SARS-CoV-2 Reinfection within the first 3 months of COVID-19 Recovery in A Referral Hospital, Tehran, Iran

Sara Sadr
Infectious Disease Research Institute
Melika Arab Bafrani
Infectious Disease Research Institute
Alireza Abdollahi
Infectious Disease Research Institute
SeyedAhmad SeyedAlinaghi
Infectious Disease Research Institute
Esmaeil Mohammadnejad
Infectious Disease Research Institute
Roghieh Hossienzade
Infectious Disease Research Institute
fereshteh Shahmari Golestan
Infectious Disease Research Institute
Zahra Ahmadinejad
Infectious Disease Research Institute
Mohamadreza Salehi
Infectious Disease Research Institute
Mohammad Javaherian
Infectious Disease Research Institute
Elahe Kimyaee
Infectious Disease Research Institute
Fatemeh Jafari
Infectious Disease Research Institute
Fereshteh Ghiasvand (ghiasvand_62@yahoo.com)
Infectious Disease Research Institute

Research Article

Keywords: COVID-19, Reinfection, Virus Shedding, Polymerase Chain Reaction, Seroconversion

Posted Date: March 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-271345/v1

Abstract

Objectives

Possibility of reinfection with SARS-CoV-2 changes our view on herd immunity and vaccination, and can impact quarantine policies. We performed follow-up studies on recovered patients to assess possible development of reinfections.

Method

During a 6-month period, 202 PCR-confirmed recovering COVID-19 patients entered this study. Follow-up RT-PCR tests and symptoms assessment were performed one month after the initial Positive results. patients who tested negative were tested again one and three months later. The Serum IgG and IgM levels were measured in the last follow-up session.

Results

In the first two follow-up sessions, 82 (out of 202) and 44 patients participated with four and three asymptomatic patients testing positive. In the last session, 32 patients were tested and four were positive, three of them were mildly symptomatic and all of them were positive for IgG.

Conclusion

A positive RT-PCR in a recovering patient may represent reinfection. While we did not have the resources to prove reinfection by genetic sequencing of the infective viruses, we believe presence of mild symptoms in the three patients who tested positive over 100 days after becoming asymptomatic, can be diagnosed as reinfection. The IgG may have abated the symptoms of the reinfection, without providing complete protection.

Introduction

Since November 2019, when the first case of infection with the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was diagnosed, this virus has infected more than 103 million people worldwide and has caused more than 2 million deaths (1). No curative drug or specific treatment has been approved to have considerable efficacy against this infection; but, currently over 200 candidate vaccines have been acknowledged by the World Health Organization (WHO) (2–4).

Our experience with other respiratory diseases, such as those caused by influenza, respiratory syncytial virus (RSV), and seasonal respiratory infections caused by coronavirus lead us to believe that the natural immunity after an episode of infection with this virus may not cause long-time immunity against this virus (5). Many studies have shown that antibody levels in infected patients wane substantially within a few months (6, 7); and the studies that report re-infection further question the efficacy and longevity of the acquired immunity after infection with this virus. Several cases of suspected and proven cases of re-

infection with SARS-CoV-2 have been reported in patients with different levels of presented antibody response. These cases have consisted of patients in all age groups, with intact immune systems and with or without underlying diseases (8–10).

The duration of sustained antibody response after an episode of infection is generally a representative of the immune protection that can be achieved by vaccination against the same virus (11). Waning of the IgG antibodies that are naturally produced in a patient after infection with SARS-CoV-2, undermines the supposed efficacy of vaccination; especially since cases of symptomatic re-infection with replication-competent virus have been reported within the first 6 months after the initial infection (12, 13).

To this date, thousands or distinct variants of SARS-CoV-2 have been identified with over 400 variants in the spike protein, which is presumably the binding cite of neutralizing antibodies (14). Besides, in many cases of re-infection, genetic sequencing has shown that the second episode of infection has been caused with a different clade of the virus (15–18). We can assume—as is the case with infections caused by rhinoviruses and influenza—that the protective activity of antibodies is limited to each specific subtype of the virus, this can possibly explain why re-infection can occur in presence of detectable levels of IgG (19, 20). Re-infections have implications not only regarding the prospective of vaccines, but also regarding management strategies in national and international levels; since they undermine the development of herd immunity and can be grounds for changing quarantine policies for recovering patients (5, 21).

In this study we aimed to investigate the rate of symptomatic and asymptomatic re-infections with SARS-CoV-2 in recovering patients for up to four months after the initial diagnosis of Coronavirus Disease 2019 (COVID-19); and find possible risk factors that are associated with re-infection.

Methods

Study design and participants

This cross sectional study was performed for a duration of six months from May to September of 2020 on COVID-19 patients who were admitted to the Imam Khomeini Referral Hospital, Tehran, Iran.

