Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Original Article

Persistent Viral Presence Determines the Clinical Course of the Disease in COVID-19

De Chang, MD, PhD[a,*], Peng Zhao, MD[b,*], Dawei Zhang, MD[b,*], Jing-Hui Dong, MD[b,*], Zhe Xu, MD[b], Guang Yang, MD[b], Bo-Yu Li, MD[b], Hong-Xia Liu, MD[b], Bo-An Li, MD[b], Cheng-Feng Qin, MD[c], Xiao-Hua Peng, MD[d], Fu-Sheng Wang, MD[b], Li-Xin Xie, MD[a], Zhu Chen, MD[b], Charles S. Dela Cruz, MD, PhD[d,‡], Lokesh Sharma, PhD[d,‡], and En-Qiang Qin, MD[b,‡]

Beijing, China; and New Haven, Conn

What is already known about this topic? Coronavirus disease 2019 has emerged as a major pandemic. The disease manifests from mild to severe infections. Various risk factors such as advanced age and comorbidities have been identified. However, precise factors contributing to the disease severity remain unknown.

What does this article add to our knowledge? Viral clearance is a major determinant of disease pathology. Prolonged viral presence was associated with increased disease severity markers including admission to intensive care units and greater lung involvement of chest imaging.

How does this study impact current management guidelines? In the absence of antiviral therapies, anti-inflammatory therapies or other therapies that may delay viral clearance should be used with caution.

BACKGROUND: The clinical management of coronavirus disease 2019 (COVID-19) is dependent on understanding the underlying factors that contribute to the disease severity. In the absence of effective antiviral therapies, other host immunomodulatory therapies such as targeting inflammatory response are currently being used without clear evidence of their effectiveness. Because inflammation is an essential component of host antiviral mechanisms, therapies targeting inflammation may adversely affect viral clearance and disease outcome.

OBJECTIVE: To understand whether the persistent presence of the virus is a key determinant in the disease severity during COVID-19 and to determine whether the viral reactivation in some patients is associated with infectious viral particles.

METHODS: The data for patients were available including the onset of the disease, duration of viral persistence, measurements of inflammatory markers such as IL-6 and C-reactive protein, chest imaging, disease symptoms, and their durations among others. Follow-up tests were performed to determine whether the viral negative status persists after their recovery.

RESULTS: Our data show that patients with persistent viral presence (>16 days) have more severe disease outcomes including extensive lung involvement and requirement of respiratory support. Two patients who died of COVID-19 were virus-positive at the time of their death. Four patients demonstrated virus-positive status on the follow-up tests, and these patient samples were sent to viral culture facility where virus culture could not be established.

CONCLUSIONS: These data suggest that viral persistence is the key determining factor of the disease severity. Therapies that may impair the viral clearance may impair the host recovery from COVID-19. © **2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;8:2585-91)**

Key words: *COVID-19; Viral persistence; Disease severity; Viral clearance; Host recovery*

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is the third and largest coronavirus-mediated disease that emerged in the 21st century. It has infected more than 5 million people with more than 350,000 deaths.[1] The disease course of COVID-19 varies

[a]College of Pulmonary and Critical Care Medicine, Chinese PLA General Hospital, Beijing, China
[b]The Fifth Medical Center, Chinese PLA General Hospital, Beijing, China
[c]State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences (AMMS), Beijing, China
[d]Section of Pulmonary and Critical Care and Sleep Medicine, Department of Medicine, Yale University School of Medicine, New Haven, Conn
* These are first authors.
‡ These are senior authors.
This study was supported by funding from Beijing Nova Program Interdisciplinary Cooperation Project (grant no. Z191100001119021 to D.C.), China 13th Five-year National Key Grant (grant no. 2018ZX09201013 to L.X.X.), China Scholarship Council (grant no. 201809112037), and Beijing Nova Program (grant no. Z171100001117012 to D.C.).
Conflicts of interest: The authors declare that they have no relevant conflicts of interest.
Received for publication May 4, 2020; revised May 30, 2020; accepted for publication June 11, 2020.
Available online June 20, 2020.
Corresponding authors: En-Qiang Qin, MD, The Fifth Medical Center, Chinese PLA General Hospital, Beijing 100039, China. E-mail: qeq2004@sina.com. Or: Zhu Chen, MD, The Fifth Medical Center, Chinese PLA General Hospital, Beijing 100039, China. E-mail: chenzhuren264@126.com.
2213-2198

https://doi.org/10.1016/j.jaip.2020.06.015

Abbreviations used
AMMS- Academy of Military Medical Sciences
COVID-19- Coronavirus disease 2019
CRP- C-reactive protein
SARS-CoV-2- Severe acute respiratory syndrome coronavirus 2

significantly among individuals, ranging from asymptomatic infection to severe disease leading to death.[2-4] The host and pathogen determinants are not known but possibly both exacerbated inflammatory host response, or direct virus-induced damage may lead to pathology. Excessive inflammation is often attributed to disease severity as well as mortality due to COVID-19.[5-7] Surprisingly, the most susceptible population to COVID-19 remains the older individuals who are known to mount a poor inflammatory and immune response to a vast variety of viral infections as well as vaccines.[8] In this study, we aimed to identify whether inflammatory response or impaired viral clearance leading to prolonged viral presence contribute toward disease severity.

