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Two cases of false-positive COVID-19 RT-PCR results

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Abstract

Reverse-transcription polymerase chain reaction (RT-PCR) is the gold standard for the diagnosis of coronavirus disease (COVID-19) in clinical practice. False-positive results in RT-PCR assays may have serious implications for patients. We aimed to investigate false-positive RT-PCR tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We performed a retrospective case series analysis. Among 46 patients who were diagnosed with COVID-19 by RT-PCR and admitted in our hospital during our study period, two patients were scheduled for surgery and underwent RT-PCR testing for SARS-CoV-2 for routine pre-operative screening. Subsequent RT-PCR retest and antibody test results for SARS-CoV-2 were consistently negative for both patients. Although COVID-19 diagnostic tests are highly specific, false-positive results can occur if there is a low pre-test probability. Our study emphasizes that clinicians should deal with unexpected positive results with diligence and caution. We considered the initial RT-PCR results of the two patients to be false positive. It is important to interpret test results with caution, especially when patients are asymptomatic with no exposure or circumstances that could increase infection-related risks.

Keywords: COVID-19, SARS-CoV-2, reverse-transcription polymerase chain reaction, false positive

Introduction

Since the emergence of the coronavirus disease (COVID-19) in December 2019 in China, it has rapidly spread worldwide, and the World Health Organization declared it a pandemic on 11 March 2020 (Omori, 2020). Early detection and isolation are critical factors because no curative antiviral drugs against COVID-19 exist and vaccine distribution remains limited (Wang, 2020). The gold standard for COVID-19 diagnosis in clinical practice is the nucleic acid-based testing of nasopharyngeal swabs for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using reverse transcription-polymerase chain reaction (RT-PCR) (Wiseman, 2020). However, a major concern is the accuracy of this test. False negative results in infected persons who do not isolate possibly spread the infection to others; while false positive results label a healthy person as infected, leading to consequences such as unnecessary quarantine and contact tracing (Woloshin, 2020). RT-PCR assays in the UK were reported to have analytical sensitivities and specificities of >95% (Watson, 2020; Mayers and Baker, 2020). Though the current specificity of RT-PCR in clinical practice is unknown, preliminary reports have estimated it to be 96%–99.2% (Mayers and Baker, 2020). This study was conducted to explore false-positive results in RT-PCR tests for SARS-CoV-2 detection in a clinical setting during the COVID-19 pandemic.

Material and Methods

We conducted a retrospective case series study. We reviewed the medical records of all patients with COVID-19 admitted to the Kawanishi Municipal Hospital (KMH) between 1 March and 31 July 2020. The Ethics Committee of KMH approved the data collection and analysis (approval no. 202008). All study procedures were carried out in compliance with the relevant laws and guidelines, in accordance with the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from patients with suspected false-positive results. All patients were diagnosed using RT-PCR to detect the SARS-

CoV-2 nucleic acid from nasopharyngeal swab or sputum specimens. Epidemiological history, clinical characteristics, physical examinations, test results, treatments, and outcome data were extracted from the hospital's electronic medical records. If the admitted patients presented without an epidemiological history or symptoms and all clinical and radiological findings were negative for COVID-19, we repeated the RT-PCR test for SARS-CoV-2 to confirm the initial positive results in the same week. If the results were negative in the repeated RT-PCR test, we conducted serological tests 3 weeks after the initial positive RT-PCR test. If all serological test results were negative, we determined the initial RT-PCR result to be false positive. In cases where we suspected initial RT-PCR results to be false positives, we tested the family members to confirm that the patients did not have any unknown exposure histories using RT-PCR to detect SARS-CoV-2 nucleic acid in nasopharyngeal swab specimens.