Eligibility Criteria

Patients who had been diagnosed with COVID-19 (approved by a polymerase chain reaction (RT-PCR) test of nasopharyngeal specimens) and had been admitted based on the national criteria for hospitalization (a sustained peripheral oxygen saturation of under 93% and/or a respiratory rate of over 30 per minute or sustained nausea and vomiting and severe weakness even with normal oxygen saturation), were brought into the study upon discharge. The current national discharge criteria dictate that prior to discharge patients must have at least two consecutive afebrile days with a blood oxygen saturation of over 90%, oral intake without nausea, and improvement in weakness. The exclusion criteria were a negative real-time polymerase chain reaction (RT-PCR) result or lack of documentation of a positive result at the initial

hospitalization, and failure to perform the follow-up RT-PCR tests (due to patient-related factors such as unwillingness to cooperate).

Study initiation and follow-up sessions

All information regarding patients' admission and epidemiological data were collected, including age, sex, any past medical history (especially hypertension, diabetes, and cardiovascular disease) and medications used by the patient for previous medical issues.

The first follow-up RT-PCR study was performed one month after the initial positive RT-PCR test; at which point all patients had been asymptomatic for at least 14 days. All patients who tested negative were re-tested one and three months after the date of the first follow-up RT-PCR test (Fig. 1). A complete assessment of signs and symptoms related to COVID-19 along with serology testing for anti-SARS-CoV-2 IgG and IgM levels were also performed during the 3rd follow-up visit.

PCR

In each follow-up session, a nasopharyngeal swab sample was obtained by a trained technician and RNA extraction was performed using a Real Genomics viral nucleic acid extraction kit (Cat. No. YVN50/YVN100). The RT-PCR testing was done using the Novel Coronavirus (2019-nCOV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing), Sansure Biotech (S3102E), made in Changsha, China for qualitative detection of the ORF1ab and N genes of novel coronavirus (2019-nCOV) with a cycle threshold of less than 35 ($Ct < 35$) for positive control and $Ct > 40$ for negative control at channel FAM, ROX and CY5 (internal control) according to kit instructions.

Antibodies

To evaluate the IgG and IgM levels, a 5 cc whole blood sample was drawn from patients (without anticoagulants), and serum was derived from the specimen using centrifugation (3000xg for 10 minutes); and the Enzyme-linked Immunosorbent Assay (ELISA) method (Pishtaz Teb SARS-CoV-2 IgM and IgG-Iran) was used to test the serum antibody levels. The test was performed according to the manufacturer's brochure. For interpretation, results greater than 1.1 were considered positive and those less than 0.9 as negative. Results within the mentioned range, were reported as borderline and the test was redone on a second, fresh serum sample to confirm the initial results.

The manufacture reported diagnostic specificity and sensitivity of the test kits:

Specificity: 97.30% and Sensitivity: 79.40% for SARS-COV-2- IGM kit.

Specificity: 98.30%% and sensitivity: 91.10% for SARS-COV-2- IGG kit.

Measurements and statistical analysis

Data was analyzed using SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Quantitative variables are reported by mean and standard

deviation (SD) and qualitative variables are reported using frequency and percentage. Chi-square and Fisher's exact tests were used to assess the statistical relationships between categorical variables. The level of significance was set as P-value < 0.05 for all analyses.

Ethical considerations

Informed consent was obtained from all patients and they were advised that they can leave the study at any time point and this will not hinder their potential future visits to the hospital or in the quality of care they would receive.

Results

One month after the first positive RT-PCR (1st F/U)

A total of 202 discharged patients were contacted for follow-up laboratory studies and 82 patients, including 27 women (33%) and 55 men (64%), decided to participate in this study.

Patients had a median age of 47 ranging between 29 and 84 years old. Table 1 shows the demographic description of participants. The first RT-PCR results of 4 out of 82 patients (4.87%) were positive; although, all four patients were asymptomatic. The duration of the initial admission, the severity of the first episode of the disease, presence and type of underlying diseases, and recent history of using immunosuppressive drugs were not significantly different between patients who tested positive and those who tested negative (P value: 0.63, 0.57, 0.59, 0.61).

Two months after the first positive RT-PCR (2nd F/U)

One month after the first negative follow-up result, among the 78 patients who had tested negative, 44 patients (53.65%) continued their participation in the study. All patients at this point were asymptomatic, and 3 patients (6%) tested positive for SARS-CoV-2 RT-PCR. Additional data regarding patients who tested positive are presented in Figure 1. The patients who tested positive were not significantly different in terms of the duration of the initial admission, the severity of the disease, and need for ICU admission (P value: 0.69, 0.62, and 0.65).

Four months after the first positive RT-PCR (3rd F/U)

Three months after the first follow-up RT-PCR test, among the 41 patients who had tested negative in the previous tests, 32 patients (39.02%) continued their participation in the study. Four patients had a positive RT-PCR result (12.5%). None of the patients who tested positive were symptomatic in prior follow-up studies; but now three of them were mildly symptomatic; also, two patients had underlying medical conditions (Figure 1); and none of the patients who tested negative were symptomatic. The underlying medical conditions, recent history of using immunosuppressive drugs, severity of the disease, the duration of admission and need for ICU admission in the first episode of infection were not significantly

different between patients who had a positive RT-PCR result and those who did not (P value: 0.67, 0.59, 0.64, 0.64, 0.71).

