Prediction of in-hospital recurrence and false-negative results in patients with COVID-19 by red blood cell values on admission

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Abstract. The clinical characteristics and risk factors of patients with coronavirus disease 2019 (COVID-19) with re-positive or false-negative test results have so far remained to be determined. The present study provides a cross-sectional observational study on 134 hospitalized patients selected from Huoshenshan Hospital (Wuhan, China) using cluster sampling. A total of 68 patients had reduced red blood cell (RBC) counts, 55 a decrease in the hemoglobin concentration (HBC) and 73 a decline in hematocrit (HCT). The false-negative rate of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) RNA detection in pharyngeal swab specimens was 18.7%. The absolute lymphocyte count (ALC), RBC, HBC and HCT levels in false-negative patients were significantly higher than those in patients who tested positive for viral nucleic acids. Multivariate logistic regression analysis indicated that RBC [odds ratio (OR)=0.43, 95% CI: 0.18-0.99], HBC (OR=0.97, 95% CI: 0.94-0.99) and ALC (OR=0.43, 95% CI: 0.20-0.91) were the factors influencing the negative testing results for viral nucleic acid. The rate of re-positive patients was 16.4%. The white blood cell, RBC, HBC and HCT values in re-positive patients were lower than those in non-re-positive patients. The median (interquartile range) values for RBC, HBC and HCT of male re-positive patients were 3.95 (3.37, 4.2) x10^{12}/l, 123 (103, 133) g/l and 36.6 (31.1, 39.2)%, respectively, while the RBC, HBC and HCT of female re-positive patients were 3.54 (3.13, 3.74) x10^{12}/l, 115 (102, 118) g/l and 34.2 (28.5, 34.9)%, respectively. It was determined that RBC, HBC and HCT values had moderate accuracy in predicting SARS-CoV-2 recurrence in patients with COVID-19 using receiver operating curve analysis. The present study suggested that RBC may have an important role in the pathogenesis of COVID-19.

Introduction

In December 2019, an unexplained viral pneumonia, now known to be part of the pathology of coronavirus disease 2019 (COVID-19), emerged in Wuhan (China) (1,2). The common clinical manifestations were fever, cough and regions of ground-glass opacity on chest computed tomography (CT) scans (3).

No effective medical treatment exists for the early stages of COVID-19 (4-6). Supportive care has been indicated to be the most effective strategy during the COVID-19 outbreak. Since 90% of cases of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection are asymptomatic, it is important to evaluate the risk of patients with SARS-CoV-2 infection regarding the progression to severe forms for prompt individual treatment and medical resource management, which may prevent imposing restrictions on whole populations and facilitate the identification of high-risk populations (7,8). Therefore, the approach employed towards the risk assessment of severe SARS-CoV-2 infection is of critical significance for the effective treatment of COVID-19.

Patients were reported to have recovered from COVID-19, but in numerous cases, a subsequent PCR test indicated SARS-CoV-2 nucleic acid-positive results (9-11). Re-positive patients usually have no or mild clinical symptoms; however, their health status, infectivity and the mechanisms of acquiring re-positivity remain elusive (12,13). In the course of COVID-19 treatment, numerous patients test false-negative, but there appear to be many and complex influencing factors interfering with these

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results (14,15). Clinical practice guidelines recommend repeated PCR testing to confirm the clinical diagnosis (16). Research on COVID-19 patient populations with re-positive or false-negative test results is still limited and no relevant reference clinical risk assessment indicators exist for re-positive and false-negative patients. Furthermore, the possible infection and replication patterns of SARS-CoV-2 in humans have remained elusive. To explore the clinical characteristics and risk factors associated with acquiring re-positive or false-negative SARS-CoV-2 test results, a cross-sectional observational study of hospitalized patients with COVID-19 was performed.

Materials and methods

Study design and participants. A cross-sectional observational study was performed at Wuhan Huoshenshan Hospital (Wuhan, China). The flowchart of the study is provided in Fig. 1. The Wuhan Municipal Government assigned COVID-19 patients to Huoshenshan Hospital (Wuhan, China) and this Hospital then randomly assigned these patients to 16 infectious disease wards. To study the disease characteristics of patients with COVID-19, one specific ward was selected. The present study included patients hospitalized between February 2020 and April 2020 with fever, respiratory symptoms, and chest CT scans indicating pneumonia (Fig. S1) (17). According to the Chinese Management Guidelines for COVID-19 (version 7.0) (17), suspected cases with one of the following etiological or serological forms of evidence were diagnosed as having COVID-19 infection: i) Positive real-time fluorescent quantitative PCR (qPCR) detection of SARS-CoV-2 nucleic acids; ii) viral gene sequencing indicating high homology with SARS-CoV-2; iii) positive detection of SARS-CoV-2-specific IgM/IgG antibodies in serum on admission. When none of these three conditions was met, the patient was excluded. The hospital's workflow pattern is outlined in Data S1 and Figs. S2-S5. A total number of 134 patients in the ward were diagnosed with COVID-19 and were finally enrolled in the present study.

The present retrospective study was reviewed and approved by the Ethics Committee of Huoshenshan Hospital (Wuhan, China; approval no. HSSLL032) and written informed consent was obtained from each enrolled patient or a first-degree relative. The nucleic acid test of patients with COVID-19 who were admitted for the first time was positive. After systematic treatment, their clinical symptoms improved and at least two nucleic acid tests were negative. However, after a few days, their clinical symptoms worsened and at least two or more nucleic acid tests were SARS-CoV-2-positive; such patients were defined as re-positive patients. Patients were considered COVID-19 false-negative if they had COVID-19-related symptoms and typical imaging manifestations of COVID-19 pneumonia and multiple COVID-19 nucleic acid tests had been previously negative but they obtained a positive result in a recent nucleic acid test.

Data collection. The clinical records, clinical classification, chest CT scans, laboratory test results, treatment details and outcome data were collected from the electronic medical records of the patients. The information for all patients was collated in a standardized form. The data were then independently reviewed by two physicians.

The blood test results of the first medical evaluation after admission were collected. White blood cells (WBC), red blood cells (RBC), hemoglobin concentration (HBC), hematocrit (HCT), lymphocytes and platelets were detected using a BC-6800 Auto Hematology Analyzer and the original matching reagent (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). The levels of C-reactive protein (CRP), high-sensitivity CRP (hs-CRP), alanine aminotransferase, aspartate aminotransferase, procalcitonin, creatine kinase isoenzyme, lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (α-HBDH), albumin (ALB), cystatin C and urea nitrogen were determined using an SAL9000 fully automatic biochemical analyzer and the original matching reagent (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). Blood coagulation parameters were detected using an EXC810 fully automatic coagulation analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). SARS-CoV-2 IgM/IgG antibodies were detected by an iFlash 3000 fully automatic chemiluminescence immunoassay analyzer and the original matching reagent (Shenzhen YHLO Biotech Co., Ltd.). IL-6 was detected using a Cobas e411 analyzer and original matching reagent (Roche Diagnostics). SARS-CoV-2 nucleic acids, open reading frame 1ab (ORF1ab) and nucleocapsid (N) sequences were detected in pharyngeal swab samples via the SLAN-96P Real-Time PCR System (Shanghai Hongshi Medical Technology Co., Ltd.) and detection reagents (Hunan Shengxiang Biotechnology Co., Ltd.). All specimens were processed in the tent-type biosafety tertiary laboratory of Huoshenshan Hospital (Wuhan, China) and all operations were in strict compliance with the instructions provided by the manufacturers of the equipment and reagents. All specimens were transported and tested following the WHO Laboratory Testing Guidelines (18).

All patients underwent 128-slice CT scans using the uCT 760 CT X-ray system (United Imaging). The scans ranged from the thoracic inlet to the bottom of the lung and the scanning type was helical. The main scanning conditions were as follows: 120 kV; automatic MAs control; rotation time, 0.5 sec; field of view, 350 mm; matrix, 512x512; pitch, 1.21; and slice thickness/gap, 0.625/0.625 mm. Chest CT results were divided into three stages: Early (grade 1), advanced (grade 2) and late (grade 3) based on the Chinese Management Guidelines for COVID-19 (version 7.0) (17).

