COVID-19 reinfection: prolonged shedding or true reinfection?

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Abstract

The SARS-CoV-2 pandemic is underway and millions of people have been infected. A large number of patients with COVID-19 have recovered and been discharged. While a number of recovered patients test positive again or even have a recurrence of clinical symptoms. Some researchers believe that a positive retest is related to the long-term persistence of the virus in the body, although there is some evidence in favor of reinfection. In this study, we focus more on the possible reasons for positive retesting, antibody responses, and review of possible reinfection case reports.

Keywords: COVID-19, positive re-test, prolonged shedding, SARS-CoV-2, true reinfection

Original Submission: 29 October 2020; **Revised Submission:** 6 November 2020; **Accepted:** 6 November 2020

Article published online: 12 November 2020

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Possible reasons for positive retest

A repeat positive PCR result for coronavirus disease 2019 (COVID-19) raises many questions: does severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) persist or does reinfection occur? If it is reinfection, then controlling the pandemic will be complicated and herd immunity to the vaccine or to natural infection will be challenged.

SARS-CoV-2 reinfection remains to be fully clarified. There are numerous reports that a number of patients tested positive again after two consecutive negative PCR tests or after clinical recovery [1,2]. In some studies, this finding is attributed to PCR false-negative results at discharge, SARS-CoV-2 long-term shedding and increased virus replication as a result of discontinuation of the drug after clinical symptoms recovery [1,3,4]. In one study, it was suggested that positive retesting in recovered patients could be related to dead viruses and viral genomic fragments [5]. On the other hand, there is evidence in favour of reinfection that should be considered. A distinction must be made between prolonged shedding/reactivation and true reinfection. True reinfection has criteria that must be considered, including isolation of the complete genome of the virus (and not just genomic fragments) in the second episode. Sometimes a positive PCR test may be related to traces of the RNA genome; identification of two different virus strains in two episodes of infection based on phylogenetic analysis; proof of virus infectivity in the second episode by virus culture and evaluation of its cytopathic effect in cell culture; investigation of immune responses and their comparison in two episodes; and epidemiologic data such as reexposure history to COVID-19 patient in the second event and timing between episodes, with a longer time interval between two episodes favouring the reinfection hypothesis [3,6]. The maximum duration of SARS-CoV-2 RNA shedding in the upper respiratory tract has been reported to be 83 days [7]. Therefore, positive retesting more than 83 days after the first positive test, along with other criteria, favours confirmation of reinfection. In addition to the above-mentioned reasons, the disease's clinical data are also useful in confirming the second episode, although the second episode may be asymptomatic. A time interval where the patient is free of clinical signs between the two episodes is also necessary.

One of the features of SARS-CoV-2 infection is prolonged virus shedding. However, the sensitivity of a PCR test to diagnose infection is not high and may cause false-negative results [1]. In addition, in a number of patients, stool samples are still positive after respiratory samples have become negative, indicating prolonged shedding of the virus in the gastrointestinal tract during the course of infection [1,8,9]. Also, it has been hypothesized that the gastrointestinal tract may act as a reservoir for SARS-CoV-2 even after tests on upper respiratory tract samples become negative [1]. Because the main criteria for discharge are the improvement of clinical symptoms and two negative PCR tests, it is recommended to modify the discharge criteria to take into account the long-term shedding or later clearance of SARS-CoV-2 in the gastrointestinal tract

https://doi.org/10.1016/j.nmni.2020.100812

compared to the upper respiratory tract [7,9]. SARS-CoV-2 RNA can persist in stool specimens for approximately 5 weeks after patients' respiratory swab results have become negative [4,10], and a faecal specimen should be tested at the time of discharge. Adding a faecal specimen test at discharge partially alleviates the problem with false-negative test results; this modification can in turn help discriminate between long-term virus shedding and true reinfection.

Dynamics of antibody responses in patients with COVID-19

Humoural immune responses are the first line of defence against reinfection, and the strength and durability of these responses are related to protection [11]. The dynamics of antibody responses in COVID-19 patients are not fully understood. In one study, the overall seroconversion rate was reported to be 96.8%, but neutralizing activity and persistence were not fully understood [12]. Similarly, in the study of Zhao *et al.* [13], the overall rate of seroconversion was 100% by day 39 after disease onset, but in this study, further follow-up was not performed to evaluate the persistence. Consistent with the above studies, in another study, serum samples from 42 patients with COVID-19 were analysed 14 to 60 days after symptom onset. Seroconversion of IgM and IgG antibodies occurred in all patients, but antibody levels were markedly reduced approximately 60 days after onset of symptoms [11]. In contrast, a study was performed on sera of 343 patients with COVID-19. The average seroconversion time for IgA, IgM and IgG antibodies against receptor-binding domain (RBD) was about 12 days. The average seroreversion time of IgA and IgM antibodies was about 71 and 49 days respectively. Anti-RBD IgG antibody decreased slightly during 3 months, and seroreversion was only seen in a small number of people [14]. Another group of researchers examined the sera of 59 patients with varying degrees of disease; their results showed a positive relationship between serum neutralizing capacity and disease severity. Given that the amount of neutralizing antibodies predicts the possibility of reinfection in patients recovering from COVID-19, it has been hypothesized that asymptomatic patients may develop reinfection [15].