The serology studies showed that IgM levels were <1.1 g/L in 30 (94%) patients (negative result); and positive in 2 patients. The patients who tested positive were asymptomatic and did not have a positive RT-PCR test in any of the previous follow-up studies. The IgG levels were >1.1 g/L in 30 (94%) patients (positive result); and negative in 2 patients. The mean IgG level among patients was 7.94±3.67 g/L and ranged between 1.48-14.5 g/L. The two patients, who had a negative IgG test result, did not test positive in any of the follow-up RT-PCR tests.

All patients recovered with no further complications and none of the patients who re-tested positive required readmission or further medical treatment.

Discussion

The possibility of reinfection or reactivation of COVID-19 is concerning to the physicians and the patients. Articles that have reported these phenomena raise questions that when answered will have a pivotal impact on worldwide preventive and treatment policies. If reinfections occur, not only seemingly recovered patients can infect others, but also achieving herd immunity via natural infection or immunity after vaccination will be challenging (22).

Interpretations of a positive RT-PCR re-test

Considering the large number of people who have been diagnosed with COVID-19, few cases of reinfection have been reported. Often, for the diagnosis COVID-19 in a recovered individual a positive RT-PCR test with or without accompanying symptoms have been used. Although, in these cases different speculations for interpretation of a positive RT-PCR test can be made; each of which will have specific reverberations (23).

In some studies, a positive RT-PCR result in a recovering patient who tested negative upon resolution of their symptoms, is considered as a strong indicator of reinfection (10). Although, we need to take into account the possibility of a false result in either test, which would undermine a diagnosis of re-infection (24). A false positive test can occur in a recovering patient; or a false negative test shortly after subsidence of symptoms, followed later by a correctly positive test may mislead physicians towards a diagnosis of re-infection (25). To avoid this problem, as per WHO recommendations, in many regions the treatment protocol for COVID-19 requires two consecutive negative RT-PCR results prior to discharge (26, 27).

Also, RT-PCR as the main diagnostic tool of infection cannot distinguish between replication-competent viruses and remnants of viral fragments that are expelled from a recovering, non-contagious patient (28, 29). Viral shedding from the respiratory tract during the recovery period has been reported to last for as long as 12 weeks after the initial infection (30); thus, a significant time-gap between the first episode of

infection and the second positive RT-PCR test can clarify that the patient has passed the post-COVID-19 viral shedding stage.

The first follow-up RT-PCR

In our study, as per national protocol, no RT-PCR testing was performed upon discharge to confirm viral clearance, and the first follow-up RT-PCR study was performed 30 days after the first positive RT-PCR result in each patient, which would have been after at least 14 days of being asymptomatic. Considering the short interval between the onset of the disease and the test, no recorded negative RT-PCR result at discharge, and the fact that none of the patients who tested positive were symptomatic, we believe that the positive results show continued viral shedding rather than re-infection. The four patients who tested positive were not significantly different from those who tested negative in terms of the severity of the initial illness, duration of the first admission, age, or significant underlying conditions to suggest a risk factor for a longer viral shedding period. Other studies have reported that up to 14% of recovering asymptomatic patients who were tested and negative upon discharge, re-test positive with no sign of infection (31). Also, some studies have reported that recovering patients became symptomatic after a short symptom-free period. A 24-year-old health-worker became symptomatic and tested positive for SARS-CoV-2 within 52 days of an initial symptomatic diagnosis of COVID-19. Similar to our study, no confirmatory RT-PCR testing was performed at discharge. Serum antibodies were not detected at the beginning of the second symptomatic period, which can represent an incomplete immune response that left the infection temporarily dormant, only to be re-activated again; and even though the patient was symptomatic and had had occupational contact with infected patients during her symptom-free recovery period, re-activation was considered more probable than re-infection (32). Similar studies have reported a return of mild or even severe symptoms; but within a short time frame and without genetic analysis of the infective pathogens, they were reported to be cases of reactivation rather than re-infection (33–36).

The second follow-up RT-PCR

Out of the 44 patients who tested negative on the first test and continued their cooperation with the study, 3 patients (6.81%) tested positive 60 days after the initial positive RT-PCR test (one month after the first negative RT-PCR test). These patients again were not symptomatic and the time laps between the initial infection and this positive test is not significant enough to rule out viral shedding. Although, in case of viral shedding and/or presence of remnant viral particles, we would have expected to achieve a positive result in the previous RT-PCR test as well as the second test. Due to the negative result of the first follow-up test, we believe our results can possibly point to re-infection, but this dissonance in results can be explained in absence of re-infection. Given the limited accuracy of RT-PCR, in case of a false negative in the first follow-up test or a false positive in the second test, continued shedding or complete recovery (respectively) can be misdiagnosed as re-infection (37, 38). On the other hand, studies have shown that COVID-19 patients have a lower concentration of ACE2 monocyte expression—the endogenous entry receptor of SARS-CoV-2—and researchers have hypothesized that the virus can remain dormant in

peripheral blood mononuclear cells and cause a relapse after the respiratory system has been cleared of the virus and patient has tested negative (39–41).

