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Original Article

Clinical characteristics associated with long-term viral shedding in patients with coronavirus disease 2019

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Abstract: Background: To delineate the clinical characteristics associated with long-term viral shedding (>21 days) in patients with coronavirus disease 2019 (COVID-19). Methods: In this retrospective study, factors associated with long-term (>21 days) severe acute respiratory coronavirus 2 (SARS-CoV-2) RNA shedding were evaluated in a conhort of 609 patients from two hospitals in Wuhan. Results: The median duration of SARS-CoV-2 viral shedding was 19 days (interquartile range, 10-28 days) among all patients. There were 42% of patients having prolonged viral shedding time (>21 days), in which the longest viral shedding time was 58 days. When comparing patients with early (≤21 days) and late viral RNA clearance (>21 days), prolonged viral shedding was associated with age <65 (P=0.015), female sex (P=0.028), cough (P=0.025), fatigue (P=0.035), sore throat (P=0.013), aspartate aminotransferase (P=0.038), procalcitonin (P=0.010), albumin (P=0.003), D-dimer (P=0.011), lung involvement (P=0.014), reticular shadow (P<0.001) and lung consolidation (P=0.004). Age range (<65 years) (odds ratio [OR], 1.46 [95% CI, 1.05-2.03]) and female sex (odds ratio [OR], 1.40 [95% CI, 1.00-1.94]) were independent risk factors. Conclusions: Long-term viral shedding (>21 days) is not a rare phenomenon among COVID-19 infectious patients. Age range (<65) and female sex are independent risk factors for long-term viral shedding. Early antiviral treatment should be considered for COVID-19 patients with such risk factors. Further study should be conducted to know the infectivity of patients with long-term viral shedding in order to develop reasonable control measures.

Keywords: Coronavirus, viral shedding, risk factors, SARS-CoV-2, COVID-19

Introduction

In 2019, pneumonia of unknown causes was broken out, and it has spread over the world in a few months. Later a novel coronavirus, named SARS-CoV-2, has been identified in samples of throat swab, sputum, broncho alveolar lavage fluid, as well as feces [1]. On March 11th, 2020, WHO announced 2019 coronavirus disease (COVID-19) as a pandemic [2]. So far, coronavirus has caused two epidemics, severe acute respiratory syndrome (SARS) in 2004, and the Middle East respiratory syndrome (MERS) in the last two decades. Compared with the two coronaviruses [3], SARS-CoV-2 is more contagious and induces severer clinical symptoms [4, 5]. SARS-CoV-2 causes both pulmonary and systemic inflammation, leading to multi-organ dysfunction in regular people, and especially in subjects with hypertension as well as the aged population [6, 7]. It is essential to understand the shedding of SARS-CoV-2 virus since it is a key factor assessing the SARS-CoV-2 transmission [8].

Because coronavirus RNA detection is more sensitive than virus isolation, most studies have used qualitative or quantitative viral RNA tests as a potential marker for infectiouscoronavirus [9], For SARS-CoV, viral RNA was detected in respiratory specimens from about a third of patients as long as 4 weeks after disease onset. Similarly, the duration of MERS-CoV RNA detection in lower respiratory specimans persisted for at least 3 weeks [8]. It suggests that the SARS-CoV-2 viral nucleic acid shedding has a different pattern from previously reported virus and seemed to have longer shedding duration than SARS-CoV and MERS-CoV [8, 10]. Long-term viral shedding duration implies more infectious [11], longer isolation time and longer hospitization days [12, 13], so viral shedding duration is closely related to patients' prognosis and burden of medical resources.

At present, there are few reports focus on the duration of SARS-CoV-2 viral shedding [14-16]. In addition, the existing reports payed more attention to severe patients, which may be different from the real situation of the population. According to the report with the largest sample size so far, 80.9% of the 44,672 patients displayed symptoms considered mild [17], In the study of risk factors on viral shedding, there are only a few reports on viral shedding duration no more than 21 days and no study on longer-term viral shedding has been done.

