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Exploration of turn-positive RT-PCR results and factors related to treatment outcome in COVID-19: A retrospective cohort study

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ABSTRACT

The cause of some patients with negative RT-PCR results experienced turn-positive after treatment remains unclear. In addition, understanding the correlation between changes in clinical data in the course of COVID-19 and treatment outcomes is of great importance in determining the prognosis of COVID-19. To perform cause analysis of RT-PCR turn-positive and the effective screening factors related to treatment outcome in COVID-19. Clinical data, including clinical manifestations, laboratory tests, radiography results, treatment methods and outcomes, were retrospectively collected and analyzed from January to March 2020 in Renmin Hospitals of Wuhan University. 116 COVID-19 patients (40 in recurrent group, 29 in recovered group and 47 in unrecovered group) were recruited. In the recurrent group, white blood cell, Neutrophils, prothrombin time, activated partial thromboplastin time, CD3, CD4, CD8, ratio of CD4/CD8, IgG and C4 complement were of significant difference among the baseline, negative and turn-positive time points. CD19 and CT scan results were found notable difference between recurrent group and recovered group. Odds from CD3, CD4, CD8, CD19, IgM, C3 complement, C4 complement and CT scan results validated associations with clinical outcomes of COVID-19. The so-called recurrence in some COVID-19 patients may be due to the false-negative of nucleic acid test results from nasopharyngeal swabs. Levels of CD3, CD4, CD8, CD19, IgM, C3 complement, C4 complement and CT results were significantly correlated with the outcome of COVID-19. The cellular immunity test could be beneficial to further screen the reliability of RT-PCR test on the basis of CT images.

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Introduction

In the late 2019, outbreak of a novel coronavirus has induced a disease now officially called "the Corona Virus Disease 2019 (COVID-19)". The epidemic has been spreading to over 188 countries or regions. Since then, there have been a total of 10,000,000 confirmed cases with more than 500,000 deaths (updated on 30 June 2020) around the globe [1]. Due to a lack of specific antiviral treatments and vaccines, identifying and isolating as many potential patients as possible, especially for those with early-onset of symptoms, is of great importance for the prevention and control of COVID-19 [2], Real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assay is a nucleic acid detection-based approach to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which confer an advantage to rapid detection and specificity [3,4].

However, attributed to the limited sensitivity and specificity of the real-time RT-PCR test, inaccurate results are inevitable [5]. Clinical evidence had shown that RT-PCR results were consistently negative or weakly positive in five patients with COVID-19 [6]. Chen et al reported a case with recurrently positive SARS-CoV-2 ribonucleic acid (RNA) of oropharyngeal swab test, suggesting that recurrence could occur in those convalescents [7]. Another publication reported a death case whose RT-PCR results were consistently negative, in which SARS-COV-2 virus particles were found in lung biopsy [8]. Thus, the truth of recurrence is still a matter of discussion.

Several potential correlates of COVID-19 outcomes have been proposed in recent studies. For instance, older age, higher SOFA score, and D-dimer greater than 1 μg/mL at admission were associated with increased odds of death [9]. A study conducted in

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Supplemental data for this article can be accessed here.

Italy also suggested that older age and pre-admission hypertension were key mortality risk factors [10]. Nevertheless, in addition to recovery and death, continuous un-recovery might also be a potential clinical outcome of COVID-19. To date, there is a lack of research on the correlation of recovery and un-recovery. Therefore, it is necessary to probe into possible risk factors of this phenomenon.

As such, we attempt to review the details of 116 COVID-19 patients in Renmin Hospitals of Wuhan University and thus, perform cause analysis of RT-PCR turn-positive and the effective screening factors related to treatment outcome in COVID-19. It is expected that this could provide useful reference for the diagnosis and management of the disease.

