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Review

A systematic review of re-detectable positive virus nucleic acid among COVID-19 patients in recovery phase

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ABSTRACT

A large number of coronavirus disease 2019 (COVID-19) patients have been cured and discharged due to timely and effective treatments. While some discharged patients have been found re-positive nucleic acid again in the recovery phase. Until now, there is still a great challenge to its infectivity and the specific potential mechanism which needs further discussion. However, more intensive attention should be paid to the prognosis of recovered patients. In this review, we mainly focus on the characteristics, potential reasons, infectivity, and outcomes of re-detectable positive patients, thereby providing some novel insights into the cognition of COVID-19.

1. Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been regarded as a major public health event globally. To date, the treatment of COVID-19 has made remarkable progress, enabling a great number of patients to be cured and discharged. The criteria for discharge in China are listed as follows: 1) Temperature returned to normal for longer than 3 consecutive days; 2) Respiratory symptoms resolved significantly; 3) Improvement of acute exudative lesions of chest computed tomography (CT); 4) Two consecutive respiratory specimens tested negative for reverse transcriptase-polymerase chain reaction (RT-PCR) tests (sampling interval of at least 24 h) [http://www.nhc.gov.cn/yzygj/s7653p/20200/46c9294a7dfe4cef80dc7f5912eb1989.shtml]. A recent study reported that four medical workers aged 30–36 years who had re-detectable positive (RP) for SARS-CoV-2 within 5–13 days after being cured and discharged, indicating that some of the recovered patients may still be virus carriers, which caused widespread concern (Lan et al., 2020). However, there is currently insufficient knowledge about the characteristics of RP patients. In the manuscript, we reviewed characteristics, potential reasons, infectivity, treatment, and outcome of RP patients in order to explain this phenomenon.

2. Characteristics

According to several reports, some patients were found to be re-positive RT-PCR results of virus nucleic acid after 5–13 days of medicine discharge to re-positive RT-PCR results (Zhang et al., 2020a, 2020b). A recent study showed that 23 of 651 patients (3%) who met the discharge criteria but turned positive again during the follow-up. The median age of the RP group was 56.0 years, and there were slightly more women than men. The average duration from discharge to the test positive again was 15.0 days (Mei et al., 2020). A follow-up case of 20 discharged COVID-19 patients showed that 3 of them had positive virus nucleic acid test results again 1 week later, but the results transferred negative in another week. However, there were no significant differences in symptoms and blood routine between RP patients and other recovered normal patients (Zheng et al., 2020). RP cases have also been reported in other countries. Some studies found that recovered patients with COVID-19 could acquire immunity against the virus (Loconsole et al., 2020; Ota, 2020). Although the patient's RT-PCR test was positive after recovery, there were no symptoms or only mild symptoms, which might mean that even if their antibodies cannot prevent re-infection after recovery, they could indeed reduce the severity of the disease (Bentivegna et al., 2020). Another finding indicated that the RP patients accounted for 14.5% (38/262) of discharged patients during the follow-up period. They were characterized as young (mostly under 14 years old), asymptomatic or minor clinical symptoms, improving or stable

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chest CT imaging, and no disease progression after re-admission (An et al., 2020). In addition, the latest report showed that 10.99% of patients (20/182) detected SARS-CoV-2 RNA re-positive, all of whom carried antibodies against SARS-CoV-2, and none of them showed any recurrence of clinical symptoms (Yuan et al., 2020). These findings indicated that RP patients accounted for a certain proportion of recovery patients, although they were asymptomatic or had only mild symptoms, rigorous self-quarantine and extended follow-up may still be required for these special cases (Bongiovanni and Basile, 2020).

3. Potential reasons

Many studies have shown that RT-PCR results of most RP patients, which may not be considered as simple viral relapse or secondary infection (Xiao et al., 2020a, 2020b). The underlying mechanism of RP patients remains elusive, the specific reasons need to be further explored. Some experts speculated that the potential reasons might be related to some factors such as virology, detection of specimens, patients' condition or intra-hospital infections.

