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ORIGINAL ARTICLE

Immunosuppression and cardiovascular dysfunction in patients with severe versus mild coronavirus disease 2019: a case series

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Abstract

Objectives. As coronavirus disease 2019 (COVID-19) continues to spread globally, we aimed to describe and compare changes in the immune and cardiovascular systems of patients with mild versus severe COVID-19 at different time points during the course of disease. **Methods.** One hundred and one patients diagnosed with COVID-19 who underwent serial peripheral blood collection and chest computed tomography (CT) imaging were enrolled in this study and grouped by the severity of their illness. Changes in the immune and cardiovascular systems were analysed and compared between groups. **Results.** The study included 43 women and 58 men, with a median age of 45 years (interquartile range [IQR], 16–71). We identified spleen shrinkage in 27.7% of study patients. Ratios of spleen volume to patient (skin) volume were compared, with evidence that severe patients had more splenic shrinkage than mild patients. Lymphopenia was observed in 65.3% of patients, and 27.3% of patients had persistently low levels of lymphocytes after discharge. Tachycardia occurred mainly during the first 2 days of hospitalisation, with increases in creatine kinase–myocardial band levels in 10 (9.9%) patients and arrhythmias in 16 (15.8%) patients. **Conclusions.** In addition to pulmonary manifestations, our study demonstrated that other organ systems can also be affected during COVID-19 infection, with evidence of immunosuppression and cardiovascular dysfunction, which may contribute to increased mortality rates in critically ill COVID-19 patients.

Keywords: cardiovascular dysfunction, COVID-19, immunology, immunosuppression, immunotherapy, SARS-CoV-2

INTRODUCTION

On 30 December 2019, the Program for Monitoring Emerging Diseases reported a group of patients with pneumonia of unknown aetiology.[1,2] Through deep sequencing analysis from lower respiratory tract samples, experts identified a novel coronavirus, which the WHO named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Shortly afterwards, the pneumonia caused by SARS-CoV-2 was termed coronavirus disease 2019 (COVID-19).[3,4] On 30 January 2020, the WHO announced that COVID-19 was a global health emergency.

SARS-CoV-2 is a member of the genus β-coronavirus and is classified as group 2B. Although its gene sequence is at least 70% similar to that of SARS-CoV, the two viruses are quite different.[4,5] Evidence suggests that SARS-CoV-2 might have originated in the horseshoe bat in China, also the natural host of SARS-CoV, with evidence of person-to-person transmission.[5,6] As of 19 February 2020, 74 284 confirmed cases of COVID-19 had been reported in China, of which 14 938 patients had been cured and 2009 had died.[7] The rapid increase in the number of patients infected with SARS-CoV-2 indicates that this virus is more infectious than both SARS-CoV and Middle East respiratory syndrome-related coronavirus.

The authors' hospital, Chongqing University Three Gorges (CUTG) Hospital (also called Chongqing Three Gorges Central Hospital), is an institution designated for the treatment of patients with COVID-19 in Northeast Chongqing and receives patients from Wanzhou, Yunyang, and 10 other districts. As of 15 February 2020, 228 confirmed cases of COVID-19 had been admitted to this hospital, and 101 of these patients were enrolled in this study (Figure 1). Using medical records, we compared data between patients who were admitted to the intensive care unit (ICU) (severe group) and those who were not (mild group). We hope that our findings will provide direction for subsequent COVID-19-related research and better inform people about this emerging disease.

RESULTS

The median age of patients with severe COVID-19 disease was 51 years (IQR, 41–79), which was significantly higher than patients with more mild disease (IQR, 16–71) ($P < 0.05$). There were more male (58 [57.4%]) than female (43 [42.6%]) patients. Of all patients, 89.1% had lived in Wuhan or had been exposed to people who had tested positive for or were suspected of having SARS-CoV-2, while 10.9% had no obvious contact history. We found that 24.8% of study patients had medical comorbidities. Compared to patients with more mild disease, patients with severe COVID-19 disease had significantly higher prevalences of comorbid diabetes (5 [5.6%] vs. 5 [41.7%]) and cardiovascular disease (CVD) (1 [1.1%] vs. 2 [16.7%]), respectively ($P < 0.05$). Chief manifestations of COVID-19 included fever (76.2%), cough (76.2%), fatigue (22.8%) and myalgias (12.9%). A few patients experienced headache, retching, chest tightness, shortness of breath and/or diarrhoea (Table 1).

The mean time to diagnosis was 4.0 days for all patients (IQR, 2.5–7.0), 8.0 days for patients with severe disease (IQR, 6.0–9.0) and 4.0 days for patients with mild disease (IQR, 2.0–7.0) ($P < 0.05$). Remission times for patients with severe and mild disease were 6 days (IQR, 4.0–7.0) and 5 days (IQR, 2.0–6.0), respectively. Days to cure was 13.0 days (IQR, 10.0–18.0) for patients with severe disease and 12.0 days (IQR, 10.0–14.0) for patients with more mild disease, with a duration of illness of 19.0 days (IQR, 17.0–26.0) and 17.5 days (IQR, 15.0–20.75), respectively, and no statistically significant differences. Most patients were managed with supportive treatment combined with antiviral treatment; however, patients with more severe disease also generally received respiratory support, hormones, antibiotics, immunoglobulin (Ig) and other treatments. For patients with mild disease, the rate of treatment with antibiotics was 12.4% and with hormones was 2.2%, both of which were used significantly less frequently than in patients with more severe disease ($P < 0.05$). In patients with more severe disease, 66.7% received ventilatory assistance with a non-invasive ventilator or high-flow nasal cannula, and 33.3% were managed with invasive mechanical ventilation. In patients with mild disease, 31.5% used a nasal cannula and 2.2% used a non-invasive ventilator or high-flow nasal cannula (Table 2).

