Twelve out of 117 recovered COVID-19 patients retest positive in a single-center study of China

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ARTICLE INFO

ABSTRACT

Background: It has been reported that a fraction of recovered coronavirus disease 2019(COVID-19) patients have retested positive for SARS-CoV-2. Clinical characteristics and risk factors for retesting positive have not been studied extensively.

Methods: In this retrospective, single-center cohort study, we included adult patients ($\geq$ 18 years old) diagnosed as COVID-19 in Affiliated Yueqing Hospital, Wenzhou Medical University, Zhejiang, China. All the patients were discharged before March 31, 2020, and were re-tested for SARS-CoV-2 RNA by real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) after meeting the discharge criteria. We retrospectively analyzed this cohort of 117 discharged patients and analyzed the differences between retest positive and negative patients in terms of demographics, clinical characteristics, laboratory findings, chest computed tomography (CT) features and treatment procedures.

Findings: Compared with the negative group, the positive group had a higher proportion of patients with comorbidities (Odds Ratio(OR) =2·12, 95% Confidence Interval(CI) 0·48−9·46; $p = 0.029$), longer hospital stay (OR=1·21, 95% CI 1·07−1·36; $p = 0.008$), a higher proportion of patients with lymphocytopenia ($p = 0.036$), a higher proportion of antibiotics treatment ($p = 0.008$) and glucocorticoids treatment ($p = 0.003$). Multivariable regression showed increasing odds of positive SARS-CoV-2 retest after discharge associated with longer hospital stay (OR=1·22, 95% CI 1·08−1·38; $p = 0.001$), and lymphocytopenia (OR=7·74, 95% CI 1·70−35·21; $p = 0.008$) on admission.

Interpretation: Patients with COVID-19 who met discharge criteria could still test positive for SARS-CoV-2 RNA. Longer hospital stay and lymphopenia could be potential risk factors for positive SARS-CoV-2 retest in COVID-19 patients after hospital discharge.

Funding: Natural Science Foundation of Zhejiang Province, Medical Scientific Research Fund of Zhejiang Province, Wenzhou science and technology project

1. Introduction

Since December 2019, several cases of pneumonia of unknown etiology had been reported. [1,2] On January 7, a novel coronavirus was identified by Chinese scientists, SARS-CoV-2 (previously known as 2019-nCoV). [3] On February 11, 2020, WHO officially named this pneumonia coronavirus disease 2019 (COVID-19). [4]

Effective control measures, standardized diagnosis and treatment gradually brought the epidemic under control in China, the number of discharged patients had increased. However, it was found in clinical practice that some patients re-tested positive of SARS-CoV-2 after meeting discharge criteria. [5] Lan et al. found 4 COVID-19 patients who met discharge criteria after treatment in Zhongnan Hospital of Wuhan University, during follow-up 5−13 days after being discharged, tested positive for nucleic acid again [6]. All 4 patients were asymptomatic, with no changes compared to previous chest computed tomography (CT) examination, and no history of exposure to persons with respiratory symptoms, suspected or confirmed cases

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Research in context

Evidence before this study

We searched PubMed and Google Scholar on May 18, 2020, for articles describing the features of SARS-CoV-2 positive patients after discharge, using the search terms "COVID-19" or "SARS-CoV-2" and "positive" and "discharge". We also searched the China National Knowledge Infrastructure database and Wanfang Data using the same terms in Chinese. We found the article about positive SARS-CoV-2 after hospital discharged are mainly case reports. We identified that comparisons of epidemiology, clinical symptoms, laboratory results, and treatment between COVID-19 patients tested positive and negative for nucleic acid after meeting the discharge standards were not reported.

Added value of this study

We retrospectively analyzed this cohort of 117 discharged patients and analyze the differences in demographics and clinical characteristics, laboratory findings and chest computed tomography (CT) features and treatment between COVID-19 patients tested positive and negative for nucleic acid after meeting the discharge standards. Of all the 117 patients, nearly one third of them had at least one or more comorbidities, among which the most common were hypertension (14·5%) and diabetes (11·1%). It was significantly higher in positive group (58·3%) than that in negative group (27·6%) (p = 0·029). Similarly, the positive group had a higher proportion of patients longer hospital stay (OR=1·21, p = 0·008), a higher proportion of patients with lymphocytopenia (p = 0·036), a higher proportion of antibiotics treatment (p = 0·008) and glucocorticoids treatment (p = 0·003) compared with the negative group. Multivariable regression showed that longer hospital stay (OR = 1·22, 95% CI 1·08−1·38; p = 0·001) and lymphocyte count less than 0·8 $\times$ 10^9/L on admission (OR = 7·74, 95% CI 1·70−35.21; p = 0.008) were associated with increased odds of positive SARS-CoV-2 retest after discharge.

