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Viral kinetics and factors associated with rapid viral clearance during lopinavir/ritonavir-based combination therapy in non-severe COVID-19 patients

J.-G. DING[1], J. LI[2], L. HONG[1], X.-Q. YU[2], E.-L. YE[3], G.-Q. SUN[4], X.-X. ZHANG[2,6], L. CHEN[5,6], Q.-F. SUN[1]

[1]Department of Infectious Diseases, Ruian People's Hospital, Ruian, Zhejiang, China
[2]Research Laboratory of Clinical Virology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China
[3]Department of Endocrinology, Ruian People's Hospital, Ruian, Zhejiang, China
[4]Department of Biology, Gordon College, MA, USA
[5]Department of Gastroenterology, Ruijin Hospital North, Shanghai Jiaotong University, School of Medicine, Shanghai, China
[6]Clinical Research Center, Ruijin Hospital North, Shanghai Jiaotong University, School of Medicine, Shanghai, China

Jiguang Ding and *Jing Li* contributed equally to this work

Abstract. – OBJECTIVE: Lopinavir/ritonavir has modest antiviral activity against severe acute respiratory syndrome coronavirus 2. The aim was to investigate the viral kinetics and factors associated with viral clearance during lopinavir/ritonavir-based combination treatment in non-severe patients.

PATIENTS AND METHODS: Sixty-four patients were retrospectively enrolled. Viral RNA was detected by real-time RT-PCR assay from sputum or throat swab samples at different time points. The patterns of viral kinetics were characterized, and factors associated with rapid viral clearance, which was defined as viral RNA undetectable within two weeks, were analyzed using multivariate logistic regression analyses.

RESULTS: All patients achieved viral RNA negativity and were discharged from the hospital. Furthermore, 48 (75%) and 16 (25%) patients achieved rapid and delayed viral clearance, respectively. The lymphocyte counts of rapid viral clearance patients (1.40 [1.20-1.80] × 10^9/L) were higher, when compared to delayed viral clearance patients (1.00 [0.70-1.47] × 10^9/L) (p=0.024). The multivariate logistic analysis revealed that high lymphocyte count (≥1.3×10^9/L) is an independent factor associated with rapid viral clearance (OR=7.62, 95% CI=1.15-50.34, p=0.035).

CONCLUSIONS: The viral shedding exhibited different patterns during treatment. Immune insufficiency is responsible for the delayed viral clearance, suggesting that an immunomodulator should be considered to promote viral clearance in patients with low lymphocyte counts.

Key Words:
COVID-19, SARS-CoV-2, Antiviral therapy, Viral kinetics, Viral clearance.

Abbreviations

COVID-19: 2019 novel coronavirus disease; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; WHO: World Health Organization; HIV-1: Human immunodeficiency virus 1; SARS: Severe acute respiratory syndrome; CT: Computed tomography; SD: Standard deviation; IQR: Interquartile range; ORs: Odds ratios; CI: Confidence interval; MERS: Middle East Respiratory Syndrome; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase; BUN: Blood urea nitrogen; CK: Creatine kinase; LDH: Lactate dehydrogenase; LDL-C: Low-density lipoprotein cholesterol; WBC: White blood cell; Lym: Lymphocytes; Neu: Neutrophils; HGB: Hemoglobin; PLT: Platelets; NK: Natural killer cell; ARDS: Acute respiratory distress syndrome; LPV: Lopinavir/ritonavir; IFN: Interferon-α.

Corresponding Authors: Qingfeng Sun, MD; e-mail: sunxue0806@126.com
Li Chen, MD; e-mail: drchenli@163.com

Introduction

Since December 2019, the 2019 novel coronavirus disease (COVID-19) outbreak caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China. The epidemic rapidly spread worldwide and was characterized as a pandemic by the World Health Organization (WHO) on March 11, 2020. At present, there are no specific antiviral agents approved for SARS-CoV-2. However, few candidate drugs, including remdesivir and lopinavir/ritonavir, have demonstrated promising antiviral efficacy in COVID-19 treatment[1].

