Re-positive discharged COVID-19 patients are at low transmission risk for SARS-CoV-2 infection– a finding from recovered COVID-19 patients in Wuhan, China

Sheng Wei (shengwei@hust.edu.cn)
School of Public Health, Tongji Medical College, Huazhong University of Science and Technology
Yeqing Tong
Hubei Provincial Center for Disease Control and Prevention
Jiafa Liu
Hubei Provincial Center for Disease Control and Prevention
Xuhua Guan
Hubei Provincial Center for Disease Control and Prevention
Siquan Wang
Hubei Provincial Center for Disease Control and Prevention
Bo Yang
Hubei Provincial Center for Disease Control and Prevention
Xingxing Lu
Hubei Provincial Center for Disease Control and Prevention
Gaoming Wang
Dongxihu District Center for Disease Control and Prevention
Qinhua Chen
Wuchang District Center for Disease Control and Prevention
Dandan Xu
Hongshan District Center for Disease Control and Prevention
Shi Liu
Qiaokou District Center for Disease Control and Prevention,
Li Cai
Wuhan Center for Disease Control and Prevention
Minna Dong
Jiangxia District Center for Disease Control and Prevention
Chenghui Ding
Jiangan District Center for Disease Control and Prevention
Hui Yu
Dongxihu District Center for Disease Control and Prevention

Linlin Liu
Hubei Provincial Center for Disease Control and Prevention
Jingya Lu
School of Public Health, Tongji Medical College, Huazhong University of Science and Technology
Huanzhuo Wang
School of Public Health, Tongji Medical College, Huazhong University of Science and Technology
Zhen Zhang
School of Public Health, Tongji Medical College, Huazhong University of Science and Technology
Yizhong Yan
School of Public Health, Tongji Medical College, Huazhong University of Science and Technology
Xibao Huang
Hubei Provincial Center for Disease Control and Prevention
Tingming Shi
Hubei Provincial Center for Disease Control and Prevention
Yanan Li
School of Public Health, Tongji Medical College, Huazhong University of Science and Technology,
Yang Wu
Hubei Provincial Center for Disease Control and Prevention, Wuhan 430079
Quanhong Zhang
Health Commission of Hubei Province
Sizhe Liu
Shanghai University of Finance and Economics
Yingbo Luo
Hubei Provincial Center for Disease Control and Prevention
Jiao Huang
School of Public Health, Tongji Medical College, Huazhong University of Science and Technology

---

Article

Keywords: Re-positive, discharged, COVID-19, patients, low transmission risk, SARS-CoV-2

Posted Date: November 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-93844/v1

Abstract

Discharged COVID-19 patients have been found to be retested positive for SARS-CoV-2 (re-positive), which has widely raised concern among the public. We investigated the prevalence and transmission risk of re-positive cases in discharged COVID-19 patients and their SARS-CoV-2-specific antibody levels in Wuhan, China. Of 1065 discharged COVID-19 patients investigated, 518 (48.64%) patients were males; the mean age was 53.29 ± 14.91 years, with a median duration of 40 (IQR: 31–47) days since discharge. 63 patients were tested re-positive for SARS-CoV-2, with the re-positive prevalence to be 5.92% (95%CI: 4.50%-7.33%). The re-positive prevalence was higher in females (7.86%, 95%CI: 5.61%-10.12%) than that in males (3.86%, 95%CI: 2.20%-5.52%, *P* = 0.006). Re-positive prevalence was similar in patients tested positive and negative for IgG (6.01% vs 5.56%, *P* = 0.821) or IgM (6.38% vs 5.07%, *P* = 0.394). Illness severity and duration from illness onset to retest were not associated with the risk of positive results for SARS-CoV-2 after discharge. All 196 environmental samples collected from 49 re-positive patients were tested negative for SAR-CoV-2. Only one close contact to the re-positive patient had been tested positive for SARS-CoV-2; however, he might be a previous COVID-19 case but had not been detected before. Viral culture of 6 nasopharyngeal specimens presented no cytopathic effect of Vero E6 cells. Virus sequencing of 11 nasopharyngeal specimens indicated genomic fragments of SARS-CoV-2. 898 (84.72%) patients and 705 (66.51%) patients were tested positive for SARS-CoV-2-specific IgG and IgM, respectively. Self-report symptoms at the survey were similar, regardless of the level of antibody. All the re-positive patients and their matched non-re-positive patients were tested negative for SARS-CoV-2 four months later. These findings indicate that Testing re-positive of SARS-CoV-2 is common in discharged COVID-19 patients, but no evidence showed the transmission risk of these re-positive cases. Further isolation of recovered COVID-19 patients is unnecessary. However, only 85% recovered COVID-19 patients had SARS-CoV-2-specific antibody, which suggested discharged COVID-19 patients still had potential re-infection risk.

Introduction

Coronavirus disease 2019 (COVID-19) is an emerging infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has been spread worldwide and caused over 36 million cases and about 1 million deaths until October 9, 2020[1]. Since COVID-19 is a novel and unprecedented communicable disease, the clinical progression, and prognosis of this disease are still poorly understood [2]. In the battle against the COVID-19 epidemic, most COVID-19 patients recovered from SARS-CoV-2 infection except for some elderly patients. To prevent the potential risk of SARS-CoV-2 transmission, COVID-19 patients need to have two negative reverse transcriptase polymerase chain reaction (RT-PCR) results for SARS-CoV-2 on consecutive samples taken at least 24 hours apart before discharge from isolation in several countries [3]. However, several studies have reported that discharged COVID-19 patients have been retested positive for SARS-CoV-2 using PCR tests [4–6]. An earlier study reported that 14.5% patients had positive RT-PCR results among 25 discharged COVID-19 patients [7]. A recent study reported that 3% of the 651 discharged COVID-19 patients from a hospital in Wuhan had been retested positive for SARS-CoV-2 by RT-PCR test with a median duration of 48 days since discharge

[8]. These findings suggested that positive RT-PCR results for SARS-CoV-2 seemed common in discharged COVID-19 patients, but there is still limited evidence on the re-positive prevalence of discharged COVID-19 patients at a population level.

