Emerging Evidence on COVID-19

Evidence Brief on SARS-CoV-2 antibodies in patients that retest RT-PCR positive

Introduction

Do hospitalized SARS-CoV-2 patients that successfully recover with no subsequently positive RT-PCR tests present with SARS-CoV-2 antibody levels or seropositivity rates that differ from those that do retest positive?

Confirmed cases of recovered COVID-19 patients retesting positive after discharge raise concerns of potential reinfection or reactivation of the virus. Research comparing immune response indicators from hospitalized patients that successfully recovered from SARS-CoV-2 with no subsequent positive RT-PCR test compared to those who retested RT-PCR positive were identified to investigate possible relationships between viral RNA test positive and immune response. This report summarizes the COVID-19 literature published up to September 9, 2020, on the differences in the immune response between patients that retest positive and those who do not, as well as SARS-CoV-2 antibody dynamics in relation to the possibility for virus reinfection or reactivation.

In this review, nine studies were identified as fitting the inclusion criteria (please see the Methods section). All nine studies occurred in China. As such, all patients that were discharged from hospital had at least two consecutive negative RT-PCR tests at least 24 hours apart. After discharge, all patients were moved to separate facilities for a mandatory 14 days monitored quarantine. It should be noted that patients that retest positive after discharge are referred to as 'RP patients', however, studies use slightly different terms - retest positive, re-detectable positive, re-positive, or recurrent positive. Patients that do not retest positive are referred to as NRP.

Key Points

- The rate of retesting positive (prevalence of RP) varied from 1.87% of discharged patients (Chen et al., 2020) to 52.7% (Hu et al., 2020) for an average of 16.5% from all studies (397/2412 patients). No study found a difference in sex distribution, but four of the nine studies found RP patients to be significantly younger than NRP patients. A wider review would be needed to explore this further (Chen et al., 2020, Lu et al., 2020, Huang et al., 2020, Yang et al., 2020).
- Of six studies that reported on the positivity rate of patients for IgG or IgM antibodies, RP patients exhibited positivity rates that did not differ from the positivity rates of NRP patients. This indicates that the presence of IgG or IgM antibodies is unlikely to be predictive of retesting positive (Yang et al., 2020, Liu et al., 2020., Yuan et al., 2020, Zou et al., 2020, Huang et al., 2020, Zhu et al., 2020).
- Of the four studies that reported on the level of IgG or IgM antibodies in serum, the results are mixed. One study found that the levels of IgM and IgG antibodies were significantly lower in RP patients than

NRP patients (Chen et al., 2020). A second found no difference (Liu et al., 2020). The third found IgG to be significantly lower in RP patients but no difference in IgM levels (Hu et al., 2020). The fourth found no difference in IgG, but that IgM levels varied over time – initially RP patients had higher IgM titers (week 3 post discharge), but the levels of IgM antibodies eventually became significantly lower for RP patients compared to NRP patients (week 6-8 post discharge) (Yang et al., 2020). This suggests that lower antibody levels might play a role in retesting positive after discharge, but the evidence is not conclusive at this point.

- It is still unclear why patients retest positive. All nine studies took place in China, which enforced a mandatory 14-day quarantine following hospital discharge at separate facilities with individual rooms. Three studies that only followed patients during this period found up to 52.7% of patients retested positive (Chen et al., 2020, Hu et al., 2020, Yuan et al., 2020). One plausible explanation for retesting positive within the two-week quarantine period is a 'reactivation' of the initial infection, following incomplete clearing of the virus. It is also possible that concentration of viral RNA in samples fluctuate during clearance of the virus resulting in two false negative results leading to discharge. In Zou et al., 2020, patients retested positive less often when required to have three negative PCR tests prior to hospital discharge, instead of the usual two.
- One study demonstrated that some patients will retest positive more than once. Upon retesting positive, patients were re-hospitalized until discharged again following two consecutive negative RT-PCR tests, only to retest positive a second, third and even fourth time (Yang et al., 2020).
- Another study found that requiring three consecutive negative tests prior to discharge significantly reduced the chance of retesting positive (Zou et al., 2020). This indicates that false-negatives may play a role in retesting positive after discharge, although an additional review would need to uncover any additional literature on this topic.

Overview of the Evidence

Nine papers were identified, eight of which were cohort studies and one of which was a case control study, scored using the Newcastle-Ottawa Scale Risk of Bias Tool. The maximum score is four for selection criteria, two for comparability between groups, and three for exposure or outcome criteria. Adequate follow-up was cut off at four weeks of follow-up.