Lopinavir is a human immunodeficiency virus 1 (HIV-1) protease inhibitor that is usually combined with ritonavir as a booster. Lopinavir/ritonavir alone or in combination with interferon can inhibit protease activity and viral replication against coronavirus, both *in vivo* and *in vitro*[2,3]. Lopinavir/ritonavir has exhibited a significantly lower rate of adverse clinical outcomes, when compared to the historical control, in the treatment of severe acute respiratory syndrome (SARS) (2.4% *vs.* 28.8%, p=0.001)[4]. Lopinavir also exhibits modest antiviral activity against SRAS-CoV-2 *in vitro*[5]. A recent clinical trial (LOTUS China) revealed that lopinavir/ritonavir treatment failed to reach the primary endpoint (i.e., clinical improvement). However, in the modified intention-to-treat analysis, lopinavir/ritonavir treatment was associated with accelerated clinical recovery (16.0 days *vs.* 17.0 days) (hazard ratio = 1.39; 95% CI = 1.00-1.91), and reduced the mortality (19.0% *vs.* 27.1%) in a subgroup of patients treated within 12 days after the onset of symptoms[6]. The antiviral efficacy and the indication population of lopinavir/ritonavir treatment remain to be further confirmed.

Due to the emergency nature of the COVID-19 epidemic, lopinavir/ritonavir and other agents, including Interferon-α, and arbidol, were recommended as an antiviral option for SARS-CoV-2, despite the lack of strong evidence, according to the diagnosis and treatment guideline for novel coronavirus pneumonia released by the National Health Commission of China (5th edition)[7]. The LOTUS trial revealed that the clearance of SARS-CoV-2 RNA on throat swabs over time was similar between lopinavir/ritonavir treatment and standard care, and this is probably due to the relatively late treatment after the onset of symptoms and severe condition of the enrolled patients[6]. Therefore, the efficacy of lopinavir/ritonavir monotherapy remains to be further evaluated. A recent study[8] revealed that lopinavir/ritonavir and arbidol combination therapy exhibits a higher rate of SARS-CoV-2 RNA negativity and earlier viral clearance, when compared with lopinavir/ritonavir monotherapy, in non-severe patients. In addition, interferon is a broad-spectrum antiviral agent, which has been used in combination with other antiviral drugs for emerging viral infection, for which no specific antiviral drugs exist at present[9]. Previous studies have suggested that combination therapy might be a beneficial strategy for COVID-19 patients. Therefore, the present study aimed to analyze the viral kinetics, and determine the factors associated with viral clearance in non-severe COVID-19 patients who received lopinavir/ritonavir-based combination therapy.

Patients and Methods

Patients

From January 21, 2020 to February 20, 2020, 74 COVID-19 patients were diagnosed and received treatment in Ruian People's Hospital. For all patients, COVID-19 was confirmed by reverse transcription-PCR assay, according to the WHO interim guidance[10] and the diagnosis and treatment guidelines for the novel coronavirus pneumonia released by the National Health Commission of China[7]. Among these patients, 64 patients were classified with non-severe COVID-19. These patients received lopinavir/ritonavir-based therapy and were retrospectively enrolled for analysis. Four severe patients and six non-severe patients without antiviral therapy were excluded. All patients received general support care and antiviral regimens, as follows lopinavir/ritonavir (AbbVie Inc. North Chicago, IL, USA) (400 mg/100 mg, twice per day) and Interferon-α (Tianjin Sinobioway Biomedicine Co., Ltd. China) (500 MIU, aerosol inhalation, twice per day) for 10 days or more, when the viral load remained detectable. Some patients additionally received arbidol (200 mg, thrice per day). Antibiotic treatment and oxygen support were applied for some patients, when necessary. The present investigation was approved by the Ethics Committee of Ruian People's Hospital (Approval No. YJ20200013).

