Re-positive nucleic acid test in COVID-19 Patients: A systematic review

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) test could be positive for a second time in some cases, after recovery from their first coronavirus disease 2019 (COVID-19) episode. However, causes of this re-positive test has not been fully understood. In this paper, the original studies with re-positive COVID-19 patients in their study population were discussed. Methods: Scopus, PubMed, Embase, and Cochrane databases were searched and the retrieved records underwent title/abstract and full-text screenings. Three independent researchers extracted the data of the studies. Results: The systematic search yielded 16 case-control, cohort, and cross-sectional studies. Data of 10,475 patients were included; 489 (4.67%) of them classified as re-positive. The female percentage and mean age for re-positive and non-re-positive groups were 56.82% and 50.0 ± 18.0, and 55.91% and 46.6 ± 17.1, respectively. The rate of symptom presentation upon second positive test turned out to be 20.0%, with the most common symptoms being cough (68.2%), fever (31.8%), and fatigue (22.7%). Hypertension (15.9%) accounted for the most common underlying disease in the re-positive group. In 33/46 (71.7%) of the re-positive patients, computed tomography (CT) abnormalities were resolved to some extent. Conclusion: A re-positive SARS-CoV-2 test is possible within a few weeks after recovery, although it does not necessarily indicate a re-infection. Other reasons could lead to a re-positive test such as reactivation of the virus that persisted in the body from the previous COVID-19 episode and testing errors. Clinical features such as symptoms and imaging could assist in identifying re-infections.

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Running title: Re-positive PCR in COVID-19

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Conclusion: A re-positive SARS-CoV-2 test is possible within a few weeks after recovery, although it does not necessarily indicate a re-infection. Other reasons could lead to a re-positive test such as reactivation of the virus that persisted in the body from the previous COVID-19 episode and testing errors. Clinical features such as symptoms and imaging could assist in identifying re-infections.

Keywords: *COVID-19; SARS-CoV-2; Re-positive; Re-infection; Polymerase Chain Reaction*

Introduction

Since the discovery of the highly infectious Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019, countries around the globe have been on a red alert (1). By the end of January 2021, more than 100 million SARS-CoV-2 infected cases and more than 2 million and 50 thousand fatal cases have been reported across the world (2). Although the disease resolves in most cases, some experience worse outcomes such as requiring intensive care (e.g., mechanical ventilation) or death (3).

The body's immune response to an external pathogen (a virus, in this case) consists of two main lines of defense: the innate and the adaptive immune systems. The innate immune system, which mainly consists of neutrophils, macrophages, and natural killer cells, acts non-specifically on viruses and infected cells and is the first to arrive on the infected site. Type I and Type III interferons produced by infected cells also contribute to the immune cascade and the anti-viral response. On the other hand, adaptive immunity can produce an antigen-specific response, but takes more time to become activated. Adaptive immunity, itself, is comprised of two arms: the cellular and humoral arm. Cytotoxic T lymphocytes are the most important cell types involved in cellular immunity and can destroy infected cells using specific cytotoxic substances like granzymes and perforin. In humoral immunity, B-lymphocytes play the main role by producing antibodies that contribute to neutralization and destruction of the virus (either by opsonization or activating the complement system). Both innate and adaptive immune systems can form some kind of memory that facilitates future responses to pathogens.

An immune response against SARS-CoV-2 occurs similarly. The innate immune system produces inflammatory cytokines such as IL-6 and TNF-α, causing the adaptive immune cells to recruit and secrete more

cytokines, further strengthening the immune response against the virus. Adaptive immune cells, especially T cells, are the mainstay of the body's defense mechanism against COVID-19 (4, 5). According to a study, CD8+ cells comprise 80% of the population of infiltrating cells in COVID-19 (6). Humoral response against SARS-CoV-2 acts by the production of antibodies that prevent the attachment of the virus to its target cells (i.e. ACE2+ cells) (4, 7, 8). Though the degree of cellular and humoral responses are usually correlated, in some cases, the antibody response is absent despite T-cell activity (9). This is either due to less severity of the disease, which diminishes the need for a humoral response or due to the persistence of cellular immunity memory at the time when antibodies are no longer detectable.

It has been shown that preliminary immune response to SARS-CoV-2 (such as anti-viral interferon and inflammatory cytokine secretion) is relatively suppressed and delayed, compared to other viral pneumonia (including SARS-CoV, another coronavirus that was the responsible pathogen for SARS disease), which may attribute to the severity of the disease (4, 10, 11). COVID-19's relatively long incubation period could also be explained by this phenomenon (12). However, in some cases, the immune response leads to oversecretion of pro-inflammatory cytokines (i.e. cytokine storm), which is an important mechanism in acute respiratory distress syndrome (ARDS), lung damage, and mortality of COVID-19 (13).

