Retrospective analysis of the effect of current clinical medications and clinicopathological factors on viral shedding in COVID-19 patients

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Abstract. The aim of the present study was to identify the risk factors associated with prolonged shedding in patients with coronavirus disease 2019 (COVID-19), and to evaluate the effects of current clinical and clinicopathological factors on viral shedding in patients. A total of 186 COVID-19 inpatients were enrolled in this multicentre retrospective analysis. Detailed clinical data of each patient were collected, and the factors that affected the duration of viral shedding were retrospectively analysed. The median duration of viral shedding in the 186 COVID-19 patients was 13 days. The median duration of viral shedding was 12 days in non-severe patients, and 17 days in severe patients, and there was a significant difference between the two groups (P<0.001). Multi-factor regression analysis suggested that the onset-hospitalization interval [odds ratio (OR), 1.27; 95% confidence interval (CI), 1.15-1.41; P<0.001] and comorbidity with a chronic disease (OR, 2.43; 95% CI, 1.14-5.17; P=0.021) were independent risk factors for prolonged viral shedding, whereas lopinavir/ritonavir (LPV/r) was an independent protective factor (OR, 0.28; 95% CI, 0.11-0.75; P=0.011). Spearman's rank correlation analysis showed that the onset-drug interval was positively correlated with the duration of viral shedding (r=0.446; P<0.0001). Umifenovir, and low and short courses of glucocorticoids were not associated with prolonged viral shedding. The prolonged viral shedding was the initial causative factor of persistent aggravation of the patient's conditions. The interval between presentation of symptoms and hospitalization as well as complications with a comorbid chronic disease were independent risk factors for prolonged viral shedding. LPV/r shortened the duration of viral shedding, and the smaller the interval between presentation and LPV/r onset was, the faster viral shedding occurred.

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Introduction

Coronavirus disease 2019 (COVID-19), caused by infection from severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), has become a global pandemic and a serious global public health emergency (1). The prevention and control of COVID-19 is a considerable challenge being faced by numerous governments worldwide, and this is complicated by the high transmission efficiency, high mortality rate, general susceptibility of the population, and the absence of effective drugs and vaccines (2,3). As the pathology of COVID-19 is similar to that of Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV infection (4,5), drugs identified to be effective for these previous diseases, as well as antivirals screened from *in vitro* experiments, are

being assessed or used clinically without sufficient clinical evidence. These drugs include IFN-α and ribavirin, as well as lopinavir/ritonavir (LPV/r) and Umifenovir as antiviral drugs. Several studies have described the effects of antiviral on the duration of viral shedding (6-8) and other studies have assessed the differences in the duration of viral shedding between patients with different degrees of severity of infection (9). Recently, obesity was identified as a risk factor for increased COVID-19 prevalence, severity and lethality (10), and modulation of zinc status may be beneficial in the management COVID-19 (11). However, these studies did not consider the effects of both clinical and clinicopathological factors on the duration of viral shedding. In the present study, detailed data on inpatients with definite clinical outcomes between January 20, 2020 and March 20, 2020 was collected and reviewed the duration of viral shedding in COVID-19 infected patients in order to evaluate the impact of current clinical and clinicopathological factors on viral shedding. Clinicopathological factors refers to the patient's general characteristics and medical history that are not related to this hospitalization, such as sex, age, comorbid chronic diseases and onset-hospitalization interval.

Materials and methods

Study design. Data on SARS-CoV-2 nucleic acid-positive hospitalized patients were collected between January 20, 2020 and March 20, 2020. Reverse transcription polymerase chain reaction (RT-PCR) was used to test for SARS-CoV-2 nucleic acids from respiratory tract secretions and throat swab specimens. The patients underwent a RT-PCR test every 3 days during the first week of hospitalization, and a nucleic acid test every day after a week of hospitalization. A retrospective analysis of the SARS-CoV-2 shedding duration and the effect of currently used drugs on the duration of viral shedding was performed. This study was exempt from the need to obtain patient consent due to the retrospective nature of the study, and was approved by the Medical Ethics Committee of Zhengzhou University (approval no. 2020-KY-162).