Data Collection

The clinical information of all enrolled patients was retrieved from the hospital history system, including the demographic data, source of in-

fection, time of incubation, time of illness onset, time of hospital admission, duration of antiviral treatment, time for undetectable viral load, and duration of hospitalization. Comorbidities, including hypertension, diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease and malignant tumor, were recorded. Furthermore, the applications of intranasal oxygen inhalation and medication regimen were also recorded. Respiratory and urinary tract and blood bacterial co-infections were detected within 48 hours of hospital admission. The symptoms, body temperature and adverse events were recorded daily.

SARS-CoV-2 RNA Detection

SARS-CoV-2 RNA was detected using the TaqMan probe targeting ORF1ab, N and E gene by real-time RT-PCR assay and expressed in cycle threshold (Ct) (Shanghai BioGerm Medical Biotechnology Co., Ltd., China). The amplification products for genes with Ct value less than 38 were considered as positive. Sputum samples were preferentially taken, when available. Otherwise, throat swab samples were taken for analysis at baseline, and every 2-3 days until hospital discharge. From February 10, viral RNA from feces samples were simultaneously detected with the last respiratory tract samples collected.

Outcome Measurement and Definition

Patients who met the following criteria[7] were defined as clinically cured and ready for discharge from the hospital: (1) normal temperature that lasted longer than three days, (2) resolved respiratory symptoms, (3) substantially improved acute exudative lesions on the chest computed tomography (CT) images, and (4) consecutively negative RT-PCR test results, twice (with at least a one-day interval). The patterns of viral clearance were divided according to the viral load at two weeks during the antiviral treatment: rapid viral clearance group and delayed viral clearance group. Rapid viral clearance was defined as viral RNA undetectable within two weeks, while delayed viral clearance was defined as viral RNA that remained detectable after two weeks.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median ± interquartile range (IQR), and categorical variables were expressed in number (%). These values were compared by Student's *t*-tests or Mann-Whitney test, as appropriate. The Kaplan-Meier method was applied to illustrate the viral clearance during antiviral treatment. A two-piecewise linear regression model was applied to examine the threshold effect of lymphocyte counts on rapid clearance using a smoothing function. The threshold level (turning point) for lymphocyte counts, as an independent factor associated with viral clearance, was determined using the trial and error method, including the selection of turning points along with a predefined interval, and the turning point that gave the maximum model likelihood. A likelihood ratio test was conducted to compare the one-line linear regression model with the two-piece-wise linear model. Then, multivariate logistic regression analysis was applied to identify factors that were independently associated with rapid viral clearance. Graphs were plotted using GraphPad Prism 7.0 (La Jolla, CA, USA). All data analyses were performed using the R software (version 3.6.2) and EmpowerStates software (www.empowerstats.com, X&Y solutions, Inc. Boston MA, USA). A two-sided p-value of <0.05 was considered statistically significant.

Results

Demographic and Clinical Characteristic of Patients

For all patients, the median age was 43.0 (35.5-57.0) years old, and 32 (50.0%) patients were male. Furthermore, 26 (40.6%) patients came from Wuhan. The median incubation time was 5.0 (4.0-7.0) days, and the time from the onset of illness to hospitalization was 3.0 (2.0-5.0) days.

The most common comorbidity was hypertension, which occurred in 10 (15.62%) patients. Furthermore, 53 (82.80%) patients had a fever, and the median temperature was 37.9°C (37.5-38.7°C). The lymphocyte counts at enrollment slightly decreased (1.35 [1.08-1.80] $\times 10^9$/L). The median oxyhemoglobin saturation was 97.60% (96.75-98.45%). Fifty-two (81.25%) patients exhibited pneumonia changes on the CT scan at enrollment.

Therapeutic Regimens and Treatment Outcomes

Forty-nine (76.56%) patients received the lopinavir/ritonavir, arbidol and interferon-α regimen, and 15 (23.44%) patients received the lopinavir/ritonavir and interferon-α regimen. The

median duration of antiviral treatment was 12.5 (10.0-16.3) days. Thirty-eight (59.38%) patients received oxygen therapy.