Statistical analysis. Descriptive statistical methods were used to summarize and analyze the data obtained. Categorical variables were expressed as n (%), continuous variables as the median and interquartile range, as appropriate. When the data of two independent samples did not follow a normal distribution, the Mann-Whitney U test was used; multiple groups of samples were compared with the Kruskal-Wallis test. Proportional data were compared with categorical variables using the Pearson χ^2 test. Continuity correction was adopted when continuous random variables approached discrete random variables. However, in the case of limited data volumes, comparison was performed using Fisher's exact test. Next, receiver operating characteristic (ROC) curves were generated to analyze the predictive accuracy of various risk

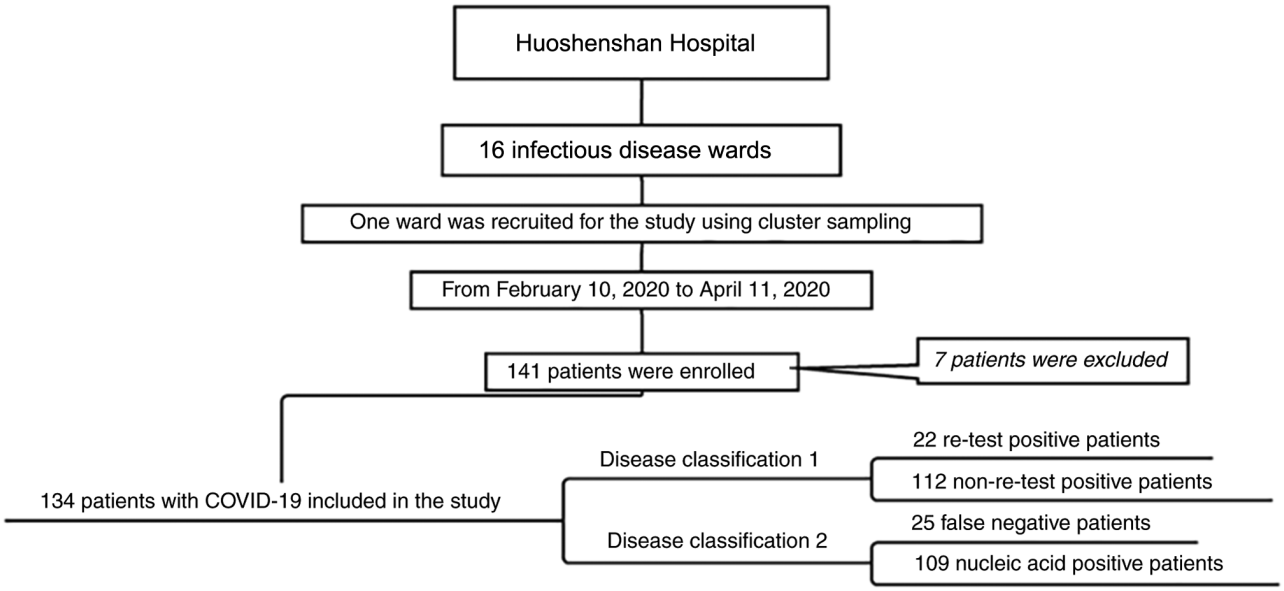


Figure 1. Flow chart of patient recruitment and movement of the patients in the present study. Disease classification was based on the CT images. COVID-19, coronavirus disease 2019.

factors. Furthermore, multivariate logistic regression analysis was utilized to assess the risk factors related to false-negative detection of viral nucleic acids. P<0.05 was considered to indicate statistical significance. All statistical analyses were performed using SPSS software, version 25.0 (IBM Corp.).

Results

Baseline characteristics. The data of 134 patients (67 females and 67 males) with COVID-19 who were hospitalized at Huoshenshan Hospital (Wuhan, China) were analyzed in the present study. The median age of the patients was 63 (51.69) years, with a minimum age of 21 years and a maximum age of 91 years. Of these 134 patients, 33 (24.6%) had a history of epidemic exposure, including direct exposure to the epidemic site and family collective infection; 103 (76.9%) had underlying comorbidities [44 (32.8%) had hypertension, 10 (7.5%) had coronary heart disease, 28 (20.9%) had diabetes and 41 (30.6%) had other comorbidities (20 patients with hypertension or coronary heart disease or diabetes; furthermore, chronic bronchitis, Parkinson's disease, hepatitis B virus (HBV), sequelae of cerebral infarction, prostatic cancer, breast cancer, gallstones, old pulmonary tuberculosis, lumbar disc herniation, nephrolithiasis, rheumatoid arthritis, duodenal ulcer, glaucoma, hepatic cysts, cataract, schizophrenia and hypothyroidism were present)]. A total of 38 patients were in the early stage according to their chest CT images, as a small number of localized patchy or ground-glass opacities were visible (Fig. 2A). Furthermore, 90 patients were in the advanced stage of the disease, the diagnosis of which was established based on their chest CT images exhibiting multiple bilateral lung ground-glass and patchy opacities (Fig. 2B). A total of six patients were in the late stage of the disease according to their chest CT images, in which large areas of consolidated opacities were observed (Fig. 2C). According to the results of the laboratory tests, 68 (50.7%; 37 males and 31 females) patients had reduced numbers of RBC, 55 (41%; 33 males and 22 females) patients had decreased HBC; 73 (54.5%; 43 males and 30 females) patients had lower HCT; 42 (31.3%) patients had a neutrophil-to-lymphocyte ratio >3.13; and 105 (78.4%) patients had varying degrees of ALB reduction (data not shown). The chest CT grades of the patients were as follows: 38 (28.3%) patients were classified as grade 1 (early stage); 90 (67.2%) as grade 2 (advanced stage); and six (4.5%) as grade 3 (late stage) (Table I). Representative CT images of the different stages of the disease are provided in Fig. 2. The common symptoms of these patients were recorded: Median fever maximum temperature of 38.0 (37.6, 38.8)˚C; symptoms of myalgia or fatigue in 75 (56%); dry cough in 105 (78.4%); dyspnea in 82 (61.2%); diarrhea in 15 (11.2%); and acute respiratory distress syndrome in 12 (9%) of the patients (Table I). The major therapeutic methods were antiviral treatment (n=96, 71.6%), antibiotic treatment (n=81, 60.4%), use of hormones (n=47, 35.1%), use of immunostimulant drugs (n=38, 28.4%) and traditional Chinese medicine (Lotus Qingwen capsules; Shijiazhuang Yiling Pharmaceutical Co., Ltd.) antiviral therapy (n=82, 61.2%). As of April 11, 2020, 73 (54.5%) of the patients had been discharged, 54 (40.3%) remained in hospital and seven (5.2%) had died (Table I).

Analysis of the laboratory findings of re-positive/non-re-positive patients. Of the 134 patients with COVID-19 included in the present study, 22 (16.4%) were re-positive and 112 (83.6%) were non-re-positive (Table I). The WBC (P<0.05), RBC (P<0.05), HBC (P<0.05) and HCT (P<0.05) in the re-positive patients were significantly lower than those in the non-re-positive patients. A total of nine re-positive patients were positive for the ORF1ab gene and 10 re-positive patients were positive for the N gene (Table I). Positivity for the ORF1ab gene (P<0.01) and N gene (P<0.01) in re-positive patients was significantly more frequent than in non-re-positive patients. The parameters of erythrocytes in re-positive patients