- Three prospective cohort studies
  - Clinical, immunological and virological characterization of COVID-19 patients that test re-positive for SARS-CoV-2 by RT-PCR (Lu et al., 2020), score: 4, 0, 3.
  - Recurrence of positive SARS-CoV-2 viral RNA in recovered COVID-19 patients during medical isolation observation (Yuan et al., 2020), score: 4, 0, 1.
  - Viral RNA level, serum antibody responses, and transmission risk in discharged COVID-19 patients with recurrent positive SARS-CoV-2 RNA test results: a population-based observational cohort study (Yang et al., 2020), score: 4, 0, 2.

  - Three retrospective cohort studies
    - The production of antibodies for SARS-CoV-2 and its clinical implication (Hu et al., 2020), score: 4, 0, 1.
    - Clinical features of COVID-19 convalescent patients with re-positive nucleic acid detection (Zhu et al., 2020), score: 4, 0, 2.
    - The issue of recurrently positive patients who recovered from COVID-19 according to the current discharge criteria: investigation of patients from multiple medical institutions in Wuhan, China (Zou et al., 2020), score: 3, 0, 1.
  - Two cohort studies (unclear if prospective or retrospective)
    - Recurrent positive SARS-CoV-2 - immune certificate may not be valid (Liu et al., 2020), score: 4, 0, 1.
    - Kinetics of SARS-CoV-2 Positivity of Infected and Recovered Patients: A Single Center COVID-19 Experience and Potential Implications (Huang et al., 2020), score: 4, 0, 2.
  - One case-control study
    - Clinical Characteristics of Recurrent-positive Coronavirus Disease 2019 after Curative Discharge: a retrospective analysis of 15 cases in Wuhan China (Chen et al., 2020), score: 2, 0, 3.

- Limitations
  - All nine studies occurred in China, with no other jurisdictions producing similar studies.
  - The sample size in some of the studies was small; two studies had fewer than 100 patients (Hu et al., 2020, Zhu et al., 2020) and three studies had fewer than 200 patients (Chen et al., 2020, Liu et al., 2020, Yuan et al., 2020).
  - The duration of follow-up varied greatly. Many studies only followed patients during the mandatory, centralized two-week quarantine that follows discharge from hospital in China, while others followed patients for many weeks afterward. There were also a wide range of RT-PCR testing intervals, spanning from a single test near the end of the 14-day quarantine period, to testing every three to five days. In combination, these limitations casts some doubt on the prevalence of retesting positive and average time to retest positive following discharge.
- Data Gaps
  - All studies that met the inclusion criteria were from China where all cases were hospitalized, caution in extrapolation of these results to other settings is recommended.
  - This review does not consider other factors that may be predictive or contribute to retesting positive following hospital discharge, including immune cell counts, comorbidities, severity of disease upon initial hospitalization, and more. These are topics for potential future reviews.
  - In this series of studies, 'reactivation' or a series of false negatives are both plausible reasons for patients retesting PCR positive shortly after discharge. A separate review and more research is needed to explore the reason and mechanism by which patients are retesting positive.

TABLE 1: SEROLOGICAL CHARACTERISTICS OF DISCHARGED HOSPITALIZED COVID-19 CASES WHO RETESTED RT-PCR POSITIVE COMPARED TO THOSE THAT DID NOT

Reference	Report Details	Key Findings
Lu et al., 2020 Prospective cohort study Guangdong, China. Jan 23 - Feb 19, 2020	This followed 619 discharged patients and serology was the main outcome of this study. 288 patients had serological testing a median of 35 days post symptom onset (range = 23 to 47 days). Patients were followed for up to 66 days.	- Neutralizing antibody titers for RP and NRP patients were not significantly different 14 days post hospital discharge. - This study had the largest cohort that was followed for the longest period of time, recording a RP incidence rate of 14% (87/619 patients). - RP patients in this study were significantly younger than NRP patients. Sex distribution did not differ between groups. - Patients were followed for 66 days post discharge, and experience reactivation on day 10 on average (tested on day 7 and 14 only).
Yang et al., 2020 (Preprint) Prospective cohort study Shenzhen, China Feb 1 – May 5, 2020	This study followed 479 discharged patients. Serology is main outcome of this study, with serum specimens collected on the 1st, 3rd, 7th, and 14th days of each of the post-discharge 14-day quarantine period. Patients were followed up to 90 days.	- RP and NRP patients did not differ in rates of testing positive for IgG antibodies (99% and 98%, respectively). Serum levels of IgG antibodies also did not differ between groups at any point after disease onset. -RP and NRP patients did not differ in rates of testing positive for IgM antibodies (37% and 50%, respectively). Serum levels of IgM antibodies differed between groups at different points post-disease onset: In week 3, RP patients had significantly higher levels of IgM, while in weeks 6 through 8, RP patients had significantly lower IgM levels. -The incident rate of RP in this study was 19% (93/479 patients). In addition, 45 (9%) experience multiple RP events: two (n=32, 7%), three (n=9, 2%), or four (n=4, 1%) RP events. - RP patients in this study were significantly younger than NRP patients (34 years compared to 45 years). Sex distribution did not differ between groups. - Patients were followed for 90 days post discharge, and experience reactivation on day 8 on average. An average of 46 days elapsed between disease onset and the final RP event for each patient.