For virology of SARS-CoV-2, it may be related to the biological characteristics of the virus. Viral residue, intermittent viral release, and periodic changes of virus replication are generally considered as the main factors (An et al., 2020). A pathological examination of a patient who reached the discharge standard but died of sudden cardiac arrest found that SARS-CoV-2 virus still remained in the lung cells and caused lung pathological changes. Although the results of three nucleic acid tests were negative for the patient, there was viral residue in the lungs, so even if the patient was discharged, we supposed that the virus would transfer positive again after a period of time (Yao et al., 2020). In addition, it may be linked to the diversity of SARS-CoV-2 genomic and the characteristics of repeated mutations (van Dorp et al., 2020). In other words, we lack a comprehensive understanding of SARS-CoV-2, which may be continuously or repeatedly positive during the course of the disease (Chen et al., 2020).

For detection of specimens, it may be related to the collection methods, processing procedures, and detection methods (Chen et al., 2020). Differences in sample types, improper nucleic acid extraction, insufficient viral level or inappropriate sample pretreatment will lead to false-negative detection results by PCR method at a certain rate (Pan et al., 2020; Xie et al., 2020; Zou et al., 2020). This may cause COVID-19 patients whose virus has not been completely clearance to reach the current discharge criteria. Then after discharge, the virus will continue to replicate at a lower level, making this part of patients re-detected positive again once viral loads rise to the detection level. Meanwhile, the virus mainly concentrates in the lower respiratory tract and the lung, so false-negative tests may appear when collecting throat swabs (Zhou et al., 2020). In addition, initial studies reported that the SARS-CoV-2 RNA could be detected in the feces of 81.8% recovered patients (54/66), even in those with negative throat swabs (Ling et al., 2020). And later studies revealed that the viral RNA can persist in fecal samples for nearly 5 weeks after the patients' respiratory specimens detected negative (Wu et al., 2020a, 2020b). Other studies have also demonstrated the importance of rectal swab-testing, which should be taken into consideration (Wölfel et al., 2020; Xu et al., 2020). Due to the possible presence of SARS-CoV-2 in the digestive tract, the current methods of discharge criteria for oral/nasopharyngeal swab virus detection are not accurate (Liu et al., 2020a, 2020b). Therefore, using more sensitive detection methods and collecting different samples to test will be a more effective way to overcome false-negative detection (Zhang et al., 2020a, 2020b).

For patients' condition, it may be related to the underlying diseases, degree of infection, and treatment methods, among which hypertension and diabetes are the most common underlying diseases (Liu et al., 2020a, 2020b). Once infected with SARS-CoV-2, the underlying diseases will be more difficult to control, leading to more complications and dysfunction of more organs and immune system (Hussain et al., 2020). Ultimately, the hospital stay will be prolonged, and patients are more likely to relapse or infection after discharge due to their lower immune function. Also, some studies have indicated that the use of antiviral drugs may affect the host's cellular immunity. Although virus can be cleared by antiviral drugs in the initial phase, patients' immune function decreased. Once antiviral therapy discontinued, virus will tend to be activated due to lack of normal cellular immunity, which may be regarded as one of the reasons for recurrence of SARS-CoV-2, but it still needs more evidence to verify that (Balachandar et al., 2020; Wu et al., 2020a, 2020b).

For intra-hospital infections, a previous study found that about 41.3% (57/138) of patients were infected with SARS-CoV-2 in the hospital, including 12.3% (17/138) are patients and 29% (40/138) are healthcare workers (Wang et al., 2020). The cause of infection by healthcare workers and inpatients might be related to the spread of hospital-related viruses, such as the hands of healthcare workers, thermometers, sphygmomanometers, and stethoscopes (Wee et al., 2020). Therefore, early isolation of suspected inpatients of COVID-19 and maintaining social distance between hospitalized patients are very important during the continuous outbreak, which may be regarded as a great significance to reduce the possibility of hospital transmission (Hoe Gan et al., 2020). Therefore, we should pay close attention to the risk of intra-hospital infections which may be the possible implications for patients to be re-infected and for healthcare workers.

