
Chronological Changes of Viral Shedding in Adult Inpatients with COVID-19 in Wuhan, China

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Summary The serial viral loads of SARS-CoV-2 in patients with COVID-19 revealed viral replication and shedding in different sites during the treatment. The resurgence of SARS-CoV-2 informed that a stricter discharge criterion should be taken.

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Abstract

Background In December 2019, the coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) broke out in Wuhan. Epidemiological and clinical characteristics of patients with COVID-19 have been reported, but the relationships between laboratory features and viral load has not been comprehensively described.

Methods Adult inpatients (≥ 18 years old) with COVID-19 who underwent multiple (≥ 5 times) nucleic acid tests with nasal and pharyngeal swabs were recruited from Renmin Hospital of Wuhan University, including general patients ($n=70$), severe patients ($n=195$) and critical patients ($n=43$). Laboratory data, demographic and clinical data were extracted from electronic medical records. The fitted polynomial curve was used to explore the association between serial viral loads and illness severity.

Results Viral load of SARS-CoV-2 peaked within the first few days (2-4 days) after admission, then decreased rapidly along with virus rebound under treatment. Critical patients had the highest viral loads, in contrast to the general patients showing the lowest viral loads. The viral loads were higher in sputum compared with nasal and pharyngeal swab ($p=0.026$). The positive rate of respiratory tract samples was significantly higher than that of gastrointestinal tract samples ($p<0.001$). The SARS-CoV-2 viral load was negatively correlated with portion parameters of blood routine and lymphocyte subsets, and was positively associated with laboratory features of cardiovascular system.

Conclusions The serial viral loads of patients revealed whole viral shedding during hospitalization and the resurgence of virus during the treatment, which could be used for early warning of illness severity, thus improve antiviral interventions.

Key words coronavirus disease 2019, laboratory features, viral load, viral shedding

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Introduction

In early December 2019, the type of pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) broke out in Wuhan, China [1, 2]. The World Health Organization (WHO) has characterized the coronavirus disease 2019 (COVID-19) as a pandemic[3]. As of May 11, 2020, over 4.3 million confirmed cases and more than 280,000 deaths were reported globally, including 82919 cases and 4633 deaths in China, including 54214 cases and 3869 deaths in Wuhan [4].

Recently, researchers have described the clinical characteristics and risk factors of COVID-19[5, 6]. Details of the laboratory test and virological course of COVID-19 have not yet been comprehensively investigated. Here, we report findings from a comprehensive analysis of viral load and laboratory findings to reveal viral replication and shedding during hospitalization. Chronological changes in viral shedding of SARS-CoV-2 after admission in individual patients, overall patients and patients grouping by illness severity (general, severe and critical) were shown by polynomial fitting curves. Meanwhile, serum albumin was decreased in high viral load patients, and serum glucose, corrected calcium were increased in high viral load patients. Of particular note, serum CKMB, MYO, ultra-TnI and NT-proBNP were significantly increased in high viral load patients, indicating that high viral loads were more likely to damage cardiovascular system. We also investigated the link between viral load and laboratory findings. Our data will provide general tendency of viral replication and shedding, which could help better guiding the clinical treatment.

Methods

Study design and participants

A total of 308 adult inpatients (≥ 18 years old) from Renmin hospital of Wuhan University between January 11 to February 21, 2020 were enrolled in this retrospective study. Each patient was diagnosed as COVID-19 according to the WHO interim guidance [7] and underwent multiple (at least 5 times with nasal and pharyngeal swab) nucleus acid testing. A confirmed COVID-19 case was defined as a positive result from the real-time reverse transcriptase polymerase chain reaction (qRT-PCR) assay according to the manufacturer's protocol (Shanghai GeneoDx Biotechnology, Shanghai, China) of nasal and pharyngeal swab specimens[8]. The clinical outcomes, such as, discharges, mortality, were followed up to March 6, 2020. The study was approved by the Research Ethics Commission of Renmin Hospital (Approval No. WDRY2020-K066).

Data collection

Data were extracted from electronic medical records and laboratory information system using a standardized form. All data were checked by two researchers (M-Y X and M-J L) independently for accuracy.

Laboratory procedures

Complete blood count, coagulation testing were assessed at Sysmex XN-9000 and CS-5100 (Sysmex, Japan), assessments of myocardial enzyme, liver and renal function, electrolytes were performed at ADVIA centaur XP immunoassay system and ADVIA 2400 chemistry system (Siemens, German), measurement of procalcitonin, N-terminal pro brain natriuretic peptide (NT-proBNP) were performed at cobas e 601 system (Roche, Switzerland), C-reactive protein was assessed at i-Chroma system (Boditech, Korea), arterial blood gas analysis was detected at GEM3000 (Instrumentation Laboratory, USA), lymphocyte subsets and cytokines

were assessed using the BD FACSCalibur™ Flow Cytometer (Becton, Dickinson and Company, USA).