The median hospitalization day was 14.0 (10.8-17.0) days, and all (100.00%) patients were discharged from the hospital with negative viral RNA, twice, from the respiratory tract (Table I).

Patterns of Viral Clearance

The mean viral load (Ct value) at enrolment was 30.87 (27.33-33.75), which steadily decreased following the antiviral treatment (Figure 1). The median time from RNA positivity to RNA negativity was 11.0 (7.0-14.3) days, with the longest duration of viral RNA positivity of 38.0 days in one patient. Furthermore 48 (75%) and 16 (25%) patients achieved rapid and delayed viral clearance, respectively, as confirmed twice in specimens obtained from the respiratory tract. Thus, all patients eventually achieved viral RNA negativity before discharge from the hospital (Figure 2).

The viral clearance pattern did not differ between antiviral regimens with or without arbidol (p=0.092). The duration of viral clearance was positively correlated with the time from the onset of illness to hospitalization in the *post hoc* subgroup of patients admitted to the hospital at seven days after illness onset (r=0.668, p=0.035). The duration of viral clearance was positively correlated with the duration of hospitalization (r=0.631, p<0.001) (Figure 3).

Factors Associated With Viral Clearance

The lymphocyte counts in the delayed viral clearance group were lower than that in the rapid

Table I. Demographic and clinical characteristics of all enrolled patients.

	Median (IQR)/N (%)
Age (years)	43.0 (35.5-57.0)
Gender (M/F)	32/32
Incubation period (days)	5.0 (4.0-7.0)
Days from illness onset to hospitalization	3.0 (2.0-5.0)
Viral load (Ct value)	30.87 (27.33-33.75)
ALT (U/L; range: 9-50)	18.00 (14.00-32.25)
AST (U/L; range: 15-40)	21.50 (17.75-31.25)
LDH (U/L; range: 120-250)	208.00 (173.75-245.25)
WBC ($\times 10^9$/L; range: 3.5-9.5)	4.45 (3.65-5.78)
Lym ($\times 10^9$/L; range: 1.1-3.2)	1.35 (1.08-1.80)
Neu ($\times 10^9$/L; range: 1.8-6.3)	2.60 (1.87-3.55)
PLT ($\times 10^9$/L; range: 125-350)	183.50 (147.75-219.50)
CD3+T cell (/μL; range: 690-1760)	1068.00 (847.50-1374.50)
CD4+T cell (/μL; range: 410-884)	587.00 (482.50-769.00)
CD8+T cell (/μL; range: 190-658)	359.00 (297.00-460.00)
NK (/μL; range: 90-536)	315.00 (206.00-421.00)
Hypertension	10 (15.62%)
Diabetes mellitus	6 (9.38%)
Digestive system disease	4 (6.25%)
Respiratory system disease	2 (3.12%)
Malignancy	1 (1.56%)
Cardiovascular and cerebrovascular diseases	1 (1.56%)
Smoking	4 (6.25%)
Top body temperature (°C)	37.9 (37.5-38.7)
ARDS	0 (0.00%)
Pneumonia at admission	52 (81.25%)
Oxygen therapy	38 (59.38%)
Antiviral treatment	
LPV+IFN	15 (23.44%)
LPV+arbidol+IFN	49 (76.56%)
Antiviral duration (days)	12.5 (10.0-16.3)
Days from RNA positive to negative (days)	11.0 (7.0-14.3)
Duration of hospitalization (days)	14.0 (10.8-17.0)

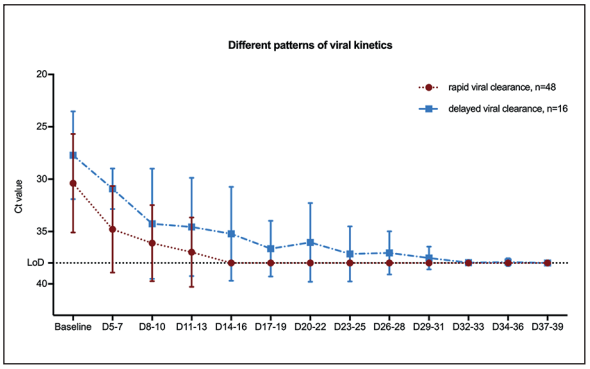

Figure 1. The viral kinetics in COVID-19 patients with rapid and delayed viral clearance during the antiviral treatment.

