cohort Study

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A prospective cohort study was conducted for adults with a diagnosis of with coronavirus disease 2019 (COVID-19). Convalescent blood samples were obtained 4, 6, and 11 months after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The seropositivity of anti-spike antibody was maintained in all patients (100%) until 11 months after COVID-19 diagnosis. Neutralizing antibody levels against wild-type SARS-CoV-2 gradually decreased but remained positive in >50% of patients 11 months after diagnosis: in 98.5% (67 of 68) at 4 months, 86.8% (46 of 53) at 6 months, and 58.8% (40 of 68) at 11 months. However, cross-neutralizing activity against the Beta and Delta variants was attenuated 2.53-fold and 2.93-fold, respectively, compared with the wild-type strain.

Keywords. antibody; COVID-19; neutralizing activity; SARS-CoV-2; variants of concern.

The longevity of antibody (Ab) responses elicited by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is an important issue concerning public health. It may contribute to the assessment of reinfection risk, measurement of herd immunity, and establishment of vaccination strategies. As the coronavirus disease 2019 (COVID-19) pandemic continues for a long time, notable SARS-CoV-2 variants harboring multiple substitutions in the spike (S) protein have emerged in the United Kingdom (B.1.1.7; Alpha), South Africa (B.1.351; Beta), Brazil (P.1; Gamma), and India (B.1.617.2; Delta) [1]. Thus, it is necessary to compare the duration and cross-neutralizing activity of Abs produced after natural SARS-CoV-2 infection or vaccination. Previously, our group investigated the anti-SARS-CoV-2 nucleocapsid (N) Ab and neutralizing Ab (NAb) against wild-type SARS-CoV-2 until 6 months after infection [2]. In the current study, we aimed to (1) assess the kinetics of anti-S, anti-N, and NAb up to 11 months after infection, (2) compare Ab responses according to disease severity, and (3) evaluate NAb against variants of concern, using convalescent serum samples from patients during the first wave of the pandemic.

METHODS

A prospective cohort of adult patients diagnosed with COVID-19 was designed. In the enrolled patients, COVID-19 was confirmed by reverse-transcription polymerase chain reaction. In addition, 4 PCR-negative persons involved in a COVID-19 outbreak were included in the cohort because they demonstrated seropositivity 4 months after exposure, by the neutralization test. Immunocompromised patients were excluded. The severity of COVID-19 was categorized into asymptomatic, symptomatic/nonpneumonic, and pneumonic groups, according to clinical manifestations. Convalescent blood samples were obtained 4, 6, and 11 months after SARS-CoV-2 infection. This study was approved by the Institutional Review Board of the Korea University Guro Hospital (no. 2020GR0130). All participants provided written informed consent.

Anti-S and anti-N Abs were measured using the Elecsys Anti-SARS-CoV-2 S assay (Roche) and the SARS-CoV-2 IgG assay (Abbott Laboratories), respectively, according to the manufacturers' protocols. The maximum detection limit of the anti-SARS-CoV-2 S assay was 2500 U/mL; therefore, titers > 2500 U/mL were regarded as 2500 U/mL.

A plaque reduction neutralization test was performed using wild-type SARS-CoV-2 (hCoV/Korea/KCDC03/2020), B.1.1.7 lineage (hCoV-19/Korea/KDCA51463/2021), B.1.351 lineage (hCoV-19/Korea/KDCA55905/2021), and B.1.617.2 lineage (hCoV-19/Korea119861/KDCA/2021) to measure NAbs against each strain. The mixture of serum dilution/virus (40 plaque-forming units per well) was incubated at 37°C for 2 hours and then added to the plate seeded with Vero E6 cells and incubated at 37°C for 1 hour, followed by the addition of 0.5% agarose (Lonza). After 2–3 days of incubation, the cells were fixed with 4% paraformaldehyde and stained to visualize the plaques. A 50% reduction in plaque count was calculated for the median neutralizing titer (ND50) using the Spearman-Karber formula, and ND50 ≥1:20 was considered positive.