Lymphocytopenia occurred in 65.3% of all study patients, including in all severe patients and in 60.7% of patients with mild disease ($P < 0.05$). C-reactive protein levels were elevated in both groups, with 114.76 $\mu g\ L^{-1}$ (IQR, 77.57–136.95) in the severe group and 7.80 $\mu g\ L^{-1}$ (IQR,

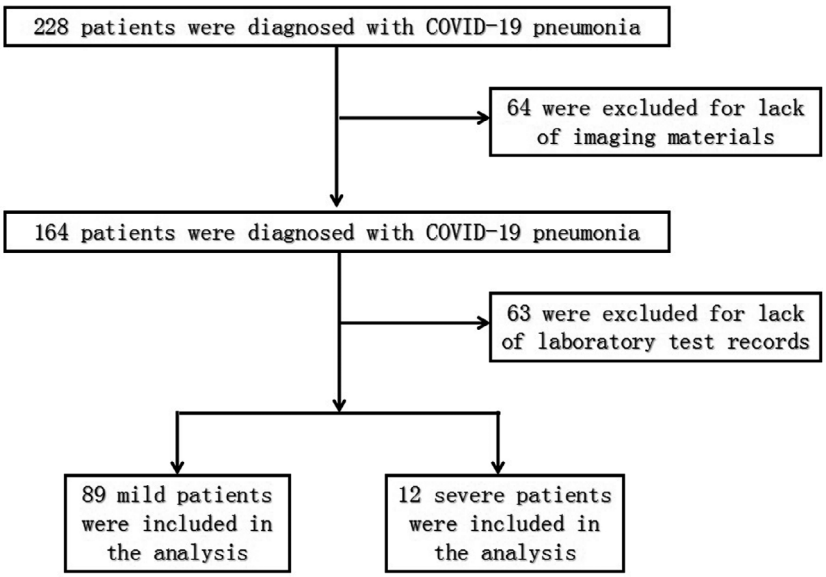

Figure 1. Study Enrolment. Two hundred and twenty-eight patients were diagnosed with coronavirus disease 2019 at our institution, and 101 of these patients were enrolled in this study. Since 64 patients had not been diagnosed at our hospital, we could not obtain their chest computed tomography images from different time points; therefore, they were excluded. In addition, 63 patients were excluded because of a lack of consistent laboratory testing. Of the 101 patients enrolled in this study, 12 patients were treated in the ICU and diagnosed with severe pneumonia, while 89 patients were diagnosed with mild or no pneumonia.

2.02–30.73) in the mild group ($P < 0.05$). Procalcitonin levels were slightly higher in patients with more severe disease than more mild disease; however, the overall increase was not significant. Prothrombin times, activated partial thromboplastin times, D-dimer levels and lactate dehydrogenase (LDH) levels were significantly higher in patients with more severe disease than in patients with more mild disease ($P < 0.05$). Albumin levels were significantly lower in the severe disease group than in the mild disease group ($P < 0.05$). There were no significant differences in platelet counts, haemoglobin, alanine aminotransferase, aspartate aminotransferase or total bilirubin levels between the two groups.

Lung imaging abnormalities were found in 95% of all patients, with more severe patients having a median of five affected lobes (IQR, 2–5) and more mild patients having a median of three affected lobes (IQR, 0–5) ($P < 0.05$) (Table 3). Splenic atrophy was identified in 27.7% of patients and significantly more commonly in patients with more severe disease (50% vs 23.6%, $P < 0.05$; Table 3). Figure 2 demonstrates this splenic atrophy in computed tomography (CT) imaging of a 79-year-old woman (Patient No. 1) with a history of CVD. CT imaging obtained from this patient on the seventh day after admission and on the day of discharge showed marked shrinkage of the spleen. In Figure 3, CT imaging shows shrinkage of the spleen in a 52-year-old man (Patient No. 2) with type 2 diabetes, viral hepatitis type B for several years and resolved tuberculosis.

To fully demonstrate changes in patients' viscera, we used a Monaco system (Monaco Treatment Planning System version 5.11.03, Elekta CMS, Maryland Heights, MO, USA), which is typically utilised during radiotherapy planning to show the three-dimensional outline of the spleen. Using Patient No. 1's CT scan obtained on the day of admission, this system showed that the spleen had become elongated and flattened (Figure 4a, b). Patient No. 2's CT scan obtained on the admission day showed irregular shrinkage of the spleen (Figure 4c, d). Figure 5a, b demonstrates splenic shrinkage and flattening in a CT scan obtained on the day of admission in a 78-year-old woman (Patient No. 3) with a history of hypertension, CVD and chronic obstructive pulmonary disease for several years. Finally, Figure 5c, d demonstrates a round and full spleen in CT imaging obtained on the day of admission in Patient No. 4, one of the patients in the mild

Table 1. Demographics and baseline characteristics of COVID-19 patients

	All patients (n = 101)	Severe patients (n = 12)	Mild patients (n = 89)	P[a]
Age, years	45 (16–71)	51 (41–79)	45 (16–71)	0.006
Sex				
Women	43 (42.6%)	6 (50.0%)	37(41.6%)	--
Men	58 (57.4%)	6 (50.0%)	52 (58.4%)	--
Any comorbidities	25 (24.8%)	7 (58.3%)	18 (20.2%)	0.012
Diabetes	10 (9.9%)	5 (41.7%)	5 (5.6%)	< 0.001[a]
Hypertension	10 (9.9%)	2 (16.7%)	8 (9.0%)	0.740
Cardiovascular disease	3 (3.0%)	2 (16.7%)	1 (1.1%)	0.030[a]
Lung diseases	3 (3.0%)	2 (16.7%)	2 (2.2%)	0.068
Chronic liver disease	5 (5.0%)	2 (16.7%)	3 (3.4%)	0.110
Malignancy	2 (2.0%)	0	2 (2.2%)	1
Others	5 (5.0%)	1 (8.3%)	4 (4.5%)	0.900
Epidemiological	90 (89.1%)	11 (91.7%)	79 (88.7%)	0.840
Living/travelling in epidemic area	67 (66.3%)	6 (50.0%)	61 (68.5%)	0.342
Contact positive patients	21 (20.8%)	5 (41.7%)	16 (18.0%)	0.129
Contact with suspected patients in epidemic area	2 (2.0%)	0	2 (2.2%)	1
No clear contact history	3 (3.0%)	2 (16.7%)	1 (1.1%)	0.037
Incubation period, days	5 (4–7.75)	4 (3.25–5.25)	5 (4–7.75)	
Signs and symptoms				
Fever,	77 (76.2%)	10 (83.3%)	67 (75.3%)	0.790
37.3–38.0	36 (35.6%)	5 (41.7%)	31 (34.8%)	0.886
38.1–39.0	27 (26.7%)	4 (33.3%)	23 (25.8%)	0.839
> 39.0	14 (13.9%)	1 (8.3%)	13 (14.6%)	0.884
No fever	13 (12.9%)	0	13 (14.6%)	--
Fever unknown	11 (10.9%)	2 (16.6%)	10 (11.2%)	--
Cough	77 (76.2%)	12 (100.0%)	65 (73.0%)	0.060
Fatigue	23 (22.8%)	3 (25.0%)	20 (22.5%)	0.830
Myalgia	13 (12.9%)	1 (8.3%)	12 (13.5%)	0.960
Pharyngalgia	18 (17.8%)	1 (8.3%)	17 (19.1%)	0.600
Headache	27 (26.7%)	7 (58.3%)	20 (22.5%)	0.020
Retching	10 (9.9%)	2 (16.7%)	8 (9.0%)	0.330
Chest tightness and shortness of breath	15 (14.9%)	3 (25.0%)	12 (13.5%)	0.530
Haemoptysis	0	0	0	--
Diarrhoea	11 (10.9%)	1 (8.3%)	10 (11.2%)	0.840
Fear of cold	11 (10.9%)	2 (16.7%)	9 (10.1%)	0.840
Palpitation	1 (1.0%)	0.0 (0.0%)	1 (1.1%)	1