For this reason, we focused on the analysis of the clinical features of 609 patients with COVID-19. We aimed at elucidating clinical characters associate with long-term viral shedding (>21 days) and to identify risk factors influencing the long-term persistence of viral shedding.

Methods

Study participants and data collection

In this retrospective analysis, we included 609 patients with COVID-19 diagnosed in two hospitals, the Wuhan Huoshenshan Hospital and Guanggu District, Hubei maternal and Child Health Hospital, from January 22, 2020 to March 30, 2020. COVID-19 was diagnosed according to WHO interim guidance [18]. At the time of admission, all patients confirmed to be positive by nucleic acid detection of SARS-CoV-2 virus in throat swab. Only when the virus nucleic acid test of throat swab is negative for two consecutive times (the sampling interval is not less than 24 hours), will the release and discharge be considered [19]. The ethics committee of the Naval Hospital of Eastern Theater of PLA approved the study. According to the duration of SARS-CoV-2 viral shedding, patients were classified into two groups: one group was patients that had persistent negative viral detection results ≤21 days after illness onset, and another group was patients with long-term viral shedding >21 days after illness onset.

The data collected in the study were extracted from the electronic medical records of 609 patients with COVID-19 by demography, clinical symptoms, laboratory indicators, chest imaging findings and treatment results. The data sheet is based on a COVID-19 based diagnosis and treatment protocol. Duration of viral shedding was considered the number of days from symptom onset to persistent negative detection of throat swab specimens except for asymptomatic patients whose viral shedding duration was counted from the first positive detection of throat swab specimens. All subsequent samples from the same patients were then tested until two consecutive samples were negative with the first negative sample defining the duration. The research team of experienced clinicians from Changzheng Hospital, Second Military Medical University analysed patients' medical records. A trained team of physicians and researchers independently entered and cross checked data in a computerised database.

All cases were diagnosed and classified according to the New Coronavirus Pneumonia Diagnosis Program (6th edition) published by the National Health Commission of China [20]. Clinical manifestations consist of four categories, mild, moderate, severe and critical. The mild clinical symptoms were mild with no pulmonary inflammation on imaging or without symptoms of respiratory infections. The moderate is the majority, showing symptoms of respiratory infections such as fever, cough, and sputum, and pulmonary inflammation on imaging; when symptoms of dyspnea appear, including any of the following: shortness of breath, RR ≥30 bpm, blood oxygen saturation

≤93% (at rest), PaO_2/FiO_2 ≤300 mmHg, or pulmonary inflammation that progresses significantly within 24 to 48 hours >50%, it was classified as severe; respiratory failure, shock, and organ failures that require intensive care were critically ill.

Laboratory procedures

Methods for laboratory confirmation of SARS-CoV-2 infection were carried out according to the manufacturers instructions [Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing), Shanghai ZJ Bio-Tech Co., Ltd., Shanghai, China]. Detailed diagnosis process has been reported previously [21]. Briefly, the throat swab samples of the subjects were collected and the total RNA was extracted by direct-zol RNA miniprep kit. 5 μL RNA was used for q-PCR detection. The reaction mixture contains 19 μL of reaction buffer, 1 μL of enzyme solution, and 5 μL of RNA template. The RT-PCR assay was conducted under the following conditions: 10 min at 45°C, 3 min at 95°C, and 45 amplification cycles at 95°C (15 s) and 58°C (30 s). A cycle threshold value of 43 or less than 43 was defined as a positive test result, and a cycle threshold value more than 43 was defined as a negative test.

Routine blood examinations were complete blood count, serum biochemical tests (including liver and kidney function, creatine kinase) and cytokine tests.