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Statistical analyses were performed with SPSS software for Windows (version 20.0; IBM). The geometric mean titer (GMT) and 95% confidence interval (CI) were calculated for each group. Ab titers were compared at each time point among case patients, with serial results at the 3 time points, using the Friedman test. The Wilcoxon signed rank test was used to analyze differences in Ab titers between 2 time points and to compare NAb titers against wild-type and variant viruses. Kruskal-Wallis test and Mann-Whitney *U* tests were used to compare the 3 groups and 2 disease severity groups, respectively. Statistical significance was set at $P < .05$. Graphs were generated using GraphPad Prism software, version 9.1.2 (GraphPad Software).

RESULTS

A total of 191 convalescent serum samples were collected from 68 patients (mean age, 49.4 years; range 28–69 years) who had COVID-19 diagnosed from February to April 2020; of the 68 patients, 8 (11.8%) were asymptomatic, 29 (42.6%) had a nonpneumonic illness, and 31 (45.6%) had pneumonia. The timing of convalescent serum samples after COVID-19 diagnosis was as follows: for the time point of 4 months, the median duration after diagnosis (interquartile range) was 113 (107.5–120.75) days; for the 6-month time point, the median was 184 (183–185) days; and for the 11-month time point, the median was 325 (323–327) days.

The seropositivity rates of anti-S Ab were maintained in all patients until 11 months after COVID-19 diagnosis: in 100% at 4 months (68 of 68), 6 months (55 of 55), and 11 months (68 of 68). Positive rates for anti-N Ab gradually declined after infection: 79.4% (54 of 68) at 4, 65.5% (36 of 55) at 6, and 20.6% (14 of 68) at 11 months. NAb against the wild-type SARS-CoV-2 gradually decreased but remained positive in >50% until 11 months after diagnosis: in 98.5% (67 of 68) at 4, 86.8% (46 of 53) at 6, and 58.8% (40 of 68) at 11 months. Ab titers at each time point were significantly different ($P = .04$ for anti-S Ab; $P < .001$ for both anti-N Ab and NAb. The anti-N Ab and NAb levels declined as time elapsed after infection; the GMT for anti-N Ab was 3.56 (95% CI, 3.11–4.09) at 4 months, 1.93 (1.57–2.38) at 6 months, and 0.62 (.48–.81) at 11 months; for ND50, the values were 239.73 (190.34–301.95) at 4, 114.13 (78.84–165.22) at 6, and 35.47 (24.16–52.07) at 11 months. However, anti-S Ab titers did not decrease at 6 (199.41 [95% CI, 139.08–285.91]) and 11 (193.84 [131.75–285.19]) months after infection, compared with titers at 4 months (166.40 [121.59–227.71]) (Figure 1A–1C).

Ab titers were higher in proportion to disease severity at 11 months after infection (Supplementary Figure 1A–1C). The pneumonic group showed higher GMTs than the nonpneumonic group for anti-S Ab (254.69 vs 83.61, respectively; $P = .005$), anti-N Ab (0.77 vs 0.26, $P < .001$), and NAb (47.08 vs 20.40; $P = .02$). In addition, anti-S and -N Ab titers at 4 and 6 months after SARS-CoV-2 infection were higher in the pneumonic than in the nonpneumonic group (Supplementary Table 1).