Currently, there are no approved therapies for COVID-19, and initial approaches to enhance the viral clearance using ritonavir/lopinavir therapies failed to show any impact on viral clearance and the disease course.[9] The other antiviral therapies that are being used include remdesivir and chloroquine based on their *in vitro* activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[10] However, their antiviral efficacy and beneficial effects in COVID-19 remain to be known. However, a wide range of anti-inflammatory therapies are currently being used in the clinical management of COVID-19 despite a lack of knowledge regarding their beneficial effects on the disease.[11,12] Understanding whether the persistent viral presence or early inflammatory response guides the disease prognosis can provide critical clinical tools in the management of the disease. In this study, we demonstrate the role of persistent viral presence in contributing to the disease severity where the early inflammatory response was not related to the viral presence of adverse outcomes during the disease.

Re-emergence of the virus after clinical recovery has also been a major concern and could pose a significant challenge in preventing the spread of the disease.[13] Currently, it is not clear what drives the re-emergence of the virus in some patients and whether these patients are infectious at this stage of the disease.

METHODS

Source of the data

Data were obtained from 69 patients admitted at the Fifth Medical Center of Chinese PLA General Hospital from early January to the end of March 2020 and discharged between January 28 and April 30 except 3 patients who remained hospitalized until April 20, 2020. Two patients died of COVID-19 and were excluded from the analysis. Epidemiological history and the day of onset of symptoms were noted on their chart with other demographic information upon their presentation to the hospital. The viral quantitative PCR was performed either at the China Center for Disease Control or in the hospital laboratory where the presence of virus was confirmed during the patients' admission and stay in the hospital every 2 days.[2] Routine measurements and laboratory tests were performed including markers of inflammation such as C-reactive protein (CRP) and IL-6. Measurement of D-dimer was performed in the blood samples. D-dimer is a fibrin derivative that is generated by its cleavage in the presence of plasmin.[14] D-dimer is a well-established marker of pulmonary embolism as well as deep vein thrombosis.[14,15] Patients received standard of care as per guidelines by the Ministry of Public Health of China. Glucocorticoids were administered to the patients with progressive deterioration of oxygenation indicators, rapid progression of pathology in chest imaging, and overactivation of the inflammatory response.[16] All the patients were discharged from the hospital once their symptoms resolved, and they had 2 consecutive viral quantitative PCR tests showing the absence of the virus performed at least 24 hours apart. Patients were asked to follow up after their discharge, and their throat swabs were tested again for the presence of the virus. If positive, these samples were sent to the State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences (AMMS) to confirm the presence of the live virus. Virus cultivation attempts were performed in Vero monolayers seeded in a 24-well plate. Briefly, throat swabs from patients who were positive for SARS-CoV-2 by RT-quantitative PCR were immersed, and serially diluted samples were inoculated into Vero monolayers. Cells were monitored daily for cytopathic effect for 5 days, and viral replication in cell culture was detected by RT-quantitative PCR or plaque assay. All experiments involving SARS-CoV-2 were performed in the BSL-3 facility at the AMMS.

Case definitions

All the patients in this study were confirmed to have SARS-CoV-2 infection, detected by viral quantitative PCR assay. The disease severity was classified as severe and nonsevere on the basis of clinical parameters as described earlier.[17] Two patients remained asymptomatic despite the presence of virus for 27 and 54 days, respectively, and they were included in the nonsevere group.

Cutoff values for inflammatory parameters

The laboratory values were defined to be abnormal when they are out of their normal prescribed range. These ranges for various parameters are as follows: IL-6, 0 to 7 pg/mL; CRP, 0.068 to 8.2 mg/L; white blood cells, 3.97 to 9.15 $\times$ 10^9/L; neutrophils, 2 to 7 $\times$ 10^9/L; leucocytes, 0.8 to 4.0 $\times$ 10^9/L; eosinophils, 0.02 to 0.5 $\times$ 10^9/L; total T cells, 690 to 2540/μL; CD4, 410 to 1590/μL; CD8, 190 to 1140/μL; and D-dimer, less than 0.55 mg/L. The values were defined as low or high if they were below or above the indicated values.[4,18] The levels of cytokines and D-dimer were measured using chemiluminescence immunoassay assay kits (CRP from Beckman, Carlsbad, Calif; IL-6 from Roche Diagnostics, Mannheim, Germany; and D-dimer from Siemens Healthineers, Marburg, Germany).

Separation in 2 different groups based on the duration of viral presence

To understand the impact of viral persistence on disease severity, we divided the groups into 2 on the basis of days of viral persistence. The start day of the presence of viral was counted from the day of onset rather than the first positive test result because the day of presentation to the hospital may be affected by various nonbiological factors. The 2 deceased patients were excluded from the analysis because they were viral positive at their time of the death, and negative viral status was never reached. Our 2 groups were designated on the basis of viral clearance days where 16 days was set as the cutoff that divided our patient population into 2 almost-even proportions (32 and 35 each group in short and long viral persistence groups, respectively).