Statistical analysis

Categorical variables were presented as numbers and percentages. Continuous variables were presented as mean and standard deviation if they were normally distributed or median and interquartile range (IQR) if they were not. We compared proportions for categorical variables by using the χ^2 test. Continuous variables normally distributed was compared with one-way ANOVA and which non-normally distributed was compared with Mann-Whitney U test. The continuous variables were transformed into dichotomous variables by using the normal range of indicators. Age and gender were independent variables, and viral shedding time >21 days was dependent variable. Multiple logistic regression model was used to study the effect of age and gender on long-term virus shedding. We considered a two sided *P* value below 0.05 to be statistically significant. Statistical analyses were done using the SPSS software (version 20.0), unless otherwise indicated.

Results

Demographics and baseline characteristics of patients

We analyzed the clinical characteristics of 609 patients with COVID-19. The median duration of SARS-CoV-2 viral shedding of all patients was 19 days (IQR, 10-28 days). The maxium duration of viral shedding in this study was as long as 58 days. 357 (58.6%) of the patients were aged <65 years, 252 (41.4%) were aged 65 years and older. The median age was 61 years (IQR, 50-69 years; **Table 1**). More than half of the 609 patients (358, 58.8%) were female. Of the 265 patients with viral duration over 21 days after illness onset, the median duration of viral shedding was 29 days (IQR, 25-35 days). The median age of patients was 59 years (IQR, 48-68 years; **Table 1**). Compared with viral shedding short-term group (≤21 days), the long-term viral shedding group showed a significantly younger age composition (P=0.015), predominantly distributed in ages <65 years (170, 64.2%). Besides, the proportion of female sex in long-term viral shedding group (169, 63.8%) is significantly higher than that in short-term viral shedding group (189, 54.9%), P=0.028. 317 of the 609 patients (52.1%) had underlying diseases-192 (31.5%) had hypertension, 76 (12.5%) had diabetes mellitus, 54 (8.9%) had heart disease, 30 (4.9%) had respiratory system disease, 22 (3.6%) had chronic kidney or liver system disease, 25 (4.1%) had cerebrovascular disease and 24 (3.9%) had digestive system disease. Of the 265 patients with viral duration over 21 days after illness onset, 129 (48.7%) had underlying diseases: 76 (28.7%) patients had hypertension, 37 (14%) had diabetes mellitus, 19 (7.2%) had heart disease, 12 (4.5%) had digestive system disease, 10 each had respiratory system disease (3.8%), chronic kidney or liver system disease (3.8%) and cerebrovascular disease (3.8%). The most common symptoms of all patients at illness onset were cough (355, 58.3%), fever (333, 54.7%), fatigue (249, 40.9%), and shortness of breath (153,

Table 1. Presenting characteristics of patients with coronavirus disease 2019

Variable	Total	Viral RNA shedding duration		*P* value
	n=609	≤21 d n=344	>21 d n=265	
Median (IQR) age, years	61 (50.69)	62.5 (50.71)	59 (48.68)	0.030
<65 years	357 (58.6)	187 (54.4)	170 (64.2)	0.015
≥65 years	252 (41.4)	157 (45.6)	95 (35.8)	
Sex				
Female	358 (58.8)	189 (54.9)	169 (63.8)	0.028
Male	251 (41.2)	155 (45.1)	96 (36.2)	
Comorbidites				
Unaccompanied disease	292 (47.9)	156 (45.3)	136 (51.3)	0.144
hypertension	192 (31.5)	116 (33.7)	76 (28.7)	0.184
Diabetes mellitus	76 (12.5)	39 (11.3)	37 (14.0)	0.331
Heart disease	54 (8.9)	35 (10.2)	19 (7.2)	0.196
Respiratory system disease	30 (4.9)	20 (5.8)	10 (3.8)	0.249
cerebrovascular	25 (4.1)	15 (4.4)	10 (3.8)	0.717
Digestive system disease	24 (3.9)	12 (3.5)	12 (4.5)	0.513
Chronic kidney, liver system disease	22 (3.6)	12 (3.5)	10 (3.8)	0.852
Signs and symptoms				
Cough	355 (58.3)	187 (54.4)	168 (63.4)	0.025
Fever (temperature ≥37.0°C)	333 (54.7)	182 (52.9)	151 (57.0)	0.317
Fatigue	249 (40.9)	128 (37.2)	121 (45.7)	0.035
Shortness of breath	153 (25.1)	89 (25.9)	64 (24.2)	0.627
chill	110 (18.1)	53 (15.4)	57 (21.5)	0.052
Chest tightness	107 (17.6)	64 (18.6)	43 (16.2)	0.445
Sputum production	99 (16.3)	53 (15.4)	46 (17.4)	0.518
Asymptom	74 (12.2)	48 (14.0)	26 (9.8)	0.121
Myalgia	45 (7.4)	25 (7.3)	20 (7.5)	0.896
Sore throat	44 (7.2)	17 (4.9)	27 (10.2)	0.013
diarrhea	25 (4.1)	14 (4.1)	11 (4.2)	0.960
Nausea	24 (3.9)	13 (3.8)	11 (4.2)	0.815
Clinical classification				
Mild	38 (6.2)	22 (6.4)	16 (6.0)	0.973
Moderate	473 (77.7)	268 (77.9)	205 (77.4)	
Severe	81 (13.3)	44 (12.8)	37 (14.0)	
Critical	17 (2.8)	10 (2.9)	7 (2.6)	