Yuan et al., 2020 Prospective cohort study Shenzhen, China Before Apr 21, 2020	This prospective cohort study followed 182 discharged patients. Serology is one of the main outcomes of this study, with 147 patients submitting for serological testing at some point after discharge. Patients were followed for 14 days.	- RP and NRP patients did not differ in rates of testing positive for IgG antibodies (100% and 99.2%, respectively). - RP and NRP patients did not differ in rates of testing positive for IgM antibodies (71.4% for both). -The incident rate of RP in this study was 11% (20/182 patients). However, only a subset had serology results (14 RP patients and 133 NRP patients). - RP patients were not significantly younger in this study, however, patients under 18 years of age were overrepresented in the RP group. Sex distribution did not differ between groups. - Patients were followed during the mandatory 14-day quarantine following hospital discharge, and retested on day 7 and 14 of quarantine.
Zhu et al., 2020 Retrospective cohort study Zhejiang, China Before Apr 2, 2020	This retrospective cohort study followed 98 discharged patients. Serology was part of a wide range of factors examined, with testing measuring temporal changes in antibody levels. The exact timing of tests is not stated. The follow-up period is at least 17 days.	- In this study, 35.5% of RP patients tested positive for both IgG and IgM antibodies, compared to 8.6% of NRP patients. 58.8% of RP patients tested positive for IgG and negative for IgM antibodies, compared to 44.4% of NRP patients. Two RP and one NRP patients tested negative for both IgG and IgM antibodies. The groups were not determined to be significantly different. - The incident rate of RP in this study was 17% (17/98 patients). - Neither age nor sex was found to differ between RP and NRP patients. - Patients were followed for at least 17 days following discharge, with the average time to RP being 7 days.
Hu et al., 2020 (Preprint) Retrospective cohort study Chongquin, China Jan 23 - Mar 3, 2020	This study followed 221 hospitalized patients. Serology was the main outcome, with serum samples taken every 3 days post-symptom onset. Only 74 patients were discharged and followed for	- Patients that experienced RP had post-discharge IgG levels of 8.94 on average, compared to 20.19 in NRP patients, which is significantly different. Levels are expressed as a ratio of the chemiluminescence signal to the cutoff value (S/CO). - RP and NRP patients did not have significantly different post-discharge IgM levels (0.90 compared to 1.39, respectively). - Reports the highest RP incidence rate of the ten studies (39/74, or 52.7%). No average time to reactivation was stated. - No age/sex differences between RP and NRP patients. - Patients were followed for up for 14 days.

