

Longitudinal assessment of anti-SARS-CoV-2 immune responses for six months based on the clinical severity of COVID-19

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Summary: Anti-SARS-CoV-2 neutralizing antibodies persisted in more than 85% of patients with COVID-19 until six months after diagnosis. Older age, prolonged viral shedding and accompanying pneumonia were strongly associated with sustained antibody response in patients with COVID-19.

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Abstract

There is insufficient data on the longevity of immunity acquired following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We aimed to evaluate the duration of SARS-CoV-2-specific humoral and cellular immunity according to the clinical severity of coronavirus disease 2019 (COVID-19). The study population comprised asymptomatic (n=14), symptomatic/non-pneumonic (n=42), and pneumonic (n=41) patients. The anti-SARS-CoV-2 IgG and neutralizing antibody (NAb) titers lasted until six months after diagnosis, with positivity rates of 66.7% and 86.9%, respectively. Older age, prolonged viral shedding and accompanying pneumonia were more frequently found in patients with sustained humoral immunity. SARS-CoV-2 specific T-cell response was strongly observed in pneumonic patients and prominent in individuals with sustained humoral immunity. In conclusion, most (> 85%) patients carries NAb until six months after diagnosis of SARS-CoV-2 infection, providing insights for establishing vaccination strategies against COVID-19.

Keywords: SARS-CoV-2, COVID-19, longevity, humoral immunity, cellular immunity

Since December 2019, more than 71 million people, globally, have been diagnosed with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, resulting in 1,608,648 deaths as of December 15, 2020 [1]. A perspective that the coronavirus disease 2019 (COVID-19) pandemic wane with the acquisition of herd immunity, supports the formulation of strategies aimed at gradually increasing the degree of herd immunity through the maintenance of social activities and improving the level of protection for high-risk groups. In fact, in some European countries such as Sweden, England, and the Netherlands, this type of a strategy was implemented during the early stages of the COVID-19 pandemic. Considering the basic reproduction number (2.5) of SARS-CoV-2 infection, more than 60% of the population should have immunity against SARS-CoV-2 for pandemic suppression [2]. However, information on the longevity of the immunity acquired after SARS-CoV-2 infection is very limited.

The titers of antibodies against SARS-CoV-2 are known to peak approximately 2-6 weeks after infection [3-6]. The longevity of neutralizing antibodies (NAbs) against SARS-CoV-2 was reported as 84.4% at > 90 days after symptom onset in COVID-19 patients [6]. Another study showed that the levels of anti-SARS-CoV-2 antibodies rapidly declined from 37 days to 86 days after symptom onset with a half-life of 36 days in mild patients [7]. According to the longevity of the humoral immunity acquired after SARS-CoV-2 infection, the long-term patterns of the COVID-19 pandemic may differ, and vaccination strategies should be altered accordingly. Therefore, longitudinal dynamics of immunity level following SARS-CoV-2 infection should be investigated.

Recently, SARS-CoV-2 reinfection case has been reported at an interval shorter than five months from the first infection [8]. Thus, there is a need for studies focusing on the characteristics of early decay groups in which sufficient antibody levels are not maintained after SARS-CoV-2 infection. In addition, it is important to investigate whether anti-SARS-

CoV-2 humoral immunity is correlated with T-cell immunity among patients who have recovered from COVID-19 because T-cell immunity can contribute to alleviation of the severity of viral infection.

In this study, we primarily aimed to assess the longevity of the acquired-immunity, according to the clinical severity of SARS-CoV-2 infection, and to evaluate the risk factors of poor anti-SARS-CoV-2 humoral immune response. Additionally, we assessed SARS-CoV-2-specific T-cell immunity in patients across diverse disease severities and their close contacts.

METHODS

Study population

A prospective cohort was constructed to investigate the longitudinal immune response of adult patients with COVID-19. COVID-19 was confirmed using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Convalescent blood was collected prospectively, and the sampling time was divided into period 1, within 8 weeks after diagnosis; period 2, 9-20 weeks after diagnosis; and period 3, 22-27 weeks after diagnosis.

Data on comorbidities, body mass index (BMI), symptoms related to COVID-19, presence of pneumonia, and oxygenation during the treatment of SARS-CoV-2 infection were investigated. The cycle threshold (Ct) value of qRT-PCR for SARS-CoV-2 at diagnosis was collected retrospectively. The severity of COVID-19 was categorized into asymptomatic, symptomatic/non-pneumonic, and pneumonic groups according to the clinical manifestations. Obesity was defined as a BMI ≥ 25.0 kg/m², according to the recommendation of the World Health Organization for the Asia-Pacific region [9]. The isolation duration was adopted as the viral shedding duration. The criteria for COVID-19 patients' release from isolation included two consecutive negative results of SARS-CoV-2 qRT-PCR at least 24 hours apart during

early periods of the pandemic [10]. On June 25, 2020, the criteria for the release from isolation were revised. Since then, negative SARS-CoV-2 qRT-PCR results are not required for release from isolation if more than 10 days have elapsed since the diagnosis of COVID-19, with symptomatic improvement [10]. All the subjects in this study were discharged before the revision of isolation release criteria.

