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 the 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 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 patient was supposed to be false positive by the instrument, possibly due to contamination. 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, for instance if the duplicate PCR test is partially positive or the CT value is high.

Keywords: Human coronavirus; Coronavirus disease 2019; Severe acute respiratory syndrome coronavirus 2

Manuscript

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 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 (**Fig. 1**). The amplification test was repeated and the results were identical. Although the result of PCR test was partially positive, the patient was diagnosed with COVID-19 and hospitalized to a designated medical institution.

The patient appeared fine, and her vital signs were stable. Laboratory examination showed a slight elevation of serum C-reactive protein (2.23 mg/dL), and chest computed tomography revealed no evidence of pneumonia. Considering this case as high-risk because of her underlying disease and long-term treatment with steroid therapy, administration of remdesivir was initiated. At this point, we suspected that the result of the PCR test for SARS-CoV-2 was 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 negative for SARS-CoV-2 but positive for HCoV-NL63, a conventional seasonal coronavirus causing the common cold. Based on these test results, the patient was diagnosed with seasonal coronavirus infection, but not COVID-19. After consultation with the local healthcare center, the patient was discharged from the hospital on the second day of admission. Only one dose of remdesivir was administered, and no adverse effects were observed. After discharge, no manifestation of COVID-19 development was reported.

Clinical differentiation of COVID-19 from other respiratory infectious diseases is very challenging because COVID-19 causes a wide variety of manifestations, such as cold-like symptoms and fatal pneumonia [5]. Thus, physicians worldwide rely on nucleic acid detection tests for diagnosis.

In this case, only N1 domain of SARS-CoV-2 was amplified reproducibility and later it turned out to be HCoV-NL63. Thus we suspected a cross-reaction between HCoV-NL63 and Ampdirect™ 2019-nCoV 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’-TTA CAA ACA TTG GCC GCA AA-3’; and N2 reverse primer: 5’-GCG 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, 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 and requires 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. When a laboratory diagnosis is unclear, as in the duplicate PCR test is partially positive or the CT value is high, repeated testing using different testing devices or approaches is essential for the definitive diagnosis of the disease.

Declaration of interest

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Informed consent

Patient written informed consent for publication was obtained.

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Figure Legends

Fig 1. The amplification plot of salivary polymerase chain reaction.

Using the Ampdirect™ 2019-nCoV detection kit (Shimadzu Corporation, Japan) and Applied BiosystemsTM QuantStudioTM 5 (Thermo Fisher Scientific), only N1 (red line), but not N2 (blue line), was amplified from the patient's saliva. The green lines denote the internal control.