TABLE I. Demographic and clinical characteristics of patients based on duration of viral persistence

Characteristic	All patients (n = 67)	Short-term group (n = 32)	Long-term group (n = 35)	*P* value
Virus-shedding peroid (d)		0-16	>16	
Age (y), n (%)				**.0006**
0-14	2 (2.99)	2 (6.25)	0 (0)	
14-59	49 (73.13)	28 (87.5)	21 (60)	
≥60	16 (23.88)	2 (6.25)	14 (40)	
Sex, n (%)				.2182
Male	38 (56.72)	21 (65.63)	17 (48.57)	
Female	29 (43.28)	11 (34.38)	18 (51.43)	
Symptoms, n (%)				
Fever	55 (82.09)	29 (90.63)	26 (74.29)	.1138
Cough	40 (59.70)	13 (40.63)	27 (77.14)	.2338
Sputum	17 (25.37)	5 (15.63)	12 (34.29)	.0977
Headache	6 (8.96)	6 (18.75)	2 (5.71)	.1389
Sore throat	4 (5.97)	1 (3.13)	3 (8.57)	.615
Fatigue	25 (37.31)	13 (40.63)	12 (34.29)	.6219
Diarrhea	6 (8.96)	5 (15.63)	1 (2.86)	.096
Dyspena	13 (19.4)	3 (9.38)	10 (28.57)	.0651
Diagnosis, n (%)				.0139
Nonsevere	49 (73.13)	28 (8.75)	21 (60)	
Severe	18 (26.87)	4 (0.125)	14 (40)	
Admission to ICU	3 (4.48)	0 (0)	3 (8.57)	.2402
Chest imaging, n (%)				**.0275**
Normal	10 (14.93)	8 (25)	2 (5.71)	
Unilateral lung	2 (2.99)	1 (3.13)	1 (2.86)	
Bilateral lung	55 (82.09)	23 (71.88)	32 (91.43)	
Treatment, n (%)				
Use of high-flow oxygen	8 (11.94)	2 (6.25)	6 (17.14)	.2624
Ventilation	9 (13.43)	2 (6.25)	7 (20)	.1534
Systemic glucocorticoid	27 (40.30)	11 (34.38)	16 (45.71)	.4555
Others (d), mean (95% CI)				
Onset to admission	9.09 (7.01-11.11)	4.9 (3.92-5.89)	12.91 (9.58-16.25)	**<.0001**
Hospitalization	20.7 (17.8-23.6)	15.03 (12.04-18.02)	25.89 (21.62-30.15)	**<.0001**

ICU, Intensive care unit.
Bold indicates statistical significance ($P < .05$).

Patients with viral reactivation

Four of the 67 patients were found to be positive during the follow-up visits. Each patient was followed up at the 7th and 14th day after discharge; if the patient's virus test result of throat swab turned out to be positive, the patient will be tested every 2 to 3 days until 2 consecutive viral quantitative PCR tests showed negative results. These tests were performed for every patient after their recovery and discharge from the hospital. These patients were further asked to be quarantined at their homes, and the throat swabs were sent to the AMMS where these swabs were inoculated in Vero cells to measure cytopathy and viral load in the cell culture as a marker of the presence of a viable virus. If no cytopathy was observed, supernatants were collected and added to a new well and this process was repeated 3 times to ensure the detection of even low viral burden in these samples. The viral presence was detected by both cytopathy and viral quantitative PCR and was deemed negative if none of these 2 parameters was positive. The culture success rate is more than 90% of the infected patients in the laboratory using this protocol from samples obtained during active disease. Patients were re-tested every 2 to 3 days again until the quantitative PCR test results turned negative.

Statistical analysis

Data were analyzed from 67 patients. The data between groups were compared using the χ^2 test (for trend) or Fisher exact test for categorical variables or t test. Data were analyzed using GraphPad Prism (version 8.4.2).

RESULTS

Demographic and clinical characteristics of 2 groups

The basic demographic characteristics of the patient population are listed in Table I. Fever was the most common symptom present in 82% of the subjects and was not significantly different among the 2 groups. Similarly, other common symptoms such as cough and fatigue were not significantly different among the 2 groups. In contrast, dyspnea was higher in the long-term viral persistent group compared with the short-term group (9% vs 29%; $P = .06$). Similarly, the use of ventilation or high-flow oxygen or glucocorticoids was not different among the 2 groups. We also determined if the use of glucocorticoids was a major contributor to the viral persistence