Implications of all the available evidence

Patients with COVID-19 may still retest positive for SARS-CoV-2 RNA even after meeting discharge criteria. Longer hospital stay or lymphocytopenia may be risk factors for COVID-19 patients to retest SARS-CoV-2 RNA positive after discharge from hospital. It should be noted that there is no evidence for infectiousness of the patients who retest positive, however, isolation and active RT-PCR retest are still recommended for discharged patients.

during home isolation. [6,7] At present, the mechanism that causing COVID-19 patients positive result for SARS-CoV-2 nucleic acid test after discharge is still unclear, and there are few relevant reports. Our purpose is to compare and analyze the differences in demographics and clinical characteristics, laboratory findings and chest CT features and treatment between COVID-19 patients tested positive and negative for nucleic acid after meeting the discharge standards, to explore the possible risk factors of positive SARS-CoV-2 retest in COVID-19 patients after discharge.

2. Methods

2.1. Study design and participants

It's a retrospective, single-center cohort study of 117 patients with COVID-19. All the patients were hospitalized in the isolation ward from January 2020 to March 2020 and followed up in the outpatient department of Affiliated Yueqing Hospital of Wenzhou Medical University after discharge. Affiliated Yueqing Hospital of Wenzhou Medical University, a tertiary university hospital with 1100 beds, located in Wenzhou, Zhejiang Province, China, the endemic areas of COVID-19. It is responsible for the treatments for COVID-19 assigned by the government. Inclusion criteria were age 18 years or older and hospital admission with a diagnosis of COVID-19 according to World Health Organization interim guidance. [8] Exclusion criteria were pregnant or lactating women, uncontrolled diseases of the blood and cardiovascular system, liver or kidney, history of mental disorders, drug abuse or dependence. As of March 31, 2020, a total of 117 patients who were hospitalized as confirmed of COVID-19 in Affiliated Yueqing Hospital of Wenzhou Medical University were discharged after meeting the hospital discharge criteria and retested real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) in the outpatient department within 1−2 weeks, of which 12 (10·3%) were found positive for SARS-CoV-2 RNA and 105 (89·7%) were found negative (Fig. 1).

Our study was approved by the Ethics Committee of Affiliated Yueqing Hospital of Wenzhou Medical University (YQYY202000002), along with a waiver of informed consent. This study was obtained from patients involved before enrollment when data were collected retrospectively.

2.2. Procedures

All the patients with COVID-19 selected in this study need to meet the diagnostic criteria according to the World Health Organization interim guidance. [8] Laboratory confirmation of SARS-CoV-2 was done by real-time RT-PCR of sputum, throat-swab specimens or feces collected from patients. For the patients with expectoration, sputum test was carried out, while for the patients without expectoration, throat swab specimens and feces were taken. Among the 12 patients retested positive for SARS-CoV-2, 8 were performed with sputum specimens, and the other 4 were performed with feces. Laboratory confirmation by real-time RT-PCR was done in two different institutions: the Chinese CDC laboratory and in Affiliated Yueqing Hospital of Wenzhou Medical University. RT-PCR detection reagents were provided by Shanghai BioGerm Medical Biotechnology Co., Ltd. The
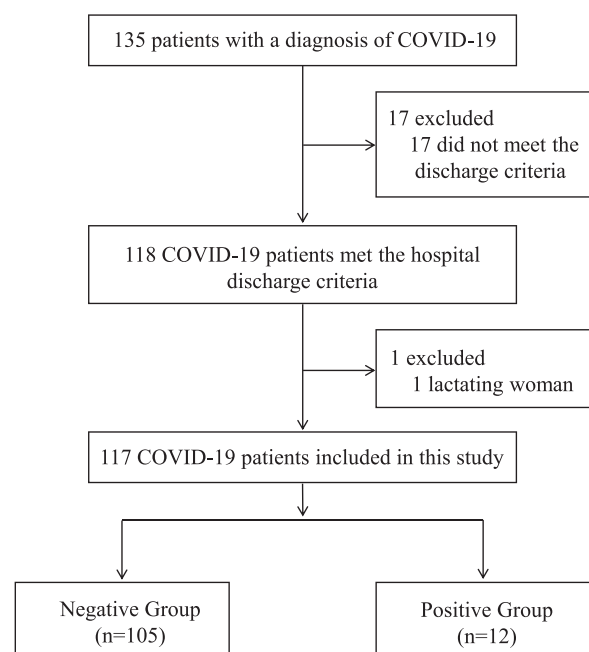