Presence of typical symptoms, which cannot be explained by any diagnosis other than SARS-CoV-2 infection is also a strong indicator of active infection, which may represent re-infection or re-activation of the initial infection (23). Positive RT-PCR tests in absence of significant symptoms in a recovering patient has also been reported in many studies (18, 42). In some reported cases, symptoms were present, although less severe than the first episode (43). Our findings include asymptomatic and mildly symptomatic patients who re-tested positive for SARS-CoV-2. None of our cases had symptoms as or more severe than the first episode of infection. Contrasting our results, the majority of other reports have described more severe symptoms in patients who re-tested positive (15–17, 44) and researchers have hypothesized that a selection bias towards testing and confirming re-infection in symptomatic patients (45) and/or a primed and heightened immune response upon the second course of infection can be the reason why most cases of re-infection pertain to patients with more intense symptoms upon second exposure and infection (15, 44).

The strongest proof of an episode of re-infection can be achieved by a positive viral culture (23, 46) and/or genetic sequencing of the infective virus in both episodes of infection to confirm that the second episode is caused by a different clade or lineage of the virus. Tillett et al, reported a case of re-infection in a 25-year-old male, who recovered from a RT-PCR-confirmed episode of COVID-19, only to become symptomatic again after a 30-day symptom-free period. Genetic sequencing showed a distinct genetic difference between the two SARS-CoV-2 specimen, indicating two separate instances of infection with genetically different variants of the virus (15). Similar cases of re-infection with a genetically different clade of the virus within 6 months of an original episode of COVID-19 have been reported (16–18).

Unfortunately, genetic sequencing is not readily available or performed and based on Center for Disease Control and Prevention (CDC) recommendations in absence of genetic proof of infection with a different clade of the virus, a positive RT-PCR test that has been obtained after the first 90 days of the onset of the initial infection can be considered indicative of re-infection. Although, a positive RT-PCR test after two consecutive negative results, especially if accompanied by typical symptoms, can be defined as re-infection even within the first 90 days of the first episode of infection (23).

The third follow-up RT-PCR

According to the recommended CDC definitions (30), re-infection is a probable diagnosis for the three symptomatic patients who tested positive in the last RT-PCR screening test; and it is less likely in case of the one asymptomatic patient. The third follow-up RT-PCR screening was performed 120 days after the initial diagnosis of COVID-19 on 32 patients who had tested negative on both of the previous tests. Even though we did not perform viral cultures to prove presence of replication-competent virus, the mild symptoms that these patients experienced can be considered as indicators of re-infection. Although, the symptoms could have been caused by re-activation of dormant infection and release of viruses from body reservoirs. A similar process involving latent infection of cells followed by transcription of viral

genome has also been suggested, which would result in reactivation of the virus from a latent to a lytic stage after a symptom-free period, causing a resurgence of COVID-19 symptoms (41); but the long time gap between the two positive RT-PCR results makes re-activation an unlikely diagnosis (23). These four patients were not significantly different from those who tested negative in terms of past medical history or severity of the initial episode of COVID-19. Also, we did not find any specific risk factors that could help us to distinguish patients who are more susceptible to re-infection from those who are not.

Humoral response

The normal human response in COVID-19 is comprised of both humoral and cellular responses and production of CD4 + and CD8 + cells. Both insufficient and overactive immune responses have been reported in these patients (47). Production of protective cytokines and IFN-gamma, which is mediated by the activation of the CD4 + and CD8 + cells, plays an important role in containing and resolution of the infection (48). The dynamics of the antibody response in COVID-19 patients is not completely known; and different studies have reported different rates of seroconversion among these patients. Zhao et al (49) and Liu et al (50) reported seroconversion in all infected patients respectively by 39 and 14 days after the onset of the infection. Liu et al also reported that by the 60th day IgM antibodies were undetectable in about one-third of the patients and the IgG titers had decreased substantially (50). Based on another study, even though recently discharged patients have a high level of humoral immunity against the virus, IgG and neutralizing antibodies start to decreases within 2 to 3 months after the infection (13). In another study the seroconversion rate for IgG, IgM and IgA was ~ 90% and most patients seroreverted within 75 days; although IgG levels remained detectable for over 90 days after the symptom onset in more than 99% of patients (51). Multiple other studies have also concluded that the humoral immunity against this virus could be short-lived (52). Contrasting these studies, our results showed that 94% of patients were positive for neutralizing antibodies (IgG) 120 days after the onset of symptoms. Our results are in line with the results of an Icelandic population study that reported a 91% seropositivity four months after the initial diagnosis of COVID-19 (6). In evaluation of the results of these studies, we should take into account the dynamics and natural process of the humoral response. In case of many other viral infections—where seroconversion is sustained as seromaintenance and immunity—we see a temporary decrease of antibody levels during the first few months of infection/inoculation (53), and since the emergence of COVID-19 is recent, we cannot judge the efficacy of the humoral response to this virus in long term and a rebound increase in antibody levels can be expected (52).