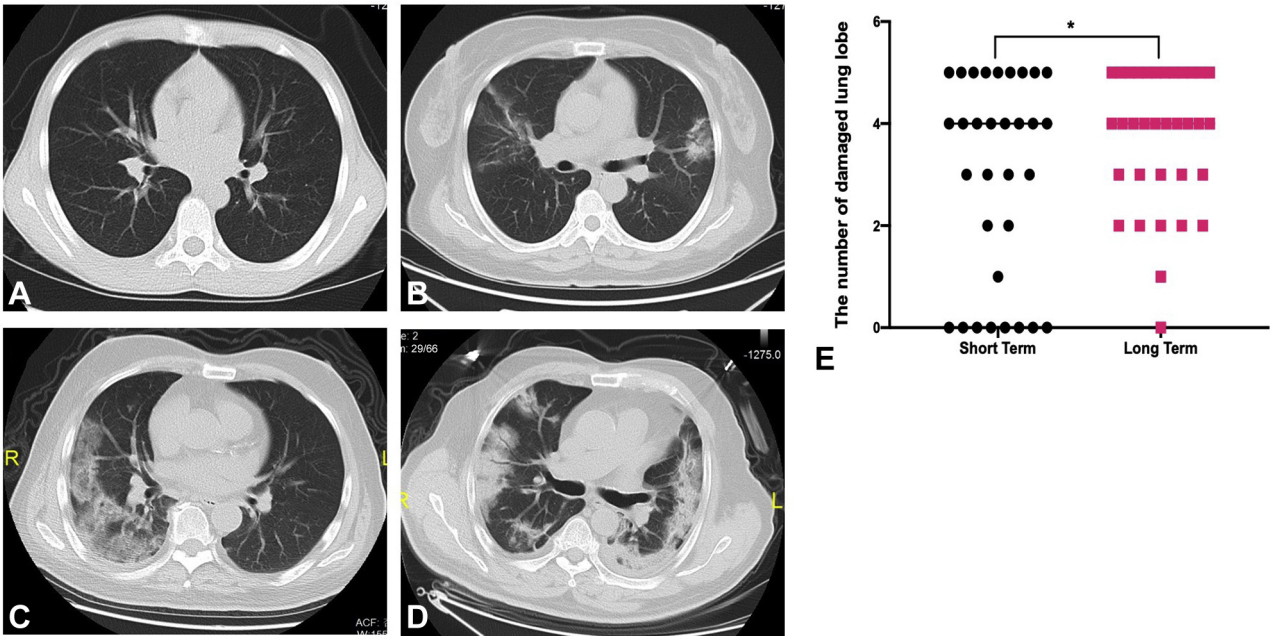

FIGURE 1. Chest CT scans of patients with COVID-19 based on the duration of the viral presence. Representative chest CT scans from patients with (**A** and **B**) short-term or (**C** and **D**) long-term viral presence. (**E**) The quantification of the number of lobes involved in each patient between 2 groups (*$P < .05$). *CT,* Computed tomography.

or other markers of disease severity. Our data indicate the glucocorticoid use did not significantly contribute to the disease severity marker (see Table E1 in this article's Online Repository at www.jaci-inpractice.org).

Based on the disease severity, a significantly higher number of severe cases were noted in the long-term viral persistence group compared with the short-term viral presence group (12.5% in the short-term vs 40% in the long-term; $P = .0139$). Three of the total 14 severe cases in the long-term group were admitted to intensive care units, whereas no one from the short-term group required intensive care unit admission. Two of the patients with confirmed SARS-CoV-2 remained asymptomatic throughout their positive viral periods.

The 2 patients included a 79-year-old woman and a 50-year-old man. Both of them reported hypertension as comorbidity. Both had critical disease and were treated with glucocorticoids. The male and female patients died at day 17 and 28 posthospital admission, respectively.

Imaging evidence

To further evaluate the impact of viral persistence on lung injury, the computed tomography scans were obtained, and the number of lobes that showed abnormalities in each patient were quantified by 2 independent investigators. A significantly higher number of lobes were involved in patients having long-term viral presence compared with those who cleared the virus within 16 days postsymptom onset (mean ± SEM, 2.938 ± 0.3505 in the short-term group vs 3.706 ± 0.2294 in the long-term group) (Figure 1, *A* and *B*). Only 1 of the subjects in the long-term group had no evidence of pathological changes in any of the lobe, whereas 7 patients in the short-term group had no radiological evidence of the pathology. In contrast, all 5 lobes were involved in 9 patients in the short-term group, but 12 patients had all 5 lobes of lung involvement in the long-term group. These data suggest an essential role of viral presence in causing lung pathology. Both the dead subjects demonstrated lung involvement by chest computed tomography where involvement of all 5 lobes appeared in chest computed tomography.

Laboratory markers of inflammation

Inflammatory response to viral infection is an essential part of viral clearance in the lung. However, uncontrolled inflammation can lead to cytokine storm and septic shock with a potential grave prognosis. We analyzed the level of inflammatory markers in these patients and compared them among 2 groups on the basis of duration of viral persistence. Our data show that the percentage of patients showing elevated levels of IL-6 were similar in the 2 groups in the first week when data were available for a maximum number of patients (Table II). Most patients had elevated values of IL-6 especially in the short-term group (60%). Surprisingly, only 43% of the patients in the long-term viral persistence group had elevated levels of IL-6, although this did not reach statistical significance. Similarly, levels of other markers of inflammation such as CRPs were not different among the 2 groups. However, levels of D-dimer, a marker that is associated with disease severity in COVID-19, was significantly elevated in a higher number of patients within the long-term group in both first (12.5% vs 37.14%; $P = .0261$) and second weeks (18.75% vs 60%; $P = .001$) of the hospital admission. The values of these inflammatory cytokines are presented in Tables E2 in this article's Online Repository at www.jaci-inpractice.org.