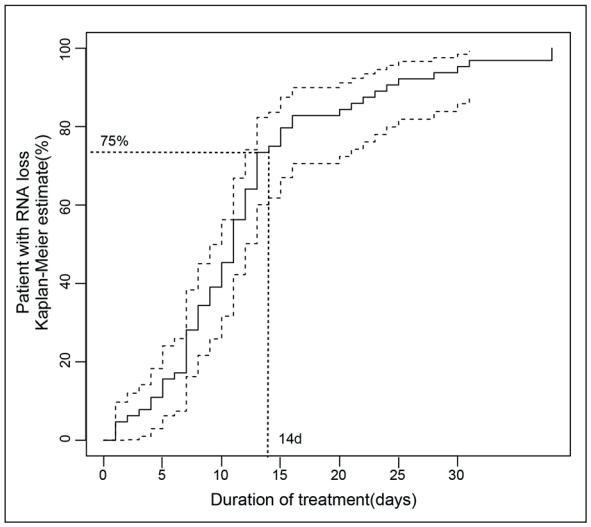

Figure 2. The Kaplan-Meier plots for the time to virus clearance during antiviral treatment in COVID-19 patients. The dotted line presents the 95% confidence interval.

viral clearance group (1.00 [0.70-1.47] $\times 10^9$/L *vs.* 1.40 [1.20-1.80] $\times 10^9$/L) (p=0.031). The serum potassium level was higher in the rapid viral clearance group than in the delayed viral clearance group (3.79 [3.57-4.06] *vs.* 3.42 [3.21-3.77], p=0.017). The baseline viral loads in the delayed viral clearance group were higher than those in the rapid viral clearance group (28.63 [25.52-30.87] *vs.* 31.58 [27.96-34.52]), although the difference was not statistically significant (p=0.086) (Table II).

To further examine the threshold effect of lymphocyte counts on the pattern of viral clearance, a smoothing function was applied, and a nonlinear relationship between the lymphocyte counts and rapid clearance odds was found (adjusted for viral load, serum potassium, Low-density lipoprotein cholesterol [LDL-C] and top body temperature). The odds ratios (ORs) of rapid clearance changed with the lymphocyte counts down to the turning point (lymphocyte counts=1.3×10^9/L). With lymphocyte counts of ≥1.3×10^9/L, the relationship between lymphocyte counts and rapid viral clearance was not significant (p=0.613). However, the ORs for rapid viral clearance increased with lymphocyte counts of <1.3×10^9/L. Thus, the rate of rapid viral clearance was higher in the lymphocytes count ≥1.3×10^9/L group (33/39, 84.62%), compared with the lymphocyte count <1.3×10^9/L group (15/25, 60.00%) (p=0.027).

The univariate logistic regression revealed that higher lymphocytes count (OR=3.77, 95% confidence interval [CI]= 1.20-11.82; p=0.023) and serum potassium level (OR=7.63, 95% CI=1.44-40.39; p=0.017) were associated with rapid viral clearance. To avoid overfitting, five variables with p-values of <0.1 were included in the multi-

