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Antibody responses to SARS-CoV-2 nucleocapsid and spike proteins in hospitalized patients with COVID-19: A multicenter, retrospective, cross-sectional study in Japan

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ABSTRACT

Background: There are many commercially available automated assays for assessing coronavirus disease 2019 (COVID-19) immune responses; however, owing to insufficient data, their validities remain unknown. Here, we examined antibody responses during acute-phase COVID-19 using four assays that detect anti-spike protein IgM (S-IgM), anti-nucleocapsid protein IgG (N-IgG), anti-spike protein total Ig (S-total Ig), and anti-spike protein IgG (S-IgG).

Methods: We measured antibody levels in 1154 serum samples collected from 286 hospitalized patients with confirmed COVID-19 by a gene amplification method between February and December 2020 in Japan. Sera from 860 healthcare workers were used as negative controls.

Results: The antibody positivity rates increased on week 2, peaked, and then started to plateau by the beginning of week 3 after symptom onset. On week 1, there were some significant differences in seropositivity rates between assays (p = 0.032): 14.9% (11.0%—19.4%) for S-IgM and 8.9% (6.0%—12.7%) for N-IgG. The seropositivity for the S-total Ig (10.6% [7.3%—14.6%]) assay was considerably better than that for the S-IgG (6.9% [4.3%—9.4%]).

Abbreviations: LAMP, loop-mediated isothermal amplification; CI, confidence interval; RT-PCR, reverse transcription polymerase chain reaction; COVID-19, coronavirus disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IQR, interquartile range.

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1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)—identified in December 2019 in Wuhan, Hubei, China—causes coronavirus disease (COVID-19), which ranges in severity from an asymptomatic infection to lethal viral pneumonia. Rapid, highly accurate diagnostic tests, which are important for controlling the COVID-19 pandemic, have been developed. The standard test for diagnosing acute-phase SARS-CoV-2 infection is a genetic test that detects SARS-CoV-2 RNA by reverse transcription polymerase chain reaction (RT-PCR) or loop-mediated isothermal amplification (LAMP) in nasal or pharyngeal swabs or saliva specimens. However, these tests may generate false-negative results even during an acute-phase symptomatic illness [1]. Furthermore, although some individuals infected with SARS-CoV-2 are asymptomatic [2–4], they can potentially spread the infection. Thus, genetic tests cannot ascertain the actual state of the infection in some populations [5]. Hence, many manufacturers have developed a wide range of SARS-CoV-2 antibody tests, and many worldwide studies have been performed using homemade or research-use only enzyme-linked immunosorbent assays [5–8]. The abovementioned assays can detect a wide variety of immunoglobulin (Ig) subclasses, such as IgA, IgM, IgG, and total Ig that targets proteins like nucleocapsid or spike protein. These serological antibody tests have a variety of potential uses, including the diagnosis of acute conditions, determination of disease prevalence in certain populations, and understanding antibody production in the vaccination-induced immune response. There have been several reports on antibody testing for patients in the acute phase of COVID-19 [5–8]; however, studies on antibody levels during the early post-onset period, i.e., the first few days after the onset of COVID-19 symptoms, are limited. Therefore, it is important to understand the trends in antibody levels among patients during the acute phase of COVID-19.

This study aimed to determine the antibody response to SARS-CoV-2 nucleocapsid and spike proteins using four automated immunosassays to detect IgM, IgG, and total Ig antibodies among patients with COVID-19 in Japan.

2. Patients and methods

2.1. Study design and setting

This multicenter retrospective cross-sectional study was conducted in the following four academic teaching hospitals that treated patients with COVID-19 in Japan: Shonan Fujisawa Tokushukai Hospital (Kanagawa), Shonan Kamakura General Hospital (Kanagawa), Chiba-Nishi General Hospital (Chiba), and Yao Tokushukai General Hospital (Osaka). In Japan, the first case of COVID-19 was diagnosed in January 2020, and the first wave of infection spread nationwide shortly thereafter. At this time, we began accepting patients with COVID-19. In April 2020, the government declared a state of emergency as the first wave peaked in mid-April and ended at the end of May. The second wave peaked in mid-August and ended in September.

