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COMMENTARY

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Retest positive for SARS-CoV-2 RNA of "recovered" patients with COVID-19: Persistence, sampling issues, or re-infection?

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Abstract

"Retest Positive" for severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) from "recovered" coronavirus disease-19 (COVID-19) has been reported and raised several important questions for this novel coronavirus and COVID-19 disease. In this commentary, we discussed several questions: (a) Can SARS-CoV-2 re-infect the individuals who recovered from COVID-19? This question is also associated with other questions: whether or not SARS-CoV-2 infection induces protective reaction or neutralized antibody? Will SARS-CoV-2 vaccines work? (b) Why could some recovered patients with COVID-19 be re-tested positive for SARS-CoV-2 RNA? (c) Are some recovered pwith atients COVID-19 with re-testing positive for SARS-CoV-2 RNA infectious? and (d) How should the COVID-19 patients with retest positive for SARS-CoV-2 be managed?

KEYWORDS

coronavirus < virus classification, disease control, immunity/immunization < epidemiology, persistent infection < infection, SARS coronavirus < virus classification

In December 2019, coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) infection emerged in Wuhan, China, and has spread rapidly worldwide. There are more than 4.7 million confirmed cases and more than 313 thousand confirmed death in 216 countries by 16th May 2020. Many countries take very different strategies to control this outbreak including asymptomatic, mild and sever patients. In China, strict quarantine is needed for all the confirmed cases. All patients with COVID-19 need to meet criteria of recovery before hospital discharge[1]: (a) normal temperature for more than 3 days, (b) no respiratory symptoms, (c) substantially improved acute exudative lesions on chest computed tomography images, (d) two consecutively reverse transcription-polymerase chain reaction (RT-PCR) tests negative for SARS-CoV-2 RNA more than 24 hours. However, the recovered (discharged) COVID-19 patients with retest positive for SARS-CoV-2 RNA have recently been reported.[1-9] Specifically, A new report on 25 February 2020 indicated that 14% of discharged patients were tested positive for SARS-CoV-2 RNA in Guangdong province. On 2 February 2020, a woman patient with COVID-19 became positive for SARS-CoV-2 RNA again during her quarantine after hospital discharge because of two consecutively negative results on 28 and 30 January, respectively.[7] A study from Zhongnan Hospital of Wuhan University suggested that four COVID-19 patients who met criteria for hospital discharge became positive for SARS-CoV-2 RNA after 5 to 13 days of discharge.[1] A single center study reported 38 out of 262 of recovered patients with COVID-19 (14.5%) became positive for SARS-CoV-2 RNA by 10 March 2020, during 14 days of further quarantine or isolation.[8] A cohort study of

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414 confirmed patients with COVID-19 in a hospital from 11 January to 23 April 2020 also suggested that 16.7% COVID-19 patients re-tested positive for SARS-CoV-2 RNA one to three times after discharge, during 14 days of strict quarantine.[9] Another single center study reported that 8 out of 108 confirmed patients with COVID-19 from 10 February to 13 April 2020 became SARS-CoV-2 positive and were re-admitted in hospital.[6] This "Retest Positive" for SARS-CoV-2 from the discharged COVID-19 has attracted extra attention and triggered numerous discussions. In this commentary, we discuss the following questions: (a) Can SARS-CoV-2 re-infect the individuals who recovered from COVID-19? This question is also associated with other questions: whether or not SARS-CoV-2 infection induces protective reaction or neutralized antibody? Will SARS-CoV-2 vaccines work? (b) Why could some recovered patients with COVID-19 be re-tested positive for SARS-CoV-2 RNA? (c) Are some recovered COVID-19 patients with re-testing positive for SARS-CoV-2 RNA infectious? (d)How should the COVID-19 patients with retest positive for SARS-CoV-2 be managed?

1 | WHY DID SOME RECOVERED COVID-19 PATIENTS BECOME RETEST POSITIVE FOR SARS-CoV-2 RNA?