Method

Study design and participants

The present retrospective study involved the clinical data of 116 COVID-19 patients from January to March 2020 in Renmin Hospitals of Wuhan University. Nasopharyngeal swab collections were used to perform SARS-CoV-2 RT-PCR test in all patients. Participants were divided into three groups according to outcomes of clinical treatment. I. recurrent group: those who turned positive of RT-PCR test again after two successive negative outcomes. II. recovered group: those who were absolutely recovered after two negative results of RT-PCR test. III. unrecovered group: those who experienced positive results continuously. In our study, COVID-19 was identified by both chest CT image and RT-PCR test, according to the Chinese management guideline for COVID-19 (version 6.0) which was established by National Health Commission of the People's Republic of China [4].

Data collection

Clinical data at three time points of baseline, negative results of RT-PCR test, turn positive results of RT-PCR test in the recurrent group were recorded. While two time points of baseline-negative or baseline-positive in recovered group and un-recovered group were registered. The clinical data, inclusive of clinical manifestations, laboratory tests, radiography results, treatment methods, and outcomes, were carefully collected from electronic medical records and analyzed by our research team. Information included laboratory examination and demographic data including blood routine (such as white blood cells, lymphocytes, erythrocyte, hemoglobin, and platelet), blood biochemistry (such as alanine aminotransferase, aspartate aminotransferase, albumin, creatinine), coagulation (such as prothrombin time, active part thrombin time, D-dimer), cellular immunity, humoral immunity, and computed tomography. Two researchers (XS, QC) checked the data independently, with discrepancies resolved by a third independent reviewer (YX). The study was carried out in accordance with the WMA Declaration of Helsinki. Informed consent was waived due to the retrospective nature of the study and analysis using anonymous clinical data (ethics approval no. WDRY 2020-k171).

Statistical analysis

Single-factor analysis of variance (ANOVA), paired sample *t*-test and repeated measures ANOVA was used to compare baseline, intra-group differences and inter-group differences. Categorical variables were expressed as percentage (%) and measured by Chi-square tests or Fisher's exact test where appropriate. The factors related to treatment outcome were analyzed by logistics regression analysis. Variables were chosen for multivariable analysis on the basis of univariable analysis (with p value under 0.10). Previous studies suggested that abnormal changes occurred in cellular and humoral immunity results [11]. Therefore associated lab findings including CD3, CD4, CD8, CD4/CD8, CD19, CD16 and CD56, IgM, IgG, C3, and C4 complements were taken into account without regard to the p value in univariable analysis.

A two-sided α of less than 0.05 was considered statistically significant. All the statistical analyzes were performed using the IBM SPSS software (version 24.0), unless otherwise indicated.

Result

The present study included a total of 116 patients. They were divided into three groups by different treatment outcomes: recurrent group (n = 40), recovered group (n = 29), and unrecovered group (n = 47). The mean age of all the patients was 43.52, and most patients were female (75%). Details of characteristics and baseline results in each group were depicted in Table 1. Continuous variables at baseline were compared among the three groups using single-factor ANOVA. No significant difference was found in almost all the continuous variables except for PT (prothrombin time) (P = 0.027) (Table 1). Fisher's exact test was performed to estimate the differences in the severity of CT images and Chi-square tests were performed to estimate the

Table 1. Characteristics and baseline of participants.

