Vital Surveillances

Factors Associated with SARS-CoV-2 Repeat Positivity — Beijing, China, June–September 2020

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ABSTRACT

Introduction: Repeat positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) following COVID-19 initial viral clearance (re-positivity) poses a public health management challenge. The objective was to determine factors associated with neutralizing antibody (Nab) level and re-positivity among patients infected with a single strain SARS-CoV-2.

Methods: During a single strain SARS-CoV-2 cluster in Beijing, China, longitudinal individual clinical, virological, and immunological data were collected from 368 infections from June 13 to September 22, 2020. Factors associated with Nab level and re-positivity were analyzed using generalized estimating equations.

Results: A total of 353 (96%) SARS-CoV-2 infections had demographic, clinical, and laboratory data available. Among the 353 infections, 55 (15.5%) were re-positive, and blood draws were taken from 346 individuals (98.0%) during hospitalization and/or during the follow-up period. Symptoms were milder for the second-time admission for the re-positives, although 36.4% of re-positives presented with radiographic appearance of pneumonia manifestation. Compared to non-re-positive patients, NAb titers were lower among re-positives; NAb was positively associated with clinical severity. Samples from the lower respiratory tract manifested higher viral load than that from the upper respiratory tract. Multivariable analysis showed re-positivity was positively associated with being female [odd ratio (OR)=1.7, 95% confidence interval (CI) 1.1–2.8] and being aged <18 years (OR=5.2, 95% CI 1.5–18.1); having initially asymptomatic infection (OR=13.7, 95% CI 1.6–116.3); and negatively associated with a higher NAb level (OR=0.9, 95% CI 0.5–1.7).

Conclusions: NAb may be important for sustained viral clearance. Lower respiratory tract infection was associated with higher viral load among all infections when compared to upper respiratory tract infection. Continuous lower respiratory and intermittent upper respiratory viral shedding among COVID-19 infections may occur.

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory disease 2019 (SARS-CoV-2) was first identified in December 2019 (*1*). The initial wave of infection was followed by 56 consecutive days without any newly confirmed infections in Beijing; however, an infection confirmed on June 11, 2020 initiated the second wave of a COVID-19 outbreak (*2*). The second wave ended in one month, with a total of 368 confirmed infections [335 (91%) symptomatic infections and 33 (9%) asymptomatic infections]. All infections from the outbreak were directly or indirectly epidemiological linked to one market, the Xinfadi (XFD) wholesales market, from the same transmission chain and all caused by the B.1.1 strain (*2*). The outbreak provided an opportunity to deeply study important aspects of COVID-19 such as re-positivity.

Repeat positivity of SARS-CoV-2 following clearance and recovery (hence "re-positive") poses a public health management challenge. Re-positivity raises concerns for local health agencies because of the transmission potential of re-positive infections whereby the re-positive infection may expose others and cause a cluster of infections or a widespread outbreak. Re-positivity is distinctive from re-infection. Re-infection is defined as epidemiological exposure to an infectious source with evidence of infection from another virus strain. Re-positivity or recurrence has no epidemiological exposure or evidence of infected of another viral strain infection (*3*). Previous studies on re-positivity included infections from different transmission chains with potential different viral strains. The XFD outbreak provided an opportunity to study re-positivity on the same viral strain.

One way to determine the cause of re-positive infections may include monitoring the viral load of re-positive and non-re-positive infections. Viral load has a likely non-linear and complex association with clinical severity and infectiousness of infection at specific timepoints (*4*–*5*). There is conflicting evidence, however, about factors associated with viral load among SARS-CoV-2 infections.

Another way to determine the cause of re-positive infections may include assessing for differences in neutralizing antibody (NAb) titer among re-positive and non-re-positive infections. Despite yet undefined immunological correlates of protection, NAb titer is the current gold standard biomarker of an immune response to COVID-19. NAb is considered to play significant role in the clearance of SARS-CoV-2 and is utilized as a potential treatment, as it is a major component of convalescent plasma for many infectious diseases (*6*). Whether re-positives are associated with low levels of NAb is unknown.

Although several studies have explored the causes of re-positivity (*7*–*10*), few studies have incorporated analysis on SARS-CoV-2 re-positivity with a comprehensive review of demographic, clinical, and laboratory data combined with NAb and viral dynamics. The objective of this analysis was to determine factors associated with NAb levels and re-positivity among patients admitted to one hospital and infected with a single strain SARS-CoV-2 cluster in Beijing, China.

METHODS

All discharged patients were required to be quarantined in hotels for another 14 days. Nucleic acid tests were required for follow-up reexaminations on Day 14 and Day 28 after being discharged. If tested positive after discharge, the patient was re-admitted and quarantined again in the hospital, defined as being re-positive. The testing-readmission cycle stopped only when both the 14-day and 28-day post-discharge tests were negative.

Deidentified data of the patients were abstracted and collected from Beijing Ditan Hospital. Repeated blood draws were collected during and/or after the hospitalization of the XFD outbreak infection cases in Ditan Hospital from June 25 to August 28, 2020. Blood draws were required to be taken with a 3-day turn around or during reexamination days. Real time reverse transcript polymerase chain reaction (rtRT-PCR) was performed to obtain the cycle threshold (Ct) values for viral loads of the XFD outbreak infections from June 13 to September 22, 2020. Viral loads were collected from recorded repeated nasopharyngeal, throat (together as "NP swabs"), and sputum samples from deep cough for rtRT-PCR during the patient's hospitalization. The lowest Ct value among all samples for each person was obtained for each sample type (e.g., the lowest Ct value from all collected NP swab samples), and the average of Ct values among different groups were shown in Table 1. Individuals' repeated measures of Ct values were included on analysis of re-positivity. Distribution of samplings was shown in Supplementary Figure S1 and Supplementary Figure S2 (available in http://weekly.chinacdc.cn). More detailed methods on data information, NAb and viral load measurements are in Supplementary Materials (available in http://weekly.chinacdc.cn).

