Current Medical Science
DOI https://doi.org/10.1007/s11596-021-2434-y
41(6):1096-1104,2021

Prolonged SARS-CoV-2 Viral Shedding in Patients with COVID-19 was Associated with Delayed Initiation of Arbidol Treatment and Consulting Doctor Later: A Retrospective Cohort Study*

Xin-liang HE, Ya-ya ZHOU, Wei FU, Yu-e XUE, Meng-yuan LIANG, Bo-han YANG, Wan-li MA, Qiong ZHOU, Long CHEN, Jian-chu ZHANG#, Xiao-rong WANG#
Department of Respiratory and Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

[Abstract] Objective: To study data about SARS-CoV-2 virus shedding and clarify the risk factors for prolonged virus shedding. **Methods:** Data were retrospectively collected from adults hospitalized with laboratory-confirmed coronavirus disease-19 (COVID-19) in Wuhan Union Hospital. We compared clinical features among patients with prolonged (a positive SARS-CoV-2 RNA on day 23 after illness onset) and short virus shedding and evaluated risk factors associated with prolonged virus shedding by multivariate regression analysis. **Results:** Among 238 patients, the median age was 55.5 years, 57.1% were female, 92.9% (221/238) were administered with arbidol, 58.4% (139/238) were given arbidol in combination with interferon. The median duration of SARS-CoV-2 virus shedding was 23 days (IQR, 17.8–30 days) with a longest one of 51 days. The patients with prolonged virus shedding had higher value of D-dimer (P=0.002), IL-6 (P<0.001), CRP (P=0.005) and more lobes lung lesion (P=0.014) on admission, as well as older age (P=0.017) and more patients with hypertension (P=0.044) than in those the virus shedding less than 23 days. Multivariate regression analysis revealed that prolonged viral shedding was significantly associated with initiation arbidol ≥8 days after symptom onset [OR: 2.447, 95% CI (1.351–4.431)], ≥3 days from onset of symptoms to first medical visitation [OR: 1.880, 95% CI (1.035–3.416)], illness onset before Jan. 31, 2020 [OR: 3.289, 95% CI (1.474–7.337)]. Arbidol in combination with interferon was also significantly associated with shorter virus shedding [OR: 0.363, 95% CI (0.191–0.690)]. **Conclusion:** Duration of SARS-CoV-2 virus shedding was long. Early initiation of arbidol and arbidol in combination with interferon as well as consulting doctor timely after illness onset were helpful for SARS-CoV-2 clearance.
Key words: SARS-CoV-2; viral shedding; risk factors; antiviral treatment; arbidol

Outbreak of the novel coronavirus disease-19 (COVID-19) caused by SARS-CoV-2 has been rapidly spreading worldwide. Up to September 8, 2020, more than 27 256 723 people with 891 308 deaths have been infected by SARS-CoV-2 globally[1]. Building programs and strategies including isolation and careful management based on the duration of virus shedding and clinical characteristics of patients with COVID-19 is very important to control transmission of the SARS-CoV-2 infection[2].

Viral replication in respiratory cells which is related with virus shedding is the important factor contributing to the pathogenesis of COVID-19[3, 4], more importantly, virus shedding which is associated with disease severity and course[5] was the most important factors in guiding isolation of patients with COVID-19 and evaluating the risk of transmission. There are many factors contributing to the prolonged respiratory virus shedding, such as delayed initiation of antiviral treatment[6–8], the corticosteroid administration[6, 8], major comorbidities[7–9], older age[8], duration of respiratory symptoms[7] and severity[5]. By now, the therapeutic antivirals for COVID-19 including arbidol, interferon, ribavirin, and chloroquine phosphate have

Xin-liang HE, E-mail: herbert1111@163.com
#Corresponding authors, Xiao-rong WANG, E-mail: rong-100@163.com; Jian-chu ZHANG, E-mail: zsn0928@163.com
*This project was supported by Fundamental Research Funds for the Central Universities (No. 2020kfyXGYJ034, No. 2020kfyXGYJ009).
Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11596-021-2434-y) contains supplementary material, which is available to authorized users.

been recommended to continue the clinical trial[10] and FDA authorized remdesivir for emergency use for severe patients with COVID-19[11]. In a retrospective cohort study for 504 patients, arbidol showed lower mortality and faster lesion absorption than ostalmovir and lopinavir groups[12]. Remdesivir was better than placebo in reducing the time to recovery in adults with COVID-19[13]. Existing research on hydroxychloroquine showed no benefit with shortening COVID-19 viral shedding[14–16]. Besides, recent research showed that among COVID-19 patients who were receiving either invasive mechanical ventilation or oxygen alone, the use of dexamethasone reduced 28-day mortality[17]. However, few studies have evaluated risk factors for prolonged SARS-CoV-2 virus clearance and the effects of delayed treatment on viral shedding in patients with COVID-19.

Here, we conducted a retrospective cohort study on 238 hospitalized patients with laboratory-confirmed COVID-19 during January–May 2020 to identify the detail virus shedding, the clinical characteristics associated with the prolonged virus shedding and the risk factors for prolonged SARS-CoV-2 virus clearance, additionally, we estimated the effects of antivirals on the SARS-CoV-2 virus clearance as well.

1 MATERIALS AND METHODS

1.1 Study Design and Participants

This was a retrospective observational cohort study of patients with confirmed COVID-19 in Wuhan Union Hospital. All patients were confirmed COVID-19 by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) according to WHO guidance[18].

Ethical approval was waived by the institutional review board of the hospital since we collected and analyzed all data from the patients according to the policy for public health outbreak investigation of emerging infectious diseases issued by the National Health Commission of the People's Republic of China.