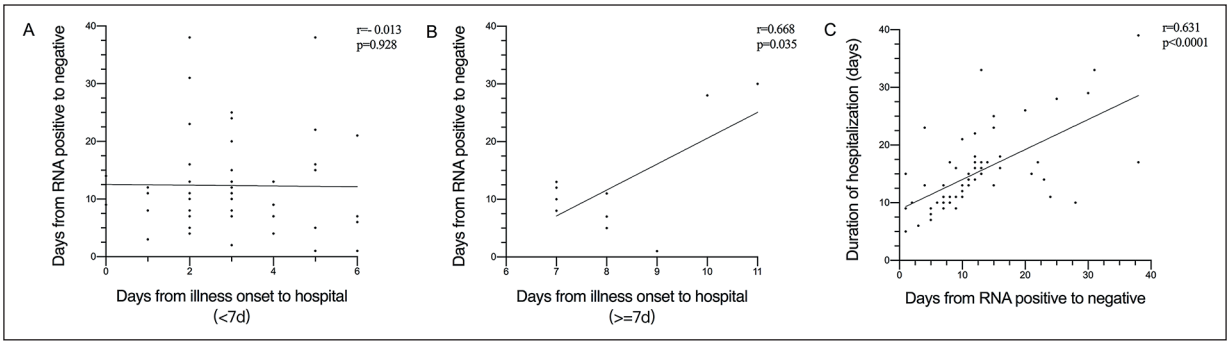

Figure 3. The correlation between time from illness onset to hospitalization and the duration of viral clearance stratified by less than seven days (**A**) and more than seven days (**B**). The duration of viral clearance was positively correlated with duration of hospitalization, indicating that delayed viral clearance is associated with longer hospitalization (**C**).

Table II. Demographic and clinical characteristics between rapid clearance and delayed clearance.

	Rapid clearance (n=48)	Delayed clearance (n=16)	*p*-value
Age (years)	43.00 (35.50-57.00)	44.50 (37.50-58.75)	0.550
Gender (M/F)	25/23	7/9	0.564
Antiviral treatment			
LPV+IFN/LPV+arbidol+IFN	14/34	1/15	0.092
Incubation period (days)	6.0 (4.0-8.0)	5.0 (4.0-6.3)	0.488
Days from illness onset to hospitalization	3.0 (2.0-5.0)	4.0 (2.8-5.0)	0.251
Viral load (Ct value)	31.58 (27.96-34.52)	28.63 (25.52-30.87)	0.086
Top body temperature(°C)	37.75 (37.30-38.60)	38.65 (37.70-38.90)	0.050
Pneumonia at admission	37 (77.08%)	15 (93.75%)	0.170
ALT (U/L; range:9-50)	17.50 (13.75-33.50)	21.00 (15.00-26.50)	0.367
AST (U/L; range:15-40)	21.00 (17.75-28.75)	24.50 (18.50-33.50)	0.876
BUN (mmol/L; range: 3.6-9.5)	3.96 (3.36-4.38)	3.75 (3.12-4.57)	0.963
CK (U/L; range: 50-310)	81.00 (49.75-105.75)	64.50 (54.50-92.00)	0.529
LDL-C	2.45 (1.89-2.73)	2.56 (2.29-3.12)	0.070
D-dimer	0.22 (0.13-0.42)	0.22 (0.13-0.28)	0.200
LDH (U/L; range:120-250)	204.00 (175.50-238.25)	214.00 (165.00-263.50)	0.138
K+ (mmol/L; range: 35-53)	3.79 (3.57-4.06)	3.42 (3.21-3.77)	0.017
WBC ($\times10^9$/L; range:3.5-9.5)	4.65 (3.77-6.00)	4.10 (3.40-4.65)	0.170
Lym ($\times10^9$/L; range:1.1-3.2)	1.40 (1.20-1.80)	1.00 (0.70-1.47)	0.031
HGB (g/L; range: 130-175)	137.00 (126.75-152.00)	131.00 (122.25-138.25)	0.120
PLT ($\times10^9$/L; range:125-350)	197.00 (154.50-223.25)	166.50 (146.25-176.00)	0.071
CD4+T cell (/μL; range:410-884)	588.00 (507.00-794.00)	535.50 (433.75-724.75)	0.497
CD8+T cell (/μL; range:190-658)	385.00 (326.00-460.00)	234.00 (192.25-353.75)	0.247
NK (/μL; range:90-536)	331.00 (207.00-426.00)	264.00 (198.50-382.25)	0.881

Data are shown as *n* (%) and median (IQR).

variate logistic analysis. These results show that high lymphocyte count (i.e., ≥1.3×10^9/L) was the only independent factor associated with rapid viral clearance (OR=7.62, 95% CI [1.15, 50.34], *p*=0.035) (Table III).