Demographic information, clinical laboratory information, clinical classification, Ct (representing viral load), treatments and NAb levels were included for univariate and multivariate analyses. Variables that were agreed upon within the research group and met the criteria of a *P*-value <0.1 by univariate analyses were selected for multivariate analysis. Generalized estimating equations (GEE) with assigned time groups were then performed to analyze potential factors associated with repeated measures of NAb and re-positivity of nucleic acid tests, with a significance level of 0.05. Data analysis and visualization were conducted using Stata/IC Version 16.0 (StataCorp, College Station, Texas).

Ethical approval and informed consent requirements were waived by the Institutional Review Board and Human Research Ethics Committee of the Beijing Center for Disease Prevention and Control (Beijing CDC) because this study was considered a continuation of the public health investigation associated with an emerging infectious disease.

RESULTS

A total of 368 SARS-CoV-2 infections were confirmed during the XFD outbreak, among which 353 (96%) had demographic, clinical, and laboratory data available. Among the 353 infections, 55 (15.5%) were re-positive, and blood draws were taken from 346 individuals (98.0%) for a total of 869 samples from blood draws collected during hospitalization and/or during the follow-up period.

The median age of all infections was 43 years [range: 1.6 to 86.0, interquartile range (IQR): 30.3–51.0].

TABLE 1. Demographic and clinical characteristics of patients admitted to Ditan Hospital with SARS-CoV-2 infections during the Xinfadi (XFD) outbreak, Beijing, China, June 25 to August 28, 2020 (n=353).

Variables	Re-positive	Non-re-positive	*P* (re-positivity)	Log2 highest NAb	*P* (NAb)	Total
Clinical severity (%)						
Asymptomatic	10 (18.2)	20 (6.7)	<0.001**	6.7 (6.6)	<0.001**	30 (8.5)
Mild	9 (16.4)	46 (15.4)	0.879	7.2 (6.6)	<0.001**	55 (15.6)
Moderate	35 (63.6)	214 (71.8)	0.069§	8.1 (8.0)	<0.001**	249 (70.5)
Severe and Critical	1 (1.8)	18 (6.0)	0.086§	10.2 (10.6)	<0.001**	19 (5.4)
Age (years old) (median)						41.6 (43.0)
0–18	3 (5.5)	6 (2.0)	0.200	7.7 (7.9)	<0.001**	9 (2.5)
19–30	13 (23.6)	64 (21.5)	0.217	7.2 (6.8)	0.464	77 (21.8)
31–40	12 (21.8)	62 (20.8)	0.151	7.5 (7.6)	0.745	74 (20.9)
41–60	25 (45.5)	139 (46.6)	0.035¶	8.1 (8.0)	0.588	164 (46.5)
>60	2 (3.6)	27 (9.1)	0.006¶	8.9 (10.0)	0.110	29 (8.2)
Gender (%)						
Male	24 (43.6)	172 (59.1)	0.055§	7.9 (8.0)	0.283	196 (56.6)
Female	31 (56.4)	119 (40.9)	<0.001**	8.1 (8.0)		150 (43.4)
Highest body temperature (℃) (median)	38.1 (38.1)	37.7 (37.6)	0.013¶		<0.001**	37.7 (37.7)
Treatment (%)						
Interferon	11 (20.0)	15 (5.03)	<0.001*	7.7 (7.6)	0.149	26 (7.4)
Traditional Chinese Medicine	37 (67.3)	140 (47.0)	0.009§	8.0 (8.0)	0.860	177 (50.1)
Chloroquine phosphate	10 (18.2)	44 (14.8)	0.658	7.6 (7.6)	0.017¶	54 (15.3)
Favipiravir	13 (23.6)	83 (27.9)	0.279	8.1 (8.0)	0.412	96 (27.2)
Convalescent plasma	3 (5.5)	11 (3.7)	0.456	9.6 (10.0)	<0.001**	14 (4.0)
Steroid	0 (0.0)	3 (1.01)	0.207	9.5 (10.0)	0.034¶	
Duration of 1st time hospitalization (median)	24.5 (24.0)	27.24 (26.5)	0.016¶		<0.001**	26.8 (26.0)
Laboratory data						
WBC (10^9/L)	5.1 (4.5)	7.6 (5.3)	0.046¶		0.013¶	7.2 (5.2)
Lymphocytes (10^9/L)	1.7 (1.6)	2.0 (1.7)	0.144		0.002**	1.9 (1.7)
Hemoglobin (g/L)	139.4 (142.0)	141.6 (143.0)	0.210		<0.001**	141.2 (143.0)
CD4	655.5 (594.0)	732.4 (644.0)	0.160		0.087§	719.7 (633.0)
CD8	396.8 (380.0)	476.6 (395.0)	0.108		0.036¶	463.4 (392.0)
CD4/CD8	1.8 (1.7)	1.9 (1.5)	0.819		0.131	
Kidney function abnormality* (%)	0 (0.0)	1 (0.3)	0.303		0.338	
Liver function abnormality† (%)	5 (9.1)	23 (7.7)	0.428		<0.001**	
Cycle threshold (Ct)						
N gene (NP)	31.3 (32.8)	30.7 (32.1)	0.269		0.001¶	29.0 (29.8)
N gene (Sputum)	27.4 (26.7)	26.4 (27.3)	0.360		0.338	26.5 (27.2)
Log2 highest NAb level (median)	8.1 (8.0)	8.0 (8.0)	0.821			
Interval between disease onset and hospitalization	9.3 (8.0)	8.2 (7.0)	0.014¶		0.004¶	8.4 (7.0)
Mental condition			<0.001**			
Re-positive (median)(%)				8.1 (8.0)	0.479	55 (15.5)
Non-re-positive (median)(%)				8.0 (8.0)		291 (82.4)
Total	55	298		8.0 (8.0)		353

Note: *P*-values were determined through single logistic regressions. Highest neutralizing antibody is the geometric mean among available samples. The lowest Ct values were taken for each person and each sample type.

Abbreviations: WBC=white blood cell; N=neutrophil (10^9/L); ALT=alanine aminotransferase (U/L); AST=aspartate aminotransferase (U/L); Cr=creatinine (μmol/L).

* Kidney function abnormality is defined as increasing Cr of the normal reference values for general population from Ditan Hospital.

† Liver function abnormality is defined as increasing both ALT and AST of the normal reference values for general population from Ditan Hospital.

§ *P*<0.1.