1.2 Data Collection

Demographic information, comorbidities, symptoms, date of symptom onset, radiological images, laboratory results on admission, and treatment data of patients with COVID-19 were extracted from electronic medical records.

1.3 Laboratory Procedures

To identify SARS-CoV-2 infection, throat swab samples were collected from all patients on admission and tested by RT-PCR according to the protocol described by us previously[19] (Supplementary). For patients, throat swab specimens were collected for SARS-CoV-2 PCR examination every other day after admission. We defined the duration of viral detection as the number of days from illness onset until the day of the first negative RT-PCR specimen when two consecutive negative specimens were tested. In our current analysis, prolonged viral shedding was defined as viral shedding ≥23 days (median duration of SARS-CoV-2 RNA shedding of 238 patients).

1.4 Statistical Analysis

We described continuous variables by using median values with interquartile range (IQR), and categorical variables by using absolute or relative frequencies. Mann Whitney *U* test for analysis of continuous variables and the χ^2 test or Fisher exact test for analysis of discrete variables were used in bivariate analyses. To identify risk factors associated with prolonged duration of SARS-CoV-2 RNA shedding, we included indicators that were significantly different between the two group (viral shedding ≥23 *vs.* <23 days) except of length of hospital stay to evaluate the factors associated with virus shedding by univariable regression analysis, and then the significantly contributing factors in univariable regression analysis were included to identify risk factors of prolonged SARS-CoV-2 virus shedding by multivariable logistic regression models. We used Kaplan-Meier survival analysis to estimate the cumulative SARS-CoV-2 RNA-negativity rate and the stratified log-rank statistic to compare the difference of SARS-CoV-2 clearance between patients with and without risk factors.

The statistical analyses and graphics were performed using IBM SPSS 22.0 (SPSS Inc.,USA) and R 3.6.0 (The R Foundation for Statistical Computing, Austria). For all the analyses, $P<0.05$ was considered to be statistically significant and all tests were 2-tailed, unless otherwise indicated.

2 RESULTS

2.1 Demographics and Characteristics

Among 242 adult patients who were confirmed COVID-19 and hospitalized in Wuhan Union Hospital from January 15, 2020 to May 15, 2020, 4 non-survivors who were positive for SARS-CoV-2 RNA until death (supplementary table 1) were excluded, 238 patients with COVID-19 were enrolled in the final analysis. The median age was 55.5 years (IQR, 35–67.3 years), and 136 patients (57.1%) were female. Fever and cough were the most common symptoms on admission. A total of 88 patients (37.0%) had comorbidities and the most common comorbidities were hypertension (67, 28.2%), diabetes (22, 9.2%), and cardiovascular disease (13, 5.5%), followed by chronic lung disease (8, 3.4%), digestive system disease (5, 2.1%), nervous system disease (3, 1.3%), hypothyroidism (3, 1.3%), chronic kidney disease (2, 0.8%). The median time from admission to discharge was 20 days (IQR, 14–26 days), and the median time from illness onset to first medical visitation was 3 days (IQR, 1–6.3 days). Among the 238 patients, 92.9% (221/238) were given

Table 1 Demographic and clinical characteristics of patients with COVID-19 on admission

Characteristics	Total (*n*=238)	Viral shedding ≥23 days (*n*=128)	Viral shedding <23 (*n*=110)	*P* value
Days of viral shedding	23 (17.8–30)	30 (25–34)	17 (14–20)	<0.001
Age, years	55.5 (35–67.3)	60 (39.3–68.8)	51 (32–66)	0.017
<65	150 (6.3)	73 (57)	78 (70.9)	
≥65	88 (37)	55 (43)	32 (29.1)	
Gender				0.107
Male	102 (42.9)	61 (47.7)	41 (37.3)	
Female	136 (57.1)	67 (52.3)	69 (62.7)	
Current smoker	7 (2.9)	2 (1.6)	5 (4.5)	0.361
Date of illness onset				0.027
Before January 31*	190 (79.8)	109 (85.2)	82 (74.5)	
After January 31	48 (20.2)	19 (14.8)	29 (25.5)	
Disease severity status				0.394
Mild/General	202 (84.9)	111 (86.7)	91 (82.7)	
Severe/Critical	36 (15.1)	17 (13.3)	19 (17.3)	
Symptoms				
Fever	188 (79)	98 (76.6)	90 (81.8)	0.323
Cough	154 (64.7)	86 (67.2)	68 (61.8)	0.320
Sputum	75 (31.5)	38 (29.7)	37 (20.9)	0.515
Myalgia	62 (26.1)	39 (30.5)	23 (20.9)	0.095
Dyspnea	101 (42.4)	58 (45.3)	43 (39.1)	0.335
Fatigue	114 (47.9)	62 (48.4)	52 (47.3)	0.858
Pharyngalgia	33 (13.9)	16 (12.5)	17 (15.5)	0.513
Digestive symptoms	73 (30.7)	36 (28.1)	37 (33.6)	0.360
Highest temperature (°C)	38.5 (38–39)	38.5 (38.1–39)	38.5 (37.9–39)	0.383
≥39.0	57 (23.9)	29 (22.7)	28 (25.5)	
<39.0	181 (76.1)	99 (77.3)	82 (74.5)	
Length of fever, days	10 (6–14)	13 (8– 20)	9 (5–12)	<0.001
Comorbidities	88 (37)	54 (42.2)	34 (30.9)	0.073
Hypertension	67 (28.2)	43 (33.6)	24 (21.8)	0.044
Cardiovascular disease	13 (5.5)	7 (5.5)	6 (5.5)	0.389
Diabetes	22 (9.2)	9 (7)	13 (11.8)	0.205
Chronic lung disease	8 (3.4)	4 (3.1)	4 (3.6)	0.828
Chronic kidney disease	2 (0.8)	2 (1.6)	0 (0)	0.190
Nervous system disease	3 (1.3)	1 (0.8)	2 (1.8)	0.477
Digestive system disease	5 (2.1)	4 (3.1)	1 (0.9)	0.236
Immune system disease	2 (0.8)	2 (1.6)	0 (0)	0.190
Hypothyroidism	3 (1.3)	2 (1.6)	1 (0.9)	0.654
Co-infections	11 (4.6)	8 (6.3)	3 (2.7)	0.198
Treatment in hospital				
Supplemental oxygen required	105 (44.1)	53 (41.4)	52 (47.3)	0.363
Nasal cannula	86 (36.1)	42 (32.8)	44 (40)	
Mask	7 (2.9)	6 (4.7)	1 (0.9)	
NMV/IMV/ECMO	12 (5.1)	5 (3.9)	7 (6.4)	
Antibacterial	229 (96.2)	122 (95.3)	107 (97.3)	0.431
Corticosteroids	54 (22.7)	22 (17.2)	32 (29.1)	0.011
Dose, hydrocortisone equivalents per day, mg	150 (133–200)	165 (129–200)	144 (134–166)	0.406
Length of usage, days	3 (1–8)	5 (1.8–9)	2 (1–4.8)	0.058
Cumulative dose of hydrocortisone equivalents, mg	400 (200–1300)	697 (265–1450)	300 (196–734)	0.023
Antiviral treatment				
Arbidol monotherapy	82 (34.4)	58 (45.3)	24 (21.8)	<0.001
Interferon monotherapy	3 (1.3)	2 (1.6)	1 (0.9)	1.000
Lopinavir/ritonavir monotherapy	1 (0.4)	1 (0.8)	0 (0)	1.000
Arbidol-interferon combination	139 (58.4)	62 (48.4)	77 (70)	0.001
Interferon-lopinavir/ritonavir combination	5 (2.1)	3 (2.3)	2 (1.8)	1.000