SARS-CoV-2 RNA Load From Feces Samples

The viral RNA load from feces samples was detected at the time of the second sampling of the respiratory tract in 52 patients. Viral RNA

Table III. Factors associated with rapid viral clearance.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Viral Load (Ct value)	1.14 (0.98, 1.34)	0.086	1.10 (0.91, 1.32)	0.319
Top body temperature(°C)	0.44 (0.20, 1.00)	0.050	0.43 (0.13, 1.44)	0.172
Lym ($\times10^9$/L)				
< 1.3	Reference		Reference	
≥ 1.3	3.77(1.20, 11.82)	0.023	7.62 (1.15, 50.34)	**0.035**
PLT ($\times10^9$/L)	1.01 (1.00, 1.02)	0.071		
Potassium (mmol/L)	7.63 (1.44, 40.39)	0.017	2.52 (0.23, 27.12)	0.446
LDL-C	2.46 (0.93, 6.49)	0.070	3.90 (0.90, 16.94)	0.070
Antiviral treatment				
LPV+IFN	Reference			
LPV+arbidol+IFN	0.16 (0.02, 1.35)	0.092		

was found in the feces samples of 13 (25%) patients, regardless of the negativity in the respiratory tract samples. The viral load in the respiratory tract samples was not different between fecal RNA positive patients and negative patients (28.74 [26.27-33.37] *vs.* 30.32 [25.67-33.73], $p>0.05$).

Discussion

Evidence from Middle East Respiratory Syndrome (MERS) and influenza have shown that rapid viral clearance contributes to the improvement of clinical outcomes, while delayed viral clearance is associated with longer hospitalization and poor outcomes[11,12]. A recent study[13] of severe COVID-19 patients, in which SARS-CoV-2 RNA was persistent until death in patients who died of the disease, further confirmed the relationship between prolonged viral clearance and poor outcomes. The present study investigated the viral kinetics of SARS-CoV-2 during the lopinavir/ritonavir and interferon-α combination treatment, and analyzed the factors associated with viral clearance in non-severe patients. All patients achieved viral RNA clearance and were discharged from the hospital. However, viral clearance exhibited different patterns during treatment. Three-quarters of patients achieved viral clearance within two weeks, while the viral RNA remained detectable after two weeks in one-quarter of patients who exhibited a higher baseline viral load. Low lymphocyte count was the independent factor associated with delayed viral clearance during the lopinavir/ritonavir and interferon-α combination treatment. These present findings indicated that systematic immunomodulator therapy should be considered to fasten the viral clearance in patients with high viral load and low lymphocyte counts.

Based on the findings of the present study, as well as those of previous studies[6,13,14], several factors are associated with the viral clearance of SARS-CoV-2 during antiviral treatment. First, it has been reported that high viral load is associated with the prolonged viral clearance of human coronavirus in patients who received hematopoietic cell transplantation[14]. In the present study, the baseline viral load in delayed viral clearance patients was higher than those in rapid viral clearance patients, although the difference was not statistically significant. Therefore, the impact of viral load on the viral clearance of SARS-CoV-2 needs to be further verified in a larger cohort. Second, a recent study[13] revealed that the median duration of viral clearance of SARS-CoV-2 in severe patients was 22.0 (18.0-24.0) days, which was double of that (11.00 [7.00-14.25] days) in non-severe patients, as observed in the present study. This difference indicates that severe pneumonia may be associated with the delayed viral clearance from the respiratory tract. Third, in the LOTUS trial, lopinavir/ritonavir failed to accelerate clinical recovery and reduce mortality in patients who received treatment at 12 days after onset of illness[6]. In the present report, the median duration of viral clearance was associated with the time of illness onset to hospitalization in the subgroup of patients who subsequently received treatment (>7 days). Thus, timely therapy may help to shorten the duration of viral clearance. Finally, and most importantly, the present research demonstrated that lower lymphocyte count was associated with delayed viral clearance, suggesting the contribution of host immune response to the viral clearance, as subsequently described in detail.