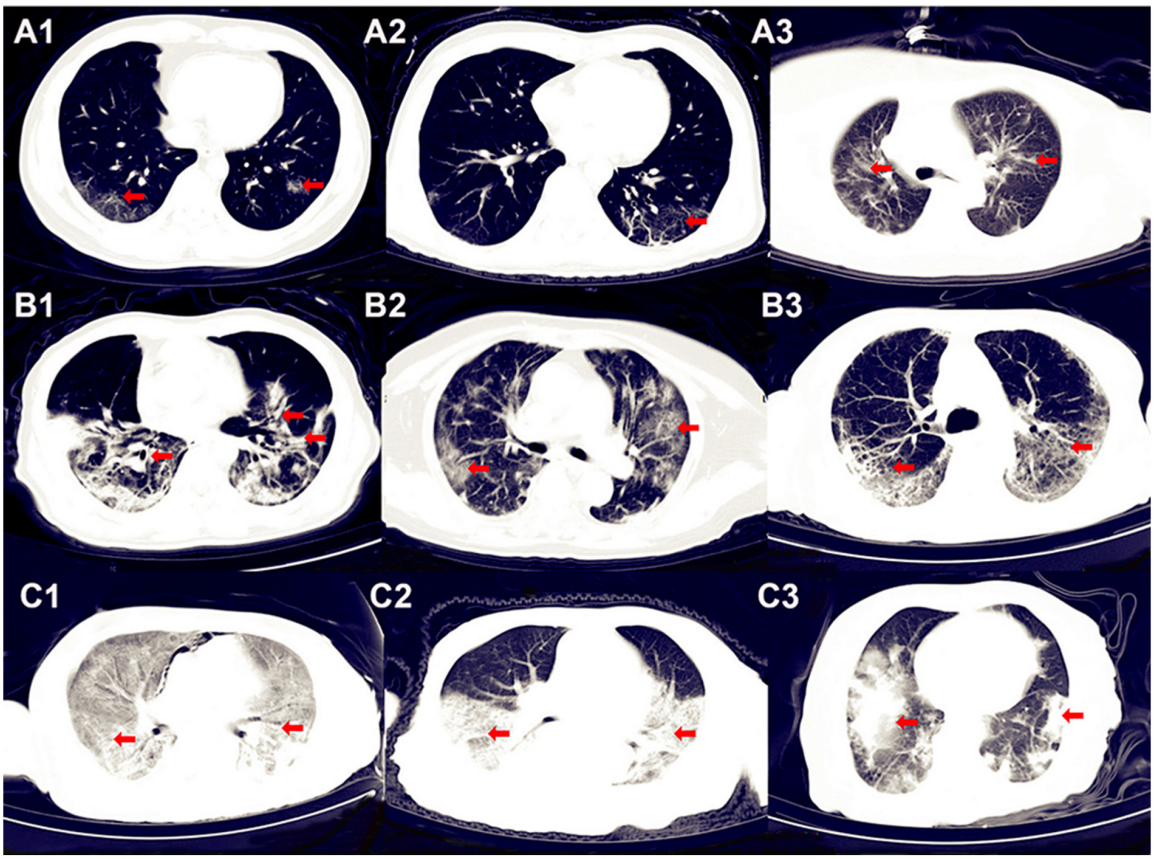


Figure 2. Typical manifestations of COVID-19 in patients with different chest CT grades. (A) Early stage of the disease. (A1) Patient 1: Bilateral patchy ground-glass opacities; (A2) Patient 2: Bilateral patchy ground-glass opacities, prominent on the left; (A3) Patient 3: Bilateral ground-glass opacities that are scattered. (B) Advanced stage of the disease. (B1) Patient 4: Multiple bilateral patchy ground-glass opacities and partial consolidation; (B2) Patient 5: Multiple bilateral ground-glass opacities; (B3) Patient 6: Multiple bilateral patchy ground-glass opacities and the lesions in the middle and outer regions exhibited reticular changes. (C) Late stage of the disease. (C1) Patient 7: Diffuse bilateral patchy opacities and patchy consolidation of lower lobes of both sides; (C2) Patient 8: Numerous bilateral patchy consolidations; (C3) Patient 9: Multiple bilateral patchy consolidations, prominent on the outer region. The red arrows point at the focus of COVID-19 pneumonia. COVID-19, coronavirus disease 2019.

(11 males and 11 females) and non-re-positive patients were quantitatively analyzed by sex (Table II). Among the males, the RBC count of the re-positive patients [4.0 (3.4, 4.2) x10^{12}/l] was significantly lower (P<0.05) than that of the non-re-positive patients [4.4 (3.9, 4.8) x10^{12}/l], the HBC of the re-positive patients [123 (103, 133) g/l] was significantly lower (P<0.05) than that of the non-re-positive patients [131 (122, 147) g/l] and the HCT of the re-positive patients [36.6 (31.1, 39.2)%] was significantly lower (P<0.05) than that of the non-re-positive patients [39.5 (36.1, 42.9)%]. Among the female patients, the RBC count [3.5 (3.1, 3.7) x10^{12}/l] of the re-positive patients was significantly lower (P<0.05) than that of the non-re-positive patients [3.9 (3.6, 4.3) x10^{12}/l], the HBC [115 (102, 118) g/l] of the re-positive patients was significantly lower (P<0.05) than that of the non-re-positive patients [122 (110, 130) g/l] and the HCT [34.2 (28.5, 34.9)%] of the re-positive patients was significantly lower (P<0.05) than that of the non-re-positive patients [36.4 (33.1, 38.8)%]. The values of the mean corpuscular volume, RBC distribution width, mean corpuscular hemoglobin (MCH), and MCH concentration were within the normal range, with no statistically significant differences between the re-positive and non-re-positive groups (Table II).

ROC curves were generated to evaluate the ability of RBC and WBC counts to distinguish between re-positive and non-re-positive patients (Fig. 3). For male re-positive patients, the area under the ROC curve (AUC) for RBC, HBC and HCT was 0.7175 (95% CI, 0.5758-0.8593), 0.7054 (95% CI, 0.5614-0.8493) and 0.7054 (95% CI, 0.5617-0.8490), respectively (Fig. 3D). The predictive values for recurrence of SARS-CoV-2 positivity in males were as follows: RBC <4.465x10^{12}/l provided a sensitivity of 100% (95% CI, 74.12-100%), a specificity of 44.64% (95% CI, 32.39-57.59%) and a likelihood ratio of 1.806; HBC <135.5 g/l provided a sensitivity of 100% (95% CI, 74.12-100%), a specificity of 44.64% (95% CI, 32.39-57.59%) and a likelihood ratio of 1.806; and HCT <40.3% provided a sensitivity of 100% (95% CI, 74.12-100%), a specificity of 42.86% (95% CI, 30.77-55.86%) and a likelihood ratio of 1.75 (Fig. 3A and D). To distinguish female re-positive from female non-re-positive patients, the AUC values of the ROC curves for RBC, HBC and HCT were 0.7297 (95% CI, 0.5799-0.8795), 0.7135 (95% CI, 0.5765-0.8504) and 0.7427 (95% CI, 0.6028-0.8826), respectively (Fig. 3D). In terms of the predictive value for SARS-CoV-2 recurrence, RBC <3.745x10^{12}/l provided a sensitivity of 81.82% (95% CI, 52.30-96.77%), a specificity of 64.29% (95% CI, 51.19-75.54%) and a likelihood ratio of 2.291; HBC <118.5 g/l provided a sensitivity of 90.91% (95% CI, 62.26-99.53%), a specificity of 62.5% (95% CI, 49.41-73.99%)

Table I. Clinical and laboratory data, treatments and outcomes for re-positive and non-re-positive patients.

Item	Total (n=134)	Re-positive patients (n=22)	Non-re-positive patients (n=112)	P-value
Clinical characteristics				
Sex (M/F)	67/67	11/11	56/56	0.652
Age (years)	63 (51, 69)	60 (52.5, 67.3)	63.5 (51, 70)	0.663
Epidemiological history	33 (24.6)	7 (31.8)	26 (23.2)	0.569
False-negative	25 (18.7)	0 (0)	25 (22.3)	0.010
Comorbidities				
Hypertension	44 (32.8)	10 (45.5)	34 (30.4)	0.309
Coronary heart disease	10 (7.5)	2 (9.1)	8 (7.1)	0.858
Diabetes	28 (20.9)	3 (13.6)	25 (22.3)	0.264
Other	41 (30.6)	7 (31.8)	34 (30.4)	0.867
Signs and symptoms				
Tmax,˚C	38.0 (37.6, 38.8)	38.3 (37.6, 39.0)	38 (37.6, 38.7)	0.319
Dry cough	105 (78.4)	15 (68.2)	90 (80.4)	0.054
Myalgia or fatigue	75 (56)	11 (50)	64 (57.1)	0.27
Dyspnea	82 (61.2)	12 (54.5)	70 (62.5)	0.214
Diarrhea	15 (11.2)	3 (13.6)	12 (10.7)	0.823
ARDS	12 (9)	1 (4.5)	11 (9.8)	0.365
Disease severity classification				0.051
Common	39 (29.1)	10 (45.5)	29 (25.9)	
Severe	85 (63.4)	11 (50)	74 (66.1)	
Critical	10 (7.5)	1 (4.5)	9 (8)	
Stages of chest CT				0.366
1	38 (28.3)	7 (31.8)	31 (27.6)	
2	90 (67.2)	15 (68.2)	75 (67)	
3	6 (4.5)	0 (0)	6 (5.4)	
Laboratory parameters (reference range)				
RBC, $x10^{12}$/l (3.8-5.8)	4.0 (3.7, 4.5)	3.7 (3.3, 4.0)	4.0 (3.7, 4.6)	0.002
HBC, g/l (115-175)	124 (115, 135.3)	117 (102.8, 125)	126 (116.5, 139)	0.005
HCT, % (35-50)	37.1 (34, 39.8)	34.8 (31, 37.2)	37.6 (34.8, 41)	0.003
WBC, $x10^9$/l (3.5-9.5)	5.7 (4.4, 7.0)	4.9 (3.9, 6.1)	5.9 (4.6, 7.1)	0.025
NEUT, % (40-75)	60.8 (54.4, 71.3)	59.9 (52.2, 68)	61.3 (54.9, 72.4)	0.36
MONO, % (3-10)	7.6 (6.3, 9.0)	7.4 (6.6, 8.9)	7.6 (6.3, 9.2)	0.68
LYM, % (20-50)	27.1 (19.7, 33.3)	29.9 (23.2, 34.8)	26.4 (17, 32.6)	0.96
ALC, $x10^9$/l (1.1-3.2)	1.4 (1.0, 1.9)	1.3 (1.1, 1.8)	1.5 (1.0, 1.9)	0.77
NLR	2.3 (1.6, 3.6)	2.0 (1.5, 2.9)	2.3 (1.7, 4.2)	0.18
MCV, fl (82-100)	92.6 (89.5, 95.4)	93.6 (91.8, 95.8)	92 (89, 95.4)	0.056
RDW, % (10.9-15.4)	12.8 (12.3, 13.3)	12.8 (12.2, 13.6)	12.8 (12.3, 13.3)	0.993
MCH, pg (27-34)	31.2 (30, 32)	31.5 (30.2, 32.1)	31.1 (29.9, 32)	0.412
MCHC, g/l (316-354)	336 (331, 342)	334 (328.8, 340.3)	336 (331.3, 342.8)	0.223
PLT, $x10^9$/l (125-350)	222.5 (178.5, 283.5)	208.5 (162.8, 253.3)	225.5 (182, 302.5)	0.08
FIB, g/l (2-4)	3.0 (2.6, 3.5)	2.9 (2.7, 3.1)	3.1 (2.6, 3.6)	0.333
D-D, mg/l (<0.5)	0.5 (0.2, 1.4)	0.4 (0.2, 1.8)	0.5 (0.2, 1.4)	0.81
CK isoenzyme, IU/l (0-24)	8.5 (6.7, 11.2)	8.2 (7.0, 9.3)	8.7 (6.7, 11.7)	0.529
ALT, IU/l (7-40)	22.1 (16.1, 42.1)	20.4 (12.0, 40.1)	22.2 (16.3, 43.2)	0.347
AST, IU/l (7-45)	19.1 (15.3, 30.5)	17.4 (15.5, 30.7)	19.2 (15.2, 30.3)	0.764
LDH, IU/l (120-250)	171.8 (148, 249.6)	171.3 (151.6, 238.9)	171.8 (146, 252.6)	0.871
α-HBDH, IU/l (72-182)	141.4 (120.5, 202.2)	138.9 (126, 199.8)	141.5 (120, 203.1)	0.925
Cystatin C, mg/l (0.54-1.15)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	0.698
Urea nitrogen, mmol/l (2.6-7.5)	4.2 (3.5, 5.5)	4.5 (3.7, 5.7)	4.2 (3.4, 5.4)	0.378
Procalcitonin, ng/ml (<0.15)	0.04 (0.03, 0.06)	0.03 (0.03, 0.05)	0.04 (0.03, 0.06)	0.717