Currently, it is unclear whether immunity develops after recovery from COVID-19. Although multiple scenarios have been proposed, it is yet to be determined which one turns out to be true (14). Experience from the previous SARS outbreak indicates that while anti-SARS-CoV antibody titers are significantly reduced a year after infection and are detectable in only 50% of the infected population after 4 years (15), SARS-CoV related memory T cells could be detected even at six to eleven years post-infection (16, 17). Also, it has been shown that humoral short-term immunity (IgM and IgA-mediated) appears through the first week from the onset of symptoms; however, long-lasting antibodies (IgG) become apparent after 14 days (4, 18, 19). IgG titers remain detectable at least two weeks after discharge, while high neutralizing antibody levels are only expected to be seen in newly recovered cases (20). According to a study conducted in Singapore, T cells of all patients who have recovered from COVID-19 can express anti-SARS-CoV-2 activity up to 28 days after negative PCR, most notably against nucleocapsid protein-1 (NP-1) and NP-2 of the virus (21). While a high number of anti-NP T cells are observed in newly recovered cases, anti-receptor-binding domain (RBD) T lymphocytes persist for a longer duration, though the number of these T cells is significantly lower than that of the anti-NP population in early recovery (20). Moreover, central memory CD4+/total CD4+ T cell ratio increases in recovered cases (22), although patients with severe disease have been reported to demonstrate lower percentages of memory CD4+ cells (23). On the other hand, declined number of T lymphocytes in peripheral blood of the subjects might cause impaired production of memory cells (24) and consequently a failure in cellular immune memory creation.

Still, we are aware that re-infection of COVID-19 exists and some patients have re-tested positive for SARS-CoV-2 even after full recovery from the disease (i.e., resolution of symptoms and negative PCR). In this systematic review, we have gathered and summarized available literature regarding re-positivity of SARS-CoV-2 tests. Our goal was to determine the epidemiology of recurrent COVID-19 positive PCR and to provide an overview of post-infectious immunity to the disease.

Methods

We conducted this systematic review to explore and investigate the validity of reported re-infected cases worldwide. For this purpose, we carried out systematic research in Pubmed, Embase, Scopus, and Cochrane databases. We present the final search strategy with the letter "C" below:

1. [reinfection] (Title/Abstract) OR [reinfected] (Title/Abstract) OR [second infection] (Title/Abstract) OR [re-positive] (Title/Abstract) OR [re-positive] (Title/Abstract) OR [recurrent] (Title/Abstract) OR [recurrence] (Title/Abstract) OR [relapse] (Title/Abstract) OR [relapsing] (Title/Abstract) OR [persistent PCR positive] (Title/Abstract) OR [persistent positive PCR] (Title/Abstract)

2. [COVID-19] (Title/Abstract) OR [SARS-CoV-2] (Title/Abstract) OR [Novel Coronavirus] (Title/Abstract) OR [2019-nCoV] (Title/Abstract)
3. [A] AND [B]

Upon retrieving the desired search results, three researchers screened the articles in a two-step process. The first step of this process involved general inclusion/exclusion criteria based on the title, abstract, and keywords of each study.

The main exclusion criteria in this phase were the following:

1) Abstracts and conference-abstracts without full-text articles

2) Ongoing projects and clinical trials yet to be published

3) Lack of published original data, including review articles, or editorials without providing any original data

4) Case-reports, case-series, and pre-prints

5) Irrelevant to the aims and scope of the study

6) The smaller study in the studies with a high suspect of population overlap between them

In the second phase, we carefully read through the full-text of the articles and included the eligible studies. Finally, peer-reviewed cross-sectional, clinical trials, case-control, and cohort studies from the beginning of January 2020 until late July 2020 were included in our study.

Results

After searching all of the prior mentioned databases, we came up with a total of 661 retrieved results. Of these, 282 were duplicates and were thus removed. After the initial screening of the remaining 379 articles, 67 articles were eligible to enter the full-text screening process. Finally, following the careful implementation of the inclusion/exclusion criteria mentioned above, we encountered 16 eligible articles to enter the study. The schematic view of the study selection process is provided in **Figure 1** .