Specimens, including nasal and pharyngeal swab, sputum, stool, urine, blood, eye secretion, bronchoalveolar lavage fluid, vaginal secretion, cerebrospinal fluid and milk, collected at multiple time points were used for testing the *ORF1ab* gene of SARS-CoV-2 by qRT-PCR[8]. The cycle threshold (Ct) values of qRT-PCR were collected, which were inversely proportional and exponential correlated with copies number of the virus. We defined the degree of severity of COVID-19 (general, severe and critical) according to the Chinese management guideline for COVID-19 (version 7.0)[9, 10]. Common cases defined as showing fever and respiratory symptoms with radiological findings of pneumonia. Severe cases meet any of the following criteria: respiratory distress (≥ 30 breaths/min), or oxygen saturation $\leq 93\%$ at rest, or arterial partial pressure of oxygen (PaO₂)/ fraction of inspired oxygen (FiO₂) ≤ 300 mmHg, or chest imaging showing an obvious lesion progression within 24-48 hours $>50\%$. Critical cases met any of the following criteria: respiratory failure and requiring mechanical ventilation, shock, or exhibiting other organ failure that requires ICU care[9, 10].

Treatment

According to the Chinese management guideline for COVID-19 (version 7.0), patients were received antiviral therapy, respiratory support, circulatory support, blood purification treatment, immunotherapy and traditional Chinese medicine treatment. Antiviral therapy included α -interferon (5 million U, atomization inhalation twice daily), lopinavir/ritonavir (200 mg/50mg per pill for adults, two pills each time, twice daily), ribavirin (500 mg each time for adults, twice or three times of intravenous injection daily), chloroquine

phosphate/hydroxychloroquine (500 mg bid for 7 days for adults aged 18-65 with body weight over 50 kg; 500 mg b.i.d. for Days 1&2 and 500 mg qid for days 3-7 for adults with body weight below 50 kg), arbidol (200 mg tid for adults)[9, 10]. The chloroquine phosphate/hydroxychloroquine treatment has started to be administered since February 19, 2020.

Statistical analysis

Continuous variables were expressed as medians and interquartile ranges, categorical variables were summarized as counts and percentages. A two-sided α of less than 0.05 was considered statistically significant. All analyses were performed with SPSS software (version 19.0). The polynomial fitting models and curves were performed by Python (version 3.6), with R-square as criterion to evaluate these models.

Results

Epidemiologic Features

The viral load testing of SARS-CoV-2 was performed for each patient during hospitalization, and those patients (n=308) who underwent at least 5 times nucleus acid tests with nasal and pharyngeal swab were enrolled in the study. At the conclusion of the study period, 16 patients died of COVID-19, 82 patients were discharged, and 210 patients remained in the hospital. The median age of 308 patients was 63.0 years (IQR 52.0–71.0), and male ratio was 49.0% (Table 1). Fever (87.0%) and cough (67.2%) were the most common symptoms, followed by shortness of breath (42.5%) and fatigue (30.2%) (Table 1). 70 patients were defined as common cases, 195 patients were severe, and 43 patients were critically ill. Accompanied with exacerbation of COVID-19, the counts and percentages of basophil, eosinophils, lymphocyte, T lymphocyte, T

helper (Th)(CD4+) lymphocytes, T suppressor (Ts) (CD8+) lymphocytes were significantly decreased (Table.1). Besides, severe coagulopathy and evaluated myocardial enzyme were major characteristics of critical patients (Table.1). The average Ct values of *ORF1ab* gene of SARS-CoV-2 were lower in deceased patients, and high viral loads of SARS-CoV-2 (Ct values <30) were observed in nearly half of deceased patients (6/16), suggesting that high viral load of SARS-CoV-2 was an importance feature for deceased patients (Table.S1). Elevated myocardial enzyme, severe coagulopathy, abnormal liver and renal functions were major laboratory features of critical patients (Table.S1). Notably, significant elevated IL-6, lactate dehydrogenase (LDH), and significant reduced lymphocyte and lymphocyte subsets were another features of deceased patients, which were agreement with previous study (Table.S1)[5].

Viral Load in Clinical Specimens

There were 2475 specimens collected from 308 patients with COVID-19 during hospitalization. Although bronchoalveolar lavage fluid specimens showed the highest positive rate, 50% (6/12) with median Ct value 38.2 (IQR 34.4-39.5), the accuracy was limited to the small size samples. Nasal and pharyngeal swab specimens showed 40% (665/1663) positive rate with median Ct value 36.9 (IQR 34.5-38.5), followed by sputum (34.4% (161/468), median Ct value: 37.1(IQR 32.6-38.7), urine (14.3% (1/7) and stool (13.5% (37/273), median Ct value: 34.0 (IQR 27.9-36.4). No SARS-CoV-2 was detected for blood, eye secretion, cerebrospinal fluid, milk and vaginal secretions specimens.