The infectiousness of the re-positive discharged COVID-19 patients has been concerned with the increasing reports of positive cases retested among discharged COVID-19 patients. Four re-positive discharged COVID-19 health worker patients in Wuhan had not infected their family members after returning home [9]. Similar findings from China and Korea have also indicated the low transmission risk of these re-positive COVID-19 survivors to their close contacts. Based on these findings, WHO and CDC of several countries have updated the criteria for releasing COVID-19 patients from isolation in May, 2020 [10–13]. The updated recommendation documented that it was unnecessary to isolate COVID-19 patients after discharge, considering the laboratory supplies, equipment and personnel were limited in the widespread transmission areas. However, all of these guidelines admitted that the currently limited evidence could not ensure absolute non-infectiousness for discharged COVID-19 patients.

Another key concern for discharged COVID-19 patients is the level of the SARS-CoV-2-specific antibody. Recovered COVID-19 patients were assumed to have antibodies to protect them against the re-infection with SARS-CoV-2. However, several recovered COVID-19 patients have been not found to develop SARS-CoV-2 specified antibodies [14]. Two COVID-19 cases with re-infection of SARS-CoV-2 were reported in Hong Kong and the USA by whole genome sequencing [14,15]. Interestingly, IgG against SARS-CoV-2 was undetectable after diagnosis during the second episode. Recent studies have suggested that the T-cell responses also played a key role in the protective immunity induced by SARS-CoV-2. However, the SARS-CoV-2-specific antibody level is still one of the primary endpoints of SAS-CoV-2 vaccine development [16,17]. Therefore, it is still a crucial concern on the level of SAS-CoV-2-specified antibody among discharged COVID-19 patients.

In this study, we performed a cross-sectional survey to assess the re-positive prevalence and the SARS-CoV-2-specific antibody levels in discharged COVID-19 patients in Wuhan, China. We also investigated the transmissibility risk among the re-positive discharged COVID-19 patients. Furthermore, a follow-up investigation were conducted for the re-positive patients and non-re-positive patients frequency-matched by age, gender, district and disease severity 4 months later. The present study could provide direct evidence for the transmission risk of re-positive discharged COVID-19 patients and suggestions for the additional medical care policy for the discharged COVID-19 patients.

Methods

Study setting and participants

A cross-sectional survey on the prevalence of re-positive RT-PCR test for SARS-CoV-2 and the SARS-CoV-2-specific antibody levels in discharged COVID-19 patients has been conducted in Wuhan city, China. The

survey was performed in confirmed COVID-19 cases who had already returned home after discharge from designated hospital or Fangcang shelter hospital.

To investigate the difference of prognosis between re-positive and matched non-re-positive discharged COVID-19 patients in the cross-sectional survey, a follow-up investigation was conducted on September 2-4, 2020. All re-positive discharged COVID-19 patients determined in the cross-sectional survey were frequency-matched by age, gender, district, and disease severity to non-re-positive COVID-19 patients. This study was reviewed and approved by the institutional review board of Tongji Medical College, Huazhong University of Science and Technology (IORG0003571). Verbal informed consent was obtained from every participant.

Sample size and sampling

We calculated the sample size assuming the expected re-positive prevalence of 10% after recovery and a maximal error of 2%. Using a 5% level of significance, a design effect of 1.1 for cluster sampling and a 10% non-response rate, we estimated that 1047 confirmed COVID-19 cases were to be interviewed. A two-stage cluster sampling design was used to select the sample. A cluster was defined as a community. Two districts (Jiangan and Qiaokou) and the other district (Jiangxia) were randomly selected from nine districts with high prevalence and four districts with low prevalence of COVID-19, respectively. Then communities with ≥50 confirmed COVID-19 cases were selected from the streets ranking top three in the number of confirmed COVID-19 cases in Jiangan and Qiaokou districts. All communities in the street where the number of confirmed COVID-19 cases was highest in Jiangxia district were also selected. Eventually, confirmed discharged COVID-19 cases from the 8 sampled communities were enrolled in this study.

In the follow-up investigation, 65 re-positive case and 130 non-re-positive COVID-19 patients frequency-matched by age, gender, district, and disease severity were enrolled in the study.

Procedure

The confirmed COVID-19 cases were interviewed on April 1-April 15, 2020, using a questionnaire to collect their information on the demographic characteristics, date of onset, date of diagnosis, date of hospitalization, date of discharge, illness severity, self-report symptoms and medical comorbidity. A nasopharyngeal swab for each participant was collected, followed the standard protocol recommended by the Chinese Centre for Disease Control and Prevention (CDC) [18]. All nasopharyngeal swabs were maintained in the universal transport medium for later laboratory tests for detecting SARS-CoV-2. Blood samples were also collected from each participant to test the IgM and IgG levels against SARS-CoV-2.

If the discharged patients were tested positive for SARS-CoV-2, house environment sampling was performed to investigate the SARS-CoV-2 RNA in their living house. Moreover, the epidemiology

investigation for their close contacts was conducted by the CDC staff members, and the nasopharyngeal swabs and blood samples were also collected from their close contacts to detect the SARS-CoV-2 RNA and SARS-CoV-2-specific IgM and IgG. In addition, virus sequencing and viral culture had been performed to assess the transmissibility of the re-positive discharged patients.

For those re-positive cases and their matched non-re-positive discharged COVID-19 patients in the cross-sectional survey, a field investigation were performed to collect information of their health status. The nasopharyngeal swabs were also collected to detect the SARS-CoV-2 RNA with RT-PCR test.

RT-PCR test for SARS-CoV-2 RNA

RNA was extracted from nasopharyngeal swabs using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA), and then were detected by reverse transcriptase polymerase chain reaction (RT-PCR) assay using the commercial kits (DAAN Gene, Guangzhou, China) for SARS-CoV-2 in a designated virology laboratory [19]. The commercial kit was used for the qualitative detection of the open reading frame 1ab (ORF 1ab) and the specific conserved sequence of coding nucleocapsid protein N gene of SARS-CoV-2. Primers of RT-PCR testing for SARS-CoV-2 were according to the recommendation by the Chinese CDC. All PCR tests were performed on the Applied Biosystems ^{TM} 7500 Real-time PCR System (ThermoFisher Scientific, CA, USA). Cycle threshold (Ct) values <37 were defined as positive, while Ct values >40 were defined as negative for both ORF 1ab and N genes. Samples with Ct values from 37 to 40 required a second test. Discharged COVID-19 patients with positive results for both genes or two consecutive positive results for one gene were defined as SARS-CoV-2 re-positive; otherwise, they were defined as SARS-CoV-2 negative.

