MINI-REVIEW

Re-positive coronavirus disease 2019 PCR test: could it be a reinfection?

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Abstract

The coronavirus disease 2019 (COVID-19) outbreak started in December 2019 and rapidly spread around the globe as a major health threat. Several reports on re-positive cases subsequent to discharge from hospitals caught our attention. We aimed to highlight RT-qPCR positivity re-detection after discharge from isolation, with special consideration of the possible reasons behind it. We found that re-positive RT-qPCR assays for severe acute respiratory syndrome coronavirus 2 after previous negative results might be attributed to false-negative laboratory results and prolonged viral shedding, rather than to re-infection. These findings are encouraging and should be validated in a larger cohort.

Keywords: Coronavirus disease 2019, CT scan, False-negative, Low viral load, RT-qPCR, Severe acute respiratory syndrome coronavirus 2
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Introduction

The coronavirus disease 2019 (COVID-19) outbreak started in December 2019, spread around the globe, and has become an unprecedented major health issue. As of 3 July 2020, COVID-19 has been responsible for 12 964 809 confirmed cases, including 570 288 fatalities across 216 countries, and the number of cases is still increasing rapidly [1]. Symptoms of COVID-19 include fever, cough, shortness of breath, headache, sore throat, fatigue, loss of taste or smell, nausea, vomiting and diarrhoea [2]. Most cases of COVID-19 are mild, whereas some individuals (14%) develop more severe forms of disease requiring oxygen therapy in hospital, and about 5% need intensive care unit admission [3]. In severe cases of COVID-19, complications such as acute respiratory distress syndrome, sepsis, septic shock and multiorgan failure have been reported [4]. In the mild form of COVID-19, individuals are usually admitted to the hospital to receive standard treatment, and if their condition improves, they will be discharged according to the protocols and guidelines issued by local health authorities. According to the guidelines, they discharge patients with no fever for >3 days and after at least two consecutive negative results of real-time reverse transcription quantitative PCR (RT-qPCR) testing, and no symptoms at the time of discharge from hospital [5]. Several reports on re-positive cases subsequent to discharge from hospitals in China and other countries caught our attention. Here, we report our review on these reports. We aimed to highlight RT-qPCR positivity re-detected after discharge from isolation, with special consideration of the possible reasons behind it.

Reports on re-positive PCR assay after discharge

The phenomenon of re-positive PCR for COVID-19 has been widely reported as an emerging global pandemic control challenge. One of the largest case series of re-positive COVID-19 was reported by the Korea Centres for Disease Control and Prevention (KCDC), in which they conducted an extensive epidemiological investigation involving 285 re-positive cases and 790 contacts. During their routine screening on asymptomatic

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patients, KCDC reported a high detection of re-positive cases of 44.7% (126 out of 284) among the asymptomatic patients [6].

Zhang et al. in Guangdong, China, investigated the clinical and laboratory characteristics of seven patients who were readmitted because of re-positive PCR assays. While being isolated in the hospital, four were positive for rectal swabs only, two were positive for throat swabs, and one had positive throat and rectal swabs [7]. Another study by Li et al. in Chongqing, China focused on identifying the 19 patients who had positive RT-qPCR results after being discharged [8]. In Guangzhou, China, Chen et al. reported that 41 women were tested positive after two consecutive negative results [9]. Luo et al. from Wuhan, China, reported a case involving a woman aged 58 years with persistent fluctuating results for COVID-19 test [10]. Another report on fluctuating results was presented in a study by Xing et al. in Wuhan, China involving two cases [11]. From a study in Chongqing, China, Chen et al. reported the results of four patients, three of whom had positive results for nasopharyngeal swabs, and one had positive result for anal swab 3 days after discharge [12]. In Shenzhen, China, a study found that 20 of 182 asymptomatic patients (10.99%) were positive after initial negative results for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA [13]. A case report described a 41-year-old man from Chengdu in China, who, despite having recovered from COVID-19, was readmitted with positive nasal swabs, sputum and stool; however, the RT-qPCR results of throat swabs turned out to be negative [14]. Wang et al. identified cases from Shenzhen, China, in which recurrent positive results accounted for 8.3% (35 out of 420) of cases [15]. Another study conducted in Shanghai, China, reported that 11 patients (16.7%) in the convalescent stage had persistent positive stool results for viral RNA [16]. A case report describing a 72-year-old woman from South Korea highlighted persistent positive RT-qPCR results 6 days after two negative results; although she had completely recovered after the second positive test [17]. Another study from South Korea noted that five individuals out of 55 (9%) had reactivation of SARS-CoV-2, in whom four had mild symptoms and one was asymptomatic [18]. Ravioli et al. conducted a study in Switzerland on the identification of two old women with underlying heart diseases. They had positive test results after 18 and 21 days of two consecutive negative results for nasopharyngeal swabs [19]. On 17 April 2020, a case report from South Korea highlighted that 163 out of 7829 patients (2.1%) were tested positive and most of them (66.9%) were women [20]. Another case report from Italy identified a 48-year-old man who had a severe form of COVID-19. He recovered and was discharged after testing negative using RT-qPCR, but IgM and IgG anti-SARS-CoV-2 antibodies were detected. Over time, he developed dyspnoea and chest pain, and became positive when re-tested [21]. Dou et al. presented a case report from Jiangsu, China, in which a 56-year-old man and his daughter (21 years old) were diagnosed with COVID-19 and were discharged after negative results. However, 17 days later, both had positive results for nucleic acid swab tests [22]. Lan et al. in Wuhan, China, presented a report of four medical professionals who had positive test results after two negative assay results. RT-qPCR tests were repeated 5–13 days later, and all tested positive [23]. Zheng et al. reported three cases of individuals whose COVID-19 improved and who were discharged 1 week later; they tested positive for nasopharyngeal and saliva swabs during the first follow up, but with mild symptoms [24]. A summary of the previous reports is shown in Table 1.