There are several possibilities why the recovered patients with COVID-19 became retest positive for SARS-CoV-2 RNA: First, two consecutively RT-PCR tests of pharyngeal swabs might be false-negative before the patient was discharged from the hospital, since overall positivity of RT-PCR for SARS-CoV-2 in COVID-19 was round 30% to 40%.[10] The sampling procedures of pharyngeal swabs, quality of sampling tube, sample storage temperature and time, transportation process of samples, and quality of detection reagents (kits) might result in the false-negative tests. Second, some COVID-19 patients did not completely meet the discharge criteria. The interval time between the viral RNA tests before discharge and the actual discharge date went too long, and viral test was not repeated right before discharge according to the requirements of the guideline of diagnosis and treatment. Third, positive signal of viral RNA might be from the "dead" viruses or viral gene fragments without active viral replications. Finally, viral clearance might be varied from the patient to patient with pre-existing conditions. For example, 48% COVID-19 patients had a comorbidity (such as hypertension, diabetes, etc), 44.9% patients received glucocorticoid therapy, and most COVID-19 patients with critical conditions were older than 50 years and above.[11,12] All these might delay virus clearance.

2 | ARE THE PATIENTS WITH RETEST POSITIVE FOR SARS-CoV-2 INFECTIOUS?

Theoretically, COVID-19 patients with active viral replication are infectious. As most already know, China has a very powerful and restrictive quarantine and follow-up strategies for all patients with COVID-19, suspected cases, and asymptomatic individuals. In the 13 discharged patients with retest positive for viral RNA in Guangdong province on 25 March 2020, follow-up results demonstrated no new infected cases from 104 close contacts to the original patients. There was no single family member being infected by the four recovered COVID-19 patients with retest positive for SARS-CoV-2 RNA, who were discharged from Zhongnan Hospital of Wuhan University,[1] suggesting a relative low or no infectivity of those recovered patients with retest positive for viral RNA. Researchers in Hong Kong have followed up more than 10 recovered, discharged COVID-19 patients with retest positive for SARS-CoV-2 and failed to isolate SARS-CoV-2 virus by cell culture in the P3 laboratory due to low viral loads or no live viruses from the samples. A recent study[13] suggested that the SARS-CoV-2 can replicate actively in upper respiratory tract tissues in the early stage with high infectivity. In the later stage, the viral load was relative low in upper respiratory tract.

3 | RE-INFECTION WITH SARS-CoV-2?

Host immune response to pathogens may prevent progression to severe illness or reinfection by the same pathogens. Many studies have shown that recovered patients with COVID-19 have antibodies to SASR-CoV-2,[14-17] some patients have very low levels of neutralizing antibodies. These raised possibilities for possible reinfection of SARS-CoV-2 and antibody dependent enhancement.[18-20] As we discussed above, the discharged patients with COVID-19 in China and elsewhere were re-testing positive for SARS-CoV-2 RNA. It remains unclear whether the convalescing patients have risks for an "reinfection." A recent animal study[18] may help understanding this situation. They used the SARS-CoV-2-infected monkeys for this study. They found that viral replication in nose, pharynx, lung and gut, moderate interstitial pneumonia at 7 days postinfection. After the symptoms were alleviated and the specific antibody tested positively, they rechallenged half of infected monkeys with the same dose of SARS-CoV-2 strain. They did not observe viral loads in nasopharyngeal and anal swabs and viral replication in all primary tissues at 5 days post-reinfection. Thus, SARS-CoV-2 infection may protect from subsequent re-exposures.[18] Previous study showed that immunoglobulin G (IgG) antibody peaked at month 4 after the onset of SARS, IgG antibodies persisted for 16 months in all patients. In patients with COVID-19, antibodies were also detected in patients' blood after being infected by SARS-CoV-2, the immunity lasted for at least 7 days following remission of symptoms.[21] SARS-CoV-2-specific neutralizing antibodies were detected in patients from day 10 to 15 after the onset of the disease and remained thereafter. The titers of these antibodies among these patients correlated with the spike-binding antibodies targeting S1, receptor-binding domain, and S2 regions, although antibody titers were variable in different patients.[16] Therefore, it is needed for further studies in animal and human for long time follow-up to rule out the possibility for re-infection of SARS-CoV-2.

4 | MANAGEMENT

First, the discharge criteria in Novel Coronavirus Pneumonia Diagnosis and Treatment Protocol (7th edition, trial) (http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml.) must be strictly followed. Patients with COVID-19 (who received glucocorticoid therapy, had comorbidities, were older than 65) may extend the length of hospital stay because of the prolonged clearance of viruses. These discharged patients with COVID-19 also should be under quarantine management and health monitoring for 14 days as described in the seventh edition of guidance, instead of "self-monitoring for 14 days" described in previous editions of the Guidance. Finally, combination of serology tests for immunoglobulin M and IgG and viral RNA might be also helpful for surveillance and decision making for discharge of patients with COVID-19.[17,22]

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