Table I. Continued.

Item	Total (n=134)	Re-positive patients (n=22)	Non-re-positive patients (n=112)	P-value
IL-6, ng/ml (<7)	2.3 (1.5, 5.4)	2.3 (1.6, 3.1)	2.4 (1.5, 5.7)	0.838
CRP, mg/l (0-4)	2.5 (1.0, 18.3)	2.2 (0.9, 13.6)	2.8 (1.0, 20.5)	0.651
hs-CRP, mg/l (0-4)	2.5 (1.0, 10)	2.2 (0.9, 10)	2.8 (1.0, 10)	0.841
ALB, g/l (40-55)	37 (33.4, 39.4)	36.9 (33.3, 39)	37.1 (33.3, 39.5)	0.38
ORF1ab gene (+/-)	12 (8.9)	9 (40.9)	3 (2.7)	0.002
N gene (+/-)	13 (9.7)	10 (45.5)	3 (2.7)	0.002
SARS-CoV-2 IgM, U/ml (<10)	27.2 (12.5, 56)	34.2 (8.9, 61.3)	23.3 (13.1, 55.5)	0.83
SARS-CoV-2 IgG, U/ml (<10)	89.3 (68.4, 170.7)	137.3 (72.3, 183.9)	87.5 (66.1, 170.3)	0.969
Treatment				
Antiviral therapy				0.037
Arbidol	69 (51.5)	11 (50)	58 (51.8)	
Oseltamivir	13 (9.7)	2 (9.1)	11 (9.8)	
Ribavirin	9 (6.7)	5 (22.7)	4 (3.6)	
Ganciclovir	2 (1.5)	0 (0)	2 (1.8)	
Chloroquine diphosphate	1 (0.7)	1 (4.5)	0 (0)	
Lopinavir/Ritonavir tablets	1 (0.7)	1 (4.5)	0 (0)	
Interferon	1 (0.7)	1 (4.5)	0 (0)	
Use of antibiotics	81 (60.4)	10 (45.5)	71 (64.4)	0.652
Use of hormones	47 (35.1)	6 (27.3)	41 (36.6)	0.844
Immune enhancement therapy	38 (28.4)	9 (40.9)	29 (25.9)	0.091
Lotus Qingwen capsules	82 (61.2)	10 (45.5)	72 (64.3)	0.149
Outcome				0.204
Discharged	73 (54.5)	8 (36.4)	65 (58)	
Hospitalized	54 (40.3)	14 (63.6)	40 (35.7)	
Died	7 (5.2)	0 (0)	7 (6.3)	

Values are expressed as the median (interquartile range) or n (%). M, male; F, female; Tmax, maximum body temperature; NEUT, neutrophils; MONO, monocytes; LYM, lymphocytes; ALC, absolute lymphocyte count; WBC, white blood cell count; RBC, red blood cells; MCV, mean corpuscular volume; NLR, NEUT to LYM ratio; HCT, hematocrit; RDW, RBC volume distributing width; MCH, mean corpuscular hemoglobin; MCHC, MCH concentration; PLT, platelets; HBC, hemoglobin concentration; FIB, fibrinogen concentration; D-D, D-dimer concentration; CK, creatine kinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LD, lactate dehydrogenase; α-HBDH, α-hydroxybutyrate dehydrogenase; hs-CRP, high-sensitivity C-reactive protein; ALB, albumin; ORF1ab, open reading frame 1ab; N, nucleocapsid; ARDS, acute respiratory distress syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

and a likelihood ratio of 2.424; and HCT <35.25% provided a sensitivity of 90.91% (95% CI, 62.26-99.53%), a specificity of 64.29% (95% CI, 51.19-75.54%) and a likelihood ratio of 2.545 (Fig. 3B and D). The AUC value of the ROC curve for WBC for re-positive patients was 0.6514 (95% CI, 0.5182-0.7845) and in terms of the value for the prediction of SARS-CoV-2 recurrence, WBC <5.45x10^9/l provided a sensitivity of 68.18% (95% CI, 47.32-83.64%), a specificity of 62.5% (95% CI, 53.26-70.91%) and a likelihood ratio of 1.818 (Fig. 3C).

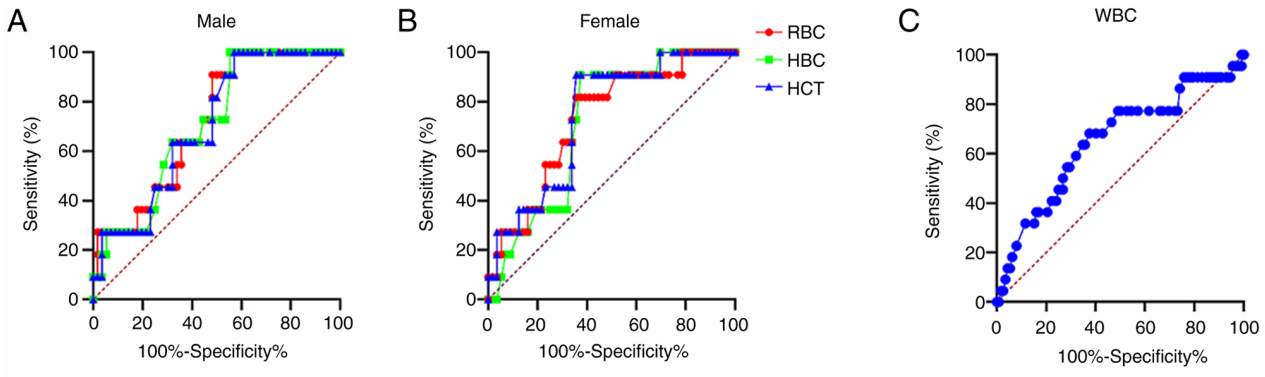
Analysis of laboratory findings of false-negative/nucleic acid-positive patients. As presented in Table III, of the 134 patients with COVID-19 included in the present study, 25 (18.7%) had false-negative viral nucleic acid tests and 109 (81.3%) had positive nucleic acid tests. The values of absolute lymphocyte count (ALC; P<0.01), ALB (P<0.05), RBC (P<0.05), HBC (P<0.05) and HCT (P<0.05) in the patients with false-negative SARS-CoV-2 nucleic acid test results were significantly higher than those of the patients who tested positive for SARS-CoV-2 nucleic acids. The D-dimer concentration, LDH, α-HBDH, cystatin C, CRP and hs-CRP in the patients with a false-negative viral nucleic acid test were significantly lower than those in the viral nucleic acid-positive patients (P<0.05; Table III).