(Continued to the next page)

(Continued from the last page)

Characteristics	Total (*n*=238)	Viral shedding ≥23 days (*n*=128)	Viral shedding <23 (*n*=110)	*P* value
Miss	8 (3.4)	2 (1.6)	6 (5.5)	0.194
Onset of symptoms to use arbidol, days	8 (5–14)	11 (7–16)	7 (2–11)	<0.001
Onset of symptoms to use interferon, days	11 (7–17)	13 (10–22)	8 (6–12.8)	<0.001
Onset of symptoms to first medical visitation, days	3 (1–6.3)	5 (2–8)	2 (0.8–4)	<0.001
Onset of symptoms to first admission, days	10 (6–16.3)	14 (9–21)	7 (5–10.3)	<0.001
Length of hospital, days	20 (14–26)	25 (18–27)	16 (11–21)	<0.001

Data are median (IQR) or *n* (%). *P* values were calculated by Mann-Whitney *U* test, χ^2 test, or Fisher's exact test, as appropriate. Values in boldface indicate statistical significance (*P*<0.05). IQR, interquartile range; NMV, noninvasive ventilation; IMV, invasive ventilation; ECMO, extracorporeal membrane oxygenation. *January 31 was included in the before January 31 group.

arbidol, and 61.7% (147/238) were given interferon. The median time from illness onset to arbidol initiation was 8 days (IQR, 5–14 days) and interferon initiation was 11 days (IQR, 7–17 days). Among them, arbidol monotherapy was given in 82 (34.4%); arbidol in combination with interferon in 139 (58.4%); interferon monotherapy in 3 (1.3%); interferon in combination with lopinavir/ritonavir in 5 (2.1%); and lopinavir/ritonavir monotherapy in 1 (0.4%); and miss in 8 (3.4%) (table 1).

2.2 SARS-CoV-2 Viral Shedding and Comparison of Characteristics of Patients with Prolonged and Short Viral Shedding

The median duration of viral shedding was 23 days (IQR, 17.8–30 days) from illness onset. The longest observed duration of viral shedding was 51 days, whereas the shortest was 9 days. We found that SARS-CoV-2 RNA was cleared in 23 patients (9.7%) within 14 days, and 14 patients (5.9%) with up to 40 days (fig. 1).The data of patients with viral shedding <23 days and ≥23 days groups were compared (table 1). There was no significant difference between the two groups in sex, comorbidities (except hypertension), symptoms (except length of fever), co-infection, antibacterial therapy or supplemental oxygen required. In patients with prolonged viral shedding, incidence of hypertension was higher (33.6%, *P*=0.044), age was older (*P*=0.017), time of fever was longer (*P*<0.001), hospital stay was longer (*P*<0.001) and first medical visitation was later (*P*<0.001) than those with viral shedding <23 days. More patients with prolonged viral shedding had onset symptoms before January 31, 2020 than those with viral shedding <23 days (85.2%, *P*=0.027). Less patients with prolonged viral shedding received corticosteroids (17.2%, *P*=0.011), but the cumulative doses of corticosteroid usage were significantly greater (*P*=0.023) than those of patients with viral shedding <23 days. The dose of corticosteroids per day (165 mg *vs.* 144 mg) and use length of corticosteroids (5 days *vs.* 2 days) were increased in prolonged viral shedding group (*P*=0.406, *P*=0.058). The median time from illness onset to initiation of arbidol or interferon therapy was 11 days (IQR, 7–16 days) *vs.* 7 days (IQR, 2–11 days) (*P*<0.001) for arbidol and 13 days (IQR, 10–22 days) *vs.* 8 days (IQR, 6–12.8 days) (*P*<0.001) for interferon in patients with virus shedding ≥23 *vs.* <23 days. In prolonged viral shedding group, the percentage of patients using arbidol in combination with interferon was significantly lower (*P*=0.001). As summarized in table 2, elevated C-reactive protein (CRP), interleukin-4 (IL-4), IL-6 and D-dimer, and increased lobes lesions in