Detection of SARS-CoV-2-specific IgM and IgG

The serum SARS-CoV-2-specific antibodies (IgM and IgG) of the discharged COVID-19 patients were detected using magnetic chemiluminescence enzyme immunoassay (MCLIA) kits supplied by Bioscience Co. (Chongqing, China) following the manufacturer's instructions. The details for detection have been described elsewhere [20,21]. Simply, SARS-CoV-2-specific IgM/IgG could conjugate with FITC-labeled recombinant antigens, containing the nucleoprotein and a peptide from the spike protein of SARS-CoV-2. They could be immobilized on anti-FITC antibody-labeled magnetic particles. The interaction between SARS-CoV-2-specific IgM/IgG and the anti-human IgM/IgG antibody conjugated to alkaline phosphatase. The chemiluminescence signals derived from alkaline phosphatase catalyzing substrate solution were used to detect the SARS-CoV-2-specific IgM/IgG. The tests were performed on Axceed 260 (Bioscience Co., Chongqing, China), an automated magnetic chemiluminescence analyzer. The antibody level was expressed as the chemiluminescence signal value divided by the cutoff value (S/CO). IgM or IgG was defined as positive if the S/CO value was ≥1.0; otherwise, it was regarded to be negative.

Environment sampling

For the re-positive discharged COVID-19 patients in the cross-sectional survey, their house environment sampling was performed to investigate environmental contamination and potential transmission risk Environmental samples were taken at four sites, including the surface of the bedside table, the door handle of the bathroom, the washbasin and the squatter or sitter toilet. Swabs moistened in the universal transport medium were used to collect surface samples by swabbing approximately 25 cm^2 areas (or all surface if areas are less than 25 cm^2) of each site. After sampling was completed, swabs were deposited in the universal transport medium for testing SARS-CoV-2.

Virus sequencing

The nasopharyngeal swabs of 11 re-positive COVID-19 patients were selected to extract total RNA using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA). Sequencing libraries were then constructed with a NEBNext Ultra II Directional RNA Kit (NEB, USA) in accordance with the manufacturer's instructions. Libraries were sequenced on a Miseq 3000 platform (Illumina) in a paired-end (150 bp) run. Reads from Illumina sequencing were assembled using Geneious (v.11.0.3) and MEGAHIT (v.1.2.9) and aligning mapped reads to the reference of SARS-CoV-2 (MN908947) for evaluating the genome coverage.

Viral culture for SARS-CoV-2

Nasopharyngeal swabs collected from 6 re-positive discharged patients were kept in sterile tubes with the universal transport medium to which DMEM containing 2.5% FBS were added. They were then centrifuged at 3000g for 15 min to remove cellular debris. Vero E6 cells were seeded on a 24-well plate at $4´10^5$ cells/well in Dulbecco modified Eagle medium (DMEM) containing 2.5% FBS and maintained in an air–liquid interface incubated at 37°C with 5% carbon dioxide one day prior to infection. Vero E6 cells were inoculated with 250ul processed samples at 37°C for one hour, then 500ul DMEM containing 2.5% FBS was added to the cell culture. Every 3-4 days, 250ul fresh culture medium was added to the cell culture. The cells were monitored daily for cytopathic effects (CPE) by light microscopy for 6 days. Every two days, 50ul cell culture supernatant was collected for RNA extraction and SARS-CoV-2 detection using RT-PCR.

Statistical analysis

Data were presented as absolute numbers and proportions for categorical variables, and as mean ± standard deviation for continuous variables. The Chi-square test or Fisher's exact test was used to compare the re-positive prevalence of discharged COVID-19 patients in different groups. Factors associated with re-positive RT-PCR results for SARS-CoV-2 in discharged COVID-19 patients were analyzed using logistic regression, and a multivariable model was built including all variables in the univariable analyses. The Kruskal-Wallis test was used to analyze the differences in the level of SARS-CoV-2-specific IgM and IgG among different groups. All statistical analyses were performed with SAS statistical software version 9.4 (SAS Institute Inc), and plots were draw using R Project version 3.6.1 (http://cran.r-project.org). The analyses were performed using two-sided tests. $P<0.05$ was considered to be statistically significant.

Results

Characteristics of 1065 discharged COVID-19 patients

A total of 1065 discharged COVID-19 patients have been included in the present study, of which 518 (48.64%) were males. The mean age of these patients was 53.29±14.91 years, with 420 (39.44%) patients aged ≥60 years. The age and gender of the participants were similar to that of the COVID-19 cases in Wuhan (Supplementary Table 1). The mean interval from illness onset to diagnosis of COVID-19, hospitalization and retest was 8.76±7.97 days, 10.73±9.05 days and 72.83±9.75 days, respectively. The mean interval between discharge and retest was 39.66±11.52 days. 184 (17.28%) individuals had experienced severe or critical COVID-19 (Table 1). 229 (21.50%) patients had self-reported symptoms. The most common symptoms were chest stuffiness (9.01%), cough (6.85%), and dyspnea (2.63%) (Supplementary Table 2). About one-third (366, 34.37%) patients had comorbidities, with 24.13% and 11.64% suffered from cardiovascular disease and diabetes, respectively (Supplementary Table 3). There were 108 (10.14%) patients tested negative for both SARS-CoV-2 and SARS-CoV-2-specific IgG/IgM after recovery. Nine (0.85%) re-positive discharged COVID-19 cases had been tested negative for SARS-CoV-2-specific IgG (Supplementary Table 4).