In our study, the four patients who had a positive result in RT-PCR screening 120 days after the initial diagnosis of COVID-19, were also positive for neutralizing antibodies; and although they theoretically may have prevented a severe episode of re-infection and caused a lack of any symptoms in one RT-PCR-positive patient, we cannot know for sure if those levels are high enough to be completely protective in each patient (23). In a similar study Zhang et.al reported re-infection in 6 recovered COVID-19 patients that was caused by viruses from lineages different than the first infection. They reported that all these patients had varied levels of neutralizing antibodies and concluded that presence and even maintenance of the humoral response cannot rule out the possibility of re-infection (54). We also believe that the two

patients who did not have sufficient levels of IgG (< 1.1 g/L), have been protected from an episode of re-infection by a strong cellular immune response, even within an epidemic situation.

Genetic sequencing of the respiratory samples, can rule out a false positive in RT-PCR testing and determine if the infection is caused by a different subclass of the virus. In cases of re-infection with a clade of the virus different from the first episode, even protective levels of IgG may not be effective against the new strain of the virus (23). So, we hypothesize that high levels of neutralizing antibodies do not make the diagnosis of re-infection unlikely, unless there is genetic proof that the positive RT-PCR results are related to the same strain of virus that caused the first episode; in which case re-activation/relapse would be a much more likely diagnosis.

Limitations

One of the limitations of our study was that a considerable number of patients, who participated, dropped out before completion of the study and that this study was only performed in one medical center. We did not perform viral genetic sequencing to prove infection with a different clade of the virus, or viral cultures to prove presence of replication-competent virus as opposed to residual viral fragments in case of viral shedding. The diagnosis of re-infection in our study is exclusively based on RT-PCR results, typical symptoms and the long time gap between the two positive RT-PCR results. And, since we did not measure the viral load in patients who tested positive, the diagnosis of re-infection in our study is as certain as is the specificity of the RT-PCR test; which due to our limited resources could not be repeated 24 hours after each session in order to further avoid false results. We were also unable to perform required serology testing from the beginning of the study, so we cannot investigate the changes of antibody levels throughout the course of the disease.

Conclusion

Based on current diagnostic guidelines, re-infection associated with mild symptoms was detected in 3 out of 82 patients; barring possible false positive results. We cannot confirm re-infection without positive viral cultures. We also believe that the quantity of antibodies that are produced against this virus can be sustained longer than the initial studies suggest, although the protective abilities of these antibodies against infection with the same or a different subtype of the virus needs to be studied further. The emergence of new vaccines against this virus, is a considerable achievement with limited guaranteed outcomes, because the intricacies of the long-term immune response against this virus is not fully known, and further studies on cases of supposed re-infection are needed to clarify the probability and underlying risk factors of re-infection.

Abbreviations

CDC Center for Disease Control and Prevention

COVID-19 Coronavirus Disease 2019

RT-PCR Real-Time Polymerase Chain Reaction

SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2

WHO World Health Organization

PMH Past Medical History

Declarations

Ethics approval and consent to participate

This study was also approved by the Tehran University of Medical Sciences (TUMS) Ethics board committee (Ethics code: IR.TUMS.VCR.REC.1399.076)

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

None.

Funding

This research has been supported by Tehran University of Medical Sciences & health services grant [grant number: 99-1-101-47279]. Authors also would like to appreciate the support and constructive comments of the methodologist(s) research development office, Imam khomeini Hospital complex, Tehran, Iran.

Authors' contributions

FSG, MJ and EK performed data gathering and curation. SS and EM performed the statistical analyses. MS, RH, and SS wrote the primary draft of the paper. MA, AA, and FJ edited the manuscript for its intellectual contents. FG, MJ, and ZA supervised the project. All authors read and approved the fnal manuscript.

Acknowledgements

The authors would like to thank the participants in this study for their cooperation and understanding, without whom this study would have never been possible.

