The value of early positive nucleic acid test and negative conversion time of SARS-CoV-2 RNA in the clinical outcome of COVID-19 patients

Yongzhen Zhai (zyz200084@sina.com)
Department of Infectious Disease, Shengjing Hospital of China Medical University
https://orcid.org/0000-0002-7665-9683

Xin Zang
Department of Infectious Disease, Shengjing Hospital of China Medical University

Liangkun Xiong
Department of Hepatobiliary Surgery, Renmin Hospital of Wuhan University

Junyao Zhu
Department of Infectious Disease, Shengjing Hospital of China Medical University

Fangfang Zhao
Department of Infectious Disease, Fujian Medical University Affiliated First Quanzhou Hospital

Shihong Wang
Department of Paediatrics, Renmin Hospital of Wuhan University

Wenhui Zeng
Department of Hepatobiliary Surgery, Renmin Hospital of Wuhan University

Kaihuan Yu
Department of Hepatobiliary Surgery, Renmin Hospital of Wuhan University

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Abstract

Background

The outbreak of coronavirus disease (COVID-19) poses a great threat to the global public health. At present, the number of new confirmed COVID-19 cases and new deaths is increasing worldwide. The strategy of comprehensive and scientific detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, the virus that causes COVID-19) through real-time reverse transcriptase polymerase chain reaction (RT-PCR) for special populations and environments provides great support for the prevention and control of this pandemic in China. Our study focused on determining the factors associated with the length of time from symptom onset to the first positive nucleic acid test of throat swabs in COVID-19 patients, evaluating the effect of early positive nucleic acid detection on the disease severity and its significance in prognosis, and predicting the factors associated with the time from positive SARS-CoV-2 RNA test to negative conversion in COVID-19 patients.

Methods

This study included 116 hospitalized patients with COVID-19 from January 30, 2020 to March 4, 2020 in Wuhan, China. Throat swabs samples were collected for real-time reverse transcriptase polymerase chain reaction (RT-PCR) test of SARS-CoV-2 RNA, and all patients included in this study were positive for this test.

Results

Multivariate Cox proportional hazards model showed that disease severity and the duration of disease before admission to hospitals were protective factors for the time from symptom onset to positive nucleic acid detection, and the time from positive nucleic acid test to negative conversion was a risk factor for the time from symptom onset to positive nucleic acid detection. Meanwhile, the time from symptom onset to positive nucleic acid detection was an independent risk factor for the prolonged negative conversion of SARS-CoV-2 virus.

Conclusions

Patients with more severe disease and longer duration of disease before admission to hospitals had a shorter time from symptom onset to positive nucleic acid test. Prolonged time from symptom onset to positive nucleic acid test was an independent risk factor for the prolonged negative conversion time of SARS-CoV-2 virus, and the severity of the disease had no correlation with negative conversion time of SARS-CoV-2 virus.

Background

Coronavirus disease (COVID-19) is a new infectious disease caused by a newly discovered coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since the outbreak in Wuhan in December 2019, the pandemic has spread to the world rapidly. At present, the number of new confirmed cases and new deaths is increasing worldwide. According to the World Health Organization (WHO), the number of new COVID-19 cases worldwide has been declining for seven consecutive weeks. However, the number of new deaths per day has not shown a clear downward trend. As of July 2, a total of 183 million cases of laboratory-confirmed SARS-CoV-2 infection and more than 3.96 million deaths were registered worldwide. Detection of SARS-CoV-2 included etiological and serological examination of this virus. Among the etiological examination methods, the detection of SARS-CoV-2 nucleic acid by reverse RT-PCR is still the gold standard for the diagnosis of COVID-19. Due to its capability of early diagnosis, high sensitivity and specificity, RT-PCR is widely used in the fields of suspected case diagnosis, population screening, and health monitoring of staff (1, 2). Two consecutive negative nucleic acid tests for respiratory pathogens (sampled at ≥ 24 hours apart) are one of the important criteria to define a patient's recovery. Therefore, negative conversion of SARS-CoV-2 RNA is essential to confirming whether a patient meets the criteria for discharge. Previous studies have shown that advanced age, more comorbid underlying diseases, fever and corticosteroid therapy use, are risk factors for prolonged nucleic acid conversion(3–5). Recently, the emergence of new SARS-CoV-2 lineage B.1.617 has been associated with a surge in the number of infections in India(6). Mutations and recombination which lead to the emergence of novel lineages of SARS-CoV-2 can reduce the sensitivity of RT-qPCR and cause false negative results in throat swab detection(7). However, only a few studies have explored the factors influencing the length of time from the onset of symptoms to the first nucleic acid test positive and the value of early positive nucleic acid test on the clinical outcome of patients. In this study, we retrospectively assessed the clinical characteristics of mild and severe COVID-19 patients in Wuhan, explored the factors associated with the length of time from symptom onset to the first positive nucleic acid test of throat swabs and also investigated the risk factors for prolonged conversion time from positive to negative nucleic acid test, as well as the effect of early positive nucleic acid test on the severity of disease and its significance in prognosis of patients, so as to promote the early diagnosis and treatment of patients and improve the prognosis of patients.