Re-positive prevalence and risk factors associated with re-positive RT-PCR results for SARS-CoV-2 in discharged COVID-19 patients

63 out of 1065 discharged COVID-19 patients have been tested re-positive for SARS-CoV-2, with re-positive prevalence to be 5.92% (95%CI: 4.50%-7.33%). Most re-positive COVID-19 patients were characterized by Ct values of the ORFlab gene (79.37%) and N gene (76.19%) to be 35-39 (Extended Data Fig. 1). Age of the re-positive COVID-19 patients (mean age, 50.65±14.31 years) were similar to negative discharged patients (mean age, 53.45±14.94 years, *P*=0.148). The re-positive prevalence of discharged COVID-19 patients was higher in females (7.86%, 95%CI: 5.61%-10.12%) than that in males (3.86%, 95%CI: 2.20%-5.52%, *P*=0.006). The median interval from discharge to retest was 39 (range, 19-58) days in re-positive discharged patients and 40 (range, 7-90) days in negative discharged patients (Fig. 1). The re-positive prevalence was higher in COVID-19 patients with 28-41 days between discharge and retest (8.58%, 95%CI: 5.94%-11.23%) than that in those with the interval from discharge to retest<28 days (3.77%, 95%CI: 0.81%-6.74%) and ≥42 days (4.21%, 95%CI: 2.40%-6.02%). The re-positive prevalence was 6.53% (95%CI: 4.52%-8.54%), 4.35% (95%CI: 2.04%-6.66%) and 6.52% (95%CI: 2.95%-10.09%) in COVID-19 patients with mild, moderate and severe/critical illness, respectively. Re-positive prevalence was similar in patients tested positive and negative for IgG (6.01% vs 5.56%, *P*=0.821) or IgM (6.38% vs 5.07%, *P*=0.394) (Table 1). In multivariable analyses, female sex (adjusted OR=2.18, 95%CI, 1.25-3.81) was significantly associated with re-positive result for SARS-CoV-2 in discharged COVID-19 patients. Compared with patients aged 60 years or above, those aged below 40 years were at higher risk for testing re-positive for SARS-CoV-2 (adjusted OR=2.29, 95%CI, 1.10-4.77) (Table 2).

Transmissibility of re-positive discharged COVID-19 patients

196 environment samples have been collected from 49 (77.78%) re-positive discharged COVID-19 patients. All the environment samples have been tested negative for SARS-CoV-2 with RT-PCR tests. Of the 63 re-positive COVID-19 patients, 12 (19.05%) lived alone after discharge from hospital without close contacts. The other 51 re-positive COVID-19 patients had 91 close contacts, with 25 and 9 individuals had been diagnosed as confirmed COVID-19 cases and clinically COVID-19 cases during the epidemic. There was only one person tested positive for SARS-CoV-2 with RT-PCR test from the 57 close contacts to the 51 re-positive COVID-19 patients. He was also tested positive for SARS-CoV-2-specific IgM and IgG (S/CO value for IgM, 1.22; S/CO value for IgG, 3.31) at the same time. This person had been quarantined as a close contact to a confirmed COVID-19 case (his father, a re-positive case) in February 2020. He had been tested negative of SARS-CoV-2 several times in the duration of isolation. But he reported mild diarrhea one week before the time when his father was diagnosed as a confirmed COVID-19 case. Considered his antibody level and disease history, he was inferred to be a previous COVID-19 case who had not been detected on February 2020.

After three generations of Vero E6 cells culture, indirect immunofluorescence assay presented no cytopathic effect upon infection with the nasopharyngeal specimens from 6 re-positive discharged COVID-19 cases. Nasopharyngeal swabs collected from 11 re-positive COVID-19 with different Ct values of the ORFlab gene and N gene were further used for sequencing. Except for one specimen failing sequencing, the other 10 specimens had been detected for virus fragments of SARS-CoV-2, with the coverage of the complete sequence to be 1.8%-35.8% (Table 3).

SARS-CoV-2-specific IgG/IgM of discharge COVID-19 patients

Blood samples were obtained from 1060 (99.53%) patients to test the antibody levels against SARS-CoV-2. The mean S/CO value of SARS-CoV-2-specific IgG in these patients was 17.00±28.04. It was higher in patients aged ≥60 years (median 9.90, IQR: 3.24-30.29) than that in those aged 40-59 years (median 5.64, IQR: 1.82-16.01) and patients aged <40 years (median 3.38, IQR: 0.78-7.85). The SARS-CoV-2-specific IgG level was similar between re-positive discharged patients and negative discharged patients (P=0.633) (Fig. 2A). Of the 1060 patients, 898 (84.72%) patients were tested positive for SARS-CoV-2-specific IgG. Self-reported symptoms at the survey were similar in patients tested negative and positive for SARS-CoV-2-specific IgG (Supplementary Table 5). Multivariable analyses showed that patients with old age, long interval between illness onset to diagnosis of COVID-19 and positive result for SARS-CoV-2-specific IgM had increased probability for positive result of SARS-CoV-2-specific IgG (Supplementary Table 6).

The mean S/CO value of SARS-CoV-2-specific IgM after recovery was 6.16±13.15. It was similar in different groups of age, illness severity and RT-PCR results for retesting SARS-CoV-2 (all P>0.05) (Fig. 2B). 705 (66.51%) of 1060 discharged patients were tested positive for SARS-CoV-2-specific IgM. Symptoms at the survey were also similar in patients tested negative and positive for SARS-CoV-2-specific IgM (Supplementary Table 5). Females were at lower risk for positive result for SARS-CoV-2-specific IgM

(adjusted OR, 0.74, 95%CI, 0.56-0.98). Compared to patients with <70 days from illness onset to survey retest, those with 70-77 days interval (adjusted OR, 1.47, 95%CI, 1.04-2.06) were at increased risk for testing positive for SARS-CoV-2-specific IgM (Supplementary Table 6).

Follow-up results for the re-positive COVID-19 patients and their matched non-re-positive COVID-19 patients

Total 48 re-positive cases and 90 matched non-re-positive recovered COVID-19 patients had been investigated on September 2-4, 2020, all of them were negative for SARS-CoV-2 with RT-PCR test. The self-report symptoms of the re-positive cases and 90 matched non-re-positive recovered COVID-19 patients at the investigation were similar (Supplementary Table 7).

Discussion

In the present study, we have reported the re-positive prevalence for SARS-CoV-2 in discharged COVID-19 patients in Wuhan, China. We found that 5.92% discharged COVID-19 patients in communities had been tested re-positive for SARS-CoV-2 with a median duration of 40 days since discharge. The females and younger discharged COVID-19 patients had higher re-positive prevalence than males and older did. The duration from onset to retest or illness severity was not associated with the risk of re-positive results for SARS-CoV-2 after discharge. The results of viral culture, virus sequencing, environment sampling and investigation of close contacts suggested there was no transmission risk of these re-positive discharged COVID-19 patients. The SARS-CoV-2-specific IgM and IgG levels had not reflected the status of viral RNA and self-reported symptoms. Only about 85% people have IgG antibody after discharge. The follow-up investigation suggested that there were no difference for the prognosis of re-positive cases and the matched non-re-positive cases four months later.

