Received: 2 January 2021 | Revised: 3 March 2021 | Accepted: 17 March 2021

DOI: 10.1002/jmv.26949

SHORT COMMUNICATION

JOURNAL OF MEDICAL VIROLOGY WILEY

Back to normal; serological testing for COVID-19 diagnosis unveils missed infections

Tomoya Tsuchida[1] | Yuko Nitahara[2] | Shotaro Suzuki[1] | Yuko Komase[3] | Katherine Candray[2] | Yasutoshi Kido[2] | Yu Nakagama[2] | Yukitaka Yamasaki[4] | Mitsuru Imamura[1] | Kimito Kawahata[1] | Hiroyuki Kunishima[4] | Shigeki Fujitani[5] | Masamichi Mineshita[1] | Takahide Matsuda[1]

[1]Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Japan

[2]Department of Parasitology and Research Center for Infectious Disease Sciences, Graduate School of Medicine, Osaka City University, Osaka, Japan

[3]Department of Respiratory Internal Medicine, St. Marianna University School of Medicine, Yokohama-City Seibu Hospital, Yokohama, Japan

[4]Department of Infectious Diseases, St. Marianna University School of Medicine, Kawasaki, Japan

[5]Department of Emergency and Critical Care Medicine, St. Marianna University School of Medicine, Kawasaki, Japan

Correspondence
Yasutoshi Kido, Department of Parasitology and Research Center for Infectious Disease Sciences, Graduate School of Medicine, Osaka City University, 1-4-3 Asahimachi Abeno-ku, 545-8585 Osaka, Japan.
Email: kido.yasutoshi@med.osaka-cu.ac.jp

Disclosures: Yasutoshi Kido and Yu Nakagama report ownership of equity of Quantum Molecular Diagnostics, an Osaka City University spinout. Quantum Molecular Diagnostics targets infectious diseases to develop and provide innovative diagnostics and is engaged in the codevelopment of the serological assay along with Mokobio Biotechnology R&D, USA. The other authors declare that there are no conflict of interests.

Funding information
COVID-19 Private Fund, Grant/Award Numbers: the Shinya Yamanaka laboratory, CiRA, Kyoto University; Japan Agency for Medical Research and Development, Grant/Award Numbers: JP20he1122001, JP20jk0110021, JP20nk0101627, JP20wm0125003

Abstract

Background: The gold standard for coronavirus disease (COVID-19) diagnosis has been the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA by nucleic acid amplification testing (NAAT). On the other hand, serological testing for COVID-19 may offer advantages in detecting possibly overlooked infections by NAAT.

Methods: To evaluate seroconversion of NAAT-negative pneumonia patients, immunoglobulin M (IgM) and IgG targeting the spike protein of SARS-CoV-2 were semiquantified by an immunofluorescence assay. Seroconversion was confirmed by another serological method, targeting the nucleocapsid protein.

Results: Eight suspected but unconfirmed COVID-19 pneumonia patients (median age, 39 years; range, 21–55) were included. The median period between symptom onset and NAAT sample collection was 6 days (2–27 days). None of them had tested positive for SARS-CoV-2 by NAAT. In contrast, all eight patients revealed seropositivity with the two serological methods, indicating actual seroconversion against SARS-CoV-2. The median period between onset and blood sampling was 26.5 days (7–51 days).

Conclusion: Eight patients with COVID-19 pneumonia, initially tested negative for SARS-CoV-2 by NAAT, were finally confirmed of the diagnosis by serological testing. To cover the whole spectrum of this heterogenous infectious disease, serology testing should be implemented to the multitiered diagnostic algorithm for COVID-19.

KEYWORDS
COVID-19, diagnosis, immunoglobulin, SARS-CoV-2, serologic tests, viral tropism