A previous modelling study for the influenza virus indicated that both antiviral therapy and immune response could affect viral kinetics, involving the processes that the free cells were infected by the virus and the virus was shed from the infected cells[15]. Antiviral therapy can suppress viral replication and prevent the progeny virus from infecting the free cells. To et al[16] revealed that a slower decline in nasopharyngeal viral load is associated with higher mortality in severe pandemic H1N1 influenza virus infection, highlighting the importance of antiviral therapy in mediating the imbalance between viral replication and immune induced lung injury. Recent kinetics studies[17,18] have revealed that the SARS-CoV-2 viral load in the respiratory tract peaked at approximately 5-6 days after the onset of illness, and persisted for two weeks in some patients. In the present study, the median time from viral RNA positivity to negativity was 11 days, indicating that active intervention with antiviral agents may fasten the natural history of the virus clearance period.

Immune response also contributes to viral clearance. In the present study, regardless of the antiviral therapy, the viral loads were still persistently detectable after two weeks of antiviral treatment in more than a quarter of patients. The multivariate analysis revealed that lymphocyte

count of <1.3×10^9/L at enrollment was the independent factor for delayed viral clearance, indicating that immune clearance is indispensable for virus clearance. Viral clearance requires a complex immune response initiated by resident respiratory tract cells and innate immune cells, and the adaptive immune response is ultimately responsible for complete viral clearance[19]. In a mice model, Zhao et al[20] reported that T cells played a crucial role in SARS-CoV clearance. The enhancement of the number of virus-specific CD8 T cells resulted in robust T cell response, earlier virus clearance, and increased survival. These results suggest that immune insufficiency might be responsible for the delayed viral clearance, and that an immunomodulator should be considered to promote viral clearance. At present, a randomized controlled trial is ongoing in China to evaluate the efficacy and safety of lopinavir/ritonavir and thymosin-a1 combination therapy in the treatment of COVID-19 (Registration No. ChiCTR2000029541).

MERS-CoV has been proven to transmit through the oral-fecal route by infecting intestinal cells[21]. Viral RNA can be detected from the stool samples, with a prevalence of 26.7-53%[22], indicating that infected patients can potentially shed this pathogen through the respiratory and fecal-oral routes. Of note, SARS-CoV-2 RNA was detected in 13 patients whose viral loads in the respiratory tract were negative twice, even though they have not received the antiviral treatment. Lan et al[23] reported that four patients with COVID-19 had positive RT-PCR test results within 5-13 days after hospital discharge, suggesting that at least a proportion of the so-called "recovered" patients may be virus carriers. Although it remains unclear whether SARS-CoV-2 can infect intestinal cells by oral-fecal transmission, it is worth to continue to isolate convalescent patients after discharge.

There were some limitations in this paper. First, the antiviral activity of lopinavir/ritonavir in SARS-CoV-2 remains controversial. Hence, the possibility that combination antiviral therapy or synergizes with the immune response could contribute to viral clearance was not specifically investigated in the present study. However, the results of the present study provided a landscape of viral clearance in non-severe patients and highlighted the importance of host immune response in the viral clearance. Second, there was no controlled group in the present study, since most of these patients were treated with antiviral agents, according to the Chinese guidelines, except for six patients who were admitted to the hospital in the early stage of the epidemic. Third, the sequential viral RNA was not always detected from the same sample type. However, it has been shown that there is consistency in viral loads between the throat swab and sputum samples[18].

Conclusions

The viral kinetics of SARS-CoV-2 during the lopinavir/ritonavir treatment exhibits various patterns. Immune insufficiency with low lymphocyte count is responsible for the delayed viral clearance. These present findings suggest the consideration of systematic immunoregulatory therapy to fasten viral clearance. This has great clinical implications for the treatment and epidemic control of COVID-19.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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