To identify the optimal specimen for SARS-CoV-2 testing, we compared the positive rate and Ct values of nasal and pharyngeal swabs, sputum and stool from the same patient at the same time. The positive rate of nasal and pharyngeal swabs and sputum were similar,

significantly higher than the rate in stool samples ($p < 0.001$). The paired Wilcoxon test showed that Ct values of sputum specimens were lower than nasal and pharyngeal swab specimens (Fig.1A, $p = 0.026$; Table S2), indicating sputum samples contained higher viral load compared with nasal and pharyngeal swab samples. Although there was no significant difference in viral load between nasal and pharyngeal swabs and stool (Fig.1B, $p = 0.581$), the positive rate of stool samples (13.5%) was lower than that in nasal and pharyngeal swab samples (40.0%) (Table S2). Notably, the Ct values of positive stool samples, 33.8 (26.2-38.7) were lower than that in positive nasal and pharyngeal swab samples, 38.3 (35.5-39.2), revealing viral load in stool samples were much higher than nasal and pharyngeal swab samples in the patients with gastrointestinal infection (Table S2). Of 5 patients showing a 100-fold viral load in stool samples (Fig.S1B, J-L), 3 of 5 patients had gastrointestinal symptoms (1/3 with constipation, 2/3 with diarrhea), a patient had sigmoid colon cancer, and another one had no gastrointestinal symptoms. Our data supported that viral shedding of gastrointestinal infection also was the feature of patients with COVID-19 [11].

Chronological Changes in Viral Shedding After Hospital Admission

We observed the viral excretion from respiratory and gastrointestinal tracts in all patients by a chronological series during hospitalization. The changes of Ct values in the serial nasal and pharyngeal swab, sputum and stool samples could approximately reflect the changes of viral load in these patients. We analyzed the viral load in specimens (include nasal and pharyngeal swab, sputum and stool) obtained from the 308 inpatients after admission by polynomial fitting models using Python. The R-square serves as the criterion to evaluate the best models. Our polynomial fitting curves suggested that the highest viral loads (inversely related to Ct value)

were detected a few days after admission and then decreased accompanied with treatment (Fig.2B). Although the overall viral load was reduced, the viral load could rebound within several days (day7, 16, 22 after admission) (Fig.2B). The chronological series Ct values of hospitalized patients showed similar change, viral loads decreased rapidly after admission, but rebounded in day 7-10, day 15-18 and day 20-22 (Fig. 2A). The viral load of critical patients was much higher than general patients and severe patients in the early stage of hospitalization, and then decreased rapidly under treatment (Fig.2C). The overall viral load of general patients was lower than severe patients (Fig.2C), but the rebound of viral was more common. On day 30 of treatment, the Ct values approximated to 40, indicating that the virus was molecularly undetectable in most patients.

The viral shedding in different types of specimens appeared to be diverse. The average viral load of sputum was the highest, followed by nasal and pharyngeal swab and stool (Fig.2D). Interestingly, different specimens from same patients were various (Fig.S1A-L). Sixteen of thirty-one patients showed higher viral loads in sputum than that in nasal and pharyngeal swab (Fig.S1A, C-I). For the majority patients, the positive rate in stool samples was lower than that in nasal and pharyngeal swabs. However, some patients (Patient 2, 10, 11 and 12) showed higher viral load in stool (Fig.S1B, J-L), which indicated that viral shedding in gastrointestinal tract was the major exposure in these patients. Notably, 42.5%(17/40) of patients were consecutively tested for negative at least twice and turned to positive in the next detection (Fig.S1D, G and J), which indicated that a stricter discharge criterion should be performed in nuclei acid test to prevent viral rebound outside hospital.

Viral Load and Laboratory Characteristic

Next, we analyzed the associations of viral load with clinical features. High viral load cases had higher neutrophil counts (3.62×10^9 ; $p=0.0001$), lower lymphocytes counts (1.33×10^9 ; $p=0.0034$), as well as eosinophils counts (0.09×10^9 ; $p=0.0004$), and basophils counts (0.40×10^9 ; $p=0.0053$) (Table 2). The number of T cells were significantly decreased in patients with COVID-19, which was more evident in the high viral load cases ($783/\mu\text{L}$; $p<0.0001$) compared with the low viral load group (Table.2). The decline of Th cells and Ts cells were more pronounced in high viral load cases, suggested T cells were shown to be more affected by SARS-CoV-2. Notably, the myocardial enzymes, include creative kinase MB (CKMB), myoglobin (MYO), ultra-troponin I (ultra-TnI) and N-terminal pro-brain natriuretic peptide (NT-proBNP), were significantly increased in high viral group, confirmed that cardiovascular was an important target of SARS-CoV-2. Besides, elevated serum albumin, hypophosphatemia and hypercalcemia were observed in high viral load group. Elevated LDH was another feature of high viral load cases, which indicated poor prognosis in COVID-19 patients[5].

Discussion

This retrospective study reported the series viral loads of the 308 hospitalized patients with COVID-19. The respiratory tract samples tested positive more frequent for SARS-CoV-2 than the gastrointestinal tract samples. The lower respiratory tract samples showed higher viral load than the upper respiratory tract samples (Fig.1A). Human ACE2 protein was the receptor to facilitate viral into target cells[12], and alveolar epithelial type II cells (AECII) were the major ACE2-expressing cells[13], suggesting that these cells can serve as the major target cells for viral invasion, resulting in a higher viral load in lower respiratory tract samples. The sputum sample was a better source in monitoring viral replication in respiratory tract. While ACE2

stained positive mainly in the cytoplasm of gastrointestinal epithelial cells[11], SARS-CoV-2 could be detected in gastrointestinal tract samples. Although the positive rate of stool samples was lower, five patients (Fig.S1B, J-L) showed higher viral loads in stool samples compared with the respiratory tract samples, and stool samples remained to be detectable after nasopharyngeal swabs turned negative. These findings suggested that the gastrointestinal tract samples were of great importance for treatment efficiency evaluation.