References

1. Organization WH. WHO Coronavirus Disease (COVID-19) Dashboard 2021 [updated February 4th, 2021. Available from: https://covid19.who.int/.
2. Shang W, Yang Y, Rao Y, Rao X. The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines. npj Vaccines. 2020;5(1):1-3.
3. Ayotte K, Gerberding J, Morrison JS. Ending the Cycle of Crisis and Complacency in US Global Health Security: A Report of the CSIS Commission on Strengthening America's Health Security: Center for Strategic & International Studies; 2020.
4. WHO. Draft landscape of COVID-19 candidate vaccines. World Health Organisation. 2020.
5. Cohen JI, Burbelo PD. Reinfection with SARS-CoV-2: Implications for Vaccines. Clinical Infectious Diseases. 2020.
6. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral immune response to SARS-CoV-2 in Iceland. New England Journal of Medicine. 2020;383(18):1724-34.
7. Crawford KH, Dingens AS, Eguia R, Wolf CR, Wilcox N, Logue JK, et al. Dynamics of neutralizing antibody titers in the months after SARS-CoV-2 infection. MedRxiv. 2020.
8. Dickson M, Mathews R, Menon GG. ANALYSIS OF COVID-19 REINFECTION RATES AND ITS UNDERLYING CAUSES: A SYSTEMATIC REVIEW. 2020.
9. Krishna E, Pathak VK, Prasad R, Jose H, Kumar MM. COVID-19 reinfection: Linked possibilities and future outlook. Journal of Family Medicine and Primary Care. 2020;9(11):5445.
10. Osman A, Al Daajani M, Alsahafi A. Re-positive coronavirus disease 2019 PCR test: could it be a reinfection? New Microbes New Infect. 2020.
11. Plotkin SA. Correlates of protection induced by vaccination. Clinical and vaccine immunology. 2010;17(7):1055-65.
12. Seow J, Graham C, Merrick B, Acors S, Steel KJ, Hemmings O, et al. Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection. MedRxiv. 2020.
13. Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nature medicine. 2020;26(8):1200-4.
14. Koyama T, Platt D, Parida L. Variant analysis of SARS-CoV-2 genomes. Bulletin of the World Health Organization. 2020;98(7):495.
15. Tillett RL, Sevinsky JR, Hartley PD, Kerwin H, Crawford N, Gorzalski A, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. The Lancet Infectious Diseases. 2021;21(1):52-8.
16. Prado-Vivar B, Becerra-Wong M, Guadalupe JJ, Marquez S, Gutierrez B, Rojas-Silva P, et al. COVID-19 Re-Infection by a Phylogenetically Distinct SARS-CoV-2 Variant, First Confirmed Event in South America. First Confirmed Event in South America(September 3, 2020). 2020.

17. Van Elslande J, Vermeersch P, Vandervoort K, Wawina-Bokalanga T, Vanmechelen B, Wollants E, et al. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain. Clin Infect Dis. 2020;10.
18. Scaria V. Asymptomatic reinfection in two healthcare workers from India with genetically distinct SARS-CoV-2. 2020.
19. Mo H, Zeng G, Ren X, Li H, Ke C, Tan Y, et al. Longitudinal profile of antibodies against SARS-coronavirus in SARS patients and their clinical significance. Respirology. 2006;11(1):49-53.
20. Payne DC, Iblan I, Rha B, Alqasrawi S, Haddadin A, Al Nsour M, et al. Persistence of antibodies against Middle East respiratory syndrome coronavirus. Emerging infectious diseases. 2016;22(10):1824.
21. SeyedAlinaghi S, Oliaei S, Kianzad S, Afsahi AM, MohsseniPour M, Barzegary A, et al. Reinfection risk of novel coronavirus (CoVID-19): A systematic review of current evidence. World journal of virology. 2020;9(5):79.
22. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C, Hausner MA, et al. Rapid decay of anti–SARS-CoV-2 antibodies in persons with mild Covid-19. New England Journal of Medicine. 2020;383(11):1085-7.
23. Yahav D, Yelin D, Eckerle I, Eberhardt CS, Wang J, Cao B, et al. Definitions for coronavirus disease 2019 reinfection, relapse and PCR re-positivity. Clin Microbiol Infect. 2020.
24. Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, Zambrano-Achig P, Del Campo R, Ciapponi A, et al. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. PloS one. 2020;15(12):e0242958.
25. Luo A. Positive SARS-Cov-2 test in a woman with COVID-19 at 22 days after hospital discharge: A case report. Journal of Traditional Chinese Medical Sciences. 2020;7(4):413-7.
26. Organization WH. Criteria for releasing COVID-19 patients from isolation: scientific brief, 17 June 2020. World Health Organization; 2020.
27. Tao W, Wang X, Zhang G, Guo M, Ma H, Zhao D, et al. Re-detectable positive SARS-CoV-2 RNA tests in patients who recovered from COVID-19 with intestinal infection. Protein & cell. 2020:1-6.
28. Kang H, Wang Y, Tong Z, Liu X. Retest positive for SARS-CoV-2 RNA of “recovered” patients with COVID-19: Persistence, sampling issues, or re-infection? Journal of medical virology. 2020;92(11):2263-5.
29. Chen Y, Bai W, Liu B, Huang J, Laurent I, Chen F, et al. Re-evaluation of retested nucleic acid-positive cases in recovered COVID-19 patients: Report from a designated transfer hospital in Chongqing, China. Journal of infection and public health. 2020;13(7):932-4.
30. Control CfD, Prevention. Duration of isolation and precautions for adults with COVID-19. 2020. 2020.
31. Lu J, Peng J, Xiong Q, Liu Z, Lin H, Tan X, et al. Clinical, immunological and virological characterization of COVID-19 patients that test re-positive for SARS-CoV-2 by RT-PCR. EBioMedicine. 2020;59:102960.