RT-PCR assay

RT-PCR testing for patients diagnosed at KMH was performed using nasopharyngeal swabs collected using FLOQSwabs (COPAN, Brescia, Italy). The swabs were placed in normal saline and transported at 4°C to the Hyogo Prefectural Institute of Health Sciences, Hyogo, Japan. Samples were analysed within 48 h at the institute. RNA was extracted from 140 µL of the supernatant using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). The RT-PCR assays were performed according to the manual of the National Institute of Infectious Diseases (NIID) (National Institute of Infectious Diseases, 2019). A real-time one-step RT-PCR was performed using the TaqMan™ Fast Virus 1-Step Master Mix #4444436 (Thermo Fisher Scientific, Waltham, MA, USA), TaqMan primer-probe customized N and N2 sets (NIID), and QuantStudio 12K Flex Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). We used this method of retesting to confirm the initial positive results when patients were from other facilities.

For patients diagnosed at other facilities and transferred to KMH, we inquired with the initial facility for detailed information on the RT-PCR method used only when our results determined the initial positive results to be false. For Case 1, nasopharyngeal swabs were collected using FLOQSwabs (COPAN, Brescia, Italy). The swabs were placed in a transport medium (viral transport medium) and transported at 4°C to a commercial laboratory (BML Inc., Tokyo, Japan). Samples were analysed within 48 h at BML. RNA was extracted from 200 µL of the supernatant using the MagNA Pure 96 External Lysis Buffer (Roche, Basel, Switzerland) and MagNA Pure 96 DNA and Viral NA SV Kit (Roche, Basel, Switzerland). The analysis was performed using the Light Cycler® Multiplex RNA Virus Master (Roche, Basel, Switzerland), Light Mix® Modular SARS-CoV (COVID-19) E-gene (Roche, Basel, Switzerland), and MagNA Pure 96 system (Roche, Basel, Switzerland). In Case 2, nasopharyngeal swabs were collected using polyester swabs, 1PX1503P (Japan Cotton Buds, Tokyo, Japan). The swabs were placed in normal saline and transported at 4°C to a

laboratory in our hospital. Samples were analysed within 1 h. RNA was extracted from 140 µL of the supernatant using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). The analysis was performed using the THUNDERBIRD Probe One-step qRT-PCR kit (Toyobo, Osaka, Japan), both CoV (COVID-19) E-gene (Roche, Basel, Switzerland) and TaqMan primer-probe customized N2 (NIID) sets, and Eppendorf realPlex4 Master cycler (Eppendorf, Hamburg, Germany).

Anti-SARS-CoV-2 antibody assay:

For serological testing, we first conducted simplified immunochromatographic assays to detect immunoglobulin G (IgG) and immunoglobulin M (IgM) against SARS-CoV-2 (KURABO industries, Osaka, Japan). We also conducted an electrochemiluminescence immunoassay (ECLA) and three chemiluminescent immunoassays (CLIAs) at Osaka University Hospital, according to the manufacturers' instructions. We used the Elecsys anti-SARS-CoV-2 assay and Cobas e 411 analysers (Roche, Basel, Switzerland) for ECLA. Elecsys assay can detect several types of SARS-CoV-2 antibodies, primarily IgG; a cut-off index (single sample/cut-off) ≥ 1.0 indicated a positive result (Muench, 2020). For CLIA, we used the Alinity SARS-CoV-2 IgG assay with the Alinity analyser (Abbott Diagnostics, Chicago, USA) (Bryan, 2020), SARS-CoV-2 Total Assay with the Centaur XPT analyser (Siemens, München, Deutschland), (Nguyen, 2020) and ACCESS SARS-CoV-2 IgG assay with the Access 2 analyser (Beckman Coulter, Brea, USA) (Péré, 2020). We followed the manufacturers' instructions and recommended positive cut-offs for all three assays, namely ≥ 1.40 for Alinity, ≥ 1.0 for Centaur XPT, and ≥ 1.0 for Access 2.