Fig. 1. Study flow diagram.

primers sequences were as follows: forward primer 5′-CCCTGTGGGTTTTACACTTAA-3′; reverse primer 5′- ACGATTGTGCAT-CAGCTGA-3′. Conditions for the amplifications were 50 °C for 15 min, 95 °C for 3 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 30 s. Other respiratory viruses were all be excluded, including influenza A virus (H1N1, H3N2, H7N9), influenza B virus, respiratory syncytial virus, parainfluenza virus, adenovirus, SARS coronavirus, and MERS coronavirus. The selected patients need to complete the examination when they are on admission, including complete blood count, procalcitonin, hypersensitive C-reactive protein, T lymphocyte subsets, coagulation profile, serum biochemical tests (including liver and renal function, creatine kinase and lactate dehydrogenase), myocardial enzymes, arterial blood gas analysis and chest CT scan. COVID-19 patients who met the discharge criteria were isolated after discharge and asked to be reviewed by RT-PCR in Affiliated Yueqing Hospital of Wenzhou Medical University within 1−2 weeks. Patients with positive RT-PCR were divided into positive group and patients with negative RT-PCR were divided into negative group.

2.3. Data collection

We collected demographic, epidemiological, clinical, laboratory, radiological characteristics, and treatments data from electronic medical records database of Affiliated Yueqing Hospital of Wenzhou Medical University. Clinical data were followed from January 2020 to March 2020. Three researchers (HY, CZ and LY) reviewed the data collection forms to double check the data collected independently.

2.4. Definitions

The exposure history was defined as the exposure with a confirmed COVID-19 infection or to Wuhan and surrounding areas within 14 days before onset or had been in contact with people from Wuhan or surrounding areas within 14 days before onset. Fever was defined as ear temperature of at least 37.3 °C. Lymphocytopenia was defined as the lymphocyte absolute value count $< 0.8 \times 10^9$/L. All the following criteria had to be met for hospital discharge: (1) the patient's body temperature returned to normal for more than 3 days, (2) respiratory symptoms significantly improved, (3) pulmonary imaging shows obvious absorption of inflammation, (4) two consecutive respiratory tract pathogen test negative for RT-PCR(sampling interval ≥ 24 h). [9]

2.5. Statistical analysis

Statistical analyses were performed using IBM SPSS (Statistical Package for the Social Sciences) Statistics Software (version 23.0; IBM, New York, USA). The measurement data were tested for normality and homogeneity of variance. Continuous variable of normal distribution was presented as mean±standard deviation (minimum-maximum) and compared by unpaired two-tailed Student's t -test. Continuous variable of skewed distribution was expressed as medians (interquartile ranges) and compared by Mann-Whitney U test. Categorical variables were expressed as number (%) and compared by χ^2 test or Fisher's exact test between positive group and negative group. To explore the risk factors of positive SARS-CoV-2 retest, univariable and multivariable logistic regression models were used. We excluded variables from the univariable analysis if their between-group differences were not significant. $p < 0.05$ was considered statistically significant.

2.6. Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors (XH, YD and JL) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

The mean age of 117 patients was 48.2 ± 13.5 years, ranging from 18 to 93 years, 65 (55.6%) patients were males and 10 (9.3%) patients had a current smoking history (Table 1). Of all the 117 patients, nearly one third of them had at least one or more comorbidities, among which the most common were hypertension (14.5%) and diabetes (11.1%). It was significantly higher in positive group (58.3%) than that in negative group(27.6%) (p = 0.029). A history of contacting (direct or indirect) with people from Wuhan was documented in 98/117 (83.8%) patients. The median length of hospital stay for all patients was 15 days (interquartile range (IQR) 12.0−18.0). The median length of hospital stay in the positive group was 19 days (IQR 14.3−27), and the median length of stay in the negative group was 14.0 days (IQR 12.0−17.5), the median length of hospital stay in the positive group was significantly longer than the negative group (p = 0.008) (Table 1). The most common symptoms were cough (95 [81.2%] of 117 patients) and expectoration (78 [66.7%]), followed by fever (43 [36.8%]), abdominal pain or diarrhea (26 [22.2%]), and fatigue (21 [17.9%]). Less common symptoms were myalgia (10 [8.5%]), headache (9 [7.7%]), stuffy or runny nose (5 [4.3%]), and dyspnea (3 [2.6%]). There was no statistical difference in clinical symptoms between positive group and negative group ($p > 0.05$) (Table 2).

