Clinical Features and Lymphocyte Subsets in Recovered Covid-19 Patients With Prolonged Viral Rna Shedding Duration

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Case Report

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Abstract

Background: The 2019 novel coronavirus disease (COVID-19) spread in many countries.Data about viral shedding duration, particularly the prolonged ones of the pathogen SARS-Coronavirus-2 (SARS-CoV-2) is scarce. The longest viral RNA sheddingduration reported previously was 37 days. Herein, we report the clinical and immunologic features ofrecovered COVID-19cases with a medium viral RNA shedding duration of 44 days.

Cases presentation: Nine laboratory-confirmed COVID-19 cases from Wuhan with viral RNA shedding duration more than 30 days were included in our study,5 of them were moderate.Althoughinflammatory markers were significantlyhigher, the medium duration in severepatients was similar to that in moderate patients (44.5days vs. 43.6days). Severepatients showed higher NK cells levels, although the T cells and B cells were lower as compared with moderate patients. Contrary to previous reports in influenza, prolonged viralshedding time did not cause poor prognosis in this study.

Conclusions: There could be characteristic immunological dysfunction in COVID-19 patients with prolonged viral shedding durationand interestingly, prolonged viral shedding duration seemed not to be related with poor prognosis.

Background

SARS-Coronavirus-2 (SARS-CoV-2) is the focus of global attention which attributed to an outbreak of a severe respiratory illness since December 2019 in Wuhan, China. This severe respiratory illness originated in Wuhan City in the Hubei province in China and is capable of rapid community transmission (1).

The level and duration of SARS-CoV-2 virus replication are important factors in assessing the risk of trans mission and guiding decisions regarding isolation of patients. A previous study reported that the median duration of viral shedding was 20 days in survivors (2). The longest duration of viral shedding was 37 days in survivors. It has been reported in seasonal or pandemic influenza viral shedding duration that the longer duration of viral RNA shedding was correlated with worse patient prognosis (3–4). It remains unclear whether the SARS-CoV-2 RNA shedding duration affected progression of COVID-19 or not. Here, we report the clinical and immunologic features of a series of COVID-19 cases with prolonged viral RNA shedding duration (the median duration was 44 days).

Cases Presentation

Methods and Patients

This retrospective study enrolled 9 laboratory-confirmed COVID-19 adult inpatients admitted to Wuhan Tongji Hospital from February 09 to March 09, 2020. Diagnosis and clinical classification of COVID-19 were according to the Chinese management guideline for COVID-19 (version 7.0) and the World Health Organization interim guidance. Severe and critically ill COVID-19 patients were identified by reviewing and analyzing admission logs and histories of all available electronic medical records and patient care resources by two physicians. This study was approved by the institutional review boards at Wuhan Tongji Hospital and the First Affiliated Hospital of Soochow University. As an emerging infectious disease, written informed consent was exempted.

Data Collection

Demographic characteristics (age and gender), clinical features (comorbidities, laboratory findings, diseases severity, treatments and outcomes) were recorded. Patients were followed-up from admission to persistent negative detection of pharyngeal swabs specimens. The SARS-CoV-2 RNA shedding duration was defined as the interval between illness onset and the date of the last pharyngeal swab with a positive finding. Patients were followed-up from admission to 28 days in hospital, hospital discharge, or death, whichever came first.

Statistical analysis

Continuous data with normal distribution were presented as mean ± standard deviation. Frequency data were expressed as proportions. Data were analyzed using SPSS 25.0.

Results

Total of 9 adult COVID-19 inpatients were included. The median age was 64.3 years old (34–87 yrs). 6 cases were male. The most popular comorbidity was hypertension (5/9). The most common symptoms were fever (6/9) and cough (5/9). All patients were survived at 28-days after admission, with 5 cases discharged. 2 patients were improved in hospital and 2 patients remained receiving invasive machinery ventilation (IMV) and continuous renal replacement therapy (CRRT).