32. Bonifácio LP, Pereira APS, Balbão VdMP, Fonseca BALd, Passos ADC, Bellissimo-Rodrigues F. Are SARS-CoV-2 reinfection and Covid-19 recurrence possible? a case report from Brazil. Revista da Sociedade Brasileira de Medicina Tropical. 2020;53.
33. Ye G, Pan Z, Pan Y, Deng Q, Chen L, Li J, et al. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. Journal of Infection. 2020;80(5):e14-e7.
34. Ravioli S, Ochsner H, Lindner G. Reactivation of COVID-19 pneumonia: a report of two cases. The Journal of infection. 2020.
35. Kang YJ, Joo H. South Korea's COVID-19 infection status: From the perspective of reconfirmation after complete recovery. Journal of Pure and Applied Microbiology. 2020;14.
36. Loconsole D, Passerini F, Palmieri VO, Centrone F, Sallustio A, Pugliese S, et al. Recurrence of COVID-19 after recovery: a case report from Italy. Infection. 2020:1-3.
37. Anwar H, Khan QU. Pathology and Therapeutics of COVID-19: A Review. International Journal of Medical Students. 2020;8(2):113-20.
38. Xie C, Lu J, Di Wu LZ, Zhao H, Rao B, Yang Z. False negative rate of COVID-19 is eliminated by using nasal swab test. Travel medicine and infectious disease. 2020.
39. Zhang D, Guo R, Lei L, Liu H, Wang Y, Wang Y, et al. COVID-19 infection induces readily detectable morphologic and inflammation-related phenotypic changes in peripheral blood monocytes. Journal of leukocyte biology. 2020.
40. Elberry MH, Ahmed H. Occult SARS-CoV-2 infection; a possible hypothesis for viral relapse. Medical hypotheses. 2020;144:109980.
41. Mondal R, Deb S, Lahiri D, Shome G. Recurrence of COVID-19: Treading the Fine Line Between Relapse and Re-infection. International Journal of Medical Students. 2020;8(3):311-3.
42. To KK-W, Hung IF-N, Ip JD, Chu AW-H, Chan W-M, Tam AR, et al. COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. Clinical infectious diseases. 2020.
43. Chen X, Pan Z, Yue S, Yu F, Zhang J, Yang Y, et al. Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. Signal transduction and targeted therapy. 2020;5(1):1-6.
44. Larson D, Brodniak SL, Voegtly LJ, Cer RZ, Glang LA, Malagon FJ, et al. A Case of Early Re-infection with SARS-CoV-2. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2020.
45. Iwasaki A. What reinfections mean for COVID-19. The Lancet Infectious Diseases. 2021;21(1):3-5.
46. Gousseff M, Penot P, Gallay L, Batisse D, Benech N, Bouiller K, et al. Clinical recurrences of COVID-19 symptoms after recovery: viral relapse, reinfection or inflammatory rebound? Journal of Infection. 2020;81(5):816-46.
47. Chen Z, Wherry EJ. T cell responses in patients with COVID-19. Nature Reviews Immunology. 2020;20(9):529-36.

48. Channappanavar R, Fett C, Zhao J, Meyerholz DK, Perlman S. Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. Journal of virology. 2014;88(19):11034-44.
49. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clinical infectious diseases. 2020;71(16):2027-34.
50. Liu A, Li Y, Peng J, Huang Y, Xu D. Antibody responses against SARS-CoV-2 in COVID-19 patients. Journal of medical virology. 2021;93(1):144-8.
51. Iyer AS, Jones FK, Nodoushani A, Kelly M, Becker M, Slater D, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. Science immunology. 2020;5(52).
52. Baumgarth N, Nikolich-Žugich J, Lee FE-H, Bhattacharya D. Antibody Responses to SARS-CoV-2: Let's Stick to Known Knowns. The Journal of Immunology. 2020;205(9):2342-50.
53. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. New England Journal of Medicine. 2007;357(19):1903-15.
54. Zhang J, Ding N, Ren L, Song R, Chen D, Zhao X, et al. COVID-19 reinfection in the presence of neutralizing antibodies. National Science Review. 2021.

Table

Table 1: Demographic data of participants

	1sr F/U	2nd F/U	3rd F/U
Number of patients	82	44	32
Female	25	16	12
Male	57	28	20
Age	52±14	49±16	47±16
Underlying disease[1]:			
DM	12	9	5
HTN	27	21	13
Cardiovascular dis.	5	2	0
Chronic pulmonary dis.	3	1	1
Malignancies	2	0	0
AIDS	1	1	0
Smoker	11	8	7
Recent use of immunosuppressive agents	1	0	0

1. No patients in our study had a history of ESRD, chronic liver disease, neurological disorders, morbid obesity, transplantation or any other immune-compromising condition other than AIDS.

