Systemic Inflammatory Cytokines Associate With SARS-COV-2 Viral Shedding Time in Covid-19 Inpatients

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Research

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Abstract

Background: Since December 2019, coronavirus disease 2019 (COVID-19), as an infectious disease with cytokine storm, has become an emerging global challenge. To assess the duration of SARS-CoV-2 viral shedding and associated risk factors in COVID-19 patients.

Methods: COVID-19 patients with interleukin (IL)-1b, soluble interleukin-2 receptor (sIL-2R), IL-6, IL-8, IL-10 and tumor necrosis factor (TNF)- α cytokines data consecutively admitted to Tongji Hospital from January 27, 2020 through February 5, 2020 were enrolled and been followed up until March 24, 2020. We utilized Kaplan-Meier method and Cox proportional hazards regression analysis to assess the duration of viral shedding and risk factors affecting virus clearance.

Results: 246 inpatients with laboratory confirmed COVID-19 were enrolled. The median duration of viral shedding was 24 days, ranging from 6 to 63 days. Age, severity of COVID-19, albumin, lactate dehydrogenase (LDH), D-dimer, ferritin and sIL-2R were associated with duration of viral shedding. Administration of lopinavir-ritonavir, arbidol, oseltamivir and intravenous immunoglobulin did not shorten viral shedding time. Multivariate cox regression analysis revealed that sIL-2R, LDH and severity of COVID-19 were independent factors associated with duration of viral shedding. At stratified analysis, the viral shedding time was positively correlated with age, sIL-2R and LDH in non-corticosteroid subgroup, while negatively correlated with lymphocyte count in corticosteroid group.

Conclusions: The present study demonstrated that elevated sIL-2R, increased LDH and severe status were related to prolongation of viral shedding in COVID-19 inpatients. Further research is urgent to investigate the mechanism of immune reaction involved in the virus clearance process and aim to the optimal antiviral therapy.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), presents a global challenge. Up to May 4, 2020, more than 3 million confirmed cases of COVID-19 have been reported, of which more than 239,000 have died.¹ As SARS-CoV-2 is an emerging virus, there is no effective anti-viral drug and vaccine to treat this disease. Supportive therapy is the main pattern. Whether corticosteroids are beneficial or lead to delayed virus shedding remains controversial. Previous studies found that the SARS-CoV-2 persist longer with higher load in severe patients.² Our previous studies found that high cytokine levels, such as soluble interleukin-2 receptor (sIL-2R), interleukin (IL)-6, IL-10 and tumor necrosis factor (TNF)- α were significantly associated with severe COVID-19.³ The inflammatory storm caused by SARS-CoV-2 plays an important role in the deterioration of the disease.⁴ However, the association between duration of viral shedding and inflammatory factors was limited.

The assessment of risk factors for delayed virus shedding will benefit the treatment and management of COVID-19. The goal of the present study was to conduct a large population-based study to describe the

data for duration of viral shedding, and assess the association between duration of viral shedding and relevant characteristics, including age, gender, smoking history, severity of the disease, comorbidities, inflammatory factors and treatment method.

Methods

Study design and participants

This study is an observational study of hospitalized patients with COVID-19 enrolled at Sino-French New City Branch of Tongji Hospital, Huazhong University of Science and Technology in Wuhan January 27, 2020 through February 5, 2020. Tongji hospital, HUST is one of the major nationally designated hospitals for COVID-19 in Wuhan. COVID-19 was diagnosed according to WHO interim guidance.⁵ Only the cases with complete cytokines data, including IL-1b, sIL-2R, IL-6, IL-8, IL-10 and TNF- α , were included in the analysis. Patients were divided into severe COVID-19 group and non-severe COVID-19 group according to 2019 clinical practice guideline from IDSA and ATS for diagnosis and treatment of adults with community-acquired pneumonia.⁶ Laboratory confirmed case of COVID-19 was defined as a positive result to next-generation sequencing or real-time reverse-transcriptase polymerase chain reaction assay for nasopharyngeal or pharyngeal swab specimens.⁷ Patients were followed up from the date of admission to March 24, 2020. Viral shedding was defined as SARS-CoV-2 nucleic acid test turned negative for two consecutive times (minimum 24h sampling interval). Duration of viral shedding was defined as the duration from illness onset to the date of first negative result of viral shedding. This study was approved by Institutional Review Board of Tongji Hospital, Huazhong University of Science and Technology. Written informed consent was waived owing to the rapid emergence of this infectious disease.