¶ *P*<0.05.

** *P*<0.001.

The median age for re-positives was 40.0 years old, whereas the median age for non-re-positives was 43.0 years old. The re-positive group had a lower percentage of male (43.6%) compared to female (56.4%) cases (Table 1). Generally, no descriptive symptoms appeared to have significant differences between re-positive group and non-re-positive group (Supplementary Table S1, available in http://weekly.chinacdc.cn/). The duration of the first-time admission was shorter in the re-positive groups than non-re-positive groups. None of the lowest Ct values from different samples presented significant differences (Table 1). Symptoms were generally milder during the second-time admission for the re-positive population. None of the re-positives were classified as severe or critical infection during their second-time admission. Comparing the first-time and the second-time admission among re-positives, infections at second-time admission presented lower percentage of radiographic appearance of pneumonia manifestation (63.6% *vs.* 36.4%, $P<0.001$) and clinical symptoms (78.2% *vs.* 3.6%, $P<0.001$).

The seroconversion rate of NAb against the same virus strain was 98.9% (349/353). Longer follow-up time was observed in the re-positives than non-re-positives (Supplementary Figure S1, available in http://weekly.chinacdc.cn/). To examine factors associated with NAb dynamics, clinical classifications, age groups, underlying diseases, re-positivity, gender, highest body temperature, liver function abnormality, different treatments, and lowest Ct values from NP samples met selection criteria after consultation, were

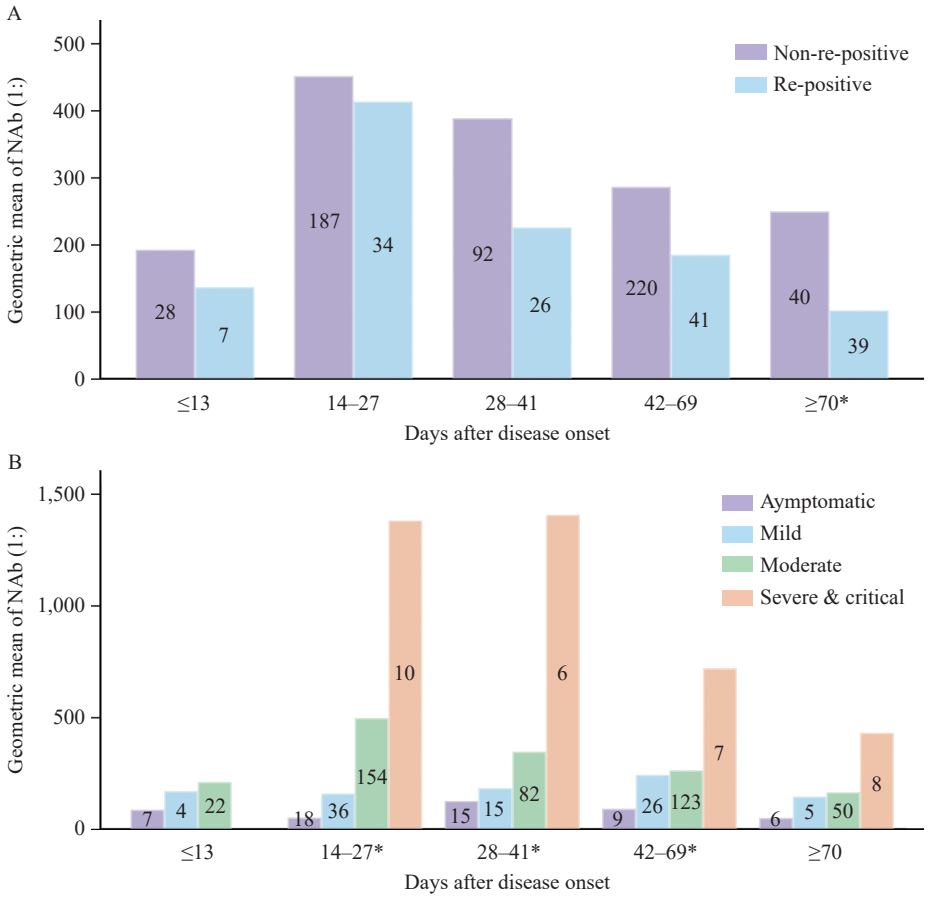

FIGURE 1. Geometric mean of neutralizing antibody level of COVID-19 infections by days after disease onset. (A) Geometric mean of neutralizing antibody level of SARS-CoV-2 infections comparing re-positives with non-re-positives. (B) Geometric mean of neutralizing antibody level of COVID-19 infections by clinical classifications.
* Indicates significant difference among comparison groups (P=0.035 for ≥70 between re-positives and non-re-positives; P=0.013, P=0.009, and $P<0.001$ for day 14–27, 28–41, and 42–69 among clinical classifications, respectively). Number in the middle of each bar represents the numbers of samples of each group (E.g., the geometric mean was calculated from 28 samples for ≤13-day after disease onset group among re-positives).
Abbreviation: NAb=neutralizing antibody.

included in the multivariable GEE model. Overall, antibody in re-positives was lower than that in non-re-positives (Figure 1A). Clinical severities were positively associated with NAb titer (Asymptomatic: β =−1.5, 95% CI −2.6 to −0.4; Mild: β =−1.1, 95% CI −2.1 to −0.2; Moderate: β =−0.9, 95% CI −1.8 to −0.1; reference: severe and critical) (Supplementary Table S2, available in http://weekly.chinacdc.cn), adjusting for other covariates (Figure 1B). Infections age between 19 to 40 (Age 19–30: β =−0.7, 95% CI −1.1 to −0.2; Age 31–40: β =−0.5, 95% CI −1.0 to 0.0; reference: >60) and having received traditional Chinese medicine (β =−0.5, 95% CI −0.9 to −0.1) had lower antibody titers. Body temperature (β =0.6, 95% CI 0.3 to 0.8), hepatic dysfunction (β =1.1, 95% CI 0.4 to 1.8) were also positively associated with NAb titer (Supplementary Table S2).