In this study, the viral load in patients with COVID-19 was found to have peaked within the first few days after admission, and then decreased rapidly along with virus rebound after treatment. The serial copy numbers of SARS-CoV-2 were associated with disease severity, the critically ill patients had the highest viral load peak and the general patients showed the lowest average viral load (Fig.2C), which is similar to SARS[14]. Toward the end of this period virus was intermittently detectable from clinical samples, and the viral shedding in stools persisted longer than respiratory samples in portion patients, suggesting a strict discharge criterion should be performed to avoid transition by the fecal-oral route, i.e., their respiratory tract samples and gastrointestinal tract samples should be monitored till testing for negative SARS-CoV-2 at least two times continuously. In a previous study, SARS-CoV-2 was detected in 1% (3/307) blood samples[15]. Though limited by a small sample size of 31 blood samples, we were not able to detect SARS-CoV-2 in blood by qRT-PCR, suggesting that the virus probably did not cause viremia. The average viral load decreased rapidly under treatment, but could rebound within 1-3 weeks (i.e., day7, 16, 22 after admission) (Fig. 2B), and a similar rebound pattern was observed in individual patients (Fig. 2A). The phenomenon may be due to that AECII cells were the major ACE2-expressing cells. Therefore, clear coronavirus particles were

observed in both bronchiolar epithelial cells marked by cilia and AECII cells[16], and the anti-viral can act on upper respiratory tract and most part of lower respiratory tract, but bronchioli terminals and alveolar epithelial type II cells may be hardly affected. The surviving coronavirus particles in bronchioli terminals and AECII cells may result in SARS-CoV-2 rebound in COVID-19 patients, and aerosol delivery treatment may minimize the viral load rebound. Because of the side effects, the course of anti-viral treatment was not longer than 10 days, drug resistance may result in the viral rebound at the end course of anti-viral treatment (i.e., day7, 16, 22 after admission), suggesting that the anti-viral medicine should be changed to inhibit virus.

Abnormal complete blood count was a notable feature of COVID-19 patients, with decreased basophil, eosinophils and lymphocyte in peripheral blood. Besides, the count of basophil, eosinophils, lymphocyte, CD3 T lymphocyte, CD4 T lymphocyte, CD8 T lymphocyte was significantly negatively associated with viral load in our study. A pathological result of three COVID-19 cases reported that cell degeneration and necrosis were observed in spleen and all three cell lines decreased in bone marrow[17]. Recently, we detected high viral load of SARS-CoV-2 in bone marrow from an 84-year-old woman with severe leukopenia (data not published) in Renmin Hospital of Wuhan University. We reasoned that immune cells or organs were candidate targets of SARS-CoV-2, which needed more studies to confirm. In addition to the lung, ACE2 is also highly expressed in the heart, playing vital role in the cardiovascular system[18]. Although SARS-CoV-2 mainly invades the lung, it can also cause myocardial injury. Cardiac complications, including heart failure, arrhythmia and myocardial infarction, were major comorbidities in COVID-19 patients[5, 8]. Our findings also supported that

cardiovascular failure was one of the most frequently reported underlying mortality causes for COVID-19 patients[19]. Taken together, that replication of SARS-CoV-2 resulted in severe cardiac complications and monitoring the laboratory features (i.e., CKMB, MYO, ultra-TnI, Nt-ProBNP, and calcium) was helpful for the early screening of critical illness and treatment of COVID-19. LDH was found in all human cells, especially in myocardial and liver cells. Meanwhile, LDH was induced upon T cell activation[20] and modulated the inflammatory response in macrophages[21]. LDH elevation was an important feature in COVID-19 patients[5, 22], and might be a predictive feature in COVID-19 patients.

More importantly, personal protective measures and disinfections should be taken to protect health care workers/ household contacts of individuals who reside in the hospital for protracted periods or who are discharged from the hospital according to the qRT-PCR results.

NOTES

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Conflict of interest

The authors declare that they have no competing interests.

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Table 1: Demographic, clinical, laboratory findings of patients with COVID-19 at the end of study

	General(n=70)	Severe (n=195)	Critical (n=43)	p value
Age, years	58.5 (44.5-67.2)	63 (52.0-71.0)	69 (60.0-81.0)	<0.0001
Sex				
Male	34 (48.6%)	87 (44.6%)	30 (69.8%)	0.0115
Female	36 (51.4%)	108 (55.4%)	13 (30.2%)	
Signs and symptoms No. (%)				
Fever	60 (85.7%)	170 (87.2%)	38 (88.4%)	0.9141
Cough	52 (74.3%)	128 (65.6%)	27 (62.8%)	0.3347
Shortness of breath	23 (32.9%)	84 (43.1%)	24 (55.8%)	0.0548
Fatigue	17 (24.3%)	62(31.8%)	14 (32.6%)	0.4699
Diarrhea	10 (14.3%)	27(13.8%)	4 (9.3%)	0.7030
Myalgia	4 (5.7%)	19 (9.7%)	4 (9.3%)	0.5876
Respiratory support				
Oxygen therapy	45 (64.3%)	74 (37.9%)	0 (0%)	<0.0001
High-flow oxygenation	19 (27.1%)	121(62.1%)	27 (62.8%)	<0.0001
Invasive mechanical ventilation	0 (0%)	0 (0%)	13(30.2%)	<0.0001
ECMO	0 (0%)	0 (0%)	3 (7.0%)	0.002
Anti-viral treatment				
Arbidol	65(92.8%)	157(80.5%)	41(95.3%)	0.006
Ribavirin	21(30.0%)	85(43.6%)	25(58.1%)	0.012

