Viral Load Dynamics and Clinical Disease Severity in Patients with SARS-CoV-2 infection

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Viral Load Dynamics and Clinical Disease Severity in Patients with SARS-CoV-2 infection

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Summary

OBJECTIVE

To study the viral loads at different stages of disease progression in patients infected with the 2019 severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2).

DESIGN

Retrospective case series.

SETTING

A designated hospital for severe COVID-19 patients in the Zhejiang Province, China.

PARTICIPANTS

96 patients admitted to hospital with laboratory confirmed SARS-CoV-2 infection. Data were collected from 19 January 2020 to 12 March 2020.

MAIN OUTCOME MEASURES

More than 3,000 respiratory, stool, serum and urine samples were collected and detection from patients after admission. SARS-CoV-2 RNA viral load was measured, and the relationship between clinical data and disease severity was analyzed. Clinical data, collected using a standardised case report form, such as symptoms, underlying diseases, and treatment.

RESULTS

All patients were confirmed by testing respiratory specimens, and 54.22% of the patients had positive in stool and 39.36% had positive in serum. The median virus duration in stool (21 days, IQR 17-29 days) was significantly longer than in respiratory (18 days, IQR 13-28 days) and serum samples (16 days, IQR, 13-20 days) (p<0.001). The median virus duration in the respiratory samples of severe patients (21 days, IQR, 14.5-28.25 days) was significantly longer than in mild patients 13.5 days (IQR, 9.5-20.5 days) (p<0.001). In the mild group, the viral loads peaked in the second week from disease onset, whereas, viral load continued to be high during the third week in the severe group. Age and sex were identified as independent factors of viral duration.

CONCLUSION

Our study suggests that respiratory samples are still the most effective for detecting SARS-CoV-2, while it is necessary to strengthen the management of stool samples in

the prevention and control of the epidemic, especially in the later stage of the disease. As SARS-CoV-2 persists longer with higher load, and peaks later in the respiratory tissue of severe patients, the rational use of antiviral drugs is needed to shorten the duration of the virus and reduce the occurrence of severe cases.

Keywords: SARS-CoV-2, COVID-19, Viral Load, Dynamics

WHAT IS ALREADY KNOWN ON THIS TOPIC

Since first recognized in December 2019, COVID-19 has now affected more than 100,000 patients globally, and the numbers are still increasing rapidly.

Trends in viral load in upper respiratory tract samples were described in a small sample.

In addition to respiratory samples, viral nucleic acids were also detected in feces, blood and urine.

Studies with larger sample size would be required to understand on the clinical progression with respect to the viral load dynamics and how different factors may affect viral load.

WHAT THIS STUDY ADDS

In this manuscript, we present the differences by screening more than 3,000 respiratory, stool, serum and urine samples from 96 COVID-19 patients, and present a detailed acount of the virus duration and viral loads in different sample types.

Our data showed that, in mild patients, the viral loads peaked during second week from disease onset, whereas high viral loads continued to be detected during the third week from illness onset in severe patients. We also found that sex and age were key factors in viral duration.

Our findings suggests that respiratory samples are still the most effective for detecting SARS-CoV-2, whilst it is necessary to strengthen the management of stool samples in the prevention and control of the epidemic, especially during later stage of disease. As SARS-CoV-2 persists longer with higher load, and peaks later in the respiratory tissue of severe patients, the rational use of antiviral drugs is needed to shorten the duration of the virus and reduce the occurrence of severe cases.

Introduction

A novel human coronavirus first detected during an unexplained cluster of pneumonia cases in Wuhan, China in December 2019 [1-2], as of March 2, 2020, has been reported in 62 countries with more than 80,000 confirmed cases and 2,900 deaths [3]. A predominant number of cases have occurred in China [4], with early clinical characterisation showing that 13.8% of those infected developed further ARDS, progressing to severe pneumonia, which led to death.

Viral load measurements from tissue samples are indicative of active virus replication and are used routinely in monitoring severe viral respiratory track infections (RTIs), including clinical progression, response to therapy, cure, and relapse [5-7]. Zou *et al* described changes in viral load in the upper respiratory samples of 18 Coronavirus Disease 2019 (COVID-19) patients [8], showing that the viral loads were equally high among asymptomatic and symptomatic patients. However, the viral load dynamics in lower respiratory and other tissue samples, and the relationship between viral load and disease severity is unknown - information that are important for the formulation of disease control strategies and clinical treatment.

We systematically estimated the viral loads in over 3000 samples of different types, collected from patients diagnosed with the novel human Coronavirus (named SARS-CoV-2) after admission, and analyzed the correlation between virus load in different sample types and disease severity.

Methods

Study design

This was a cross-sectional study of patients with laboratory-confirmed Coronavirus Disease 2019 (COVID-19) admitted to the First Affiliated Hospital, School of Medicine, Zhejiang University from 19 Jan 2020 to 12 March 2020. The First Affiliated Hospital is a major general hospital with 3000 beds and serves as a designated hospital for severe COVID-19 patients in the Zhejiang Province. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of the First Affiliated Hospital of Zhejiang University.

Sample collection and laboratory confirmation

After admission, respiratory specimens, serum, stool and urine were collected daily whenever possible to determine the amount of SARS-CoV-2 viral RNA by PCR analysis. Sputum samples were collected from respiratory tract of patients with sputum, and saliva after deep cough was collected from patients without sputum. Blood samples were collected in a special whole blood collection tube and urine and stool samples were collected in a special sterile container. All medical personnel operated under three levels of safety protection during sampling. In this study, a total of 1637 respiratory samples were collected, on average 16 samples per patient (range, 2-32 samples). 732 stool samples, 6 samples per patient (range, 1-18 samples). 612 serum samples, 5 samples per patient (range, 1-11 samples), and 135 urine samples, 1 sample for each patient (range, 1-4 samples).