Positive rates for NAb at 11 months after SARS-CoV-2 infection were 58.8% (40 of 68) against the wild-type virus, 72.1% (49 of 68) against the Alpha variant, and 26.5% (18 of 68) against the Delta variant. The neutralizing activities of convalescent serum were significantly diminished against the Delta variant (10.80 [95% CI, 8.11–14.37]) compared with the wild-type virus (31.63 [23.02–43.47]) and the Alpha variant (37.07 [29.07–47.27]) ($P < .001$). Titers of NAb against the wild-type virus and the Alpha variant did not differ significantly

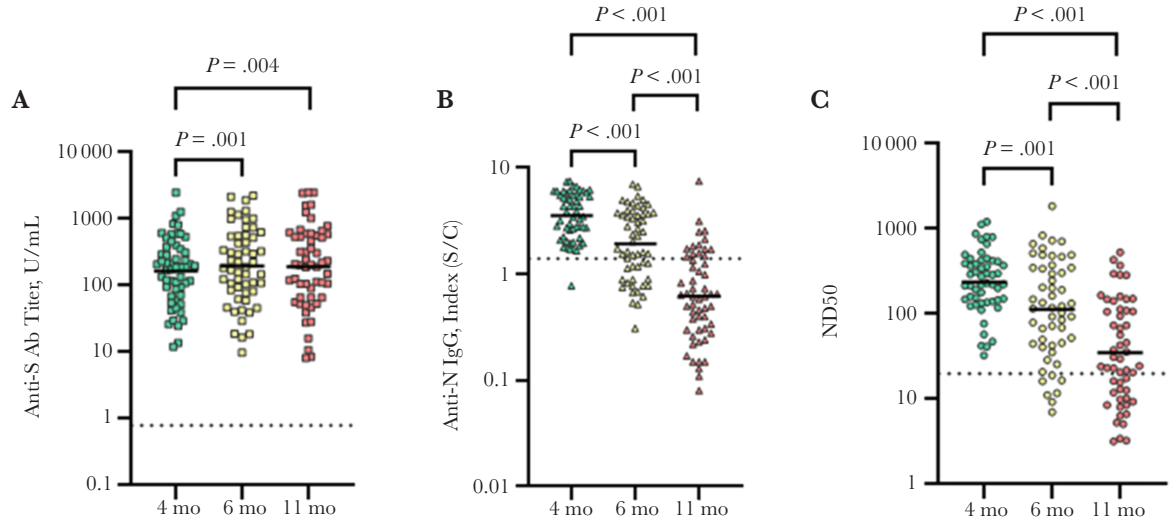

Figure 1. Anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) antibody (Ab) (*A*), anti-SARS-CoV-2 nucleocapsid (N) immunoglobulin G (IgG) (*B*), and the median neutralizing titer (ND50) for neutralizing antibody (*C*) 4, 6, and 11 months after coronavirus disease 2019 diagnosis. Solid lines represent geometric mean titer; dotted lines, cutoff values. Abbreviation: S/C, signal-to-cutoff ratio.

($P = .77$). Furthermore, 36 serum samples with ND50 ≥20 to the wild-type virus were evaluated for their neutralizing activities against the Beta variant, and they showed weakened cross-neutralization ($P < .001$) (Figure 2A and 2B).

DISCUSSION

In the current study, we found seropositivity rates of 100% for anti-S Ab, 20.6% for anti-N Ab, and 58.8% for NAb 11 months after SARS-CoV-2 infection. The pneumonic group showed higher levels of Abs than the nonpneumonic and asymptomatic groups at 4, 6, and 11 months. Similar to our study findings, the longer persistence of anti-S Ab after SARS-CoV-2 infection has been reported [3–5]. Nabs were detected in 76% of patients 8 months after COVID-19 diagnosis [4]. Although NAbs persist in a significant number of patients up to 12 months after SARS-CoV-2 infection, it is unclear whether this indicates protective immunity against reinfection. Immune correlates of protection of SARS-CoV-2 have not yet been established. It is necessary to evaluate the duration of immunity and the level of herd immunity based on immune correlates of protection .