Data collection. The age, sex, clinical symptoms, onset-drug interval, onset-hospitalization interval, therapeutic drugs and prognosis of each patient were collected. The median age of the 186 patients was 46.5 years (age range, 5-94 years). A total of 105 (56.5%) cases were males and 81 (43.5%) were females. Onset was defined as the earliest time when clinical symptoms appeared. The duration of viral shedding was defined as the time from onset to the last positive test for SARS-CoV-2. The criteria for severe cases (including critical) was defined as follows: Breathing significantly faster, >30 times per minute in a resting state; blood oxygen saturation <90%, or blood oxygen saturation notably decreased in the analysis of arterial blood gas; and lung image showing >50% apparent progression of lesions within 24-48 h. Non-severe cases were diagnosed with mild respiratory infection symptoms (defined as fever <39˚C, no breathing difficulties, blood oxygen saturation ≥90%), without visible radiological changes of the chest. The application of drugs was defined as patients treated with a drug for ≥3 days.

Statistical analysis. All statistical analysis was performed using SPSS version 21.0 (SPSS, Inc.). Categorical variables were expressed as percentages. Normally distributed continuous variables are shown as the mean ± standard deviation, and were compared using a Student's t-test. Non-normally distributed continuous variables are presented as the median, and a rank sum test was used for comparison. Significant risk factors identified by univariate analyses were further analysed by multivariate logistic regressions to identify independent risk factors associated with a prolonged duration of SARS-CoV-2 shedding [odds ratio (OR), 95% confidence interval (CI)]. Rank correlation analysis was used to analyse the correlation between onset-drug interval of LPV/r and the duration of viral shedding. A two-sided $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics and treatments. A total of 133 non-severe cases (71.5%) and 53 (28.5%) severe cases were enrolled in this study, including 10 deaths (5.4%). A total of 54 patients (29.0%) had a comorbid chronic disease, and 31 patients exhibited ≥1 comorbid disease, including 29 with hypertension, 18 with diabetes, 15 with coronary heart disease and 7 with chronic lung disease. The median onset-hospitalization interval for the 186 patients was 5 days [interquartile range (IQR) 2-8 days]. LPV/r (adults, 400 mg/100 mg bid po; children, 200 mg/50 mg bid po) was administered to 158 patients (84.9%) during hospitalization. A total of 140 patients had a clear onset-drug interval, with a median period of 7 days (IQR, 4-10 days). Umifenovir (200 mg tid po) was administered to 44 (23.7%) patients. A total of 18 patients (9.7%) were treated with ribavirin (500 mg bid ivggt) during hospitalization. A total of 30 patients (16.1%) were treated with a corticosteroid during hospitalization, at doses of ranging from 40-120 mg; however, although the highest dose was 120 mg per day, the majority of patients received <80 mg/day.

Duration of viral shedding. The duration of SARS-COV-2 shedding was 3-40 days, and the median period was 13 days (IQR, 10-19 days). The median duration of viral shedding in non-severe case was 12 days (IQR, 8-17 days), and the longest duration was 39 days. The median duration of viral shedding in severe case was 17 days (IQR, 12-23 days), and the longest duration was 40 days; there was a significant difference ($P<0.001$) between the two groups. In 5 deaths, the nucleic acid test remained positive. The different characteristics of the COVID-19 patients with the duration of viral shedding are outlined in Table I.

Analysis of influencing factors on the duration of viral RNA shedding. As the median duration of SARS-CoV-2 shedding was 13 days, a 14-day cut-off was used. A 12 or 13-day cut-off was not appropriate as the 12 or 13-day cut-off was not statistically significant, although there was a difference. This study focused on analysing the factors influencing viral shedding duration (≤14 and >14 days). With the duration of viral shedding as a dependent variable, univariate analysis of sex, age, onset-hospitalization interval, whether the patient had a comorbid chronic disease, ribavirin use, lopinavir/ritonavir

Table I. Characteristics of COVID-19 patients and the duration of viral shedding.