Timing of testing positive from discharge

Taking all of these studies together, the median time of being tested positive from discharge was 12 days (range 1–37 days) [6–10,14,18,19,21–23].

Symptoms of re-positive cases

Most patients had mild symptoms [20]. Some had cough, sore throat [6]; dyspnoea, chest pain [22]; and fever, cough, dyspnoea, sore throat and fatigue [18].

Contact tracing of re-positive case

For all the reported re-positive cases, no studies have reported any evidence of contact with suspected or confirmed cases [7,23,24]. The KCDC investigated 285 re-positive cases and 790 contacts. Over a 14-day duration of contact tracing, 27 of the contacts were positive, of which 24 (88.9%) were previously confirmed cases, whereas the remaining three (11.1%) newly confirmed cases were contacts who had been exposed to the re-positive cases [6].

Results of the presence of anti-SARS-CoV-2 antibodies in re-positive cases

Several studies have investigated the presence of antibodies in individuals testing as re-positive. The KCDC reported that 96% of the 23 re-positive samples were found to be positive for neutralizing antibodies [6]. Another study reported that IgM and IgG anti-SARS-CoV-2 antibodies were detected [21].

Real-time RT-PCRs

Real-time RT-PCR has become a popular molecular tool employed to detect coronavirus. In principle, PCR is used to amplify the specific target gene sequence into a huge number of copies using sequence-specific primers and a DNA polymerase enzyme [25].