Characteristics	Recurrent Group (n = 40)	Recovered Group (n = 29)	Un-recovered Group (n = 47)	p Value
Age (years), mean ± SD	39.85 ± 14.88	42.21 ± 13.92	47.45 ± 16.95	0.07
Gender				
Male, *n* (%)	7 (17.5%)	8 (27.6%)	14 (29.8)	0.391
Female, *n* (%)	33 (82.5%)	21 (72.4)	33 (70.2)	
Complete Blood Count				
WBC, 10^9/L	5.28 ± 1.56	5.05 ± 1.97	5.33 ± 2.15	0.823
Neutrophils, 10^9/L	3.16 ± 1.41	2.96 ± 1.79	3.09 ± 1.61	0.882
Lymphocytes, 10^9/L	1.58 ± 0.62	1.56 ± 0.58	1.66 ± 0.85	0.821
RBC, 10^12/L	4.20 ± 0.49	4.32 ± 0.46	4.28 ± 0.55	0.603
HB, g/L	125.53 ± 16.20	129.19 ± 16.35	130.09 ± 15.47	0.392
PLT, 10^9/L	230.75 ± 79.92	206.16 ± 69.29	199.13 ± 64.28	0.110
Liver Function Test				
ALT, U/L	24.83 ± 23.55	22.62 ± 19.75	23.96 ± 17.38	0.905
AST, U/L	23.80 ± 12.98	23.25 ± 13.57	24.53 ± 10.08	0.886
TBIL, μmol/L	10.29 ± 7.12	9.72 ± 3.53	9.55 ± 4.83	0.814
DBIL, μmol/L	3.08 ± 1.90	3.22 ± 1.15	3.20 ± 1.70	0.925
ALB, g/L	40.52 ± 3.69	40.06 ± 3.62	40.14 ± 4.55	0.869
Urea, mmol/L	4.52 ± 2.58	4.13 ± 1.18	4.21 ± 1.42	0.521
Cr, μmol/L	62.50 ± 56.53	58.14 ± 14.04	58.98 ± 16.46	0.854
Urea/Cr, ratio	0.078 ± 0.020	0.072 ± 0.019	0.073 ± 0.023	0.380
Coagulation Test				
PT, sec	11.72 ± 0.68	11.68 ± 0.53	11.29 ± 0.73	0.006
APTT, sec	29.48 ± 1.97	29.38 ± 3.42	29.25 ± 2.46	0.917
D-Dimer, mg/L	0.82 ± 1.09	0.98 ± 1.61	0.52 ± 0.72	0.202
Cell-mediated Immunity				
CD3+/μL	1018.11 ± 478.35	1079.80 ± 524.23	969.15 ± 477.05	0.632
CD4+/μL	565.00 ± 285.05	646.55 ± 301.64	554.60 ± 303.81	0.390
CD8+/μL	361.52 ± 188.88	380.43 ± 224.87	354.56 ± 190.61	0.857
CD4+/CD8+, ratio	1.70 ± 0.49	1.91 ± 0.67	1.73 ± 0.78	0.379
CD19+/μL	173.69 ± 98.99	226.46 ± 117.04	200.82 ± 148.56	0.226
CD16+&CD56+/μL	180.00 ± 119.35	174.19 ± 81.26	215.43 ± 136.64	0.242
Humoral Immunity				
IgM, g/L	1.09 ± 0.34	1.26 ± 0.61	1.06 ± 0.44	0.145
IgG, g/L	12.00 ± 3.22	12.06 ± 3.05	11.86 ± 2.95	0.956
C3 complement, g/L	0.87 ± 0.15	0.83 ± 0.15	0.86 ± 0.17	0.605
C4 complement, g/L	0.26 ± 0.08	0.22 ± 0.08	0.24 ± 0.09	0.221
CT				
Abnormal/n*	39/40	29/29	45/47	0.785

Note: SD; WBC: white blood cell; HB: hemoglobin;PLT: platelet;ALT: alanine transaminase;AST: aspartate aminotransferase;TBIL: total bilirubin;DBIL: direct bilirubin;ALB: albumin; PT: prothrombin time;APTT: activated partial thromboplastin time;CD: cluster of differentiation; CT: computed tomography.

differences in gender composition at baseline. There also were no statistically significant differences in severity of CT images (P = 0.785) and gender composition (P = 0.391) among the three groups (Table 1). In all, there was no overall significant difference among baselines of the three groups.

Intra-comparison in recurrent group

Continuous variables at the three time points were compared in recurrent group using single-factor ANOVA. Fisher's exact test was performed to estimate the differences in the CT values. The results showed that the significant differences at the three time points were in WBC (white blood cell) (P = 0.009), Neutrophils (P = 0.035), PT (P = 0.000), APTT (activated partial thromboplastin time) (P = 0.000), CD3 (cluster of differentiation 3) (P = 0.007), CD4 (P = 0.017), CD8 (P = 0.001), CD4/CD8 (P = 0.005), IgG (P = 0.018), and C4 complement (P = 0.001) (Table 2). At baseline, PT, APTT, C4 complement was the highest and CD3, CD4, CD8 were the lowest. Meanwhile, WBC, NEU, and IgG were the highest and the CD4/8 ratio was the lowest at the turn-negative timepoint. Severity of CT images was calculated by Fisher's exact probability method and the differences between the three time points were significant (P = 0.000) (Table 2). Continuous improvement in CT results from baseline to negative and from negative to positive was identified.