Materials And Methods

Study Design and subjects

We retrospectively analyzed the clinical characteristics of 116 laboratory-confirmed COVID-19 patients admitted to the East Campus of Renmin Hospital of Wuhan University from January 30, 2020 and March 4, 2020. The East Campus of Renmin Hospital of Wuhan University was one of the medical institutions designated for COVID-19 by the National Health Commission, responsible for the treatment of severe COVID-19 patients in China. In addition, retrospective analysis of clinical data posed no potential risk to the patients.

Data Collection

Respiratory tract samples were tested by RT-PCR, and all patients participating in this study showed positive test results. Patients under 18 years of age, pregnant women, and those transferred to other hospitals during hospitalization were excluded. So, a total of 116 patients were included in this study. Patients' demographics, laboratory test results, and the assessment of disease severity at admission were obtained from electronic medical records. Specifically, laboratory parameters included complete blood count (CBC), biochemical parameters, immune function, and coagulation function. According to the SARS-CoV-2 Diagnosis and Treatment Guidelines (6th edition) issued by the National Health Commission, all patients met the clinical diagnostic criteria with the classification of disease severity. Two researchers independently reviewed the data collection forms to verify the accuracy of the data.

Statistical Analysis

Continuous variables were expressed as mean and standard deviation (SD) or median and interquartile range (IQR), and dichotomous variables were expressed as number of cases and percentage (n,%). The t test was used for the variables in normal distribution in the two groups of data, the wilcoxon rank sum test was used for non-normal distribution in the two groups of data; the analysis of variance was used for the variables that conform to the normal distribution in the four groups of data, the Kruskal-Wallis H test was used for the variables that do not conform to the normal distribution in the four groups of data, and Bonferroni correction method was used for pairwise comparison of the variables with different test results; Chi-square test was used for the dichotomous variables, and pairwise comparison was conducted for the variables with different test results. Univariate and multivariate analysis on the factors affecting the time from symptom onset to positive nucleic acid test and the conversion time from positive to negative nucleic acid test. Data were analyzed using R software, and $p < 0.05$ was considered statistically significant difference.

Results

Association between clinical characteristics and laboratory findings and the time from symptom onset to the first positive nucleic acid test

As shown in Table 1, patients were divided into two groups according to the time from symptom onset to the first positive nucleic acid test. 61 patients were diagnosed with positive nucleic acid within 1 week after onset, who were defined as the non-prolongation group. Of these 61 patients, 23 (37.70%) had severe disease and 38 (62.30%) had mild disease. The remaining 55 patients were diagnosed after more than 7 days and were defined as the prolongation group, in which the proportion of severe patients was 58.18% (32 patients), and the rate of severe disease in the prolongation group was significantly higher than that in the patients from the non-prolongation group ($p < 0.05$). The demographics and laboratory findings were compared between the two groups. The mean age of the patients in the prolongation group was 61.53 years, higher than that in the non-prolongation group (56.46 years), but the difference was not

statistically significant ($p = 0.063$). The median conversion time from positive to negative nucleic acid test in the prolongation group was 29 days (IQR,16.0-43.5), which was significantly higher than that in the non-prolongation group (23 days, IQR,15.0-37.75, $p < 0.05$). In addition, the duration of disease before admission was significantly higher ($p < 0.05$) in the prolongation group. In terms of blood routine examination, the lymphocyte count of patients in the prolongation group was significantly lower than that in the non-prolongation group, however, the comparison of other parameters between these groups showed no statistically significant difference ($p > 0.05$). Similarly, no significant difference of lymphocyte subsets and biochemical parameters ($p > 0.05$) was observed between the two groups.

Table 1
Comparison of clinical characteristics and laboratory findings between the two groups

	⩽7 days	≥ 7 days	*P* value
Sex, n (%)			
Male	42 (68.85)	29 (52.73)	0.075
Female	19 (31.15)	26 (47.27)	
Disease Severity, n (%)			
Mild	38 (62.30)	23 (41.82)	0.027
Severe	23 (37.70)	32 (58.18)	
Age (years)	56.46 ± 13.71	61.53 ± 15.42	0.063
Length of hospital stay (days)	36.09 ± 15.28	39.25 ± 16.01	0.281
Median conversion time from positive to negative nucleic acid test (days)	23.00(15.00,37.75)	29.00(16.00,43.50)	0.041
Median time from onset of symptom to hospital admission (days)	8 .00(6.00, 12.00)	13.00 (9.50, 15.00)	< 0.001
White blood cells ($\times 10^9$/L)	5.65 (4.50, 7.81)	5.65 (4.36, 7.11)	0.477
Neutrophils ($\times 10^9$/L)	3.83 (2.61, 6.43)	3.89 (2.84, 5.75)	0.810
Lymphocytes ($\times 10^9$/L)	1.12 (0.83, 1.55)	0.94 (0.64, 1.22)	0.027
Platelets ($\times 10^9$/L)	219.34 ± 87.23	243.4 ± 85.61	0.137
CD3 + T cell counts (cells/uL)	683.00 (405.00, 926.00)	517 (315, 821)	0.091
CD4 + T cell counts (cells/uL)	380.00 (237.00, 626.00)	346 (202, 491)	0.179
CD8 + T cell counts (cells/uL)	229.00(125.00,336.00)	169.00 (101.50 270.50)	0.084
CD4 + /CD8 + ratio	1.85 (1.23, 3.05)	1.85 (1.37, 2.82)	0.636
CD19 + B cell counts (cells/uL)	159.00 (106.00, 250.00)	132.00 (95.00, 199.00)	0.201
NK cell counts (cells/uL)	96.00 (71.00, 170.00)	92.00 (57.00, 147.00)	0.360
D-dimer (μg/mL)	0.82 (0.40, 2.61)	0.95 (0.43, 3.68)	0.713
Albumin (g/L)	35.92 ± 4.28	35.48 ± 3.79	0.561