Viral RNAs were extracted using the MagNA Pure LC 2.0 (Roche, Basel, Switzerland), and quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using a China Food and Drug Administration (CFDA) approved commercial kit specific for SARS-CoV-2 detection (BoJie Co., Ltd., Shanghai, China). The detection limit of the ORFab1 RT-PCR assays was approximately 100 copies per mL. Specimens with Ct values ≤38.0 were considered positive, specimens with a Ct >38.0 were repeated, specimens with repeated results of Ct values >38 were considered positive, and specimens with undetectable Ct values after repeated tests were considered negative. Viral load was calculated by plotting Ct values onto the standard curve constructed based on the standard product.

Data collection

The clinical data included demography, medical comorbidities, date of symptom onset, symptoms and signs, timing of antiviral therapy, progression and resolution of clinical illness. Medical comorbidities documented included diabetes mellitus, heart disease, chronic lung disease, renal failure, liver disease, human immunodeficiency virus infection, cancer, and receipt of immunosuppressive therapy, including corticosteroids. We considered that the symptoms started when any of fever, cough,

chills, dizziness, headache, and fatigue appeared. The severity of illness was evaluated according to the sixth edition Guideline for Diagnosis and Treatment of SARS-CoV-2 issued the National Health Commission of the People's Republic of China [9]. According to the Guideline for Diagnosis and Treatment of SARS-CoV-2 issued by the National Health and Family Planning Commission of China, patients who have tested negative for SARS-CoV-2 for two consecutive days are considered SARS-CoV-2 negative and will not be tested further. By the end of March 12, 16 (16.7%) patients in severe group were still hospitalized, including 2 cases continued to be positive for the SARS-CoV-2.

Statistical analysis

For most variables, descriptive statistics, such as the mean standard deviation (SD; for data with normal distribution), median with interquartile range (IQR; for data with skewed distribution), and proportion (%), were calculated. Statistical comparisons between the mild and severe groups were evaluated by t-test, analysis of variance, Mann-Whitney U tests, Kruskal-Wallis tests where appropriate. To explore the variation of virus load across the days since symptoms onset, we firstly calculated the median of virus load each day, then smooth lines were fitted using loess method. In this part of analysis, only the patients whose virus load were monitored more than five times were included. Multivariate linear regression analysis was used to analyze the risk factors for viral duration. All statistical analyses were performed using R software package, versions 3.6.2. A P value <0.05 was considered significant.

Results

Patient characteristics

The clinical characteristics of 96 patients (22 mild and 74 severe) is shown in Table 1. The median age was 55 years (IQR 44.3-64.8). Hypertension (36.5%) and diabetes mellitus (11.5%) were the most common underlying disease. Majority of the patients developed fever (88.5%) and cough (56.3%). 78 (81.3%) patients received glucocorticoids and 33 (34.4%) received antibiotic treatment. All patients were treated with antiviral therapy comprising interferon α inhalation, Arbidol, Favipiravir,

Lopinavir/ritonavir and Darunavir/cobicistat. Among them, 63 (65.6%) patients commenced antiviral treatment within 5 days from illness onset and 29 (30.2%) 5 days after illness onset. 30 (40.5%) severe patients were admitted to ICU. The laboratory findings are shown in Table S1.

SARS-CoV-2 detection rates during disease progression and between sample types

All of the patients were confirmed by testing respiratory specimens. 54.22% of these patients were positive for viral nucleic acid in stool specimens and 39.36% in serum specimens. Rates of SARS-CoV-2 detection in the respiratory samples gradually decreased from 95.45% in the first week of symptom onset to 54.39% in week four, with subsequent respiratory samples turning negative, while the positive rate in stool samples and serum samples gradually increased from week 1 and decreased from week 3. In addition, the rate of detection in blood samples in severe patients was higher than in mild patients (44.44% vs. 22.73%) but there was no statistical difference, which was not reflected in stool. A urine sample collected from a critically ill patient on day 10 was positive (Table 2).

Correlation between viral duration and disease severity

The median virus duration in stool specimens (21 days, IQR 17-29 days) was significantly longer than that of respiratory specimens (18 days, IQR 13-28 days) and serum specimens 16 days (IQR, 13-20 days) (p<0.001) (Fig.1A). In the respiratory samples, the median virus duration in severe patients (21 days, IQR, 14.5-28.25 days) was significantly longer than in mild patients 13.5 days (IQR, 9.5-20.5 days) (p<0.001) (Fig.1B), while no significant difference was observed in the virus duration in stool and serum specimens among patients with different severities (Fig.1C, Fig.1D).

Correlation between viral load and disease severity

We found significant differences in viral load among different sample types, with respiratory samples having the highest viral load, followed by fecal samples and blood samples having the lowest (Fig.2A). In respiratory samples, severe patients had significantly higher viral loads than mild patients (Fig.2B). Fecal and blood samples showed no statistically significant difference in viral loads between mild and severe

patients (Fig.2C, Fig.2D).

Using a loess regression analysis (see *Methods*), we found that in the mild group, the viral load was greater during the initial stages of the disease, reached the peak in the second week from disease onset, followed by lower loads (Fig.3 Green line), whereas, in the severe group, the viral load continued to be high during the third week from disease onset, and maintained a high viral load (Fig.3 Red line). The viral load of fecal samples also increased early, but more slowly (Fig.3 Blue line).

Factors associated with viral duration

We performed multivariate linear regression to identify risk factors associated with viral duration (Table 3). Age and sex were identified as independent factors of viral duration, whereas infected in Wuhan, currently smoking and underlying disease were not independent factors. We stratified the patients according to the severity of the disease and found that in the severe group, the duration of the virus was significantly longer in male than female, and significantly longer in patients over 60 than in patients under 60 (Fig.4).