2.2. Participants and sample collection

The study enrolled all consecutive adult patients with COVID-19 who were admitted between February and December 2020 to any of the four hospitals mentioned above who met the following inclusion criteria: (1) over the age of 20 years; (2) confirmed to have SARS-CoV-2 infection by real-time PCR or LAMP using respiratory tract, nasal, pharyngeal, and/or saliva specimens; and (3) have surplus serum specimens available. When multiple samples were available from the same patient for the periods described below, the one that was collected first was used: every 3 days from the day of onset to day 29 post-onset, and every 6 days from days 30–41 post-onset.

The clinical COVID-19 status of each patient was classified using a five-category ordinal scale based on the maximum respiratory support required by a patient, which was a modified version of the 8-point ordinal scale of the National Institute of Allergy and Infectious Diseases [9]. The categories were as follows: 1, hospitalized, not requiring supplemental oxygen; 2, hospitalized, requiring supplemental oxygen; 3, hospitalized, requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both; 4, hospitalized, requiring extracorporeal membrane oxygenation, invasive mechanical ventilation, or both; and 5, death.

As negative controls, we used surplus serum samples that were collected during the annual medical checkups of medical staff at Shonan Fujisawa Tokushukai Hospital to determine the validity of the assays. The checkups were conducted between June 1, 2020 and July 30, 2020, a few months after the first wave of the COVID-19 pandemic, which peaked in mid-April 2020 in Japan. Any individual who was formerly diagnosed or suspected to have COVID-19 was excluded.

2.3. Antibody measurement

We measured the levels of antibodies against SARS-CoV-2 using four automated assays for (1) anti-spike protein IgM,
(2) anti-nucleocapsid protein IgG, (3) anti-spike protein total Ig, and (4) anti-spike protein IgG. Anti-spike protein IgM and anti-nucleocapsid protein IgG antibodies were analyzed using the Architect SARS-CoV-2 IgM and SARS-CoV-2 IgG assays, respectively, which employ chemiluminescent microparticle immunoassay technology (Abbott, Abbott Park, Illinois, USA). The assays were designed to detect IgM and IgG antibodies against the spike and nucleocapsid proteins, respectively, of SARS-CoV-2 in serum or plasma samples. The results are reported as an index (i.e., the ratio of the chemiluminescent signal for the samples and a calibrator, S/C), and the manufacturer-recommended positivity cutoff index values were 1.00 S/C for IgM and 1.40 S/C for IgG. The latter two antibodies, anti-spike protein total Ig and anti-spike protein IgG, were respectively analyzed using the Dimension EXL SARS-CoV-2 Total Antibody and IgG Antibody with LOCI (luminescent oxygen channeling immunoassay technology) assays (Siemens, Washington, D.C., USA), which are designed to detect the spike protein receptor-binding domain on the surface of SARS-CoV-2. The results are reported as an index (the name of the unit, QUAL, is the first letters of the words qualitative, unit, analysis, and LOCI), and the manufacturer-recommended positivity cutoff index value for both assays is 1000 QUAL.

2.4. Ethical approval and data collection and analysis

This study was performed in accordance with the Declaration of Helsinki and was reviewed and approved by the Tokushukai Group Ethics Committee (approval no. TGE01500-008, July 1, 2020) and the Institutional Review Boards or Ethics Committees of other participating facilities. Tacit informed consent was obtained from patients with COVID-19 via an opt-out notice on the institutional homepage; patients who refused to provide their consent were excluded. All medical staff in Shonan Fujisawa Tokushukai Hospital whose samples were used as negative controls provided written informed consent before study participation. For all enrolled patients, clinical information was obtained from electronic medical records. Some data for the Abbot anti-nucleocapsid protein IgG assay were presented in a previous study [10].

Data analysis and visualization were performed using R (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org). Continuous variables are expressed as the median with interquartile range (IQR) for non-normally distributed data and were compared using the Mann-Whitney U test. Confidence intervals were calculated using the modified Wald method. Categorical variables are presented as numbers (percentages) and compared using Fisher’s exact test. P values < 0.05 were considered statistically significant.