Data are median number (IQR) or n (%).
COVID-19, coronavirus disease 2019; IQR, interquartile range.
[a]Difference among severe and mild groups.

disease group. Respective spleen volumes in these patients were 89.1 cm^3, 212.0 cm^3, 50.7 cm^3 and 152.6 cm^3 (Figure 6a–d). Spleen volume-to-patient skin) volume ratios were smaller in patients with more severe disease than in those with more mild disease (Figure 7).

The incidence of COVID-19-related complications was 58.3% in patients with more severe disease and 20.2% in patients with more mild disease. Out of the 12 patients with severe disease, nine (75%) developed acute respiratory distress syndrome (ARDS), while there were no cases of ARDS or acute myocardial injury in patients with more mild disease. Incidences of secondary infections, electrolyte disorders and hypoproteinaemia were significantly higher in patients with more severe disease than in those with mild disease ($P < 0.05$). The median hospital stay was 11 days in both groups, with no statistically significant difference ($P < 0.05$; Table 2).

Twenty-four patients experienced tachycardia occurring mainly during the first 2 days of hospitalisation, with a higher heart rate in patients with more severe disease. During the second week of hospitalisation, most patients had

Table 2. Diagnosis and treatment of patients infected with COVID-19

	All patients (n = 101)	Severe patients (n = 12)	Mild patients (n = 89)	P^a
Symptom onset to be diagnosed, days	4.0 (2.5–7.0)	8.0 (6.0–9.0)	4.0 (2.0–7.0)	0.006
Cure time, days	13.0 (10.0–14.5)	13.0 (10.0–18.0)	12.0 (10.0–14.0)	0.640
Remission time, days	5.0 (2.0–6·0)	6.0 (4.0–7.0)	5.0 (2.0–6.0)	0.380
Complications	25 (24.8%)	7 (58.3%)	18 (20.2%)	0.119
Acute respiratory distress syndrome	9 (8.9%)	9 (75%)	0	< 0.001[a]
Acute cardiac injury	2 (2.0%)	2 (16.7%)	0	< 0.001[a]
Acute kidney injury	3 (3.0%)	2 (16.7%)	1 (1.1%)	0.030
Secondary infection	11 (10.9%)	5 (41.7%)	6 (6.7%)	0.002[a]
Shock	3 (3.0%)	3 (25%)	0	0.001[a]
Liver injury	6 (6.0%)	3 (25%)	3 (3.4%)	0.020
Electrolyte disturbance	4 (4.0%)	3 (25%)	1 (1.1%)	0.004[a]
Hypoproteinaemia	10 (9.9%)	8 (66.7%)	2 (2.2%)	< 0.001[a]
Treatment				
Kaletra	85 (84.2%)	6 (50%)	78 (87.6%)	0.004[a]
Kaletra and antiviral therapy	17 (15.8%)	6 (50%)	11 (12.4%)	0.004[a]
Antibiotic therapy	28 (34.4%)	12 (100%)	16 (12.4%)	< 0.001[a]
Use of corticosteroid	11 (10.9%)	9 (75%)	2 (2.2%)	< 0.001[a]
Replacement therapy				
Oxygen support				
Nasal cannula	40 (39.6%)	12 (100%)	28 (31.5%)	< 0.001[a]
Non-invasive ventilation or high-flow nasal cannula	10 (9.9%)	8 (66.7%)	2 (2.2%)	< 0.001[a]
Invasive mechanical ventilation	3 (3.0%)	4 (33.3%)	0	0.001[a]
Prognosis				
Discharge	98 (97.03%)	9 (75%)	89 (100%)	0.001[a]
Length of stay, days	11 (5–20)	13 (8–20)	11 (5–20)	0.350

The measurement data are represented by the median (IQR) and are compared with the Mann–Whitney *U*-test, and the chi-square test is performed for the counting data.

Data are median number (IQR) or *n* (%).

COVID-19, coronavirus disease 2019; IQR, interquartile range.

[a]Difference among severe and mild groups.

recovered from fever; however, tachycardia was still widespread among patients with severe disease (Supplementary figure 1), with some patients experiencing tachycardia even in the absence of fever. Creatine kinase–myocardial band (CK-MB) levels were increased in 10 patients, including in one case whose CK-MB/CK ratio was < 4%, indicating skeletomuscular damage. In another four cases, the CK-MB/CK ratio increase ranged from 4% to 30%, indicating myocardial damage. An elevated troponin I level was also observed in one patient with severe disease. Elevated brain natriuretic peptide (BNP) levels were found in three patients, all of whom had severe disease. There were 16 patients (15.8%) who experienced arrhythmias, including atrioventricular block (6/16), bundle branch block (6/16), premature beat (2/16), atrial escape (1/16) and ST segment depression (1/16), with conduction block being the most common manifestation of this finding.