Review of reinfection studies

There are only a few studies on reinfection, but they have raised many questions. A study of reinfection in an animal model found that an initial infection with SARS-CoV-2 provided protection against SARS-CoV-2 reexposure, and reinfection did not occur [16]. Virus replication in monkeys' noses, lungs and intestines was observed 5 days after infection. After symptoms improved, the monkeys were reexposed to the same dose of the SARS-CoV-2, and no recurrence of infection was observed. Although there are few reports of reinfection, more focus should be placed on it.

In a case report in Hong Kong, 142 days after the first positive PCR test, a 33-year-old immunocompetent patient tested positive again via acid test during an airport screening. During the first episode, the patient had fever, cough, sputum production and headache, but during the second episode, the patient was asymptomatic. The second PCR test was positive on 15 August 2020, and he was hospitalized on 16 August. C-reactive protein was slightly elevated, and the patient had hypokalaemia. Serum samples were negative for anti–SARS-CoV-2 IgG 10 days after symptom onset in the first episode and 1 to 3 days after hospitalization in the second episode; IgG seroconversion was observed from the fifth day after hospitalization in the second event. The patient had two different virus strains in the two episodes of infection [17]. Laboratory parameters in the disease's second bout, IgG seroconversion, increased C-reactive protein and identification of two different virus clades during the two events provide evidence in support of the reinfection hypothesis. In addition, the relatively long time interval between the two events also favours reinfection. Virus culture was ongoing at the time of publication.

Similarly, another study in the United States diagnosed a second infection in July 2020, 140 days after the primary infection in March. The patient was symptomatic for both bouts of disease. Laboratory and radiographic findings showed that disease severity the second time was less than the first. About 140 days after the primary infection and recovery, the patient was exposed to people with cough. The patient then experienced dyspnoea, cough and weakness, followed by a positive retest for COVID-19. The patient recovered with remdesivir and dexamethasone treatment. Genomic analysis showed that virus strains isolated in the two distinct episodes were not the same. The strain isolated in March was similar to the strain imported from Asia, while the July strain had a D614G mutation that was similar to the European strain. The time interval between the two infections was consistent with a dominant strain change in the United States (D614G circulated in July as the dominant strain), which supports the reinfection hypothesis (vs. intrahost virus evolution). Epidemiologic data (history of exposure to people with cough), clinical features, laboratory findings and phylogenetic data were all in favour of reinfection. Humoral immune responses were also examined; it was concluded that poor humoral immune responses or their decrease over time is one of the

reasons that a person may be infected with SARS-CoV-2 multiple times [18].

Another case report described a 46-year-old man who had symptoms on 12 May 2020 and who tested positive via PCR on 20 May, then negative on 3 June. In late July, he had close contact with a COVID-19 patient, and he became symptomatic 2 days later. A few days after the onset of symptoms, on 22 July, the test was positive again. The patient had symptoms in both episodes, although the second episode was more severe. The virus strains belonged to two different clades. IgG/IgM antibodies were evaluated on 16 May; IgM was positive and IgG was negative in the first infection, whereas both IgM and IgG antibodies were detected in the second infection [19]. Evidence including epidemiologic data, clinical findings, positive PCR retest, two different virus clades and antibody responses was compatible with reinfection. However, one of the important limitations of this study is that virus culture was not performed.

The first case of possible reinfection in Belgium showed a symptomatic reinfection 93 days after the first mild symptomatic infection. The time interval between the two infection events was about 3 months. Genome sequencing showed that the first strain was a lineage B.1.1 SARS-CoV-2 virus and that the second strain was related to lineage A. The authors of this study noted that according to a number of previous studies, antibody responses in mild infections are lower than in severe infections; also, according to some data, the disease of 20% of people does not seroconvert at all. Therefore, these populations are susceptible to reinfection [20].

In accordance with the above studies, the first case of reinfection in Turkey was described in a 23-year-old woman with two symptomatic episodes 116 days apart. Although the authors claimed reinfection, the study had several drawbacks: genomic analysis was not performed, and antibodies were not screened in the first infection. Antibodies in the second episode at about 24 days after symptom onset were slightly positive [21].

India also published cases confirming reinfection. Gupta *et al.* [22] reported possible reinfection in two patients in India. The patients were healthcare workers who were identified during routine screening; they were asymptomatic in both episodes. The interval between the two episode in each case was more than 3 months. In each episode, a different virus strain was detected, but antibody analysis and virus culture were not performed.

Conclusion

Some researchers believe that a positive retest for SARS-CoV-2 may be explained by reactivation or relapse of the infection; others emphasize the reinfection hypothesis. In fact, however, a retest result of SARS-CoV-2 positivity should be interpreted correctly thanks to a false-negative test result at discharge, prolonged virus shedding, a rebound in virus replication after drug discontinuation and reinfection. According to the cases described above as well as the evidence confirming reinfection, although reinfection may occur, its incidence is low. However, the reinfection rate may be underestimated as a result of asymptomatic infections in one or both episodes.

Conflict of interest

None declared.

Acknowledgements

We thank all the healthcare staff involved in diagnosing COVID-19.

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