TABLE 1. Summary of the reports on PCR re-positive COVID-19 cases

No.	First author	Country/date	*n*	Male, *n* (%)	Age (years)	Type of sample	Timing of re-positive from discharge	Symptomatic/ asymptomatic	Severity	Ct value: below/ Above 30	Main findings and/or conclusions
1	KCDC [6]	Korea, May 2020	285	31 (33.3%) of viral culture	—	—	1–37 (14.3)	126/158	—	8/68	No infectivity
2	Zhang et al. [7]	China, Jan–Feb 2020	7	6 (85.7%)	10 months – 35 years	Throat, rectal swabs	7–11	4/3	Mild (85.7%)	—	Recovered patients may still be virus carriers, longer positive rectal swab
3	Li et al. [8]	China, Feb 2020	19	12 (63.2)	48 (18–71)	Throat	1–10 (4.4)	0/19	Mild (78.9%)	2/17	Longer positive throat swabs represent non-infectious virus
4	Chen et al. [9]	China, Feb 2020	1	1 female	46	Oropharyngeal	2	0/1	Mild	—	False negative
5	Luo [10]	China, Mar 2020	1	1 female	58	Throat	22	0/1	No symptoms	—	Incomplete clearance of the virus, false negative
6	Xing et al. [11]	China, Feb 2020	2	1 (50%)	20, 40	Throat	2–3	0/2	No symptoms	—	Recovered patients may have a small amount of virus
7	Chen et al. [12]	China, Jan–Feb 2020	4	2 (50%)	12, 29, 38, 49	Nasopharyngeal, anal swabs	3	0/4	No symptoms	—	False-negative or -positive results do not mean there is live virus
8	Yuan et al. [13]	China, Jan–Feb 2020	20	7 (35%)	41.5 (1–72)	Nasopharyngeal, anal swabs	7, 14	0/20	No symptoms	—	Recovered patients might still carry virus
9	Li et al. [14]	China, Feb 2020	1	1 (100%)	41	Nasal swabs, sputum and stool	18	1/0	Mild symptoms	—	Some patients may have a long repeatable process
10	Wang et al. [15]	China, Jan–Mar 2020	35	15 (42%)	32 (21–45)	Nasopharyngeal, anal swabs	10 (7–16)	0/35	No symptoms	—	Persistent virus in the body, patients still in a recovery process
11	Ling et al. [16]	China, Feb 2020	11	28 (42.4%) from all investigated patients	44 (34–62)	Stool	2–22	—	—	—	Virus may be transmitted through the digestive tract or be re–transmitted through aerosols
12	Chae et al. [17]	South Korea	1	1 female	72	Nasopharyngeal,	6	—	—	—	Reconsidered discharging patients based on mismatched radiologic and PCR results
13	Ye et al. [18]	China, Feb 2020	5	2 (40%)	27–42	Respiratory tract	4–17	4/1	Mild symptoms	—	Reactivation
14	Ravioli et al. [19]	Switzerland	2	2 females	77, 81	Nasopharyngeal	18, 25	2/0	Severe symptoms	—	Reactivation assumed. Re-infection unlikely
15	Kang et al. [20]	South Korea, Apr 2020	163	53 (33.1%)	(20–29) most of them	Nasopharyngeal	13.5 (1–35)	61	Mild symptoms	—	Reactivation
16	Loconsole et al. [21]	Italy, May 2020	1	1 (100%)	48	Nasopharyngeal	30	1	Moderate symptoms	—	Reactivation
17	Dou et al. [22]	China, Jan–Feb 2020	2	1 (50%)	21, 56	Throat, anal swabs	17	—	—	—	False negative
18	Lan et al. [23]	China, Jan–Feb 2020	4	2 (50%)	31–36	Throat swabs	5–13	0/4	No symptoms	—	Some of the recovered patients may be virus carriers.
19	Zheng et al. [24]	China, Jan–Feb 2020	3	—	—	Salivary and faecal	7	0/3	No symptoms	—	Positivity is unlikely due to reinfection

Viral load and test results

Accurate detection and measurement of viral load are crucial for clinical practice and decision-making. RT-qPCR could be used to directly quantify viral load by observing the fluorescence signal that proportionally increases with the amount of nucleic acid. This test serves to confirm the positivity of a case under investigation based on a specified threshold of detected fluorescence and a certain number of PCR cycles. A high cycle threshold (Ct) value indicates low viral load. A Ct value of 40 is a cut-off point commonly used in many laboratories.

Sensitivity and accuracy of real-time RT PCR

Many researchers reported that sensitivity and specificity of the real-time RT-PCR test vary greatly and lack consistency. A systematic review has revealed rates of false negatives between 2% and 29% (sensitivity of 71%–98%) [26], possibly as the result of differences in personnel competency level, standards of laboratory practice, nucleic acid extraction method used, targeted DNA sequence, probe and primer design, sampling procedures, timing for peak viral load in the patient, and sampling site during specimen collection. Some researchers

reported that sputum is the most accurate specimen, followed by nasal swabs, and throat swabs are least suitable for the diagnosis of COVID-19 [27]. Another study found that the sensitivity of bronchoalveolar lavage samples was 93%, sputum samples 72%, nasal swabs 63% and throat swabs were the least suitable, at 32% [28].

Validation of different PCR techniques

There are different real-time RT-PCR assays commonly used for targeting on different SARS-CoV-2 genomic regions, including ORF8 regions, ORF1b, spike (S), nucleocapsid (N), envelope (E) genes, or RNA-dependent RNA polymerase [29]. These gene-specific primers may also affect the results of the tests through the variation in targeted viral RNA sequences. Limit of detection of COVID-19 tests can be validated by applying intact virus to yield better detection of actual samples compared with using the nucleotide sequence. Therefore, improved PCR techniques with higher amplification efficiency are now routinely used, such as the addition of a second primer pair or a multiple-target gene amplification, and the use of probing primer sets that are designed to minimize misdetection.

Limitations of RT-PCR

The RT-PCR test detects the genetic material of the virus, but it does not differentiate between live and dead virus. Therefore, the reference standard for detection of live virus is viral culture. Another limitation of the test is the false-negative result that may be attributed to a low level of viral RNA that does not reach the limit of detection of the test. Hence, despite a negative result, there remains the possibility of undetected infection.