Re-positives received on average more NP swabs than non-re-positive groups during their first-time hospitalization (*P*=0.001). The distribution of sampling time of each infection is illustrated in Supplementary Figure S2. Generally, sputum sample presented lower Ct values than NP samples, whereas no observed difference between re-positives and non-re-positives (Figure 2). Considering 236 infections with both NP and sputum samples, sputum samples presented lower Ct values than NP samples among both re-positives (N gene: 25.5 *vs.* 27.4, *P*=0.002) and non-re-positives (N gene: 25.7 *vs.* 27.8, *P*<0.001). Comparing the first- and second-time admission among re-positives, significant difference in Ct values was observed among all re-positives with NP samples (N gene: 27.4 *vs.* 28.3, *P*=0.009).

Repeated measures of NAb, clinical classifications, age groups, comorbidities, gender, different treatments, time difference between disease onset and hospitalization, mental condition, and liver function state met selection criteria and, after consultation, were included in the multivariate GEE model analyzing factors associated with re-positivity. Multivariable

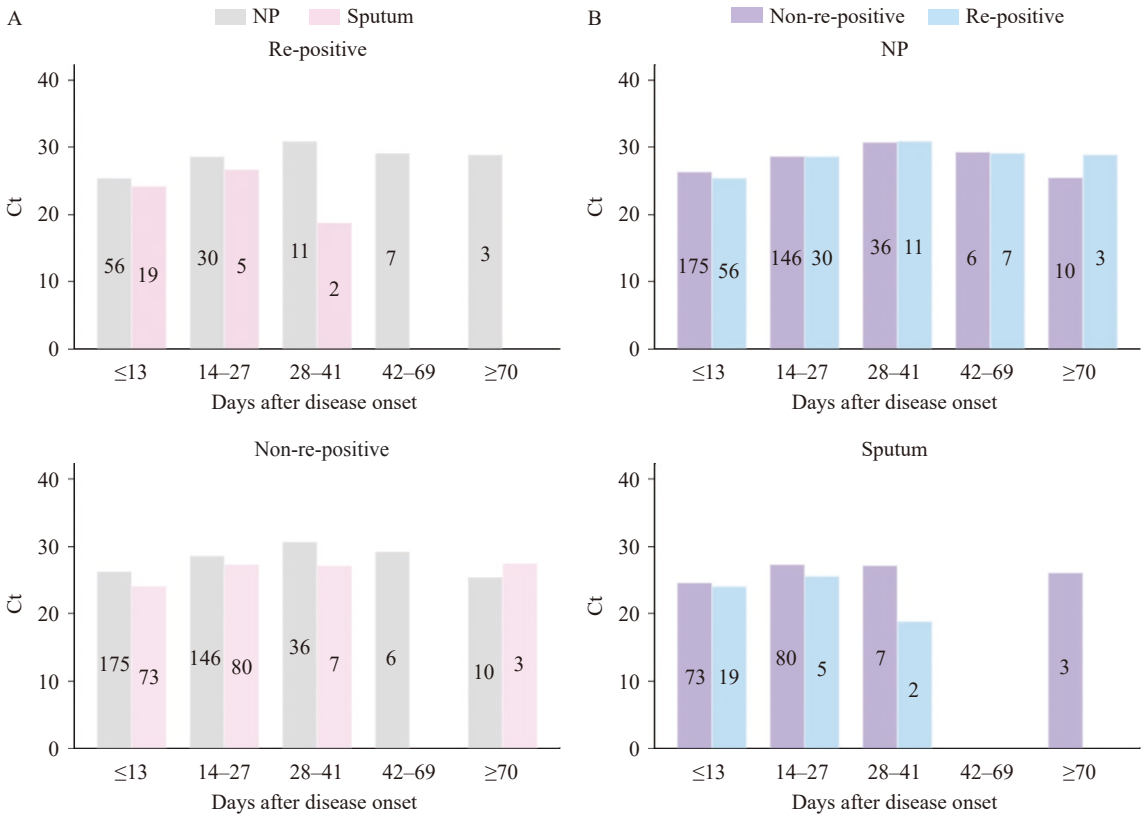

FIGURE 2. Ct values for all COVID-19 infections with upper (NP) and lower respiratory (sputum) tract specimens by days after disease onset. (A) Ct values comparison of upper and lower respiratory tract specimens among re-positives and non-re-positives. (B) Ct values comparison of re-positives and non-re-positives by specimen types (NP and sputum).
Note: Number in the middle of each bar represents the numbers of samples of each group; (e.g., the average Ct value is from 56 samples for ≤13-day after disease onset group among re-positives).
Abbreviation: Ct=cycle threshold; NP=nasopharyngeal.

analysis showed re-positivity was associated with being female (OR=1.7, 95% CI 1.1–2.8) and aged <18 years (OR=5.2, 95% CI 1.5–18.1), having initially asymptomatic infection (OR=13.7, 95% CI 1.6–116.3), having received traditional Chinese medicine (OR=2.1, 95% CI 1.2–3.6) or interferon (OR=7.6, 95% CI 3.7–15.7) or convalescent plasma (OR=15.9, 95% CI 2.4–107.6), liver function abnormality (OR=3.6, 95% CI 1.5–3.6), and was negatively associated with age 41–60 years (OR=0.1, 95% CI 0.0–0.8) and higher NAb level (OR=0.9, 95% CI 0.8–1.0) (Table 2).

CONCLUSION

A certain proportion of recovered populations subsequently test positive repeatedly after two consecutively negative PCR tests. In this study, the re-positive rate was 15.5%, which was similar to other reported studies (*8*,*11*), although the re-positive rates varied from study to study with a range from 3.3% to 30.7% (*7*). Re-positives appeared with some recurrence but less severe clinical symptoms during the second-time positivity. Moreover, consistent with other studies, a higher percentage of re-positivity was observed in females and younger age groups (*12*–*13*).

TABLE 2. Odds ratios of re-positive status associated with selected factors using generalized estimating equations (GEE) among patients admitted to Ditan Hospital with SARS-CoV-2 infections during the Xinfadi (XFD) outbreak, Beijing, China, June 25 to August 28, 2020 (n=349).