Cohorts accounted for the most observed type of articles in our study, with 14 included papers. one cross-sectional and one case-control study were also eligible to enter this systematic review. All the data reported in the articles are gathered from three distinct countries of China (14 articles), South Korea (1 article), and Brunei (1 article) (**Figure 2**). Two studies from South Korea provided the whole country's data using official South Korean governmental statistics (South Korea Center for Disease Control and Prevention). Therefore, we excluded the study with the smaller population due to their high possibility of population overlap (**Figure 1**).

The details of the included studies are discussed in **Table 1** . The included articles yield a total of 10,475 studied individuals. Of these participants, a sum of 489 (4.67%) patients were considered to be re-positive cases (**Figure 3**).

Considering the articles reporting gender prevalence, the overall population sums up to 1275 patients, including 695 (54.51%) females and 580 (45.49%) males. Among the studies reporting gender prevalence of both re-positive and non-re-positive subgroups, total females and total males are 405/723 (56.02%) and 318/723 (43.98%), respectively. In the re-positive subgroup, the female and male numbers are 50/88 (56.82%) and 38/88 (43.18%), sequentially. In the non-re-positive subgroup, females and males account for 355/635 (55.91%) and 280/635 (44.09%) of the population (**Figure 4**).

To understand the mean age of different groups, we combined the studies that published the age data for total, re-positive, and non-re-positive groups. The mean age for the total population, the re-positive subgroup, and the non-re-positive subgroup were 47.5 $\pm$ 16.9, 50.0 $\pm$ 18.0, and 46.6 $\pm$ 17.1, respectively.

Other than the details presented in **Table 1** , PCR conversion time (days between symptom onset to first negative PCR test), hospital stays (days) during the first hospitalization, days between last negative test and first re-positive PCR, second-time clinically symptomatic patients among re-positive patients, second-time clinical symptoms, comorbidities, days to second negative PCR, second-time chest CT changes among re-positive patients, number of re-positives infecting close contacts, number of re-positive patients with positive IgM and IgG, number of non-re-positive patients with positive IgM and IgG, and mean/median IgM and IgG levels for the included articles are demonstrated in **Supplementary Table 1** .

Combining all studies which reported symptom status among re-positive cases, the rate of symptom presentation upon second positive test turned out to be 20.0%. Among those, observed symptoms included cough (68.2%), fever (31.8%), throat pain (9.1%), fatigue (22.7%), chest discomfort (4.5%), chest expectoration (4.5%), itchy throat (4.5%) and constipation (4.5%).

The most common comorbidities in re-positive patients according to available data were hypertension (15.9%), diabetes (3.2%), cerebrovascular disease (3.2%), depression (3.2%), tuberculosis in mediastinal lymph nodes (1.6%), hepatopathy (1.6%), chronic lymphocytic leukemia (1.6%), and pregnancy (1.6%).

Two studies reported the average number of days from second positive to second negative, being 2.73 ± 2.03 (25) and 1.83 ± 0.22 (26) (combined mean $= 2.32 \pm 1.56$). The only re-positive patient in Qiao et al.'s study (27) stayed in the hospital for approximately 17 days after her second admission.

When comparing CT scans obtained upon first discharge and second positive PCR of a total of 46 patients, thirteen (28.3%) showed no improvement of lesions since initial discharge, while in the other 33 patients (71.7%), CT lesions were resolved to some extent.

Out of 64 patients whose antibody data were available, 55 (85.9%) had positive IgG and 35 (54.7%) had positive IgM titers, based on each study's definition of "positive". Some of the studies had also gathered antibody information of their non-re-positive patients. When combining available data, 93.9% of non-re-positive patients had positive IgG profile, showing a significant difference with that of re-positive patients (85.9%, $p = 0.03$), while 53.5% were positive for IgM, which is not statistically different to that of re-positive patients (54.7%, $p = 0.86$).

Discussion

COVID-19 continues to spread among people and every day, new aspects of the disease are discovered. Furthermore, a new strain has recently been discovered in the United Kingdom with a more rapid spread rate (28). Despite the importance of identifying re-infected cases, there are still limited data on the rate of re-infection and the characteristics of the patients who test re-positive for SARS-CoV-2 for the second time.

By gathering existing literature data, our results showed that approximately 4.5% of patients might test positive again after disease resolution and negative PCR. However, in a single study, this number reached as high as 50% (29). Although many of the re-positive cases were discovered upon routine checkups in the first or second week after discharge, a few number of them tested re-positive after the 14-day mark, even as late as 35 days after first recovery (30).

More than 70% of patients show CT improvement upon re-positive PCR, compared with first discharge scans. Zhu et al. (31) reported that at the time of first discharge, at least 50% of the lung lesions were resolved in 54.3% and 29.4% of non-re-positive and re-positive patients, respectively ($p = 0.029$), and concluded that a slow rate of lung lesion absorption could lead to a higher chance of re-positive PCR.

