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.

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Brief According to the current discharge criteria, approximately 20% of patients will become recurrently positive. However, most of such patients could be detected by an additional RT-PCR test. These data will provide a reference to reevaluate the current criteria for discharge.

Abstract

The current discharge criteria for COVID-19 require that patients have two consecutive negative results for RT-PCR detection. Here, we observed that recurrently positive RT-PCR test results in patients with three consecutive negative results (3xNegRPos, 5.4%) were significantly decreased compared with those in patients with two consecutive negative results (2xNegRPos, 20.6%); such patients reported positive RT-PCR test results within 1 to 12 days after meeting the discharge criteria. These results confirmed that many recovered patients could show a positive RT-PCR test result, and most of these patients could be identified by an additional RT-PCR test prior to discharge.

Background

Since December 2019, an outbreak of coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 virus has been reported in Wuhan, Hubei, China[1,2]. SARS-CoV-2 is highly contagious humans, and it has affected more than 100 countries worldwide, resulting in an international pandemic [3]. As of 30 March 2020, 638,146 cases and 30,039 fatalities have been reported globally [4].

To date, the Chinese clinical guidance for COVID-19 pneumonia diagnosis and treatment has been updated and is now in the 7th edition[5]. Between the 3rd and 7th editions of the guidelines, the discharge criteria remain unaltered, and all the following conditions had to be met: the patient has a normal temperature, resolved respiratory symptoms and improved CT images, and RT-PCR tests reveal two consecutively negative detection results for SARS-CoV-2 in respiratory tract specimens within an interval of at least 24 h[5-7]. The core criterion according to the guidelines is whether the RT-PCR results become negative.

The Guangdong Provincial Centers for Disease Control and Prevention reported that approximately 14% of discharged patients had positive RT-PCR test results after hospital discharge. Besides, Lan found that four recovered COVID-19 patients had positive RT-PCR test results within 5 to 13 days of leaving the hospital [8]. However, there has been no large-scale and multicenter study of this phenomenon. Does the evidence support the discharge of COVID-19 patients after two consecutively negative RT-PCR detections of SARS-CoV-2? Could more recurrently positive cases be detected after increasing the number of tests for patients who meet discharge criteria? Do the clinical manifestations of recurrently positive patients display a tendency toward aggravation? When does recurrence occur? Which factor(s) increase the recurrence rate? Is there an association between viral antibodies and recurrence?

In this study, 257 inpatients with confirmed COVID-19 from multiple medical institutions in Wuhan, China, were reviewed retrospectively. After meeting the discharge criteria, all patients who were not discharged at the time underwent at least one additional RT-PCR detection. We analyzed the differences in the recurrence rates, medical conditions, clinical symptoms, therapeutic schedules and serum-specific antibodies among these patients.

Methods

Study design

This retrospective cohort study included 257 inpatients with confirmed COVID-19 who were admitted from January 1 to March 10, 2020 at Renmin Hospital of Wuhan University, Tongren Hospital of Wuhan University, and the Central Theater General Hospital of the Chinese People's Liberation Army. All patients met the discharge criteria[5] and underwent at least one additional nucleic acid detection before hospital discharge. All data was deidentified to protect patient privacy. The experimental procedures used in this study were approved by all three hospitals (License No.: WDRY2020-K110; KY2020-032; [2020]026-1).

Data collection

The records for patients diagnosed with COVID-19 were screened in an electronic case system by physicians with extensive clinical experience. The information we recorded included medical histories, symptoms and signs, laboratory results, and the main therapeutic strategies used.

RT-PCR test

RT-PCR tests were performed on throat swabs using 2019-nCoV test kits (Wondfo, China). The molecular technique mainly focused on the detection of the open reading frame lab (ORFlab) and nucleocapsid protein (N) regions of the SARS-CoV-2 genome, and the CT value was determined according to the manufacturer's instructions. Additionally, any suspicious results resulted in retesting.