Results

A total of 46 patients with positive RT-PCR results for SARS-CoV-2 were admitted to KMH during the study period. The median age of the patients was 50 (range 1–92) years. Twenty-six patients (56.5%) had household exposures. Samples collected for RT-PCR testing were nasopharyngeal swabs (45 cases) or sputum (1 case). Among the 46 patients, 12 (26.0%) were asymptomatic and 34 (74.0%) were symptomatic. Among the 12 asymptomatic patients, ten had household exposures. The most frequent symptoms were fever > 37.5 °C (n=18, 52.9%), followed by cough (n=11, 32.4%). Other less common symptoms were loss of taste or smell (n=6, 17.6%), diarrhoea (n=6, 17.6%), vomiting (n=3, 8.8%), and dyspnoea (n=3, 8.8%).

Two of the 46 patients had false-positive RT-PCR results for SARS-CoV-2. Both patients were scheduled for surgery at other hospitals. They had undergone RT-PCR testing for SARS-CoV-2 as part of the routine pre-operative screening and tested positive. They were subsequently admitted to KMH for COVID-19 treatment.

Case 1

A 40-year-old woman with a history of asthma, sleep apnea syndrome, and Crohn's disease tested positive for COVID-19 in a hospital and was transferred to KMH. The cycle threshold (Ct) values were 36.76 and 36.14, and the patient's condition was diagnosed as COVID-19. The patient took regular medications, including mesalamine, probiotics (Clostridium butyric and Bifidobacterium sp.), adalimumab, escitalopram oxalate, lamotrigine, and flunitrazepam. RT-PCR retests of the nasopharyngeal swabs were conducted on hospital days 1 and 4, and the test results were negative for both. The first antibody test was conducted at KMH on hospital day 4 using an immunochromatography kit (KURABO industries, Osaka, Japan), and the test results were negative. On hospital day 6, the patient presented with mild dyspnoea and wheezing, but the peripheral capillary oxygen saturation (SpO2) level remained at 95%. However, the patient recovered from all the symptoms within a day without any treatment. We attributed the mild symptoms to the patient's comorbidity of asthma rather than to COVID-19. The patient was discharged on hospital day 7 without any treatment. Patient was asymptomatic except for a mild asthma attack. ECLA and CLIA were conducted at the Osaka University Hospital after 13 days. All serological test results were negative for Ig M and Ig G. Additionally, the patient's family also tested negative for SARS-CoV-2.

Case 2

A 60-year-old woman with a history of hypertension, hyperlipidaemia, and a tumor of the acoustic nerve tested positive for COVID-19 in another hospital and was transferred to KMH. The Ct values were 33.64 and 33.05 for the N2 set; the initial E-gene PCR assay result was negative. The hospital confirmed the test results to be positive; hence, the patient was transferred to KMH. The patient was taking regular medications, including nifedipine and rosuvastatin calcium. RT-PCR retests of the

nasopharyngeal swabs were conducted on hospital days 6 and 7; the test results were negative for both. The first antibody test was conducted at KMH on hospital day 6 using an immunochromatography kit (KURABO industries, Osaka, Japan), and the test results were negative. The patient was discharged on hospital day 8 without any treatment or symptoms. ECLA and CLIA were conducted at the Osaka University Hospital after 14 days. All serological tests were negative for Ig M and Ig G. The patient's family also tested negative for SARS-CoV-2.

The laboratory and chest computed tomography findings on admission for both cases are shown in Table 1 and Fig. 1, respectively. The indices of ECLA and CLIA are shown in Table 2.

Discussion

We came across two false-positive SARS-CoV-2 RT-PCR test results. Both patients tested positive during pre-operative screening. They did not have any symptoms or radiological manifestations that suggested the diagnosis of COVID-19 throughout their hospitalization. Additionally, all RT-PCR and serological retest results were negative. Cases 1 and 2 were discharged without any treatment and complications on hospital days 7 and 8, respectively. Our hospital, KMH, is a designated priority medical institute for COVID-19 treatment in the Hyogo prefecture. During the study period (March 2020 to July 2020), 46 COVID-19 patients were admitted; among them, two cases were identified as false positives. This suggests that even with a highly specific test such as RT-PCR, false positives can occur when performed on patients with no history of exposure or symptoms of COVID-19, for example, during pre-operative screening.