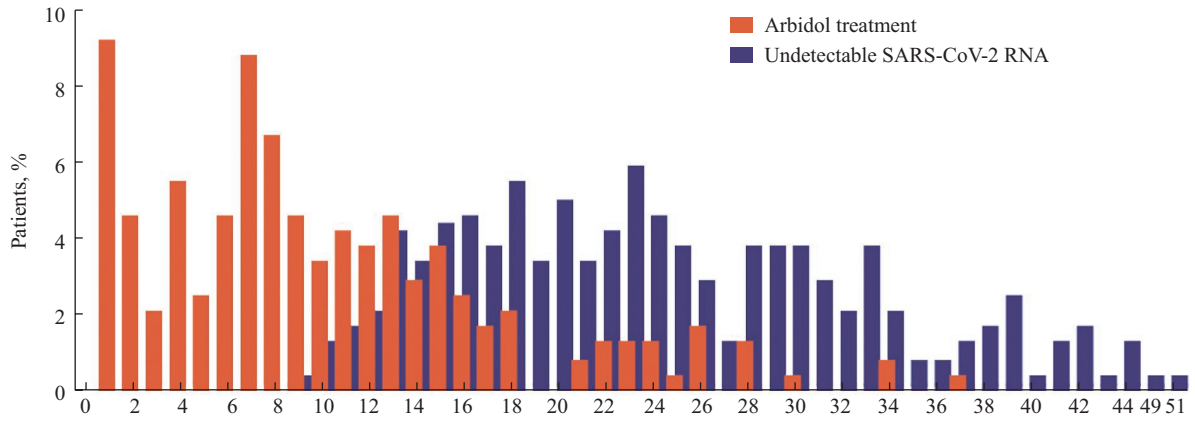

Fig. 1 Distribution of arbidol treatment and proportion of patients with undetectable SARS-CoV-2 RNA by day after onset of symptom Among 238 patients, 221 patients (92.9%) received arbidol treatment, and 17 patients (7.1%) did not receive arbidol treatment.

Table 2 Laboratory and radiographic findings of patients on admission

	Total (*n*=238)	Viral shedding ≥23 days (*n*=128)	Viral shedding <23 (*n*=110)	*P* value
Hematologic results				
WBC, 10^9/mL	238	5.3 (4.1–6.7)	5.0 (3.8–6.8)	0.203
Neutrophils, 10^9/mL	238	3.3 (2.6–4.7)	3.3 (2.4–4.6)	0.522
Lymphocytes, 10^9/mL	238	1.2 (0.9–1.6)	1.0 (0.8–1.5)	0.051
Lymphocytes <0.8×10^9/mL	238	21 (16.4)	27 (24.5)	0.119
T lymphocytes				
CD3+ (%)	194	74.1 (69.0–80.3)	74.1 (72.0–79.1)	0.656
CD4+ (%)	194	44.6 (40.2–51.0)	44.6 (37.9–45.9)	0.223
CD8+ (%)	194	24.9 (19.6–28.1)	24.9 (23.2–29.8)	0.081
CD4+/CD8+, ratio	194	2.0 (1.6–2.5)	1.9 (1.3–2.1)	0.054
Biochemical results				
ALT >40 U/L	52/238 (21.8)	29 (22.7)	23 (20.9)	0.745
AST >40U/L	35/238 (14.7)	20 (15.6)	15 (13.6)	0.666
LDH >245 U/L	82/234 (35)	46 (36.8)	36 (33.0)	0.546
Urea nitrogen >8.2 mmol/L	6/238 (2.5)	5 (3.9)	1 (0.9)	0.221
Creatinine >133 μmol/L	2/238 (0.8)	2 (1.6)	0 (0)	0.501
Infection-related markers				
CRP >8.0 mg/L	102/219 (46.6)	62 (55.9)	40 (37.0)	0.005
PCT >0.5 μg/L	3/222 (1.4)	1 (0.9)	2 (1.9)	0.610
IL-2 >4.1 pg/mL	5/196 (2.6)	4 (3.7)	1 (1.1)	0.382
IL-4 >3.2 pg/mL	12/196 (6.1)	11 (10.2)	1 (1.1)	0.013
IL-6 >2.9 pg/mL	154/213 (72.3)	114 (95.8)	40 (42.6)	<0.001
IL-10 >5.00 pg/mL	30/196 (15.3)	19 (17.6)	11 (12.5)	0.325
TNF-α >23.00 pg/mL	7/196 (3.6)	5 (4.6)	2 (2.3)	0.619
IFN-γ >18.00 pg/mL	1/196 (0.5)	1 (0.9)	0 (0)	
D-dimer >0.5μg/L	79/219 (36.1)	51 (45.9)	28 (25.9)	0.002
Chest CT images				0.014
Normal	7/224 (3.1)	3 (2.4)	4 (4.0)	
One lung lobs lesion	40/224 (17.9)	14 (11.4)	26 (25.7)	
More lung lobs lesion	177/224 (79)	106 (86.2)	71 (70.3)	

Data are median (IQR), *n* (%) or *n*/*N* (%). *P* values were calculated by Mann-Whitney *U* test, χ^2 test, or Fisher's exact test, as appropriate. Values in boldface indicate statistical significance (*P*<0.05). WBC, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; PCT, procalcitonin; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α; IFN-γ: interferon-γ; CT, computed tomography. Reference range of laboratory test are summarized in Supplementary table 4.

lung CT were significantly related with the prolonged viral shedding of COVID-19.