Data are median (IQR), n (%), unless otherwise stated. For statistical analyses, Mann-Whitney U test of multiple independent samples was done for continuous variables and χ^2 test or Fisher's exact test was done for categorical variables. All of the signs and symptoms were statistically present prior to admission.

25.1%). There were 74 (12.2%) asymptomatic patients in all cases. Among the 265 patients who had viral duration over 21 days, the most common symptoms at onset of illness were cough (168, 63.4%), fever (151, 57.0%), fatigue (121, 45.7%), shortness of breath (64, 24.2%) and chill (57, 21.5%). Cough (P=0.025), fatigue (P=0.035) and sore throat (P=0.013) were more frequent in patients with viral shedding duration >21 days than those with viral shedding duration ≤21 days.

Moreover, as is shown in clinical classification in all patients, those with moderate disease account the majority proportion (473, 77.7%). 38 (6.2%), 81 (13.3%) and 17 (2.8%) patients had mild disease, severe disease and critical disease respectively. Similar proportion can be

found in patients with viral duration over 21 days.

Laboratory parameters of patients

In blood routine, over 90% patients showed normal white blood cell count, platelet count, neutrophil count and monocyte count, except that 16.2% patients showed lymphopenia (lymphocyte count <1.1×10^9/L; **Table 2**). No obvious difference can be found between short-term viral shedding group and long-term viral shedding group.

In blood biochemistry study, more than 90% patients also had normal aspartate aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, creatine kinase-MB, creatine kinase, hypersensitive troponin I, serum creatinine and blood urea nitrogen levels. 63 (10.7%) and 112 (24.3%) patients had elevated alanine aminotransferase (>55 U/L) and procalcitonin (>0.05 ng/mL) respectively. 96 (16.3%) patients had decreased albumin (<35 g/L). In inflammation indicators, levels of interleukin-6, C-reactive protein and D-dimer increased in 39 (11.4%), 79 (13.3%) and 133 (30.9%) patients. Significant difference can be found between short-term and long-term groups in aspartate aminotransferase (P= 0.038), procalcitonin (P=0.010), albumin (P= 0.003) and D-dimer (P=0.011). Only 17 (6.5%), 43 (19.1%), 29 (11.2%) and 48 (24.6%) patients in long-term group developed abnormal aspartate aminotransferase, procalcitonin, albumin and D-dimer which were less frequent than those in short-term group 38 (11.6%), 69 (29.4%), 263 (79.7%) and 85 (36.0%) respectively.