Factors	Viral shedding duration after illness onset		P-value
	≤14 days, n=115	>14 days, n=71	
Age, years, mean ± standard deviation	44.35±17.44	51.39±18.15	0.009[b]
Sex, n (%)			0.98
Male	65 (56.5)	40 (56.3)	
Female	50 (43.5)	31 (43.7)	
Onset-hospitalization interval, days (IQR)	4 (2-7)	7 (3-11)	<0.001
Comorbid chronic disease, n (%)			0.005[b]
Yes	25 (21.7)	29 (40.8)	
No	90 (78.3)	42 (59.2)	
Ribavirin use, n (%)			0.002[b]
Yes	5 (4.3)	13 (18.3)	
No	110 (95.7)	58 (81.7)	
Lopinavir/ritonavir use, n (%)			<0.001
Yes	106 (92.2)	52 (73.2)	
No	9 (7.8)	19 (26.8)	
Umifenovir use, n (%)			0.028[a]
Yes	21 (18.3)	23 (32.4)	
No	94 (81.7)	48 (67.6)	
Corticosteroid use, n (%)			0.007[b]
Yes	12 (10.4)	18 (25.4)	
No	103 (89.6)	53 (74.6)	

[a]P<0.05, [b]P<0.01. IQR, interquartile range; COVID-19, coronavirus disease 19.

use, Umifenovir use and corticosteroid use were defined as independent variables and logistic stepwise multiple regression analysis was performed.

Univariate analysis showed that the factors significantly associated with prolonged viral shedding were age, onset-hospitalization interval, comorbid chronic disease, use of LPV/r, use of ribavirin, use of Umifenovir and use of a corticosteroid. However, sex was not associated with prolonged viral shedding.

For the multivariate analysis, four parameters were included in the final logistic regression model: Onset-hospitalization interval, comorbid chronic disease, ribavirin use and LPV/r use. Multiple regression analysis suggested that the onset-hospitalization interval (OR, 1.27, 95% CI, 1.15-1.41; P<0.001), comorbid chronic disease (OR, 2.43, 95% CI, 1.14-5.17; P=0.021) and ribavirin use (OR, 5.97, 95% CI, 1.77-20.09; P=0.004) were independent risk factors for prolonged viral shedding. LPV/r was an independent protective factor (OR, 0.28, 95% CI, 0.11-0.75; P=0.011) that could shorten the duration of viral shedding (Table II).

Relationship between duration of SARS-CoV-2 shedding and onset-drug interval of LPV/r. A total of 140 patients were administered LPV/r, and the onset-drug interval was positively correlated with the duration of viral shedding (rank correlation test r=0.446; P<0.0001). The longer the onset-drug interval of LPV/r was, the longer the duration of viral shedding was (Fig. 1).

Discussion

COVID-19 is a newly discovered infectious disease with no specific drug treatments and asymptomatic patients have been identified as a considerable source of infection (12). At present, several drugs are being used based on previous experience in the treatment of similar coronavirus epidemics such as SARS and MERS; however, the efficacy of these drugs require urgent verification. Therefore, in the present study, a multicentre retrospective analysis of 186 patients was performed to explore the effects of therapeutic drugs and clinicopathological factors on the duration of SARS-CoV-2 viral shedding.

The duration of viral shedding is used as an index to measure infectivity and evaluate the efficacy of antiviral drugs (13-15). The median duration of SARS-CoV-2 shedding was 13 days (IQR, 10-19 days), with a maximum of 40 days. The results showed that the duration of SARS-CoV-2 shedding was generally long; this indicated a long period where a person is potentially infectious, and thus requires prolonged isolation, in which case, the course of antiviral treatment should be extended accordingly. The median viral shedding period for severe patients was 17 days (IQR, 12-23 days), which was significantly longer than the 12 days (IQR, 8-17 days) of non-severe patients. Since viral load is the initial factor exhibiting persistent aggravation, early antiviral therapy is very important in theory.