Chloroquine	23(32.8%)	98(50.3%)	29(67.4%)	0.001
phosphate/hydroxychloroquine				
Lopinavir/ritonavir	0 (0%)	15(7.7%)	20(46.5%)	<0.0001
Outcome				
Discharged	18 (25.7%)	59 (30.3%)	5 (11.6%)	0.0429
Hospitalization	52 (74.3%)	136 (69.7%)	22 (51.2%)	0.0221
Death	0 (0%)	0 (0%)	16 (37.2%)	<0.0001
Cycle threshold values	37.36(35.09-38.80)	36.93(34.27-38.67)	36.85(33.34-38.61)	0.1328
Complete blood count				
WBC, $\times 10^9/L$	5.71(4.93-7.41)	6.01(4.64-8.24)	8.03(5.64-12.37)	<0.0001
Neu, $\times 10^9/L$	2.93(2.29-3.71)	3.37(2.22-4.63)	4.33(2.58-7.03)	<0.0001
Neu percentage, %	54.90(47.60-62.30)	61.65(52.68-69.60)	67.90(53.05-78.00)	<0.0001
LYM, $\times 10^9/L$	1.75(1.36-2.12)	1.35(1.08-1.76)	1.32(1.00-1.76)	<0.0001
LYM percentage, %	30.70(24.33-36.60)	24.90(18.05-31.15)	18.60(12.10-26.95)	<0.0001
Mono, $\times 10^9/L$	0.54(0.43-0.74)	0.53(0.41-0.73)	0.63(0.48-1.09)	<0.0001
Mono percentage, %	9.80(8.30-12.60)	9.30(7.60-11.50)	9.10(7.45-12.50)	0.0494
EOS, $\times 10^9/L$	0.11(0.05-0.19)	0.11(0.06-0.18)	0.11(0.02-0.19)	0.3172
EOS percentage, %	2.20(1.05-3.30)	2.00(0.90-3.13)	1.80(0.20-2.60)	0.0012
BASO, $\times 10^9/L$	0.04(0.02-0.05)	0.03(0.02-0.05)	0.03(0.01-0.04)	0.0033
BASO percentage, %	0.70(0.40-0.90)	0.50(0.30-0.80)	0.30(0.20-0.70)	<0.0001
RBC, $\times 10^{12}/L$	3.98(3.67-4.45)	3.85(3.37-4.24)	3.85(3.42-4.39)	<0.0001
	121.00(110.00-			
Hb, g/L	131.00)	115.00(103.00-125.00)	116.00(101.00-130.00)	<0.0001

	215.00(163.00-			0.5369
PLT, $\times 10^9$ /L	261.00)	225.00(172.00-274.00)	217.00(149.00-266.00)	
CRP, mg/L	4.90(4.90-4.90)	4.90(4.90-12.00)	6.20(4.90-29.10)	<0.0001
PCT, ng/mL	0.04(0.03-0.05)	0.05(0.04-0.08)	0.08(0.05-0.15)	<0.0001
IL6, pg/mL	10.58(2.17-120.25)	12.41(3.46-95.00)	15.90(6.33-103.50)	0.3803
Coagulation				
PT, s	11.20(10.90-11.50)	11.20(10.80-11.80)	11.70(11.20-12.40)	<0.0001
PTINR	0.95(0.93-0.98)	0.95(0.92-1.01)	1.00(0.95-1.06)	<0.0001
Ptact, s	98.50(93.20-104.20)	98.50(88.50-106.20)	90.00(79.80-98.80)	<0.0001
APTT, s	25.60(24.70-27.20)	25.40(24.00-27.30)	26.00(24.70-29.60)	0.0006
DDimer, ng/mL	0.59(0.33-1.01)	1.17(0.56-2.71)	2.42(1.32-4.93)	<0.0001
FDP, μ g/mL	1.40(0.56-3.01)	3.57(1.51-8.57)	7.87(4.52-13.77)	<0.0001
FIB, g/L	2.86(2.48-3.48)	3.33(2.70-4.42)	3.41(2.79-4.55)	<0.0001
TT, s	17.90(17.30-18.50)	18.00(16.90-19.00)	17.70(16.80-18.80)	0.1932
Lymphocyte Subsets				
	203.00(153.00-			
B cells (CD3 -CD19+) / μ l	244.00)	160.00(109.00-230.50)	206.00(129.00-317.00)	<0.0001
B cells (CD3 -CD19+) %	12.04(9.91-14.84)	12.61(9.09-16.90)	16.98(12.34-24.74)	<0.0001
	1188.00(935.00-			
T cells (CD3+CD19 -) / μ l	1440.00)	910.00(687.00-1184.50)	811.00(621.00-1099.00)	<0.0001
T cells (CD3+CD19 -) %	71.26(66.74-76.40)	71.76(63.83-77.38)	66.50(63.55-71.00)	<0.0001
	203.00(133.00-			
NK cells (CD16+CD56+) / μ l	281.00)	151.00(103.50-232.00)	125.00(88.50-231.25)	<0.0001