Data collection

The demographic data were obtained by face-to-face or telephone interview. Laboratory examination at admission, treatment during hospitalization and outcome data were extracted from electronic medical records. Patient data were cross-checked for consistency before final data entry and then entered into a computerized database.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR). Categorical variables were expressed as number (%). Survival curves were performed by Kaplan-Meier method and validated by Log-rank, Breslow and Tarone-Ware tests. The time-dependent ROC curve analysis was conducted and the area under the curve (AUC) value was used to evaluate the predictive effect of laboratory findings. Adjusted hazard ratios (HRs) along with 95% confidence intervals (CIs) were calculated by using the Cox proportional hazards regression model. Age, diabetes, lymphocyte count, albumin, sIL-2R and usage of systemic corticosteroids were chosen for multivariate cox regression analysis on the basis of univariate cox regression analysis and clinical significance. The correlation between duration of viral shedding and

age, sIL-2R, LDH and lymphocyte count was analyzed by Spearman's rank correlation. When the P value was <0.05, the difference was regarded as statistically significant. All statistical tests were two tailed. Overall statistical analysis was performed by the software statistical package for social science version 23.0 (SPSS Inc., Chicago, IL, USA) and R software for Windows, version 3.6.1.

Results

Patient Characteristics

A total of 246 patients with COVID-19 were enrolled January 27, 2020 through February 5, 2020. Median age of the patients was 58 years (IQR 47-67). 126 (51.2%) patients were male. 22 (9.0%) patients were current smokers. 72 (29.3%) patients had hypertension, 45 (18.3%) patients had diabetes. 16 (6.5%) patients had coronary heart disease. 16 (6.5%) patients had tumor or impaired immune system disease. 14 (5.7%) patients had underlying respiratory disease.

118 (48.0%) patients were classified as severe cases and 128 (52.0%) patients were categorized into non-severe cases. Concentrations of lactate dehydrogenase (LDH) were increased in 163 (67.4%) of 242 patients. 95 (40.9%) of 232 patients had D-dimer over 1mg/L. Levels of erythrocyte sedimentation rate were increased in 175 (73.5%) of 238 patients. 201 (82.4%) of 244 patients had high-sensitive C-reactive protein concentrations greater than 10 mg/L. 20 (9.3%) of 215 patients had procalcitonin over 0.5 ng/mL. 179 (75.5%) of 237 patients had ferritin levels greater than 400mg/L. Serum levels of IL-1b, sIL-2R, IL-6, IL-8, IL-10 and TNF- α were increased in 16.7%, 52.8%, 70.3%, 6.5%, 28.0% and 59.3% patients, respectively.

113 (45.9%) of 246 patients were treated with low dosage (prednisone-equivalent dose of less than 1mg/Kg per day) of systemic corticosteroids, 32 (13.0%) of 246 patients were given high dosage (prednisone-equivalent dose of at least 1mg/Kg per day) of systemic corticosteroids. Demographic and clinical characteristics of patients in this study were listed in Table 1 in the Online Repository.

Viral shedding time

By March 24, 2020, 215 (87.4%) patients were recorded with the date when the SARS-CoV-2 nucleic acid test turned negative. Two (0.8%) patients lost follow-up after transferred to other medical centers. 26 (10.6%) patients died before the virus turned negative. 2 (0.8%) patients could not have the SARS-CoV-2 nucleic acid test because of their critically ill condition. The mean follow-up period is 53 (49-58) days.

The overall median duration of viral shedding was 24 days. The shortest duration of viral shedding was 6 days from illness onset, whereas the longest one was 63 days. One patient was persistent positive until the end of follow-up date.

By the analysis of Kaplan-Meier method, the severity of disease, old age, low albumin level, high LDH, high D-dimer, high ferritin, high sIL-2R and low lymphocyte count were associated with longer duration of viral shedding time (Table 2, Figure 1). Gender, smoking history, comorbidity, and administration of

lopinavir-ritonavir, arbidol, oseltamivir and intravenous immunoglobulin were not associated with duration of viral shedding (Table 2).

The median duration of viral shedding was 24 days in patients without systemic corticosteroids, 23 days in patients treated with low dose (prednisone-equivalent dose of less than 1mg/Kg per day) and 27 days in patients administrated with high dose (prednisone-equivalent dose of at least 1mg/Kg per day) systemic corticosteroids. Low dose of systemic corticosteroids did not prolong viral shedding compared with no use of systemic corticosteroids (Log-rank, $P=0.986$; Breslow, $P=0.677$; Tarone-Ware, $P=0.822$). High dose of systemic corticosteroids use may associate with prolonged viral shedding compared with no use of systemic corticosteroids (Log-rank, $P=0.023$; Breslow, $P=0.015$; Tarone-Ware, $P=0.014$).