	7 days	≥ 7 days	*P* value
Alanine aminotransferase (U/L)	28.00 (16.00, 50.00)	27.00 (19.00, 42.00)	0.715
Aspartate aminotransferase (U/L)	30.00 (21.00, 48.00)	30.00 (19.00, 40.00)	0.281
Alkaline phosphatase (U/L)	60.00 (51.00, 79.00)	64.00 (50.00, 92.00)	0.521
γ-glutamyl transpeptidase (U/L)	34.00 (19.00, 69.00)	30.00 (21.00, 56.00)	0.678
Total bilirubin (μmol/L)	10.70 (8.90, 13.80)	10.70 (7.70, 15.75)	0.923
Serum creatinine (μmol/L)	61.00(50.00, 72.00)	63.00 (50.00, 71.00)	0.894
Lactate dehydrogenase (U/L)	266.00 (203.00, 391.00)	298.00 (229.00, 370.00)	0.682
C-reactive protein (mg/L)	37.50 (17.20, 73.60)	37.50(11.95, 59.65)	0.463

As shown in Table 2, univariate Cox regression analysis indicated that the prolonged time from positive to negative nucleic acid test was a risk factor for the time from symptom onset to positive nucleic acid test ($p = 0.018$), while disease severity ($p = 0.044$), disease duration before admission ($p < 0.001$), age ($p = 0.04$), and platelet count ($p = 0.035$) were protective factors for the time from symptom onset to positive nucleic acid test. In order to systematically analyze the factors affecting the length of time from symptom onset to first positive nucleic acid in patients, disease severity, disease duration before admission, age and platelet (PLT) were included in the multivariate Cox regression model as independent variables based on the analysis results of univariate Cox regression and our expertise on COVID-19. Multivariate analysis showed that disease severity (HR = 0.572; 95% CI 0.348–0.942; $p = 0.028$) and disease duration before admission (HR = 0.853; 95% CI 0.805–0.904; $p < 0.001$) were protective factors for the time from symptom onset to positive nucleic acid test, and the time from positive to negative nucleic acid test (HR = 1.030; 95% CI 1.020–1.040; $p < 0.001$) was a risk factor for the time from symptom onset to positive nucleic acid test.

Table 2
Univariate and multivariate Cox regression model analysis results of the time from symptom onset to positive nucleic acid test

	Univariate model		Multivariate model	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Disease Severity	0.675 (0.461–0.989)	0.044	0.572 (0.348–0.942)	0.028
Conversion time from positive to negative acid test	1.010 (1.000-1.020)	0.018	1.030(1.020–1.040)	0.001
Sex	0.756 (0.520–1.100)	0.142		
Age	0.988 (0.976–0.999)	0.040	1.010 (0.995–1.030)	0.192
Time from onset of symptom to hospital admission	0.891 (0.851–0.934)	0.001	0.853 (0.805–0.904)	0.001
Length of hospital stay	1.010 (0.994–1.020)	0.340		
White blood cells	0.990 (0.935–1.050)	0.732		
Neutrophils	1.020 (0.997–1.030)	0.104		
Lymphocytes	1.070 (0.743–1.530)	0.730		
Platelets	0.998 (0.995-1.000)	0.035	0.999 (0.996-1.000)	0.307
CD3 + T cell counts	1.000	0.801		
CD4 + T cell counts	1.000 (0.999-1.000)	0.912		
CD8 + T cell counts	1.000(0.999-1.000)	0.515		
CD4 + /CD8 + ratio	0.947 (0.801–1.120)	0.526		
CD19 + B cell counts	1.000 (0.999-1.000)	0.260		
NK cell counts	1.000	0.010		
D-dimer	1.000 (0.989–1.010)	0.924		

	Univariate model		Multivariate model	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Alanine aminotransferase	1.000 (0.997–1.010)	0.416		
Aspartate aminotransferase	1.010 (0.998–1.020)	0.142		
Alkaline phosphatase	0.996 (0.990-1.000)	0.245		
γ-glutamyl transpeptidase	1.000 (0.998-1.000)	0.467		
Albumin	1.010 (0.965–1.060)	0.621		
Total bilirubin	0.985 (0.955–1.020)	0.329		
Serum creatinine	1.000 (0.994–1.01)	0.616		
Lactate dehydrogenase	1.000 (0.999-1.000)	0.905		
C-reactive protein	0.997 (0.994-1.000)	0.097		