Discussion

We have systematically described the clinical characteristics of 96 COVID patients and described the dynamic changes of SARS-CoV-2 viral loads and disease progression using 3116 samples of multiple types, revealing the dynamic interaction between SARS-CoV-2 replication and clearance by host defence mechanisms. We found that the pathogen detection rate and viral load was significantly higher in respiratory samples, when compared to serum, stool and urine, indicating that respiratory samples were still the most reliable sample type for detecting novel coronavirus. The median viral duration in the respiratory samples was 18 days, which was consistent with the median duration of 20 days for MERS [10]. Peak viral shedding of SARS occurred after approximately 10–12 days from symptom onset [11-12], which is similar to the peak observed for SARS-CoV-2 in our study. Consistent with earlier reports of SARS-CoV-2 [13], we found that there were differences in the duration of virus in patients with different severities, with severe cases showing a significantly higher viral load than in mild cases, which suggests that viral load can be used to assess prognosis.

While *Zou* et al showed that the peak load of SARS-CoV-2 in upper respiratory specimens was during the early stages of the disease [8], however, we find that the virus shedding of the lower respiratory specimens virus was longer, and peak viral shedding occurred after approximately 2 weeks from symptom onset. These findings have significance for effective epidemic control and prevention as it suggests strict management of the whole disease process of patients with SARS-CoV-2. In this study, we also found that the viral load in the severe group was significantly higher than that in the mild group, suggesting that high viral load may be a risk factor for severe disease. Therefore, the rational use of antiviral drugs during the treatment process, effectively shortening the occurrence of the virus, may help to reduce the incidence of severe disease.

Active replication of SARS-CoV in the gut has been demonstrated through live virus isolation [14]. During 2003, the RNA prevalence of SARS in stool samples was so high that testing of stool was proposed as a reliable and sensitive way to routinely diagnose the disease [15-16], whereas MERS RNA was found in only 14.6% of stool-associated samples, with rather low RNA concentration [17]. In this study, we detected SARS-CoV-2 in 54.22% of patients' stool samples and found that, when compared to respiratory samples, the stool samples had longer virus duration and peaked in viral load later, and we also have isolated more than 10 SARS-CoV-2strains from the stool samples. Based on this study, we think the role of fecal excretion for spread of SARS-CoV-2 cannot be ignored, however the significance of high detection in stool samples in the prevention and control of SARS-CoV-2 epidemic requires comprehensive and careful evaluation. We rarely found urine-associated SARS–CoV-2 RNA in this study, although patients with SARS-CoV showed viral RNA detection rates up to 50% in urine [15-16].

A clear difference from SARS and SARS-CoV-2 were the detection of viral RNA in serum. There is evidence of SARS-CoV replicating in circulating lymphocytes, monocytes, macrophages and dendritic cells, albeit at low levels [18-20]. Up to 79% of serum samples were found to contain SARS-CoV RNA during the first week of illness, and around 50% during the second week [21-23]. The rates were similar in MERS-CoV

[17]. In this study, we found that the detection rate of SARS-CoV-2 in blood was only 39.36%, and we did not detect virus in a bone marrow sample from a critically ill patient.

Sex-dependent increase in disease severity after pathogenic CoV infection were reported in SARS and MERS [24, 25], which was also found in SARS-CoV-2 [26]. In this study, we found that the duration of virus in male was significantly longer than that in female. Our results shed light on the causes of disease severity in male in terms of the duration of the virus. In addition to differences in immune status between the sexes, it has also been reported to be related to differences in hormone levels between male and female [27]. In this study, we also found a correlation between age and viral duration, which partly explains the high rate of severe illness in elderly patients. This is partly because of immunosenescence [28]. Another reason is that older people have higher levels of ACE2 in their alveoli [29], which are thought to be receptors for novel coronaviruses.

Limitations of this study

There are several limitations to our study. Firstly, this study is a single center cross-sectional analysis, the sample size was insufficient to compare treatment effects in different subgroups, which could lead to the unbalanced distribution of confounders when we evaluate viral shedding and viral load. Secondly, viral load is influenced by many factors, specimen collection quality directly affects the discretion of the viral load, so the study of the viral load is only partly reflect the amount of virus in the body, in addition, although we try to collect all kinds of samples for testing in patients with every day, but not all of the sample type could be collected from the patients, especially the stool and urine samples. Thirdly, PCR, cannot distinguish non-viable virus and does not reflect the replication level of the virus in different tissue. However, PCR has higher sensitivity, is easier to operate, and is comparable to several other studies [30, 31]. Finally, it has been reported that virus may be detected in some patients 10 days after discharge [32]. Although this phenomenon was not found in the follow-up patients in this study, we suggest that some patients may have residual virus in their lungs after discharge.

Conclusion

Respiratory samples are still the most effective samples for detecting novel coronavirus nucleic acid. The duration of fecal samples is significantly longer than that of respiratory and serum, it is necessary to strengthen the management of stool samples in the prevention and control of the epidemic, especially in the later stage of the disease. Compared to mild patients, SARS-CoV-2 persisted longer, had a higher load, and peaked later in the respiratory tract of severe patients. Therefore, it is necessary to carry out strict management in each stage of severe cases to prevent the spread of the virus, and use antiviral drugs rationally to shorten the duration of the virus and reduce the occurrence of severe cases.

Notes

Contributors.