Selected factors	OR (95% CI)	*P*
Neutralizing antibody level*	0.9 (0.8, 1.0)	0.046†
Clinical classifications		
Asymptomatic	13.7 (1.6, 116.3)	0.017†
Mild	8.0 (1.0, 64.3)	0.050§
Moderate	6.9 (1.0, 50.3)	0.055§
Severe and critical	Ref	
Age group (years old)		
0–18	5.2 (1.5, 18.1)	0.010†
19–30	0.9 (0.5, 1.7)	0.683
31–40	1.3 (0.7, 2.4)	0.405
41–60	0.1 (0.0, 0.8)	0.034†
>60	Ref	
Gender, female	1.7 (1.1, 2.8)	0.030†
Treatment-convalescent plasma, yes	15.9 (2.4, 107.6)	0.005†
Treatment-TCM¶, yes	2.1 (1.2, 3.6)	0.007†
Treatment-interferon, yes	7.6 (3.7, 15.7)	<0.001**
Hepatic dysfunction††, yes	3.6 (1.5, 3.6)	0.005†
Underlying disease(s)§§, yes	0.7 (0.4, 1.3)	0.250
Ct N gene (NP)¶¶	0.6 (0.3, 1.3)	0.197
Interval between disease onset and hospitalization	1.0 (0.9, 1.0)	0.181
Mental condition***	0.5 (0.2, 1.3)	0.175

Note: Odds ratio (OR), 95% confidence intervals (95% CI) and corresponding *P*-values were identified.
* Repeated measures of neutralizing antibody levels.
† *P*<0.05.
§ *P*<0.1.
¶ TCM denotes for traditional Chinese Medicine.
** *P*<0.001.
†† Hepatic dysfunction is defined as increasing both ALT, Alanine aminotransferase (U/L) and AST, Aspartate aminotransferase (U/L) of the normal reference values for general population from Ditan Hospital. Reference range: ALT, 5–40 U/L; AST, 8–40 U/L
§§ Underlying diseases include any underlying disease that the infection reported at first time admission.
¶¶ Individuals' lowest Ct values for N gene from nasopharyngeal and throat swabs, by PCR
*** Mental condition identified by physician at first day of hospital admission, 5 levels (1−5) from poor to normal, with reference to the worse mental state.

NAb is considered to play a significant role in the clearance of SARS-CoV-2 (*6*). In our study, NAb levels for both re-positive and non-re-positive groups followed the expected patterns of antibody (*14*). Similar to other studies, severity is highly correlated with NAb levels (*14–17*) and may affect liver function or body temperature. Lower NAb levels were observed for re-positive group than in non-re-positive group. Classified clinical severities of the patients in the re-positive group were generally milder, and milder people would generate relatively lower antibody level (*14–16*). Antibodies were produced at relatively lower levels in asymptomatic patients, affecting the overall clearance of the virus. Indeed, our results presented an overall lower NAb level in the re-positive group. Furthermore, the use of convalescent plasma, traditional Chinese medicine, and interferons might have potential effects on re-positivity. However, the treatment used may also be related to specific symptoms and severity; in other words, it may represent reverse causality or may be confounders.

Nevertheless, re-positive remains controversial terminology with respect to the COVID-19 pandemic. Researchers have proposed hypotheses regarding the re-positive phenomenon, including re-infection with another strain, secondary infection with the first virus strain, prolonged viral shedding, intermittent virus shedding, and laboratory errors (*8*,*18*). In our study, the possibility of being infected by another virus strain could be excluded because all included infections in this study were from the same outbreak (the XFD outbreak), and there was no other circulating virus from June to September (i.e., sampling time) in Beijing. Moreover, it was not likely to be laboratory error for all re-positive infections, as 55 tested positive repeatedly for these infections and 2 assays from different manufactures used for PCR tests to increase specificity. The re-positives generally had milder symptoms compared to the non-re-positives. As no obvious upper respiratory symptoms manifested in re-positives, our evidence suggests intermittent upper-respiratory tract viral shedding may have occurred. Particularly, they may test negative twice consecutively during their first hospitalization period, but it could be hypothesized that they were still shedding virus in the lower respiratory tract as higher viral load was found in sputum, and 20 out of 55 (36.4%) still showed pneumonia symptoms by computed tomography (CT) during the second hospitalization for re-positives. It could be hypothesized that the re-positive population did not fully recover from the infection and needed further treatment by the date of their first discharge. Indeed, no CT scan diagnosis was required for discharged from hospital for COVID-19, and few samples from the lower-respiratory tract were collected during the second hospitalization for re-positives. Despite this, whether the twice negative tests for re-positives during their first time hospitalization were false negatives need further investigation. In addition, as statistically lower viral loads were observed during second-time hospital admission, lower infectivity amongst re-positives during their second hospital admission could also be hypothesized (*19*).

The lower NAb levels among re-positives suggest that the probability of being re-positive may be reduced by boosting the NAb levels, such as through vaccination. Together with the alternating viral shedding in upper respiratory tract and lower NAb level, re-positives may not be detected before their first discharge. Despite hypothesized lower infectivity, a few case studies did propose prolonged and intermittent infectious viral shedding by isolation of infectious virus in some re-positive infections (*20–21*). Under the stringent management policy of COVID-19 infections in China, re-positives were not able to transmit to other people, even with lower transmission rates as re-positives were more likely to have milder symptoms (*19*,*22*). Nevertheless, the potential infectivity of re-positives could not be neglected.

This study was subject to some limitations. Despite the evidence revealed, further laboratory examination is needed to test viral infectivity. Ct values may also be related to techniques of obtaining sample by different health personnel. Sputum specimens may only be collected among those who were symptomatic. In addition, the infectiousness of SARS-CoV-2 is highly dependent on the specific variant. Infectiousness for the virus strain (B.1.1) in the XFD market cannot fully represent the others as the hypotheses generated from this analysis does not imply the same effect could occur for all other strains. More evidence on viral load and infectivity should be verified on different virus strains. Additionally, due to the fact the limited number of infections during the XFD outbreak, the results may not be widely generalizable.

In conclusion, our results revealed the associated factors and implications with re-positivity. Lower overall NAb level may affect the clearance of virus among re-positives. Intermittent viral shedding among COVID-19 infections may occur, and it may instead be that re-positivity is an indicator of a lack of recovery from infection.