People in close contact (household members and colleagues working in the same closed space for more than eight hours per day) with the patients with SARS-CoV-2 infection, who had negative SARS-CoV-2 PCR results, were recruited for comparison. Workers in a call center in which the COVID-19 outbreak occurred were included in the present study [11]. Although these people were PCR-negative after close contact with the patients with COVID-19, serological tests were performed to identify PCR-negative SARS-CoV-2 infections. Of them, three people showed seropositivity in the SARS-CoV-2 immunoglobulin class G (IgG) assays as well as neutralization tests, performed using sera obtained 16 weeks after the exposure to patients with SARS-CoV-2 infection. These persons were classified as asymptomatic patients.

The study protocol was approved by the Institutional Review Board of the Korea University Guro Hospital (approval number: 2020GR0130). All participants provided written informed consent.

Measurements of humoral immunity

The levels of antibodies against SARS-CoV-2 were measured using the anti-SARS-CoV-2 nucleocapsid IgG assay (Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's protocol. This assay is a chemiluminescent microparticle immunoassay that is

designed to detect IgG antibodies against the nucleocapsid protein of SARS-CoV-2 [12].

Results were reported as the index (S/C) through division of the sample result by the calibrator result; the cutoff was 1.40.

A plaque reduction neutralization test (PRNT) was performed using wild-type SARS-CoV-2 (BetaCoV/Korea/KCDC03/2020) to measure the level of NAbs against SARS-CoV-2. Sera were four-fold diluted from 1:20 to 1:20,480. The mixture of serum dilution/wild type SARS-CoV-2 (40 PFU/well) were incubated at 37°C for 2 h. The mixture was added to each well of a 24-well plate seeded with Vero E6 cells (1.8×10^5 cells/well) and incubated at 37°C for 1 h, followed by addition of 1 ml of 0.5% agarose (Lonza, Basel, Switzerland) on the medium. After 2 to 3 days of incubation, the cells were fixed with 4% paraformaldehyde and stained with crystal violet to visualize the plaques. All assays were performed in duplicate for each serum. Reduction in plaque count of 50% was calculated for the median neutralizing titer (ND50) using the Spearman-Kärber formula, and ND50 value of 1:20 or higher was considered positive [13].

SARS-CoV-2-specific IFN- γ ELISpot assays

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral whole blood via standard Ficoll-Paque (Cytiva, Marlborough, MA, USA) density gradient centrifugation.

Direct *ex vivo* IFN- γ ELISpot assays were performed using PBMCs. Polyvinylidene difluoride (PVDF) plates (Millipore) were coated with anti-IFN- γ coating antibody (2 μ g/ml) (Thermo scientific) overnight at 4°C. After washing, they were blocked with PBS containing 1% BSA for 2 h and RPMI medium containing 5% FBS for 1 h at room temperature (RT). PBMCs (300,000 cells per well) were cultured for 24 h in RPMI medium containing 5% FBS in the presence of DMSO as a negative control, phytohemagglutinin (PHA, 1:100, Thermo

scientific) as a positive control, or overlapping peptide (OLP) pools covering spike (S), nucleocapsid (N), and membrane (M) proteins of SARS-CoV-2 (Miltenyi Biotec). After washing, biotinylated anti-IFN- γ detection antibody (0.25 μ g/ml) (Thermo scientific) was added to the plates. After 2 h of incubation at RT and washing, streptavidin-alkaline phosphatase (SA-AP, 1:5,000, Thermo scientific) containing 1% BSA/PBS/Tween was added to the plates. After 1 h incubation at RT, development solution from the AP Conjugate Substrate Kit (Bio-Rad) was added to the plates and incubated for 5 min at RT. Finally, the plates were washed with distilled water and dried in the dark room at RT overnight. The spot-forming cells (SFCs) in each well were counted using an automated ELISpot reader (AID GmbH). The number of specific spots was calculated by subtracting the mean number of spots in the DMSO control well from the mean number of spots in the OLP pool-stimulated well. We performed IFN- γ ELISpot assays in duplicate and calculated an average.