4. Infectivity

Theoretically, the infectivity of patients is determined by the existence of the virus in different body fluids, secretions, and excreta (Ling et al., 2020). And the viral infectivity mainly depends on its reproduction state (Wölfel et al., 2020). In a study from South Korea, no active virus was discovered in samples from RP patients (Kang, 2020). This means that re-positive virus nucleic acid does not indicate infectivity. This also can explain that although SARS-CoV-2 RNA can be detected in RP patients, no cases of infection have been reported so far. For example, all close contacts of RP patients were tested negative for nucleic acid and showed no suspicious clinical symptoms (An et al., 2020). Another case report showed that there was no significant change in chest CT of RP patients and no family members were infected, which suggested that RP patients have no or lower infectivity (Lan et al., 2020). However, the infectivity of RP patients is still needed to be verified by more studies and more cases. Furthermore, it is important and necessary to continue epidemiological follow-up on RP patients in order to monitor their health status and explain their infectivity.

SARS-CoV-2 RNA can be detected not only in the respiratory tract, but also in blood, digestive tract and feces (Holshue et al., 2020). Some reports further indicate that some patients with COVID-19 were still positive in anal swab samples even after nasal or throat swab test turned negative (Zhang et al., 2020a, 2020b). However, the detection of viral nucleic acid in anal swabs might not necessarily mean the presence of live virus in feces, and it might not have infectivity too. A recent study showed that 21.2% (46/217) of patients detected positive SARS-CoV-2 RNA in anal swabs, but they could not isolate the live virus (Lin et al., 2020). What's more, several studies had found that the positive rate and duration of SARS-CoV-2 RNA in anal swabs were higher than those in nasal or throat swabs during the recovery phase, but the viral load was relatively low, which indicated that SARS-CoV-2 might have weak ability of active infection and replication in gastrointestinal tract (Xu et al., 2020; Zhang et al., 2020a, 2020b). Therefore, compared to nasal or throat swab positive subjects, persistent anal swab positive patients have lower infectivity. However, there is no conclusive evidence whether these patients will transmit the virus to other people, we should still be aware of the potential route of fecal-oral transmission and take relevant preventive measures (Kipkorir et al., 2020).

5. Treatment and outcome

According to recent research, RP patients usually complete negative-conversion again 2–3 weeks later, and they could heal themselves without any antibiotics or antiviral drugs, which might be related to the body's recovery immunity (An et al., 2020). In other words, even if sometimes the virus nucleic acid tested by RT-PCR is positive in the recovery phase of COVID-19, it will not cause a more serious condition, and antiviral therapy may not be required in most patients. RT-PCR results will turn negative again within a few days as immunity function recovered (Zhang et al., 2020a, 2020b). For these cases, observational therapy can be used instead of antiviral drug therapy for asymptomatic RP patients.

A recent study showed that the recovered patients acquired relatively stable and sustained immunity after being infected with SARS-CoV-2. All the subjects produced $CD4^+$ T cell responses to the spike protein on the surface of the SARS-CoV-2, which also provided theoretical support for the vaccine under development (Grifoni et al., 2020). The detectable and sustained high levels of IgM indicate that the acute phase of SARS-CoV-2 infection, but IgG suggests that the body has enough immune protection against the SARS-CoV-2 and IgG can persist a very long time (Xiao et al., 2020a, 2020b). It has been reported that the results of IgM were negative but IgG were positive when three patients were discharged from the hospital. And these results were still the same when they were re-admitted to the hospital as virus nucleic acid transferred positive again (Fu et al., 2020). Therefore, detection of virus nucleic acid combined with antibody is useful for determining disease status, treatment and outcome.

6. Conclusion

It has been over half a year since the COVID-19 epidemic spread around the world. Although a lot of patients from different countries have gradually recovered, it is very important to follow up with the patients who recovered from the infection. There are still some unknowns in the face of recovered patients. In this situation, it is necessary to understand the characteristics of RP patients and determine if they are potential threats to the public (Bongiovanni and Basile, 2020). According to current reports, RP patients account for a certain proportion of recovered patients, but most of their symptoms are asymptomatic or mild and can heal themselves without any treatment. Pre-symptomatic and asymptomatic carriers may be infectious, although there have been no reports of recovered patients infecting others, we should consider that carriers in the recovery period may also transmit the virus (Rothe et al., 2020).