Blood leukocyte counts and lymphocyte counts

The blood cell counts for most of the patients were in the normal range including total white blood cell counts and neutrophils in the blood. However, as described before, a significant

TABLE II. Comparison of inflammatory markers between short-term and long-term viral persistent groups

		Short-term group		Long-term group		
Inflammatory markers	**Week**	**High**	**Normal**	**High**	**Normal**	***P* value**
IL-6	First week	19 (59.38)	13 (40.63)	15 (42.88)	20 (57.14)	.2242
	Second week	4 (12.5)	28 (87.5)	5 (14.29)	30 (85.71)	.6245
CRP	First week	16 (50)	16 (50)	15 (42.88)	20 (57.14)	.628
	Second week	5 (15.63)	27 (84.38)	8 (22.86)	27 (77.14)	.5445
D-dimer	First week	4 (12.5)	28 (87.5)	13 (37.14)	22 (62.86)	**.0261**
	Second week	6 (18.75)	26 (81.25)	21 (60)	14 (40)	**.001**

Values are n (%).
Bold indicates statistical significance ($P < .05$).

TABLE III. Comparison of blood leukocyte counts and lymphocyte counts in 2 groups

			Short- term			Long- term		
Counts	**Time**	**High**	**Normal**	**Low**	**High**	**Normal**	**Low**	***P* value**
White blood cell	First week	1 (3.13)	30 (93.75)	1 (3.13)	4 (11.43)	23 (65.71)	8 (22.86)	**.0261**,* .1734†
	Second week	4 (12.5)	24 (75)	4 (12.5)	5 (14.29)	28 (80)	2 (5.71)	.4155,* >.9999†
Neutrophils	First week	1 (3.13)	25 (0.78)	6 (18.75)	3 (8.57)	27 (77.14)	5 (14.29)	.7500,* .6151†
	Second week	1 (3.13)	31 (96.88)	0 (0)	5 (14.29)	28 (80)	2 (5.71)	.2377,* .1968†
Lymphocytes	First week	0	28 (87.5)	4 (12.5)	0	25 (71.42)	10 (28.57)	.138*
	Second week	0	30 (93.75)	2 (6.25)	0	25 (71.42)	10 (28.57)	**.0247***
Eosinophils	First week	0	16 (50)	16 (50)	0	17 (48.6)	18 (51.4)	>.9999*
	Second week	0	28 (87.5)	4 (12.5)	0	29 (82.9)	6 (17.1)	.7364*
$CD4^+$ T cells	First 10 d	0	21 (65.63)	11 (34.38)	0	13 (37.14)	22 (62.86)	**.028***
$CD8^+$ T cells	First 10 d	0	28 (87.5)	4 (12.5)	0	21 (60)	14 (40)	**.0139***
Total T cells	First 10 d	0	25 (78.13)	7 (21.88)	0	18 (51.43)	17 (48.57)	**.0403***

Values are n (%).
Bold indicates statistical significance ($P < .05$).
**P* value between low and normal values between 2 groups.
†*P* value between high and normal value between 2 groups.

proportion of patients had lymphocytopenia, especially in those in the long-term viral persistent group (first week, 4 of 32 patients in the short-term group and 10 of 35 patients in the long-term group had lymphocytopenia; second week, 2 of 32 patients in the short-term group and 10 of 35 patients in the long-term group had lymphocytopenia). $CD4^+$ T-cell counts were lower than normal in only 34% of the subjects in the short-term group compared with 63% in the long-term viral presence group within the first 10 days of the hospitalization ($P = .028$). Similar trends were observed for $CD8^+$ T cells where only 12.5% in the short-term group had lower CD8 T-cell counts compared with 40% of the subjects who had lower CD8 T-cell counts in the long-term group ($P = .0139$). Similar trends were also observed for total T cells (Table III).

Clinical characteristics of patients with viral reactivation

All the patients except 3 who are still in hospital were discharged after symptomatic recovery and confirming viral negative status (tests performed at least twice 24 hours apart). As part of the regular follow-up, patients who were discharged were followed up every week to obtain their throat swabs. Four of the swabs from these 64 patients turned positive at various time points after their confirmed negative status (Figure 2). The throat swabs were sent to the AMMS laboratory to test whether the quantitative PCR positive test results are indicative of the presence of a live pathogen. The swabs were plated on Vero cells to measure cytopathy and viral replication using quantitative PCR of cell lysates after infection. We failed to detect any cytopathy or presence of viral replication in the infected cells. The time course of these patients is demonstrated in Figure 2. Individually, none of these patients had viral presence for more than 22 days, suggesting that the re-emergence of viral positivity is not associated with impaired ability to clear the initial infection.