TL and YC were coprincipal investigators, designed and supervised the study, and wrote the grant application (assisted by SZ). KX, XL, GW, JZ, QF, HC, YQ, and JS had roles in recruitment, data collection, and clinical management. SZ, JF, FY, BF, BL, QZ, GX, SL, RW, XY, WC, QW, DZ, YL, RG, ZM, SL, YX, YG, JZ, and HY did clinical laboratory testing and analysis. SZ, FY, BF, YC, and TL drafted the Article, and all authors contributed to review and revision and have seen and approved the final version.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Table 1. Demographics and clinical characteristics of Patients with SARS-CoV-2 infection

Variables	Total（n=96)	Mild (n=22)	Severe (n=74)	*P* values
Demographics				
Age, y, median [IQR]	55 (44.3-64.8)	47.5 (36.8-56.3)	57 (47.5-66)	**0.010**
Male sex	58 (60.4)	9 (40.9)	49 (66.2)	**0.033**
Infected in Wuhan	28 (29.2)	2 (9.1)	26 (35.1)	**0.018**
Underlying diseases				
Hypertension	35 (36.5)	4 (18.2)	31 (41.9)	**0.042**
Diabetes mellitus	11 (11.5)	1 (4.5)	10 (13.5)	0.436
Heart disease	7 (7.3)	0 (0)	7 (9.5)	0.302
Lung disease	4 (4.2)	0 (0)	4 (5.4)	0.571
Liver disease	3 (3.1)	1 (4.5)	2 (2.7)	0.546
Renal disease	1 (1.0)	0 (0)	1 (1.4)	1.000
Malignancy	1 (1.0)	0 (0)	1 (1.4)	1.000
Immune compromise	1 (1.0)	0 (0)	1 (1.4)	1.000
Symptoms				
Fever	85 (88.5)	17 (77.3)	68 (91.9)	0.131
Cough	54 (56.3)	1 (4.5)	8 (10.8)	0.639
Sputum	26 (27.1)	12 (54.5)	42 (56.8)	0.854
Chest distress	12 (12.5)	7 (31.8)	19 (25.7)	0.592
Dizziness	7 (7.3)	2 (9.1)	10 (13.5)	0.854
Headache	4 (4.2)	0 (0)	7 (9.5)	0.302
Nausea	5 (5.2)	0 (0)	4 (5.4)	0.571
Vomiting	2 (2.1)	2 (9.1)	3 (4.1)	0.322
Diarrhea	10 (10.4)	0 (0)	2 (2.7)	1.000
Myalgia	19 (19.8)	0 (0)	10 (13.5)	0.154
Fatigue	9 (9.4)	6 (27.3)	13 (17.6)	0.485
Treatment				
Gamma globulin	53 (55.2)	4 (18.2)	49 (66.2)	**<0.001**
Glucocorticoids	78 (81.3)	9 (40.9)	69 (93.2)	**<0.001**
Antibiotic treatment	33 (34.4)	1 (4.5)	32 (43.2)	**0.001**
Antiviral treatment	96 (100)	22 (100)	74 (100)	-
Day of illness to antiviral therapy				
≤5day	63 (31.3)	14 (18.2)	49 (35.1)	0.098
>5day	29 (30.2)	8 (36.4)	21 (28.4)	0.575
Disease severity				
Bilateral pulmonary infiltrates	80 (83.3)	12 (54.5)	68 (91.9)	**<0.001**
Invasive mechanical ventilation	10 (10.4)	0 (0)	10 (13.5)	0.154
ECMO	5 (5.2)	0 (0)	5 (6.8)	0.586
Intensive care unit admission	30 (31.3)	0 (0)	30 (40.5)	**<0.001**

Bold texts indicate *P*<.05.

Abbreviation: IQR, interquartile range.

Table 2. The detection of SARS-CoV-2 in mild and severe patients at different stages after onset in different sample types.

	Sample types	After admission (n/N, %)	Days since onset of symptoms				*p* values
			1st week (n/N, %)	2nd week (n/N, %)	3rd week (n/N, %)	4th week (n/N, %)	
All	Respiratory	96/96, 100	42/44, 95.45	74/90, 82.22	64/89, 71.91	31/57, 54.39	**<0.001**
	Stool	45/83, 54.22	4/10, 40.00	28/53, 52.83	32/62, 51.61	17/44, 38.64	0.451
	Serum	37/94, 39.36	4/33, 12.12	19/84, 22.62	19/85, 22.35	5/46, 10.87	0.228
	Urine	1/65, 2.50	0/10, 0	1/53, 4.00	0/21, 0	0/19, 0	/
Mild	Respiratory	22/22, 100	11/12, 91.67	15/21, 71.43	9/19, 47.37	4/9, 44.44	**0.038**
	Stool	12/22, 54.55	2/4, 50.00	8/16, 50.00	10/16, 62.50	5/8, 62.50	0.892
	Serum	5/22, 22.73	0/9, 0	3/19, 15.79	2/17, 11.76	0/7, 0	0.671
	Urine	0/19, 0	0/3, 0	0/15, 0	0/7, 0	0/3, 0	/
Severe	Respiratory	74/74, 100	31/32, 96.88	59/69, 85.51	55/70, 78.57	27/48, 56.25	**<0.001**
	Stool	33/61, 54.10	2/6, 33.33	20/37, 54.05	22/46, 47.83	12/36, 33.33	0.308
	Serum	32/72,44.44	4/24, 16.67	16/65, 24.62	17/68, 25.00	6/39, 15.38	0.570
	Urine	1/33, 3.03	0/7, 0	1/38, 5.00	0/14, 0	0/16, 0	/

Bold texts indicate $P<.05$.

Table 3. Multivariate linear regression analysis of factors associated with viral duration in patients with SARS-CoV-2 infection

Variable	β (95% CI)	*P* Value
Age	0.135 (0.009-0.261)	0.039
Sex	5.667 (1.782 -9.552)	0.005
Infected in Wuhan	2.451 (-1.424-6.325)	0.218
Current smoking	-4.034 (-9.478-1.410)	0.149
Underlying disease	1.009 (-3.225-5.245)	0.641

Abbreviations: CI, confidence interval.