Our study suggested that the re-positive result for SARS-CoV-2 was common among discharged COVID-19 patients. Several large hospital-based studies reported that the proportion of testing re-positive in discharged COVID-19 patients was about 3% to 10.0% depended on the days since discharge [8,22]. Our study found that females and young people had higher re-positive prevalence in discharged COVID-19 patients in Wuhan. However, one study in Guangzhou (outside of Wuhan) demonstrated that only the duration of viral shedding and Ct value were associated with retest positive event [23]. These studies have not found the association between the severity of COVID-19 and the risk of retest positive.

Currently, the mechanisms on re-positive results for SARS-CoV-2 in discharged COVID-19 patients has not been well established. First, the RT-PCR test might be false negative just before discharge. The RT-PCR sensitivity for nasopharyngeal swab in COVID-19 patients was only 38% according to the early study in Wuhan [24]. Later studies also reported similar findings [25,26]. Second, PCR test re-positive suggested these discharged patients were still in recovery rather than re-infection or recurrence. Studies on re-positive COVID-19 patients demonstrated that these patients had improved clinical symptoms and indicators compared with that during their first hospitalization [4,27]. Third, current evidence did not support the

discharged COVID-19 patients are at risk for “re-infection”. An animal model study documented Rhesus macaques infected with SARS-CoV-2 after recovery from initial SARS-CoV-2 infection did not show any viral replication in all primary tissues, clinical symptoms and histopathological changes [28]. Further long-time follow-up studies are needed to illustrate the underlying mechanisms of test re-positive.

Since re-positive test for SARS-CoV-2 was common in discharged COVID-19 patients, it raised questions on the infectivity of the re-positive discharged COVID-19 patients. Evidence from our study suggested that the transmission risk of these re-positive discharged COVID-19 patients was low. Detection of viral RNA in re-positive COVID-19 patients did not necessarily mean that they were infectious and able to transmit the virus to close contacts. A study has studied the relationship between cycle threshold (Ct) values for SARS-CoV-2 with RT-PCR test and the infectivity of respiratory samples and found that the SARS-CoV-2 infectivity to Vero cell lines was only observed from RT-PCR confirmed positive samples with $Ct < 24$ [29]. The Ct values of all re-positive nasopharyngeal samples in our study were high than 24 and the cytopathic effect was not observed in Vero cell culture. Furthermore, we found that self-reported symptoms were not different between re-positive discharged patients and negative discharged patients. One study has investigated chest CT findings from re-positive discharged COVID-19 patients and found no specific clinical symptoms of them [30]. All of these indicated that re-positive discharged COVID-19 patients in Wuhan might have no transmission risk to others.

Our study showed that the level of antibodies against SARS-CoV-2 was not associated with the status of viral RNA tests in discharged COVID-19 patients. It suggested that serology tests for discharged COVID-19 patients could not identify the patients is free of SARS-CoV-2 infection or not when molecular testing was not available. According to recommendation [31], the serology test seems to play a different role in the diagnosis and recovery stage in COVID-19 development. The value of the serology test for recovered COVID-19 still needs more evidence. According to the findings from T cell responses to SARS-CoV-2 in Humans, $CD4^+$T cell responses to spike of the virus were correlated with the magnitude of the anti-SARS-CoV-2 IgG titers [32]. The IgG antibody level has also been found to be predictive of in vitro neutralizing capacity [33]. All these evidence suggested that the recovered COVID-19 patients with IgG antibody may have both neutralizing antibody and T-cell responses against the virus re-infection. But it is still unknown whether the recovered people without detectable IgG antibody face the risk of SARS-CoV-2 re-infection.

Based on our findings from discharged COVID-19 patients in Wuhan, here are some recommendations for COVID-19 prevention. First, the re-positive cases have low transmission risk for SARS-CoV-2 spread. There is no need to isolate recovered COVID-19 patients for 14 days after discharge. However, if a discharged COVID-19 patient has new onset symptoms, he/she may suffer the COVID-19 recurrence. Such recurrent COVID-19 case may cause potential community outbreak. Considering this point, it is necessary to follow-up and conduct surveillance on the health of discharge COVID-19 patients. Second, for the screening and management of imported COVID-19 cases, it is unnecessary to isolate all passengers with RT-PCR test positive for SARS-CoV-2. If a passenger has no symptom but positive RT-PCR test and positive IgG antibody, he/she is referred to a recovered COVID-19 patient and do not need quarantine or isolation. It

will help retard the medical overwhelmed and save limited medical resources in COVID-19 epidemic areas. Third, it is common that the recovered COVID-19 patients are tested negative for SARS-CoV-2-specific IgG. That means the recovered COVID-19 patients also need to take steps to prevent getting COVID-19. More evidence are needed for the re-infection risk of recovered COVID-19 patients.

Although it is the first population-based study on the re-positive prevalence for SARS-CoV-2 among the COVID-19 epidemic center in China, there are still some limitations in the present study. First, cluster sampling was used to recruit discharged COVID-19 patients from communities. The age and gender of the participants were similar to that of the COVID-19 cases in Wuhan, but the proportion of severe/critical patients were smaller in this study. The enrolled subject may not be a well representative sample of recovered COVID-19 patients in Wuhan. Second, we identified the SAS-CoV-2 RNA by nasopharyngeal swabs, but studies have demonstrated that the positive findings from saliva or rectal swabs from recovered COVID-19 patients were higher. The false-negative PCR test from nasopharyngeal swabs might not reflect the infectious status of discharged COVID-19 patients. Third, the present study is a cross-sectional study. The associations between the related factors and the status of viral RNA, antibody might not be causal. The prospective study is still on going to validate our findings.

In conclusion, this study suggested that testing re-positive for SARS-CoV-2 was common in discharged COVID-19 patients, but no evidence showed their transmission risk. However, not all recovered COVID-19 patients have SARS-CoV-2-specific IgG antibody, which suggested the potential re-infection risk of discharged COVID-19 patients.

Declarations

Competing interests

The views expressed in this study are those of the authors and do not represent the official position of their organizations. All of authors declare no competing interests.