Although Japan experienced the COVID-19 outbreak during the study period, the situation was better than that in the US, Europe, and other countries. The mean number of confirmed cases reported in a day in Japan during the study period was 234 (0.19 per 100,000) (Japanese Ministry of Health, Labour and Welfare, 2020; Péré, 2020). On April 7, 2020, the Government declared a state of emergency in five prefectures, including Hyogo, where our hospital is located (Prime Minister of Japan and his Cabinet, 2020). Hospitals and surgeons were advised to postpone or cancel scheduled elective surgeries as per the government's "basic policies" issued at the time of declaration (Prime Minister of Japan and his Cabinet, 2020). Under such epidemiological and social circumstances, some hospitals in Japan started pre-operative screening with RT-PCR tests. However, considering the low prevalence of infection among asymptomatic general cohorts, false positive results were unavoidable, even with high specificity tests. Therefore, the results of our study should be accepted as a natural consequence.

Even in a test with high specificity, positive results cannot determine infection if the pre-test probability is low. Clinicians should diligently handle positive results in asymptomatic patients. The pre-test probability will steadily decrease when the proportion of screening asymptomatic cases increases (Surkova, 2020). The government of Japan assumes that the RT-PCR assays' sensitivity and specificity for SARS-CoV-2 are 70% and 99.9%, respectively (Japanese Cabinet Secretariat, 2020). The positive predictive value was 41% with the test, assuming a pre-test probability of 0.1%. This pre-test probability value (prevalence) was derived from the peak incidence and duration of infection (Japanese Ministry of Health, Labour and Welfare, 2020). However, a pre-test probability of 0.1% is very high for asymptomatic general cohorts because most confirmed COVID-19 cases have some symptoms and/or exposure histories. Mizumoto et al. reported that the estimated asymptomatic proportion was 17.9% on board the Diamond Princess Cruise ship in Japan (Mizumoto, 2020). If people had no exposure, they would not seek medical care or undergo tests. With a lower pre-test probability than the government's assumption, the real world's positive predictive value would be very low. Patients unlikely to be infected with COVID-19 should not be tested based on the clinician's discretion to prevent false-positive results from affecting clinical management and patients' normal lives.

This study has two major limitations. First, it was difficult to determine a generalized specificity or positive predictive value for RT-PCR because this was a single-center case series study. Although we found two false-positive cases among the 46 RT-PCR confirmed COVID-19 patients admitted at our hospital, the true rate of false positives remains unknown. Further studies are needed to determine the specificity or positive predictive value of RT-PCR. Second, no false-negative results were identified in this study. There is no gold standard for testing the SARS-CoV-2 infection yet. Therefore, it is possible that the confirmation test results were all false negatives, and the initial RT-PCR test result was true. However, neither case had any exposure histories, related symptoms, or radiological manifestations. The confirmation RT-PCR tests were conducted twice, and both results were negative. Antibody tests performed using four different laboratory methods and one simplified kit consistently yielded negative results. We believe that the antibody test results are sufficiently reliable (Bryan, 2020; Muench, 2020; Nguyen, 2020; Péré, 2020). In addition, we tested the household members/families of the patients using RT-PCR, and all of them tested negative for SARS-CoV-2.

Conclusion

Considering all results, we concluded that the initial RT-PCR results were indeed false-positive results. Two of 46 patients among the RT-PCR tests were identified as false positive for SARS-CoV-2. This suggests that we should interpret the test results with caution if patients are asymptomatic and have no exposure histories or complications that raise the pre-test probability.