Table S1. Clinical laboratory characteristics of Patients with SARS-CoV-2 infection

Laboratory findings, median [IQR]	Total （n=96)	Mild (n=22)	Severe (n=74)	*P* values
Leukocyte count, mm^3	5.7 (3.9-9.3)	5.3 (3.9-7.6)	6.2 (3.9-10.8)	0.191
Lymphocyte count, mm^3	0.8 (0.5-1.2)	1.2 (0.8-1.5)	0.7 (0.5-1.1)	**0.002**
Monocytes count, mm^3	0.4 (0.2-0.5)	0.5 (0.3-0.7)	0.3 (0.2-0.5)	**0.005**
Hemoglobin, g/L	135 (121.8-149)	134 (126-144)	135 (121-150)	0.850
Platelet count, mm^3	187 (143-237.5)	187 (159-266)	181 (140-227)	0.374
Albumin, g/L	39.1 (34.5-43.6)	42.4 (37.9-46.1)	37.9 (33.9-42.7)	**0.004**
Alanine transaminase, IU/L	21 (15-30.8)	24 (16-45)	21 (14-27.5)	0.395
Aspartate transaminase, IU/L	22 (18-34)	19 (16-34)	23 (18-34.5)	0.236
Creatinine, μmol/L	76 (61.3-89.8)	62 (54-88)	79 (65-91.5)	**0.021**
Blood urea nitrogen, mmol/L	5 (3.9-6.8)	4 (3.2-5)	5.4 (4.2-7.9)	**0.001**
Creatine kinase, IU/L	69 (49-122.8)	62.5 (42.8-86.3)	78 (52-138)	**0.043**
Creatine kinase-MB, IU/L	20 (17-24)	17 (15.8-19.5)	22 (17-24.5)	**0.012**
Lactate dehydrogenase, IU/L	261.5 (211.8-341.3)	205 (176-224.3)	292.5 (227.3-355.8)	**<0.001**
Cardiac troponin I, ng/mL	0.004 (0.002-0.009)	0.002 (0.001-0.003)	0.004 (0.002-0.01)	**<0.001**
Potassium, mmol/L	3.8 (3.5-4.1)	3.6 (3.5-4.2)	3.8 (3.5-4.1)	0.458
Sodium, mmol/L	139 (137-141)	140 (138-141)	138 (136-141)	0.063
Fibrin, g/L	4.5 (4-5.5）	4.0 (2.8-4.3)	4.7 (4.2-5.5)	**0.001**
D-dimer, ug/lFEU	370 (214-673)	236 (85-391)	429 (260.5-787.5)	**0.002**
C-reactive protein, mg/L	27.1 (10.8-54.8)	8 (2.2-21.9)	39.9 (16.2-63.7)	**<0.001**
Procalcitonin, ng/mL	0.06 (0.04-0.1)	0.05 (0.02-0.07)	0.07 (0.04-0.1)	**0.023**
Interleukin-2, pg/mL	0.5 (0.4-1.5)	0.7 (0.4-1.3)	0.5 (0.4-1.5)	0.939
Interleukin-4, pg/mL	0.9 (0.7-0.9)	0.9 (0.9-0.9)	0.9 (0.7-0.9)	**0.042**
Interleukin-6, pg/mL	22.5 (10.7-58.3)	18 (6-46.4)	32.7 (12.3-60.5)	0.137
Interleukin-10, pg/mL	5.4 (3-8)	3.7 (2.6-7.5)	5.9 (3-8.4)	0.170
Tumor necrosis factor-α, pg/mL	7.4 (3.3-38)	13.8 (6.1-43)	6.1 (3.3-38)	0.490
Interferon-γ, pg/mL	9.2 (5.1-28.6)	12.2 (5.2-38.5)	9 (4.6-26.3)	0.548
$CD45^+$ lymphocytes, μL	684.5 (404.3-1205)	1295.5 (764-1774)	629.5 (339.8-1013.3)	**0.027**
$CD3^+$ lymphocytes, μL	336.5 (208.8-780.3)	856 (335.5-1382.3)	317 (201.3-661)	**0.041**
$CD4^+$ lymphocytes, μL	182.5 (85.5-463.5)	498.5 (165.3-740.8)	171.5 (83.3-372.3)	**0.033**
$CD8^+$ lymphocytes, μL	150 (81.8-303.3)	310 (152.8-458.8)	138 (79-227.3)	**0.035**
$CD19^+$ lymphocytes, μL	116.5 (70.3-180.8)	139.5 (113.3-243)	108 (67.8-180.3)	0.297
$CD56^+$ lymphocytes, μL	103.5 (68.3-205.3)	215 (161.8-281.8)	96.5 (60.3-195.8)	**0.015**

Figure 1. Duration of SARS-CoV-2 in different sample types. (A) Distribution of positive samples by day of symptom onset. (B), Comparison of virus duration of SARS-CoV-2 in respiratory samples between mild and severe cases. (C), Comparison of virus duration of SARS-CoV-2 in stool samples between mild and severe cases. (D), Comparison of virus duration of SARS-CoV-2 in serum samples between mild and severe cases.