3. Results

3.1. Patient clinical characteristics

Among the 1609 patients with COVID-19 admitted to the four academic teaching hospitals between February 1, 2020 and December 31, 2020, 1323 were excluded based on the following criteria: (1) aged <20 years; (2) diagnosed using SARS-COV-2 antigen test only, without genetic testing; (3) surplus serum specimens not available; and (4) informed consent not provided. The remaining 286 patients who met the criteria were included in the analysis. The median age of the enrolled patients was 67 years (IQR, 51–77 years), and 62.2% of patients were men. At admission, the clinical COVID-19 status of 259 patients required and did not require oxygen administration during hospitalization, respectively. Patient clinical characteristics are summarized in Table 1.

### Table 1 – Baseline characteristics and clinical data of study patients with COVID-19.

| Characteristics | Total (N = 286) |
|-----------------|----------------|
| **Sex, male, n (%)** | 149 (52.1) |
| **Age (years), median (IQR)** | 66 (51–77) |
| **Height (cm), median (IQR)** | 160.0 (153.9–165.0) |
| **Weight (kg), median (IQR)** | 61.0 (51.0–70.3) |
| **Body mass index, median (IQR)** | 23.0 (20.4–25.7) |
| **Race/region, n (%)** | |
| Japanese | 280 (97.9) |
| Asians outside of Japan | 4 (1.4) |
| Westerners (Caucasians) | 2 (0.7) |
| **Comorbidities and smoking history, n (%)** | |
| Smoker | 128 (44.8) |
| Hypertension | 97 (33.9) |
| Diabetes mellitus | 49 (17.1) |
| Dyslipidemia | 59 (20.6) |
| Chronic obstructive pulmonary disease | 8 (2.8) |
| Bronchial asthma | 29 (10.1) |
| Interstitial pneumonia | 4 (1.3) |
| Autoimmune/rheumatic disorders | 9 (3.1) |
| Cerebrovascular disease | 14 (4.9) |
| Cardiovascular disease | 28 (9.8) |
| **NEWS on day 1, median (IQR)** | 1 (0–2) |
| **Illness score on the 5-point ordinal scale at admission and the most severe illness score, n (%)** | |
| 1 | 259 (90.6) 232 (81.1) |
| 2 | 24 (8.4) 37 (12.9) |
| 3 | 3 (1.0) 13 (4.5) |
| 4 | 0 (0) 2 (0.7) |
| 5 | 0 (0) 2 (0.7) |
| **Symptoms associated with COVID-19, n (%)** | |
| Fever ≥37 °C | 200 (69.9) |
| Cough | 131 (45.8) |
| Dyspnea | 43 (15.0) |
| Taste and/or smell disorder | 71 (24.8) |
| Diarrhea | 39 (13.6) |
| **Pneumonia on chest roentgenogram/CT during hospitalization, n (%)** | 260 (90.9) |
| **Laboratory data on admission, median (IQR)** | |
| Lymphocyte count (μL) | 1032.4 (798.7–1392.0) |
| Lactate dehydrogenase (IU/L) | 215 (190–260) |
| C-reactive protein (mg/dL) | 0.93 (0.28–3.97) |
| Ferritin (ng/mL) | 212.9 (129.0–390.6) |
| D-dimer (μg/mL) | 0.81 (0.59–1.39) |
| **Treatment for COVID-19** | |
| Corticosteroid, n (%) | 60 (21.0) |
| Favipiravir, n (%) | 57 (19.9) |
| Remdesivir, n (%) | 15 (5.2) |
| Tocilizumab, n (%) | 1 (0.3) |

Abbreviations: COVID-19, coronavirus disease-2019; NEWS, National Early Warning Score; CT, computed tomography; IQR, interquartile range.
3.2 The positivity rates of all four assays at different time points after symptom onset

We collected and tested 1282 serum samples from 286 patients with COVID-19 during hospitalization and outpatient follow-up, and 227 patients had sequential samples available. Thus, the analysis was performed on samples collected from these 227 patients (n = 1154), excluding duplicate samples collected from the same patient during the same time period. The positivity rates of all four assays were low in the first week after symptom onset, increased rapidly in the second week, and peaked and started to plateau by the third week (Fig. 1). The seropositivity rates of the four assays are summarized in Table 2.