Only three studies (26, 32, 33) performed contact tracing for their patients and checked whether they infected any other close contacts, which none of them did, suggesting an inactive disease, rather than a second infection. Although, out of eight re-positive patients in Wang et al.'s study, only two had contact with other people in their observation period (a total of seven close contacts), which is a small number, thus

further investigations are required to determine the transmissibility of SARS-CoV-2 in re-positive cases. These results are consistent with Wolfel et al.'s findings, which stated that live virus could not be extracted from SARS-CoV-2 samples eight days after onset of symptoms, despite high levels of viral load (34).

It is a matter of debate whether the presence of anti-SARS-CoV-2 antibodies can immunize a patient from a second infection. Based on the results of our study, many patients had positive antibody profiles and yet, tested positive again. Non-re-positive patients had significantly higher rates of positive IgG titers, while IgM positivity was alike between re-positive and non-re-positive groups. As a result, we believe that a re-positive PCR after the first recovery is less likely in a patient with a positive IgG profile, whereas a positive IgM titer does not affect the likelihood of testing positive a second time. Nonetheless, there are some important limits to this analysis. Different studies had different methods for antibody measurement (e.g., time from disease resolution to sample collection) and reporting (e.g., definition of a "positive" antibody titer). Therefore, further studies are necessary to establish a correlation between antibody levels and the probability of a second positive nucleic acid test.

Although RT-PCR is the gold standard of SARS-CoV-2 detection (35), it is not completely sensitive; sensitivity of PCR tests could range from 98% down to 60.2% (36). Improper sampling is one of the reasons that may cause false negatives (37). False-negative results not only could account for underestimated rates of re-infection, but could also lead to a false assumption of recovery. As a result, re-positive PCR may not necessarily indicate a second infection, but rather due to the same virus that had not been cleared at all in the first place. Since samples for PCR could be obtained from nasopharyngeal swabs, oral swabs, sputum, or fecal swab, some experts suggest sampling from as many sites as possible to reduce the probability of false negatives (29). Nevertheless, it is assumed that PCR from sputum specimen has the highest sensitivity for SARS-CoV-2 detection, followed by nasal swab and oral swab (37, 38). On the other hand, although the presence of virus might be missed due to false negatives, not all positive tests indicate SARS-CoV-2 infection, as contamination or a cross-reaction with another organism's genome could falsely turn a test positive (39). Therefore, a second positive PCR after testing negative might be due to false negative or false positive results of PCR and does not necessarily indicate a second infection. However, in the study by Li et al., patients had negative RT-PCR at least four times with one-day intervals between testings, which highly reduced the chance of false-negative result (37). Li et al. stated that there is a chance that the virus might have replicated and increased its viral load due to immunologic alterations throughout the disease course (37). Prolonged intermittent viral shedding is another explanation for negative PCR periods, as Wong et al. (26) indicate in their study, and has been previously described in SARS (40). Ye et al. also believe that second positive tests for SARS-CoV-2 in their study were subsequent to virus reactivation, rather than a second acquired infection (41). Immunosuppression due to corticosteroid administrations might also be a contributing factor (41). Some patients might not be able to fully clear the virus from their body and still test positive, even two to three months after infection (42). Also, remnants of SARS-CoV-2 have been observed in pulmonary tissue of a ready-for-discharge patient despite negative PCR (43), which might point to another source for virus re-emergence.

Being infected a second time after full recovery usually requires contact with another infected patient. However, most of the re-positive patients in our gathered studies tested positive in their post-discharge observation or quarantine period, and therefore a transmitted infection is unlikely. Nevertheless, an analysis of 8922 cases in South Korea (30) after the termination of their quarantine period (which requires two negative PCR tests with at least 24 hours in between) revealed that 292 (3.3%) patients tested positive again. The study challenges the re-activation or second infection argument, stating that re-positive tests are mainly due to technical limitations and the inability of current testing methods to differ viral nucleic acid remnants of dead viruses from an active virus. The grounds on which Kang makes these claims are acuity of SARS-CoV-2 and the fact that it is not a chronic infection, high chances of immunity after the first infection based on previous data from other coronaviruses, absence of documented disease transmission from re-positive patients to other individuals, and negative in-vitro viral activity tests of re-positive samples (30).