Association between clinical characteristics and laboratory findings and the length of negative conversion time of SARS-COV-2 virus in patients

As shown in Table 3, patients were divided into four groups according to the length of conversion time from the first positive to negative nucleic acid test. Of all the patients in these groups, 23 experienced less than 2 weeks of this conversion time, 40 experienced 2–4 weeks, 26 experienced 4–6 weeks, and the remaining 27 experienced more than 6 weeks. The basic characteristics of patients and laboratory findings were compared among these groups which showed that the most common symptom on admission was fever (75.86%), followed by cough (62.93%), fatigue (21.55%), Shortness of breath (21.55%), and diarrhea (10.34%) dyspnea (9.48%). The most common comorbidity was hypertension (25.86%), followed by diabetes (16.38%), and cardiovascular disease (12.07%), however, no significant differences were found these symptoms and comorbidities among these groups ($p > 0.05$).

Table 3
Comparison of clinical characteristics and laboratory findings among the four groups

	< 2 weeks	2–4 weeks	4–6 weeks	≥ 6 weeks	*P* value
Sex, n (%)					0.880
Male	12 (52.17)	23 (57.50)	13(50.00)	13 (48.15)	
Female	11 (47.83)	17 (42.50)	13(50.00)	14 (51.85)	
Disease Severity, n (%)					0.709
Mild	13 (56.52)	26 (65.00)	14 (53.85)	18 (66.67)	
Severe	10 (43.48)	14 (35.00)	12 (46.15)	9 (33.33)	
Age (years)	64.74 ± 14.32	56.15 ± 14.58	60.73 ± 14.8	56.07 ± 14.1	0.091
Symptoms and signs, n (%)					
Fever	17 (73.91)	29 (72.5)	19 (73.08)	23 (85.19)	0.640
Cough	15 (65.22)	28 (70.00)	12 (46.15)	18 (66.67)	0.240
Shortness of breath	8 (34.78)	6 (15.00)	8 (30.77)	3 (11.11)	0.092
Dyspnea	2 (8.70)	2 (5.00)	3 (11.54)	4 (14.81)	0.578
Fatigue	8 (34.78)	5 (12.50)	7 (26.92)	5 (18.52)	0.178
Diarrhea	3 (13.04)	4 (10.00)	3 (11.54)	2 (7.41)	0.924
Comorbidities, n (%)					
Hypertension	8 (34.78)	6 (15.00)	7 (26.92)	9 (33.33)	0.239
Diabetes	5 (21.74)	7 (17.50)	4 (15.38)	3 (11.11)	0.781
Cardiovascular and cerebrovascular disorders	4 (17.39)	2 (5.00)	4 (15.38)	4 (14.81)	0.398

[a] indicates $p < 0.05$ in the pairwise comparison with the < 2 weeks group;

[b] indicates $p < 0.05$ when compared with the 2 to 4 weeks group;

[c] indicates $p < 0.05$ when compared with the 4 to 6 weeks group;

[d] indicates $p < 0.05$ when compared with the ≥ 6 weeks group.

	≤2 weeks	2–4 weeks	4–6 weeks	≥ 6 weeks	*P* value
Other comorbidities	10 (43.48)	19 (47.50)	10 (38.46)	7 (25.93)	0.346
Median time from symptom onset to positive nucleic acid test (days)	4 (0, 8)	5(2.5,8.25)	6.5 (3.5, 10)	9 (6, 14.5)[a]	0.007
Median time from onset of symptom to hospital admission (days)	7 (5.5, 13)	10 (7, 10.5)	12 (8.25, 14.75)	12 (8.5, 14.5)[b]	0.020
White blood cells ($\times 10^9$/L)	5.51 (4.37, 7.70)	5.37 (4.42, 6.74)	6.23 (4.61, 10.06)	5.65 (4.49, 7.12)	0.507
Neutrophils ($\times 10^9$/L)	3.89 (2.98, 5.86)	3.46 (2.74, 5.08)	4.38 (2.93, 9.16)	3.83 (2.44, 5.83)	0.481
Lymphocytes ($\times 10^9$/L)	1.05 ± 0.44	1.14 ± 0.61	1.06 ± 0.46	1.16 ± 0.58	0.816
Platelets ($\times 10^9$/L)	231.70 ± 74.90	229.38 ± 100.81	220.92 ± 76.62	241.44 ± 87.01	0.864
CD3 + T cell counts (cells/uL)	510.00 (323.50, 854.50)	602.50 (358.70, 927.20)	631.00 (342.50, 830.00)	670.00 (435.00, 926.50)	0.818
CD4 + T cell counts (cells/uL)	381.00 (217.50, 495.00)	352.50 (221.00, 551.00)	377.00 (220.00, 562.00)	443.00 (216.00, 528.00)	0.960
CD8 + T cell counts (cells/uL)	118.00 (91.50, 261.00)	249.00 (129.75, 337.50)	194.00 (76.00, 305.50)	207.00 (134.00, 319.00)	0.151
CD4 + /CD8 + ratio	2.53 (1.62, 3.40)	1.60 (1.07, 2.16)[a]	2.44 (1.26, 3.23)	1.85 (1.20, 3.09)	0.037
CD19 + B cell counts (cells/uL)	143.00 (113.00, 212.50)	118.00 (87.00, 177.25)	156.00 (86.00, 245.25)	159.00 (104.50, 273.50)	0.495

[a] indicates $p < 0.05$ in the pairwise comparison with the < 2 weeks group;

[b] indicates $p < 0.05$ when compared with the 2 to 4 weeks group;

[c] indicates $p < 0.05$ when compared with the 4 to 6 weeks group;

[d] indicates $p < 0.05$ when compared with the ≥ 6 weeks group.