Comparison between recurrent group and recovered group

In comparison of the values in between recurrent group and recovered group, a robust increase in the CD19 + count was demonstrated in recurrent group using repeated measurement ANOVA (F = 4.884, P = 0.031) (Appendix Material S1). Meanwhile, significant severity of CT images was found in the negative time of recurrent group by $\chi 2$ test (P = 0.000) (Appendix Material S2).

Table 2. Intra group comparison of recurrent group.

Characteristics	Baseline (n = 40)	Negative (n = 40)	Positive (n = 40)	P value
Complete *Blood* Count				
WBC, 10^9/L	5.28 ± 1.56	6.41 ± 2.07	5.59 ± 1.30	0.009
Neutrophils, 10^9/L	3.16 ± 1.41	3.97 ± 1.99	3.21 ± 1.09	0.035
Lymphocytes, 10^9/L	1.58 ± 0.62	1.77 ± 0.55	1.72 ± 0.43	0.273
RBC, 10^12/L	4.20 ± 0.49	4.02 ± 0.42	4.02 ± 0.49	0.125
HB, g/L	125.53 ± 16.20	121.29 ± 12.86	119.78 ± 24.19	0.353
PLT, 10^9/L	230.75 ± 79.92	241.74 ± 67.46	232.38 ± 49.41	0.730
Liver Function Test				
ALT, U/L	24.83 ± 23.55	35.00 ± 30.93	34.88 ± 24.32	0.147
AST, U/L	23.80 ± 12.98	22.53 ± 8.93	26.53 ± 11.32	0.268
TBIL, μmol/L	10.29 ± 7.12	12.07 ± 5.33	10.28 ± 4.40	0.276
DBIL, μmol/L	3.08 ± 1.90	3.08 ± 1.51	2.99 ± 2.50	0.973
ALB, g/L	40.52 ± 3.69	39.24 ± 3.25	40.46 ± 2.64	0.139
Urea, mmol/L	4.52 ± 2.58	4.29 ± 2.17	4.27 ± 2.37	0.873
Cr, μmol/L	62.50 ± 56.53	54.94 ± 37.64	58.48 ± 40.43	0.760
Urea/Cr, ratio	0.078 ± 0.020	0.082 ± 0.022	0.077 ± 0.021	0.513
Coagulation Test				
PT, sec	11.72 ± 0.68	10.57 ± 0.38	10.50 ± 0.44	0.000
APTT, sec	29.48 ± 1.97	26.85 ± 1.87	27.34 ± 2.18	0.000
D-Dimer, mg/L	0.82 ± 1.09	0.54 ± 0.43	0.52 ± 0.49	0.140
Cell-mediated Immunity				
CD3+/μL	1018.11 ± 478.35	1228.08 ± 300.22	1262.44 ± 295.75	0.007
CD4+/μL	565.00 ± 285.05	663.65 ± 200.09	711.41 ± 194.51	0.017
CD8+/μL	361.52 ± 188.88	477.54 ± 129.98	462.30 ± 121.81	0.001
CD4+/CD8+, ratio	1.70 ± 0.49	1.48 ± 0.39	1.62 ± 0.33	0.005
CD19+/μL	173.69 ± 98.99	195.80 ± 69.13	189.49 ± 51.61	0.114
CD16+&CD56+/μL	180.00 ± 119.35	213.96 ± 97.20	209.37 ± 79.60	0.198
Humoral Immunity				
IgM, g/L	1.09 ± 0.34	1.28 ± 0.50	1.23 ± 0.54	0.152
IgG, g/L	12.00 ± 3.22	13.37 ± 2.91	11.46 ± 3.00	0.018
C3 complement, g/L	0.87 ± 0.15	0.88 ± 0.15	0.87 ± 0.14	0.882
C4 complement, g/L	0.26 ± 0.08	0.21 ± 0.07	0.20 ± 0.06	0.001
CT				
Abnormal/n*	39/40	24/40	10/40	0.000

Note: SD; WBC: white blood cell; HB: hemoglobin;PLT: platelet;ALT: alanine transaminase;AST: aspartate aminotransferase;TBIL: total bilirubin;DBIL: direct bilirubin;ALB: albumin; PT: prothrombin time;APTT: activated partial thromboplastin time;CD: cluster of differentiation; CT: computed tomography.