Serologic detection

The expression of IgM and IgG antiviral antibodies in serum samples was detected by an automatic chemiluminescence immunoassay technique according to the manufacturer's instructions. This detection system was developed based on the RBD of the recombinant S polypeptide (rS) of SARS-CoV2 and recommended by National Health Commission of China. The specificity and reliability of this kit have been confirmed by Spicuzza and Li [9,10].The analyzer automatically calculated the levels of IgM or IgG (AU/ml) against SARS-CoV-2 according to the RLU and the built-in calibration curve. A result of >10.0 AU/ml was considered positive, while a result of < 10.0 AU/ml was considered negative.

Statistical analysis

Continuous and categorical variables are presented as the mean/median and frequency/percentage, respectively. The T test was used for intergroup comparisons among groups with normal distributions, and the Mann-Whitney U test was used for intergroup comparisons among groups with skewed distributions. The Pearson χ^2 test or Fisher's exact test was performed for the comparison of enumerated data, and the Wilcoxon rank sum test

was used for the comparison of ranked data. Statistical analysis was performed using SPSS software V.20.0, and a bilateral p value < 0.05 was considered statistically significant.

Results

The comparison of the recurrently positive rate

A total of 257 patients with confirmed COVID-19 were included in this study. Among the 257 cases with 2 consecutive negative detections, there were 204 cases without recurrence (2xNegRNeg) and 53 cases (20.6%) with recurrence of positive results (2xNegRPos) (Fig 1a).

Among 37 cases with 3 consecutive negative detections, there were 35 cases without recurrence (3xNegRNeg) and 2 cases (5.4%) with recurrence of positive results (3xNegRPos). Among the 5 cases with 4 consecutive negative detections, there were 5 cases without recurrence (4xNegRNeg) and no cases with recurrence of positive results (4xNegRPos) (Fig 1a).

The frequency of recurrently positive RT-PCR results was significantly lower in those with 3 consecutive negative results (5.4%) than in those with only 2 consecutive negative results (20.6%) (χ^2=4.925, p=0.026<0.05). Thus, we suppose that it is more effective for the detection of recurrently positive patients that an additional RT-PCR test be administered under the current discharge criteria. Because there were so few cases, a statistical analysis was not carried out to compare the frequency of a recurrent positive RT-PCR result in those with 4 consecutive negative results compared to those with 3 or 2 negative results.

The comparison of medical conditions

The median age of the included patients with the recurrence of positive RT-PCR results in the 2xNegRPos group was 60.37 years and ranged from 22 years to 98 years, and males (n=111) comprised 55.4% of the patients, while females (n=91) comprised 44.6%. The median age of the patients in the 2xNegRNeg group was 62.19 years and ranged from 29 years to 87 years, and males (n=23) comprised 43.4% of the patients, while females (n=30) comprised 56.6%. No significant difference in age or gender was found between the 2xNegRPos group and the 2xNegRNeg group (Fig 1b, $p > 0.05$).

In addition, the overall disease duration of patients also showed no significant difference between the 2xNegRPos group (27.68 ± 0.60 days) and the 2xNegRNeg group (25.32 ± 1.17 days) (Fig 1b, $p > 0.05$). Hypertension, diabetes and coronary heart disease were the most common underlying diseases, followed by other respiratory diseases, cerebrovascular disease, and malignant tumors (Fig 1b, $p > 0.05$).

The comparison of clinical symptoms

The main clinical symptoms in COVID-19 patients were fever (37.3℃-38℃), cough (mostly dry cough), and fatigue. Other symptoms included dyspnea, diarrhea, chest pain, myalgia, and headache. There was no significant difference in symptoms between the 2xNegRPos group and the 2xNegRNeg group (Fig 1b, $p > 0.05$).

In addition, the patients in the 2xNegRPos group reported positive RT-PCR test results again within 1 to 12 days after meeting the discharge criteria, with a median of 3 days and an average of 4.6 days (Fig 2a). Of these 53 patients, 2 patients developed clinical symptoms;

one of the patients developed a cough, while the other patient had diarrhea. The rest of the patients with a recurrently positive result showed no clinical symptoms.