DISCUSSION

SARS-CoV-2 enters the host cell by binding the S protein on the virus surface to the angiotensin-converting enzyme 2 (ACE2) on the cell surface. In addition to lung expression, ACE2 is expressed widely in other human organs, including the heart, kidneys and digestive organs.[8] The virus is sensed by Toll-like receptor-7 (TLR-7), which is present in the endosome via ACE2.[9,10] Activation of TLR-7 leads to production of tumor necrosis factor-α, α-interferon, interleukin (IL)-12 and IL-6. Normally, this activation leads to the formation of CD8 and CD4 T cells, which results in formation of B cells and antibody production.[11,12] If the immune response is abnormally activated, however, cytokine storm, ARDS and diffuse organ involvement can be induced.[13] The cytokine storm created by SARS-CoV-2 can lead to ARDS and/or multiorgan dysfunction or failure, which threatens the lives of COVID-19 patients.[14,15]

Table 3. Laboratory findings and imageological tests of patients infected with COVID-19

	All patients (n = 101)	Severe patients (n = 12)	Mild patients (n = 89)	P[a]
White blood cell count, × 10^9 L^{-1}	5.1 (3.9–7.0)	5.55 (4.25–7.65)	5.0 (3.9–6.9)	0.588
< 3.5	17 (16.8%)	2 (16.7%)	15 (16.9%)	0.170
3.5–9.5	76 (75.2%)	8 (66.7%)	68 (76.4%)	--
> 9.5	8 (7.9%)	2 (16.7%)	6 (6.7%)	--
Neutrophil count, × 10^9 L^{-1}	3.53 (2.53–4.48)	4.64 (3.30–6.61)	3.47 (2.51–4.36)	0.060
Lymphocyte count, × 10^9 L^{-1}	0.91 (0.67–1.40)	0.59 (0.36–0.66)	0.96 (0.78–1.48)	< 0.001[a]
< 1.1	66 (65.3%)	12 (100%)	54 (60.7%)	0.001
≥ 1.1	35 (34.7%)	0 (0%)	35 (39.3%)	--
Haemoglobin, gL^{-1}	134 (122–152)	129 (121–143)	136 (123–152)	0.600
Platelet count, × 10^9 L^{-1}	179 (140–235)	155 (133–226.5)	184 (144–237)	0.552
< 100	3 (3.0%)	0 (0%)	3 (3.4%)	1
≥ 100	98 (97.0%)	12 (100%)	86 (96.6%)	--
Prothrombin time, s	11.0 (10.4–11.5)	11.75 (11.28–12)	11 (10.2–11.3)	0.001[a]
Activated partial thromboplastin time, s	26.4 (24.7–29.7)	64.05 (29.68–96.13)	26.3 (24.5–28.9)	< 0.001[a]
D-dimer, mgL^{-1}	0.32 (0.19–0.52)	0.82 (0.46–1.32)	0.29 (0.19–0.49)	< 0.001[a]
Albumin, gL^{-1}	40.4 (36.8–42.8)	35.2 (32.55–36.43)	40.9 (37.9–43)	< 0.001[a]
Alanine aminotransferase, UL^{-1}	19.5 (13.8–33.3)	19.5 (10.35–29.25)	19.5 (14.8–35.5)	0.250
Aspartate aminotransferase, UL^{-1}	23.2 (16.8–32)	32.65 (25.38–35.25)	22.4 (16.4–30.3)	0.005
≤ 40	87 (86.1%)	10 (83.3%)	77 (86.5%)	< 0.001[a]
> 40	14 (13.9%)	2 (16.7%)	12 (13.5%)	--
Total bilirubin, mmol L^{-1}	8.8 (5.9–13.7)	10.9 (8.05–14.4)	8.7 (5.7–13.3)	0.270
Potassium, mmol L^{-1}	4.05 (3.75–4.41)	4.07 (3.45–4.32)	4.05 (3.76–4.41)	0.065
Sodium, mmol L^{-1}	138 (137.0–141)	135 (134.75–137)	139 (137.0–141)	< 0.001[a]
Creatinine, μmol L^{-1}	67 (57–75)	67 (56.75–69.75)	67 (57–76)	0.520
≤ 133	101 (100%)	12 (100%)	89 (100%)	--
> 133	0 (0%)	0 (0%)	0 (0%)	--
Creatine kinase, UL^{-1}	57 (39.75–85.25)	60.5 (40.25–141)	57 (39.75–80.25)	0.613
≤ 185	94/100 (94%)	11 (91.7%)	83/88 (94.3%)	0.855
> 185	6/100 (6%)	1 (8.3%)	5/88 (5.7%)	--
Lactate dehydrogenase, UL^{-1}	221 (183–273)	301.5 (262.8–338.8)	216 (182–258)	< 0.001[a]
≤ 245	64 (63.4%)	2 (16.7%)	62 (69.7%)	0.001[a]
> 245	37 (36.6%)	10 (83.3%)	27 (30.3%)	--
Procalcitonin, ng mL^{-1}	0.04 (0.03–0.069)	0.099 (0.0675–0.212)	0.04 (0.029–0.06)	< 0.001[a]
< 0.1	91 (90.1%)	6 (50.0%)	84 (94.4%)	< 0.001[a]
≥ 0.1 to < 0.25	10 (9.9%	5 (41.7%	5 (5.6%	--
≥ 0.5	1 (1.0%)	1 (8.3%)	0 (0.0%)	--
C-reactive protein, μg L^{-1}	10.47 (2.91–49.55)	114.76 (77.57–136.95)	7.80 (2.02–30.73)	< 0.001[a]
CT abnormal	96 (95.0%)	12 (100%)	84 (94.4%)	1
No. of affected CT segments, lobes	3 (2–5)	5 (2–5)	3 (1–5)	0.002[a]
No. of spleen atrophy	28 (27.7%)	6 (50%)	21 (23.6%)	0.003[a]

Data are median number (IQR) or n (%).
COVID-19, coronavirus disease 2019; IQR, interquartile range.
[a]Difference among severe and mild groups.

Analysis of autopsy biopsy specimens has found that an abnormal host immune response and an inflammatory cytokine storm caused by increased alveolar exudates may hinder alveolar gas exchange in COVID-19 patients, perhaps partially accounting for the high mortality of patients with more severe disease.[16] Splenic atrophy has also been reported during pathological examinations of COVID-19 patients;[17,18] however, most changes in spleen volume cannot be measured by pathology directly. Therefore, in this study, we imported patient CT imaging into a Monaco system, delineated the target organ slice by slice, designed the target area for radiotherapy and estimated the volume of the target organ, thereby effectively demonstrating splenic changes.[19-21]

In our study, we identified splenic shrinkage in 27.7% of patients. In addition to comparisons of lymphocyte levels in COVID-19 patients, changes in spleen volumes and ratios of spleen volume to patient (skin) volume may also predict changes in the immune status of patients with mild and more severe disease. The splenic changes we identified were consistent with the changes in lymphocyte levels, perhaps providing a new direction for immune system evaluation.