DISCUSSION

In this retrospective study, we demonstrate that the prolonged presence of the virus is a vital determinant of the disease severity and should be considered during therapeutic interventions. Currently, there are no approved antiviral therapies for COVID-19. However, various therapies that target inflammation are being used without clear evidence of their benefits.[19] Inflammation including those mediated by IL-6 is essential for helping viral clearance, and a decrease in overall inflammation may contribute to impaired viral clearance.[20] These data presented here warrant careful investigation of the impact of anti-inflammatory therapies such as those targeting IL-6 on viral clearance kinetics to ensure these therapies do not impair host antiviral mechanisms.

Our data show that elevated inflammation measured by the circulating markers such as IL-6 or CRP was not a common

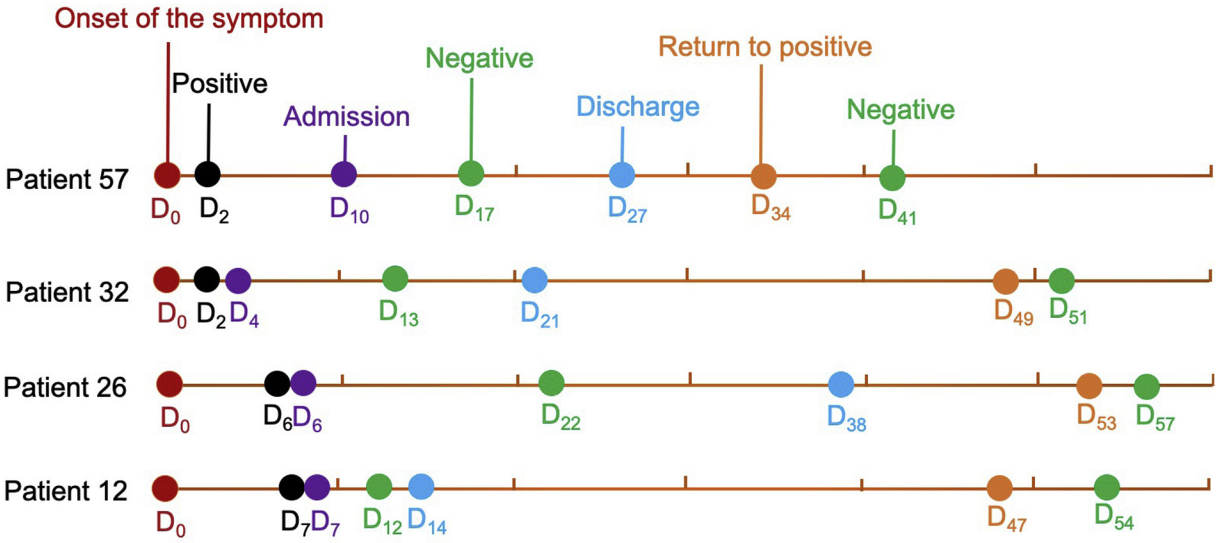

FIGURE 2. Timeline of events in patients with re-emergence of viral positivity postrecovery from COVID-19. Four patients who were discharged from the hospital turned out to be virus-positive by virus RT-PCR testing of the throat swab. The onset of the symptom, first virus-positive day, day of admission, first negative viral test result, and day of discharge are shown as dots in different colors. Orange dots represent the reactivation of the virus followed by the disappearance of second viral positivity by green dots.

feature of the patient population with long-term viral persistence. Indeed, a trend toward a lower fraction of patients having elevated IL-6 was observed during the early course of the disease in the group with impaired viral clearance. In agreement with IL-6, a similar fraction of the population had elevated CRP in both the groups. Similarly, the number of total circulating white blood cells and neutrophils remained in the regular or lower range for most of the patients. These data suggest either lung-specific inflammatory response[21] that is not reflected in the circulation or SARS-CoV-2–mediated mechanisms that may actively suppress the host inflammatory response. However, the involvement of other inflammatory cytokines that are not measured here cannot be ruled out. It is also possible that the deleterious effects of inflammation are observed only during the late stage of the disease when the infectious viral burden is already significantly decreased. Long-term consequences of the elevated inflammatory response are recently reported in children where some children show inflammatory response manifested as Kawasaki disease.[22]

Elevation of D-dimer is known to be a major risk factor for the disease severity of COVID-19.[5] In our study, we demonstrate that patients with persistent viral presence show significantly elevated levels of D-dimer in both the first and second weeks of hospitalization, suggesting that viral presence corroborates with previously reported disease severity markers. At the same time, the elevated level of D-dimer may suggest an involvement of the vascular system in mediating COVID-19 pathogenesis. Indeed, new disease phenotypes such as Kawasaki disease strongly suggest the role of vasculature inflammation in children as a chronic consequence after the viral clearance.[5,22] Further studies are required to understand whether using anti-inflammatory therapies such as targeting IL-6 can provide any potential benefit during Kawasaki disease.