Comparison between recurrent group and un-recovered group

In comparison of the values in the baseline-positive time in un-recovered group, PLT count (F = 6.817, P = 0.011) and D-dimer (F = 12.471, P = 0.001) were higher in the positive time of recurrent group using repeated measurement ANOVA (Appendix Material S3). There was no significant difference in severity of CT images by $\chi 2$ test (P = 0.533) (Appendix Material S4).

Factors related to clinical outcomes

Logistic regression models were applied to explore the risk factors associated with outcome of treatment, in which recovered group and un-recovered group were considered as favorable and unfavorable endings, respectively. Among the included parameters, the increase of CD3 (P = 0.005), IgM (P = 0.001), C3 complement (P = 0.009) and severity of CT images (P = 0.009) in patients seemed to be more conducive to the occurrence of recovery. Conversely, the increase of CD4 (P = 0.010), CD8 (P = 0.024), CD19 (P = 0.001), and C4 complement (P = 0.006) was prone to make the outcome inclined to the disease state (Table 3).

Discussion

This retrospective cohort study identified the underlying explanations for "turn positive" in patients with COVID-19 and explored related risk factors for the outcomes of treatment. The three groups were comparable at baseline with regard to characteristics. Compelling evidence has uncovered that the total number of WBC of the disease is reduced and proportion of "WBC $< 4 \times 10^9$ per L" is statistically lower in COVID-19 patients with critical or mortal conditions [12]. In the recurrent group, patient's WBC and NEU levels were found to be higher when their RT-PCR tests turned negative. It indicates the improvement of the patient's condition when ruling out deterioration of the disease based on clinical manifestation. Meanwhile, decrease in ratio of CD4 to CD8, and rise on levels of IgM and IgG are strongly consistent with viral infection. Recent data pinpoint that serological responses, including viral-specific immunoglobulin M (IgM) and

Table 3. Risk factors associated with clinical outcomes.

	Univariable analysis		Multivariable analysis	
Laboratory test	OR (95%CI)	p value	OR (95%CI)	p value
Age (years)	1.022 (0.991–1.054)	0.166		
Gender (vs male)	1.114 (0.399–3.109)	0.837		
Complete B*lood* Count				
WBC, 10^9/L	1.022 (0.782–1.335)	0.873		
Neutrophils, 10^9/L	0.961 (0.708–1.305)	0.798		
Lymphocytes, 10^9/L	1.725 (0.710–4.189)	0.229		
RBC, 10^12/L	0.714 (0.289–1.764)	0.466		
HB, g/L	0.989 (0.957–1.023)	0.527		
PLT, 10^9/L	1.000(0.994–1.006)	0.997		
Liver Function Test				
ALT, U/L	0.996 (0.976–1.017)	0.704		
AST, U/L	0.991 (0.949–1.036)	0.699		
TBIL, μmol/L	0.977 (0.886–1.077)	0.641		
DBIL, μmol/L	0.955 (0.725–1.259)	0.745		
ALB, g/L	0.997 (0.924–1.076)	0.944		
Urea, mmol/L	1.109 (0.707–1.741)	0.652		
Cr, μmol/L	0.995 (0.958–1.033)	0.790		
Urea/Cr, ratio	3803.25(0–7.7*10^{12})	0.451		
Coagulation Test				
PT, sec	1.978 (0.708–5.532)	0.193		
APTT, sec	0.944 (0.745–1.196)	0.631		
D-Dimer, mg/L	1.170 (0.614–2.227)	0.633		
Cell-mediated Immunity				
CD3+/μL	0.999 (0.997–1.001)	0.239	0.953 (0.922–0.986)	0.005*
CD4+/μL	1.000 (0.997–1.002)	0.769	1.037 (1.009–1.066)	0.010*
CD8+/μL	0.998 (0.995–1.001)	0.261	1.051 (1.007–1.096)	0.024*
CD4+/CD8+, ratio	1.128 (0.533–2.388)	0.753	1.396 (0.062–31.477)	0.834
CD19+/μL	1.006 (1.000–1.013)	0.052	1.042 (1.018–1.067)	0.001*
CD16+&CD56+,/μL	1.000 (0.995–1.004)	0.851	0.997 (0.987–1.008)	0.618
Humoral Immunity				
IgM, g/L	0.036 (0.004–0.309)	0.002	0.001 (0.000–0.061)	0.001*
IgG, g/L	0.717 (0.539–0.954)	0.023	0.801 (0.512–1.256)	0.334
C3, g/L	0.371 (0.013–10.436)	0.560	0.000 (0.000–0.075)	0.009*
C4, g/L	1.3*10^{5}(0.583–3.1*10^{10})	0.061	2.5*10^{12} (3467–1.7*10^{21})	0.006*
CT (vs positive)	0.302 (0.078–1.170)	0.083	0.010 (0.000–0.317)	0.009*