In the first week after symptom onset (i.e., days 0–6), analysis using Fisher’s exact test indicated significant differences in the seropositivity rates between the Abbott assays for detecting IgM (both IgM only and total Ig) and IgG antibody levels alone, (p = 0.032): 14.9% (11.0–19.4%) for anti-spike protein IgM and 8.9% (6.0–12.7%) for anti-nucleocapsid protein IgG. The seropositivity rate of the Siemens assay for detecting anti-spike protein total Ig (10.6% [7.3–14.6%]) was higher than that of the assay for detecting anti-spike protein IgG (6.9% [4.3–10.4%]), although the difference was not statistically significant (p = 0.150).

Among patients with available sera collected 21 days after symptom onset, 13 patients (median age 51.0 years [IQR 39–73 years]; 53.8% were men) did not show seroconversion for at least one of the four assays at any point during the 0–41 day period after symptom onset. One patient did not show seroconversion in any of the assays, two patients did not show seroconversion for three assays (negative for anti-spike protein IgM, anti-spike protein total Ig, and anti-spike protein IgG), three patients for two assays (negative for anti-spike protein IgM and anti-spike protein total Ig: two patients; anti-nucleocapsid protein IgG and anti-spike protein total Ig: one patient), and seven patients for one assay (negative for anti-spike protein IgM: three patients, anti-nucleocapsid protein IgG: two patients, and anti-spike protein total Ig: two patients).

3.3 Dynamic changes in antibody levels

Although all assays are intended to be qualitative tests, the quantitative antibody levels over time are summarized in Table 2, and the temporal changes in antibody levels are shown in Fig. 2. For all four assays, antibody levels were generally low during the first week after symptom onset. During the second week, anti-spike protein IgM, anti-nucleocapsid protein IgG, and anti-spike protein total Ig levels showed a rapid increase, whereas anti-spike protein IgG levels showed a slower increase. Anti-spike protein IgM levels peaked during the third week and decreased after the fourth week, whereas anti-nucleocapsid protein IgG, anti-spike protein total Ig, and anti-spike protein IgG antibody levels peaked during the fifth week and started decreasing after the sixth week.

To determine whether disease severity was related to antibody levels, we used sera sampled within 13 days after symptom onset and more than 14 days after symptom onset to compare antibody levels between individuals with disease severity categories 1–2 (269 patients) and 3–5 (17 patients) on
the day when maximum respiratory support was required (Fig. 3). All assays showed significant differences in antibody levels within or after 14 days of symptom onset, except for anti-spike protein IgM antibody levels after 14 days of symptom onset.

### 3.4. Specificity of the four assays: seroprevalence among healthy medical staff

As negative controls, we enrolled 862 medical staff who underwent an annual medical checkup, and who were never suspected and/or confirmed to have COVID-19 (median age, 34 years [IQR 28–46 years]; 28.8% were men). Among the tested staff, three patients were positive for anti-spike protein IgM, one patient was positive for anti-nucleocapsid protein IgG, one patient was positive for anti-spike protein total Ig, and one patient was positive for anti-spike protein IgG. The calculated specificities (95% confidence intervals) for the anti-spike protein IgM, anti-nucleocapsid protein IgG, anti-spike protein total Ig, and anti-spike protein IgG assays were 99.7% (99.0–99.9%), 99.9% (99.3–100%), 99.9% (99.3–100%), and 99.9% (99.3–100%), respectively. The serum samples from each of the four tested antibody assays by time of collection.