Emerging SARS-CoV-2 variants and their global expansion have been a serious threat to public health. Variants of concern are associated with increased transmissibility, enhanced disease severity, and considerable reduction in NAb activity elicited by previous infection or vaccination [1]. The Alpha variant, harboring N501Y and P681H substitutions and H69/V70 deletion in the S protein, showed increased transmissibility. The Beta variant contains K417N, E484K, and N501Y mutations, and the Delta variant has L452R and P681R substitutions in the S protein [6]. Convalescent serum samples from wild-type SARS-CoV-2–infected patients showed no loss of neutralizing activity against the Alpha variant but a 9.4-fold loss against the Beta variant [7].

Compared with the early strain, neutralizing activity for the Delta variant was reduced 2.7-fold in serum samples from patients during the first wave of COVID-19 [8]. Consistent with previous studies, neutralizing activities against the Beta (2.53-fold) and Delta (2.93-fold) variants were also significantly attenuated in the current study. The relatively smaller fold reduction might be due to the expansion of NAb breadth with B-cell maturation over time. It is necessary to evaluate the cross-neutralizing activity according to the time since vaccination or recovery from wild-type SARS-CoV-2 infection. In addition, the fold decrease of NAb titer against the Beta variant was smaller than expected compared with the Delta variant. As for the Beta variant, only patients whose ND50 was maintained at ≥20 by 11 months were selected (n = 36) to evaluate cross-neutralizing activity; memory B cells thus might be better matured, thereby producing Abs with higher affinity and diversity.

The current study has a limitation. Genotypic analysis of SARS-CoV-2 was not performed. However, it was presumed that the patients were infected with the original Wuhan strain, considering the time of diagnosis (February to April 2020). The first variant of concern, the Alpha variant, was reported to emerge in the United Kingdom in September 2020 [1].

In conclusion, anti-SARS-CoV-2 NAbs were durable up to 11 months after diagnosis but showed diminished cross-neutralizing activity against the Beta and Delta variants. In vaccine recipients and patients recovered from COVID-19, the vaccination strategy (optimal timing of booster/revaccination) should be carefully established, considering the reduction of

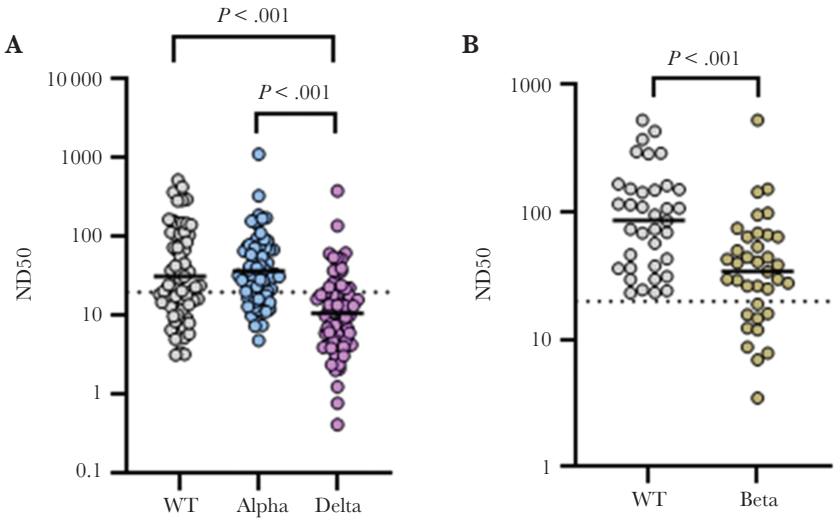

Figure 2. *A,* Neutralizing antibody titers of serum samples 11 months after infection against wild-type (WT) severe acute respiratory syndrome coronavirus (SARS-CoV-2), the Alpha variant, and the Delta variant. *B,* Neutralizing antibody titers of serum samples 11 months after infection against the WT strain and the Beta variant; samples with median neutralizing titer (ND50) ≥22.0 to the WT virus were included in this comparison. Solid lines represent geometric mean titers; dotted lines, cutoff values.

protective immunity over time and cross-neutralizing activity against variant viruses.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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