DM (Diabetes mellitus), HTN (Hypertension0, AIDS (Acquired immunodeficiency syndrome), ESRD End-Stage Renal Disease)

Figures

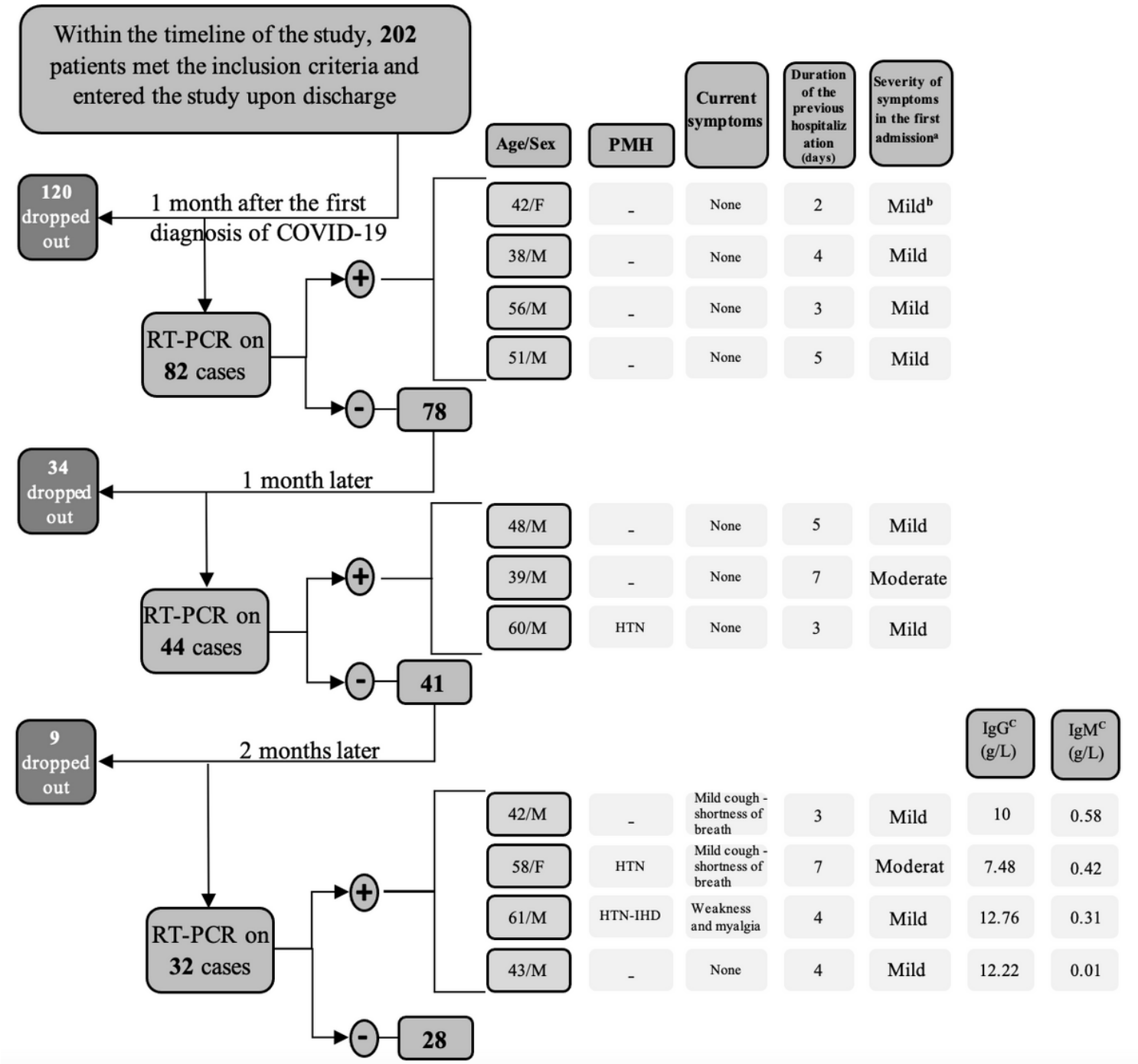

Figure 1

Detailed information on RT-PCR-positive patients along the study time-line a. patients with a sustained peripheral oxygen saturation (O2Sat) of under 90% were considered severe cases, patients with an O2Sat of between 90 and 93% were considered moderate, and patients with higher levels of O2Sat were mild. b. Patients with a high O2Sat were admitted if they experienced other considerably impairing symptoms, which in case of all these patients were severe nausea and vomiting or excessive weakness. c. Levels lower than 1.1 g/L are considered negative.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- HighlightsRE.docx