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SUPPLEENTARY MATERIALS

Supplementary Methods

Definitions and examination criteria

Five clinical severity categories of SARS-CoV-2 infections were defined in China: asymptomatic, mild, moderate, severe, and critical. Asymptomatic infections were designated in individuals who had no symptom but tested positive for SARS-CoV-2 by nucleic acid tests. Disease onset of asymptomatic infections was defined as the day that the infection tested positive for the first time. Mild infections were individuals with mild clinical symptoms and no pneumonia sign on radiography image. Moderate infections were those patients with fever or respiratory symptoms, and radiographic appearance of pneumonia manifestation. Severe infections were individuals who have respiratory distress (respiratory rate >30 breaths/min), oxygen saturation (SpO_2) <93% on room air. Critical infections were individuals in respiratory or circulatory failure and requiring mechanical ventilation, circulatory support, or other organ failure that required ICU admission and monitoring.

In China, any positive SARS-CoV-2 infection was required to be quarantined in a hospital, and the patient could be discharged only if two consecutive (>24 hours apart) nucleic acid tests with negative results had been observed. All discharged patients were required to be quarantined in hotels for another 14 days. Nucleic acid tests were required for follow-up reexaminations on Day 14 and Day 28 after being discharged. Once tested positive after being discharged, the patient was re-admitted and quarantined again in hospital, defined as being re-positive. The testing-readmission cycle stopped only when both the 14-day and 28-day post-discharge tests were negative.

Data and laboratory method

1. Data source and ethics

Deidentified data of the patients were extracted and collected from Ditan Hospital, an infectious-disease-oriented hospital in Beijing that managed the majority of SARS-CoV-2 infections in Beijing.

Ethical approval and informed consent requirements were waived by the Institutional Review Board and Human Research Ethics Committee of the Beijing Center for Disease Prevention and Control (Beijing CDC) because this study was considered a continuation of the public health investigation associated with an emerging infectious disease.

2. Neutralizing antibody (NAb)

Repeated blood draws were collected during and/or after the hospitalization of the XFD outbreak infection cases in Ditan Hospital from June 25 to August 28, 2020. Blood draws were required to be taken with a 3-day turnaround or during reexamination days (14 and 28 day). NAb was determined with a modified cytopathogenic neutralization assay based on live SARS-CoV-2 and was assessed by NAb titer. For calculation, antibody titers were assigned values on 2 to the 'x' times, respectively. A titer with $\geq 1:4$ is defined as seropositive.

3. Viral load (Ct values)

Real time RT-PCR was performed to obtain the Ct values for viral loads of the XFD outbreak infections from June 13 to September 22, 2020. Viral loads were collected from recorded repeated nasopharyngeal, throat (together as "NP swabs") and sputum samples from deep cough (quality check by nurse and laboratory technician) for RT-PCR during the patient's hospitalization. Reagents for RT-PCR tests were from Da An Gene Co., Ltd. and Beijing Applied Biological Technologies Co., Ltd. Both assays were performed to determine any nucleic acid positivity. Reaction system and amplification conditions were performed according to the manufacturers' instruction (Da An Gene Co., Ltd. and Beijing Applied Biological Technologies Co., Ltd.). All available Ct values for determining viral load were abstracted from 7500 software (version 2.3, Thermo Fisher Scientific, Beijing, China).

The lowest Ct value among all samples for each person was obtained for each sample type (E.g., lowest Ct value from all collected NP swab samples), and the average of Ct values among different groups were shown in Supplementary Table S1. Individuals' repeated measures of Ct values were included on analysis of re-positivity.

4. Clinical/Laboratory data

Data from blood routine examinations, including levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), from liver function tests, creatinine level (Cr) from kidney function tests, and computerized tomography (CT) images were collected from the clinical charts and tests results (Supplementary

Table S1).

5. Statistical analysis

Demographic information, clinical laboratory information, clinical classification, cycle threshold (representing viral load), treatments and NAb levels were included for uni- and multivariate analyses. Variables that were agreed upon within the research group and met the criteria of a *P*-value <0.1 by univariate analyses were selected for multivariate analysis. Generalized estimating equations (GEE) with assigned time groups were then calculated to analyze potential factors associated with repeated measures of NAb and re-positivity of nucleic acid tests. Data analysis and visualization were conducted using Stata/IC (version 16.0, StataCorp, College Station, Texas).

Supplementary Results

Patients with underlying diseases fell into 11 classifications according to ICD-11 by World Health Organization (WHO), including 2 (0.57%) with diseases of the blood or blood-forming organs, 6 (1.73%) with endocrine, nutritional, or metabolic diseases, 1 (0.29%) with mental, behavioral, or neurodevelopmental disorders, 1 (0.29%) with diseases of the nervous system, 35 (10.12%) with diseases of circulatory, 8 (2.31%) with diseases of the respiratory system, 12 (3.47%) with diseases of the digestive system, 1 (0.29%) with diseases of the skin, 2 (0.58%) with diseases of the musculoskeletal system or connective tissue, 5 (1.45%) with diseases of genitourinary system, 3 (0.87%) with symptoms, signs, or clinical findings that were not classified elsewhere. In addition, 264 (76.3%) had no underlying diseases.

SUPPLEMENTARY TABLE S1. Demographic and clinical characteristics associated with re-positive (*vs.* non-re-positive) status among 346 COVID-19 infections with blood samples during a secondary outbreak, Beijing, China, June to September 2020.