	< 2 weeks	2–4 weeks	4–6 weeks	≥ 6 weeks	*P* value
NK cell counts (cells/uL)	95.00 (66.50, 132.00)	105.00 (72.50, 174.75)	59.00 (40.25, 125.00)	109.00 (78.50, 172.50)	0.074
D-dimer (μg/mL)	1.05 (0.62, 4.51)	0.70 (0.36, 2.42)	0.84 (0.36, 3.21)	0.82 (0.48, 2.70)	0.370
Alanine aminotransferase (U/L)	27.00 (20.50, 42.50)	29.00 (16.75, 42.00)	24.50 (16.75, 50.75)	27.00 (18.00, 44.00)	0.966
Aspartate aminotransferase (U/L)	30.00 (20.00, 45.00)	29.50 (20.75, 39.25)	31.50 (19.25, 43.00)	34.00 (19.50, 45.00)	0.938
Alkaline phosphatase (U/L)	77.00 (57.00, 90.50)	59.00 (48.75, 84.50)	59.00 (50.50, 72.75)	65.00 (51.00, 86.50)	0.264
γ-glutamyl transpeptidase (U/L)	40.00 (20.00, 57.00)	30.50 (21.00, 68.25)	31.00 (20.25, 38.00)	31.00 (20.00, 57.00)	0.925
Total bilirubin (μmol/L)	11.30 (8.05, 16.05)	10.50 (7.83, 16.43)	10.90 (8.32, 13.57)	10.30 (8.80, 14.00)	0.960
Serum creatinine (μmol/L)	65.00 (55.50, 77.50)	63.50 (49.17, 70.00)	56.50 (47.25, 69.50)	59.00 (49.00, 69.00)	0.571
Lactate dehydrogenase (U/L)	300.00 (230.50, 381.50)	261.50 (206.70,328.20)	307.50 (244.00, 378.00)	242.00 (210.50, 442.00)	0.454
C-reactive protein (mg/L)	50.90 (28.70, 92.45)	37.5 (17.18, 62.73)	37.50 (5.78, 63.09)	37.5 (17.00, 48.10)	0.213

[a] indicates $p < 0.05$ in the pairwise comparison with the < 2 weeks group;

[b] indicates $p < 0.05$ when compared with the 2 to 4 weeks group;

[c] indicates $p < 0.05$ when compared with the 4 to 6 weeks group;

[d] indicates $p < 0.05$ when compared with the ≥ 6 weeks group.

The median time from symptom onset to positive nucleic acid test was 9 days (IQR, 6.0-14.5) in patients with the conversion time of more than 6 weeks from positive to negative nucleic acid test, which was significantly longer than that in patients with the conversion time of less than 2 weeks (4 days, IQR, 0–8.0) ($p < 0.05$). In addition, patients with the conversion time of more than 6 weeks, had longer disease

duration before admission, compared with patients with the conversion time of 2 to 4 weeks ($p < 0.05$). In terms of the blood routine examination, there were no statistically significant differences in white blood cell count, neutrophil count and lymphocyte count among these groups ($p > 0.05$). As for immunological parameters, the CD4/CD8 ratio was lower in patients with conversion time of 2 to 4 weeks than in patients with conversion time of less than 2 weeks ($p < 0.05$). However, CD3 + T cell, CD4 + T cell, and NK cell counts did not show statistically significant differences between these groups ($p > 0.05$).

As shown in Table 4, univariate Cox regression analysis indicated that the time from symptom onset to positive nucleic acid test ($p = 0.019$), disease duration before admission ($p = 0.004$), length of hospital stay ($p < 0.001$), and duration of virus shedding ($p < 0.001$) were significantly correlated with the negative conversion time of SARS-CoV-2 virus. Combined with the results of univariate Cox regression analysis and our expertise in COVID-19, the time from symptom onset to positive nucleic acid test, duration disease before admission, length of hospital stay, duration of virus shedding, and age were included as independent variables in the multivariate Cox regression model, and the results showed that the time from symptom onset to positive nucleic acid test (HR = 1.010; 95% CI 1.005–1.020; $p = 0.0282$) was an independent risk factor for prolonged negative conversion from positive to negative nucleic acid test.