Possible explanations for positive SARS-CoV-2 RT-qPCR after negative results

Reactivation of the virus

Ye et al. suggested the possibility of viral reactivation [18] and proposed three categories of risk factors: host immunity status, virological factors, and type and degree of immunosuppression [18]. Another study suggested that some individuals could be virus carriers after recovery [23]. Additionally, Li et al. found that most of the investigated cases were asymptomatic, and with low viral loads. Therefore, they attributed this phenomenon to low viral load rather than to the reactivation of SARS-CoV-2 [8]. In the study conducted by the KCDC, 108 re-positive cases were found to have negative results for viral cell culture. Further investigation on 76 re-positive cases using RT-qPCR revealed that most patients (89.5%) were positive at cycle threshold values > 30, indicating low viral loads, which were undetected. However, interpretation of these findings was limited; it could not explain the actual viral load in either the patients or the collected samples. They also found that 23 (96%) tested positive for neutralizing antibodies [6]. Another study found evidence of positive IgM and IgG in 8 of 16 patients [8], indicating the presence of active immunity and ongoing infection.

Persistent infection

Dou et al. confirmed the presence of significant lesions detected on serial CT images that were not resolved in re-positive cases [22]. Prolonged viral shedding was detected using respiratory swabs in a 71-year-old woman 60 days after the onset of symptoms, and 36 days after symptoms had subsided [30]. Researchers have suggested certain factors that may be associated with protracted viral shedding, including gender, delayed admission and individuals requiring mechanical ventilation [31]. Therefore, prolonged viral shedding may explain persistent infection in re-positive cases.

New infection with the same strain

This hypothesis seems to be unwarranted because all investigated patients were self-quarantined at home and were not exposed to or in contact with confirmed cases, as stated in a previous study [22].

New infection with another strain

Some evidence suggests that the virus is evolving. Some strains might coexist, such as the European, North American and Asian strains, with the possibility of different mutation patterns [32].

Laboratory errors (false-negative/positive, or sample contamination)

Early diagnosis and treatment of COVID-19 is the fundamental approach for the prevention and control of this health crisis. Hence, clinical manifestations alone cannot accurately diagnose COVID-19, because many individuals are asymptomatic or have mild or clear respiratory symptoms. Nucleic acid assays have the ability to detect viruses using rapid and validated methods. Particularly, PCR assay is considered the reference standard for the investigation of viruses. RT-qPCR is considered one of the most commonly used methods to detect SARS-CoV-2 [33–35]. However, the RT-qPCR method could not differentiate between infectious and non-infectious RNA [19], and it has a certain risk of false-negative results due to low levels of viral load. After false-negative results were identified in a case report in China, investigators performed re-testing using RT-qPCR for throat swab specimens that had yielded positive results [36]. Xie et al. reported five symptomatic patients with false-negative RT-qPCR but typical findings of ground-glass appearance were detected using computed

tomography (CT) scans [37]. The remaining three patients had negative throat swabs but positive rectal swabs, so they needed to continue their quarantine [7]. A case report from China involving a 58-year-old woman with COVID-19 indicated fluctuations in her results from positive to negative [10]. Another case of fluctuating results involved a patient in whom test results changed from negative to positive repeatedly [11]. Another study investigated patients using RT-qPCR for SARS-CoV-2 and found a high false-negative rate of 12.5% (48 out of 384 assays) [38]. Differences in results from different sample sites have been reported. Some evidence suggests the possibility of viral shedding in faeces for long durations, extending into the 5th week after respiratory samples became negative [16,39,40]. Differences in respiratory swab results were observed in a 49-year-old man. His sputum tested positive for much longer than throat swab detection [41]. Another case report involved a 41-year-old man from Chengdu, China, who was readmitted after recovery from COVID-19. His nasal swabs, sputum and stool samples tested positive, while his throat swabs were negative [14]. Therefore, it is possible for re-positive results to be persistent infections, as patients could be tested falsely negative at discharge.

Infection with other respiratory viruses

When a patient develops symptoms again after being discharged and tested negative, there is a possibility of new infection with other types of influenza virus or coronavirus species. A study of 93 individuals identified new infections in two with adenovirus (2.2%) and one with bocavirus (1.1%) [6].

Conclusions

We conclude that re-positive RT-qPCR assays for SARS-CoV-2 after prior negative results might be attributed to false-negative laboratory results and prolonged viral shedding, rather than to re-infection. Considering the significance of this ongoing global public health emergency, it is necessary to carry out large-scale, multicentre studies to better understand the issue of potential SARS-CoV-2 recurrence in individuals with COVID-19. Prevention of re-positive testing is a fundamental measure in containing the outbreak, in addition to proper diagnosis and treatment. We would suggest that health authorities need to consider the importance of maintaining social distancing, even after treating the infection and discharging the patient, and to encourage patients to comply with strict post recovery home isolation for at least 2 weeks. Moreover, they should consider adding RT-qPCR testing for rectal swabs and low-dose CT to the criteria for patient discharge. Finally, there is a need to re-assess the guidelines for patient discharge.

CRediT author statement

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