Time-dependent ROC curve analysis

The time-dependent ROC curve analysis was conducted and the AUC value was used to evaluate the predictive effect of albumin, LDH, D-dimer, ferritin, sIL-2R. As shown in Figure 2, the AUC for albumin was 0.646, 0.708 and 0.665 at 20, 40, and 60 days, respectively; the AUC for LDH was 0.627, 0.747 and 0.762 at 20, 40, and 60 days, respectively; the AUC for D-dimer was 0.648, 0.714 and 0.710 at 20, 40, and 60 days, respectively; the AUC for ferritin was 0.628, 0.652 and 0.683 at 20, 40, and 60 days, respectively; the AUC for sIL-2R was 0.658, 0.660 and 0.710 at 20, 40, and 60 days, respectively.

Univariate and multivariate cox regression analysis

As shown in Table 3, age, severity of COVID-19, albumin, LDH, D-dimer, ferritin, sIL-2R and administration of high dose systemic corticosteroids were significantly associated with duration of viral shedding in univariate cox regression analysis ($P<0.05$). Ten variables, including age, severity of COVID-19, diabetes, lymphocyte count, albumin, LDH, D-dimer, ferritin, sIL-2R and usage of systemic corticosteroids were chosen in multivariate cox regression analysis on the basis of univariate cox regression analysis and clinical significance. In the final model, sIL-2R, LDH and severity of COVID-19 were independent factors associated with duration of viral shedding. sIL-2R >710 U/mL (HR=0.719; $P=0.031$; 95%CI 0.533-0.971), LDH >250 U/L (HR=0.691; $P=0.022$; 95%CI 0.503-0.949) and severe status of COVID-19 (HR=0.712; $P=0.032$; 95%CI 0.522-0.971) were related to prolongation of viral shedding (Table 3).

Stratified analysis

According to whether systemic corticosteroid was used, the correlation between duration of viral shedding and age, sIL-2R, LDH and lymphocyte count was stratified analyzed. As shown in Figure 3, when systemic corticosteroid was not used, duration of viral shedding was positively correlated with age ($r_s=0.316$, $P=0.002$), sIL-2R ($r_s=0.259$, $P=0.012$) and LDH ($r_s=0.290$, $P=0.005$), but not lymphocyte count ($r_s=-0.069$, $P=0.508$). When systemic corticosteroid was used, duration of viral shedding was positively correlated with sIL-2R ($r_s=0.193$, $P=0.033$) and negatively with lymphocyte count ($r_s=-0.184$, $P=0.044$), but not age ($r_s=-0.034$, $P=0.714$) and LDH ($r_s=0.165$, $P=0.074$).

Discussion

Little do we know about the duration of viral shedding of COVID-19, a novel infectious disease. As far as we know, this study proposed the longest follow-up duration of the viral shedding in COVID-19. Our result revealed that the viral shedding time ranged from 6 to 63 days, which was wider than previous study in Wuhan with a range of 8 to 37 days⁸ and another study performed in Shanghai with a range of 2 to 22 days.⁹ The longer duration of viral shedding time in this study may result from a higher severity of the patients recruited in Tongji hospital and a longer duration of follow-up time.

In univariate cox regression analysis, age was significantly associated with duration of viral shedding. Elderly patients experience a marked decline of immune function. The age-dependent defects in T-cell and B-cell function and the excess production of type 2 cytokines could lead to a deficiency in control of viral replication.^{8,10} Diabetes was the most common comorbidity in elderly patients with COVID-19. Previous study found that COVID-19 patients with diabetes were at higher risk of severe pneumonia, release of tissue injury-related enzymes, excessive uncontrolled inflammation response, hypercoagulable state and rapid deterioration.¹¹ However, there was no difference in viral shedding time between patients with or without diabetes in our study. Larger sample studies are needed to explore virus clearance ability of patients with diabetes. In the present study, although the P value of Log-rank test is greater than 0.05, but the P values of Breslow and Tarone-Ware tests were less than 0.05, lymphocyte count $< 0.5 \times 10^9/L$ may be associated with delayed virus shedding. Lymphocytes play a crucial role in immune system. Lymphopenia was commonly observed in severe COVID-19 illness.⁸ Analyze of lymphocyte subsets demonstrated that absolute numbers of T lymphocytes, CD4+ and CD8+ T cells decrease in nearly all the patients, and were markedly lower in severe cases.¹²