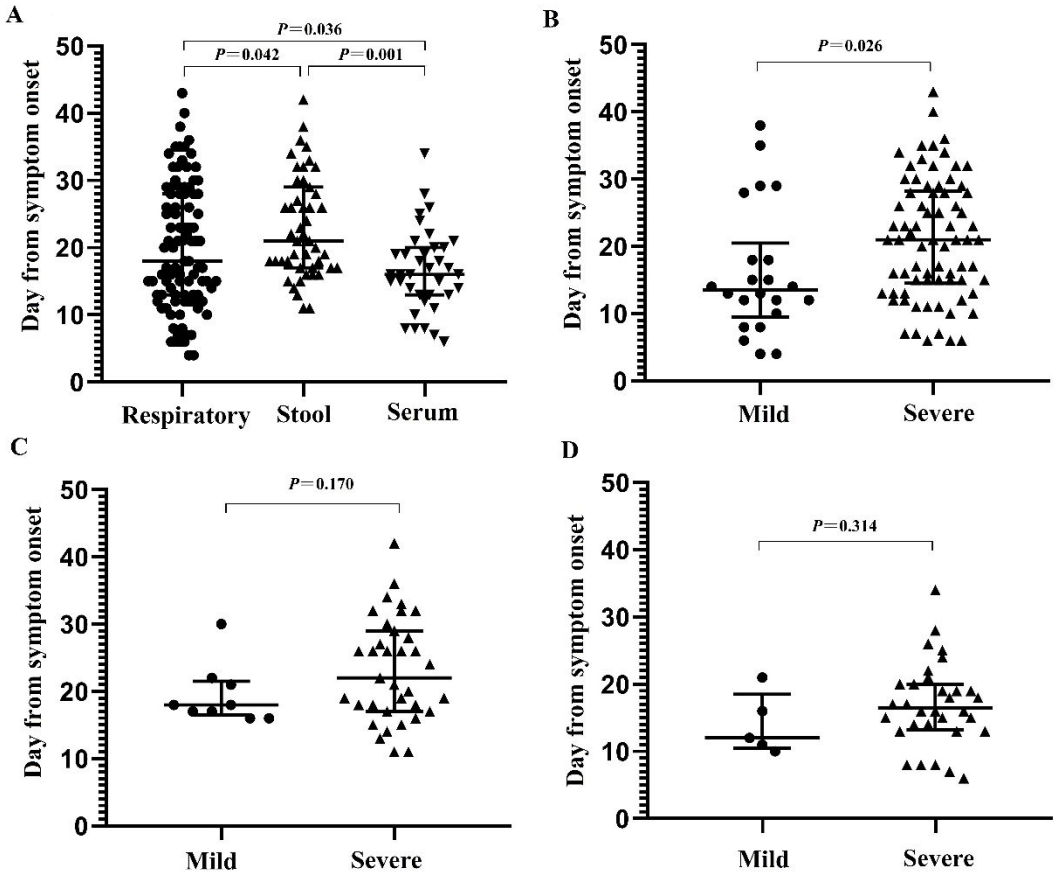


Figure 2. SARS-CoV-2 viral load in different sample types (A) Virus load of SARS-CoV-2 in different sample types. (B), Comparison of virus load of SARS-CoV-2 in respiratory samples between mild and severe cases. (C), Comparison of virus load of SARS-CoV-2 in stool samples between mild and severe cases. (D), Comparison of virus load of SARS-CoV-2 in serum samples between mild and severe cases.

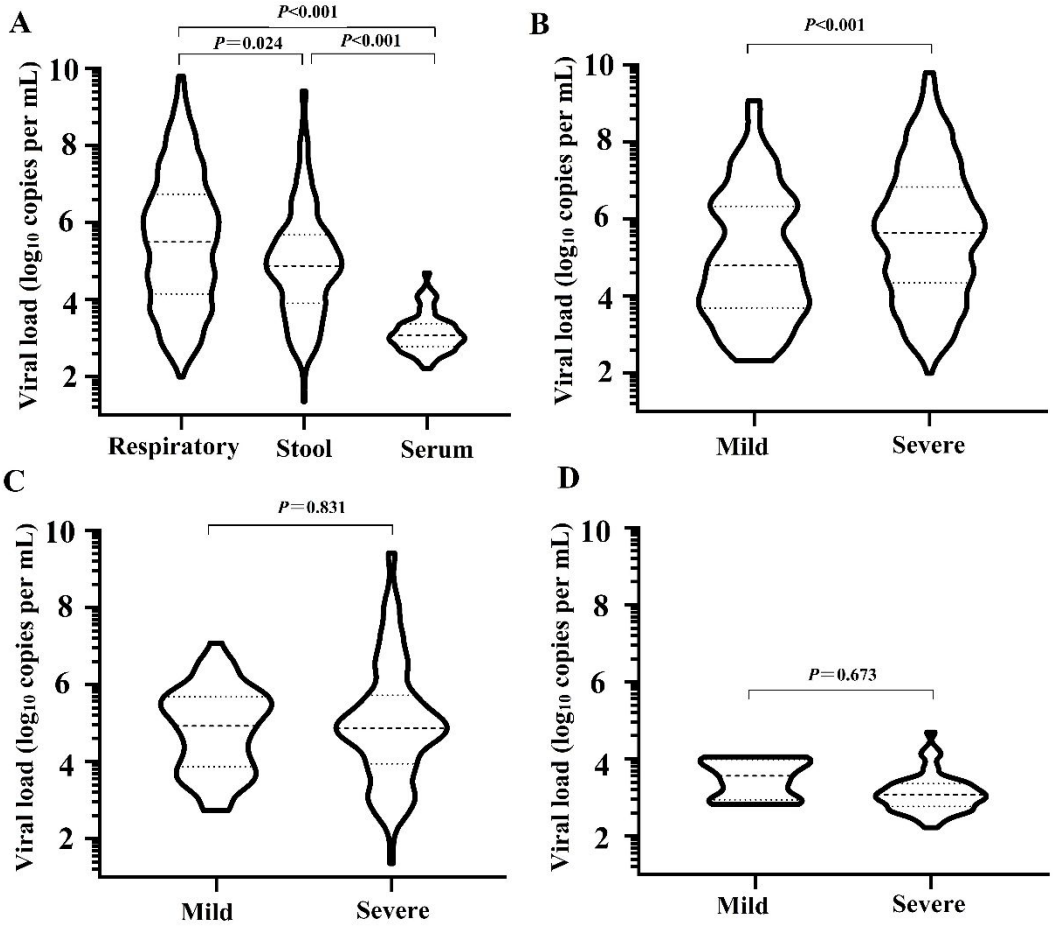


Figure 3. Smooth lines were fitted using loess method to explore the variation of virus load of SARS-CoV-2 across the days since symptoms onset. (Green line) Respiratory viral load in mild patients with SARS-CoV-2 infected. (Red line), Respiratory viral load in severe patients with SARS-CoV-2 infected. (Blue line), Stool viral load in patients with SARS-CoV-2 infected.