Note: SD; WBC: white blood cell; HB: hemoglobin;PLT: platelet;ALT: alanine transaminase;AST: aspartate aminotransferase;TBIL: total bilirubin;DBIL: direct bilirubin;ALB: albumin; PT: prothrombin time;APTT: activated partial thromboplastin time;CD: cluster of differentiation; CT: computed tomography.

IgG antibodies, have potential significance for evaluating the severity and prognosis of COVID-19 [11,13].

Intriguingly, significant alterations on CD19 count occur in favorable outcome. It indicates CD19 is likely to be a critical marker to identify the results of RT-PCR test. It is widely believed that CD19 is a biomarker for B lymphocyte development due to its high expression on all B cells [14]. We found that patients in the recurrent group experienced an increase in CD19 levels when RT-PCR test showed negative results, compared with the recovered patients. Furthermore, CD19 level was comparable in the three tine points of the recurrent group. Thus, elevated CD19 level caused by activated B cell response to viral infection may provide explanations for the false-negative RT-PCR results for SARS-CoV-2 detections. More importantly, there were no significant differences in laboratory examinations between recurrent group and un-recovered group, expect for PLT level, which was still in the normal range. These results demonstrated that the so-called recurrence may indeed be the un-recovered status. However, larger samples are warranted to prove our speculation because of the complicated mechanisms of immune systems.

During viral infection with SARS-CoV-2, the production of specific antibodies against the virus is consistent in most patients, except for immunodeficient patients. Our data showed a positive association among cell-mediated immunity (CD3, CD4, CD8, CD19 count), humoral immunity (IgM, C3, C4 level) and outcome of treatment. Detection of IgM antibody provides the first line of humoral immunity, and indicates a recent exposure to SARS-CoV-2 [13]. A recent study of SARS-CoV found that activation of C3 complement exacerbates disease in SARS-CoV-associated acute respiratory distress syndrome (ARDS) [15]. Moreover, C3-deficient mice infected with SARS-CoV exhibited less respiratory dysfunction, which suggests the role of C3 in the inflammatory lung complications of SARS-CoV-2 infection [16]. Although our data reinforce the idea that cell-mediated immunity and humoral immunity could be regarded as a hallmark for SARS-CoV-2 infection, causal relationship and more specific mechanisms are necessary to be explored.

Notably, CT test results seem to be more convincing to explain the so-called recurrence. In a study conducted in China, about 81% of the patients with negative RT-PCR results but positive chest CT scans were re-classified as highly likely or probable cases with COVID-19, indicating a higher sensitivity of CT results to identify the disease [17]. Albeit with the significant difference in CT results between recurrent group and recovered group, patients in the recurrent group during the period of baseline-negative-positive showed continuous improvement in the CT findings. Accordingly, for patients with negative RT-PCR tests, depending on self-control CT results to assess the reliability of RT-PCR was not sufficient. Based on our available data, CD19 + count and CT scan findings should be interpreted altogether in order to screen "false-negative" RT-PCR test results more accurately. In addition, our evaluation in CT results of "recurrent" and "unrecovered" groups showed no statistical difference. Consequently, it is speculated that the patients in the recurrent group are more likely to be consistently infected and presented with false negative in RT-PCR results. The extents to which CT scans could provide accurate diagnosis of COVID-19 in different stages of the disease await further investigation.