Author contributions

SW and JH had designed the study and had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JFL, BY, XL, GW, QC, DX, SL, LC, MD, CD, HY, LL, JYL, HW, ZZ, YY, XH, TS, YNL, YW, QZ, SZL and YBL collected the data. YT, JH, SQW and XL analyzed the data. JFL, XG, SQW, BY, XL, GW, QC, DX, SL and LC consulted on and interpreted the results. YT, XG and JH drafted the manuscript. All authors critically revised the manuscript and gave final approval for the version to be published.

Acknowledgement

This work is supported by the National Key Research and Development Program of China (2020YFC0861100), Hubei Provincial Emergency Science and Technology Program for COVID-19 (2020FCA49), the Fundamental Research Funds for the Central Universities (2020kfyXGYJ066) and Hubei Medical Youth Reserve Talent Program (2019). We acknowledge all health-care workers involved in the assistance with field investigation administration and data collection of discharged COVID-19 patients in Wuhan and we thank Dr. Zengli Shi (Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China) for her help in the experiment design and laboratory work.

References

1. WHO. Weekly Operational Update on COVID-19–9 October 2020. Vol. 2020 (2020).
2. Wiersinga, W.J., Rhodes, A., Cheng, A.C., Peacock, S.J. & Prescott, H.C. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *Jama* (2020).
3. WHO. Criteria for releasing COVID-19 patients from isolation. Vol. 2020 (2020).
4. Cao, H., Ruan, L., Liu, J. & Liao, W. The clinical characteristic of eight patients of COVID-19 with positive RT-PCR test after discharge. *Journal of medical virology* (2020).
5. Jiang, M., *et al.* Recurrent PCR positivity after hospital discharge of people with coronavirus disease 2019 (COVID-19). *The Journal of infection* (2020).
6. Xing, Y., *et al.* Post-discharge surveillance and positive virus detection in two medical staff recovered from coronavirus disease 2019 (COVID-19), China, January to February 2020. *Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* **25**(2020).
7. Yuan, J., *et al.* PCR Assays Turned Positive in 25 Discharged COVID-19 Patients. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* (2020).
8. Mei, Q., *et al.* Assessment of patients who tested positive for COVID-19 after recovery. *The Lancet. Infectious diseases* (2020).
9. Lan, L., *et al.* Positive RT-PCR Test Results in Patients Recovered From COVID-19. *Jama* **323**, 1502–1503 (2020).
10. USA CDC. Duration of Isolation and Precautions for Adults with COVID-19. Vol. 2020 (2020).
11. Korean CDC. Findings from Investigation and Analysis of re-positive cases. May 19, 2020., Vol. 2020 (2020).
12. WHO. Clinical management of COVID-19 (Interim Guidance). Vol. 2020 (2020).
13. European CDC. Guidance for discharge and ending of isolation of people with COVID-19. Vol. 2020 (2020).
14. To, K.K., *et al.* COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* (2020).

15. Larson, D., *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).
16. Yang, J., *et al.* A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. *Nature* (2020).
17. Zhu, F.C., *et al.* Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet (London, England)* **396**, 479–488 (2020).
18. Chinese CDC. Notice on the issuance of technical documents related to the prevention and control program for pneumonia infected with noval coroavirus. Vol. 2020 (2020).
19. Xu, Y., *et al.* Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nat Med* **26**, 502–505 (2020).
20. Long, Q.X., *et al.* Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* **26**, 845–848 (2020).
21. Long, Q.X., *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* (2020).
22. Deng, W., *et al.* Positive results for patients with COVID-19 discharged form hospital in Chongqing, China. *BMC infectious diseases* **20**, 429 (2020).
23. Zheng, J., *et al.* Incidence, clinical course and risk factor for recurrent PCR positivity in discharged COVID-19 patients in Guangzhou, China: A prospective cohort study. *PLoS neglected tropical diseases* **14**, e0008648 (2020).
24. Liu, R., *et al.* Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Clinica chimica acta; international journal of clinical chemistry* **505**, 172–175 (2020).
25. Liu, K., Chen, Y., Lin, R. & Han, K. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. *The Journal of infection* **80**, e14-e18 (2020).
26. Wang, W., *et al.* Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *Jama* **323**, 1843–1844 (2020).
27. Chen, M., *et al.* Clinical characteristics of rehospitalized patients with COVID-19 in China. *Journal of medical virology* (2020).
28. Deng, W., *et al.* Primary exposure to SARS-CoV-2 protects against reinfection in rhesus macaques. *Science (New York, N.Y.)* **369**, 818–823 (2020).
29. Bullard, J., *et al.* Predicting infectious SARS-CoV-2 from diagnostic samples. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* (2020).
30. Li, C., *et al.* Chest CT study of fifteen COVID-19 patients with positive RT-PCR retest results after discharge. *Quantitative imaging in medicine and surgery* **10**, 1318–1324 (2020).
31. Peeling, R.W., *et al.* Serology testing in the COVID-19 pandemic response. *The Lancet. Infectious diseases* (2020).

32. Grifoni, A., *et al.* Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* **181**, 1489–1501.e1415 (2020).
33. Ng, D.L., *et al.* SARS-CoV-2 seroprevalence and neutralizing activity in donor and patient blood. *Nature communications* **11**, 4698 (2020).

Tables

Table 1. Re-positive prevalence by different characteristics in 1065 discharged COVID-19 patients in Wuhan.

Characteristics	Number of patients	Re-positive prevalence (95%CI)
Overall	1065	5.92% (4.50-7.33)
Age group, years		
0-39	203	8.87% (4.96-12.78)
40-59	442	5.66% (3.50-7.81)
≥60	420	4.76% (2.73-6.80)
Gender		
Male	518	3.86% (2.22-5.52)
Female	547	7.86% (5.61-10.12)
Days from onset to diagnosis		
<5	384	5.47% (3.19-7.74)
5-9	283	6.36% (3.52-9.20)
≥10	398	6.03% (3.69-8.37)
Days from onset to hospitalization		
<5	301	3.65% (1.53-5.77)
5-9	257	6.23% (3.27-9.18)
≥10	507	7.10 (4.86-9.34)
Days from onset to retest		
<70	338	6.21% (3.64-8.79)
70-77	368	5.98% (3.56-8.40)
≥78	359	5.57% (3.20-7.94)
Days from discharge to retest		
<28	159	3.77% (0.81-6.74)
28-41	431	8.58% (5.94-11.23)
≥42	475	4.21% (2.40-6.02)
Severity		
Mild	582	6.53% (4.52-8.54)
Moderate	299	4.35% (2.04-6.66)
Severe or critical	184	6.52% (2.95-10.09)
Self-reported symptoms at the survey		
None	836	5.86% (4.27-7.45)
1	167	5.99% (2.39-9.59)
≥2	62	6.45% (0.34-12.57)
Comorbidities		
None	699	6.15% (4.37-7.93)
≥1	366	5.46% (3.14-7.79)
IgG		
Negative	162	5.56% (2.03-9.08)
Positive	898	6.01% (4.55-7.77)
IgM		
Negative	355	5.07% (2.79-7.35)
Positive	705	6.38% (4.58-8.19)

Table 2. Factors associated with re-positive RT-PCR results for SARS-CoV-2 in discharged COVID-19 patients.