Statistical analysis

Statistical analyses were performed using SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA) and the Statistical Analysis System software 9.4 (SAS Institute, Cary, NC, USA). The geometric mean titer (GMT) with its 95% confidence interval (CI) was calculated for the comparison of the anti-SARS-CoV-2 IgG titers and ND50 titers employing the PRNT. Median with interquartile range (IQR) is shown for the number of SFCs by SARS-CoV-2-specific IFN- γ ELISpot assay as a certain value of SFC was zero. For continuous variables, an independent t-test for parametric tests and a Mann-Whitney U-test for non-parametric tests were conducted for between-group comparisons. The Kruskal-Wallis test was used for the comparison of variables across three or more groups. The chi-square test was performed for the analysis of categorical variables. For the comparison of the categorical

variables of the asymptomatic, symptomatic/non-pneumonic, and pneumonic groups, a chi-square test for trend (linear by linear association) was performed. A *P* value <0.05 was considered statistically significant.

RESULTS

Characteristics of the patients with SARS-CoV-2 infection

A total of 97 patients who had recovered from SARS-CoV-2 infection were enrolled. The mean age of the study population was 46.7 years (Table 1). Ct values for the RdRp gene of SARS-CoV-2 at diagnosis were available in 92 (94.8%) patients; the mean was 24.4. Data on viral shedding duration of SARS-CoV-2 were available in 84 (86.6%) patients, and SARS-CoV-2 was detected for an average of 29.7 days in the respiratory specimens of the patients. Of the 97 patients, 14 (14.4%) were asymptomatic and 42 (43.3%) were symptomatic without pneumonia. Pneumonia developed in 41 (42.3%) patients, nine (9.3%) of whom received oxygen therapy. The pneumonic group had complaints of fever, myalgia, and nausea/vomiting more frequently than the symptomatic/non-pneumonic group.

Longitudinal profile of anti-SARS-CoV-2 IgG and NAb

Median day (IQR) of each sample collection periods was 44.5 (28.8-56.0) for period 1, 112.5 (100.8-120.0) for period 2, and 184.0 (182.0-185.0) days for period 3 (Figure 1-A). The seropositivity rate of anti-SARS-CoV-2 IgG gradually decreased over time: 91.7% (11/12) in period 1, 77.7% (73/94) in period 2, and 66.7% (42/63) in period 3. The anti-SARS-CoV-2 IgG GMTs were 3.64 in period 1, 2.38 in period 2, and 1.96 in period 3. Compared to the value in period 1, the anti-SARS-CoV-2 IgG GMT decreased by 34.6% in period 2 and by 46.2% in period 3.

The NAb positivity rates against SARS-CoV-2 were 100% (12/12) in period 1, 97.8% (91/93) in period 2, and 86.9% (53/61) in period 3. The NAb GMTs were 343.53 in period 1, 189.50 in period 2, and 109.02 in period 3. Compared to the value in period 1, the GMT of the NABs against SARS-CoV-2 was reduced by 44.8% in period 2 and 68.3% in period 3.

Patients aged over 50 years showed a higher titer of anti-SARS-CoV-2 IgG than those aged < 50 years in period 2 (3.05 vs. 2.0, $P = 0.02$); however, the anti-SARS-CoV-2 IgG titer did not differ in period 3 (Table 2). Patients with comorbidities showed higher NAb titer than otherwise healthy patients in period 2 (292.89 vs. 166.90, $P = 0.02$). The NAb titer in period 3 was higher in patients with a prolonged viral shedding duration compared to those with a viral shedding duration < 21 days (132.70 vs. 60.19, $P = 0.04$). Obesity was not associated with the anti-SARS-CoV-2 antibody levels.

A total of 166 sera were tested by both SARS-CoV-2 IgG assays and PRNT against SARS-CoV-2. The results of IgG titer and ND50 titer were not well correlated (Spearman correlation coefficient, 0.48).

Antibody response according to the clinical severity

In period 1, although rather higher in the pneumonic group, there was no significant difference in the anti-SARS-CoV-2 IgG and NAb titers among the asymptomatic, symptomatic/non-pneumonic, and pneumonic groups: IgG, 3.49 (95% CI, 2.66-4.58) in the asymptomatic group, 3.12 (95% CI, 1.46-6.68) in the symptomatic/non-pneumonic group, and 6.65 (95% CI, 1.52-29.14) in the pneumonic group ($P = 0.29$); NAb, 373.62 (95% CI, 145.25-961.07) in the asymptomatic group, 241.75 (95% CI, 91.25-640.51) in the

symptomatic/non-pneumonic group, and 1,035.9 (95% CI, 449.75-2,385.97) in the pneumonic group ($P = 0.17$) (Figure 1-B).