For all patients, the median viral RNA shedding duration was 44 days (30–62 days). The median viral RNA shedding duration in severe or critical patients was similar to that in moderate patients (44.5 days *vs.* 43.6 days). The severe and critical patients were older, have a lower lymphocyte count (0.58×10^9 /L *vs.* 1.72×10^9 /L) and ALB levels (29.75 g/L vs. 40.06 g/L), a higher levels of hs-CRP (89.33 mg/L vs. 2.56 mg/L), interleukin 6 (35.62 pg/ml vs. 2.27 pg/ml), d-dimer (1.67 μg/ml vs. 0.36 μg/ml), cTnI (91.03 pg/ml vs. 2.10 pg/ml), and lactate dehydrogenase (402.25 U/L vs. 182.40 U/L).

We also detected the lymphocyte subsets levels in peripheral blood samples. As compared with moderate COVID-19 cases, the severe and critical patients showed a lower levels of $CD3^+CD19^-$ T cells (675.75 N/μl *vs.* 1370.40 N/μl), $CD3^-CD19^+$ B cells (130.25 N/μl *vs.* 275.00 N/μl), $CD3^+CD4^+$ Treg (380.25 N/μl *vs.* 734.80 N/μl) and $CD3^+CD8^+$ Ts cells (283.50 N/μl *vs.* 561.40 N/μl). Interestingly, severe and critical patients have a higher levels of $CD3^-CD16^+CD56^+$ NK

cells (318.25 N/μl *vs.* 232.20 N/μl). All patients showed bilateral lung lesion in CT findings (patchy shadowing or ground-glass opacity), 2 patients showed pleural effusion.

Discussion

Our study focused on the clinical features and immunologic manifestations of COVID-19 patients with prolonged viral RNA shedding duration. Duration of infectious virus replication are crucial factors in evaluating the transmission risk (1). The median shedding duration was 44 days, which was far longer than that reported in previous study (2). In this study, the longest viral RNA shedding duration was 62 days. It might be the longest viral shedding duration of COVID-19 to date. The difference might be due to the epidemiological feature of patients enrolled, which were enrolled form the designated hospital for COVID-19 patients of severe or critical illness in Wuhan, the most serious epidemic region in China. Delayed hospital admission was associated with prolonged viral shedding duration, as they received insufficient treatment when outside of hospital. We need more data to determine transmission dynamics and inform our screening practices (5).

Previous reports about influenza A (H7N9) and respiratory syncytial virus revealed that prolonged viral shedding duration associated with risk of complications and poor prognosis (3–4). Contrarily, all patients in our study remain survived although their viral RNA shedding duration exceeded 30 days. It indicated that prolonged viral RNA shedding was not always associated with poor prognosis. This might indicate the specificity of SARS-CoV-2. The stabilizing mutation falling in the nsp2 protein could account for COVID-2019 high ability of contagious, while the destabilizing mutation in nsp3 proteins could suggest a potential mechanism differentiating COVID-2019 from SARS (6–7). The molecular divergence of SARS-CoV-2 evolved into two major types with different characteristics (7). Furthermore, the diversity of mortality in different region of China might be due to the virological dynamics and epidemiological characteristics (8).

Although the inflammatory markers were obviously different (such as hs-CRP and interleukin 6) between severe patients and common patients, the median duration of viral RNA shedding was similar. We did not find this relationship between viral shedding and disease severity. The balance between viral load and immune system was one of the most important factors determined diseases progression or not. SARS-CoV-2 infection can activate innate and adaptive immune responses (9). However, uncontrolled inflammatory innate responses and impaired adaptive immune responses contributed to both locally and systemically harmful tissue damage. Although the severe and critical patients showed obvious lymphocytopenia and dysregulation of lymphocytes subsets, their NK cells levels were higher than common patents. This might be helpful for immune responses against SARS-CoV-2 infection. The differentiation of naive CD4 + T-cells into effector and memory subsets is one of the most fundamental facets of T-cell-mediated immunity (10). Additionally, the antivirus treatment might affect the disease progression.