Corticosteroids could suppress cytokine storm of COVID-19, but also inhibit immune response and holds the possibility to delay viral clearance. In severe acute respiratory syndrome and Middle East respiratory syndrome, corticosteroids were not routinely recommended.¹³ Whether corticosteroids are beneficial or lead to poor clinical outcomes in COVID-19 remains controversial. Our study revealed that for the comparison between high dose systemic corticosteroids and no use of systemic corticosteroids, although the P value of Log-rank test is greater than 0.017, the P values of Breslow and Tarone-Ware tests were less than 0.017. High dose systemic corticosteroids may associate with prolonged viral shedding compared with no use of systemic corticosteroid. This may due to the impaired immune response aggravated by high-dose systemic corticosteroids, but also could be an indicator of severe condition when high dose corticosteroid would be used. Further research is needed to clarify the role of systemic corticosteroid in viral shedding of COVID-19. The present study also identified that administration of lopinavir-ritonavir did not shorten viral shedding time (Log Rank, $P > 0.05$; Breslow, $P > 0.05$; Tarone-Ware, $P > 0.05$). Our result was consistent with previous clinical trial that the percentage of patients with detectable viral RNA for SARS-CoV-2 at various time points were similar between the lopinavir-ritonavir group and the standard-care group.¹⁴ Oseltamivir is an antiviral used to treat influenza virus. December and January were the high incidence period of influenza in Wuhan previously. Co-infected with influenza

virus was likely existed in COVID-19 patients. Although oseltamivir was prescribed to 39% patients, our result showed that oseltamivir did not affect duration of viral shedding. Thus, we speculate that the seasonal influenza may not affect the viral shedding of SARS-Cov-2. Arbidol is a broad-spectrum antiviral, which can inhibit numerous enveloped or non-enveloped RNA or DNA viruses. Previous study showed arbidol treatment potentially improved discharging rate and decrease the mortality rate of COVID-19.¹⁵ While, this study indicated that arbidol did not shorten duration of viral shedding. High quality clinical research is needed to verify the clinical efficacy of arbidol on COVID-19 patients.

In our study, virus shedding was delay in severe patients. Previous study suggests high viral load is associated with COVID-19 severity.¹⁶ The increased viral load may contribute to the longer viral shedding in severe cases of COVID-19. The other reason may due to the impaired immune since the severe COVID patients presented with significantly decreased lymphocyte. In previous studies, continuously decrease of serum albumin is observed in critical cases, which was associated with poor clinical outcome in hospitalized patients.¹⁷ Albumin can reflect the basic nutrition status. Over consumption of COVID-19 and inadequate nutrition intake may lead to hypoalbuminemia. This study demonstrated low level albumin associated with delayed viral shedding in consistent with previous study finding that albumin level was significantly correlated with SARS-CoV-2 viral load.¹⁶ Therefore, nutrition support is very important in the healthcare of COVID-19. LDH was a glycolytic enzyme, which widely exist in many organs and tissues, especially in fast growing tumors. LDH was often regarded as an indicator of organ injury and tumor burden.¹⁸ Previous studies found that high level LDH were common in severe cases and correlated with severe acute lung injury.^{12,16} The present study revealed that high level LDH was associated with prolongation of viral shedding time, which may be due to the severe lung injury. Our study also found that elevated D-dimer level was associated with delayed viral shedding. Serum D-dimer can reflect fibrinolytic activities and was also an inflammatory biomarker.¹⁹ Previous studies found that severe cases were commonly complicated with coagulopathy, markedly elevated D-dimer was related to poor prognosis of severe COVID-19.²⁰