Table 4
Univariate and multivariate cox regression model analysis results of the conversion time from positive to negative nucleic acid test

	Univariate model		Multivariate model	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Disease severity	1.120 (0.771–1.640)	0.544		
Time from symptom onset to positive nucleic acid test	1.040 (1.010–1.080)	0.019	1.010 (1.005–1.020)	0.028
Sex	0.769 (0.528–1.120)	0.172		
Fever	0.725 (0.471–1.120)	0.145		
Cough	0.857 (0.582–1.260)	0.436		
Shortness of breath	1.310 (0.836–2.040)	0.241		
Dyspnea	0.754 (0.403–1.41)	0.376		
Fatigue	1.090 (0.700–1.700)	0.698		
Diarrhea	1.080 (0.586–1.980)	0.813		
Hypertension	1.000 (0.659–1.530)	0.990		
Diabetes	1.510 (0.922–2.490)	0.101		
Other comorbidities	1.140 (0.774–1.670)	0.514		
Age	1.010 (0.996–1.020)	0.179	1.000 (0.984–1.020)	0.192
Time from onset of symptom to hospital admission	0.937 (0.896–0.980)	0.004	1.000 (0.944–1.060)	0.602
Length of hospital stay	0.921 (0.905–0.938)	0.001	1.000 (0.979–1.020)	0.669
White blood cells	1.01 (0.949–1.070)	0.808		

	Univariate model		Multivariate model	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Neutrophils	1.000 (0.985–1.020)	0.865		
Lymphocytes	0.869 (0.615–1.230)	0.426		
Platelets	0.999 (0.997-1.000)	0.251		
CD3 + T cell counts	1.000 (0.999-1.000)	0.504		
CD4 + T cell counts	1.000 (0.999-1.000)	0.457		
CD8 + T cell counts	1.000 (0.999-1.000)	0.646		
CD4 + /CD8 + ratio	0.999 (0.865–1.150)	0.984		
CD19 + B cell counts	1.000 (0.999-1.000)	0.560		
NK cell counts	0.999 (0.998-1.000)	0.385		
D-dimer	1.000 (0.989–1.010)	0.869		
Alanine aminotransferase	1.000 (0.995–1.010)	0.944		
Aspartate aminotransferase	0.998 (0.989–1.010)	0.604		
Alkaline phosphatase	1.000 (0.994–1.010)	0.767		
γ-glutamyl transpeptidase	0.999 (0.995-1.000)	0.492		
Albumin	1.000 (0.959–1.050)	0.842		
Total bilirubin	1.010 (0.977–1.040)	0.664		
Serum creatinine	0.999 (0.995-1.000)	0.513		
Lactate dehydrogenase	1.000 (0.999-1.000)	0.803		

	Univariate model		Multivariate model	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
C-reactive protein	1.000 (1.000-1.010)	0.078		

Association between clinical characteristics and laboratory findings and disease severity in patients

As shown in Table 5, the relevant parameters between mild and severe patients were compared. The mean age of severe patients was 70.49 years, which was significantly higher than that of mild patients (51.49 years, $p < 0.05$). Among the mild patients, 50.70% were male, slightly lower than that in severe patients (55.56%), but the difference between the two groups was not statistically significant. The median time from symptom onset to the first positive nucleic acid test was 5.0 days (IQR, 2.5-8.0) in mild patients, significantly shorter than 8 days (IQR, 4.0–12.0) in severe patients ($p = 0.047$). The median conversion time from the first positive to negative nucleic acid test was 24 days (IQR, 15.5–41.5) and 25 days (14.0–38.0) in mild and severe patients, respectively ($p = 0.671$). In terms of the blood routine examination, the white blood cell count and neutrophil count were increased to varying degrees, and the lymphocyte count was decreased ($p < 0.05$) in severe patients than in mild patients. As for the immunological indicators, CD3 + T cells and CD8 + T cells were significantly lower in severe patients ($p < 0.05$), compared with those in mild patients, and there were no statistically significant differences in CD4 + T cells and NK cell counts between the two groups ($p > 0.05$). In terms of the biochemical parameters, albumin concentration was significantly higher in mild patients than that in severe patients ($p < 0.05$). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (γ-GGT) in severe patients were slightly higher than those in mild patients, but the difference was not statistically significant between the two groups ($p > 0.05$).

Table 5
Comparison of clinical characteristics and laboratory findings between mild and severe patients with COVID-19

	Mild	Severe	*P* value
Sex, n (%)			
Male	36 (50.70)	25 (55.56)	0.610
Female	35 (49.30)	20 (44.44)	
Age (years)	51.49 ± 12.30	70.49 ± 9.90	<0.001
Length of hospital stay (days)	37.00 (23.50, 46.00)	40 (31, 47)	0.309
Median time from symptom onset to positive nucleic acid test (days)	5 (2.5, 8)	8 (4, 12)	0.047
Median conversion time from positive to negative nucleic acid test (days)	24 (15.5, 41.5)	25 (14, 38)	0.671
Median time from onset of symptom to hospital admission (days)	10 (7, 14)	10 (7, 14)	0.776
White blood cells ($\times 10^9$/L)	5.48 (4.13, 6.82)	6.49 (4.75, 9.00)	0.026
Neutrophils ($\times 10^9$/L)	3.45 (2.43, 4.70)	4.88 (3.26, 7.47)	0.002
Lymphocytes ($\times 10^9$/L)	1.14 (0.80, 1.55)	0.93 (0.61, 1.13)	0.009
Platelets ($\times 10^9$/L)	235.27 ± 89.86	223.62 ± 82.58	0.484
CD3 + T cell counts (cells/uL)	684.00 (431.50, 958.50)	503.00 (306.00, 723.00)	0.011
CD4 + T cell counts (cells/uL)	394.00 (223.50, 613.50)	333.00 (197.00, 476.00)	0.119
CD8 + T cell counts (cells/uL)	259.00 (130.50, 354.00)	132.00 (96.00, 222.00)	0.001
CD4 + /CD8 + ratio	1.66 (1.15, 2.66)	2.16 (1.47, 3.33)	0.020
CD19 + B cell counts (cells/uL)	158.00 (106.00, 248.50)	137.00 (85.00, 193.00)	0.150
NK cell counts (cells/uL)	98.00 (69.50, 180.50)	92.00 (49.00, 136.00)	0.182
D-dimer (μg/mL)	0.59 (0.34, 1.42)	1.63 (0.71, 5.36)	<0.001
Albumin (g/L)	37.14 ± 3.97	33.47 ± 3.04	<0.001