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Conflict of Interest

None to declare

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Table 1. Laboratory findings

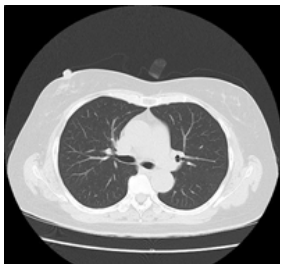
Parameter	Case 1	Case 2
WBC (/μL)	8610	6500
Neutrophil (%)	57.8	61.1
Lymphocyte (%)	31.6	30.9
Monocyte (%)	8	6.3
Eosinophil (%)	2.1	1.4
Basophil (%)	0.5	0.3
RBC (× 10 4/μL)	454	477
Haemoglobin (g/dL)	13.9	14.2
Platelet (g/dL)	22.9	24.3
AST (U/L)	30	20
ALT (U/L)	53	21
ALP (U/L)	126	196
TB (mg/dL)	0.6	0.5
LDH (U/L)	230	205
BUN (mg/dL)	17	16
CRE (mg/dL)	0.65	0.71
TP (g/L)	7.9	6.7
CRP (mg/L)	0.28	0.03
Procalcitonin (ng/L)	0.03	≤0.02
PT (%)	128.4	89.1
D-D dimer (μg/mL)	0.6	0.6

WBC, white blood cells; RBC, red blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CRE, creatinine; TP, total protein; CRP, C-reactive protein; PT, prothrombin time.

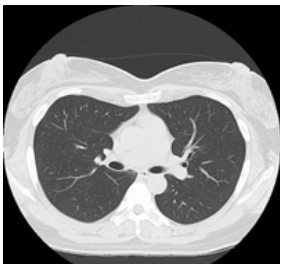
Table 2. Results of serological tests

Assay	Analyzer	Cut-off	Case 1	Case 2
Elecsys Anti-SARS-CoV-2 (Roche)	Cobas e 411	(-): <1.0 (+): ≥1.0	0.123	0.081
Alinity SARS-CoV-2 IgG (Abbott)	Alinity	(-): <1.4 (+): ≥1.4	0.02	0.01
SARS-CoV-2 Total (Siemens)	Centaur XPT	(-): <1.0 (+): ≥1.0	<0.05	0.47
Access SARS-CoV-2 IgG (Beckman Coulter)	Access 2	(-): ≤0.8 (±): 0.8< ~ <1.0 (+): ≥1.0	0.02	0.01

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IgG, immunoglobulin.



a



b

Fig. 1. Computed tomography on admission a) Case 1, b) Case 2 There were no abnormal findings