Inflammatory cytokines would be highly associated with severity and immune status of COVID-19. The present study indicated that increased levels of ferritin and sIL-2R were related to prolongation of viral shedding time. Hyperferritin, a marker of macrophage activation, reflect the activation of reticuloendothelial system.²¹ As an acute-phase reactant, ferritin level can be used as an indicator to reflect the degree of inflammation of inpatients with COVID-19. IL-2R is composed of three subunits: α , β , and γ_c . While β and γ_c subunits are constitutively active, the α subunit is induced and expressed only after mononuclear cell activation.^{22,23} The sIL-2R is generated in “activation-dependent” manner, it is cleaved from IL-2R α protein once T cells are activated.^{22,23} Therefore, sIL-2R is used as a classical hallmark of T cell activation.²³ It can also be released from activated B cells, monocytes and dendritic cells.²³ Significant increase of sIL-2R are seen in autoimmune disease, organ transplant rejection and in many T cell and B cell neoplasms.²⁴ In autoimmune disease, sIL-2R level associated with disease activity and reflects immune system activation.²⁵ In clinic, sIL-2R is also an important diagnostic and disease

marker in hemophagocytic syndromes/hemophagocytic (HPS/HLH).^{22,23} High levels of sIL-2R are associated with poor prognosis, and falling sIL-2R corresponds to disease remission or treatment response in HPS/HLH.²³ A cytokine profile resembling secondary hemophagocytic lymphohistiocytosis (sHLH) was seen in COVID-19.²⁶ sIL-2R can be used as a biomarker of immune system activation in patients with COVID-19 and predict the prognosis of COVID-19.

The duration of viral shedding was correlated with viral load, host immune response and treatment. Administration of systemic corticosteroid may impair immune response and delay viral clearance. When systemic corticosteroid was not used, the duration of viral shedding was correlated with age. The viral shedding time was longer in elder patients. However, when systemic corticosteroid was used, the duration of viral shedding was not correlated with age. Similarly, the duration of viral shedding was correlated with LDH when systemic corticosteroid was not used. The viral shedding time was longer in patients with elevated LDH. But when systemic corticosteroid was used, the predict effect of LDH disappeared due to the impairment of systemic corticosteroid. When systemic corticosteroid was not used, the duration of viral shedding was not correlated with lymphocyte count. In cases administrated with systemic corticosteroid, the duration of viral shedding was negatively correlated with lymphocyte count. This may because high dose corticosteroid would be used in severe cases, in whom lymphopenia was very common, and viral shedding time was longer. sIL-2R was positively associated with duration of viral shedding no matter whether systemic corticosteroid was used or not. The result suggests that sIL-2R was an independent factor for predicting viral shedding time. It is a key factor for the virus inducing immune reaction in COVID-19 patients. The delayed viral shedding may associate with high virus load which stimulates the IL-2R related immune activation.

To our knowledge, this is the most detailed study of duration of viral shedding and its association with cytokines in COVID-19 patients. However, there are some limitations to this study. Firstly, there was potential false negative in SARS-CoV-2 nucleic acid test. Secondly, due to limited medical resources during the outbreak, only patients with severe symptoms could be admitted to hospital and most patients were severe cases. The durations of viral shedding from illness onset in this study tended to be very long and may differ from those in all population of COVID-19 patients. Duration of viral shedding in asymptomatic patients need to be further investigated. Finally, this report is a descriptive study, further research is urgent to investigate the mechanism of immune reaction involved in the virus clearance process.

Conclusions

The median duration of viral shedding was 24 days. Low dose of systemic corticosteroids did not prolong viral shedding compared with no use of systemic corticosteroids. The current antiviral treatments including lopinavir-ritonavir, arbidol, oseltamivir and intravenous immunoglobulin did not shorten viral shedding time. sIL-2R >710 U/mL, LDH>250 U/L and severe COVID-19 were related to prolongation of viral shedding. Optimal antiviral treatment strategy is urgently needed in the future.

Abbreviations

COVID-19: Coronavirus disease 2019; IL: Interleukin; sIL-2R: Soluble interleukin-2 receptor; TNF: Tumor necrosis factor; LDH: Lactate dehydrogenase; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; IQR: Interquartile range; AUC: Area under the curve; HRs: Hazard ratios; CIs: Confidence intervals; HPS/HLH: Hemophagocytic syndromes/hemophagocytic; sHLH: Secondary hemophagocytic lymphohistiocytosis

Declarations

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Authors' Contributions

MX, SX and MZ conceived and designed the study. JS and MX contributed to the literature search and writing of the report. MY, KW, YT, YZ, LQ and JS contributed to data collection. JS, XLi, MX, HR, ZY, CZ, JY and CC contributed to data analysis and data interpretation. XLiu, HR and MX contributed to revision of the manuscript. *Contributed equally.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Commission of Tongji Hospital (TJ-C20200122).

Consent for publication

Not applicable.

Competing interests

HR is member of and receives funding from the German Lung Centre (DZL) and of the Universities Giessen and Marburg Lung Centre (UGMLC). The other authors declare that they have no competing interests.