Imaging characteristics of patients with COVID-19

As shown in **Table 3**, abnormalities on chest radiographs were found in 93.2% patients. Bilateral lung involvement was much more common (76.5%) than unilateral lung involvement (13.7%). Predominantly CT patten of infected patients on admission were ground-glass opacities (502, 89.5%) or reticular shadow (215, 38.3%) or pulmonary fibrosis (60, 10.7%) or lung consolidation (35, 6.2%). The majority of patients (70.4%) had lesion range less than 50%. Only 27 (4.8%) patients had lesion range up to 75%-100%.

Significantly difference was found in lung involvement (P=0.014), reticular shadow (P< 0.001) and lung consolidation (P=0.004) between short-term and long-term groups. Bilateral lung involvement is more common in long-term group (207, 84.5%) than short-term group (239, 75.6%). The frequence of reticular shadow was higher in the short-term group (145, 45.9%) than that in the long-term group (70, 28.6%). Similar trend could be found in constituent ratio of lung consolidation.

Treatment and outcome of patients with COVID-19

129 (21.2%) patients received oxygen treatment during hospitalization (**Table 4**). Only 2 (0.3%) patients received invasive mechanical ventilation. 397 (65.2%) received arbidol hydrochloride capsule treatment. There was no significant difference in oxygen therapy, mechanical ventilation and antiviral arbidol treatment between short-term and long-term groups.

Risk factors for long-term SARS-CoV-2 RNA shedding

The group with the duration of viral shedding (>21 days) is considered as the dependent variable. The indicators (P<0.10) in the univariate analysis were taken into consideration as covariates. Considering the effect of the inconsistent course of the patient's admission and subjective factors, we excluded the serum, imaging indicators and unconfirmed self-reported indicators. Age and gender were selected as covariates in the Logistic regression analysis, and the forward stepwise method was adopted to screen the covariables. Multivariate analysis indicated that age range (<65 years) (odds ratio [OR], 1.46 [95% CI, 1.05-2.03], P=0.025) and female sex (odds ratio [OR], 1.40 [95% CI, 1.00-1.94], P=0.048) were independent risk factors for long-term SARS-CoV-2 RNA shedding (**Table 5**).

Discussion

The present study comprehensively described the clinical features among 609 patients with different duration of viral shedding. Of importance, there are several risk factors positively correlated with long-term COVID-19 viral shedding (>21 days).

Table 2. Laboratory findings of patients on admission with coronavirus disease 2019

Variable	Total	Viral RNA shedding duration		*P* value
	n=609	≤21 d n=344	>21 d n=265	
Blood routine				
White blood cell count, $\times10^9$ per L (3.5-9.5)				
<3.5	33/598 (5.5)	15 (4.4)	18 (6.9)	0.441
3.5-9.5	544/598 (91.0)	309 (92.0)	235 (89.7)	
>9.5	21/598 (3.5)	12 (3.6)	9 (3.4)	
Platelet count, $\times10^9$ per L (125-350)				
≤350	569/598 (95.2)	321 (95.5)	248 (94.7)	0.691
>350	29/598 (4.8)	15 (4.5)	14 (5.3)	
Neutrophil count, $\times10^9$ per L (1.8-6.3)				
<1.8	24/598 (4.0)	11 (3.3)	13 (5.0)	0.473
1.8-6.3	539/598 (90.1)	307 (91.4)	232 (88.5)	
>6.3	35/598 (5.9)	18 (5.3)	17 (6.5)	
Lymphocyte count, $\times10^9$ per L (1.1-3.2)				
<1.1	97/598 (16.2)	52 (15.5)	45 (17.2)	0.274
1.1-3.2	494/598 (82.6)	282 (83.9)	212 (80.9)	
>3.2	7/598 (1.2)	2 (0.6)	5 (1.9)	
Monocyte count, $\times10^9$ per L (0.1-0.6)				
≤0.6	565/598 (94.5)	317 (94.3)	248 (94.7)	0.869
>0.6	33/598 (5.5)	19 (5.7)	14 (5.3)	
Blood biochemistry				
Alanine aminotransferase, U/L (0-55)				
0-55	526/589 (89.3)	296 (89.4)	230 (89.1)	0.914
>55	63/589 (10.7)	35 (10.6)	28 (10.9)	
Aspartate aminotransferase, U/L (5-34)				
5-34	534/589 (90.7)	291 (88.4)	243 (93.5)	0.038
>34	55/589 (9.3)	38 (11.6)	17 (6.5)	
Alkaline phosphatase, U/L (40-150)				
≤150	585/588 (99.5)	327 (99.4)	258 (99.6)	0.708
>150	3/588 (0.5)	2 (0.6)	1 (0.4)	
Procalcitonin, ng/mL (0-0.05)				
≤0.05	348/460 (75.7)	166 (70.6)	182 (80.9)	0.010
>0.05	112/460 (24.3)	69 (29.4)	43 (19.1)	
Albumin, g/L (35-52)				
<35	96/590 (16.3)	67 (20.3)	29 (11.2)	0.003
≥35	494/590 (83.7)	263 (79.7)	231 (88.8)	
Total bilirubin, umol/L (3.4-20.5)				
≤20.5	557/590 (94.4)	315 (95.5)	242 (93.1)	0.212
>20.5	33/590 (5.6)	15 (4.5)	18 (6.9)	
Direct bilirubin, umol/L (0-8.6)				
≤8.6	554/582 (95.2)	314 (96.3)	240 (93.8)	0.151
>0-8.6	28/582 (4.8)	12 (3.7)	16 (6.2)	
Creatine kinase-MB, U/L (0-3.1)				
≤3.1	356/373 (95.4)	186 (94.9)	170 (96.0)	0.596
>3.1	17 (4.6)	10 (5.1)	7 (4.0)	
Creatine kinase, U/L (<190)				