In the present study, 44 patients (23.7%) were treated with Umifenovir (200 mg tid po), and the administration

Table II. Multivariate analysis of factors associated with the duration of viral shedding of COVID-19.

	Multivariable analysis			Stepwise analysis		
Variable	OR	95% CI	P-value	OR	95% CI	P-value
Age	1.02	1.01-1.04	0.010	-	-	-
Sex	1.01	0.56-1.83	0.980	-	-	-
Onset-hospitalization interval	1.22	1.11-1.33	<0.001	1.27	1.15-1.41	<0.001[c]
Comorbid chronic disease	2.49	1.30-4.75	0.006	2.43	1.14-5.17	0.021[a]
Using ribavirin	4.93	1.68-14.51	0.004	5.97	1.77-20.09	0.004[b]
Using lopinavir/ritonavir	0.23	0.10-0.55	0.001	0.28	0.11-0.75	0.011[a]
Using Umifenovir	2.15	1.08-4.26	0.029	-	-	-
Using corticosteroid	2.92	1.31-6.50	0.009	-	-	-

[a]P<0.05, [b]P<0.01, [c]P<0.001. OR, odds ratio; CI, confidence interval.

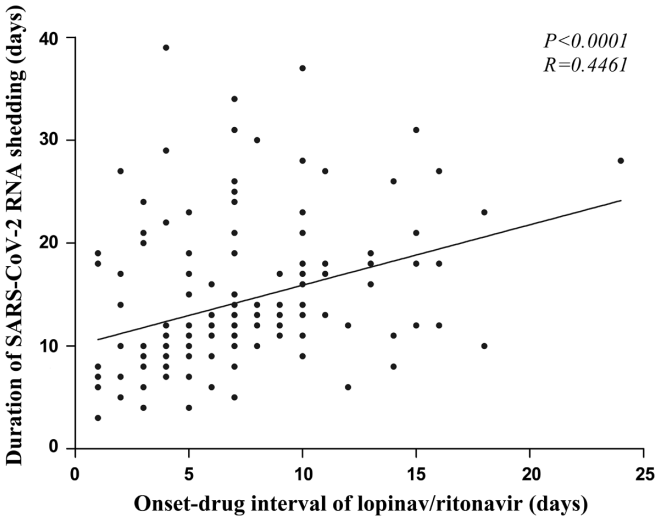

Figure 1. Relationship between duration of SARS-CoV-2 shedding and onset-drug interval of lopinavir/ritonavir. SARS-CoV2, severe acute respiratory syndrome-coronavirus 2.

of Umifenovir was relatively early. Multivariate analysis showed that Umifenovir did not affect SARS-CoV-2 shedding. This finding suggests that Umifenovir has no beneficial anti-SARS-CoV-2 effect.

The results of multivariate analysis showed that ribavirin was a high-risk factor for prolonging the time of viral shedding. This was an unexpected result that differed from a previous study (16), and this may have been caused by data bias. Additional clinical trials, particularly randomized controlled clinical trials, are required to further evaluate the effect of ribavirin on patients with SARS-CoV-2.

As the most widely used anti-inflammatory and immunosuppressive agents in clinical practice, corticosteroids serve an important role in the treatment of critically ill patients (17). However, whether and how to use them for the treatment of COVID-19 remains controversial. Previous studies have shown that corticosteroids may prolong the duration of viral shedding and may facilitate adverse effects such as secondary infection and delirium; however, corticosteroids has been shown to also reduce mortality in patients with severe COVID-19 (18-20). The present study found no evidence suggesting that corticosteroid treatment prolonged viral shedding time. In China, a low dose and short course of corticosteroid therapy is generally used on severe and critical COVID-19 patients (21). In the present study, 30 patients were treated with 40-120 mg corticosteroids during hospitalization. The dose is generally <80 mg and the course of treatment is ~7 days. Therefore, for critically ill patients, small doses and short courses of a corticosteroid may be considered under special circumstances.