Regarding the reactivation or reinfection of SARS-CoV-2 will be a major public health problem, as it may greatly promote the spread of the virus in the population. Given the false-positive rate of RT-PCR detection and other potential reasons, patients who relapsed are actually more likely to have persistent viral infection(Kang et al., 2020). In this regard, research suggests that the virus should be cultured and genetically identified to determine the infectivity of RP patients and to distinguish whether the patients tested positive during recovery are re-infected or relapsed (Hoang et al., 2020). In addition, regarding the potential risk of fecal-oral transmission, we recommend that it is necessary to screen all discharged patients for gastrointestinal viruses and to carry out close monitoring and early intervention for patients with positive anal swab tests. Due to the higher risk of potentially asymptomatic COVID-19 cases, we recommend that patients avoid mixing, always wear surgical masks and no visitation allowed. For COVID-19 patients with underlying diseases or other complications, the time to discharge should be extended due to the prolonged virus clearance time. In addition, all recovered patients are subject to home isolation for at least 14 days after being discharged. In summary, the current public health emergency requires more and urgent investigations of recovery cases to contain the epidemic.

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Declaration of Competing Interest

The authors declared that they have no conflict of interests.

References

An, J., et al., 2020. Clinical characteristics of the recovered COVID-19 patients with re-detectable positive RNA test. medRxiv. https://doi.org/10.1101/2020.03.26.20044222.
Balachandar, V., et al., 2020. Follow-up studies in COVID-19 recovered patients - is it mandatory? Sci. Total Environ. 729, 139021.
Bentivegna, E., et al., 2020. New IgM seroconversion and positive RT-PCR test after exposure to the virus in recovered COVID-19 patient. J. Med. Virol. https://doi.org/10.1002/jmv.26160.
Bongiovanni, M., Basile, F., 2020. Re-infection by COVID-19: a real threat for the future management of pandemia? Infect. Dis. (Lond.) 52, 581–582.
Chen, D., et al., 2020. Recurrence of positive SARS-CoV-2 RNA in COVID-19: A case report. Int. J. Infect. Dis. 93, 297–299.
van Dorp, L., et al., 2020. Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. Infect. Genet. Evol. 83, 104351.
Fu, W., et al., 2020. Letter to the Editor: Three cases of re-detectable positive SARS-CoV-2 RNA in recovered COVID-19 patients with antibodies. J. Med. Virol. https://doi.org/10.1002/jmv.25968.
Grifoni, A., et al., 2020. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell 181, 1–13.
Hoang, V.T., et al., 2020. Recurrence of positive SARS-CoV-2 in patients recovered from COVID-19. J. Med. Virol. https://doi.org/10.1002/jmv.26056.
Hoe Gan, W., et al., 2020. Preventing intra-hospital infection and transmission of COVID-19 in healthcare workers. Saf. Health Work 11, 241–243.
Holshue, M.L., et al., 2020. First Case of 2019 Novel Coronavirus in the United States. N. Engl. J. Med. 382, 929–936.
Hussain, A., et al., 2020. COVID-19 and diabetes: Knowledge in progress. Diabetes Res. Clin. Pract. 162, 108142.
Kang, H., et al., 2020. Retest positive for SARS-CoV-2 RNA of "recovered" patients with COVID-19: persistence, sampling issues, or re-infection? J. Med. Virol. https://doi.org/10.1002/jmv.26114.
Kang, Y.J., 2020. South Korea's COVID-19 Infection Status: from the perspective of Re-positive after Viral Clearance by Negative Testing. Disaster Med. Public Health Prep. https://doi.org/10.1017/dmp.2020.168.
Kipkorir, V., et al., 2020. Prolonged SARS-Cov-2 RNA Detection in Anal/Rectal Swabs and stool Specimens in COVID-19 patients After Negative Conversion in Nasopharyngeal RT-PCR Test. J. Med. Virol. https://doi.org/10.1002/jmv.26007.
Lan, L., et al., 2020. Positive RT-PCR Test Results in patients Recovered From COVID-19. JAMA 323 (15), 1502–1503.
Lin, W., et al., 2020. Association between detectable SARS-COV-2 RNA in anal swabs and disease severity in patients with Coronavirus Disease 2019. J. Med. Virol. https://doi.org/10.1002/jmv.26307.
Ling, Y., et al., 2020. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. Chin. Med. J. 133, 1039–1043.
Liu, J., et al., 2020a. Detection of SARS-CoV-2 by RT-PCR in anal from patients who have recovered from coronavirus disease 2019. J. Med. Virol. https://doi.org/10.1002/jmv.25875.
Liu, W., et al., 2020b. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. Chin. Med. J. 133, 1032–1038.
Loconsole, D., et al., 2020. Recurrence of COVID-19 after recovery: a case report from Italy. Infection. https://doi.org/10.1007/s15010-020-01444-1.
Mei, Q., et al., 2020. Assessment of patients who tested positive for COVID-19 after recovery. Lancet Infect. Dis. https://doi.org/10.1016/S1473-3099(20)30433-3.
Ota, M., 2020. Will we see protection or reinfection in COVID-19? Nat. Rev. Immunol. 20, 351.
Pan, Y., et al., 2020. Potential False-Negative Nucleic Acid Testing Results for Severe Acute Respiratory Syndrome Coronavirus 2 from Thermal Inactivation of Samples with Low viral Loads. Clin. Chem. 66, 794–801.
Rothe, C., et al., 2020. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. N. Engl. J. Med. 382, 970–971.
Wang, D., et al., 2020. Clinical Characteristics of 138 Hospitalized patients With 2019 Novel Coronavirus-Infected pneumonia in Wuhan, China. JAMA 323, 1061–1069.
Wee, L.E., et al., 2020. Minimizing intra-hospital transmission of COVID-19: the role of social distancing. J. Hosp. Infect. 105, 113–115.
Wölfel, R., et al., 2020. Virological assessment of hospitalized patients with COVID-2019. Nature 581, 465–469.
Wu, F., et al., 2020a. Discontinuation of antiviral drugs may be the reason for recovered