It is unclear how the virus persists in the lung for such prolonged periods with manifestations of the disease. It seems plausible that the virus attacks host antiviral immune response as evidenced by significant depletion of $CD4^+$ and $CD8^+$ cells in those with persistent viral presence. Our data support this hypothesis that lower levels of T lymphocytes, including CD4 and $CD8^+$ cells, contribute to the lack of viral clearance, where these cells are actively targeted by the virus. Infected patients who can maintain sufficient levels of T cells can efficiently clear the virus and resolve the disease. Although previous studies have reported that viral load is not associated with the disease, these viral loads were measured in upper airways rather than lung where major pathological events take place.[23] Furthermore, recent studies have indicated the presence of angiotensin-converting enzyme 2 on T cells and the ability of SARS-CoV-2 to infect T cells.[24]

Host tolerance is the ability of the host to tolerate the virus without having significant pathological consequences to the host. Two of our patients had no clinical symptoms but were tested because of their known close contact with infected patients. These observations indicate that host tolerance is another major contributor in defining disease severity besides the host resistance (ability to clear the virus). Although only 2 patients in our cohort represented these populations with host tolerance sufficient to suppress any clinical symptoms, the precise fraction of this population can be identified only with extensive serological testing.[25]

Another critical point of discussion is regarding the re-emergence of the virus in recovered patients. There has been significant concern regarding the re-emergence of the virus in patients who have recovered from COVID-19 but whose results come positive on the quantitative PCR test. The results for 4 of our patients turned positive on their follow-up visits after confirming viral negative status and resolution of symptoms. Despite our efforts to grow these viruses, we were unable to grow these viruses, suggesting the possible presence of viral remnants rather than infective viral particles. As reported previously, the clinical features of these patients were not different among these patients.[13] Because of multiple positive quantitative PCR test

results, however, we believe that it was not due to error in the quantitative PCR giving false-positive results.

CONCLUSIONS

Our data demonstrate the urgent need for developing new antiviral therapies and at the same time reconsider the role of therapies that may adversely affect the host's ability to clear the virus. We hope that these data will provide important clinical guidance to clinicians regarding the therapeutic approaches being used to treat COVID-19.

REFERENCES

1. World Health Organization. Coronavirus disease 2019 (COVID-19) situation report − 130. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200529-covid-19-sitrep-130.pdf?sfvrsn=bf7e7f0c_4. Accessed May 30, 2020.
2. Chang D, Mo G, Yuan X, Tao Y, Peng X, Wang F, et al. Time kinetics of viral clearance and resolution of symptoms in novel coronavirus infection. Am J Respir Crit Care Med 2020;201:1150-2.
3. Chang D, Lin M, Wei L, Xie L, Zhu G, Cruz CSD, et al. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA 2020;323:1092-3.
4. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus−infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
5. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
6. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033-4.
7. Pedersen SF, Ho Y. SARS-CoV-2: a storm is raging. J Clin Invest 2020;130:2202-5.
8. Aspinall R, Del Giudice G, Effros RB, Grubeck-Loebenstein B, Sambhara S. Challenges for vaccination in the elderly. Immun Ageing 2007;4:9.
9. Cao Bin, Wanf Y. A trial of lopinavir−ritonavir in adults hospitalized with severe covid-19. 2020. N Engl J Med 2020;382:1787-99.
10. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;30:269-71.
11. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: a single center experience. J Med Virol 2020;92:814-8.
12. Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, et al. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the experience of clinical immunologists from China. Clin Immunol 2020;214:108393.
13. Ye G, Pan Z, Pan Y, Deng Q, Chen L, Li J, et al. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. J Infect 2020;80:e14-7.
14. Kyrle PA, Eichinger S. D-dimer for long-term risk prediction in patients after acute coronary syndrome: jack of all trades, or master of none? Circulation 2018;138:724-6.
15. Stein PD, Hull RD, Patel KC, Olson RE, Ghali WA, Brant R, et al. D-dimer for the exclusion of acute venous thrombosis and pulmonary embolism: a systematic review. Ann Intern Med 2004;140:589-602.
16. China National Health Commission. Chinese clinical guidance for COVID-19 pneumonia diagnosis and treatment. 7th edition. Available from: http://kjfy.meetingchina.org/msite/news/show/cn/3337.html. Accessed May 30, 2020.
17. Zhao JY, Yan JY, Qu JM. Interpretations of "diagnosis and treatment protocol for novel coronavirus pneumonia (trial version 7)". Chin Med J (Engl) 2020;133:1347-9.
18. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:507-13.
19. Kalil AC. Treating COVID-19—off-label drug use, compassionate use, and randomized clinical trials during pandemics. JAMA 2020;323:1897-8.
20. Dienz O, Rud JG, Eaton SM, Lanthier PA, Burg E, Drew A, et al. Essential role of IL-6 in protection against H1N1 influenza virus by promoting neutrophil survival in the lung. Mucosal Immunol 2012;5:258-66.
21. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med 2020;26:842-4.
22. Verdoni L, Mazza A, Gervasoni A, Martelli L, Ruggeri M, Ciuffreda M, et al. An outbreak of severe kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet 2020;395:1771-8.
23. Peiris JSM, Chu C, Cheng VC, Chan K, Hung I, Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 2003;361:1767-72.
24. Wang X, Xu W, Hu G, Xia S, Sun Z, Liu Z, et al. SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. Cell Mol Immunol 2020:1-3.
25. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis 2020;71:778-85.