2.3 Risk Factors for Prolonged Viral Shedding

To identify risk factors associated with prolonged duration of SARS-CoV-2 RNA shedding, we included indicators that were significantly different between the two groups (viral shedding ≥23 *vs.* <23 days). The results demonstrated that age older than 65 years, date of illness onset (before or after January 31, 2020), the time from illness onset to first medical visitation, hypertension, arbidol in combination with interferon, lesions in lung CT images, and the time from illness onset to arbidol treatment initiation were significantly associated with the duration of SARS-CoV-2 RNA shedding by univariable regression analysis. Other indicators including cumulative dose of corticosteroids ≥400 mg was not associated with prolonged viral shedding significantly (table 3).

The multivariable logistic regression showed the time from illness onset to arbidol initiation ≥8 days, first medical visitation after illness onset ≥3 days, and illness onset before January 31, 2020 were independent factors associated with the duration of SARS-CoV-2 RNA shedding. Importantly, arbidol in combination with interferon was also significantly associated with shorter virus shedding (table 3).

2.4 Impact of Antiviral Treatment Regimens

The median time from illness onset to arbidol initiation was 8 days (IQR, 5–14 days). The median duration of SARS-CoV-2 virus shedding was 20 days (IQR, 16–25 days) in the patients with initiation of arbidol <8 days from illness onset and 28 days (IQR, 21–33) in patients with initiation arbidol ≥8 days from illness onset. SARS-CoV-2 RNA clearance was significantly delayed in patients who received arbidol beginning ≥8 days after illness onset as compared with those in whom arbidol was started <8 days after illness onset (HR=1.675, 95% CI: 1.240–2.262, *P*<0.001; fig.

Table 3 Univariate and multivariate Logistic regression analysis for prolonged viral shedding of patients with COVID-19

	Univariate		Multivariate	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age ≥65 years old	1.836 (1.070–3.152)	0.027	1.242 (0.633–2.437)	0.528
Hypertension	1.813 (1.012–3.246)	0.045	1.411 (0.709–2.808)	0.327
Length of fever ≥10 days	1.544 (0.917–2.600)	0.102		
Date of illness onset before Jan. 31	2.054 (1.077–3.919)	0.029	3.289 (1.474–7.337)	0.004
Onset of symptoms to first medical visitation ≥3 days	2.842 (1.667–4.845)	<0.001	1.880 (1.035–3.416)	0.038
Cumulative dose of corticosteroids ≥400 mg	1.066 (0.488–2.327)	0.873		
Starting arbidol ≥8 days from illness onset	3.062 (1.791–5.234)	<0.001	2.447 (1.351–4.431)	0.003
Interferon and arbidol combination	0.403 (0.236–0.688)	0.001	0.363 (0.191–0.690)	0.002
Starting interferon ≥11 days from illness onset	1.077 (0.282–4.115)	0.913		
CRP >8.0 mg/L	0.803 (0.474–1.359)	0.413		
IL-4 >3.2 pg/mL	3.353 (0.911–12.344)	0.069		
IL-6 >2.9 pg/mL	1.382 (0.450–4.242)	0.572		
D-dimer >0.5 μg/L	1.577 (0.943–2.636)	0.082		
More lung lobs lesion in chest CT images	2.448 (1.366–4.387)	0.003	1.743 (0.894–3.396)	0.103

CI, confidence interval; OR, odds ratio; CRP, C-reactive protein; IL-4, interleukin-4; IL-6, interleukin-6; CT, computed tomography