Variables	Re-positive	Non-re-positive	*P*-value (re-positivity)	Log2 Highest NAb	*P*-value (NAb)	Total
Body mass index (median)	24.0 (23.5)	25.0 (24.7)	0.182		0.245	24.8 (24.5)
Self-medication prior admission (%)	12 (21.8)	49 (16.6)	0.439		<0.001***	61 (17.4)
Body temperature (°C) at admission (median)	36.7 (36.6)	36.9 (36.8)	0.149		<0.001***	36.9 (36.8)
Number of underlying diseases (%)						
None	42 (76.4)	222 (76.3)	0.986	7.9 (7.6)		264 (76.3)
One underlying disease	12 (23.5)	59 (20.3)	0.701	8.3 (8.0)	0.005***	71 (20.5)
Two underlying diseases	1 (2.0)	8 (2.8)	0.569	9.0 (9.0)	0.008***	9 (2.6)
Three or more underlying diseases	0 (0.0)	2 (0.7)	0.405	9.0 (9.0)	0.527	2 (0.6)
Symptoms (%)						
None	10 (18.1)	19 (6.5)	0.254	6.7 (6.6)	0.001***	29 (8.4)
Fever	27 (49.1)	137 (47.1)	0.780	8.6 (8.6)	<0.001***	164 (47.4)
Cough	16 (29.1)	84 (28.9)	0.979	8.5 (8.6)	<0.001***	100 (28.9)
Uncomfortable throat	6 (10.9)	15 (5.2)	0.167	7.9 (8.0)	0.296	21 (6.1)
Ageusia (loss of taste)	4 (7.8)	6 (2.1)	0.188	8.1 (8.0)	<0.001***	10 (2.9)
Anosmia (loss of smell)	3 (5.9)	5 (1.7)	0.181	8.3 (7.6)	0.390	8 (2.3)
Nausea	5 (7.8)	8 (2.8)	0.054	8.4 (8.0)	0.065**	13 (3.8)
Diarrhea	0 (0.0)	15 (5.2)	0.154	8.6 (9.0)	0.030**	15 (4.3)
Fatigue	10 (18.2)	50 (16.8)	0.953	8.7 (9.0)	0.008***	60 (17.0)
Total	55	298		8.0 (8.0)		353

Note: *P*-values were determined through single logistic regressions. Highest neutralizing antibody (NAb) is the geometric mean among available samples. Lowest Ct value were taken for each person and each sample type.

* *P*<0.1,

** *P*<0.05,

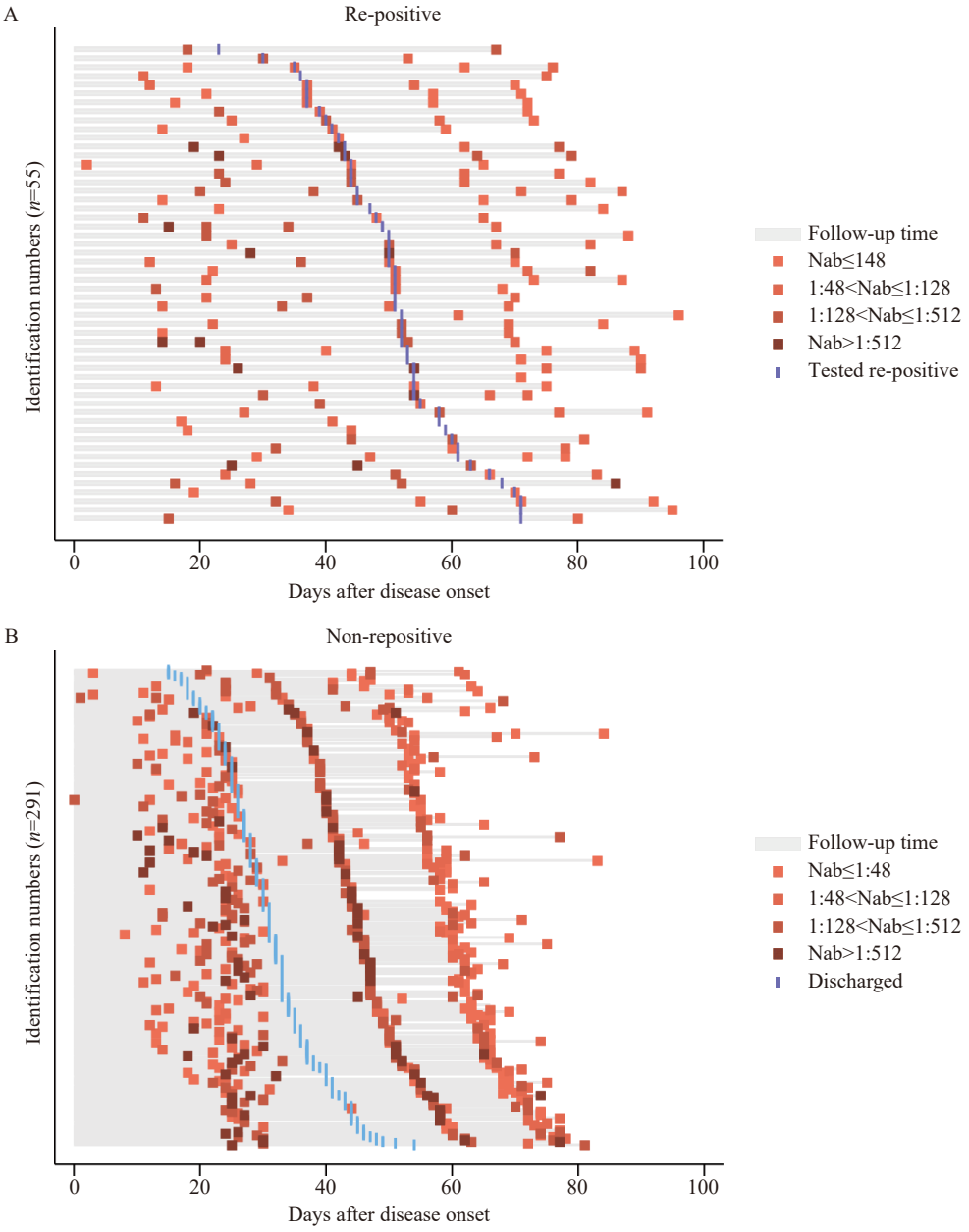
*** *P*<0.01.

Abbreviations: WBC=white blood cell; N=neutrophil; L=lymphocytes; HB=hemoglobin; ALT=alanine aminotransferase; AST=aspartate aminotransferase; Cr=creatinine.