References

1. Bryan, A., Pepper, G., Wener, M.H., et al., (2020). Performance characteristics of the Abbott architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho, Journal of Clinical Microbiology, 58(8): e00941-20. doi:10.1128/JCM.00941-20
2. Japanese Cabinet Secretariat. The Second Japanese COVID-19 Expert Meeting, July 2020. Available at <https://www.cas.go.jp/jp/seisaku/ful/bunkakai/corona2.pdf> Accessed November 23, 2020.
3. Japanese Ministry of Health, Labour and Welfare. Antibody Possession Survey Results for COVID-19, June 2020. Available at <https://www.mhlw.go.jp/content/10906000/000640184.pdf> Accessed November 23, 2020.
4. Japanese Ministry of Health, Labour and Welfare. COVID-19 cases in Japan, July 2020. Available at <https://www.mhlw.go.jp/stf/covid-19/kokunainohasseijoukyou.html> Accessed November 23, 2020.
5. Mayers, C., Baker, K., (2020). Impact of false-positives and false-negatives in the UK's COVID-19 RT-PCR testing programme. 1:3-7. Available at <https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/895843/S0519_Impact_of_false_positives_and_negatives.pdf> Accessed February 19, 2021.
6. Mizumoto, K., Kagaya, K., Zarebski, A., Chowell, G., (2020). Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020, Euro surveillance, 25(10): 2000180. doi:10.2807/1560-7917.ES.2020.25.10.2000180
7. Muench, P., Jochum, S., Wenderoth, V., et al., (2020). Development and validation of the Elecsys anti-SARS-CoV-2 immunoassay as a highly specific tool for determining past exposure to SARS-CoV-2, Journal of Clinical Microbiology, 58(10): e01694-20. doi:10.1128/JCM.01694-20
8. National Institute of Infectious Diseases. Manual for the Detection of Pathogen 2019-nCoV ver.2.9.1. Available at <https://www.niid.go.jp/niid/images/lab-manual/2019-nCoV20200319.pdf> Accessed November 23, 2020.
9. Nguyen, N.N., Mutnal, M.B., Gomez, R.R., et al., (2020). Correlation of ELISA method with three other automated serological tests for the detection of anti-SARS-CoV-2 antibodies, PLoS ONE. 15(10): e0240076. doi: 10.1371/journal.pone.0240076
10. Omori, R., Mizumoto, K., Chowell, G., (2020). Changes in testing rates could mask the novel coronavirus disease (COVID-19) growth rate, International Journal of Infectious Diseases, 94: 116-118. doi: 10.1016/j.ijid.2020.04.021
11. Péré, H., Wack, M., Védie, B., et al., (2020). Sequential SARS-CoV-2 IgG assays as confirmatory strategy to confirm equivocal results: Hospital-wide antibody screening in 3,569 staff health care workers in Paris, Journal of Clinical Virology, 132: 104617. doi: 10.1016/j.jcv.2020.104617
12. Prime Minister of Japan and His Cabinet. (COVID-19) Declaration of a State of Emergency in Response to the Novel Coronavirus Disease (April 7), 2020. Available at <https://japan.kantei.go.jp/ongoingtopics/_00018.html> Accessed November 23, 2020.
13. Prime Minister of Japan and His Cabinet. (COVID-19) Declaration of a State of Emergency in Response to the Novel Coronavirus Disease (April 16), 2020. Available at <https://japan.kantei.go.jp/ongoingtopics/_00020.html> Accessed November 23, 2020.
14. Prime Minister of Japan and His Cabinet. Basic Means to Cope with COVID-19, May 2020. Available at <https://www.kantei.go.jp/jp/singi/novel_coronavirus/th_siryou/kihon_h(4.7).pdf> Accessed November 23, 2020.
15. Surkov, E., Nikolayev's, V., Dronies, F., (2020) False-positive COVID-19 results: Hidden problems and costs, The Lancet Respiratory Medicine, 8: 1167-1168. doi:10.1016/S2213-2600(20)30453-7
16. Wang, H., Li, X., Li, T., et al., (2020). The genetic sequence, origin, and diagnosis of SARS-CoV-2, European Journal of Clinical Microbiology & Infectious Diseases, 39: 1629-1635. doi:10.1007/s10096-020-03899-4
17. Watson, J., Whiting, P.F., Brush, J.E., (2020) Interpreting a COVID-19 test result, BMJ, 369: m1808. doi:10.1136/bmj.m1808
18. Wiseman, J., D'Amico, T.A., Zawadzka, S., Anyimadu, H., (2020). False negative SARS-CoV-2 PCR – A case report and literature review, Respiratory Medicine Case Reports, 31: 101140. doi:10.1016/j.rmcr.2020.101140
19. Woloshin, S., Patel, N., Kesselheim, A.S., (2020). False negative tests for SARS-CoV-2 infection – Challenges and implications, New England Journal of Medicine, 383: e38. doi:10.1056/NEJMp2015897.
20. World Health Organization. Coronavirus disease 2019 (COVID-19) Situation (Report), vol. 101;2020. Available at <https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200430-sitrep-101-covid-19.pdf?sfvrsn=2ba4e093_2> Accessed November 23, 2020.