1 | INTRODUCTION

Human history is often described as man's endless battle against infectious diseases. None of these battles would have ever settled without the development of reliable diagnostic measures. Two distinct approaches exist in the diagnosis of an infectious disease. One approach aims to catch the pathogen by a nucleic acid or antigen detection in materials derived from biological specimens. On the other hand, serological testing analyzes the host immune response against the pathogen. Both approaches harbor unique pros and cons, and thus are often used in combination to form a multitiered diagnostic algorithm. The fundamental structure of this multitiered diagnostic approach shall be strictly followed even when we encounter a novel pathogen, as in the current pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Since the outbreak at the end of 2019, the gold standard for coronavirus disease (COVID-19) diagnosis has been the detection of the unique RNA sequences of the virus by nucleic acid amplification testing (NAAT).[1] However, NAAT has substantial limitations. Study groups have reported that the viral load of SARS-CoV-2 in the upper respiratory tracts peaks out within days from symptom onset, and the window of viral detection by NAAT may be limited up to 20 days from disease onset.[2] In contrast, the rise in antibody titer observed as early as 5 days from disease onset may last up to 4 months.[3,4] Considering these reports, serological testing for COVID-19 may offer advantages in detecting low viral load infections and in extending the window period for disease recognition as well.[5] Combining both approaches shall potentially cover the whole spectrum of the heterogenous SARS-CoV-2 infection and offer a suitable diagnostic algorithm. Herein, we report a case series of eight patients with pneumonia who, although had initially tested negative for NAAT, were finally confirmed of COVID-19 by serological testing.

2 | MATERIALS AND METHODS

Immunoglobulin M (IgM) and IgG targeting the spike protein of the virus were detected by a lateral flow immunofluorescence assay kit (SARS-CoV-2 IgM and IgG Quantum Dot Immunoassay, Mokobio Biotechnology R&D). The assay was carried out according to the manufacturer's instructions: 20 µl of undiluted sera, followed by 100 µl of running buffer provided in the kit, were applied to the assay cassette. The fluorescence signal was semiquantified by an immunofluorescence analyzer (Mokosensor-Q100, Mokobio Biotechnology R&D). To increase precision of the diagnosis, IgG seroconversion was confirmed orthogonally using another serological method,[6] targeting the nucleocapsid protein (Anti-SARS-CoV-2 NCP ELISA [IgG], Euroimmun AG). Patient sera were diluted by 1:100 and assessed in duplicates. The absorbance at the wavelength of 450 nm was measured by Varioskan LUX (Thermo Fisher Scientific). Seropositivity for both assays was determined according to cut-off values provided by the manufacturers.

2.1 | Informed consent

All patients provided written consent to participate in this study. This study was approved by the institutional review board (#2020-003).

3 | RESULTS

Eight patients (five men and three women; median age, 39 years; range, 21–55) presented with fever and mild-to-severe pneumonia at the St. Marianna University Hospital, Kanagawa, Japan, between April and June 2020. Five of the eight patients had a history of having had contacts with COVID-19 patients before the onset of symptoms. None had comorbidities. Their chest computed tomography scans all showed the typical appearance of COVID-19 pneumonia: all cases showed bilateral peripheral ground-glass opacities and/or consolidations in at least one lung segment. Other respiratory pathogens, such as *Mycoplasma pneumoniae*, Influenza A and Influenza B viruses, were ruled out by negative results of either antigen or serological tests. Half of the cases required oxygen therapy under hospitalization, including one patient who received invasive mechanical ventilation (Patient 8). All inpatients were discharged within 44 days in stable conditions. The other four patients were managed as outpatients and declared recovery within 14 days from disease onset.

Nasopharyngeal swabs, sputum, and blood samples from the patients were repeatedly collected for laboratory examinations during hospitalization or hospital visits (Figure 1). A total of 12 nasopharyngeal swab samples and one sputum sample (Patient 8) was collected from the eight patients (median 1 per patient; range, 1–3). NAAT consisted of 10 reverse transcription polymerase chain reaction tests and three loop-mediated isothermal amplification tests. Consequently, none of the NAATs turned positive during their clinical course. The median period between symptom onset and NAAT sample collection was 6 days (range, 2–27 days). Fourteen blood samples were evaluated by the serological analysis for IgG and IgM. The median period between onset and blood sampling was 26.5 days (range, 7–51 days). All eight patients revealed seropositivity for anti-spike IgM and/or IgG during their course of illness (Supporting Information Table), indicating actual seroconversion against SARS-CoV-2. Although these cases remained negative for NAAT, we considered, with the two positive results from independent SARS-CoV-2-specific antibody assays, that

