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Note

A patient with human coronavirus NL63 falsely diagnosed with COVID-19; Lesson learned for the importance of definitive diagnosis

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ABSTRACT

The gold standard for the diagnosis of coronavirus disease 2019 (COVID-19) is a nucleic acid detection test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which may occasionally reveal false-positive or false-negative results. Herein, we describe a case of a patient infected with human coronavirus NL63 (HCoV-NL63) who was falsely diagnosed with COVID-19 using the Ampdirect™ 2019-nCoV detection kit (Shimadzu Corporation, Japan) and SARS-CoV-2 Detection Kit (TOYOBO co., ltd.), and was admitted to a COVID-19 hospital ward. We suspected a cross-reaction between HCoV-NL63 and SARS-CoV-2; however, the reported genome sequences of HCoV-NL63 and N1/N2 primers for SARS-CoV-2 do not correspond. Thus, the PCR result was supposed to be a false positive possibly due to contamination or human error. Although the issue of a false-negative result has been the focus of much attention to prevent the spread of the disease, a false positive is fraught with problems as well. Physicians should recognize that unnecessary isolation violates human rights and a careful diagnosis is indispensable when the results of laboratory testing for COVID-19 are unclear. Generally, in cases such as a duplicate PCR test was partially positive, either N1 or N2 alone was positive, PCR testing for two or more target regions resulted in a positive only for single region, a high cycle threshold (>35) was obtained, a false positive should be suspected. Especially, when these conditions coincide, we should recognize the high likelihood of a false positive.

As of February 2021, the coronavirus disease 2019 (COVID-19) global pandemic, due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has continued to spread worldwide. More than 100 million incidences and two million deaths have been reported globally as of January 2021, according to the World Health Organization statistics [1]. Since COVID-19 is regulated by the Infectious Diseases Control Law as a Class II designated infectious disease in Japan, hospitalization is officially recommended to the patients when diagnosed for the purpose of preventing the disease from spreading out.

COVID-19 burdens the patients physically, socially, and psychologically; thus, the diagnosis should be as accurate as possible. Polymerase chain reaction (PCR) testing provides a diagnostic value with high accuracy; however, it can result in false-positive and false-negative results in some cases [2]. Although the issue of a false negative, as a major cause of misdiagnosis, has been the focus of much attention [3], a serious consequence of false positives should also be shared among physicians [4]. Herein, we describe a patient infected with human coronavirus NL63 (HCoV-NL63) who was falsely diagnosed with COVID-19 and admitted to a COVID-19 hospital ward.

In February 2021, a 67-year-old woman with eosinophilic granulomatous polyangiitis, who had been taking prednisone 5 mg per day for 27 years, visited her primary physician with fever of 37 °C, nasal secretions, and cough. She had no history of contact with COVID-19 patients or travel to COVID-19 endemic regions. She underwent salivary PCR examination for SARS-CoV-2 using Ampdirect™ 2019-nCoV detection kit (Shimadzu Corporation, Japan), in which two sequences specific to SARS-CoV-2, N1 and N2, as defined by the Centers for Disease Control and Prevention (the United States), were targeted as primers and probes. We used Applied Biosystems™ QuantStudio™ 5 (Thermo Fisher Scientific) and found that N1, but not N2, was amplified, with cycle
threshold (CT) values of around 40 (Fig. 1). The amplification test was repeated using the same specimen and the results were similar. For further confirmation, we also applied the sample to another PCR kit (SARS-CoV-2 Detection Kit, TOYOBO Co., Ltd.), which again resulted in a positive result for N1 alone. Despite the partially-positive results, the patient was diagnosed with COVID-19 and hospitalized to a designated medical institution.

The patient appeared fine, and her vital signs were stable on admission. Laboratory examination showed a slight elevation of serum C-reactive protein (2.23 mg/dL), and chest computed tomography revealed no evidence of pneumonia. However, this case was considered to be high-risk because of her underlying disease and long-term treatment with immunosuppressive therapy. We thus initiated remdesivir after obtaining the patient’s consent, although it was off-label use in the absence of pneumonia. At this point, we suspected that the result of the PCR test for SARS-CoV-2 was a false positive and confirmed it using other measurement methods. Applying the nasopharyngeal specimen, BD MAX™ Open System (Becton, Dickinson and Company) was negative for SARS-CoV-2. The results of the FILMARRAY® Respiratory 2.1 Panel (bioMérieux), a multiplex PCR test for the detection of respiratory pathogens, including 19 viruses (including SARS-CoV-2) and 4 bacteria, was also negative for SARS-CoV-2, while being positive for HCoV-NL63, a conventional seasonal coronavirus causing the common cold. Based on these test results, the patient was diagnosed with HCoV-NL63 infection. Thus we suspected a cross-reaction between HCoV-NL63 and Ampdirect™ 2019-nCoV detection kit and SARS-CoV-2 Detection Kit. We referred to the reported genome data of HCoV-NL63 (Accession number: NC_005831) and examined whether a corresponding sequence site can align with the sequence primers used in the test kit: N1 forward primer: 5′-GAC CCC AAA ATC AGC GAA AT-3′; N1 reverse primer: 5′-TCT GGT TAC TGC CAG TTG AAT CTG-3′; N2 forward primer: 5′-TCT GGT TAC TGC CAG TTG AAT CTG-3′; and N2 reverse primer: 5′-GGG CGA CAT TCC GAA GAA-3′. As a result, they are not identical, and mis-annealing of highly homologous sequences cannot be expected. Inquiry into the manufacture did not find any similar reports in the past. Collectively, we concluded that this case was false positive by instrument or human error, possibly due to contamination.

This case highlights the importance of accurate diagnosis of COVID-19. The disease is a designated infectious disease with high infectivity, requiring legal isolation. However, unnecessary isolation can violate human rights. Herd immunity by vaccination has yet to be developed, and the current status will continue for a while. Previous reports have referred that results of high CT values (e.g., >35), especially in a low prevalent area [6] and in case of a positive result for single target [7],
should be interpreted with careful attention. When a laboratory diagnosis is unclear, a repeated investigation using different testing devices or approaches is essential for the definitive diagnosis of the disease.

Authorship statement

All authors meet the ICMJE authorship criteria; Yuki Otsuka, Hideharu Hagiya, Yasuhiro Nakano, Daisuke Omura, Kou Hasegawa and Haruto Yamada managed the patient. Yuki Otsuka drafted the manuscript. Koji Iio was responsible for laboratory experiment. Tomoyuki Honda was a technical adviser on the genetic analysis. Fumio Otsuka supervised the work. All authors contributed to the manuscript writing.

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Informed consent

Patient written informed consent for publication was obtained.

Declaration of competing interest

None.

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