	Mild	Severe	*P* value
Alanine aminotransferase (U/L)	27.00 (1800, 45.50)	28.00 (18.00, 45.00)	0.823
Aspartate aminotransferase (U/L)	30.00 (20.00, 40.00)	31.00 (20.00, 44.00)	0.509
Alkaline phosphatase (U/L)	59.00 (49.00, 78.50)	67.00 (52.00, 92.00)	0.106
γ-glutamyl transpeptidase (U/L)	31.00 (19.50, 60.50)	32.00 (21.00, 68.00)	0.465
Total bilirubin (μmol/L)	9.90 (7.60, 12.55)	13.10 (9.50, 18.30)	0.003
Serum creatinine (μmol/L)	58.00 (46.50, 68.00)	67.00 (57.00, 75.00)	0.005
Lactate dehydrogenase (U/L)	251.00 (208.50, 325.00)	324.00 (242.00, 404.00)	0.019
C-reactive protein (mg/L)	37.50 (13.05, 56.70)	41.80 (17.20, 89.50)	0.061

Discussion

In this study, we retrospectively analyzed the clinical characteristics of 116 laboratory-confirmed COVID-19 patients, and explored the factors associated with the length of time from symptom onset to the first positive nucleic acid test of throat swabs, as well as the risk factors for prolonged conversion time from positive to negative nucleic acid test. We concluded that the prolonged time from symptom onset to positive nucleic acid detection was an independent risk factor for prolonged negative conversion time of SARS-CoV-2. Meanwhile, patients with more severe disease and longer disease duration had a shorter time from symptom onset to positive nucleic acid test; on the contrary, patients with longer conversion time from positive to negative nucleic acid test, showed a longer time from symptom onset to positive nucleic acid test.

The strategy of comprehensive and scientific detection of SARS-COV-2 in specific populations and environments through PCR method provides support for the COVID-19 pandemic prevention and control in China (8, 9). Nucleic acid screening helps identify infected individuals in a timely manner and prevents the spread of this pandemic (10). Nucleic acid detection of SARS-COV-2 virus by RT-PCR is considered the gold standard for COVID-19 diagnosis (2). At present, testing of throat swabs samples is mainly used for the diagnosis of suspected cases, population screening and staff health monitoring, but the results may be interfered by the patient's viral load, the quality of samples, the mutation and recombination of SARS-CoV-2 and other factors, leading to false negative results(7, 11). Younger patients are more likely to have false negative results in the early stages of the disease (12). Therefore, the New Coronavirus Pneumonia

Prevention and Control Protocol (6th edition) states that negative nucleic acid test result does not rule out SARS-COV-2 virus infection. Several studies have reported that RT-PCR produces false negative results, including one study from Beijing which reported a case with two consecutive false negative RT-PCR results (13). Our study found that patients who were tested positive for nucleic acid within a week from the onset of symptoms had a significantly lower rate of severe disease than those who were tested more than a week later. Therefore, there is a need to develop highly sensitive and specific test methods, to improve RT-PCR assays and serological analysis (14), in order to reduce false negative results and promote the timely diagnosis, and eventually to reduce the rate of severe patients. At the same time, our study found that disease severity was a protective factor for the time from symptom onset to positive nucleic acid test: the more severe the patients' disease, the shorter the time from symptom onset to positive nucleic acid test. The reason may be related to the significantly higher viral load in severe patients than in mild patients (15). Studies have reported that the viral load of nasopharyngeal swabs in severe patients can even reach 60 times that of mild patients (16). However, a retrospective study from New York University found that the initial viral load was significantly higher in mild COVID-19 patients than in those severe patients who required hospitalization (17). Further studies are needed to further investigate the relationship between disease severity and viral load in COVID-19 patients.