Lymphopenia was also common in COVID-19 patients in our study. This finding was consistent with study results recently published by Zhong Nanshan's team, suggesting that the virus may cause disease by attacking the immune system.[6] Wan *et al*.[15] found that levels of $CD4^+$ and CD8 T cells were generally lower in patients with severe COVID-19.[22] It has also been suggested that levels of T-cell subsets and cytokines could be used to predict the transition from mild to more severe disease. In our study, 65.3% of patients with lymphocytopenia demonstrated a return to normal levels after treatment; however, 27.3% still had low levels of lymphocytes. These patients remain at risk for reinfection with COVID-19 and other infections and therefore will require close follow-up and early intervention.

The American College of Cardiology (ACC) studied early cases of COVID-19 and found that 40% of hospitalised patients diagnosed with COVID-19 had a history of CVD and/or cerebrovascular disease.[23] Therefore, patients with potential CVD may have an increased risk of infection, and for those already diagnosed with CVD, preventive measures should be undertaken in advance. In our study, we found that middle-aged and elderly men with diabetes or CVD were more susceptible to SARS-CoV-2 infection, with a higher proportion of severe cases complicated by diabetes or CVD than mild cases.[3,24,25]

To investigate the occurrence of myocardial injury during disease progression, we retrospectively analysed heart rate, myocardial enzyme levels, troponin I, BNP, electrocardiogram and colour Doppler ultrasound data of 101 patients obtained during their hospitalisations. The serum myocardial enzyme spectrum, which is an important indicator for diagnosis of myocardial injury, includes CK, CK-MB and LDH, of which CK-MB has the highest sensitivity and specificity in clinical practice. The CK-MB/CK ratios in our patients ranged from 4% to 30%. We observed four cases of myocardial damage and one case of a patient with severe disease who had a prior history of coronary heart disease. The patient with myocardial damage in the severe group had normal myocardial enzyme levels and electrocardiogram findings at admission; however, higher CK-MB levels were noted 3 days after admission, with a CK-MB/CK ratio greater than 30%. Troponin I, CK and CK-MB levels rose obviously during the sixth day of hospitalisation. Three patients with mild COVID-19 disease and without a history of CVD showed mild increases in CK-MB within 4 days of hospitalisation. In our study, the myocardial injury rate was 3.58% (4/101). Our 101 patients had mostly mild disease; therefore, most did not receive dynamic monitoring of troponin I levels, electrocardiograms or colour Doppler ultrasounds. Accordingly, we may have underestimated the incidence of myocardial injury in our sample. As has been reported, even if COVID-19 patients do not have a history of CVD, they may experience myocardial damage and therefore require vigilant monitoring on our part, since cardiovascular complications in these patients can prove fatal.[26,27,28]

While hypertension, diabetes mellitus, CVD and other comorbidities may lead to an increased risk of mortality in patients with COVID-19, immunosenescence may also play an important role. With increased age, the number of original T cells is reduced, and memory T cells with antigen experience constitute a significant proportion of total T cells.[29,30] In the elderly, on the one hand, the immune system's ability to respond to previously exposed pathogens is strong. In children, on the other hand, larger proportions of naive T cells are present and ready to respond to new pathogens. These differences may explain

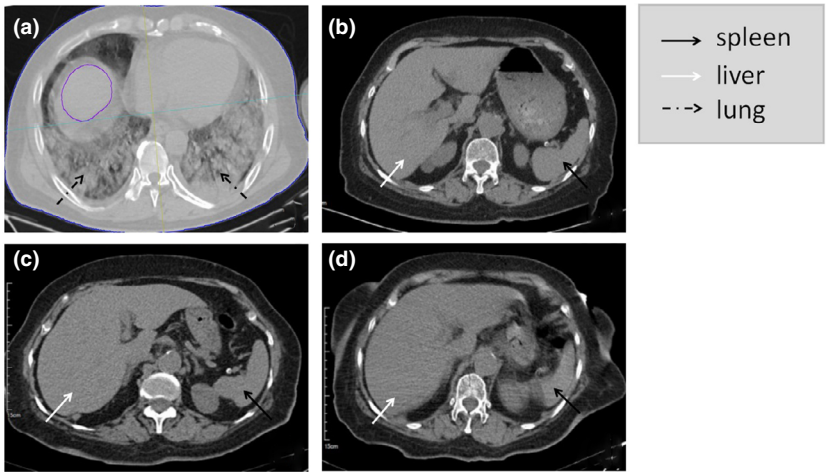

Figure 2. Computed tomography imaging changes in the spleen of Patient No. 1, a 79-year-old woman. **(a)** Pulmonary window on the day of admission, **(b)** transverse abdominal CT on the day of admission, **(c)** transverse abdominal CT on day 7 of her hospitalisation and **(d)** transverse abdominal CT on the day of death showing marked shrinkage of the spleen.

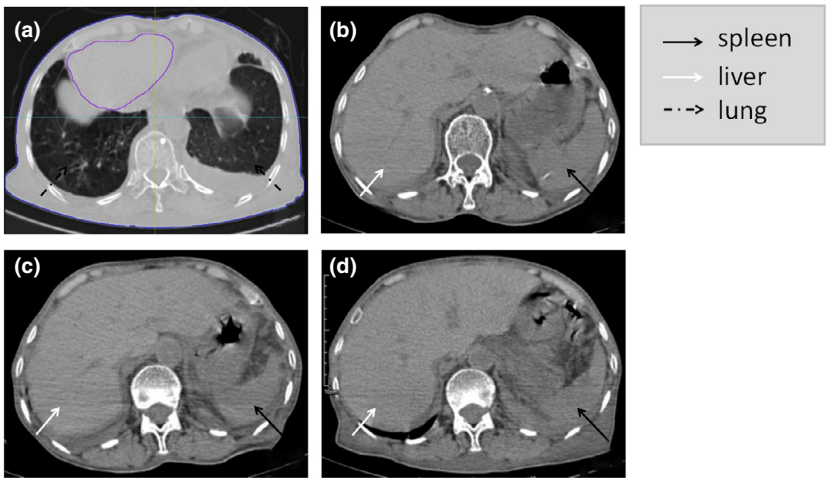

Figure 3. Computed tomography (CT) imaging changes in the spleen and liver of Patient No. 2, a 52-year-old man. **(a)** Pulmonary window on the day of admission, **(b)** transverse abdominal CT on the day of admission, **(c)** transverse abdominal CT on day 7 of his hospitalisation and **(d)** transverse abdominal CT on the day of death showing shrinkage of the spleen and liver.

why children have a lower COVID-19 incidence and related mortality than the elderly. We could therefore paralyse the cytokine cell-mediated antiviral response during cytokine storm by increasing the expression of programmed death 1 (PD-1).[31] However, thus far, our efforts to prevent the spread of COVID-19 have been inadequate.