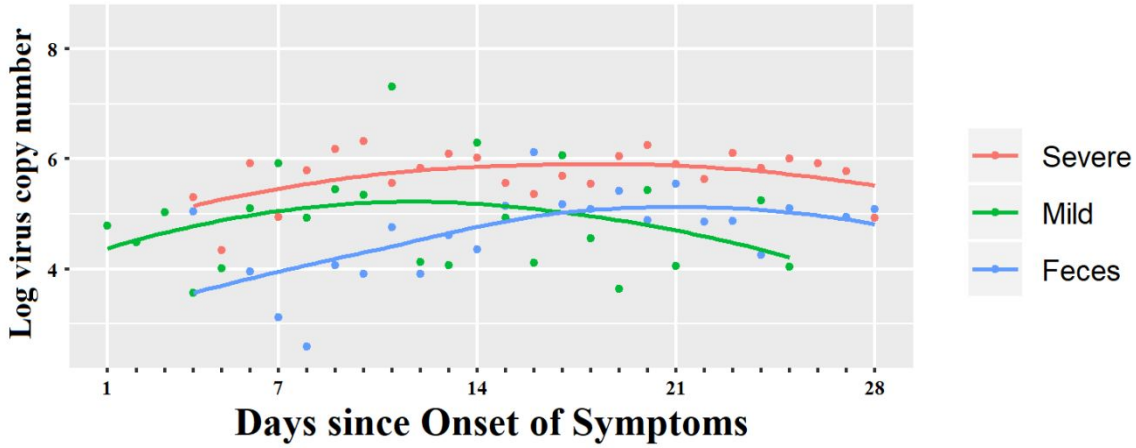
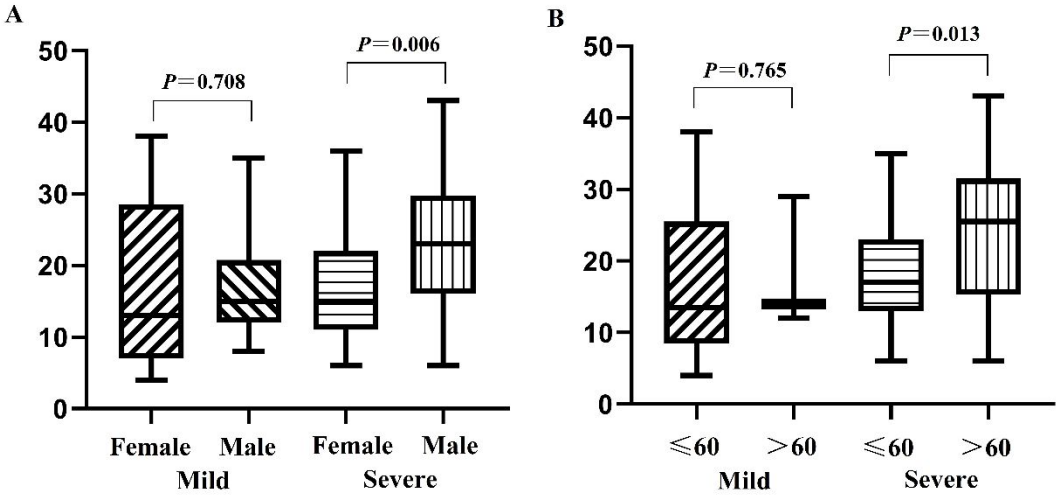
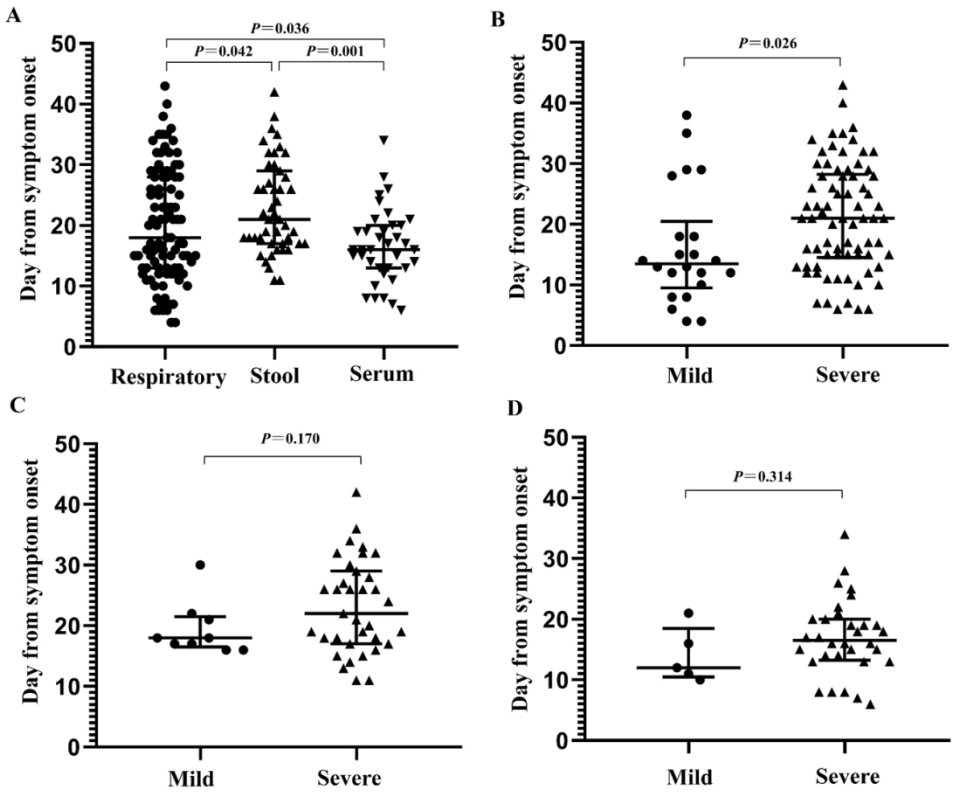


Figure 4. Effects of sex and age on the duration of the SARS-CoV-2 (A) Effects of sex on the duration of the SARS-CoV-2. (B), Effects of age on the duration of the SARS-CoV-2.