The COVID-19 prevention and control protocol was based on the detection of SARS-CoV-2 nucleic acid in samples of respiratory tract or blood by real-time fluorescent RT-PCR to determine diagnostic and discharge criteria for patients (18). However, there is no consistent standard to accurately define the duration of SARS-CoV-2 virus infection in the diagnosis and treatment of this disease in actual clinical environment. Only a few studies have analyzed the factors influencing the conversion time from positive to negative test using throat swabs samples for nucleic acid detection. Our study found that the prolonged time from symptom onset to positive nucleic acid test was an independent risk factor for prolonged negative conversion time of SARS-CoV-2 RNA, and if patients were diagnosed in time, the severity of the disease can be effectively predicted and the progression of the disease from mild to severe condition can be reduced, which is of great clinical significance for the pandemic prevention and control of COVID-19. A retrospective cohort study from Wuhan, China, found that the median duration of viral shedding in recovered patients was 20.0 days, and the longest duration of viral shedding was 37.0 days (19). However, some of the infected individuals showed persistent positive nucleic acid test result. A study from Shanghai, China, reported that four COVID-19 cases with persistent positive nucleic acid test had an average conversion time of 61 days from positive to negative nucleic acid test (20). In our study, the median conversion time from the first positive to negative nucleic acid test was 24 days and 25 days in mild and severe patients, respectively, and the conversion time in one of the severe patients was up to 75 days. Zhou et al. found that the duration of viral RNA shedding in the upper respiratory tract specimens from a 75-year-old male patient with COVID-19 reached 111 days (21). Patients infected with SARA-CoV-2 are still shedding virus after six months of treatment, and this is called a chronic carrier state in the human body. The long-term positive viral RNA test result may be due to the damage of the immune system, immune tolerance and escape, or the mutation of the virus (20, 22). Further studies are still needed to verify whether COVID-19 will form a chronic carrier state in humans.

The condition of severe patients with COVID-19 is critical and can change rapidly(23). Our results demonstrated that the white blood cell count, neutrophil count, and lymphocyte count were significantly lower in the severe group than in the mild group, suggesting that early diagnosis and intervention are very important to reduce the risk of death caused by the tendency to severe condition transformation(24). A study comparing the virus shedding time between patients hospitalized in ICU and those not hospitalized in ICU, showed that the duration of virus shedding time of blood, saliva, and nasal samples was longer in ICU patients than in non-ICU patients(25). The reason may be related to the fact that severe patients might be more likely to receive invasive mechanical ventilation (15, 26). However, Zheng et al. (27) and Zhou et al. (21) found there was no association between the virus shedding time and the severity of COVID-19. In this study, we divided the virus shedding time into two time periods: from symptom onset to positive nucleic acid test and from positive nucleic acid test to negative nucleic acid test, and the study results indicated that there was no association between the severity of the disease and the time from positive to negative nucleic acid test of the throat swab samples. Further studies revealed that there were no significant differences in the average length of hospital stay between these two groups. The severity of the disease did not affect the conversion time of nucleic acid test and the length of hospital stay, which may be due to the fact that there was no significant positive correlation between the copy number of SARS-CoV-2 viral RNA and the severity of the disease (28). And viral clearance of SARS-CoV-2 may depend mainly on the host's own immune status. Previous studies have shown that advanced age and corticosteroid therapy use are risk factors for prolonged nucleic acid conversion. Elderly COVID-19 patients often show impaired immunity, which reduces the body's ability to clear the virus (29). Glucocorticoid therapy may be effective in suppressing T-lymphocyte-mediated immune responses (4, 30). In clinical practice, some severe COVID-19 patients have a short conversion time from positive to negative nucleic acid test, however, some mild patients and asymptomatic patients with SARS-CoV-2 infection may have a longer conversion time from positive to negative nucleic acid test due to insufficient immune responses(31). Further clinical and scientific researches are still needed to explore the effect of disease severity on the time of nucleic acid conversion of SARS-CoV-2 virus.

Conclusions

Patients with more severe disease and longer duration of disease before admission to hospitals had a shorter time from symptom onset to positive nucleic acid test. Prolonged time from symptom onset to positive nucleic acid test was an independent risk factor for the prolonged negative conversion time of SARS-CoV-2 virus, and the severity of the disease had no correlation with negative conversion time of SARS-CoV-2 virus.

Abbreviations

COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; WHO: World Health Organization; IQR: Interquartile range;

RT-PCR: real-time reverse transcriptase polymerase chain reaction.

Declarations

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Authors' contributions

Yongzhen Zhai and Kaihuan Yu contributed to the conception and design of this research and finally approved the version to be submitted. Xin Zang, Liangkun Xiong, Junyao Zhu and Fangfang Zhao performed data analysis and interpretation and wrote the manuscripts. Liangkun Xiong, Shihong Wang and Wenhui Zeng contributed to data acquisition and inspection.

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Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study protocol meets the requirements of the Medical Ethics Committee of Renmin Hospital of Wuhan University. Written informed consent was waived due to the urgency of the COVID-19 pandemic.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Infectious Disease, Shengjing Hospital of China Medical University, Shenyang, 110004, China

[2]Department of Hepatobiliary Surgery, Renmin Hospital of Wuhan University, Wuhan, 430000, China.

[3]Department of Infectious Disease, Fujian Medical University Affiliated First Quanzhou Hospital, Quanzhou, Fujian 362000, China.

[4]Department of Paediatrics, Renmin Hospital of Wuhan University, Wuhan, 430000, China.

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