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Factors	Values
Age, years	58.0 (47.0-67.0)
<60	136/246 (55.3%)
≥60	110/246 (44.7%)
Sex	
Female	120/246 (48.8%)
Male	126/246 (51.2%)
Smoking history	
Never smokers	205/245 (83.7%)
Former smokers	18/245 (7.3%)
Current smokers	22/245 (9.0%)
Severity of COVID-19	
Non-severe	128/246 (52.0%)
Severe	118/246 (48.0%)
Comorbidity	
Respiratory diseases	14/246 (5.7%)
Diabetes	45/246 (18.3%)
Hypertension	72/246 (29.3%)
Coronary heart disease	16/246 (6.5%)
Tumor or impaired immune system disease	16/246 (6.5%)
Blood leukocyte count, × 10 ⁹ /L	5.2 (3.9-6.8)
≤10	229/244 (93.9%)
>10	15/244 (6.1%)
Neutrophil count, × 10 ⁹ /L	3.8 (2.6-5.3)
≤7.5	218/244 (89.3%)
>7.5	26/244 (10.7%)
Lymphocyte count, × 10 ⁹ /L	0.9 (0.6-1.1)
≥0.5	219/244 (89.8%)
<0.5	25/244 (10.2%)
Globulin, g/L	33.5 (30.7-36.8)
≤35	158/244 (64.8%)
>35	86/244 (35.2%)
Albumin, g/L	34.1 (31.4-37.2)
≥35	104/245 (42.4%)
<35	141/245 (57.6%)
Lactose dehydrogenase, U/L	298.5 (234.8-426.5)

£250	79/242 (32.6%)
>250	163/242 (67.4%)
Creatinine, µmol/L	68.0 (55.0-88.5)
£133	233/244 (95.5%)
>133	11/244 (4.5%)
D-dimer, mg/L	0.8 (0.5-1.7)
£1	137/232 (59.1%)
>1	95/232 (40.9%)
Erythrocyte sedimentation rate, mm/h	35.0 (20.0-58.0)
£20	63/238 (26.5%)
>20	175/238 (73.5%)
High-sensitive C-reactive protein, mg/L	44.6 (16.2-86.5)
£10	43/244 (17.6%)
>10	201/244 (82.4%)
Procalcitonin, ng/mL	0.1 (0-0.2)
£0.5	195/215 (90.7%)
>0.5	20/215 (9.3%)
Ferritin, µg/L	651.8 (418.3-1282.9)
£400	58/237 (24.5%)
>400	179/237 (75.5%)
IL-1β, ng/L	5.0 (5.0-5.0)
£5	205/246 (83.3%)
>5	41/246 (16.7%)
sIL-2R, U/mL	744.5 (531.3-1035.0)
£710	116/246 (47.2%)
>710	130/246 (52.8%)
IL-6, ng/L	19.6 (5.3-47.4)
£7	73/246 (29.7%)
>7	173/246 (70.3%)
IL-8, ng/L	16.3 (10.3-24.7)
£62	230/246 (93.5%)
>62	16/246 (6.5%)
IL-10, ng/L	5.4 (5.0-10.0)
£9.1	177/246 (72.0%)
>9.1	69/246 (28.0%)
TNF-α, ng/L	8.9 (7.1-11.1)

≤8.1	100/246 (40.7%)
>8.1	146/246 (59.3%)
Systemic corticosteroids	
Duration, days	4.0 (0-10.3)
Prednisone-equivalent dose, mg/Kg per day	
0	101/246 (41.1%)
<1	113/246 (45.9%)
≥1	32/246 (13.0%)
Intravenous immunoglobulin	94/245 (38.4%)
Lopinavir-ritonavir	74/246 (30.1%)
Arbidol	182/246 (74.0%)
Oseltamivir	96/246 (39.0%)

IL=interleukin. TNF=tumor necrosis factor. Data are expressed as median (IQR), n/N (%), where N is the total number of patients with available data.