≤190	280/287 (97.6)	114 (98.3)	166 (97.1)	0.518
<190	7/278 (2.4)	2 (1.7)	5 (2.9)	
Hypersensitive troponin I, pg/mL (0-34.2)				
≤34.2	356/373 (95.4)	186 (94.9)	170 (96.0)	0.596
>34.2	17/373 (4.6)	10 (5.1)	7 (4.0)	
Serum creatinine, mmol/L (64-104)				
≤104	543/569 (95.4)	298 (94.6)	245 (96.5)	0.293
>104	26/569 (4.6)	17 (5.4)	9 (3.5)	
Blood urea nitrogen, mmol/L (3.2-7.4)				
≤7.4	527/570 (92.5)	290 (91.8)	237 (93.3)	0.490
>7.4	43/570 (7.5)	26 (8.2)	17 (6.7)	
Interleukin-6, pg/mL (0-10)				
≤10	304/343 (88.6)	151 (86.8)	153 (90.5)	0.274
>10	39/343 (11.4)	23 (13.2)	16 (9.5)	
C-reactive protein, mg/L (0-10)				
≤10	513/592 (86.7)	284 (85.8)	229 (87.7)	0.491
>10	79/592 (13.3)	47 (14.2)	32 (12.3)	
D-dimer, mg/L (0-0.55)				
≤0.55	298/431 (69.1)	151 (64.0)	147 (75.4)	0.011
>0.55	133/431 (30.9)	85 (36.0)	48 (24.6)	

Data are n (%), unless otherwise stated. For statistical analyses, Mann-Whitney U test of multiple independent samples was done for continuous variables and χ^2 test or Fisher's exact test was done for categorical variables. There were many missing blood biochemical data.

Previous reports suggested that the viral clearance of SARS-CoV-2 was mainly completed in 21 days [10, 22], and researches on the associated factors of viral shedding duration are mainly within 21 days. With the development of the COVID-19, more and more patients with longer duration of viral shedding have been reported [23, 24]. Consistently, our study shows the presence of long-term viral RNA positive patients in a larger sample of cases, and the proportion is not small. To our surprise, 43.5% of the patients had viral shedding duration for more than 21 days. In addition, there are a certain number of asymptomatic patients (74, 12.2%) in our cases. This is the result of the state's active screening of close contacts. According to previous reports, asymptomatic patients are infectious [25]. Among them, 35.1% patients also had viral shedding duration over 21 days. The presence of such persistent viral RNA positive patients is a great challenge to the treatment and control of transmission. Therefore, it is very important to understand the clinical characteristics of patients with long-term viral shedding (>21 days) and the related factors.