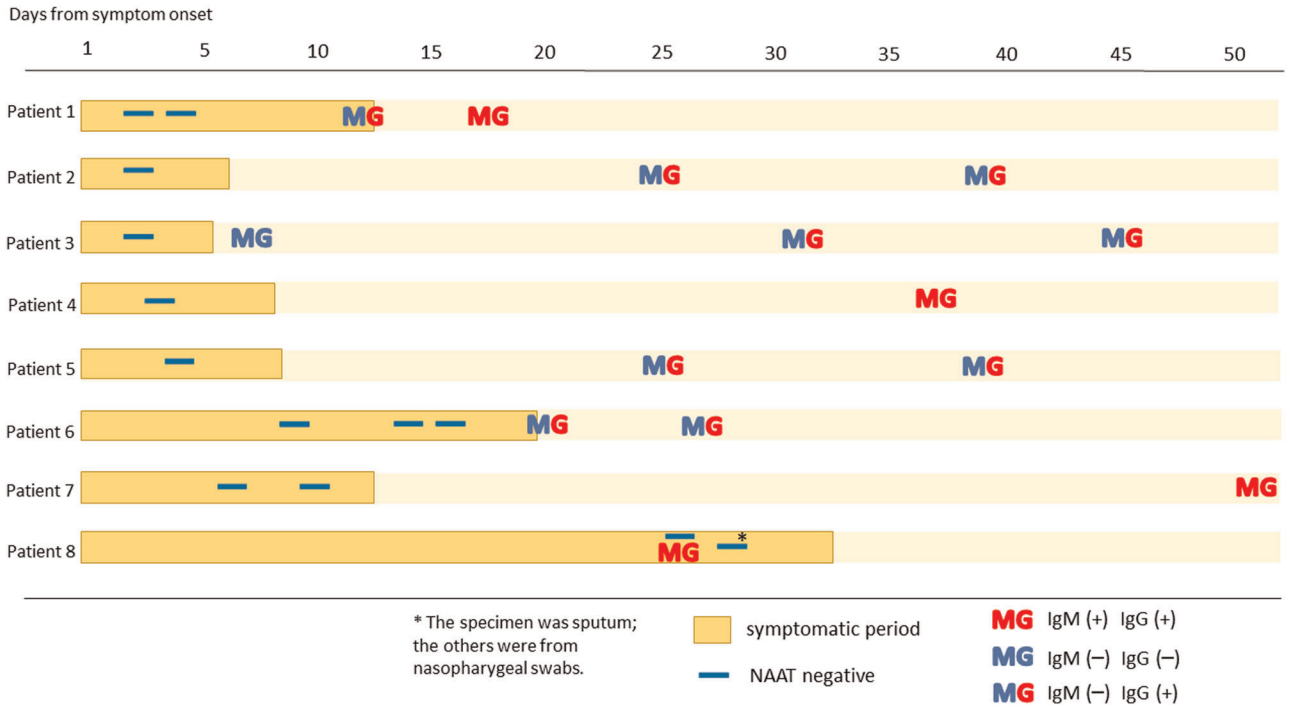

FIGURE 1 Diagnostic testing results of serologically confirmed COVID-19 patients. A total of 12 nasopharyngeal swab samples and one sputum sample (Patient 8) was collected from the eight patients (median 1 per patient; range, 1–3). NAAT consisted of 10 reverse transcription polymerase chain reaction tests and three loop-mediated isothermal amplification tests. The median period between symptom onset and NAAT sample collection was 6 days (range, 2–27 days). Fourteen blood samples were evaluated by a semiquantitative serological analysis for IgM and IgG targeting the spike protein of SARS-CoV-2 (SARS-CoV-2 IgM and IgG Quantum Dot Immunoassay, Mokobio Biotechnology R&D). Seroconversion was confirmed by another serological method (Anti-SARS-CoV-2 NCP ELISA [IgG], Euroimmun AG). The median period between onset and blood sampling was 26.5 days (range, 7–51 days). COVID-19, coronavirus disease; IgG, Immunoglobulin G; IgM, Immunoglobulin M; NAAT, nucleic acid amplification testing; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

positive predictivity is sufficiently high to declare them as "serologically confirmed COVID-19" cases.[6]

4 | DISCUSSION

Serological analyses for SARS-CoV-2 have been predominantly applied for epidemiological and surveillance purposes.[6,7] The discussion over whether applying serological testing for individual patient care shall be beneficial has not reached a clear consensus.[1,8] Considering the limited diagnostic performance of NAAT, however, serological testing should be readily considered, not exactly as an alternative but as the indispensable counterpart of the COVID-19 diagnostic algorithm.[9,10]

As the growing literature describes the nature of COVID-19, we are coming to realize the difficulty in defining the truly affected population. This is mainly due to disease heterogeneity, that is, the variety in clinical manifestations and, as well, the unique tropism of SARS-CoV-2.[2–6] Indeed, false-negative results can lead to overlooking the diagnosis and cause serious consequences, especially in vulnerable communities (e.g., health facilities).[10] To combat this situation, it is necessary to grasp the overall spectrum of the disease by implementing all different tiers of the fundamental diagnostic approaches. Following the diagnostic norms, combining NAAT and serological testing shall maximize disease recognition and is therefore essential in finding our way back to normal.