Table 2: Median duration of viral shedding in patients with COVID-19

Factors		Median duration of viral shedding	95% CI	P value (Log-rank)
Age, years	<60	23	21.6-24.4	0.011
	≥60	27	25.2-28.8	
Sex	Female	25	22.7-27.3	0.247
	Male	23	21.7-24.3	
Smoking history	Never smokers	24	22.4-25.6	0.823
	Former smokers	22	20.8-23.2	
	Current smokers	23	17.9-28.1	
Severity of COVID-19	Non-severe	22	20.8-23.2	<0.001
	Severe	27	25.9-28.1	
Comorbidity				
Respiratory diseases	Without	24	22.4-25.6	0.735
	With	27	18.9-35.1	
Diabetes	Without	24	22.4-25.6	0.716
	With	24	19.3-28.7	
Hypertension	Without	23	21.4-24.6	0.613
	With	25	21.0-29.0	
Coronary heart disease	Without	24	22.4-25.6	0.327
	With	22	20.4-23.6	
Tumor or impaired immune system disease	Without	24	22.3-25.7	0.565
	With	23	20.4-25.6	
Blood leukocyte count, × 10 ⁹ /L				
	£10	24	22.5-25.5	0.436
	>10	27	19.9-34.1	
Neutrophil count, × 10 ⁹ /L				
	£7.5	23	21.5-24.5	0.332
	>7.5	28	25.5-30.5	
Lymphocyte count, × 10 ⁹ /L				
	≥0.5	23	21.7-24.3	0.067
	<0.5	28	24.7-31.3	
Globulin, g/L				
	£35	23	21.7-24.3	0.240
	>35	26	24.2-27.8	
Albumin, g/L				
	≥35	22	20.3-23.7	0.017
	<35	25	23.1-26.9	

Lactose dehydrogenase, U/L	£250	21	19.6-22.4	0.001
	>250	26	24.4-27.6	
Creatinine, µmol/L	£133	24	22.5-25.5	0.297
	>133	22	12.8-31.2	
D-dimer, mg/L	£1	22	20.7-23.3	0.001
	>1	27	25.5-28.6	
Erythrocyte sedimentation rate, mm/h	£20	23	21.0-25.0	0.278
	>20	24	22.3-25.7	
High-sensitive C-reactive protein, mg/L	£10	22	18.3-25.7	0.172
	>10	24	22.5-25.5	
Procalcitonin, ng/mL	£0.5	23	21.4-24.6	0.258
	>0.5	26	22.0-30.0	
Ferritin, µg/L	£400	21	19.2-22.8	0.017
	>400	25	23.2-26.8	
IL-1β, ng/L	£5	23	21.4-24.6	0.998
	>5	25	21.4-28.6	
sIL-2R, U/mL	£710	22	20.8-23.2	0.002
	>710	26	24.5-27.5	
IL-6, ng/L	£7	24	21.5-26.5	0.695
	>7	24	22.0-26.0	
IL-8, ng/L	£62	23	21.5-24.5	0.405
	>62	27	21.7-32.3	
IL-10, ng/L	£9.1	23	21.5-24.5	0.347
	>9.1	26	23.5-28.6	

TNF- α , ng/L				
	≤8.1	25	22.7-27.3	0.522
	>8.1	23	21.5-24.5	
Systemic corticosteroids				
Prednisone-equivalent dose, mg/Kg per day	0	24	21.9-26.1	0.046
	<1	23	21.4-24.6	
	≥1	27	19.2-34.8	
Intravenous immunoglobulin	No	23	21.3-24.7	0.477
	Yes	24	21.8-26.2	
Lopinavir-ritonavir	No	23	21.2-24.8	0.553
	Yes	25	22.3-27.7	
Arbidol	No	22	19.9-24.1	0.628
	Yes	24	22.3-25.7	
Oseltamivir	No	25	22.3-27.7	0.088
	Yes	23	21.6-24.4	

CI=confidence interval. IL=interleukin. TNF=tumor necrosis factor. The Kaplan-Meier method was used to calculate the median duration of viral shedding and validated by Log-rank test.

Table 3: Cox regression analysis of clinical characteristics associated with viral shedding duration in patients with COVID-19