All the 117 patients were sampled for blood cell counting on admission.The mean of white blood cell, neutrophil and lymphocyte

Table 1
Demographics and baseline characteristics of COVID-19 patients.

Demographics and baseline characteristics	All patients(n = 117)	Positive group (n = 12)	Negative group (n = 105)	p value
Age, years	48.2 ± 13.5(18.0−93.0)	52.3 ± 14.4(35.0−76.0)	47.7 ± 13.4(18.0−93.0)	0.273
Sex	..	..	..	0.683
Male	65(55.6%)	6(50.0%)	59(56.2%)	..
Female	52(44.4%)	6(50.0%)	46(43.8%)	..
Current smokers	10(9.3%)	3(25.0%)	7(6.7%)	0.108
Comorbidity	36(30.8%)	7(58.3%)	29(27.6%)	0.029
Hypertension	17(14.5%)	1(8.3%)	16(15.2%)	0.833
Diabetes	13(11.1%)	0	13(12.4%)	0.356
Cardiovascular disease	6(5.1%)	0	6(5.7%)	1.000
Nervous system diseases	5(4.3%)	1(8.3%)	4(3.8%)	0.424
Carcinoma	2(1.7%)	0	2(1.9%)	1.000
Others	10(8.5%)	1(8.3%)	9(8.6%)	1.000
Exposure history	98(83.8%)	12(100.0%)	8(81.9%)	0.211
Hospital stay, days	15.0(12.0−18.0)	19.0(14.3−27.0)	14.0(12.0−17.5)	0.008

Data are mean±standard deviation (minimum-maximum), median (IQR) or n (%). p values comparing positive group and negative group are from unpaired two-tailed Student's t -test, Mann-Whitney U test, χ^2or Fisher's exact test.

Table 2
Symptoms or signs of COVID-19 patients.

Symptoms or signs	All patients(n = 117)	Positive group (n = 12)	Negative group (n = 105)	p value
Fever (temperature $\geq$37·3 °C)	43(36·8%)	6(50·0%)	37(35·2%)	0·315
Cough	95(81·2%)	10(83·3%)	85(81·0%)	1·000
Sputum	78(66·7%)	8(66·7%)	71(67·6%)	1·000
Stuffy or runny nose	5(4·3%)	0	5(4·8%)	1·000
Sore throat	19(16·2%)	3(25·0%)	16(15·2%)	0·649
Fatigue	21(17·9%)	3(25·0%)	18(17·1%)	0·783
Myalgia	10(8·5%)	2(16·7%)	8(17·1%)	0·605
Headache	9(7·7%)	0	9(8·6%)	0·595
Nausea or vomiting	15(12·8%)	3(25·0%)	12(11·4%)	0·381
Abdominal pain or diarrhea	26(22·2%)	3(25·0%)	23(21·9%)	1·000
Dyspnea	3(2·6%)	0	3(2·9%)	1·000

Data are n (%), p values comparing positive group and negative group are from χ^2, Fisher's exact test.

were $4\cdot8 \pm 1\cdot5 \times 10^9$/L, $2\cdot97\pm1\cdot15 \times 10^9$/L and $1\cdot3 \pm 0\cdot5 \times 10^9$/L in positive group, $5\cdot1 \pm 1\cdot8 \times 10^9$/L, $3\cdot1 \pm 1\cdot5 \times 10^9$/L and $1\cdot5 \pm 0\cdot6 \times 10^9$/L in negative group, respectively. Among 117 patients, 13(11·1%) showed lymphocytopenia (lymphocyte count< $0\cdot8 \times 10^9$/L), including 4 (33·3%) of 12 in positive group, 9(8·6%) of 105 in negative group and the proportion of patients with lymphocytopenia in the positive group was significantly higher than that in the negative group (p = 0·036) (Table 3). The mean CD4$^+$T count of 117 patients was $564\cdot4 \pm 241\cdot2 \times 10^6$/L, ranging from 106·0 to $1257\cdot0 \times 10^6$/L and the mean CD8$^+$T count was $378\cdot6 \pm 177\cdot7 \times 10^6$/ L, ranging from 77·0 to $945\cdot0 \times 10^6$/L. Nearly half of the patients had CD4$^+$T lymphocyte counts of less than 550×10^6/L, and 49 (41·9%) patients had CD8$^+$T lymphocyte counts of less than 320×10^6/L. The median values of procalcitonin and hypersensitive c-reactive proteins were 0·3 ng/mL (IQR 0·3−0·3) and 6·3 mg/L (IQR 5·0−24·3). The median prothrombin time and activated partial thromboplastin time were 13·0 s (IQR 12·6−13·4) and 37·3 s (IQR 35·2−41·35). The median D-dimer was 0·4 ng/mL (IQR 0·3−0·7). In blood biochemical examination results, the median alanine aminotransferase, aspartate aminotransferase and creatinine were 22·0 U/L (IQR 15·0−31·0), 25·0 U/L (IQR 19·0−33·5) and 66·1 U /L (IQR 39·0−124·0). All patients