In period 2 and 3, significant difference of anti-SARS-CoV-2 IgG and NAb values was found across the clinical severity of SARS-CoV-2 infection (Table 2). Pairwise multiple comparison analysis showed that the levels of anti-SARS-CoV-2 IgG in period 2 and 3 and those of NAb in period 3 were significantly higher in the pneumonic group than in the symptomatic/non-pneumonic group (Figure 1-C and 1-D). The pneumonic group was further divided in the oxygen therapy and non-oxygen therapy groups; there was no significant difference in the levels of humoral immunity between two groups (Supplementary Figure 1).

SARS-CoV-2-specific T cell response

The SARS-CoV-2-specific IFN- γ ELISpot assay was performed using PBMCs of the 78 patients. Most PBMCs (93.6%, 73/78) were collected in period 2. A median day (IQR) of PBMC collection of 78 patients was 113.0 (108.75-120.25) days after diagnosis, showing little difference from the value of period 2 serum collection (median [IQR], 112.5 [100.8-120.0] days).

Comparison of the number of IFN- γ -secreting cell count among the asymptomatic (n=9), symptomatic/non-pneumonic (n=35), and pneumonic (n=34) groups showed that there were significant differences in the number of SARS-CoV-2 M-specific SFCs and the sum of S-, N-, and M-specific SFCs according to clinical severity of SARS-CoV-2 infection. Pairwise multiple comparison analysis showed that the number of SARS-CoV-2 M-specific SFCs was higher in the pneumonic group than in the symptomatic/non-pneumonic group ($P < 0.001$). In addition, higher SARS-CoV-2 M-specific SFCs count was observed in the pneumonic group

than in the asymptomatic group ($P = 0.04$). Compared to the non-pneumonic group, pneumonic group showed higher sum values of S-, N-, and M-specific SFCs count ($P = 0.003$) (Figure 2, Supplementary Table 1).

Furthermore, the pneumonic group was divided in the oxygen therapy and non-oxygen therapy groups. Among the pneumonic group, there was no difference in the IFN- γ -secreting cell count according to the use of oxygen therapy (Supplementary Figure 2).

There were 31 close contacts who were revealed to be negative for both SARS-CoV-2 PCR and anti-SARS-CoV-2 IgG assay. Notably, IFN- γ -secreting cells were detected in close contacts without documented SARS-CoV-2 infection: median (IQR) S-specific SFCs, 4.0 (0-15.0); N-specific SFCs, 1.0 (0-10.0); M-specific SFCs, 6.5 (0-37.0); sum of S-, N-, and M-specific SFCs, 23.0 (3.5-57.0).

Comparison of the early decay and sustained response groups

In the sustained response group in which anti-SARS-CoV IgG was detected in period 2 and period 3, the proportion of patients aged ≥ 50 years was higher than that in the early IgG decay group (47.9% vs. 19.0% in period 2, $P = 0.02$; 61.9% vs. 28.6% in period 3, $P = 0.01$) (Table 3). Those with a prolonged viral shedding duration (≥ 21 days) tended to show sustained response with detectable NAbs until period 3, rather than early decay (negative for NAbs in period 3) (78.0% vs. 28.6%, $P = 0.02$). When the pneumonic group was compared to the symptomatic/non-pneumonic group by the highly sufficient ND50 cutoff of 1:160 at period 3, the proportion of pneumonic patients was higher in the ND50 $\geq 1:160$ group than in the ND50 $< 1:160$ group (82.6% [19/23] vs. 42.4% [14/33], $P = 0.003$).

A significantly larger number of S-, N-, and M-specific IFN- γ -secreting cells were present in the sustained response group than in the early decay group of anti-SARS-CoV-2 IgG. However, although they were relatively higher in the group with ND50 \geq 1:160, the IFN- γ -secreting cell counts were not significantly different between the groups with respect to the ND50 (\geq 1:160 vs. $<$ 1:160) (Supplementary Table 2).

DISCUSSION

In this study, the longitudinal profile of anti-SARS-CoV-2 antibodies in patients who had recovered from COVID-19 was investigated for longer than six months after diagnosis. NAbs were detected in 86.9% of patients until six months after diagnosis of SARS-CoV-2 infection. In particular, the pneumonic group showed significantly higher titers of anti-SARS-CoV-2 antibody until six months after diagnosis than mild group. The NAb and IgG antibody titers showed low correlation coefficient, which may be related to differences in the antibody targets and individual variation in the antibody affinity maturation. Moreover, a larger number of SARS-CoV-2-specific IFN- γ -secreting cells were measured in the pneumonic group. Sustained humoral immune responders showed stronger T-cell immunity compared to early antibody decay group.