The comparison of the therapeutic schedules

Most patients received antiviral therapy [oseltamivir, 244 (95.0%)], antibiotic treatment [moxifloxacin, levofloxacin, cefaclor, or azithromycin, 209 (81.3%)], immunomodulatory therapy [123 (47.9%)], corticosteroid treatment [1 mg/kg/d methylprednisolone, 98 (38.1%)], phlegm elimination therapy [tocilizumab, 204 (79.4%)], and oxygen therapy [197 (76.7%)]. Among them, 12 patients received noninvasive continuous positive airway pressure. There was no significant difference between patients in the 2xNegRPos group and those in the 2xNegRNeg group (Fig 2b, p >0.05). These findings suggest that a recurrently positive result is not associated with differences in therapeutic schedules.

The comparison of serum-specific antibodies

Serum-specific IgG and IgM antibodies against SARS-CoV2 were detected in 150 of 257 COVID-19 patients. There was no significant difference in the detection of serum-specific IgG and IgM antibodies between patients with recurrence of a positive RT-PCR result (36 cases, 2xNegRPos group) and those without recurrence (114 cases, 2xNegRNeg group) (Fig 2c, p >0.05). These results suggest that a recurrently positive result is not associated with the expression of serum-specific antibodies.

Discussion

Of the 257 patients included in this study, there were 53 patients (20.6%) with recurrence of positive SARS-CoV-2 RNA detection in the 2xNegRPos group, most of whom were asymptomatic patients. This phenomenon prompts us to question whether increasing the number of RT-PCR tests performed before discharge can effectively identify recurrently positive cases. We found that the recurrently positive rate was reduced from 20.6% after 2 negative detections to 5.4% after 3 negative detections (approximately 4-fold), and no patient tested positive after 4 consecutive negative RT-PCR results. Therefore, our study confirmed that it may be effective to detect the potential recurrence of positive test results by increasing the number of RT-PCR tests. We speculate that the main reason for recurrence of positive results is the incomplete elimination of the SARS-CoV-2 virus in vivo, which results in subsequent virus replication. Therefore, therapeutic regimens need to be improved. More effective antiviral therapies, such as remdesivir, favipiravir, lopinavir, and ritonavir, and more effective immunomodulatory therapies may enable patients to clear the virus faster, which may reduce the frequency of recurrence[11,12].

Lan reported that four recovered COVID-19 patients had positive RT-PCR test results again within 5 to 13 days of leaving the hospital [8]. We found that 53 patients in the 2xNegRPos group reported positive RT-PCR test results again within 1 to 12 days of discharge, and 2 of these patients developed clinical symptoms. The median time and mean time from meeting the discharge criteria to relapse were 3 days and 4.6 days, respectively. Mild and asymptomatic patients are potential sources of SARS-CoV-2 infection [13,14]. These results verify the importance and necessity of keeping patients who have recovered from COVID-19 in isolation for 14 days after hospital discharge according to the current discharge criteria [5-7]. Such a measure could ensure that potential recurrently positive patients are detected and

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treated in a timely fashion and that further infection of others is prevented. Additionally, it is recommended to provide access to medical staff and necessary medications to patients in isolation to address the discomfort of such patients in a timely manner.

SARS-CoV-2 was detected in stool samples of COVID-19 patients with abdominal symptoms [15]. Although there has been no clinical confirmation of fecal-oral transmission, rapid human-to-human transmission between close contacts is an important feature of COVID-19. We believe that it is also necessary to treat household waste at the isolation point as if it were in an infectious disease hospital.

The detection of SARS-CoV-2-specific IgM and IgG antibodies in serum was added to the Chinese clinical guidelines for COVID-19 pneumonia diagnosis and treatment (7th edition)[5]. In this study, it was indicated that whether a recovered patient tested positive again was not associated with the presence of IgG and IgM antibodies.

To date, this study is the largest-scale study involving multiple medical institutions that has investigated the issue of recurrently positive RT-PCR tests in patients who have recovered from COVID-19. According to the current discharge criteria, approximately 20% of patients will become recurrently positive. However, most of such patients could be detected by an additional RT-PCR test. These data will provide a reference for public health officials to reevaluate the current criteria for hospital discharge and establish more reasonable, evidence-based policies for COVID-19 prevention and treatment.

Notes

Potential conflicts of interest

We declare no competing interests.