were sampled for arterial blood gas analysis and it showed the mean of pH, partial pressure oxygen, partial pressure of carbon dioxide and blood lactic acid were 7·4 ± 0·03 (7·4−7·5), 111·0 ± 27·3 mmHg (74·5−166·0), 35·9 ± 3·7 mmHg (28·7−40·5) and 2·1 ± 0·6 mmol/L (1·0−3·4) in positive group, 7·4 ± 0·04 (7·3−7·5), 99·7 ± 24·9 mmHg (99·0−194·0), 36·4 ± 4·3 mmHg (26·8−54·8) and 1·8 ± 0·7 mmol/L (0·7−4·0) in negative group, respectively. All the patients underwent chest CT scans on admission, and abnormalities in chest CT images were detected among all patients. Of the 117 patients, 85 (72·6%) had bilateral involvement, including ground-glass opacity, infiltration, consolidation (Table 3).

All the patients had pneumonia. During hospitalization, all patients received antiviral therapy (including Oseltamivir, Abidor, Lopinavir ritonavir, α-interferon inhalation, and intravenous Ribavirin). Oxygen therapy, antibiotics and glucocorticoid therapy (Solu-Medrol of 40 mg/day) were initiated in 85 (72·6%), 31 (26·5%) and 11 (9·4%) during hospitalization (Table 4).

In this univariable logistic analysis, compared with the negative group, the positive group had a higher proportion of patients with comorbidities (Odds Ratio(OR) =2·12, 95% Confidence Interval(CI)

Table 3
Laboratory findings and chest radiographs of COVID-19 patients on admission to hospital.

Laboratory findings	All patients(n = 117)	Positive group (n = 12)	Negative group (n = 105)	p value
White blood cell count, $\times 10^9$/L	5·1 ± 1·7(1·0−12·3)	4·8 ± 1·5(2·5−6·9)	5·1 ± 1·8(1·0−12·3)	0·609
Neutrophil count, $\times 10^9$/L	3·1 ± 1·5(0·7−9·4)	2·97±1·15(1·4−4·6)	3·1 ± 1·5(0·7−9·4)	0·830
Lymphocyte count, $\times 10^9$/L	1·5 ± 0·6(0·4−3·9)	1·3 ± 0·5(0·6−2·2)	1·5 ± 0·6(0·4−3·9)	0·323
<0·8	13(11·1%)	4(33·3%)	9(8·6%)	0·036
CD4+ T lymphocytes count, $\times 10^6$/L	564·4 ± 241·2(106·0−1257·0)	514·3 ± 185·9(267·0−861·0)	570·2 ± 46·8(106·0−1257·0)	0·450
<550	61(52·1%)	8(66·7%)	53(50·5%)	0·448
CD8+ T lymphocytes count, $\times 10^6$/L	378·6 ± 177·7(77·0−945·0)	343·3 ± 135·1(148·0−522·0)	382·7 ± 181·9(77·0−945·0)	0·469
<320	49(41·9%)	7(58·3%)	42(40·0%)	0·223
Procalcitonin, ng/mL	0·3(0·3−0·3)	0·3(0·3−0·3)	0·3(0·3−0·3)	0·848
Hypersensitive C-reactive protein, mg/L	6·3(5·0−24·3)	5·6(5·0−13·8)	6·5(5·0−26·3)	0·416
Prothrombin time, s	13·0(12·6−13·4)	12·8(12·5−13·1)	13·0(12·7−13·4)	0·098
Activated partial thromboplastin time, s	37·3(35·2−41·4)	35·5(32·5−37·1)	37·5(35·4−41·8)	0·061
D-dimer, mg/L	0·4(0·3−0·7)	0·3(0·2−0·4)	0·4(0·3−0·7)	0·089
Alanine aminotransferase, U/L	22·0(15·0−31·0)	22·5(16·0−40·8)	22·0(14·5−30·5)	0·716
Aspartate aminotransferase, U/L	25·0(19·0−33·5)	22·0(17·3−33·5)	25·0(19·0−33·5)	0·559
Albumin, g/L	41·3 ± 4·8(31·8−67·1)	42·5 ± 2(37·3−44·7)	41·1 ± 4·9(31·8−67·1)	0·357
Lactate dehydrogenase, U/L	225·0(186·5−290)	205·5(168·5−248·8)	226·0(186·5−296·5)	0·110
Creatine kinase, U/L	71·0(51·5−115·5)	70·5(49·3−192·0)	71·0(51·5−111·0)	0·689
Creatinine, μmol/L	66·1 ± 15·9(39·0−124·0)	67·5 ± 16·5(48·0−110·0)	65·9 ± 15·9(39·0−124·0)	0·754
Hypersensitive troponin I, pg/mL	0·01(0·1−0·2)	0·01(0·1−0·2)	0·01(0·1−0·2)	0·519
Blood urea nitrogen, mmol/L	3·6(3·1−4·5)	4·2(3·2−4·8)	3·6(3·0−4·5)	0·261
pH	7·4 ± 0·04(7·3−7·5)	7·4 ± 0·03(7·4−7·5)	7·4 ± 0·04(7·3−7·5)	0·932
PaO$_2$, mmHg	100·8 ± 25·4(74·5−194·0)	111·0 ± 27·3(74·5−166·0)	99·7 ± 24·9(99·0−194·0)	0·143
PaCO$_2$, mmHg	36·3 ± 4·2(26·8−54·8)	35·9 ± 3·7(28·7−40·5)	36·4 ± 4·3(26·8−54·8)	0·723
Lactic acid, mmol/L	1·8 ± 0·7(0·7−4·0)	2·1 ± 0·6(1·0−3·4)	1·8 ± 0·7(0·7−4·0)	0·228
Bilateral involvement of chest radiographs	85(72·6%)	9(75·0%)	76(72·4%)	1·000