The variables with statistically significant differences obtained by grouping patients on the basis of false-negative/positive viral nucleic acid test results were analyzed by multivariate logistic regression to identify the factors that influenced nucleic acid false-negative detection. Taking a negative viral nucleic acid test result as the dependent variable, the data with statistically significant differences (e.g., RBC, HBC and ALC) were used as the independent variables for logistic regression analysis.

Table II. Blood cell parameters in re-positive and non-re-positive patients by sex.

Parameter	Reference range	Re-positive (n=22)	Non-re-positive (n=112)	P-value
RBC, x10^{12}/l				
Males	4.3-5.8	4.0 (3.4, 4.2)	4.4 (3.9, 4.8)	0.023
Females	3.8-5.1	3.5 (3.1, 3.7)	3.9 (3.6, 4.3)	0.017
HBC, g/l				
Males	130-175	123 (103, 133)	131 (122, 147)	0.32
Females	115-150	115 (102, 118)	122 (110, 130)	0.026
HCT, %				
Males	40-50	36.6 (31.1, 39.2)	39.5 (36.1, 42.9)	0.32
Females	35-45	34.2 (28.5, 34.9)	36.4 (33.1, 38.8)	0.011
MCV, fl	82-100	93.6 (91.8, 95.8)	92 (89, 95.4)	0.056
RDW, %	10.9-15.4	12.8 (12.2, 13.6)	12.8 (12.3, 13.3)	0.993
MCH, pg	27-34	31.5 (30.2, 32.1)	31.1 (29.9, 32.0)	0.412
MCHC, g/l	316- 354	334 (328.8, 340.3)	336 (331.3, 342.8)	0.223

Values are expressed as the median (interquartile range). RBC, red blood cells; MCV, mean corpuscular volume; HCT, hematocrit; RDW, RBC volume distributing width; MCH, mean corpuscular hemoglobin; MCHC, MCH concentration.



D

	AUC	95% CI	Sensitivity %	95% CI	Specificity %	95% CI	Cut-off value	Likelihood ratio	p value
RBC									
Male	0.7175	0.5758–0.8593	100	74.12–100.0	44.64	32.39–57.59	4.465	1.806	0.0233
Female	0.7297	0.5799–0.8795	81.82	52.30–96.77	64.29	51.19–75.54	3.745	2.291	0.0166
HBC									
Male	0.7054	0.5614–0.8493	100	74.12–100.0	44.64	32.39–57.59	135.5	1.806	0.0323
Female	0.7135	0.5765–0.8504	90.91	62.26–99.53	62.5	49.41–73.99	118.5	2.424	0.026
HCT									
Male	0.7054	0.5617–0.8490	100	74.12–100.0	42.86	30.77–55.86	40.30	1.75	0.0323
Female	0.7427	0.6028–0.8826	90.91	62.26–99.53	64.29	51.19–75.54	35.25	2.545	0.0114
WBC	0.6514	0.5182–0.7845	68.18	47.32–83.64	62.5	53.26–70.91	5.45	1.818	0.0251

Figure 3. ROC curve analysis of the predictive performance of RBC, HBC, HCT and WBC for re-positive patients. (A) ROC curves for RBC, HBC and HCT to distinguish between male re-positive and male non-re-positive patients. (B) ROC curves for RBC, HBC and HCT to distinguish between female re-positive and female non-re-positive patients. (C) ROC curves for WBC to distinguish between re-positive and non-re-positive patients. (D) Parameters of the predictive value of RBC, HBC and HCT regarding virus recurrence obtained from the ROC curves. ROC, receiver operating characteristic; AUC, area under the ROC curve; RBC, red blood cells; HBC, hemoglobin concentration; HCT, hematocrit; WBC, white blood cells.

The results indicated that RBC [odds ratio (OR)=0.43, 95% CI: 0.18-0.99], HBC (OR=0.97, 95% CI: 0.94-0.99) and ALC (OR=0.43, 95% CI: 0.20-0.91) were significant influencing factors for a SARS-CoV-2 false-negative nucleic acid test result (Fig. 4A). ROC curve analysis was then applied to evaluate the predictive values of RBC, HBC and ALC for SARS-CoV-2 false-negative nucleic acid test results in patients with COVID-19, providing an AUC of 0.7136 (95% CI: 0.6057-0.8215; P=0.0009; Fig. 4B). Increased RBC, HBC and ALC values led to a greater tendency to obtain a

Table III. Clinical and laboratory parameters, treatments and outcomes of false-negative/nucleic acid positive patients.

Item	Total (n=134)	False-negative patients (n=25)	Nucleic acid positive patients (n=109)	P-value
Clinical characteristics				
Sex, M/F	67/67	16/9	51/58	0.121
Age, years	63 (51, 69)	53 (43, 65.5)	64 (55, 70)	0.008
Epidemiological history	33 (24.6)	4 (16)	29 (26.6)	0.267
Re-positive patients	22 (16.4)	0 (0)	22 (20.2)	0.010
Comorbidities				
Hypertension	44 (32.8)	5 (20)	39 (35.8)	0.13
Coronary heart disease	10 (7.5)	1 (4)	9 (8.3)	0.465
Diabetes	28 (20.9)	3 (12)	25 (22.9)	0.225
Other	41 (30.6)	5 (20)	36 (33)	0.202
Signs and symptoms				
Tmax,˚C	38.0 (37.6, 38.8)	38.0 (37.4, 38.4)	38.2 (37.7, 39.0)	0.090
Dry cough	105 (78.4)	17 (68)	88 (80.7)	0.163
Myalgia or fatigue	75 (56)	14 (56)	61 (56)	0.997
Dyspnea	82 (61.2)	18 (72)	64 (58.7)	0.219
Diarrhea	15 (11.2)	1 (4)	14 (12.8)	0.206
ARDS	12 (9)	0 (0)	12 (11)	0.082
Disease severity classification				0.031
Common	39 (29.1)	11 (44)	28 (25.7)	
Severe	85 (63.4)	14 (56)	71 (65.1)	
Critical	10 (7.5)	0 (0)	10 (9.2)	
Stages of chest CT				0.095
1	38 (28.3)	10 (40)	28 (25.7)	
2	90 (67.2)	15 (60)	75 (68.8)	
3	6 (4.5)	0 (0)	6 (5.5)	
Laboratory parameters				
RBC, $x10^{12}$/l	4.0 (3.7, 4.5)	4.3 (3.9, 4.8)	4.0 (3.7, 4.4)	0.034
HBC, g/l	124 (115, 135.3)	131 (119.5, 149)	124 (114, 133)	0.02
HCT, %	37.1 (34, 39.8)	39 (36, 43.7)	36.6 (33.4, 39.6)	0.013
WBC, $x10^9$/l	5.7 (4.4, 7.0)	6.2 (5.0, 7.3)	5.7 (4.4, 7.0)	0.202
NEUT, %	60.8 (54.4, 71.3)	57.9 (52, 69.3)	61.8 (55.3, 72.4)	0.113
MONO, %	7.6 (6.3, 9.0)	7.7 (6.4, 9.3)	7.5 (6.3, 9.0)	0.528
LYM, %	27.1 (19.7, 33.3)	29.4 (21.9, 34.6)	25.6 (17.1, 33)	0.143
ALC, $x10^9$/l	1.4 (1.0, 1.9)	1.8 (1.2, 2.3)	1.3 (1.0, 1.8)	0.010
NLR	2.3 (1.6, 3.6)	1.9 (1.5, 3.2)	2.4 (1.7, 4.2)	0.136
MCV, fl	92.6 (89.5, 95.4)	92.5 (89.3, 94.9)	92.6 (89.6, 95.5)	0.728
RDW, %	12.8 (12.3, 13.3)	12.6 (12.3, 13.3)	12.8 (12.3, 13.3)	0.71
MCH, pg	31.2 (30, 32)	30.8 (29.8, 32)	31.2 (30, 32)	0.495
MCHC, g/l	336 (331, 342)	335 (331.5, 343.5)	336 (331, 341.5)	0.909
PLT, $x10^9$/l	222.5 (178.5, 283.5)	242 (184.5, 289.5)	219 (177, 284)	0.515
FIB, g/l	3.0 (2.6, 3.5)	2.9 (2.4, 3.3)	3.1 (2.7, 3.6)	0.726
D-D, mg/l	0.5 (0.2, 1.4)	0.2 (0.1, 0.5)	0.6 (0.3, 2.2)	0.001
CK isoenzyme, IU/l	8.5 (6.7, 11.2)	7.8 (6.6, 10.0)	8.8 (7.0, 11.5)	0.294
ALT, IU/l	22.1 (16.1, 42.1)	29.4 (17.3, 52.2)	21.6 (15.5, 40.4)	0.205
AST, IU/l	19.1 (15.3, 30.5)	17.6 (14.5, 33.4)	19.1 (15.4, 30.5)	0.804
LDH, IU/l	171.8 (148, 249.6)	151.5 (129.1, 194.7)	175.8 (152.2, 258.2)	0.21
α-HBDH, IU/l	141.4 (120.5, 202.2)	121.8 (104.7, 164.4)	143.3 (121.9, 208.2)	0.012
Cystatin C, mg/l	0.9 (0.8, 1.1)	0.8 (0.8, 1.0)	1.0 (0.8, 1.1)	0.025
Urea nitrogen, mmol/l	4.2 (3.5, 5.5)	4.0 (3.4, 5.0)	4.2 (3.5, 5.6)	0.281
Procalcitonin, ng/ml	0.04 (0.03, 0.06)	0.04 (0.03, 0.04)	0.04 (0.04, 0.06)	0.21

Table III. Continued.