We observed waning antibody levels with reduction rates of 46.2% for IgG and 68.3% for NAbs against SARS-CoV-2 from six weeks to six months after diagnosis. In a previous study, a four-fold decrease was observed in the NAb titers from one to four months after symptom onset [6]. Our longitudinal observation showed that the anti-SARS-CoV-2 IgG levels consistently decreased after diagnosis in those who had recovered from COVID-19. In some patients however, the levels of the NAbs against SARS-CoV-2 in period 3 increased compared to those in period 2 (Figure 1-A). Similar finding was found in other studies, where rising NAb titers 90 days after symptom onset in patients with COVID-19 [6, 7].

Neutralization titers were strongly correlated with the high affinity of antibodies in rabbits immunized with SARS-CoV-2 S antigens [14]. In this study, patients with a prolonged viral shedding showed significantly higher NAb titer at six months after diagnosis. Prolonged viral exposure might affect the affinity maturation of anti-SARS-CoV-2 antibodies. Further studies on antibody affinity maturation are required in COVID-19 patients.

The NAb titers against SARS-CoV-2 at six months after diagnosis were significantly higher in the pneumonic group than in the symptomatic/non-pneumonic group. A large proportion of the pneumonic patients showed sustained response, with the NAb titer remaining at $\geq 1:160$ until six months after diagnosis. The Food and Drug Administration recommended the use of convalescent plasma with NAb titers $\geq 1:160$ for plasma therapy [15]. Anti-SARS-CoV-2 IgG levels were initially higher in the pneumonic group, but rapidly declined compared to those in the other groups from period 1 to period 2. The observed immune response profile and waning antibody patterns are consistent with those previously noted [6, 16-18]. A profound expansion in the number of plasmablasts has been observed in association with severe SARS-CoV-2 infection [19]. It may be related to an enhanced antibody response and reduction of short-lived antibody-secreting cells in patients with severe COVID-19. There is a possibility that the clinical severity-dependent difference in the antibody levels may become narrower over a period of six months.

Older patients and symptomatic individuals were reported to have an enhanced antibody response to SARS-CoV-2 infection, while obesity has been shown to be positively associated with SARS-CoV-2 antibody levels [4, 20, 21]. Considering that these factors could affect the severity of disease, clinical severity may be strongly associated with antibody response in patients with COVID-19 [22-24]. In comparison, another study reported comparable peak antibody levels, irrespective of disease severity, although faster increase was demonstrated in association with a higher severity [25]. Depending on the disease severity, underlying

medical conditions, and patient's age, the antibody production and its persistence may be variable.

The degree of SARS-CoV-2-specific T-cell response has been shown to be robust in severe COVID-19 cases [26, 27], although viral clearance was not associated with SARS-CoV-2 T-cell immunity [27]. SARS-CoV-2 M-reactive T-cell response was higher in the pneumonic group than in the symptomatic/non-pneumonic groups. The M-protein among SARS-CoV-2 proteins stimulated a larger number of IFN- γ -secreting cells, consistent with a previous report [27]. As the central organizer of viral assembly, the M protein is the most abundantly present protein in the virion of coronaviruses, and may activate anti-SARS-CoV-2-specific T-cell immune response in accordance with disease severity [28].

In comparison, the level of SARS-CoV-2-reactive T-cell response in the asymptomatic group was not significantly different from that in the symptomatic/non-pneumonic group. Although the level was lower, SARS-CoV-2-reactive T-cell immunity was detected even in the test-negative close contacts of the patients with SARS-CoV-2 infection. This phenomenon has been demonstrated in several studies, suggestive of the presence of a cross-reactive T-cell response between SARS-CoV-2 and other common-cold coronaviruses [29, 30]. Although SARS-CoV-2 reactive T-cell immunity was detected in our asymptomatic group, the level was low and similar to that observed in the symptomatic/non-pneumonic group. Given the relatively low T-cell immunity in asymptomatic patients, the role of T-cell immunity may be quite limited in the prevention of disease development. The innate immunity including natural killer cells and interferons has a chance to be more important to suppress disease development and progression in the early stage of exposure to SARS-CoV-2.

This study has several limitations. First, the sample size was small in period 1, so statistically significant difference could not be confirmed. Second, the collection of blood samples from COVID-19 patients who had recovered was not feasible at strictly regular intervals; therefore, the sample collection period was broad. Third, anti-SARS-CoV-2 IgG was measured against nucleocapsid protein, but not for spike protein. IgG antibody binding site might be related to the low correlation between NAb and IgG antibody titers. Finally, the level of SARS-CoV-2 T-cell immunity was examined cross-sectionally only once in each participant and T-cell response against common-cold coronaviruses was not measured.