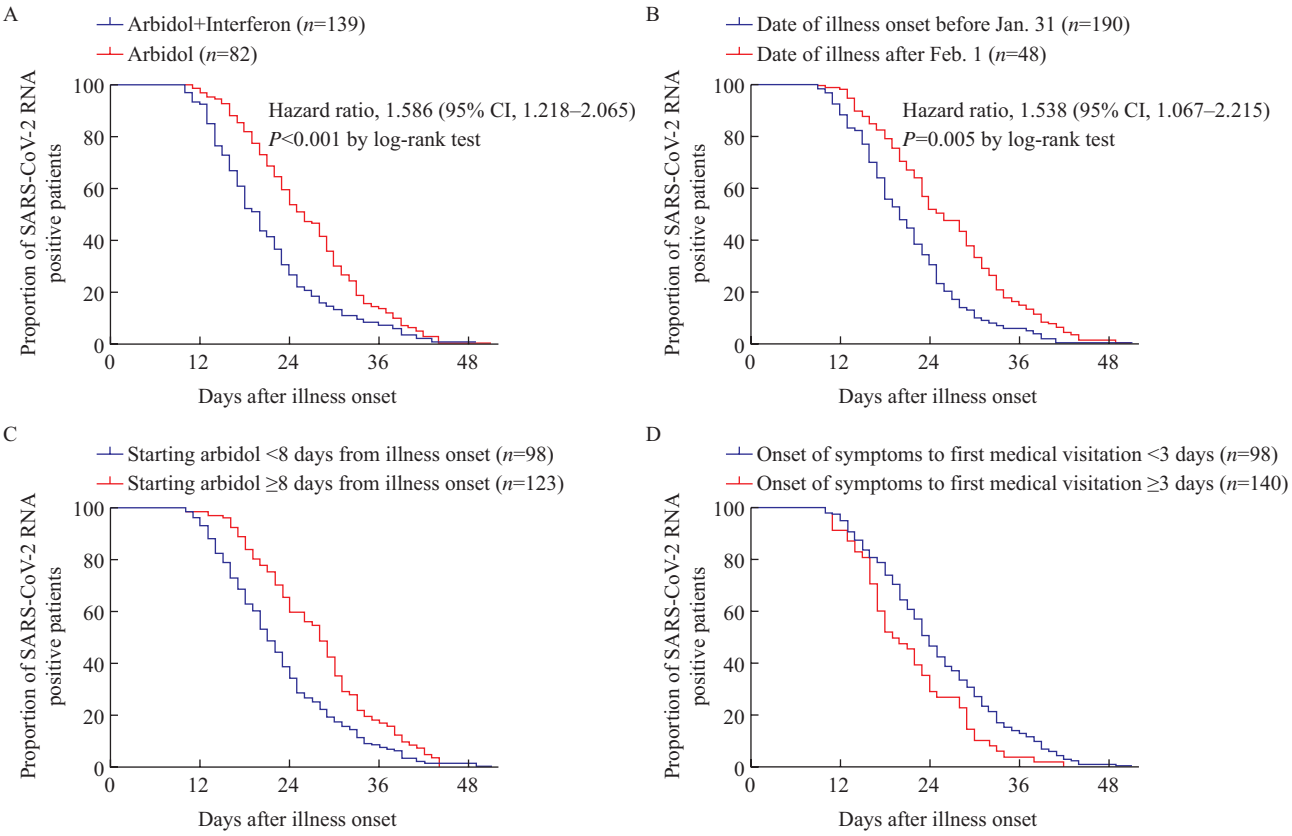

Fig. 2 A: Cumulative proportion of between patients who started arbidol therapy <8 days and ≥8 days after illness onset with detectable SARS-CoV-2 RNA, by day after onset of illness. B: Cumulative proportion of patients who firstly visited medical staff <3 days from symptoms onset *versus* ≥3 days who had detectable SARS-CoV-2 RNA, by day after onset of illness. C: Cumulative proportion of patients treated with arbidol *versus* combination of arbidol and interferon who had detectable SARS-CoV-2 RNA, by day after onset of illness. D: Cumulative proportion of patients after onset illness before Jan. 31 *versus* after Feb. 1 who had detectable SARS-CoV-2 RNA, by day after onset of illness. HR, hazard ratio; CI, confidence interval. Jan. 31 was included in before January 31 group and Feb. 1 was included in after Feb. 1 group.

2A). The characteristics of patients of the two groups were summarized in supplementary table 2. We further compared characteristics of patients within 65 years with COVID-19 who started arbidol within 7 days and more than 7 days (supplementary table 3).

As compared with arbidol monotherapy (28 days, IQR, 16–28 days), the median durations of SARS-CoV-2 shedding in patients with arbidol-interferon

combination therapy (21 days, IQR, 21.8–33 days) were significantly shortened (P<0.001). As compared with those who received arbidol-interferon combination therapy, the virus clearance was also delayed in patients given arbidol monotherapy in Kaplan-Meier survival analysis (fig. 2C). The main characteristics of patients who received arbidol monotherapy and arbidol-interferon combination therapy were summarized in supplementary table 2.

3 DISCUSSION

In the current cohort study of 238 hospitalized patients with COVID-19, we found that the duration of SARS-CoV-2 RNA shedding was long, and patients with prolonged virus shedding had higher value of D-dimer, inflammation markers, more lobes lesions in lung CT images and older age as well as more hypertension. We also found that initiation of arbidol less than 8 days after illness onset as well as combination with arbidol and interferon were helpful for SARS-CoV-2 RNA clearance. Additionally, the time from onset of symptoms to first medical visitation more than 3 days was also an independent risk factor for prolonged SARS-CoV-2 RNA detection. Interestingly, compared with illness onset after Jan. 31, 2020, illness onset before Jan. 31, 2020 was associated with prolonged SARS-CoV-2 RNA shedding.

We detected SARS-CoV-2 RNA in the respiratory tract for a median duration of 23 days, which was longer than 20 days reported by an early study[20]. The SARS-CoV-2 RNA was detected for more than 40 days in 14 patients, with a longest 51 days in a 75 years old man with severe COVID-19. The much longer SARS-CoV-2 virus shedding may contribute to the COVID-19 pandemic around the world, and indicate more time may be needed to isolate the patients with COVID-19 and the length of antiviral treatment may be longer. The prolonged virus shedding duration of A (H1N1) pdm09 and highly pathogenic avian influenza A (H5N1) virus shedding were associated with severity of disease[5, 6, 21, 22]. The SARS-CoV-2 virus shedding of the critical female patients with ARDS who received the ECMO therapy was up to 49 days and we detected the virus RNA in 4 deaths until they died (supplemental table 1). Additionally, compared to patients with virus shedding less than 23 days, more patients with prolonged virus shedding had higher values of D-dimer, IL-4, IL-6, CRP, more lobes lesions in lung images, older age and more hypertension. Previous studies have reported that the older age and D-dimer were risk factors for death and acute respiratory distress syndrome (ARDS) in COVID-19 patients[20, 23]. Besides, comorbidities, more lung lobes lesions and higher levels of inflammation markers were related with severity of COVID-19[10]. Those results combined with our present findings demonstrated that patients with prolonged virus shedding were more likely to develop to severe disease.