Item	Total (n=134)	False-negative patients (n=25)	Nucleic acid positive patients (n=109)	P-value
IL-6, ng/ml	2.3 (1.5, 5.4)	3.6 (2.0, 5.2)	2.2 (1.5, 5.7)	0.13
CRP, mg/l	2.5 (1.0, 18.3)	1.4 (0.6, 3.7)	3.1 (1.0, 27.0)	0.025
hs-CRP, mg/l	2.5 (1.0, 10)	1.4 (0.6, 3.7)	3.1 (1.0, 10)	0.018
ALB, g/l	37 (33.4, 39.4)	39 (35.4, 42.2)	36.7 (32.6, 39)	0.007
ORF1ab gene (+/-)	12 (8.9)	0 (0)	12 (11.0)	0.055
N gene (+/-)	13 (9.7)	0 (0)	13 (11.9)	0.055
SARS-CoV-2 IgM, U/ml	27.2 (12.5, 56)	16.3 (13.2, 46)	31.7 (12.2, 61.8)	0.26
SARS-CoV-2 IgG, U/ml	89.3 (68.4, 170.7)	79.1 (63.7, 163.1)	93.3 (69.7, 175.8)	0.132
Treatment				
Antiviral therapy				0.187
Arbidol	69 (51.5)	11 (44)	58 (53.2)	
Oseltamivir	13 (9.7)	3 (12)	10 (9.2)	
Ribavirin	9 (6.7)	1 (4)	8 (7.3)	
Ganciclovir	2 (1.5)	1 (4)	1 (0.9)	
Chloroquine diphosphate	1 (0.7)	0 (0)	1 (0.9)	
Lopinavir/Ritonavir tablets	1 (0.7)	0 (0)	1 (0.9)	
Interferon	1 (0.7)	0 (0)	1 (0.9)	
Use of antibiotics	81 (60.4)	12 (48)	69 (63.3)	0.825
Use of hormones	47 (35.1)	6 (24)	41 (37.6)	0.198
Immune enhancement therapy	38 (28.4)	2 (8)	36 (33)	0.032
Lotus Qingwen capsules	82 (61.2)	16 (64)	66 (60.6)	0.57
Outcome				0.027
Discharged	73 (54.5)	20 (80)	53 (48.6)	
Hospitalized	54 (40.3)	5 (20)	49 (45)	
Died	7 (5.2)	0 (0)	7 (6.4)	

Values are expressed as the median (interquartile range) or n (%). M, male; F, female; Tmax, maximum body temperature; NEUT, neutrophils; MONO, monocytes; LYM, lymphocytes; ALC, absolute lymphocyte count; WBC, white blood cell count; RBC, red blood cells; MCV, mean corpuscular volume; NLR, NEUT to LYM ratio; HCT, hematocrit; RDW, RBC volume distributing width; MCH, mean corpuscular hemoglobin; MCHC, MCH concentration; PLT, platelets; HBC, hemoglobin concentration; FIB, fibrinogen concentration; D-D, D-dimer concentration; CK, creatine kinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LD, lactate dehydrogenase; α-HBDH, α-hydroxybutyrate dehydrogenase; hs-CRP, high-sensitivity C-reactive protein; ALB, albumin; ORF1ab, open reading frame 1ab; N, nucleocapsid; ARDS, acute respiratory distress syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

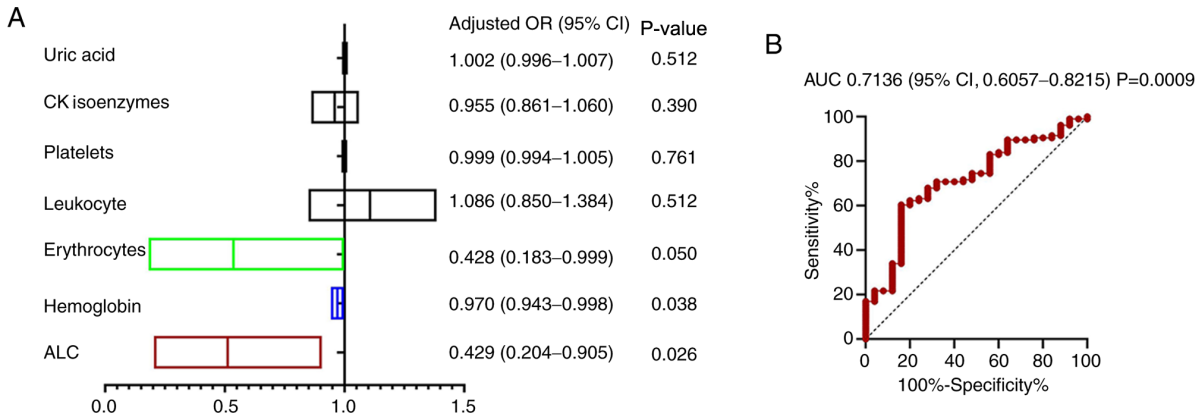


Figure 4. (A) Multivariate logistic regression to confirm the influencing factors of nucleic acid negative detection. The probability of the occurrence of a nucleic acid negative result was predicted by the regression model. According to the results, RBC, HBC and ALC were significant influencing factors for a nucleic acid negative test. (B) ROC curve for the predictive value of combined RBC, HBC and ALC regarding the probability of a nucleic acid negative result in patients with Coronavirus disease 2019. RBC, red blood cells; HBC, hemoglobin concentration; ALC, absolute lymphocyte count; HR, hazard ratio; CK, creatine kinase; ROC, receiver operating characteristic; AUC, area under the ROC curve.

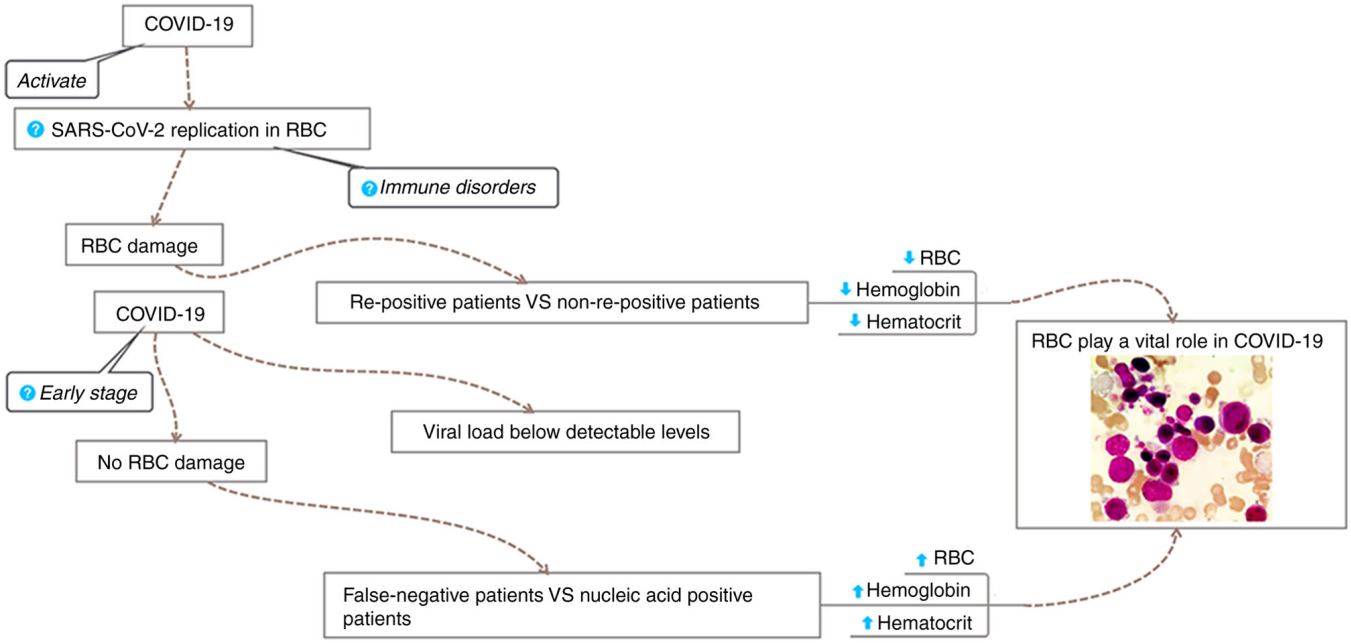


Figure 5. Schematic of the proposed mechanisms of the roles of RBCs in the pathogenesis of COVID-19. The blue question marks represent hypotheses of the present study. RBC, red blood cells; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

negative result of the SARS-CoV-2 nucleic acid tests, with a higher probability of a false-negative SARS-CoV-2 nucleic acid test result.