	the 14-day quarantine period.	
Zou et al., 2020 Retrospective cohort study Wuhan, China Jan 1 – Mar 10, 2020	This study followed 257 hospitalized patients. Serology was not main outcome of the study. It is unclear how long patients were followed for or when they underwent serological testing.	- RP and NRP patients did not differ in rates of testing positive for IgG antibodies (94.4% and 85.1%, respectively). - RP and NRP patients did not differ in rates of testing positive for IgM antibodies (52.8% and 58.8%, respectively). - The incident rate of RP in this study was 20.6% (53/257 patients). However, only a subset had serology results (36 RP patients and 114 NRP patients). - Neither age nor sex was found to differ between RP and NRP patients. - It is unclear how long patients were followed for, but were said to retest positive an average of 4.6 days post-discharge. - The goal of this study was to compare RP rates for patients with two subsequent negative PCR tests compared to three subsequent negatives to qualify for discharge. 20.6% of patients with two negative tests experience RP, compared to only 5.4% of patients with three negative tests.
Huang et al., 2020 (Preprint) Cohort study - unclear if prospective or retrospective Shenzhen, China Jan 11 - Apr 23, 2020	This study followed 414 hospitalized patients. Serology was part of a wide range of factors examined. 154 patients had serological testing at discharge from hospital. Patients were followed for four weeks.	- RP and NRP patients did not differ in rates of testing positive for IgG antibodies (100% and 99.1%, respectively). - RP and NRP patients did not differ in rates of testing positive for IgM antibodies (75.0% and 48.2%, respectively). - The incident rate of RP in this study was 16.7% (69/414 patients). - RP patients in this study were significantly younger than NRP patients. Sex distribution did not differ between groups. - Patients were followed for four weeks following discharge. Reactivation occurred on the day 10 on average, with RT-PCR testing done every 3-5 days.
Liu et al., 2020 Cohort study-unclear if prospective or retrospective Wuhan, China Mar 1 - 13, 2020	This study followed 150 discharged patients. Serology was main outcome measure, but neither timing of serology nor the duration of follow-up was noted.	- RP and NRP patients did not differ in rates of testing positive for IgG antibodies (100% and 90.6%, respectively). Serum levels of IgG antibodies also did not differ between groups (243 AU/mL and 185 AU/mL, respectively). -RP and NRP patients did not differ in rates of testing positive for IgM antibodies (45.5% and 47.5%, respectively). Serum levels of IgM antibodies also did not differ between groups (9.6 AU/mL and 8.9 AU/mL, respectively). - The incident rate of RP in this study was 7.3% (11/150 patients). - Neither age nor sex was found to differ between RP and NRP patients. - Testing at different points following discharge may have affected the results.

Chen et al., 2020 Case-control study Wuhan, China Feb 10 - Mar 31, 2020	This study examined the serology of 15 RP cases and 107 controls admitted to a single hospital. Serology was part of a wide range of factors examined. The timing of the serological testing is not clear.	- Patients experiencing RP had IgG levels of 78.53 AU/mL on average, compared to 147.85 AU/mL in NRP patients, which is significantly different. - Patients experiencing RP had IgM levels of 13.69 AU/mL on average, compared to 68.10 AU/mL in NRP patients, which is significantly different. - Reports the lowest RP incidence rate of the ten studies (2/107, or 1.9%) from the cohort in a single hospital. 15 cases from multiple sites were compared to 107 controls. - Age and sex were not matched between cases and controls. RP patients were found to be significantly younger than NRP patients (43 years compared to 60 years). There was no significant difference in sex of RP versus NRP patients. - Patients were followed up for 14 days. Reactivation occurred at day 12 post-discharge on average. However, patients were only tested near the end of the 14-day quarantine.

Methods:

A daily scan of the literature (published and pre-published) is conducted by the Emerging Sciences Group, PHAC. The scan has compiled COVID-19 literature since the beginning of the outbreak and is updated daily. Searches to retrieve relevant COVID-19 literature are conducted in Pubmed, Scopus, BioRxiv, MedRxiv, ArXiv, SSRN, Research Square and cross-referenced with the literature on the WHO COVID literature list, and COVID-19 information centers run by Lancet, BMJ, Elsevier and Wiley. The daily summary and full scan results are maintained in a refworks database and an excel list that can be searched. Targeted keyword searching is conducted within these databases to identify relevant citations on COVID-19 and SARS-CoV-2. Search terms used included: Reactivation, reinfection, reoccurrence, recurrent, in conjunction with hospitalization, discharge, antibody and immunity (including terms with similar endings and alternative spelling).

This review contains research published up to September 9, 2020.

Each potentially relevant reference was examined to confirm it had relevant data and relevant data is extracted into the review.

All studies included in this review focus on characteristics of hospitalized patients who retested positive (RP) compared to those who did not retest positive (NRP) after discharge, and also included serology data for both RP and NRP groups. Papers included in this review were either case-control or cohort studies. Review articles were excluded to avoid double counting. Case-series or case reports were also excluded as they do not present a comparison between patients that retested positive versus those that did not.