ACKNOWLEDGMENTS

This study was supported by Japan Agency for Medical Research and Development (AMED) under Grant number JP20wm0125003 (Yasutoshi Kido), JP20he1122001 (Yasutoshi Kido), JP20nk0101627 (Yasutoshi Kido), and JP20jk0110021 (Yu Nakagama). We are grateful for the COVID-19 Private Fund (to the Shinya Yamanaka laboratory, CiRA, Kyoto University). ELISA data were acquired at the Research Support Platform, Osaka City University Graduate School of Medicine. Serological tests were provided by Mokobio Biotechnology R&D, USA. This study was performed at St. Marianna University Hospital, Kanagawa and Osaka City University Graduate School of Medicine, Osaka, Japan.

AUTHOR CONTRIBUTIONS

Tomoya Tsuchida, Yuko Nitahara, Yasutoshi Kido, and Yu Nakagama designed the study; Tomoya Tsuchida, Shotaro Suzuki, Yuko Komase, Yukitaka Yamasaki, Mitsuru Imamura, Kimito Kawahata, Hiroyuki Kunishima, Shigeki Fujitani, and Masamichi Mineshita selected

patients and acquired clinical data; Tomoya Tsuchida, Shotaro Suzuki, Yuko Komase, Katherine Candray, and Yu Nakagama performed immunological assays; Tomoya Tsuchida, Yuko Nitahara, Yasutoshi Kido, and Yu Nakagama wrote the manuscript and contributed to analysis and interpretation of the data; Yukitaka Yamasaki, Hiroyuki Kunishima, Masamichi Mineshita, and Takahide Matsuda contributed to a critical discussion of the manuscript. All authors approved the final manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jmv.26949.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Tomoya Tsuchida https://orcid.org/0000-0002-8517-3941
Yuko Nitahara http://orcid.org/0000-0002-5026-478X
Yuko Komase https://orcid.org/0000-0001-5042-8476
Yasutoshi Kido https://orcid.org/0000-0003-3615-2631
Yu Nakagama https://orcid.org/0000-0001-9780-9719

REFERENCES

1. Centers for Disease Control and Prevention. Overview of testing for SARS-CoV-2 (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html. Accessed November 15, 2020.
2. Fang FC, Benson CA, Del Rio C, et al. COVID-19—lessons learned and questions remaining. *Clin Infect Dis*. 2020:ciaa1654. https://doi.org/10.1093/cid/ciaa1654
3. Guo L, Ren L, Yang S, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). *Clin Infect Dis*. 2020;71(15):778-785.
4. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral immune response to SARS-CoV-2 in Iceland. *N Engl J Med*. 2020;383(18):1724-1734.
5. 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(4):262-267.
6. Yoshiyama T, Saito Y, Masuda K, et al. Prevalence of SARS-CoV-2–specific antibodies, Japan, June 2020. *Emerg Infect Dis*. 2021;27(2):628-631.
7. Alter G, Seder R. The power of antibody-based surveillance. *N Engl J Med*. 2020;383(18):1782-1784.
8. Yamamoto S, Saito M, Nagai E, et al. Seroconversion against SARS-CoV-2 occurred after the recovery in patients with COVID-19. *J Med Virol*. 2020;93:1-3.
9. Knight D, Irizarry-Alvarado J. SARS-CoV-2 serological testing changes disease management in a PCR-negative patient. *BMJ Case Rep*. 2020;13(8):1-3.
10. Woloshin S, Patel N, Kesselheim AS. False negative tests for SARS-CoV-2 infection—challenges and implications. *N Engl J Med*. 2020;383(6):3.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Tsuchida T, Nitahara Y, Suzuki S, et al. Back to normal; serological testing for COVID-19 diagnosis unveils missed infections. *J Med Virol*. 2021;93:4549-4552. https://doi.org/10.1002/jmv.26949