Discussion

False-negative results are common during the treatment process of COVID-19. Cuñarro-López *et al* (19) reported that 38.7% of 111 obstetric patients suspected of having COVID-19 had negative PCR results. False-negatives may occur due to various factors, including the incubation period of the disease, lack of standardized procedures for sample collection, poor storage conditions, insufficient detection and analysis accuracy, and human factors (18). In the present study, the false-negative rate of SARS-CoV-2 RNA detection in pharyngeal swab specimens was determined to be 18.7%. The detection of SARS-CoV-2 RNA by PCR in pharyngeal swab specimens is the most commonly used method for COVID-19 diagnosis in clinical practice. Usually, sputum specimens are difficult to collect due to the dry cough of most patients with COVID-19. The false-negative detection results of SARS-CoV-2 RNA in patients with COVID-19 may be related to various factors, such as the condition of the sample collection site, sample quality and laboratory bias (18). Furthermore, Chen *et al* (20) indicated that SARS-CoV-2 RNA was more likely to be detected in blood and anal swabs than in pharyngeal swabs. Guidelines for laboratory testing of patients with suspected COVID-19 issued by the World Health Organization in 2020 suggest that SARS-CoV-2-positive detection rates may be higher in lower respiratory tract specimens (18). An earlier study on SARS also confirmed that the virus-positive rate of sputum specimens was higher than that of pharyngeal swabs and there was no significant association with age, respiratory symptoms and underlying diseases (21). Considering the high expression of angiotensin-converting enzyme II, which is utilized by SARS-CoV-2 as the receptor for entry into type II alveolar cells (22), it may be recommended that patients with severe disease and a false-negative SARS-CoV-2 RNA test should be re-tested using sputum samples from the lower respiratory tract. In the present study, the age, organ damage indices (D-D, LDH, α-HBDH and cystatin C) and the immune response indices (CRP and hs-CRP) of the false-negative patients were significantly lower than those of the patients who tested positive for SARS-CoV-2 RNA by PCR. In addition, the values of ALC, ALB, RBC, HBC and HCT of the false-negative patients were significantly higher than those of the positive patients and the use of immunity-enhancing drugs in the false-negative patients was lower than that in the positive patients. These results indicated that false-negative patients may have relatively normal immune function in the early stages of COVID-19 disease. Logistic regression analysis revealed that the probability of negative viral nucleic acid detection increases with the elevation of RBC and HBC values.

In the present cross-sectional study, the rate of re-positive patients was determined to be 16.4%. It was established that WBC, RBC, HBC and HCT of the re-positive patients were lower than those of the non-re-positive patients. Leukopenia has been previously confirmed in patients with recurrence of hepatitis C virus (HCV) infection (23,24), which may suggest that these patients are immunocompromised. In addition to respiratory function, RBCs also perform multiple immune functions (25,26). RBCs are the most abundant cell type in the human blood and are involved in human innate immune responses (27). HBV, HCV, HIV, Epstein-Barr virus, transfusion-transmitted virus and echovirus have been reported to cause severe bone marrow aplasia (28,29). Parvovirus B19 commonly infects pro-erythroblasts and induces transient RBC aplasia, similar to that observed in patients with chronic hemolytic anemia (28). Furthermore, parvovirus B19

infection may also be associated with pancytopenia, particularly in immunocompromised patients (28). It is noteworthy that viruses have been postulated to induce lymphocyte activation and eventually lead to apoptotic death of hematopoietic cells in the bone marrow (30). In the present study, immune enhancement therapy of re-positive patients was effective. A total number of nine patients received immunostimulant drugs, of which thymosin α 1 was most commonly used.

The present study suggested that ALC, RBC and HBC were independent predictors of negative viral nucleic acid detection. Therefore, higher values of RBC, HBC and ALC in patients with COVID-19 are associated with a higher likelihood of a negative viral nucleic acid test result. Accumulating evidence has confirmed the occurrence of lymphopenia in patients with COVID-19 (3,31-33). However, surprisingly, the present results indicated that higher RBC was associated with a lower probability of SARS-CoV-2 infection. It was confirmed that re-positive patients had significantly reduced RBC, HBC and HCT. This result may indicate that the recurrence of SARS-CoV-2 in re-positive patients leads to mild normocytic anemia. Studies have confirmed that certain viruses specifically invade vertebrate RBC, including *Orthomyxoviridae influenza* A (34,35), HIV-1 (36) and *Orthomyxoviridae isavirus* (37). In addition, an earlier study indicated that salmon erythrocytes were the main *Piscine orthoreovirus* (PRV) replicating cells in the early peak phase of the infection and cytoplasmic inclusions called 'virus factories' were observed in the erythrocytes, which were the primary sites for the formation of new virus particles (38). Erythrocytes are the main target cells for PRV in the early infection phase and blood cell infection precedes myocardial infection (39). The infected erythrocytes provide further PRV dissemination into various host tissues (40-42). In the present study, ROC curves were generated to evaluate the predictive value of the RBC count regarding re-positive patients. An AUC value of 0.9 is considered to indicate high accuracy, 0.7-0.9 moderate accuracy and 0.5-0.7 low accuracy, while 0.5 indicates a chance result (43,44). The present results suggested that RBC has moderate accuracy in predicting virus recurrence in patients with COVID-19.

Based on the present results and literature review, the following mechanisms may be hypothesized, as outlined in a schematic in Fig. 5: On the one hand, false-negative patients have an antiviral response to SARS-CoV-2 in the early phase of the disease when the viral load is below the detectable level. At this stage, the virus does not activate or destroy the erythrocytes, resulting in higher levels of RBC, HBC and HCT in the false-negative patients than those in patients who test positive for viral nucleic acids. On the other hand, immune disorders that induce viral replication in the erythrocytes may lead to re-positivity of patients, resulting in an increase in the viral load in damaged erythrocytes. This effect may account for the lower RBC, HBC and HCT levels in re-positive patients than those in non-re-positive patients.

The RBC count may be used as an important screening index to determine whether a patient is COVID-19 false-negative. Even if the detection of SARS-CoV-2 by PCR is negative, a downward trend in the RBC count of a suspected patient may indicate that the virus is being activated and is beginning to destroy RBCs. In this case, further continuous PCR and chest CT detection are required, as the aforementioned indications are strongly suggesting that the patient may have been infected. The changes in the RBC count values in COVID-19 patients that recovered from hospital treatment and whose nucleic acid test results were negative may be used to predict SARS-CoV-2 recurrence. A new decrease in the RBC count values after their previous gradual return to normal after treatment may indicate that the increase in the viral load in the body is causing RBC damage. Based on the changes in the RBC counts, treatment plans may be prepared in advance and preventive antiviral treatment may be provided to minimize the virus-induced damage to patients.

In summary, the RBC values of hospitalized patients on admission may predict the evolution of COVID-19 disease. SARS-CoV-2 infection has a relatively lower probability to recur in patients with high RBC counts and their prognosis is good. This observation suggests the important role of human erythrocytes in SARS-CoV-2 infection, which may provide the key to explaining the subsequent pathogenesis. The present study provided novel insight into hidden and evasive mechanisms of SARS-COV-2 in the human body.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BL and XGZ conceived and designed the study. BL and XJ confirm the authenticity of all the raw data. BL, YH and XPQ contributed to writing of the manuscript. XJ, HoJ, HuJ and CML collected the data. BL, YH, XPQ, GL, DWX, DLW and XJ performed the statistical analysis. All authors contributed to data acquisition, data analysis or data interpretation, and have reviewed and approved the final version of the manuscript.