NK cells (CD16+CD56+) %	12.91(8.64-17.88)	12.15(8.34-18.06)	12.53(8.00-17.39)	0.9707
	690.00(586.00-			
Th cells (CD3+CD4+) /μl	837.00)	544.00(387.50-705.00)	514.00(305.00-638.00)	<0.0001
Th cells (CD3+CD4+) %	43.63(35.96-49.55)	40.78(35.30-48.11)	36.93(30.79-42.38)	<0.0001
	404.00(291.00-			
Ts cells (CD3+CD8+) /μl	587.00)	316.00(216.50-462.50)	340.50(202.25-503.00)	<0.0001
Ts cells (CD3+CD8+) %	25.49(21.70-30.79)	25.16(19.37-31.67)	28.13(20.06-34.28)	0.1360
Th/Ts	1.82(1.15-2.42)	1.72(1.22-2.25)	1.36(0.86-1.93)	0.0031
Myocardial enzyme				
CKMB, μg/L	0.50(0.32-0.74)	0.56(0.37-0.86)	1.09(0.75-2.17)	<0.0001
MYO, μg/L	27.04(20.73-32.06)	24.64(19.45-29.36)	35.47(22.31-115.88)	<0.0001
UltraTnI, μg/L	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.03)	<0.0001
NTproBNP, ng/L	45.18(21.29-122.30)	74.18(26.83-177.80)	207.30(52.33-752.93)	<0.0001
Electrolytes				
K, mmol/L	4.17(3.95-4.44)	4.22(3.95-4.51)	4.19(3.85-4.61)	0.4036
Na, mmol/L	142.0(140.0-145.00)	142.0(140.0-146.0)	142.0(138.0-146.0)	0.2824
	107.00(104.90-			
Cl, mmol/L	107.80)	106.00(103.90-107.70)	104.10(100.80-107.60)	<0.0001
Ca(adj), mmol/L	2.34(2.21-2.48)	2.43(2.28-2.62)	2.49(2.32-2.72)	<0.0001
Mg, mmol/L	0.82(0.78-0.87)	0.85(0.79-0.89)	0.87(0.80-0.91)	<0.0001
IP, mmol/L	1.22(1.10-1.33)	1.21(1.07-1.34)	1.07(0.94-1.31)	<0.0001
Liver function				
ALB, g/L	38.20(36.40-40.80)	37.40(34.40-39.70)	35.90(31.80-38.60)	<0.0001

ALT, U/L	25.00(16.50-45.00)	31.00(18.00-50.00)	34.00(19.00-64.00)	0.0126
AST, U/L	20.00(16.00-28.00)	23.00(18.00-34.00)	27.00(20.00-42.00)	<0.0001
	183.00(168.00-			
LDH, mmol/L	204.00)	204.00(181.00-232.25)	259.00(197.00-360.00)	<0.0001
Glu, mmol/L	5.08(4.71-6.11)	5.15(4.66-6.09)	5.66(4.89-8.52)	<0.0001

Data are median (IQR), n (%), or n/N (%). p values were calculated by Mann-Whitney U test and χ^2 test. WBC, White blood cell;

Neu, Neutrophils; LYM, Lymphocytes; Mono, Monocyte; EOS, Eosinophils; BASO, Basophils; RBC, Red blood cell; Hb,

Hemoglobin; PLT, Platelet; CRP, C-reactive protein; PCT, Procalcitonin; IL6, Inter Leukin 6; PT, prothrombin time; PTINR,

international normalized ratio; PTact, activated clotting time; APTT, activated partial thromboplastin time; FDP, fibrinogen

degradation products; TT, thrombin time; FIB, fibrinogen; CKMB, creatine kinase isoenzyme; MYO, myoglobin; ultraTnI, high

sensitivity troponin I; NTproBNP, N-terminal pro-B-type natriuretic peptide; ALB, Albumin; ALT, alanine aminotransferase; AST,

aspartate aminotransferase; LDH, lactate dehydrogenase; Glu, glucose.