Data are mean±standard deviation (minimum-maximum), median (IQR) or n (%). p values comparing positive group and negative group are from unpaired two-tailed Student's t -test, Mann-Whitney U test, χ^2 or Fisher's exact test.

Table 4
Treatments of COVID-19 patients.

Treatments	All patients(n = 117)	Positive group (n = 12)	Negative group (n = 105)	p value
Oxygen therapy	85(72·6%)	9(75·0%)	76(72·4%)	0·591
Antiviral treatment	117(100·0%)	12(100·0%)	105(100·0%)	..
Antibiotic treatment	31(26·5%)	7(58·3%)	24(22·9%)	0·008
Glucocorticoids treatment	11(9·4%)	4(33·3%)	7(6·7%)	0·003

Data are n (%), p values comparing positive group and negative group are from χ^2, Fisher's exact test.

0·48−9·46; p = 0·029), longer hospital stay (OR=1·21, 95% CI 1·07−1·36; p = 0·008), a higher proportion of patients with lymphocytopenia (OR=5·95, 95% CI 1·16−30·58; p = 0·036), a higher proportion of antibiotics treatment (OR=1·22, 95% CI 0·25−5·84; p = 0·008) and glucocorticoids treatment (OR=1·11, 95% CI 0·19−6·43; p = 0·003) (Table 5). We included 117 patients with complete data for these two significant variables in the multivariable logistic regression model and found that longer hospital stay (OR=1·22, 95% CI 1·08−1·38; p = 0·001) and lymphocyte count less than 0.8×10^9/L on admission(OR=7·74, 95% CI 1·70−35·21; p = 0·008) were associated with increased odds of positive SARS-CoV-2 retest after discharge (Table 5).

4. Discussion

This retrospective, single-center cohort study identified several risk factors that might cause SARS-CoV-2 nucleic acid to be positive again with COVID-19 patients after discharge. All the 117 COVID-19 patients reported in this paper were discharged after meeting discharge criteria and were followed up for RT-PCR retest, 12 of them were positive and 105 were negative. Compared with the negative group, the positive group had a higher proportion of comorbidities, longer hospital stays, higher proportion of patients with lymphocytopenia, higher proportion of antibiotics and glucocorticoids treatment during hospitalization. Concurrently, we also found that longer hospital stay and lymphocytopenia were associated with increased odds of positive SARS-CoV-2 retest after discharge. At present, the article about positive SARS-CoV-2 after hospital discharged are mainly case reports. Compared with previous studies, we had more patients with SARS-CoV-2 positive patients after discharge. Moreover, we compared the epidemiology, clinical symptoms, laboratory findings, treatments between positive group and negative group, and found significant differences in multiple indicators.