Ethics approval and consent to participate

The present retrospective study was reviewed and approved by the Ethics Committee of Huoshenshan Hospital (Wuhan, China; approval no. HSSLL032) and written informed consent

was obtained from each enrolled patient or a first-degree relative.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, *et al*: A Novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382: 727-733, 2020.
2. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, *et al*: Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med 382: 1199-1207, 2020.
3. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, *et al*: Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 382: 1708-1720, 2020.
4. Lai CC, Shih TP, Ko WC, Tang HJ and Hsueh PR: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. Int J Antimicrob Agents 55: 105924, 2020.
5. Zhao W, Zhong Z, Xie X, Yu Q and Liu J: Relation between chest CT findings and clinical conditions of coronavirus disease (COVID-19) Pneumonia: A multicenter study. AJR Am J Roentgenol 214: 1072-1077, 2020.
6. Poland GA: SARS-CoV-2: A time for clear and immediate action. Lancet Infect Dis 20: 531-532, 2020.
7. Calina D, Docea AO, Petrakis D, Egorov AM, Ishmukhametov AA, Gabibov AG, Shtilman MI, Kostoff R, Carvalho F, Vinceti M, *et al*: Towards effective COVID19 vaccines: Updates, perspectives and challenges (Review). Int J Mol Med 46: 3-16, 2020.
8. Stancioiu F, Papadakis GZ, Kteniadakis S, Izotov BN, Coleman MD, Spandidos DA and Tsatsakis A: A dissection of SARSCoV2 with clinical implications (Review). Int J Mol Med 46: 489-508, 2020.
9. Kang YJ: South Korea's COVID-19 infection status: From the perspective of re-positive test results after viral clearance evidenced by negative test results. Disaster Med Public Health Prep 14: 762-764, 2020.
10. Simon V, van Bakel H and Sordillo EM: Positive, again! What to make of 're-positive' SARS-CoV-2 molecular test results. EBioMedicine 60: 103011, 2020.
11. Habibzadeh P, Sajadi MM, Emami A, Karimi MH, Yadollahie M, Kucheki M, Akbarpoor S and Habibzadeh F: Rate of re-positive RT-PCR test among patients recovered from COVID-19. Biochem Med (Zagreb) 30: 030401, 2020.
12. Wong J, Koh WC, Momin RN, Alikhan MF, Fadillah N and Naing L: Probable causes and risk factors for positive SARS-CoV-2 test in recovered patients: Evidence from Brunei Darussalam. J Med Virol 92: 2847-2851, 2020.
13. He S, Zhou K, Hu M, Liu C, Xie L, Sun S, Sun W and Chen L: Clinical characteristics of 're-positive' discharged COVID-19 pneumonia patients in Wuhan, China. Sci Rep 10: 17365, 2020.
14. Pan Y, Long L, Zhang D, Yuan T, Cui S, Yang P, Wang Q and Ren S: Potential false-negative nucleic acid testing results for severe acute respiratory syndrome coronavirus 2 from thermal inactivation of samples with low viral loads. Clin Chem 66: 794-801, 2020.
15. Wikramaratna PS, Paton RS, Ghafari M and Lourenco J: Estimating the false-negative test probability of SARS-CoV-2 by RT-PCR. Euro Surveill 25: 2000568, 2020.
16. Hanson KE, Caliendo AM, Arias CA, Englund JA, Lee MJ, Loeb M, Patel R, El Alayli A, Kalot MA, Falck-Ytter Y, *et al*: Infectious diseases society of America guidelines on the Diagnosis of COVID-19. Clin Infect Dis: ciaa760 2020.
17. National Health Commission of the People's Republic of China. Chinese management guideline for COVID-19 (version 7.0). http://www.nhc.gov.cn/xcs/zhengcwj/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf. Accessed March 3, 2020.
18. World Health Organization: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. Interim guidance. https://www.who.int/publications/i/item/10665-331501. Accessed January 17, 2020.
19. Cunarro-Lopez Y, Cano-Valderrama O, Pintado-Recarte P, Cueto-Hernández I, González-Garzón B, García-Tizón S, Bujan J, Asúnsolo Á, Ortega MA and De León-Luis JA: Maternal and perinatal outcomes in patients with suspected COVID-19 and their relationship with a negative RT-PCR result. J Clin Med 9: 3552, 2020.
20. Chen W, Lan Y, Yuan X, Deng X, Li Y, Cai X, Li L, He R, Tan Y, Deng X, *et al*: Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microbes Infect 9: 469-473, 2020.
21. Jeong JH, Kim KH, Jeong SH, Park JW, Lee SM and Seo YH: Comparison of sputum and nasopharyngeal swabs for detection of respiratory viruses. J Med Virol 86: 2122-2127, 2014.
22. Zou X, Chen K, Zou J, Han P, Hao J and Han Z: Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med 14: 185-192, 2020.
23. Castells L, Vargas V, Allende H, Bilbao I, Luis Lázaro J, Margarit C, Esteban R and Guardia J: Combined treatment with pegylated interferon (alpha-2b) and ribavirin in the acute phase of hepatitis C virus recurrence after liver transplantation. J Hepatol 43: 53-59, 2005.
24. Tame M, Buonfiglioli F, Del Gaudio M, Lisotti A, Cecinato P, Colecchia A, Azzaroli F, D'Errico A, Arena R, Calvanese C, *et al*: Long-term leukocyte natural alpha-interferon and ribavirin treatment in hepatitis C virus recurrence after liver transplantation. World J Gastroenterol 19: 5278-5285, 2013.
25. Siegel I, Liu TL and Gleicher N: The red-cell immune system. Lancet 2: 556-559, 1981.
26. Morera D and MacKenzie SA: Is there a direct role for erythrocytes in the immune response? Vet Res 42: 89, 2011.
27. Minasyan H: Phagocytosis and oxycytosis: Two arms of human innate immunity. Immunol Res 66: 271-280, 2018.
28. Gonzalez-Casas R, Garcia-Buey L, Jones EA, Gisbert JP and Moreno-Otero R: Systematic review: Hepatitis-associated aplastic anaemia-a syndrome associated with abnormal immunological function. Aliment Pharmacol Ther 30: 436-443, 2009.
29. Gonzalez-Casas R, Jones EA and Moreno-Otero R: Spectrum of anemia associated with chronic liver disease. World J Gastroenterol 15: 4653-4658, 2009.
30. Young NS, Calado RT and Scheinberg P: Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 108: 2509-2519, 2006.
31. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, Ma K, Xu D, Yu H, Wang H, *et al*: Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. BMJ 368: m1091, 2020.
32. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, Li J, Zhao D, Xu D, Gong Q, *et al*: Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: A retrospective review of medical records. Lancet 395: 809-815, 2020.
33. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, *et al*: Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395: 497-506, 2020.
34. Schoch C, Blumenthal R and Clague MJ: A long-lived state for influenza virus-erythrocyte complexes committed to fusion at neutral pH. FEBS Lett 311: 221-225, 1992.
35. Skehel JJ and Wiley DC: Receptor binding and membrane fusion in virus entry: The influenza hemagglutinin. Annu Rev Biochem 69: 531-569, 2000.
36. Beck Z, Brown BK, Wieczorek L, Peachman KK, Matyas GR, Polonis VR, Rao M and Alving CR: Human erythrocytes selectively bind and enrich infectious HIV-1 virions. PLoS One 4: e8297, 2009.
37. Davies AJ and Johnston MR: The biology of some intraerythrocytic parasites of fishes, amphibia and reptiles. Adv Parasitol 45: 1-107, 2000.
38. Wessel O, Krasnov A, Timmerhaus G, Rimstad E and Dahle MK: Antiviral responses and biological concequences of piscine orthoreovirus infection in salmonid erythrocytes. Front Immunol 9: 3182, 2019.
39. Finstad OW, Dahle MK, Lindholm TH, Nyman IB, Løvoll M, Wallace C, Olsen CM, Storset AK and Rimstad E: Piscine orthoreovirus (PRV) infects Atlantic salmon erythrocytes. Vet Res 45: 35, 2014.

40. Wessel O, Braaen S, Alarcon M, Haatveit H, Roos N, Markussen T, Tengs T, Dahle MK and Rimstad E: Infection with purified Piscine orthoreovirus demonstrates a causal relationship with heart and skeletal muscle inflammation in Atlantic salmon. PLoS One 12: e0183781, 2017.
41. Takano T, Nawata A, Sakai T, Matsuyama T, Ito T, Kurita J, Terashima S, Yasuike M, Nakamura Y, Fujiwara A, *et al*: Full-genome sequencing and confirmation of the causative agent of erythrocytic inclusion body syndrome in coho salmon identifies a new type of piscine orthoreovirus. PLoS One 11: e0165424, 2016.
42. Hauge H, Vendramin N, Taksdal T, Olsen AB, Wessel Ø, Mikkelsen SS, Alencar ALF, Olesen NJ and Dahle MK: Infection experiments with novel Piscine orthoreovirus from rainbow trout (Oncorhynchus mykiss) in salmonids. PLoS One 12: e0180293, 2017.
43. Fischer JE, Bachmann LM and Jaeschke R: A readers' guide to the interpretation of diagnostic test properties: Clinical example of sepsis. Intensive Care Med 29: 1043-1051, 2003.
44. Swets JA: Measuring the accuracy of diagnostic systems. Science 240: 1285-1293, 1988.