Variable		Univariate HR (95%CI)	P value	Multivariate HR (95%CI)	P value
Age, years	<60	Ref			
	≥60	0.713 (0.543-0.938)	0.016		
Sex	Female	Ref			
	Male	1.165 (0.889-1.528)	0.268		
Smoking history	Never smokers	Ref			
	Former smokers	1.094 (0.644-1.857)	0.740		
	Current smokers	0.894 (0.557-1.435)	0.642		
Severity of COVID-19	Non-severe	Ref		Ref	
	Severe	0.612 (0.465-0.806)	<0.001	0.712 (0.522-0.971)	0.032
Comorbidity					
Respiratory diseases	Without	Ref			
	With	1.101 (0.614-1.977)	0.746		
Diabetes	Without	Ref			
	With	0.937 (0.648-1.354)	0.728		
Hypertension	Without	Ref			
	With	0.927 (0.683-1.259)	0.628		
Coronary heart disease	Without	Ref			
	With	1.309 (0.746-2.298)	0.348		
Tumor or impaired immune system disease	Without	Ref			
	With	1.166 (0.677-2.007)	0.581		
Blood leukocyte count, × 10 ⁹ /L	≤10	Ref			
	>10	0.785 (0.415-1.484)	0.456		
Neutrophil count, × 10 ⁹ /L	≤7.5	Ref			
	>7.5	0.785 (0.471-1.308)	0.353		
Lymphocyte count, × 10 ⁹ /L	≤0.5	Ref			
	>0.5	0.651 (0.401-1.056)	0.082		
Globulin, g/L	≤35	Ref			
	>35	0.849 (0.638-1.130)	0.263		
Albumin, g/L	≥35	Ref			
	<35	0.729 (0.555-0.958)	0.023		
Lactose dehydrogenase, U/L	≤250	Ref		Ref	
	>250	0.634 (0.476-0.843)	0.002	0.691 (0.503-0.949)	0.022
Creatinine, μmol/L	≤133	Ref			
	>133	1.470 (0.689-3.138)	0.319		
D-dimer, mg/L	≤1	Ref			

	>1	0.621 (0.465-0.829)	0.001		
Erythrocyte sedimentation rate, mm/h	≤20	Ref			
	>20	0.849 (0.624-1.156)	0.299		
High-sensitive C-reactive protein, mg/L	≤10	Ref			
	>10	0.796 (0.565-1.121)	0.191		
Procalcitonin, ng/mL	≤0.5	Ref			
	>0.5	0.704 (0.371-1.333)	0.281		
Ferritin, µg/L	≤400	Ref			
	>400	0.696 (0.508-0.951)	0.023		
IL-1β, ng/L	≤5	Ref			
	>5	1.000 (0.698-1.431)	0.998		
sIL-2R, U/mL	≤710	Ref		Ref	
	>710	0.657 (0.501-0.863)	0.003	0.719 (0.533-0.971)	0.031
IL-6, ng/L	≤7	Ref			
	>7	0.947 (0.714-1.257)	0.708		
IL-8, ng/L	≤62	Ref			
	>62	0.781 (0.425-1.435)	0.426		
IL-10, ng/L	≤9.1	Ref			
	>9.1	0.863 (0.626-1.190)	0.369		
TNF-α, ng/L	≤8.1	Ref			
	>8.1	1.089 (0.829-1.431)	0.541		
Systemic corticosteroids					
Prednisone-equivalent dose, mg/Kg per day	0	Ref			
	<1	1.008 (0.759-1.339)	0.957		
	≥1	0.589 (0.370-0.940)	0.026		
Intravenous immunoglobulin	No	Ref			
	Yes	0.908 (0.689-1.199)	0.497		
Lopinavir-ritonavir	No	Ref			
	Yes	0.919 (0.687-1.230)	0.570		
Arbidol	No	Ref			
	Yes	0.930 (0.682-1.266)	0.643		
Oseltamivir	No	Ref			
	Yes	1.255 (0.955-1.651)	0.104		

HR=hazard ratio. CI=confidence interval. IL=interleukin. TNF=tumor necrosis factor. Adjusted hazard ratios along with 95% confidence intervals were calculated by using the Cox proportional hazards regression model.

Figures

Figure 1

Kaplan-Meier curves for duration of viral shedding compared by severity (A), age (B), albumin (C), lactate dehydrogenase (D), D-dimer (E), ferritin (F), soluble interleukin-2 receptor (G) and usage of systemic corticosteroids (H). LDH=lactate dehydrogenase; sIL-2R= soluble interleukin-2 receptor

Figure 2

The time-dependent ROC curve for predictive effect of albumin (A), lactate dehydrogenase (B), D-dimer (C), ferritin (D) and soluble interleukin-2 receptor (E) at 20 days, 40 days and 60 days. LDH = lactate dehydrogenase; sIL-2R = soluble interleukin-2 receptor

Figure 3

The correlation between duration of viral shedding and age, lactate dehydrogenase, soluble interleukin-2 receptor and lymphocyte count stratified by the usage of systemic corticosteroid. LDH=lactate dehydrogenase; sIL-2R= soluble interleukin-2 receptor.