In the total 186 patients, the median time of onset-hospitalization interval was 5 days (IQR, 2-8 days). Multivariate regression analysis found that the onset-hospitalization interval was an independent risk factor for prolonged viral shedding time. Therefore, early hospitalization of COVID-19 patients can shorten the duration of viral shedding and improve the prognosis of patients, whilst also reducing the infectivity of the patient.

Complications with a comorbid chronic disease was also an independent risk factor for prolonged viral shedding. COVID-19 patients with long-term comorbid chronic diseases may have relatively low immunity; these patients were susceptible to severe pneumonia and thus had a poor prognosis (22).

LPV has been used in combination with ritonavir (a booster) in HIV infection therapy and prevention, and it functions as an antiretroviral protease inhibitor (23). LPV/r has shown efficacy in the treatment of SARS-CoV and MERS (24-26). SARS-CoV-2 is a coronavirus similar to SARS-CoV and MERS (27); however, the effects of LPV/r on SARS-CoV-2 remains unclear. In a recent retrospective study of risk factors associated with death in hospitalized COVID-19 patients in Wuhan, Zhou *et al* (14) found no reduction in the duration of viral shedding following LPV/r treatment. In critically ill patients with COVID-19, Cao *et al* (28) found that LPV/r did not significantly accelerate clinical improvement, reduce mortality, or reduce the viral RNA load detected in the throat. These results may be due to the small sample sizes, single factor retrospective analysis, or the severity of the patients' illness. More recent studies have shown that hospital mortality rates were lower in the LPV/r group (29), and the lack of LPV/r treatment was independently associated with prolonged

SARS-CoV-2 RNA shedding (30). The multiple factor regression analysis performed in the present study showed that LPV/r shortened the duration of SARS-CoV-2 shedding. In the study by Cao *et al* (28), all the patients had severe symptoms, the number of cases was small and treatment with LPV/r started relatively later compared with the present study. In contrast, in the present study, the data on 158 patients who used LPV/r and 28 patients who did not was collected, and the median onset-drug interval was only 7 days (IQR, 4-10 days). The strengths of the present study include a larger number of cases, which consisted primarily of patients with non-severe patients and the earlier administration of antivirals.

To further evaluate the effect of early antiviral treatment with LPV/r on SARS-CoV-2 shedding, the correlation between the onset-drug interval and the duration of viral shedding was assessed, and this showed a significant positive correlation. The longer the onset-drug interval of LPV/r, the longer the duration of viral shedding; these findings suggested that early antiviral treatment was important for favourable outcomes. A previous study showed that the timing of LPV/r combined with hydroxychloroquine treatment initiation did not seem to affect the clinical course of COVID-19 patients (31), in contrast to the results of the present study. Thus, additional studies with larger cohorts are required to demonstrate the efficacy of LPV/r on COVID-19 infection.

The present study has limitations in that it was a retrospective analysis, several factors were included, and the sample size was limited. Although the research factors were screened by single factor analysis and stepwise regression, the results still require further verification in large-scale prospective studies.

In conclusion, the results of the present study showed that the duration of viral shedding of SARS-COV-2 based on treatment, and prolonged viral shedding was an important factor causing persistent aggravation of the patient's condition. The onset-hospitalization interval and complication with comorbid chronic diseases were independent risk factors for prolonged viral shedding. LPV/r application shortened the duration of viral shedding, and the earlier LPV/r was used, the shorter the duration of viral shedding was. These results suggest that COVID-19 patients should be hospitalized and receive antiviral treatment as early as possible, and the use of LPV/r is a viable option.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

YP contributed to the conception and design of study and the analysis and interpretation of data. QLi contributed to the acquisition, analysis and interpretation of data and manuscript review. XY contributed to the acquisition, analysis and interpretation of data. QLuo contributed to the interpretation of data and manuscript review. TQ, NX, QZhang, XL, XD, QZhao and LS contributed to the acquisition of data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was exempt from the need to obtain patient consent due to the retrospective nature of the study, and was approved by the Medical Ethics Committee of Zhengzhou University (approval no. 2020-KY-162).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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