The presence of prolonged viral shedding over 21 days suggests that in the early acute response, the virus has not been cleaned up. On the one hand, it is possibly due to the weak immune function that the virus can not be eliminated in a short time [26]. In our study, the overall patients were relatively old. The median age was 61 years (IQR, 50-69 years). Although our study shows that patients under 65 years old are more likely to have long-term viral shedding, these patients are mainly distributed in the 45-64 age group, accounting for 68.1% of those under 65 years old, which are still relatively old. Previous report showed that people aged 40-64 years were at greatest risk to SARS-CoV-2 [27], suggesting that their immune function has declined. In addition, our research also shows that women are more likely to have long-term viral shedding. This age group of women has been in menopause and the original innate immune advantage weakened, immune function decreased. This also explains part of the reason why women are susceptible. On the other hand, the long-term existence of the virus may be due to the immune escape and mutation of the virus

Table 3. CT findings on admission of patients with coronavirus disease 2019

Variable	Total	Viral RNA shedding duration		*P* value
	n=561	≤21 d n=316	>21 d n=245	
Lung involvement				
Unilateral	77 (13.7)	55 (17.4)	22 (9.0)	0.014
Bilateral	446 (79.5)	239 (75.6)	207 (84.5)	
Normal	38 (6.8)	22 (7.0)	16 (6.5)	
Predominantly CT pattern				
Ground-glass opacities	502 (89.5)	278 (88.0)	224 (91.4)	0.186
Reticular shadow	215 (38.3)	145 (45.9)	70 (28.6)	<0.001
Pulmonary fibrosis	60 (10.7)	34 (10.8)	26 (10.6)	0.955
Normal	38 (6.8)	22 (7.0)	16 (6.5)	0.840
lung consolidation	35 (6.2)	28 (8.9)	7 (2.9)	0.004
Blade clearance displacement	9 (1.6)	6 (1.9)	3 (1.2)	0.771
Pleural effusion	9 (1.6)	6 (1.9)	3 (1.2)	0.528
Peribronchial wall thickening	6 (1.1)	3 (0.9)	3 (1.2)	1.000
Adjacent pleural thickening	4 (0.7)	2 (0.6)	2 (0.8)	1.000
Cumulative lung segments	4 (2.10)	5 (2.10)	4 (3.8)	0.390
Atelectasis	3 (0.5)	3 (0.9)	0 (0.0)	0.344
Lesion range				
No	38 (6.8)	22 (7.0)	16 (6.5)	0.106
0-25%	249 (44.4)	131 (41.5)	118 (48.2)	
26-50%	146 (26.0)	79 (25.0)	67 (27.3)	
51-75%	101 (18.0)	69 (21.8)	32 (13.1)	
76-100%	27 (4.8)	15 (4.7)	12 (4.9)	

Data are median (IQR), n (%), unless otherwise stated. For statistical analyses, Mann-Whitney U test of multiple independent samples was done for continuous variables and χ^2 test or Fisher's exact test was done for categorical variables. CT results were lacking in 48 patients.

Table 4. Treatments of patients with coronavirus disease 2019

Variable	Total	Duration viral RNA shedding range		*P* value
	n=609	<21 d n=344	≥21 d n=265	
Oxygen treatment	129 (21.2)	69 (20.1)	60 (22.6)	0.439
Mechanical ventilation				
Non-invasive mechanical ventilation	5 (0.8)	4 (1.2)	1 (0.4)	0.287
Invasive	2 (0.3)	1 (0.3)	1 (0.4)	0.853
Arbidol Hydrochloride Capsules	397 (65.2)	227 (66.0)	170 (64.2)	0.637

Data are n (%), unless otherwise stated. For statistical analyses was done for continuous variables and χ^2 test or Fisher's exact test was done for categorical variables.