Nevertheless, this study showed that anti-SARS-CoV-2 neutralizing antibodies persisted in more than 85% of patients with COVID-19 until six months after diagnosis. Actually in the prospective cohort studies of healthcare workers who recovered from COVID-19, 83-89% of reinfections were prevented in the ensuing 6 months [31, 32]. Older age, prolonged viral shedding and accompanying pneumonia were strongly associated with sustained antibody response in patients with COVID-19. This longitudinal assessment of the anti-SARS-CoV-2 immune response may be valuable in establishing vaccination strategies and providing implications for protection against reinfection.

Note

Author contributions

JYN, ECS and JYS contributed to the conception and design of the study. JYN, JEK, ECS and JYS analyzed the data with responsibility for its integrity and prepared the manuscript. All authors contributed to acquisition of clinical and laboratory data. JYN, JEK, JSY, CSL, SYY, JYL, ECS and JYS contributed to the interpretation of data. JYN, SYH, JGY, HS, HJH, JSR, SSK, HJC, WJK and JYS contributed to statistical analysis. All authors critically revised the manuscript for intellectual content and approved the final draft for submission.

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Potential conflicts of interest.

All No reported conflicts of interest. 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.

Figure legends

Figure 1. Humoral immunity assessment of patients with COVID-19. **(A)** Anti-SARS-CoV-2 IgG titers and neutralizing antibody titers against SARS-CoV-2 on the day after diagnosis in each patient. **(B)** GMT of anti-SARS-CoV-2 IgG titers and neutralizing antibody titers at each period according to the clinical disease severity. Periods 1, 2, and 3 represent within 8 weeks, 9-20 weeks, and 22-27 weeks after diagnosis with COVID-19, respectively. The dotted line indicates the cut-off value for IgG (1:40) and neutralizing antibody (1:20). **(C)** Individual titers of anti-SARS-CoV-2 IgG according to the severity in period 2 and period 3. Line indicates GMT of IgG titers in each group. **(D)** Individual titers of neutralizing antibody against SARS-CoV-2 according to the severity in period 2 and period 3. Line indicates GMT of neutralizing antibody titers in each group. COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; GMT, geometric mean titer; AP, asymptomatic patient; NPP, symptomatic/non-pneumonic patient; PP, pneumonic patient.

Figure 2. T-cell immunity assessment of patients with COVID-19. Numbers of SARS-CoV-2 S- **(A)**, N- **(B)**, M- **(C)**, sum of S-, N-, and M- **(D)** reactive IFN- γ -secreting T-cells. Line indicates the median of SFCs in each group. COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S, spike protein; N, nucleocapsid protein; M, membrane protein; SFC, spot-forming cell; PBMC, peripheral blood mononuclear cell; AP, asymptomatic patient; NPP, symptomatic/non-pneumonic patient; PP, pneumonic patient.

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Table 1. Demographic and clinical characteristics of patients with SARS-CoV-2 infection

	Total (n=97)	Asymptomatic (n=14)	Non-pneumonic (n=42)	Pneumonic (n=41)	<i>P</i>
Sex (male), n (%)	18 (18.6)	3 (21.4)	10 (23.8)	5 (12.2)	0.51
Age, mean ± SD	46.7±8.9	51.3±10.4	44.7±8.9	47.3±8.0	0.24
Comorbidity [†]	23 (23.7)	5 (35.7)	5 (11.9)	13 (31.7)	0.03
Body mass index (kg/m ²)	23.8±4.0	23.1±3.0	23.5±4.9	24.4±3.3	0.12
≥ 25.0	32 (33.0)	4 (28.6)	12 (28.6)	16 (39.0)	0.71
Symptom					
Fever	30 (30.9)	-	10 (23.8)	20 (48.8)	0.02
Febrile sense	8 (8.2)	-	5 (11.9)	3 (7.3)	0.71 [‡]
Cough	27 (27.8)	-	14 (33.3)	13 (31.7)	0.87
Sore throat	28 (28.9)	-	16 (38.1)	12 (29.3)	0.40
Sputum	23 (23.7)	-	13 (31.0)	10 (24.4)	0.50
Rhinorrhea	17 (17.5)	-	11 (26.2)	6 (14.6)	0.19
Myalgia	29 (29.9)	-	10 (23.8)	19 (46.3)	0.04 [‡]
Chill	19 (19.6)	-	7 (16.7)	12 (29.3)	0.17
Chest pain	6 (6.2)	-	2 (4.8)	4 (9.8)	0.43 [‡]
Nausea/vomiting	8 (8.2)	-	1 (2.4)	7 (17.1)	0.03 [‡]
Diarrhea	22 (22.7)	-	10 (23.8)	12 (29.3)	0.57
Abdominal pain	4 (4.1)	-	0	4 (9.8)	0.06 [‡]
Anosmia/ageusia	38 (39.2)	-	22 (52.4)	16 (39.0)	0.22
Headache	3 (3.1)	-	1 (2.4)	2 (4.9)	0.62 [‡]
Rash	1 (1.0)	-	1 (2.4)	0	1.00 [‡]
Viral titer at diagnosis [‡]	24.4±3.6	24.8±1.6	24.5±4.0	24.3±3.6	0.70
Viral shedding duration	29.7±13.8	24.0±10.5	28.9±14.9	31.6±13.1	0.26
Oxygenation	9 (9.3)	-	-	9 (22.0)	-