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References

Chen, L., Zhang, Z.-Y., Zhang, X.-B., Zhang, S.-Z., Han, Q.-Y., Feng, Z.-P., Fu, J.-G., Xiao, X., Chen, H.-M., Liu, L.-L., Chen, X.-L., Lan, Y.-P., Zhong, D.-J., Hu, L., Wang, J.-H., & Yin, Z.-Y. (2020). Clinical Characteristics of Recurrent-positive Coronavirus Disease 2019 after Curative Discharge: A retrospective analysis of 15 cases in Wuhan China. MedRxiv, 2020.07.02.20144873. https://doi.org/10.1101/2020.07.02.20144873

Hu, Q., Cui, X., Liu, X., Peng, B., Jiang, J., Wang, X., Li, Y., Hu, W., Ao, Z., Duan, J., Wang, X., Zhu, L., Wu, G., & Guo, S. (2020). The Production of Antibodies for SARS-CoV-2 and its Clinical Implications. SSRN Electronic Journal. https://doi.org/10.2139/ssrn.3576845

Huang, J., Zheng, L., Li, Z., Hao, S., Ye, F., Chen, J., Yao, X., Liao, J., Wang, S., Zeng, M., Qiu, L., Cen, F., Huang, Y., Zhu, T., Xu, Z., Ye, M., Yang, Y., Wang, G., Li, J., … Ling, X. B. (2020). Recurrence of SARS-CoV-2 PCR Positivity in COVID-19 Patients: A Single Center Experience and Potential Implications. MedRxiv, 2020.05.06.20089573. https://doi.org/10.1101/2020.05.06.20089573

Liu, T., Wu, S., Zeng, G., Zhou, F., Li, Y., Guo, F., & Wang, X. (2020). Recurrent positive SARS-CoV-2: Immune certificate may not be valid. Journal of Medical Virology, n/a(n/a). https://doi.org/10.1002/jmv.26074

Lu, J., Peng, J., Xiong, Q., Liu, Z., Lin, H., Tan, X., Kang, M., Yuan, R., Zeng, L., Zhou, P., Liang, C., Yi, L., Plessis, L. du, Song, T., Ma, W., Sun, J., Pybus, O. G., & Ke, C. (2020). Clinical, Immunological and Virological Characterization of COVID-19 Patients That Test Re-Positive for SARS-CoV-2 by RT-PCR. EBioMedicine, 59. https://doi.org/10.1016/j.ebiom.2020.102960

Wu, J., Xia, X., Liu, H., Xia, H., Huang, W., Jia, B., & Peng, F. (2020). Clinical Characteristics and Outcomes of Discharged COVID-19 Patients with Reoccurrence of SARS-CoV-2 RNA in a County Hospital of Western Chongqing, China (SSRN Scholarly Paper ID 3576906). Social Science Research Network. https://doi.org/10.2139/ssrn.3576906

Yang, C., Jiang, M., Wang, X., Tang, X., Fang, S., Li, H., Zuo, L., Jiang, Y., Zhong, Y., Chen, Q., Zheng, C., Wang, L., Wu, S., Wu, W., Liu, H., Yuan, J., Liao, X., Zhang, Z., Lin, Y., … Hu, Q. (2020). Viral RNA Level, Serum Antibody Responses, and Transmission Risk in Discharged COVID-19 Patients With Recurrent Positive SARS-CoV-2 RNA Test Results: A Population-Based Observational Cohort Study. MedRxiv, 2020.07.21.20125138. https://doi.org/10.1101/2020.07.21.20125138

Yuan, B., Liu, H.-Q., Yang, Z.-R., Chen, Y.-X., Liu, Z.-Y., Zhang, K., Wang, C., Li, W.-X., An, Y.-W., Wang, J.-C., & Song, S. (2020). Recurrence of Positive SARS-CoV-2 Viral RNA in Recovered COVID-19 Patients During Medical Isolation Observation. Scientific Reports, 10(1), 11887. https://doi.org/10.1038/s41598-020-68782-w

Zhu, H., Fu, L., Jin, Y., Shao, J., Zhang, S., Zheng, N., Fan, L., Yu, Z., Ying, J., Hu, Y., Chen, T., Chen, Y., Chen, M., Chen, M., Xiong, Z., Kang, J., Jin, J., Cai, T., & Ye, H. (2020). Clinical Features of COVID-19 Convalescent Patients with Re-Positive Nucleic Acid Detection. Journal of Clinical Laboratory Analysis, 34(7), e23392. https://doi.org/10.1002/jcla.23392

Zou, Y., Wang, B.-R., Sun, L., Xu, S., Kong, Y.-G., Shen, L.-J., Liang, G.-T., & Chen, S.-M. (2020). The Issue of Recurrently Positive Patients who Recovered From COVID-19 According to the Current Discharge Criteria: Investigation of Patients From Multiple Medical Institutions in Wuhan, China. The Journal of Infectious Diseases. https://doi.org/10.1093/infdis/jiaa301