Table 1
The clinical and immunologic characteristics of all patients.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Average (severe+criti
Age (years)	81	81	87	55	49	72	34	66	54	80.25
Sex (M/F)	M	M	M	F	F	M	M	F	M	
Comorbidity	CHD, HPT	DM	COPD, HPT	DM, HP	–	–	HPU	–	DM, HPT	
Severity	Critical	Critical	Severe	Moderate	Moderate	Severe	Moderate	Moderate	Moderate	
First symptom	Fever Cough	Fever Cough	Cough Dyspnea	Fever Cough	Fever Fatigue	Fever	Fever Myalgia	Cough Fatigue	Cough Dyspnea	
Lab findings										
WBC (10^9 /L)	4.00	8.66	6.08	7.07	3.64	7.48	7.99	4.93	5.13	6.56
Lym (10^9 /L)	0.49	0.35	0.75	1.72	1.5	0.72	1.91	1.84	1.62	0.58
Platelet (10^9 /L)	398	213	174	266	255	268	144	188	145	263.25
Albumin (g/L)	30.40	27.50	34.10	33.40	38.50	27.00	44.90	40.10	43.40	29.75
ALT (U/L)	11	72	10	7	27	299	113	21	47	98
Cretinine (μM)	96	149	100	44	67	66	86	59	43	102.75
LDH (U/L)	247	720	344	280	167	298	166	150	149	402.25
hs-cTNI (pg/ml)	7.4	24.6	322	2.8	1.9	10.1	1.9	2	1.9	91.03
D-dimer (μg/ml)	0.56	2.04	1.81	0.82	0.22	2.27	0.22	0.25	0.28	1.67
hs-CRP (mg/L)	81.9	147.5	56.4	2.2	3.2	71.5	2.8	1.7	2.9	89.33
IL-6 (pg/ml)	16.19	54.08	25.25	1.61	2.83	46.96	1.5	2.1	3.3	35.62
PCT (ng/ml)	0.1	0.35	0.08	0.06	0.06	0.09	0.09	0.05	0.07	0.16
Lymphocyte subsets in peripheral blood (number/μl)										
$CD3^+CD19^-$T cells	347	410	771	1508	1119	1175	1608	1290	1327	675.75
$CD3^-CD19^+$B cells	101	291	18	292	263	111	224	389	207	130.25
$CD3^+CD4^+$Treg	278	281	389	846	745	573	573	1020	490	380.25
$CD3^+CD8^+$Ts	65	117	358	573	349	594	911	220	754	283.50
$CD3^-CD16^+CD56^+$NK cells	97	127	167	87	236	882	304	205	329	318.25
Treatment										
Antibiotics	Moxi	Merop	Cefop						Moxi	
Antivirus	Lopi, Arbi	Lopi, Arbi	Arbi	Arbi	Arbi	Lopi, Arbi	Lopi, Arbi	Arbi	Arbi	
CT findings										
Bilateral patchy shadowing	+	+	+		+	+				
Bilateral GGO	+	+		+			+	+	+	
Pleural effusion			+			+				
28-days outcomes	IMV CRRT	IMV CRRT	Improved	Discharged	Discharged	Improved	Discharged	Discharged	Discharged	
Virus shedding duration (d)	43	30	43	49	41	62	52	37	39	44.5

Abbreviation: CHD: coronary heart disease; COPD: chronic obstructive pulmonary disease;HPT: hypertension; HPU: hyperuricemia; DM: diabetes mellitus;Lym: ritonavir; Arbi:Arbidol;PCT: procalcitonin;IMV: invasive machinery ventilation; CRRT: continuous renal replacement therapy; Moxi: moxifloxacin; Merop: merop GGO: ground-glass opacity.

Conclusion

In conclusion, our study focuses on the clinical and immunologic features in COVID-19 patients with prolonged viral RNA shedding duration. Severe COVID-19 patients have a lower level of $CD3^{+}CD19^{-}$T cells and $CD3^{-}CD19^{+}$B cells but a higher $CD3^{-}CD16^{+}CD56^{+}$NK cells levels. Contrary to previous reports in influenza, prolonged viral shedding time did not cause poor prognosis in this study. However, further studies are needed to determine the virological dynamics.