Antiviral treatment especially early initiated antiviral treatment has been proven to shorten the viral shedding of epidemic and seasonal influenza as well as H7N9 avian influenza[6, 7, 24–26]. In this retrospective study, most patients (221/238, 92.9%) were administrated with arbidol, among them, arbidol was initiated >5 days after illness onset in most patients (fig. 1). Our results showed that initiation of arbidol treatment before the 8 days of illness onset was associated with a shorter duration of SARS-CoV-2 RNA shedding. Multivariate regression analysis revealed that delayed arbidol antiviral therapy was an independent risk factor of the prolonged SARS-CoV-2 RNA shedding, which were consistent with other study[27]. Arbidol, a small indole-derivative molecule, as a broad-spectrum antiviral drug to treat influenza and other respiratory viral infections[28, 29] was recommended to treat COVID-19[10]. The antiviral mechanism of arbidol involves inhibition of virus-mediated fusion with target membrane and a resulting block of virus entry into target cells was revealed to effectively inhibit SARS-CoV *in vitro*[30]. Additionally, arbidol showed effect to improve rate of discharging and reduce mortality of patients with COVID-19[31]. These results combined with our present findings suggested that arbidol exerts a therapeutic effect on SARS-CoV-2 infections, however, further research is needed to clarify the effects of additional arbidol treatment for COVID-19.

Interferon, which was found to inhibit SARS-CoV virus reproduction *in vitro*[32], is also a broad-spectrum antiviral drug and is recommended to treat coronavirus infection[33]. In the present study, about 147 patients (61.8%) used interferon, among them, up to 94.6% (139/147) was administrated in combination with arbidol. Our findings showed arbidol combination with interferon can help to clear SARS-CoV-2 RNA. The effects of arbidol combination with interferon treatment for COVID-19 are worth further study. Previous studies demonstrated that low to middle dose of corticosteroid can reduce the risk of death and shorten hospitalization stay in severe SARS-pneumonia and COVID-19 pneumonia[23, 34]. However, most observational studies have reported that use of corticosteroids was associated with persistent viral shedding[6, 7, 16, 35]. In the present study, 22.7% used corticosteroids and most of them used in a short duration, which was not associated with prolonged SARS-CoV-2 RNA shedding and was in accordance with other study[36]. However, the length and cumulative dose of corticosteroids usage were increased in prolonged shedding group, which demonstrated the long-term use of corticosteroids to a certain dose may have a tendency to delay viral

clearance.

Our present finding showed that the time from onset of symptoms to first medical visitation more than 8 days was an independent risk factor for prolonged SARS-CoV-2 RNA detection, which suggested consultation of a doctor as soon as possible after illness onset helped to clear SARS-CoV-2 (table 3, fig. 2B). Interestingly, illness onset before Jan. 31, 2020 was also associated with prolonged SARS-CoV-2 RNA shedding, compared with illness onset after Jan. 31, 2020 (table 3, fig. 2D). This may be associated with shortage of medical resources in January, 2020 in Wuhan and a large number of patients with COVID-19 could not consult doctor and receive treatment timely. Additionally, genomic-level and expression changes during passage of viruses through transmissibility may lead to the changes of transmissibility and pathogenicity[37].

Some limitations of this study should be acknowledged. First, the retrospective single-center design leads to missing data and unavoidable biases and the sample size is relatively small. Second, owing to limited medical resources, most patients with COVID-19 cannot visit hospital timely, leading to the laboratory tests on admission reflecting different disease stages. Third, because most of patients received the antivirals therapy, we were not able to get the natural virus shedding of patients with COVID-19. Finally, because our study did not carry out virus culture, patients with longer virus shedding may not mean to spread the virus (because patients may shed non-transmissible virus fragments, rather than viable virus). Despite these limitations, the study was designed to reflect the "real life" clinical situation. Clinical information was meticulously gathered using standard protocols by admitted medical team. This has important implications for both patient isolation decision making and guidance around the length of antiviral treatment and the choice of antivirals.

To the best of our knowledge, we found that the detectable SARS-CoV-2 RNA persisted for a median of 23 days with longest 51 days, and initiation of arbidol and/or combination with interferon as soon as possible after illness onset were helpful for clearance of SARS-CoV-2 RNA. Additionally, prolonged SARS-CoV-2 RNA shedding in the respiratory tract was independently associated with delayed medical visitation and correlated with the illness onset day (before or after Jan. 31, 2020). These results reinforce consulting a doctor and receiving treatments timely helps for SARS-CoV-2 clearance in patients with COVID-19.

Conflict of Interest Statement

The authors declare there are no conflicts of interest.

Author Xiao-rong WANG is a young member and Wan-li MA is a member of the Editorial Board for Current Medical Science. The paper was handled by the other editors and has undergone rigrous peer review process. Authors Xiao-rong WANG and Wan-li MA were not involved in the journal's review of, or decisions related to, this manuscript.

REFERENCES

1 Johns Hopkins University. Coronavirus COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE). Available at: https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6. Accessed 8 September 2020.

2 World Health Organization. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). 2020. Available at: https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf. Accessed 8 September 2020.

3 Jin Y, Yang H, Ji W, *et al*. Virology, Epidemiology, Pathogenesis, and Control of COVID-19. Viruses, 2020, 12(4):372

4 Xu Z, Shi L, Wang Y, *et al*. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med, 2020,8(4):420-422

5 Fielding JE, Kelly HA, Mercer GN, *et al*. Systematic review of influenza A(H1N1) pdm09 virus shedding: duration is affected by severity, but not age. Influenza Other Respir Viruses, 2014,8(2):142-150

6 Wang Y, Guo Q, Yan Z, *et al*. Factors Associated With Prolonged Viral Shedding in Patients With Avian Influenza A(H7N9) Virus Infection. J Infect Dis, 2018, 217(11):1708-1717

7 Ryoo SM, Kim WY, Sohn CH, *et al*. Factors promoting the prolonged shedding of the pandemic (H1N1) 2009 influenza virus in patients treated with oseltamivir for 5 days. Influenza Other Respir Viruses, 2013,7(5):833-837

8 Lee N, Chan PK, Hui DS, *et al*. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. J Infect Dis, 2009,200(4):492-500

9 Kanchana S, Kanchana S, Chuntrakul C, *et al*. Pandemic (H1N1) 2009 virus infection: persistent viral shedding after Oseltamivir treatment. J Infect, 2011,63(4):295-299

10 National Health Commission of the People's Republic of China. Diagnosis and Treatment Program of New Coronary Pneumonia (the eighth edition). 2020. Available at: http://www.nhc.gov.cn/xcs/zhengcwj/202008/0a7bdf12bd4b46e5bd28ca7f9a7f5e5a.shtml. Accessed 8 September 2020.