COVID-19 patients testing positive again. Br. J. Hosp. Med. (Lond.) 81, 1–2.
Wu, Y., et al., 2020b. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol. Hepatol. 5, 434–435.
Xiao, A.T., et al., 2020a. Profile of specific antibodies to SARS-CoV-2: The first report. J. Infect Secur. 81 (1), 147–178.
Xiao, A.T., et al., 2020b. False-negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: Rather than recurrence. J. Med. Virol. https://doi.org/10.1002/jmv.25855.
Xie, X., et al., 2020. Chest CT for Typical 2019-nCoV pneumonia: Relationship to Negative RT-PCR Testing. Radiology 296 (2), E41–E45.
Xu, Y., et al., 2020. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat. Med. 26, 502–505.
Yao, X.H., et al., 2020. Pathological evidence for residual SARS-CoV-2 in pulmonary tissues of a ready-for-discharge patient. Cell Res. 30, 541–543.
Yuan, B., et al., 2020. Recurrence of positive SARS-CoV-2 viral RNA in recovered COVID-19 patients during medical isolation observation. Sci. Rep. 10 (1), 11887.
Zhang, B., et al., 2020a. Positive rectal swabs in young patients recovered from coronavirus disease 2019 (COVID-19). J. Infect Secur. 81 (2), e49–e52.
Zhang, W., et al., 2020b. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg. Microbes Infect. 9, 386–389.
Zheng, K.I., et al., 2020. A Case Series of Recurrent Viral RNA positivity in Recovered COVID-19 Chinese patients. J. Gen. Intern. Med. 35 (7), 2205–2206.
Zhou, L., et al., 2020. Cause analysis and treatment strategies of "recurrence" with novel coronavirus pneumonia (COVID-19) patients after discharge from hospital. Chin. J. Tuberc. Respir. Dis. 43, 281–284.
Zou, L., et al., 2020. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected patients. N. Engl. J. Med. 382, 1177–1179.