[26]. The virus antibody of some patients can not play an effective role in virus clearance, suggesting that there is immune escape [23, 26]. Besides, Tang et al. showed the SARS-CoV-2 virus have evolved into two major types (named l subtype and s subtype) [28], Homg-ming Miao et al. pointed out that the virus infected by the patient may have mutation. But this is still needs to be further verified.

The intensity of inflammation and immune response is related to virus shedding. In this study, the majority of mild or moderate cases are in line with the epidemiological characteristics [17], It is worth noting that the proportion of women in mild or moderate clinical classification (146, 67.0%) is more than that of men (72, 33%), which indirectly shows that the inflammation and immune response of women

Table 5. Multivariate analysis of patients with coronavirus disease 2019

Variable		Stepwise analysis		
		Odds ratio (OR)	95% CI	p
Age	<65	1.46	1.05-2.03	0.025
	≥65	Ref	-	-
Sex	Male	Ref	-	-
	Female	1.40	1.00-1.94	0.048

The viral shedding time >21 days was taken as dependent variable. Age and sex were included as independent variables in binary logistic regression analysis.

are weak, and therefore the virus clearance is slow [29]. Blood biochemistry and chest computed tomograms results indicated that the short-term viral shedding group had stronger inflammatory and immune response. In blood biochemistry, compared with the long-term viral shedding group, abnormal aspartate aminotransferase, procalcitonin and albumin were more common in the short-term viral shedding group. These implied that inflammation and organ damage were more frequent in the short-term group. This may be partly due to the indirect damage of immune response. In addition, imaging examination has been very helpful for the early detection and diagnosis of COVID-19 [30-32]. Our imaging results showed that, reticular shadow and lung consolidation were more common in patients in short-term viral shedding group. Although there seemed to be more bilateral lung involvement in patients with long-term viral shedding, there is no difference in the lesion range between two groups. These may imply that the patients who have stronger inflammatory reaction are prone to have viral shedding. In the patients with short-term viral shedding, the proportion of pulmonary consolidation is large, considering that the virus stimulates the body to produce strong inflammatory response. This is consistent with the previous laboratory examination of the trend of inflammatory indicators, that is, patients with strong inflammatory response are more likely to have negative viral nucleic acid conversion. In patients of short-term viral shedding group, reticular shadow may be caused by increased disordered lung texture and early formation of pulmonary fibrosis, suggesting that there is a relatively strong inflammatory response before reticular shadow is formed, so the proportion of network structure shadow is larger, and the inflammatory response may be stronger in patients with short time viral shedding.

Our study has several limitations. First, this is a retrospective study, some laboratory tests (for example, blood urea nitrogen, creatinine, C-reactive protein, CT image, etc.) were not done in all the patients, and missing data or important tests might lead to bias of clinical characteristics. Second, how to define the length of time regarding long-term viral shedding has not been confirmed by relevant literature, which needs to be further clarified. Third, because this study is based on the analysis of single center data, the problems and conclusions reflected may be one-sided, and some preliminary conclusions still need to be confirmed by the joint study of multiple centers. Therefore, further study is warranted to gain a better understanding of risk factors for long-term viral shedding in patients with COVID-19, which ultimately may help to guide efforts aimed at promoting complete cure, clinical decision-making and disease transmission control.

The level and duration of infectious virus replication are important factors in assessing the risk of transmission and guiding decisions regarding isolation and treatment of patients [8]. It is of vital importance to have a deep understanding of the clinical characteristics and risk factors associated with long-term viral shedding. Age range (<65) and female sex are risk factors for long-term viral shedding. We suggested that early antiviral treatment should be considered for COVID-19 patients with such risk factors. Further study should be conducted to know the infectivity of patients with long-term viral shedding in order to optimize treatment and isolation management.

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Disclosure of conflict of interest

None.

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