In our study, most patients with COVID-19 received supportive therapy combined with antiviral therapy.[32] Our hospital adopted lopinavir/ritonavir (Kaletra, AbbVie, Inc., Lake Bluff, Illinois, USA) for treatment of confirmed cases of COVID-19 in accordance with the fifth edition of China's national treatment guidelines. This medication, containing protease inhibitors used to treat human immunodeficiency virus infection, has certain value in the treatment of SARS.[7,33] However, whether lopinavir/ritonavir plays a definitive role in the treatment of COVID-19 still requires further study.[34] Interestingly, COVID-19 patients treated at our hospital also opted for treatment with traditional Chinese medicine (TCM) at a rate of up to 91.3%.

IgG immunotherapy can also be used to neutralise the virus that causes COVID-19.[35] There is evidence that ageing is associated with

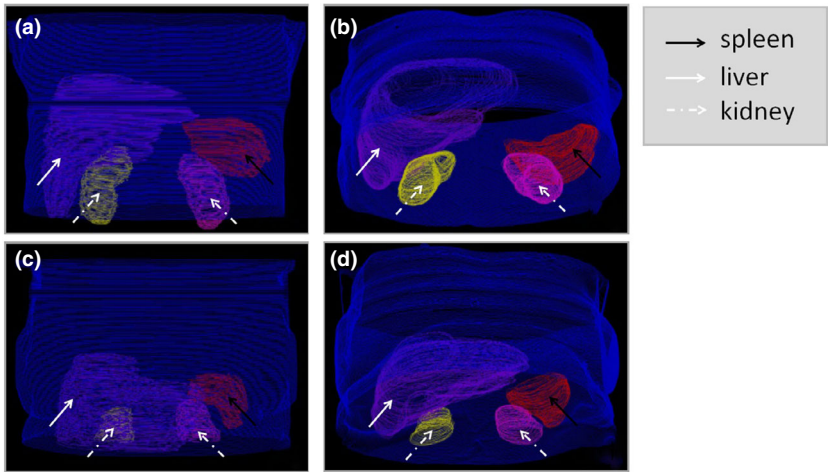

Figure 4. Three-dimensional view of the liver, spleen and kidney in deceased Patient No. 1 **(a, b)** and deceased Patient No. 2 **(c, d)**. **(a)** 3D positive view based on CT scan of the beginning of the liver; **(b)** 3D antapical view showing elongation and flattening of the spleen and liver, **(c)** 3D positive view based on CT scan of the beginning of the spleen and liver and **(d)** 3D antapical view showing the irregular shape and shrinkage of the spleen and liver.

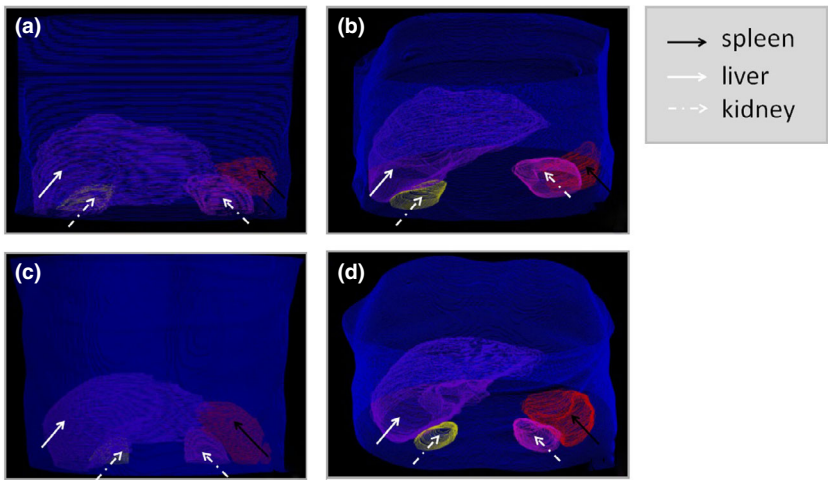

Figure 5. Three-dimensional view of the liver, spleen and kidney in deceased Patient No. 3 **(a, b)** and recovered Patient No. 4 **(c, d)**. **(a)** 3D positive view based on CT scan of the beginning of liver, **(b)** 3D antapical view showing shrinkage and flattening of the spleen on CT scan, **(c)** 3D positive view based on CT scan of the beginning of spleen and liver and **(d)** 3D antapical view showing a round and full spleen and liver.

impaired cytokine regulation and reduced T-cell banks. In addition, male gender is associated with relatively reduced antiviral immunity, and comorbidities associated with COVID-19 are associated with excessive inflammation.[14] Patients may also be more likely to develop severe SARS-CoV-2 infection if they have congenital abnormalities of the immune system.[36] In particular, abnormalities in proinflammatory cytokines, type I interferons and interferon-stimulated genes may contribute to development of cytokine storm. The current evidence suggests that this novel coronavirus inhibits the immune response to viruses and has a powerful ability to replicate in host cells.[37] There is no doubt that future research should focus on the development of an active immunisation vaccine against SARS-CoV-2.[14]

It has been demonstrated that pathogenic T cells and inflammatory monocytes induce inflammatory storms through high levels of IL-6; therefore, monoclonal antibodies targeting the IL-