For COVID-19 patients, comorbidities such as hypertension and diabetes mellitus may affect prognosis. Patients with diabetes seems to have lower immune function and are more prone to pneumonia [10]. In addition, when diabetes is complicated with infection, the body is in a strong stress state, and the blood sugar of the patients becomes more difficult to control, forming a vicious circle and leading to relapse. [11] Therefore, patients with diabetes mellitus are prone to secondary bacterial infection during hospitalization, which prolongs the duration of hospitalization, and even after discharge, they are vulnerable to COVID-19 reinfection, resulting in a positive nucleic acid test. For patients with hypertension, it may be related to the use of ACEI/ARB antihypertensive drugs such as captopril [12].

Hypertension is associated with abnormal activation of the RAS system. [13] ACE2 is widely distributed in lung, kidney, heart, pancreatic islet and other tissues, and is also expressed in the lung. [14] In an animal experiment, ACEI drugs and ARB drugs were given to rats, and ACE2 mRNA in the hearts of rats in ACEI group and ARB group increased by 4·7 and 2·8 times, respectively. [15] The results of this study indicate that ACEI/ARB drugs can cause the up-regulation of ACE2 in the heart, and it is speculated that ACEI/ARB drugs can also promote the expression of ACE2 in the lungs. Wan Y used HeLa cells for COVID-19 virus infectivity research, proving that COVID-19 must use the ACE2 receptor to enter the cell, which suggested that ACE2 may be a receptor for COVID-19. [16] Therefore, COVID-19 patients with hypertension may cause increased expression of ACE2 in the lungs when they take ACEI/ARB drugs during the illness, so that more viruses can enter the lungs through ACEI receptor, and the rate of positive test after hospital discharge was higher.

Lymphocyte count is related to the severity of COVID-19, and monitoring lymphocyte count can help early detection of severity. [17] Lymphocyte count $<0.8 \times 10^9$/L is one of the risk indicators for evaluating viral pneumonia. [18] In our study, the proportion of patients with lymphocytopenia in positive group was significantly higher than that of negative group, which suggests that the immune system damage is more common in the positive group. Another study also found that there was a significant positive correlation between lymphocyte count and virus recurrence time in COVID-19 patients left the hospital [19]. In addition, we used logistic regression and found that lymphopenia is a risk factor for SARS-CoV-2 retested positively. Lymphocytes play a key role in maintaining immunologic function of the body, participate in the immune response to viral pneumonia, remove the virus effectively, and help the body resist virus infections. [20] When there is lymphocytopenia, cell homeostasis will be destroyed, leading the body more vulnerable to external invasion. [21] Therefore, lymphocytopenia may leads to the incomplete clearance of the virus by the immune system, and the higher probability of nucleic acid retest positively. A study had reported that COVID-19 patients may have high expression of interleukin-1β (IL-1β), interferon gamma (IFN-γ), IP10 and MCP1 and other inflammatory factors and decreased lymphocyte count. [22] SARS-CoV-2 may be similar to SARS and MERS which causes activation of inflammatory factors, resulting in immune impairment and thus reduced lymphocyte count, which in turn may affect the outcome of COVID-19 patients.

The use of glucocorticoid therapy in SARS and other viral pneumonia has been controversial [23,24] In this study, the proportion of glucocorticoid users in positive group was significantly higher than that

Table 5
Risk factors of COVID-19 patients with positive SARS-CoV-2 after discharge.

	Univariable OR (95% CI)	p value	Multivariable OR (95% CI)	p value
Comorbidity	2·12(0·48−9·46)	0·325	..	..
Hospital stay, days*	1·21(1·07−1·36)	0·003	1·22(1·08−1·38)	0·001
Lymphocyte count, × 109/L	..	..	..	..
<0·8	5·95(1·16−30·58)	0·033	7·74(1·70−35·21)	0·008
Antibiotic treatment	1·22(0·25−5·84)	0·808	..	..
Glucocorticoids treatment	1·11(0·19−6·43)	0·897	..	..

OR= Odds Ratio. *Per 1 unit increase.