Contributions

YZ, BRW, and SMC designed the experiments. YZ, BRW, LS, YGK, LJS, GTL collected data. YZ and BRW analysed data. YZ, SX, and SMC interpreted the results and wrote the manuscript.

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Legend

Fig 1. The comparison of the recurrently positive test rate and clinical characteristics. a. The frequency of a recurrent positive RT-PCR test was significantly lower in those with 3 consecutive negative results than in those with only 2 negative results (p<0.05). b. No significant difference in medical conditions and clinical symptoms was found between the 2xNegRPos group and the 2xNegRNeg group.

Fig 2. Comparison of the results of RT-PCR, use of treatments and the presence of specific antibodies. a. The length of time between discharge and the detection of a recurrent positive result. 0: time at which the discharge criteria were met; green: symptom onset; red: positive RT-PCR result; blue: negative RT-PCR result. b. There was no significant difference between patients in the 2xNegRPos group and those in the 2xNegRNeg group. c. There was no significant difference in serum-specific IgG and IgM antibodies between the 2xNegRPos group and the 2xNegRNeg group.

Figure 1

a

The comparison of the recurrently positive rate

|	Totle	Negative	Positive
RT-PCR test results after 2 negative detections (2N)	257	204(79.4%)	53(20.6%)
RT-PCR test results after 3 negative detections (3N)	37	35(94.6%)	2(5.4%)[*]
RT-PCR test results after 4 negative detections (4N)	5	5(100%)	0

*2N Vs 3N, p=0.026<0.05

b

The medical conditions and clinical symptoms of patients with 2xNegRPos or 2xNegRNeg

Clinical characteristics	Groups		$t/\chi^2/Z$ value	P value
	2xNegRNeg group (n=204）	2xNegRPos group (n=53)		
Age, years	Mean (60.37); Range (22-98)	Mean (62.19); Range (29-87)	0.827	0.409
Sex, No. (%)				
Male	111(54.4)	23(43.4)	2.046	0.153
Female	93(45.6)	30(56.6)		
Comorbidity, No. (%)				
Hypertension	53(26.0)	18(34.0)	1.341	0.247
Diabetes	23(11.3)	6(11.3)	0.001	0.992
Coronary heart disease	8(3.9)	2(3.7)	0.001	1.000
Other respiratory diseases	7(3.4)	0(0.0)	—	0.351
Cerebrovascular disease	3(1.5)	2(3.8)	—	0.275
Malignant tumor	4(2.0)	0(0.0)	—	0.584
Total basic disease	91(44.6)	29(54.7)	1.727	0.189
Symptom and signs, No. (%)				
Fever	144(70.6)	42(79.2)	1.577	0.209
Fatigue	32(15.7)	8(15.1)	0.011	0.916
Cough	88(43.1)	19(35.8)	0.920	0.338
Myalgia	2(1.0)	0(0.0)	—	1.000
Anorexia	0(0.0)	0(0.0)	—	—
Dyspnoea	11(5.4)	4(7.5)	0.072	0.789
Expectoration	8(3.9)	4(7.5)	0.561	0.454
Sore throat	4(2.0)	1(1.9)	0.001	1.000
Diarrhea	7(3.4)	2(3.8)	0.001	1.000
Nausea	1(0.5)	0(0.0)	—	1.000
Dizziness	3(1.5)	0(0.0)	—	1.000
Headache	3(1.5)	2(3.8)	0.274	0.601
Vomiting	0(0.0)	0(0.0)	—	—
Abdominal pain	0(0.0)	0(0.0)	—	—
Classification, No. (%)				
Mild type	2(1.0)	0(0.0)		
General type	144(70.6)	36(67.2)	0.563	0.574
Severe type	56(27.5)	15(28.3)		
Critical type	2(1.0)	2(3.8)		
Duration from symptom onset to hospital admission, days	Mean (27.68); Range (4-58)	Mean (25.32); Range (5-39)	1.782	0.076