[†]hypertension, diabetes mellitus, cerebral infarction, asthma, cardiomegaly, hyperthyroidism, hypothyroidism, thyroid cancer, breast cancer, pituitary adenoma, autoimmune disease, ankylosing spondylitis

[‡]cycle threshold (Ct) value for RdRp gene

[‡]Fisher's Exact Test

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation

Table 2. Comparison of humoral immunity to SARS-CoV-2 according to the characteristics of patients with SARS-CoV-2 infection

	Period 2, GMT (95% CI)				Period 3, GMT (95% CI)			
	IgG	<i>P</i>	NAb	<i>P</i>	IgG	<i>P</i>	NAb	<i>P</i>
Age								
< 50 years	2.0 (1.49-2.67)	0.02	168.50 (127.87-222.05)	0.18	1.76 (1.35-2.28)	0.24	102.82 (62.56-168.98)	0.74
≥ 50 years	3.05 (2.41-3.86)		222.96 (163.90-303.30)		2.17 (1.69-2.79)		114.95 (70.82-186.59)	
Comorbidity								
No	2.21 (1.75-2.80)	0.19	166.90 (132.79-209.76)	0.02	1.88 (1.53-2.31)	0.44	93.80 (63.90-137.71)	0.14
Yes	3.01 (2.10-4.31)		292.89 (190.48-450.36)		2.20 (1.50-3.24)		166.36 (80.15-345.32)	
BMI								
< 25.0 kg/m ²	2.08 (1.61-2.69)	0.05	171.08 (132.20-221.41)	0.15	1.87 (1.49-2.35)	0.51	99.37 (62.51-157.98)	0.47
≥ 25.0 kg/m ²	3.13 (2.36-4.15)		234.88 (168.23-327.93)		2.12 (1.55-2.89)		128.47 (79.0-208.92)	
Viral shedding duration								
< 21 days	2.26 (1.65-3.09)	0.99	194.05 (130.47-288.62)	0.64	1.67 (1.20-2.34)	0.30	60.19 (29.94-121.0)	0.04
≥ 21 days	2.26 (1.71-2.98)		172.88 (131.81-226.74)		2.08 (1.66-2.60)		132.70 (89.95-195.76)	
Severity								
Asymptomatic	1.75 (1.01-3.05)	<0.01	114.58 (53.65-244.70)	0.04	1.53 (0.49-4.79)	0.03	100.34 (15.35-655.77)	0.04
Symptomatic/no n-pneumonic	1.65 (1.20-2.27)		166.47 (127.27-217.73)		1.50 (1.16-1.95)		64.85 (40.17-104.70)	
Pneumonic	3.75 (2.96-4.74)		249.15 (181.54-341.95)		2.45 (1.93-3.11)		158.55 (98.82-254.40)	

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; GMT, geometric mean titer; CI, confidence interval; IgG, immunoglobulin G; NAb, neutralizing antibody; BMI, body mass index

Table 3. Longevity of anti-SARS-CoV-2 antibodies according to the characteristics of patients with SARS-CoV-2 infection