ONLINE REPOSITORY

TABLE E1. Clinical characteristics and laboratory findings of patients based on glucocorticoid use

	Glucocorticoid use		No glucocorticoid use		
Characteristic	Short-term group (n = 11)	Long-term group (n = 16)	Short-term group (n = 21)	Long-term group (n = 19)	*P* value
Virus-shedding period (d)	13.36 ± 2.378	28 ± 8.832	10.43 ± 3.802	31.26 ± 12.02	.2389
Age (y), mean ± SD	51.82 ± 16.9	57.13 ± 16.99	34.19 ± 14.61	48.53 ± 15.5	.2111
Sex, n (%)					
Male	4 (14.81)	10 (37.04)	7 (17.5)	7 (17.5)	.4401
Female	7 (25.93)	6 (22.22)	14 (35)	12 (30)	>.9999
Diagnosis, n (%)					
Nonsevere	7 (25.93)	2 (7.41)	21 (52.5)	19 (47.5)	.267
Severe	4 (14.81)	14 (51.85)	0 (0)	0 (0)	>.9999
Admission to ICU	0 (0)	3 (11.11)	0 (0)	0 (0)	>.9999
Chest imaging, n (%)					
Normal	0 (0)	0 (0)	8 (20)	2 (5)	>.9999
Unilateral Lung	0 (0)	0 (0)	1 (2.5)	1 (2.5)	>.9999
Bilateral Lung	11 (40.74)	16 (59.26)	12 (30)	16 (40)	>.9999
Treatment, n (%)					
Use of high-flow oxygen	2 (7.41)	6 (22.22)	0 (0)	0 (0)	>.9999
Ventilation	2 (7.41)	7 (25.93)	0 (0)	0 (0)	>.9999
High IL-6, first week	10 (37.04)	10 (37.04)	9 (22.5)	5 (12.5)	.4953
High IL-6, second week	3 (11.11)	5 (18.52)	1 (2.5)	0 (0)	.4444
High CRP, first week	10 (37.04)	11 (40.74)	6 (15)	4(10)	.7043
High CRP, second week	3 (11.11)	5 (18.52)	2 (5)	3 (7.5)	>.9999
High D-D, first week	3 (11.11)	9 (33.33)	1 (2.5)	4 (10)	>.9999
High D-D, second week	5 (18.52)	15 (55.56)	1 (2.5)	6 (15)	>.9999
Leucopenia, first week	1 (3.7)	6 (22.22)	0 (0)	2 (5)	>.9999
Leucopenia, second week	3 (11.11)	1 (3.7)	1 (2.5)	1 (2.5)	>.9999
Neutropenia, first week	4 (14.81)	4 (14.81)	2 (5)	1 (2.5)	>.9999
Neutropenia, second week	0 (0)	1 (3.7)	0 (0)	1 (2.5)	>.9999
Lymphocytopenia, first week	4 (14.81)	7 (25.93)	0 (0)	3 (7.5)	.5055
Lymphocytopenia, second week	2 (7.41)	8 (29.63)	0 (0)	2 (5)	>.9999
Eosinopenia, first week	9 (33.33)	13 (48.15)	7 (17.5)	5 (12.5)	.4754
Eosinopenia, second week	4 (14.81)	4 (14.81)	0 (0)	2 (5)	.4667
Low $CD4^+$ T-cell counts	7 (25.93)	13 (48.15)	4 (10)	9 (22.5)	>.9999
Low $CD8^+$ T-cell counts	2 (7.41)	11 (40.74)	2 (5)	3 (7.5)	.5327
Low total T-cell counts	4 (14.81)	9 (33.33)	3 (7.5)	8 (20)	>.9999

TABLE E2. The levels of inflammatory markers in short-term and long-term viral persistent groups

		Short-term group, mean (95% CI)		Long-term group, mean (95% CI)		
Inflammatory markers	Week	High	Normal	High	Normal	*P* value
IL-6 (pg/mL)	First week	23.86 (14.33 to 33.38)	4.86 (3.98 to 5.74)	19.29 (11.34 to 27.24)	3.32 (2.36 to 4.28)	.3324
	Second week	19.87 (11.81 to 27.93)	2.58 (1.71 to 3.45)	23.73 (−4.24 to 51.69)	3.68 (2.99 to 4.38)	.3887
CRP (mg/L)	First week	26.51 (15.04 to 37.97)	3.59 (2.14 to 5.04)	22.35 (11.83 to 32.88)	2.63 (1.73 to 3.53)	.4577
	Second week	19.66 (6.78 to 32.53)	3.91 (3.36 to 4.46)	18.74 (11.54 to 25.93)	2.20 (1.45 to 2.96)	.5315
D-dimer (mg/L)	First week	2.81 (−0.69 to 6.31)	0.25 (0.21 to 0.30)	2.29 (0.68 to 3.90)	0.27 (0.22 to 0.32)	.5324
	Second week	4.66 (−2.85 to 12.16)	0.33 (0.29 to 0.38)	2.21 (0.75 to 3.66)	0.24 (0.18 to 0.30)	.1033