Figure 2

a
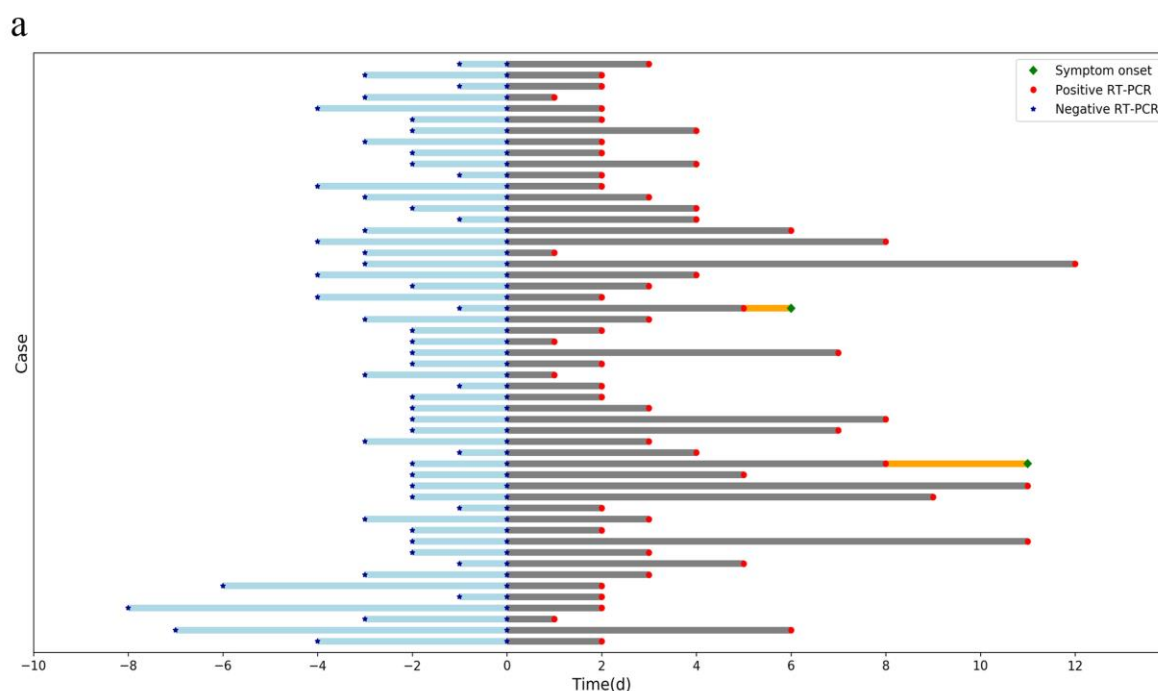
b

The main treatments used in patients with 2xNegRPos or 2xNegRNeg

Therapeutic schedules	Groups			χ2 value	P value
	Total (n=257）	2xNegRNeg group (n=204）	2xNegRPos group (n=53）		
Antiviral therapy	244(95.0)	193(94.6)	51(96.2)	0.016	0.899
Antibiotic treatment	209(81.3)	170(83.3)	39(73.6)	2.632	0.105
Corticosteroid treatment	98(38.1)	76(37.3)	22(41.5)	0.323	0.570
Immunomodulatory therapy	123(47.9)	96(47.1)	27(50.9)	0.254	0.614
Phlegm elimination therapy	204(79.4)	85(41.7)	19(35.8)	0.591	0.442
Oxygen therapy	197(76.7)	159(77.9)	38(71.7)	0.916	0.338
Noninvasive ventilation	12(4.7)	10(4.9)	2(3.8)	0.001	1.000
Invasive mechanical ventilation	0(0.0)	0(0.0)	0(0.0)	—	—
ECMO	0(0.0)	0(0.0)	0(0.0)	—	—

c

The serum specific IgG and IgM antibodies of patients with 2xNegRPos or 2xNegRNeg

Serum specific antibodies	Groups		χ2 value	P value
	2xNegRNeg group (n=114）	2xNegRPos group (n=36）		
IgG, No. (%)				
Negative	17(14.9)	2(5.6)	1.402	0.236
Positive	97(85.1)	34(94.4)		
IgM, No. (%)				
Negative	47(41.2)	17(47.2)	0.402	0.526
Positive	67(58.8)	19(52.8)		