Taking clinical features into account could help in identifying a new or re-activated infection from false positives or previous false negatives. Approximately one-third of the patients express symptoms upon re-positive. The symptoms alone do not indicate a second infection, as they have to be compared with the previous episode of infection. Worsened symptoms, signs, and health status is more likely to be a result of re-infection. Wang et al. (32) retrospectively studied 131 discharged COVID-19 patients for four weeks, of which 8 tested re-positive during this period. However, only one was deemed a recurrent infection, as the positive PCR test was accompanied by deteriorated CT as well as fever. Qiao et al. also ruled out false-positive tests resulting from viral nucleic residues, due to the presence of symptoms and imaging findings (27).

Furthermore, a similar rate of IgM positivity in re-positive and non-re-positive cases increases the chance of a testing error and makes reinfection unlikely (42). Additionally, inflammatory markers in Yuan et al.'s (44) study were within the normal range in most re-positive patients, further reducing the probability of active disease.

Chinese guidelines require two negative PCR tests with a 24-hour interval before discharging a patient. Considering the probability of a re-positive test even after two negative tests, some experts believe that the currently practiced guidelines are not practical enough for recognizing clearance of the virus from the body (25). Increasing testing intervals and utilizing other diagnostic procedures such as antibody testing could assist in achieving this goal (25). Hao et al. demonstrated that when the number of negative PCRs increases from two to three, chances of a future re-positive test reduces by approximately 70% (16.4% vs 4.8%, respectively).

Most of the above-mentioned studies do not believe that re-positives in their population are second acquired infections, but rather consequences of testing inaccuracies or re-activation of the previous disease. The main factors contributing to these views are the few numbers of days between initial negative/discharge and re-positive PCR, no transmission to close contacts in re-positive patients, and testing positive without an identifiable infection source. On the other hand, numerous case reports and case series have been published, describing patients who have tested positive many days after their first infection, making false test results and virus re-emergence unlikely. In a recent case report by Tillett et al. (45), a 25-year-old man was infected by a genetically different strain of SARS-CoV-2 almost two months after his first episode of infection. Additionally, the case also had major symptoms such as self-reported fever and dyspnea. This is the first documented and confirmed re-infection of a patient with SARS-CoV-2 in North America, which also raises doubts regarding immunity after an episode of COVID-19.

In another case presented by Wang (46), PCR of the reported case turned positive three more times, after initial discharge. The number of days in between discharges and the next positive PCR were 13, 37, and 25. The patient did not infect his close contacts, and the study does not report any symptoms upon re-positives. Moreover, ground-glass opacities were present on the patients' chest CT until the day before his 3rd discharge from the hospital. Total antibody titers were positive for SARS-CoV-2 since his second admission, further strengthening the idea that a positive antibody titer does not prevent a second positive PCR test, however, it might lead to a clinically suppressed relapse as the patient had no symptoms.

Lafaie et al. (47) provide another evidence for the role of antibodies in COVID-19 re-positive cases, where they report an 84-year-old female who presented with hypothermia and respiratory symptoms 41 days after her first positive test. She was tested positive again 4 days after the beginning of her second episode of symptoms, with relatively low cycle thresholds (17.5 and 18.1), and a worsened chest CT. Neutralizing antibodies were negative until one week after symptom onset, after which they became weakly positive. The patient, unfortunately, died 13 days after symptom onset.

On the other hand, in a case series (48) of 11 patients with re-positive PCR at least 21 days after initial symptom presentation, all cases experienced symptoms (for a median of 10 days) after a symptom-free interval, and all had COVID-19 matching CT scans. Nevertheless, six out of nine patients who underwent serologic antibody testing had positive or weakly positive antibody levels, raising doubt about the role of

antibodies in disease outcome.

Multiple other case series and case reports are also available in which re-positive patients present worsened symptoms and/or chest imaging, suggesting a relapsing disease or re-infection (49-57). Some of the patients experienced relapse symptoms after drug cessation (58, 59).

Conclusion

Patients who have passed an episode of COVID-19 might test re-positive again, and furthermore, become ill seriously. A second positive PCR after discharge or negative PCR is not uncommon, and it is most likely due to disease reactivation or testing limitations, rather than another exogenous re-infection. Further prospective studies are needed to provide reliable epidemiologic data and reveal the mechanisms and risk factors for re-positives. Although anti-SARS-CoV-2 antibodies do not seem to prevent a second positive test, they might avert severe illness in re-positive patients.

Authors' contributions

All authors have contributed to preparing the manuscript. Three authors (A.N., A.K., and S.A.) gathered the data and prepared the draft, and two authors (N.K. and N.R.) supervised and revised the manuscript.

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