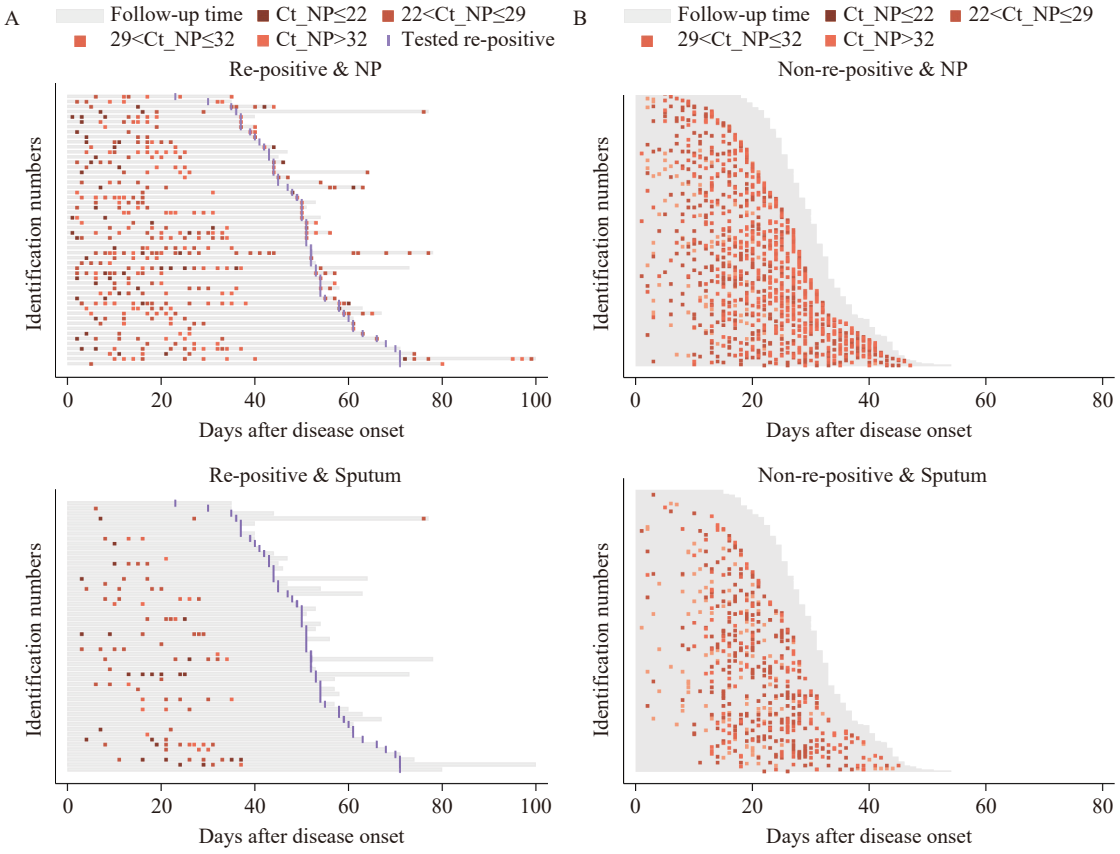
SUPPLEMENTARY TABLE S2. Selected factors and their association with neutralizing antibody (NAb) levels using generalized estimating equations (GEE), among patients admitted to Ditan Hospital with SARS-CoV-2 infections during the Xinfadi (XFD) outbreak, Beijing, China, June 25 to August 28, 2020 (n=349).

Selected factors	NAb Level Coefficient (95% CI)	*P*-value
Clinical classifications		
Asymptomatic	−1.5 (−2.6 to −0.4)	0.010*
Mild	−1.1 (−2.1 to −0.2)	0.019*
Moderate	−0.9 (−1.8 to −0.1)	0.021*
Severe and critical	Ref	
Age group (years old)		
0−18	0.1 (−1.2 to 1.3)	0.904
19−30	−0.7 (−1.1 to −0.2)	0.005*
31−40	−0.5 (−1.0 to 0.0)	0.031*
41−60	0.3 (−0.4 to 0.9)	0.432
>60	Ref	
Underlying disease(s)[†], yes	0.2 (−0.2 to 0.7)	0.297
Gender, female	0.0 (−0.3 to 0.4)	0.819
Highest Body Temperature (°C)[§]	0.6 (0.3 to 0.8)	<0.001[¶]
Treatment-convalescent plasma, yes	0.0 (−1.0 to 0.9)	0.947
Treatment-steroid, yes	0.3 (−1.4 to 2.0)	0.732
Treatment-chloroquine phosphate, yes	−0.4 (−0.9 to 0.1)	0.140
Treatment-interferon, yes	0.3 (−0.5 to 1.1)	0.404
Treatment-TCM**, yes	−0.5 (−0.9 to −0.1)	0.013*
Hepatic dysfunction[††], yes	1.1 (0.4 to 1.8)	0.001*
Re-positive, yes	−0.3 (−0.9 to 0.2)	0.214
Ct N gene (NP)[§§]	0.4 (−0.1 to 1.0)	0.137

* $P<0.05$.
[†] Underlying diseases include any underlying disease that the infection reported at first time admission.
[§] The highest body temperature during the infections' overall hospitalization time.
[¶] $P<0.001$.
** TCM denotes for traditional Chinese Medicine.
[††] Hepatic dysfunction is defined as increasing both ALT, Alanine aminotransferase (U/L) and AST, Aspartate aminotransferase (U/L) of the normal reference values for general population from Ditan Hospital. Reference range: ALT, 5−40 U/L; AST, 8−40 U/L.
[§§] The lowest Ct value (i.e., the highest viral load) for each person.



SUPPLEMENTARY FIGURE S1. Blood draw sampling time distribution of neutralizing antibodies (NAb) among all infections (N=349). (A) Blood draw sampling time distribution of neutralizing antibodies among re-positives. (B) Blood draw sampling time distribution of neutralizing antibodies among non-re-positives.
Notes: Re-positives were sorted by re-positive date, and non-re-positives were sorted by their discharged date. Follow-up time is defined as the range of day 0 (disease onset day or first-time tested positive for asymptomatic infections) and last sampling day of each infection.



SUPPLEMENTARY FIGURE S2. Nucleic acid tests sampling time distribution among infections (n=353). (A) Nucleic acid test sampling time distribution among re-positive infections. (B) Nucleic acid test sampling time distribution among non-re-positive infections.

Notes: Re-positives were sorted by re-positive date, and non-re-positives were sorted by discharged date. Follow-up time is defined as the range of day 0 (disease onset day or first-time tested positive for asymptomatic infections) and the day of discharge of each infection.