Characteristics	OR (95%CI)	*P*	Adjusted OR (95%CI)	*P*
Age group, years				
≥60	1.00		1.00	
40-59	1.20 (0.66, 2.19)	0.556	1.27 (0.67, 2.39)	0.468
0-39	1.95 (1.01, 3.77)	0.048	2.29 (1.10, 4.77)	0.028
Gender				
Male	1.00		1.00	
Female	2.12 (1.23, 3.66)	0.007	2.18 (1.25, 3.81)	0.006
Days from onset to retest				
<70	1.00		1.00	
70-77	0.96 (0.52, 1.78)	0.896	0.99 (0.52, 1.88)	0.977
≥78	0.89 (0.47, 1.67)	0.719	1.03 (0.53, 1.99)	0.933
Days from discharge to retest				
<28	1.00		1.00	
28-41	2.39 (0.99, 5.79)	0.052	2.15 (0.88, 5.29)	0.095
≥42	1.12 (0.44, 2.84)	0.810	0.95 (0.36, 2.49)	0.918
Severity				
Mild	1.00		1.00	
Moderate	0.65 (0.34, 1.24)	0.192	0.61 (0.31, 1.17)	0.134
Severe or critical	1.00 (0.51, 1.95)	0.997	0.99 (0.49, 1.99)	0.981
Self-report symptoms at the survey				
None	1.00		1.00	
1	1.02 (0.51, 2.06)	0.949	0.99 (0.48, 2.02)	0.977
≥2	1.11 (0.39, 3.18)	0.849	0.96 (0.33, 2.83)	0.946
Comorbidities				
None	1.00		1.00	
≥1	0.88 (0.51, 1.52)	0.652	1.15 (0.63, 2.09)	0.647
IgG				
Positive	1.00		1.00	
Negative	1.09 (0.53, 2.25)	0.821	1.15 (0.52., 2.54)	0.733
IgM				
Positive	1.00		1.00	
Negative	1.28 (0.73, 2.24)	0.395	1.38 (0.76, 2.51)	0.294

* The Pearson correlation coefficients of days from onset to retest and days from onset to diagnosis, days from onset to hospitalization were 0.50 and 0.46, respectively. Therefore, only days from onset to retest were included in the logistic analyses.

Table 3. Viral sequencing result of the nasopharyngeal specimens obtained from 11 re-positive discharged COVID-19 patients

Number of sample	Gender	Age (years)	Illness severity	Ct value for ORF 1ab gene	Ct value for N gene	Base number of SARS-CoV-2 genome (bp)	Coverage of SARS-CoV-2 genome with Metagenomics sequencing analysis (%)
1	Female	70	Mild	38.87	NA	538	1.8
2	Female	68	Mild	37.98	NA	3499	11.7
3	Female	37	Mild	NA	37.71	1196	4
4	Male	24	Moderate	37.72	40.1	1764	5.9
5	Male	38	Mild	37.75	37.1	10559	35.3
6	Female	65	Mild	38.78	38.91	10708	35.8
7	Female	54	Mild	28.38	27.22	4728	15.81
8	Female	20	Severe	29.19	28.99	757	2.53
9	Female	38	Mild	29.38	27.22	2976	9.95
10	Male	61	Mild	29.54	30.06	1732	5.79
11	Male	36	Mild	30.71	29.79	NA	NA

NA: not available.