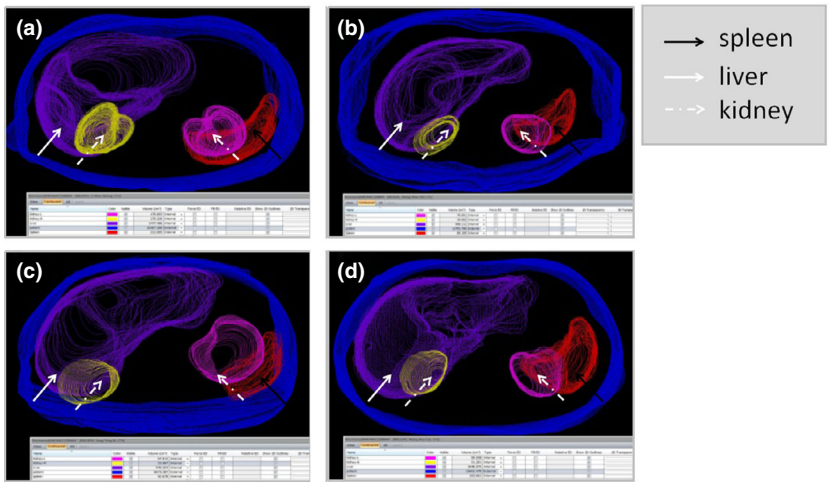

Figure 6. Spleen volumes. The contour of the liver, spleen, kidney and skin of all patients was delineated from the thoracic entrance to the lower pole plane of bilateral kidneys according to the Monaco software, and the volumes of the liver, spleen and kidneys were calculated. The spleen volumes were **(a)** 89.1 cm^3 in Patient No. 1, **(b)** 212.0 cm^3 in Patient No. 2, **(c)** 50.7 cm^3 in Patient No. 3 and **(d)** 152.6 cm^3 in Patient No. 4.

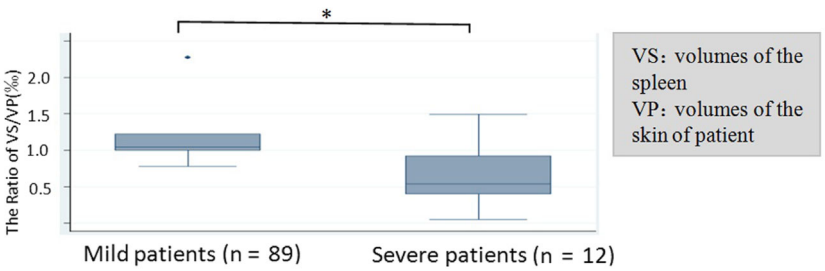

Figure 7. Ratio of spleen volume (VS) to patient volume (VP). Spleen and patient (skin) volumes were directly calculated using the volume estimation function of the software after outlines of the spleen and patient (skin) were delineated. The skin volume was delineated from the level of the carina to the lower pole of the right kidney. The ratio of spleen volume to patient (skin) volume was then calculated. Significant differences were observed between patients with mild disease (n = 89) and patients with more severe disease (n = 12). * P-value < 0.05.

6 pathway may inhibit inflammatory storms.[38] Research has also shown that intravenous administration of mesenchymal stem cells (MSCs) significantly improved inflammation in patients with severe COVID-19. As a result of the unique immunosuppressive capacity of MSCs, serum levels of proinflammatory cytokines and chemokines were significantly reduced after their administration, thus reducing monocytes and macrophages in the fragile lung.[39,40]

In conclusion, our study suggests that infection with SARS-CoV-2 tends to affect not only the respiratory system, but also the immune and cardiovascular systems. Therefore, patients with COVID-19 must be carefully monitored for immune system damage and cardiac events. Follow-up CT imaging and regular monitoring of lymphocyte counts, ECGs and myocardial enzymes may be appropriate clinical strategies for patients with COVID-19 pneumonia.

METHODS

Study design

Chongqing University Three Gorges Hospital is located in Northeast Chongqing, an endemic area for COVID-19. It is a teaching hospital for nine medical colleges and universities and is responsible for treating COVID-19 patients assigned to it by the government. As of 15 February 2020, 228 patients had been diagnosed with COVID-19 pneumonia

(confirmed by real-time polymerase chain reaction) at our institution. Because some of these patients had not been diagnosed at our hospital, we could not obtain peripheral blood collection results and chest CT scans from different time points in all patients. Therefore, only 101 patients were enrolled in this study. Relevant measurements were performed in these patients during their hospital stays, and we retrospectively analysed the results. Of the 101 patients, only 12 were treated in the ICU. Three patients did not experience clinical symptoms despite positivity by nucleic acid testing. We analysed a variety of relevant data, including demographics, clinical manifestations, examination results, diagnoses, treatments, lengths of stay, medical expenses and, most relevantly, changes in the immune and cardiovascular systems. This case series was approved by the Institutional Ethics Board of CTGU Hospital (No. 2020-2).

Case collection

We accessed the medical records, laboratory testing results, nursing records, imaging results and some paper records of the 101 study patients through the hospital data system. The data were collected into an Excel spreadsheet after being checked by two persons. We divided patients into severe or mild disease according to their discharge diagnoses. Patients were considered to have mild disease if their diagnoses were mild or no pneumonia. Patients were considered to have severe disease if they were diagnosed with dyspnoea, with a respiratory rate of ≥ 30 breaths per minute, a blood oxygen saturation of $\leq 93\%$, a ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2) of < 300, and/or evidence of lung infiltration of $> 50\%$ within 24 to 48 h of symptom onset. The criteria for COVID-19 pneumonia diagnosis and treatment plan were announced by the General Office of China National Health Commission.[15,17]

Use of radiotherapy target contour to evaluate spleen volume in patients

Splenic atrophy is a pathological finding that has been reported in some patients with COVID-19.[17,18] Splenic volume changes, however, cannot adequately be measured by pathological analysis in most patients; therefore, we used a Monaco system in this study, which is generally used to show splenic changes during radiotherapy planning.[19-21] We imported CT scans of selected patients into the Monaco treatment planning system and delineated the outline of the spleen, with a CT slice thickness of 5 mm. Spleen and patient (skin) volumes were directly calculated using the volume estimation function of the software after outlines of the spleen and patient (skin) were delineated. The skin volume was delineated from the level of the carina to the lower pole of the right kidney. Average values for spleen and patient (skin) volumes were recorded after repeating measurements three times. Subsequently, ratios of spleen volume to patient (skin) volume were calculated, and the IQRs of these ratios in the mild and severe group were compared using the Mann–Whitney *U*-test.

Patient involvement

No patients were involved in identifying the research question or the outcome measures. We did not ask patients for advice on the interpretation or the writing up of our results.