| Assay          | Sample collection, days post-onset | Positive samples/all samples, n | Seropositivity rate (95% CI) | Antibody level, mean (SE) |
|----------------|-----------------------------------|-------------------------------|-----------------------------|---------------------------|
| Abbott S-IgM   | Day 0–2                           | 6/84                          | 7.1 (2.7–14.9)              | 0.48 (0.20)               |
|                | Day 3–5                           | 26/157                        | 16.6 (11.1–23.3)            | 0.84 (0.18)               |
|                | Day 6–8                           | 65/192                        | 33.9 (27.2–41.0)            | 1.84 (0.25)               |
|                | Day 9–11                          | 141/196                       | 71.9 (65.1–78.1)            | 4.92 (0.53)               |
|                | Day 12–14                         | 120/133                       | 90.2 (83.9–94.7)            | 9.26 (0.92)               |
|                | Day 15–17                         | 84/87                         | 96.6 (90.3–99.3)            | 12.07 (1.35)              |
|                | Day 18–20                         | 49/50                         | 98.0 (89.4–99.9)            | 14.47 (2.13)              |
|                | Day 21–23                         | 47/49                         | 95.9 (86.0–99.5)            | 13.08 (2.53)              |
|                | Day 24–26                         | 64/69                         | 92.9 (84.1–97.6)            | 10.75 (1.60)              |
|                | Day 27–29                         | 80/83                         | 96.4 (89.8–99.2)            | 11.68 (1.37)              |
|                | Day 30–35                         | 24/24                         | 100.0 (85.8–100.0)          | 9.19 (1.36)               |
|                | Day 36–41                         | 28/30                         | 93.3 (77.9–99.2)            | 10.39 (2.53)              |
| Abbott N-IgG   | Day 0–2                           | 6/84                          | 7.1 (2.7–14.9)              | 0.36 (0.13)               |
|                | Day 3–5                           | 13/157                        | 8.3 (4.5–13.7)              | 0.44 (0.10)               |
|                | Day 6–8                           | 35/192                        | 18.2 (13.0–24.4)            | 0.97 (0.14)               |
|                | Day 9–11                          | 102/196                       | 52.0 (44.8–59.2)            | 2.73 (0.20)               |
|                | Day 12–14                         | 111/133                       | 83.5 (76.0–89.3)            | 5.12 (0.26)               |
|                | Day 15–17                         | 82/87                         | 94.3 (87.1–98.1)            | 5.95 (0.24)               |
|                | Day 18–20                         | 50/50                         | 100.0 (92.9–100.0)          | 6.45 (0.25)               |
|                | Day 21–23                         | 47/49                         | 95.9 (86.0–99.5)            | 5.96 (0.25)               |
| Siemens S-total IgG | Day 0–2           | 4/84                          | 4.8 (1.3–11.7)              | 5.26 (1.43)               |
|                | Day 3–5                           | 17/157                        | 10.8 (6.4–16.8)             | 825.5 (284.8)             |
|                | Day 6–8                           | 55/192                        | 28.6 (22.4–35.6)            | 1997.7 (403.6)            |
|                | Day 9–11                          | 113/136                       | 57.7 (50.4–64.7)            | 6278.1 (724.6)            |
|                | Day 12–14                         | 111/133                       | 83.5 (76.0–89.3)            | 13375.4 (1996.2)          |
|                | Day 15–17                         | 81/87                         | 93.1 (85.6–97.4)            | 19287.8 (15808.8)         |
|                | Day 18–20                         | 46/50                         | 92.0 (80.8–97.8)            | 23005.4 (1984.6)          |
| Siemens S-IgG  | Day 21–23                         | 46/49                         | 93.9 (83.1–98.7)            | 20448.2 (2336.2)          |
|                | Day 24–26                         | 66/69                         | 95.7 (87.8–99.1)            | 17614.9 (1607.1)          |
|                | Day 27–29                         | 81/83                         | 97.6 (91.6–99.7)            | 19610.7 (1554.9)          |
|                | Day 30–35                         | 22/24                         | 91.7 (73.0–99.0)            | 26235.2 (3406.9)          |
|                | Day 36–41                         | 28/30                         | 93.3 (77.9–99.2)            | 19645.8 (2578.7)          |

Abbreviations: CI, confidence interval; Ig, immunoglobulin; N-IgG, anti-nucleocapsid protein IgG; SE, standard error; S-IgM, anti-spike protein IgM; S-IgG, anti-spike protein IgG; S-total Ig, anti-spike protein total Ig.
Fig. 2 – Temporal dynamic changes in antibody levels (mean ± standard error) among hospitalized patients with COVID-19 as measured using each of the four assays. Manufacturer-recommended positivity threshold indexes are depicted as horizontal dashed lines, which are (A) 1.0 for anti-spike protein IgM, (B) 1.4 for anti-nucleocapsid protein IgG, and (C) and (D) 1000 for both anti-spike protein total Ig and IgG.