in negative group (p = 0·003), suggesting that in the treatment of COVID-19, glucocorticoids may inhibit the immune function of the body. Some scholars hold a negative attitude towards the treatment of viral pneumonia with glucocorticoids, believing that the use of glucocorticoids in viral pneumonia may inhibit the immune function of the body, make it difficult to remove residual virus, and increase the risk of secondary infection. [25] In a retrospective study, 151 (49%) of 309 MERS patients received glucocorticoid therapy, which was found to be associated with delayed clearance of MERS virus RNA from respiratory secretions(adjusted hazard ratio, 0·35; 95% CI, 0·17−0·72; p = 0·005). [26] A meta-analysis of glucocorticoid therapy for influenza showed that influenza patients treated with corticosteroids had delayed virus clearance and increased risk of hospital acquired infection. (unadjusted odds ratio, 2·74; 95% CI, 1·51−4·95), suggesting that glucocorticoids may suppress the innate immune system and affect virus clearance, increasing the risk of infection. [27] Because of the delayed clearance of viral RNA in the glucocorticoid treatment group, glucocorticoids are not recommended in the treatment of COVID-19, especially for mild disease. [28] However, some studies have shown that glucocorticoid can inhibit inflammation and excessive proliferation of fibroblasts, so as to reduce the damage of inflammatory response to the body. [29] In this study, it was found that the proportion of patients receiving antibiotics in the positive group was 58·3%, while that in the negative group was 22·9%, which was significantly higher in the negative group (p = 0·008). It may be that the glucocorticoid use led to the suppression of immune function, so the risk of secondary bacterial infection was increased, therefore, the proportion of patients receiving antibiotics in the positive group was also significantly higher than that in the negative group.

Previous studies have confirmed that after infection with the virus, the body will stimulate humoral immunity and produce specific antibodies, the antibodies will continue to exist for a period of time after curing and play a protective role on the body, helping the body to resist the secondary invasion of the virus[30,31]. But COVID-19 as a new infectious disease, the strength and duration of the protective effect of antibodies produced by the body are still unknown, after the cured patients are exposed to the virus again, if the protective ability of antibodies is weak or the duration is too short, there is still a risk of re-infection. All the patients in this study were isolated for at least 14 days after their first discharge, and the likelihood of re-exposure was very low, therefore, for the time being, we will not consider these reasons to cause nucleic acid retest positive. So far, the SARS-COV-2 RNA tests of close contacts of retested positive patients were negative, and no suspicious clinical symptoms were reported. [32]

Hospital stay is strongly associated with patient's characteristics and clinical status. In our study, increasing odds of positive SARS-CoV-2 retest after discharge was associated with longer hospital stay. Extended hospital stay appeared to increase the risk of infection, and the risk of infection increased by 1% for every day in hospital [33]. Although there is no evidence that SARS-CoV-2 has mutated, it is worth noting that the coronavirus RNA has high replication frequency and mutation rate. [34] The specific mechanism still needs further research and observation.

Our study has several limitations. First, this is a retrospective study, so we are unable to unify the methods and parts of specimen collection before hospital discharge. In clinical practice, different methods and parts of specimen collection may lead to false negative results of PCR, so the actual number of patients carrying SARS-CoV-2 may be higher than the number of PCR-positive patients. Regarding the patients' clinical symptoms, there may also be recall bias, which may lead to inaccurate symptom analysis results between the negative and positive groups. In addition, we cannot use the relative risk (RR) but OR to describe the risk factors for positive SARS-CoV-2 retest in COVID-19 patients after hospital discharge. Second, this is a single center study and cannot fully reflect the characteristics of COVID-19

patients. Due to the differences in the control measures and medical level of COVID-19 in different regions, the number of cases, the doubling time of infectious diseases and the characteristics of cases are quite different [35,36]. Third, the statistics and p values should be interpreted with caution due to the small number of positive group patients included. Nevertheless, our study depicts the difference between SARS-CoV-2 positive group and SARS-CoV-2 negative group and find two risk factors. Our study may serve as a clinical reference in categorizing the COVID-19 patients and reduce recurrence.

In conclusion, patients with COVID-19 may still retest positive for SARS-CoV-2 RNA even after meeting discharge criteria. Longer hospital stay or lymphocytopenia may be risk factors for COVID-19 patients to retest SARS-CoV-2 RNA positive after discharge from hospital. It should be noted that there is no evidence for infectiousness of the patients who retest positive, however, isolation and active RT-PCR retest are still recommended for discharged patients.

5. Contributors

XH, YD and JL conceived and designed the study, responsible for the integrity and accuracy of the data, and had full access to the study. HY and CZ contributed to drafting and writing this paper. LY took responsibility for obtaining written consent from patients, obtaining ethical approval, collecting samples, and confirming data accuracy. XY and LZ made substantial contributions to data acquisition, analysis, and interpretation. YS, ZX, XZ, XY, and LZ collected the data. All the authors had strictly revised the manuscript and agreed to be responsible for all aspects of the work, and finally approved the version to be published.

Declaration of Interests

All authors declare no competing interests.

Data sharing

With the permission of the corresponding authors, we can provide participant data without names and identifiers, but not the study protocol, statistical analysis plan, or informed consent form. Data can be provided after the Article is published. Once the data can be made public, the research team will provide an email address for communication. The corresponding authors have the right to decide whether to share the data or not based on the research objectives and plan provided.

Funding

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