11 Coronavirus (COVID-19) update: FDA issues emergency use authorization for potential COVID-19 treatment. U.S. Food and Drug Administration; 2020. Available at: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment. Accessed 1 May2020.

12 Liu Q, Fang X, Tian L, *et al*. The effect of Arbidol Hydrochloride on reducing mortality of Covid-19 patients: a retrospective study of real world date from three hospitals in Wuhan. medRxiv 2020.

13 Beigel JH, Tomashek KM, Dodd LE, *et al*. Remdesivir for the Treatment of Covid-19 -- Preliminary Report. N Engl J Med, 2020,383(10):994
14 Chen Z, Hu J, Zhang Z, *et al*. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. MedRxiv. 2020.
15 Chen J, Liu D, Liu L, *et al*. A pilot study of hydroxychloroquine in treatment of patients with moderate COVID-19. Zhejiang Da Xue Xue Bao Yi Xue Ban (Chinese), 2020,49(2):215-219
16 Tang W, Cao Z, Han M, *et al*. Hydroxychloroquine in patients with COVID-19: an open-label, randomized, controlled trial. MedRxiv. 2020.
17 RECOVERY Collaborative Group, Horby P, Lim WS, *et al*. Dexamethasone in Hospitalized Patients with Covid-19--Preliminary Report. N Engl J Med, 2021, 384(8):693-704
18 World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: interim guidance. January 28, 2020. Available at: https://www.who.int/publications-detail/clinical-management-of-severeacute-respiratory-infection-when-novelcoronavirus-(ncov)-infection-is-suspected. Accessed 8 September 2020.
19 Huang C, Wang Y, Li X, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, 2020,395:497-506
20 Zhou F, Yu T, Du R, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet, 2020,395(10229):1054-1062
21 de Jong MD, Simmons CP, Thanh TT, *et al*. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med, 2006,12(10):1203-1207
22 Gu J, Xie Z, Gao Z, *et al*. H5N1 infection of the respiratory tract and beyond: a molecular pathology study. Lancet, 2007, 370(9593):1137-1145
23 Wu C, Chen X, Cai Y, *et al*. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med, 2020,180(7):934-943
24 Treanor JJ, Hayden FG, Vrooman PS, *et al*. Efficacy and Safety of the Oral Neuraminidase Inhibitor Oseltamivir in Treating Acute Influenza: A Randomized Controlled Trial. JAMA, 2000,283(8):1016-1024
25 Aoki FY, Boivin G. Influenza virus shedding: excretion patterns and effects of antiviral treatment. J Clin Virol, 2009,44(4):255-61
26 Nicholson KG, Aoki FY, Osterhaus A, *et al*. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Lancet, 2000,355(9218): 1845-1850
27 Hu F, Yin G, Chen Y, *et al*. Corticosteroid, oseltamivir and delayed admission are independent risk factors for prolonged viral shedding in patients with Coronavirus Disease 2019. Clin Respir J, 2020,14(11):1067-1075
28 Hulseberg CE, Feneant L, Szymanska-de Wijs KM, *et al*. Arbidol and Other Low-Molecular-Weight Drugs That Inhibit Lassa and Ebola Viruses. J Virol, 2019,93(8): e02185-18
29 Blaising J, Polyak SJ, Pecheur EI. Arbidol as a broad-spectrum antiviral: an update. Antiviral Res, 2014,107: 84-94
30 Khamitov RA, Loginova S, Shchukina VN, *et al*. Antiviral activity of arbidol and its derivatives against the pathogen of severe acute respiratory syndrome in the cell cultures. Vopr Virusol, 2008,53(4):9-13
31 Wang Z, Yang B, Li Q, *et al*. Clinical Features of 69 Cases with Coronavirus Disease 2019 in Wuhan, China. Clin Infect Dis, 2020,71(15):769-777
32 Stockman LJ, Bellamy R, Garner P. SARS: systematic review of treatment effects. PLoS Med, 2006,3(9):e343
33 Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology, 2018,23(2):130-137
34 Chen RC, Tang XP, Tan SY, *et al*. Treatment of severe acute respiratory syndrome with glucosteroids: the Guangzhou experience. Chest, 2006,129(6):1441-1452
35 Arabi YM, Mandourah Y, Al-Hameed F, *et al*. Corticosteroid Therapy for Critically Ill Patients with Middle East Respiratory Syndrome. Am J Respir Crit Care Med, 2018,197(6):757-767
36 Hu Z, Lv Y, Xu C, *et al*. Clinical Use of Short-Course and Low-Dose Corticosteroids in Patients With Non-severe COVID-19 During Pneumonia Progression. Front Public Health, 2020,8:355
37 Garretson TA, Shang H, Schulz AK, *et al*. Expression- and genomic-level changes during passage of four baculoviruses derived from bacmids in permissive insect cell lines. Virus Res, 2018,256:117-1124

(Received Oct. 29, 2020; accepted Apr. 7, 2021)