	Period 2						Period 3								
	IgG (+)	IgG (-)	<i>P</i>	ND50 ≥ 1:160	ND50 <1:160	<i>P</i>	IgG (+)	IgG (-)	<i>P</i>	NAb (+)	NAb (-)	<i>P</i>	ND50 ≥ 1:160	ND50 < 1:160	<i>P</i>
Age															
< 50 years	38/73 (52.1)	17/21 (81.0)	0.02	27/54 (50.0)	27/39 (69.2)	0.06	16/42 (38.1)	15/21 (71.4)	0.01	24/53 (45.3)	5/8 (62.5)	0.46 [¶]	13/25 (52.0)	16/36 (44.4)	0.56
≥ 50 years	35/73 (47.9)	4/21 (19.0)		27/54 (50.0)	12/39 (30.8)		26/42 (61.9)	6/21 (28.6)		29/53 (54.7)	3/8 (37.5)		12/25 (48.0)	20/36 (55.6)	
Comorbidity															
No	54/73 (74.0)	18/21 (85.7)	0.38 [¶]	39/54 (72.2)	33/39 (84.6)	0.16	30/42 (71.4)	17/21 (81.0)	0.41	38/53 (71.7)	7/8 (87.5)	0.67 [¶]	16/25 (64.0)	29/36 (80.6)	0.15
Yes	19/73 (26.0)	3/21 (14.3)		15/54 (27.8)	6/39 (15.4)		12/42 (28.6)	4/21 (19.0)		15/53 (28.3)	1/8 (12.5)		9/25 (36.0)	7/36 (19.4)	
BMI															
< 25.0 kg/m ²	46/73 (63.0)	17/21 (81.0)	0.12	34/54 (63.0)	29/39 (74.4)	0.25	26/42 (61.9)	14/21 (66.7)	0.71	32/53 (60.4)	7/8 (87.5)	0.24 [¶]	13/25 (52.0)	26/36 (72.2)	0.11
≥ 25.0 kg/m ²	27/73 (37.0)	4/21 (19.0)		20/54 (37.0)	10/39 (25.6)		16/42 (38.1)	7/21 (33.3)		21/53 (39.6)	1/8 (12.5)		12/25 (48.0)	10/36 (27.8)	
Viral shedding duration															
< 21 days	18/63 (28.6)	6/21 (28.6)	1.00	15/47 (31.9)	9/36 (25.0)	0.49	10/39 (25.6)	6/20 (30.0)	0.72	11/50 (22.0)	5/7 (71.4)	0.02 [¶]	4/22 (18.2)	12/35 (34.3)	0.19
≥ 21 days	45/63 (71.4)	15/21 (71.4)		32/47 (68.1)	27/36 (75.0)		29/39 (74.4)	14/20 (70.0)		39/50 (78.0)	2/7 (28.6)		18/22 (81.8)	23/35 (65.7)	
Severity															
Asymptomatic	8/73 (11.0)	5/21 (23.8)	0.49	5/54 (9.3)	7/39 (17.9)	0.94	3/42 (7.1)	2/21 (9.5)	0.47	4/53 (7.5)	1/8 (12.5)	0.69	2/25 (8.0)	3/36 (8.3)	0.02

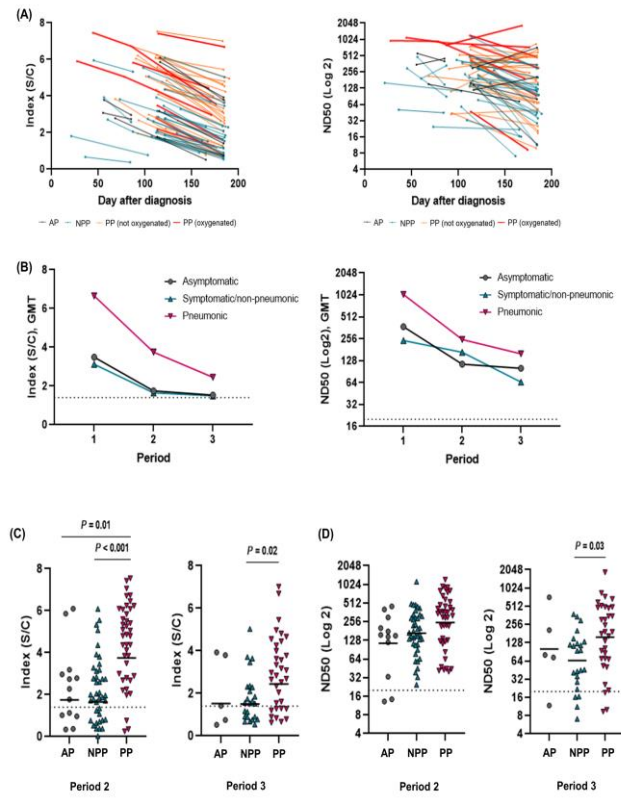
Symptomatic/ non- pneumonic	27/73 (37.0)	13/21 (61.9)	21/54 (38.9)	19/39 (48.7)	14/42 (33.3)	10/21 (47.6)	19/53 (35.8)	4/8 (50.0)	4/25 (16.0)	19/36 (52.8)
Pneumonic	38/73 (52.1)	3/21 (14.3)	28/54 (51.9)	13/39 (33.3)	25/42 (59.5)	9/21 (42.9)	30/53 (56.6)	3/8 (37.5)	19/25 (76.0)	14/36 (38.9)

[†]Fisher's Exact Test

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IgG, immunoglobulin G; ND50, median neutralizing titer; NAb, neutralizing antibody;

BMI, body mass index

Figure 1



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101

Figure 2