Abbreviations

COVID-19
The 2019 novel coronavirus disease; SARS-CoV-2:SARS-Coronavirus-2; IMV:invasive machinery ventilation; CRRT:continuous renal replacement therapy; hs-CRP:high-sensitivity C-reactive protein; RNA:Ribonucleic Acid; NK cells:natural killer cells; CD:Cluster of Differentiation; Treg:Regulatory T cells; Ts cells:suppressor T cells; CT:Computed Tomography; cTnI:cardiac troponin I.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written informed consent for publication of the clinical details and clinical images was obtained from the patient.

Availability of data and materials

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

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Author's contributions

WYZ, KW participated in the collection and check of electronic medical records and patient care resources. YYL analysed the data with the assistance from CGW. DXZ wrote the first draft, JAH and JHJ edited the final draft. All authors had read and approved the final manuscript.

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Figures

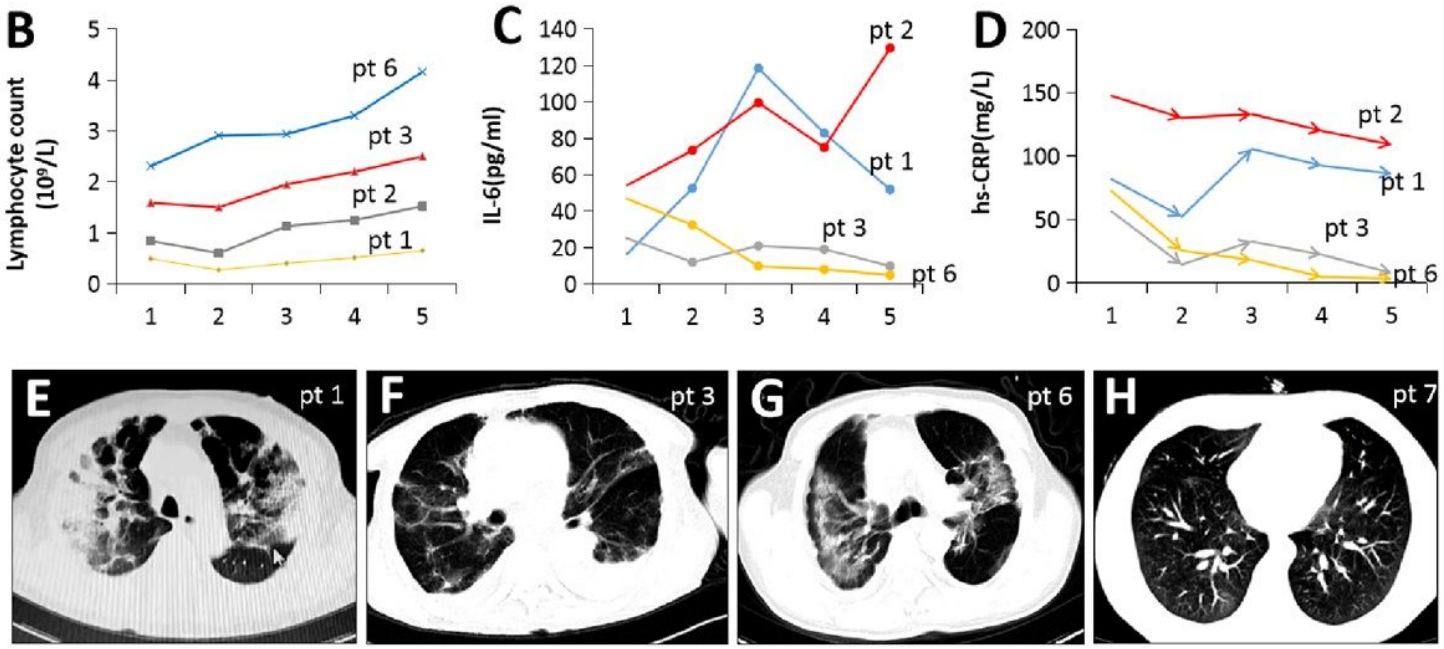

Figure 1

A: The clinical process of a moderate patient. B-D: the dynamic changes of the lymphocytes counts, hs-CRP and interleukin 6 in hospitalization. E-H: the chest CT scan of 4 patients in admission.