Additionally, in accordance with the natural history of the SARS-CoV-2 and viral load kinetic in different anatomic sites, specimens collected from diverse sites might be attributed to the false negative results. In the 205 samples collected by Wang W and colleagues, lower respiratory tract (bronchoalveolar lavage fluid, 93%) samples showed the highest testing positive for the virus, followed by sputum, nasal swabs, fibro bronchoscope brush biopsy, and pharyngeal swabs [18]. Nevertheless, collection of bronchoalveolar lavage fluid not solely requires specialized tools and operators, but also causes pain to patients. Conversely, collection of other samples such as sputum, nasal, and pharyngeal swabs would be quicker, simpler, and safer [19]. Further research is required to probe adopt appropriate strategies in clinical practice.

RNA viruses have a high mutation rate due to the lack of proofreading activity of polymerases. Thus, most RNA viruses are prone to develop resistance to drugs and escape from immune surveillance [20]. Meanwhile, genetic diversity and rapid evolution of SARS-Cov-2 have been pointed out in several studies [21]. Although the real-time RT-PCR assay was designed precisely based on the conserved regions of the SARS-Cov-2 genomes, variability causing mismatches could bring about potential false-negative results. Furthermore, prevailing notions confirmed that the SARS-CoV-2 receptor angiotensin converting enzyme 2 (ACE2) is not limited to alveolar epithelial type II cells and ciliated cells in the lung, but also highly expressed on intestinal enterocytes [22]. Recent publications indicate that SARS-CoV-2 may infect other tissues aside from the lungs [23]. Consequently, we speculated that SARS-Cov-2 may have the capacity to undergo "latent" in the intestine and transmit to other organs when patients experienced hypo-immunity or discontinued treatment albeit with the negative results detected from respiratory tract. Taken together, larger clinical samples and more convincing experimental research to explain the so-called recurrence in patients with COVID-19 are needed to be further validated.

Several limitations in the present study should be mentioned. First, due to the retrospective study design, not all laboratory tests were done in all patients, which could bring about unavoidable gap of information. Therefore, their roles might be underestimated or overestimated. Second, inadequate adherence to antivirals or supportive therapy might have also contributed to the clinical outcomes in some patients.

Conclusion

This retrospective cohort study identified the underlying explanations for "turn-positive" in patients with COVID-19 and explored risk factors related to the outcomes of treatment. Our findings revealed that "recurrence" might be caused by false-negative results from RT-PCR, which was possibly related to limitations of nasopharyngeal swabs sampling. Levels of CD3, CD4, CD8, CD19, IgM, C3 complement, C4 complement, and CT results in patients were significantly correlated with the outcome of COVID-19. For patients with negative RT-PCR tests, only counting on self-control CT scan to assess the reliability of RT-PCR was not sufficient. Indeed, CD19+ count and CT scan findings should be interpreted altogether in order to screen "false-negative" RT-PCR test results more accurately. Our speculations should be validated by prospective research with larger samples.

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Disclosure statement

All authors declare no competing interests.

Author contributions

Yong Xiao, Xiao Shi, Qian She, Qi Chen, Hong Pan, Fen Wang and Mingkai Chen had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Jin Zhang, Xiaojiao Liu, Haiyan Wu, Wenfei Jin, Ge Ke, Shuzhong Liu, Jiao Li, Jing Zhou and Dongwen Wu recruited patients and collected data. Xiao Shi and Qi Chen performed data analysis. Yong Xiao, Xiao Shi, Qian She, Qi Chen, Fen Wang, Honggang Yu, and Mingkai Chen drafted and revised the paper. All authors approved the final draft of the manuscript for publication.

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