Fig. 3 – Box plots showing the median antibody levels among hospitalized patients with COVID-19 according to disease severity using four assays for detecting (A) anti-spike protein IgM, (B) anti-nucleocapsid protein IgG, (C) anti-spike total Ig, and (D) anti-spike IgG. All cohorts were divided into two groups, <14 days and ≥14 days after symptom onset. For all assays, patients with disease severity category 3–5 showed significantly higher antibody levels than patients with category 1–2 disease severity, except for anti-spike IgM at 14 days after symptom onset. Boxes show interquartile ranges, and I bars represent the highest and lowest values. The Mann-Whitney U test was used for comparison.
the six abovementioned patients showed a positive result with only one assay, and were negative in the other three assays.

4. Discussion

We measured serum antibody levels in sera collected from healthy medical staff and acutely ill hospitalized patients with COVID-19 using four assays. All four assays showed high specificities (>99%) among the negative controls, and almost all COVID-19 hospitalized patients showed seroconversion at 3 weeks after symptom onset. Antibody levels in all assays were low during the first week and the peaked on weeks 3 and 5 after symptom onset for anti-spike protein IgM and the other three assays detecting IgG, respectively. In addition, antibody levels tended to be higher in patients with greater disease severity. In the hyperacute phase, the seropositivity rate within 1 week of symptom onset tended to be higher in the assays detecting IgM (IgM only and total Ig) than in the assays detecting IgG alone.

According to recent studies, the sensitivity for detecting IgG antibodies in patients with COVID-19 is 100% [6–8,10–12]; however, we obtained negative results for some patients, even among those who were followed up for at least 3 weeks. One patient had a negative result in all assays (probably due to a false-positive PCR result or the absence of an antibody response), and 12 patients had no seroconversion in at least one of the assays; hence, there was discordance among the assay results. Moreover, there were patients who showed no seroconversion of specific subclasses of IgM and IgG antibodies, which could be due to problems in determining the manufacturer cutoff or an inability to produce antibodies corresponding to specific antigenic proteins or globulin subclasses [13]. In the absence of a gold standard for these serological tests, it was difficult to fully evaluate these results.

Antibody levels were relatively higher in patients with severe disease, both within the first 13 days and after 14 days post-onset, which is consistent with previous study findings [14–16]. Although there was no significant difference in anti-spike protein IgM antibody levels after 14 days of symptom onset, there was a tendency for antibody levels to be higher in patients with severe disease. The mechanism underlying the positive relationship between the antibody response and disease severity is not yet clear. One possibility is that enhanced and prolonged stimulation of B-cell receptors is required to induce SARS-CoV-2–specific antibody responses. In fact, Chen et al. reported that B-cell receptor rearrangements were enhanced in patients with severe COVID-19 [15]. In other words, the increased inflammatory response associated with severe disease may promote a more robust humoral immune response, resulting in increased antibody production.

This study had several limitations. First, the specificity in this study may have been underestimated because the negative control specimens were collected after the beginning of the COVID-19 pandemic. The positive results among the negative controls could be associated with asymptomatic or very mild cases of early COVID-19. Second, this study targeted only hospitalized patients with mild to severe disease. Therefore, we did not examine asymptomatic infected patients or those with mild disease not requiring hospitalization.

To further understand the diagnostic implications of these assays on immune response, it is necessary to conduct large-scale prospective studies of patients with multiple specimens collected over time. This would clarify the changes in serum antibody levels over a long period of time after disease onset, after vaccination, and during reinfection or post-vaccination infection.