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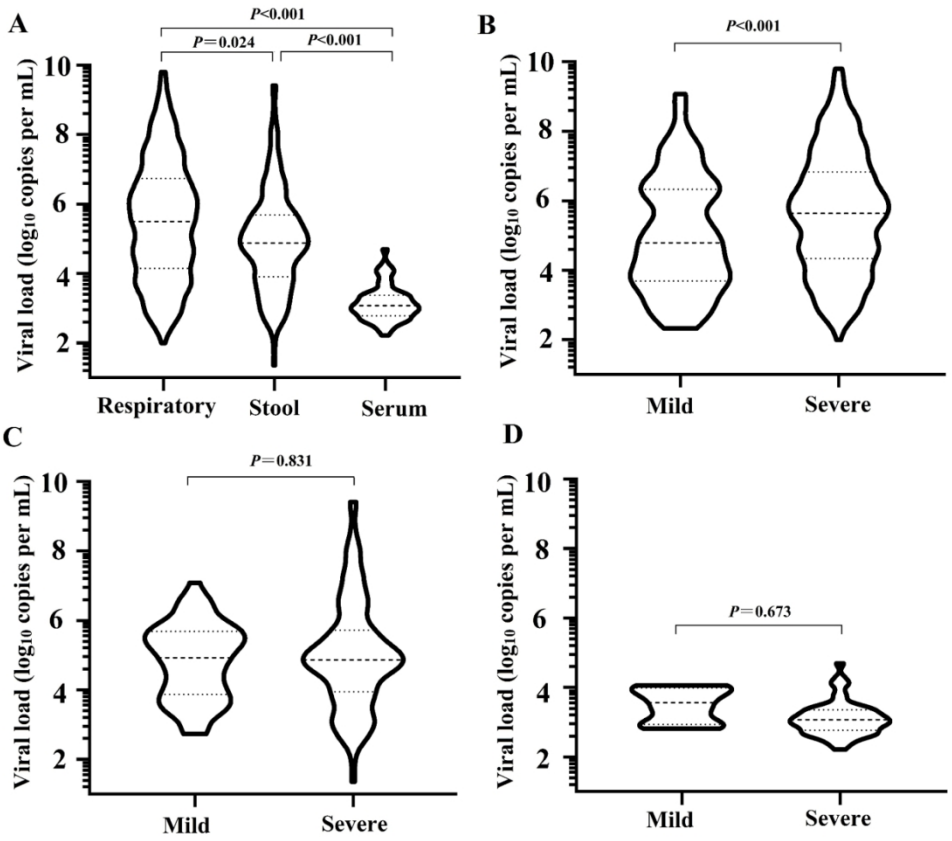

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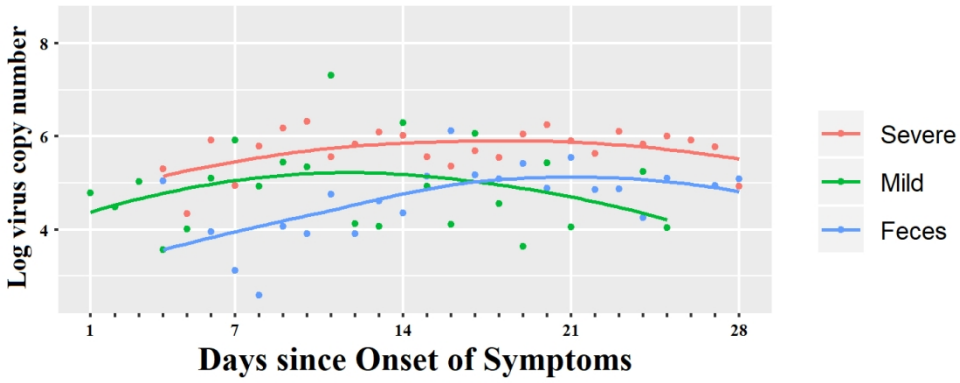

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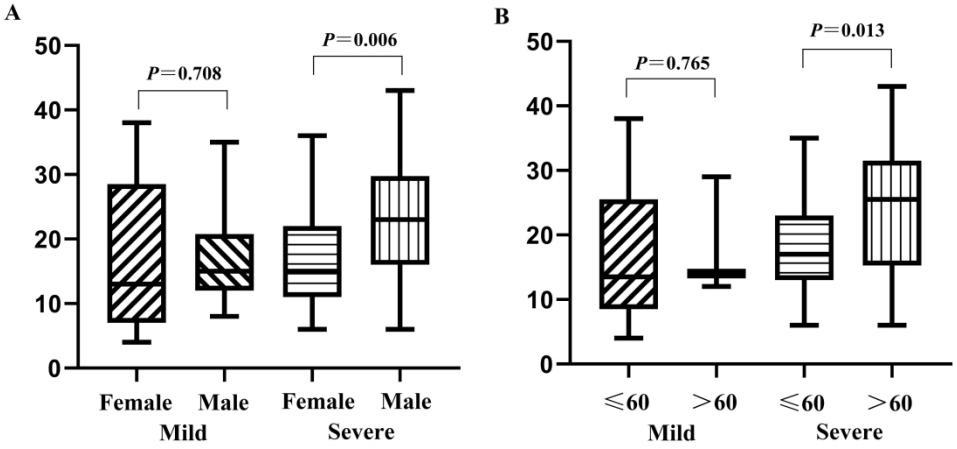

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