Figures

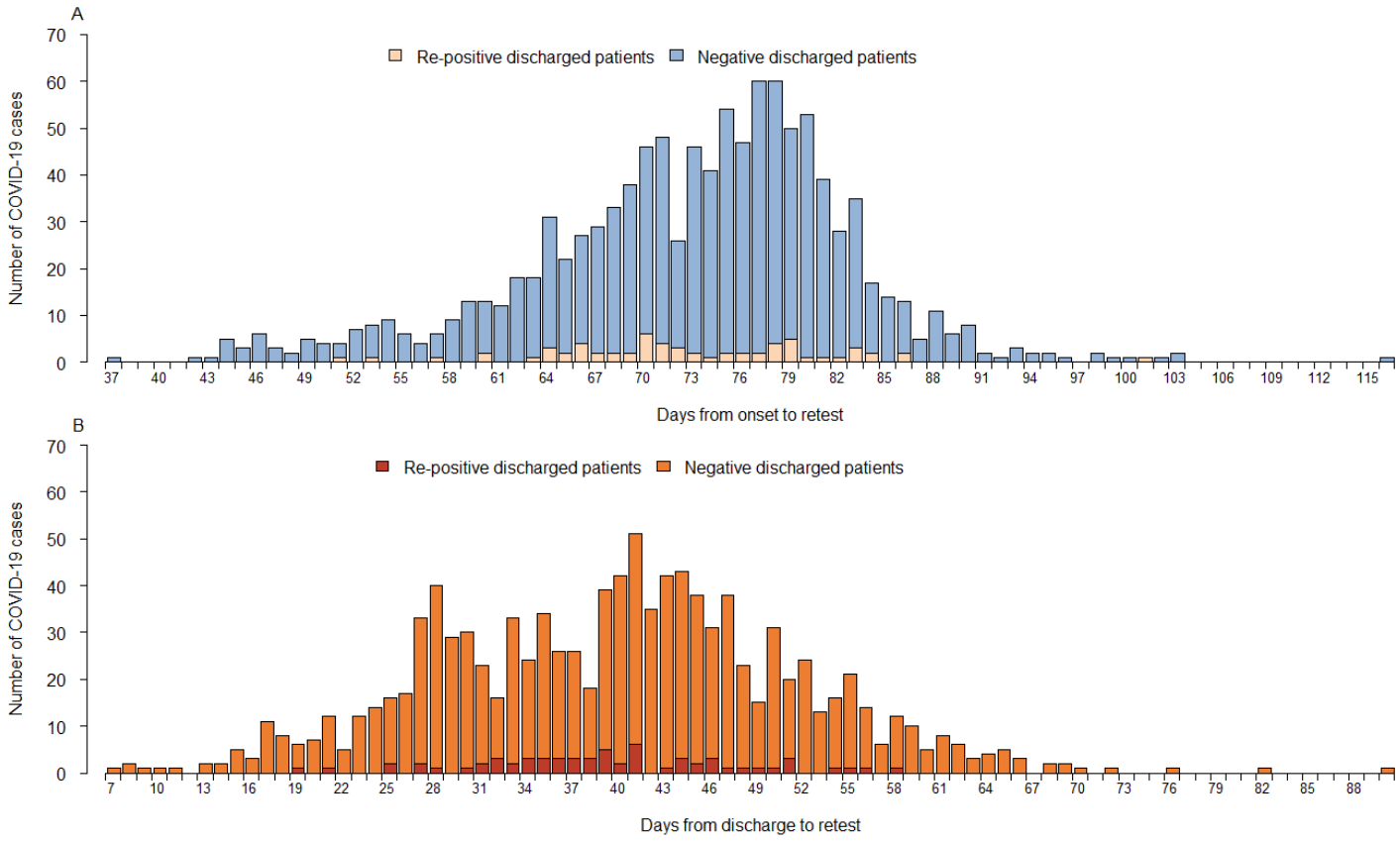

Figure 1

Daily number of discharged COVID-19 cases by days from onset to retest (A) and days from discharge to retest (B).

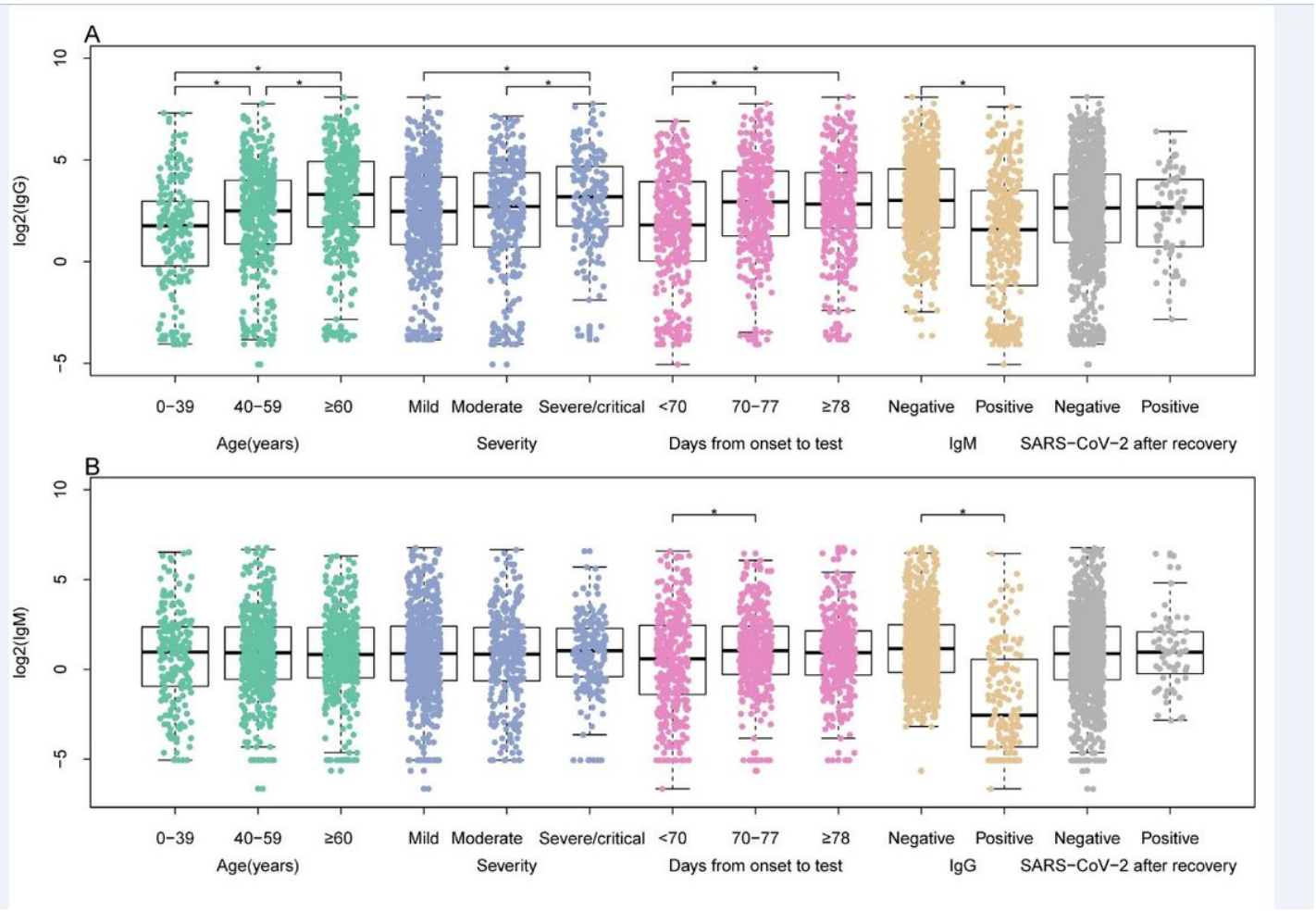

Figure 2

The level of SARS-CoV-2-specific IgG (A) and IgM (B) antibodies in discharged COVID-19 patients by age, severity, days from onset to retest and RT-PCR test for SARS-CoV-2 after discharge. The boxplots show medians (middle line), first and third quartiles (boxes), while the whiskers show 1.5× the interquartile range (IQR) above and below the box. Each value is indicated by a dot. “*” indicates $p<0.05$ for the post hoc pairwise comparisons with Dunn's test.

Supplementary Files

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

- SupplementaryTables1015NM.docx
- ExtendedDataFig.1.tif
- ExtendedDataFig.1.tif
- SupplementaryTables1015NM.docx