Statistical analysis

Continuous variables were expressed as medians with IQRs and were compared using the Mann–Whitney *U*-test. Categorical variables were expressed as numbers (%) and were compared between patients with severe or mild disease using the chi-square test or Fisher's exact test. We performed all statistical analyses using GraphPad Prism software version 6 (GraphPad Software, Inc., San Diego, California, USA). A P-value < 0.05 was considered to be statistically significant.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Hejing Bao: Conceptualization; Data curation; Resources; Writing-original draft; Writing-review & editing. **Gang Li:** Funding acquisition; Project administration; Supervision; Visualization. **Yinhua Fang:** Formal analysis; Methodology; Software; Writing-original draft. **Qin Lai:** Data curation; Investigation; Writing-original draft. **Hehong Bao:** Resources; Validation; Visualization. **Yu Zheng:** Methodology; Visualization; Writing-review & editing. **Yanjun Hu:** Conceptualization; Resources; Supervision.

REFERENCES

1. Pro M.E.D. PRO/AH/EDR>Undiagnosed pneumonia - China (HU): RFI. ProMED-mail. 2020 [cited 31 January]. Available from: https://promedmail.org/promedpost/?id=20191230.6864153.
2. 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**: 1054–1062.
3. 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.

4. Zhou P, Yang XL, Wang XG *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**: 270–273.
5. Hui DS, I Azhar E, Madani TA *et al*. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health-the latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis* 2019; **2020**: 264–246.
6. Zhu N, Zhang D, Wang W *et al*. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727–733.
7. Center for Disease Control and Prevention. 2019 novel coronavirus, Wuhan, China. 2020 [cited 24 January]. Available from: https://www.cdc.gov/coronavirus/COVID-19/about/transmission.html.
8. Jawhara S. Could intravenous immunoglobulin collected from recovered coronavirus patients protect against COVID-19 and strengthen the immune system of new patients? *Int J Mol Sci* 2020; **21**: 2272.
9. Saghazadeh A, Rezaei N. Immune-epidemiological parameters of the novel coronavirus - a perspective. *Expert Rev Clin Immunol* 2020; **16**: 465–470.
10. Totura AL, Baric RS. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. *Curr Opin Virol* 2012; **2**: 264–275.
11. Hamming I, Timens W, Bulthuis MLC *et al*. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631–637.
12. Leng ZK, Zhu RJ, Hou WF *et al*. Transplantation of ACE2-mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging Dis* 2020; **11**: 216–218.
13. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J *et al*. Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. *J Virol* 2006; **80**: 5927–5940.
14. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA* 2020; **323**: 1775–1776.
15. Wan SX, Yi QJ, Fan SB *et al*. Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. *Br J Haematol* 2020; **189**: 428–437.
16. Braun E, Sauter D. Furin-mediated protein processing in infectious diseases and cancer. *Clin Transl Immunol* 2019; **8**: e1073.
17. Office of the State Administration of Traditional Chinese Medicine, General Office of the National Health Commission. COVID-19 pneumonia treatment plan (trial version 7). 2020 [updated 4 March 2020; cited 20 March 2020]. Available from: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml.
18. Xu Z, Shi L, Wang Y *et al*. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420–422.
19. Yohanathan L, Loveday BPT, Brar N *et al*. Effect of vessel preservation on splenic volume and function in patients with spleen preserving distal pancreatectomies. *HPB (Oxford)* 2020.
20. Chediak AE, Haydar AA, Hakim A *et al*. Increase in spleen volume as a predictor of oxaliplatin toxicity. *Ther Clin Risk Manag* 2018; **14**: 653–657.
21. Son JH, Lee SS, Lee Y *et al*. Assessment of liver fibrosis severity using computed tomography–based liver and spleen volumetric indices in patients with chronic liver disease. *Eur Radiol* 2020; **30**: 3486–3496.
22. Ritchie AI, Singanayagam A. Immunosuppression for hyperinflammation in COVID-19: a double-edged sword? *Lancet* 2020; **395**: 1111.
23. Guan WJ, Ni ZY, Hu Y *et al*. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020; **382**: 1708–1720.
24. Chen N, Zhou M, Dong X *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507–513.
25. Li Q, Guan X, Wu P *et al*. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* 2020; **382**: 1199–1207.
26. Batlle D, Wysocki J, Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clin Sci (Lond)* 2020; **134**: 543–545.
27. Nakagawa S, Inoue S, Kryukov K *et al*. Rapid sequencing-based diagnosis of infectious bacterial species from meningitis patients in Zambia. *Clin Transl Immunol* 2019; **8**: e1087.
28. Thompson RN. Novel coronavirus outbreak in Wuhan, China, 2020: intense surveillance is vital for preventing sustained transmission in new locations. *J Clin Med* 2020; **9**: 498.
29. Fung SY, Yuen KS, Ye ZW, Chan CP, Jin DY. A tug-of-war between severe acute respiratory syndrome coronavirus 2 and host antiviral defence: lessons from other pathogenic viruses. *Emerg Microbes Infect* 2020; **9**: 558–570.
30. Jiankun Z, Chandra C. Toll-Like receptor signaling pathways–therapeutic opportunities. *Mediators Inflamm* 2010; **2010**: 781235.
31. Zhou Y, He C, Wang L, Ge B. Post-translational regulation of antiviral innate signaling. *Eur J Immunol* 2017; **47**: 1414–1426.
32. Chan JFW, Yuan S, Kok KH *et al*. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020; **395**: 514–523.
33. Feldmann M, Maini RN, Woody JN *et al*. Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed. *Lancet* 2020; **395**: 1407–1409.
34. Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): A Review. *JAMA* 2020; **323**: 1824–1836.
35. Teijaro JR, Walsh KB, Rice S, Rosen H, Oldstone MBA. Mapping the innate signaling cascade essential for cytokine storm during influenza virus infection. *Proc Natl Acad Sci USA* 2014; **111**: 3799–3804.

36. Heaton SM. Frontiers in antiviral therapy and immunotherapy. *Clin Transl Immunol* 2020; **9**: e1115.
37. Weiskopf D, Weinberger B, Grubeck-Loebenstein B. The aging of the immune system. *Transplant Int* 2009; **22**: 1041–1050.
38. Ahmadpoor P, Rostaing L. Why the immune system fails to mount an adaptive immune response to a Covid-19 infection. *Transpl Int* 2020; **33**: 824–825.
39. Velazquez-Salinas L, Verdugo-Rodriguez A, Rodriguez LL, Borca MV. The role of interleukin 6 during viral infections. *Front Microbiol* 2019; **10**: 1057.
40. Heaton SM. Harnessing host–virus evolution in antiviral therapy and immunotherapy. *Clin Transl Immunol* 2019; **8**: e1067.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.