5. Conclusions

The antibody levels as determined using the four tested assays peaked at 3–5 weeks post symptom onset and then declined. The assays showed very good diagnostic performance, with high specificity (>99%). Within 1 week of symptom onset, seroconversion of IgM alone or total Ig (containing IgM) was more likely than seroconversion of IgG alone. Thus, the four commercial automated assays were suitable for the detection of seropositivity by 15 days after symptom onset, and assays of IgM alone or total Ig (containing IgM) were better than assays of IgG alone as adjunct serological tests for the diagnosis of early stages of COVID-19. However, using these serological assays alone is not sufficient.

Authorship statement

All persons who meet ICMJE authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content as follows. MH: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing (Original Draft). SW, ST, KM, SH, SN, AI, KU, SK, and TK: Investigation, Resources, Writing (Review & Editing).

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Conflict of Interest

The authors have no conflicts of interest to declare.

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REFERENCES

[1] Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction–based SARS-CoV-2 tests by time since exposure. Ann Intern Med 2020;173:262–7. https://doi.org/10.7326/M20-1495.

[2] Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Sci China Life Sci 2020;63:706–11. https://doi.org/10.1007/s11427-020-1661-4.

[3] Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020;395:514–23. https://doi.org/10.1016/S0140-6736(20)30154-9.

[4] Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, et al. Presumed asymptomatic carrier transmission of COVID-19. J Am Med Assoc 2020;323:1406–7. https://doi.org/10.1001/jama.2020.2565.

[5] Kittel M, Muth MC, Zahn I, Roth HJ, Thiaucourt M, Gerhards C, et al. Clinical evaluation of commercial automated SARS-CoV-2 immunoassays. Int J Infect Dis 2021;103:590–6. https://doi.org/10.1016/j.ijid.2020.12.003.

[6] Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Cochrane COVID-19 Diagnostic Test Accuracy Group. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev 2020;6:CD013652. https://doi.org/10.1002/14651858.CD013652.

[7] La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM. Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. Reprod Biomed Online 2020;41:483–99. https://doi.org/10.1016/j.rbmo.2020.06.001.

[8] Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Harauoi LF, Johnston JC, et al. Diagnostic accuracy of serological tests for COVID-19: systematic review and meta-analysis. BMJ 2020;370:m2516. https://doi.org/10.1136/bmj.m2516.

[9] Beigel JH, Tomashek KM, Dodd LE, Mehta Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19 - final report. N Engl J Med 2020;383(19):1813–26.

[10] Hibino M, Iwabuchi S, Munakata H. SARS-CoV-2 IgG seroprevalence among medical staff in a general hospital that treated patients with COVID-19 in Japan: retrospective evaluation of nosocomial infection control. J Hosp Infect 2021;107:103–4. https://doi.org/10.1016/j.jhin.2020.10.001.

[11] Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26:845–8. https://doi.org/10.1038/s41591-020-0897-1.

[12] Padoan A, Cosma G, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. Clin Chem Lab Med 2020;58:1081–8. https://doi.org/10.1515/cclm-2020-0443.

[13] Nakano Y, Kurano M, Morita Y, Shimura T, Yokoyama R, Qian C, et al. Time course of the sensitivity and specificity of anti-SARS-CoV-2 IgM and IgG antibodies for symptomatic COVID-19 in Japan. Sci Rep 2021;11:2776. https://doi.org/10.1038/s41598-021-82428-5.

[14] To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020;20:565–74. https://doi.org/10.1016/S1473-3099(20)30196-1.

[15] Chen X, Pan Z, Yue S, Yu F, Zhang J, Yang Y, et al. Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. Signal Transduct Target Ther 2020;5:180. https://doi.org/10.1038/s41392-020-00301-9.

[16] Kowitdamrong E, Puthanakit T, Jantarabenjakul W, Prompetchara E, Suchartliwitpong W, Putcharoen O, et al. Antibody responses to SARS-CoV-2 in patients with differing severities of coronavirus disease 2019. PLoS One 2020;15:e0240502. https://doi.org/10.1371/journal.pone.0240502.