Table 2. Laboratory features in different viral load

	Negative	Low viral load	High viral load	p value
Age, years	61.00(49.00-69.00)	62.00(51.00-69.00)	62.00(51.00-70.00)	0.0008
Sex				
Male	1589 (66.0%)	402 (16.7%)	417 (17.3%)	0.6970
Female	1652 (65.3%)	445 (17.6%)	431 (17.0%)	
Complete blood count				
WBC, $\times 10^9/L$	5.86(4.74-8.03)	6.10(4.93-8.00)	5.96(4.73-8.63)	0.5529
Neu, $\times 10^9/L$	3.19(2.29-4.45)	3.52(2.62-4.93)	3.62(2.58-5.32)	0.0001
Neu percentage, %	59.60(50.75-68.50)	61.80(52.55-70.50)	62.40(54.80-74.20)	<0.0001
LYM, $\times 10^9/L$	1.41(1.10-1.85)	1.45(1.08-1.88)	1.33(1.03-1.70)	0.0034
LYM percentage, %	25.90(18.60-32.80)	25.70(17.60-32.40)	24.10(16.10-30.20)	0.0007
Mono, $\times 10^9/L$	0.53(0.41-0.72)	0.52(0.43-0.70)	0.54(0.40-0.77)	0.9536
Mono percentage, %	9.40(7.55-11.80)	8.90(7.40-10.80)	9.30(7.10-11.10)	0.0250
EOS, $\times 10^9/L$	0.11(0.06-0.19)	0.11(0.05-0.18)	0.09(0.03-0.16)	0.0004
EOS percentage, %	2.10(1.00-3.30)	2.00(0.98-3.20)	1.60(0.50-2.80)	0.0001
BASO, $\times 10^9/L$	0.03(0.02-0.05)	0.03(0.02-0.05)	0.03(0.02-0.04)	0.0053
BASO percentage, %	0.60(0.30-0.90)	0.60(0.30-0.80)	0.40(0.20-0.70)	0.0002
RBC, $\times 10^{12}/L$	3.86(3.42-4.29)	3.84(3.42-4.26)	3.80(3.38-4.26)	0.7576
Hb, g/L	116.00(104.00-127.00)	117.00(105.00-127.00)	117.00(106.00-128.00)	0.3772
PLT, $\times 10^9/L$	220.00(163.00-271.00)	222.50(177.50-265.25)	226.00(176.00-272.00)	0.2811
CRP, mg/L	4.90(4.90-11.95)	4.90(4.90-8.80)	4.90(4.90-13.10)	0.0491
PCT, ng/mL	0.05(0.04-0.09)	0.05(0.03-0.08)	0.06(0.03-0.13)	0.2811

IL6, pg/mL	9.70(3.86-56.26)	5.65(2.79-20.18)	8.00(2.90-27.70)	0.0130
Coagulation				
PT, s	11.20(10.80-11.80)	11.20(10.80-11.90)	11.30(10.90-11.90)	0.2426
PTINR	0.95(0.92-1.01)	0.95(0.92-1.02)	0.96(0.93-1.02)	0.2645
Ptact, s	98.50(88.50-106.20)	98.50(86.90-106.20)	96.90(86.30-104.20)	0.2711
APTT, s	25.70(24.30-27.70)	25.60(24.23-27.10)	25.60(24.30-27.85)	0.3962
DDimer, ng/mL	1.04(0.46-2.38)	0.84(0.39-2.23)	1.10(0.48-2.72)	0.2119
FDP, µg/mL	3.33(1.05-7.41)	2.45(0.88-7.21)	3.36(1.23-8.88)	0.2081
FIB, g/L	3.16(2.56-4.10)	3.13(2.56-4.21)	3.20(2.56-4.26)	0.7432
TT, s	17.80(16.80-18.70)	17.80(16.90-18.80)	17.90(17.00-18.80)	0.5999
Lymphocyte Subsets				
B cells (CD3 -CD19+) /µl	168.50(113.00-246.00)	177.00(116.00-254.00)	151.00(85.00-247.75)	0.0577
B cells (CD3 -CD19+) %	12.89(9.52-17.40)	13.05(10.00-17.93)	14.61(10.30-20.36)	0.0040
T cells (CD3+CD19 -) /µl	944.00(699.00-1226.50)	916.00(692.00-1132.00)	783.00(466.25-1126.00)	0.0000
T cells (CD3+CD19 -) %	71.26(64.08-76.52)	71.00(64.23-75.56)	68.24(60.38-75.99)	0.0029
NK cells (CD16+CD56+) /µl	152.00(102.00-236.50)	153.00(95.00-237.00)	135.00(79.75-221.25)	0.0265
NK cells (CD16+CD56+) %	11.83(8.17-17.37)	12.06(8.25-17.27)	13.02(8.42-19.63)	0.4014
Th cells (CD3+CD4+) /µl	571.00(405.50-730.50)	539.00(400.00-691.00)	465.00(268.00-671.25)	<0.0001
Th cells (CD3+CD4+) %	42.18(36.16-48.41)	42.58(35.51-48.58)	40.02(32.73-47.01)	0.0171
Ts cells (CD3+CD8+) /µl	333.50(216.25-462.00)	317.00(217.00-428.00)	265.00(146.50-412.00)	0.0001
Ts cells (CD3+CD8+) %	24.75(19.16-30.25)	23.83(18.71-29.59)	22.41(17.38-31.28)	0.1391
Th/Ts	1.77(1.25-2.34)	1.79(1.34-2.38)	1.71(1.24-2.48)	0.6902
Myocardial enzyme				

CKMB, µg/L	0.63(0.39-0.99)	0.65(0.40-1.12)	0.84(0.53-1.66)	<0.0001
MYO, µg/L	25.41(20.16-34.36)	26.36(21.09-38.58)	32.80(25.04-68.19)	<0.0001
UltraTnI, µg/L	0.01(0.01-0.01)	0.01(0.01-0.01)	0.01(0.01-0.03)	<0.0001
NTproBNP, ng/L	78.41(27.28-290.90)	91.43(28.00-277.98)	173.40(43.30-646.10)	0.0005
Electrolytes				
K, mmol/L	4.21(3.89-4.52)	4.16(3.87-4.47)	4.17(3.91-4.47)	0.1904
Na, mmol/L	142.00(140.00-146.00)	143.00(140.00-146.00)	142.00(140.00-146.00)	0.8553
Cl, mmol/L	106.10(103.80-107.80)	105.90(103.80-107.58)	106.00(103.20-108.00)	0.7327
Ca(adj), mmol/L	2.40(2.23-2.58)	2.40(2.25-2.59)	2.47(2.27-2.67)	<0.0001
Mg, mmol/L	0.84(0.78-0.88)	0.84(0.79-0.89)	0.85(0.79-0.90)	0.0049
IP, mmol/L	1.22(1.06-1.36)	1.22(1.07-1.35)	1.15(0.99-1.30)	<0.0001
Liver function				
ALB, g/L	37.80(34.70-40.50)	37.70(34.80-39.98)	36.30(32.90-39.20)	<0.0001
ALT, U/L	28.00(17.00-49.00)	26.00(16.00-47.00)	26.00(15.00-46.00)	0.2592
AST, U/L	23.00(17.00-33.75)	22.00(16.00-30.00)	23.00(17.00-33.00)	0.3027
LDH, mmol/L	206.00(179.00-245.00)	204.00(174.00-240.00)	220.00(187.00-287.50)	<0.0001
Glu, mmol/L	5.15(4.67-6.23)	5.24(4.70-6.41)	5.47(4.74-7.60)	0.0003

Data are median (IQR) or n (%). p values were calculated by Mann-Whitney U test and χ^2 test. The high viral load and low viral load were divided by the median of Ct values.

Figure Legends

Figure 1. The viral load in different specimens from inpatients with COVID-19. Comparison of the viral load between nasal and pharyngeal swab and sputum (A), stool (B) from the same patients in the same day. Red means nasal and pharyngeal swab samples, blue means sputum samples and yellow means stool samples.

Figure 2 Chronological changes in viral shedding after admission. Chronological changes in Ct values of *Orf1ab* genes by qRT-PCR after admission in individual patients(A), all patients (B), different severity (C) and different samples (D). The polynomial fitting curve taking R-square as criterion was performed by Python, and revealed the viral shedding since hospital admission. The Ct value is inversely related to viral load and a value of 40 means the virus is molecularly undetectable. Data were shown as average Ct values of each day(B-D) and standard deviation (B). The shaded blue area means the standard deviation of Ct values (B).

Figure 1

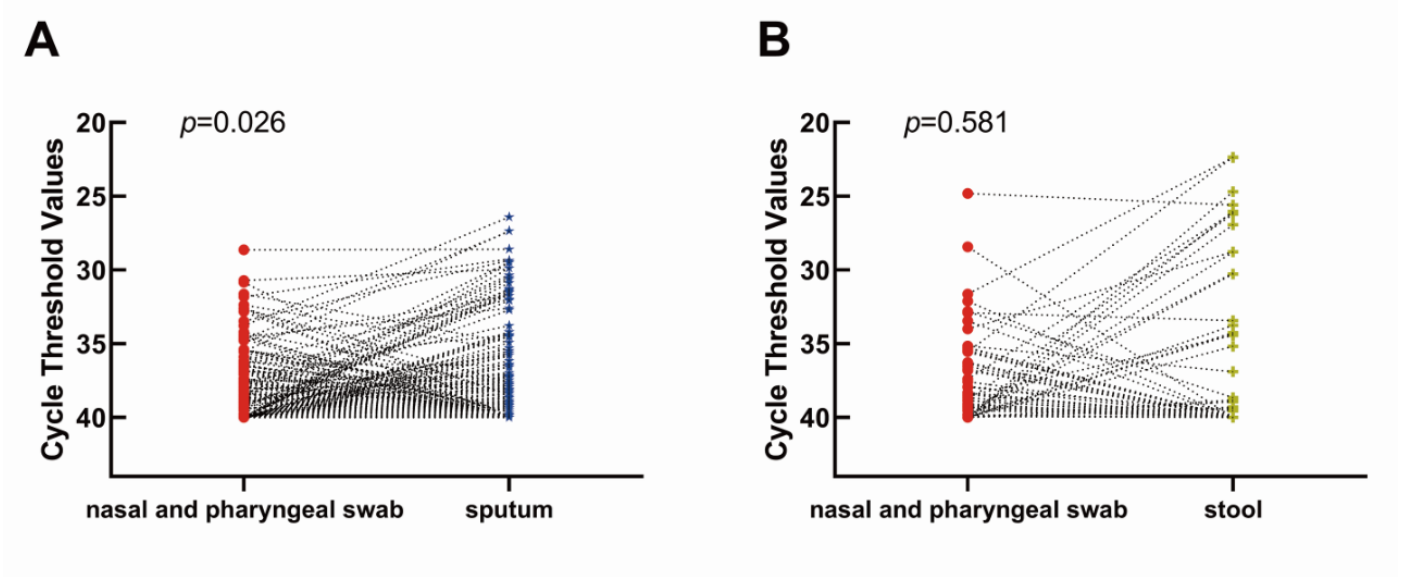


Figure 2

