Original Article

Simple prognostic factors and change of inflammatory markers in patients with severe coronavirus disease 2019: a single-center observational study

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Aim: The aim of this study was to investigate the prognostic factors and evaluate the change in inflammatory markers of patients with coronavirus disease 2019 (COVID-19) requiring mechanical ventilation.

Methods: This retrospective observational study conducted from April 1, 2020, to February 18, 2021, included 97 adult patients who required mechanical ventilation for severe COVID-19 pneumonia and excluded nonintubated patients with a positive COVID-19 polymerase chain reaction test and those who had any obvious bacterial infection on admission. All patients were followed up to discharge or death. We obtained clinical information and laboratory data including levels of presepsin, interleukin-6, procalcitonin, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody every day. Poor outcome was defined as death or receiving a tracheostomy during hospitalization, and favorable outcome was defined as discharge after extubation.

Results: Differences (median [interquartile range]) were detected in age (76 [70–82] versus 66 [55–74] years), day from the onset of first symptoms to admission for mechanical ventilation (5 [3–7] versus 10 [8–12] days), and P/F ratio (i.e., ratio of arterial oxygen concentration to the fraction of inspired oxygen) after intubation (186 [149–251] versus 236 [180–296]) in patients with poor outcome versus those with favorable outcome on admission. Serum SARS-CoV-2 antibody levels had already increased on admission in patients with favorable outcome. We determined the day from the onset of first symptoms to admission for mechanical ventilation to be one of the independent prognostic factors of patients with COVID-19 (adjusted odds ratio 0.69, confidence interval 0.56–0.85).

Conclusion: These results may contribute to understanding the mechanism of progression in severe COVID-19 and may be helpful in devising an effective therapeutic strategy.

Key words: IL-6, SARS-CoV-2 antibody, mechanical ventilation, presepsin

INTRODUCTION

CORONAVIRUS DISEASE 2019 (COVID-19) is causing enormous morbidity and mortality across the world, with the associated acute respiratory dysfunction syndrome (ARDS) being the major problem among affected patients admitted to the intensive care units.[1] COVID-19 with ARDS (CARDS) is thought to cause cytokine release syndrome, which includes the release of interleukin-6 (IL-6), tumor necrosis factor-α, and other cytokines.[2,3] CARDS is thought to occur due to a hyperinflammatory response rather than to direct viral damage, and CARDS in patients with COVID-19 may be one of the risk factors of poor outcome and the requirements for intensive care.[4] In particular, it is important for clinicians to recognize prognostic factors and changes in inflammatory markers when planning therapeutics for COVID-19. In this study, we investigated the prognostic factors and evaluated changes of inflammatory markers in patients with severe COVID-19.

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PATIENTS AND METHODS

Study design

THIS WAS A single-center, retrospective, observational study conducted on all adult patients admitted to our critical care center, including those transferred from other hospitals, during April 1, 2020, to February 18, 2021. Patients aged 18 or older who required mechanical ventilation for severe COVID-19 pneumonia were included in the

study. The criterion for intubation due to respiratory failure was a P/F ratio (i.e., ratio of arterial oxygen concentration to the fraction of inspired oxygen) <200 under oxygen inhalation, which occurred, for example, when patients could not maintain a peripheral arterial oxygen saturation of 95% on oxygen inhalation of 5 L/min by mask. The nonintubated patients with a positive COVID-19 polymerase chain reaction (PCR) test (bioMerieux Japan Ltd., Tokyo, Japan) and those with any obvious bacterial infection on admission were excluded (Fig. 1). We applied an opt-out method on the website to obtain patient consent. This observational study followed the principles of the Declaration of Helsinki and was approved by the Institutional Review Board for Clinical Research of Higashiosaka Medical Center (approval number: 02-0546-A).

Diagnosis of COVID-19

The diagnosis of COVID-19 was made according to World Health Organization interim guidance and confirmed by RNA detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using PCR testing in the clinical laboratory of the Osaka Institute of Public Health or our hospital. Samples for the PCR SARS-CoV-2 laboratory test were collected via a nasopharyngeal or oropharyngeal swab.

Treatment protocol

We administered favipiravir or remdesivir in accordance with the patient's previous hospital use or started favipiravir from admission. We also administered intravenous steroid for patients with COVID-19 pneumonia using the following treatment protocol: oral favipiravir (3600 mg on day 1, 1600 mg from days 2 to 14) or intravenous drip infusion of remdesivir (200 mg on day 1, 100 mg from day 2 to extubation or day 10), methylprednisolone (1000 mg for 3 days followed by 125 mg for 3 days and 40 mg for 3 days). We also used low-molecular weight heparin (2000 IU every 12 h) or unfractionated heparin (10,000–12,000 IU/day). Heparin was administered after mechanical ventilation was started. We used dexmedetomidine for the sedation of all patients.

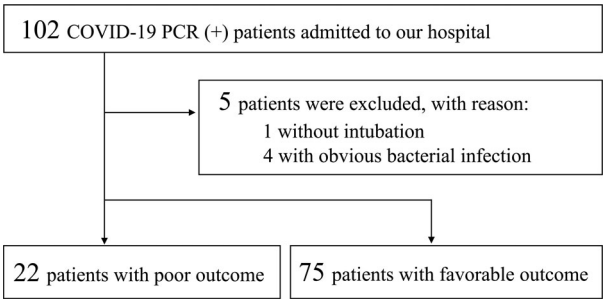


Fig. 1. Patient flow diagram. PCR, polymerase chain reaction.

Data collection

Patients were followed up until hospital discharge or death. Patient information was collected from the medical record, which included demographic characteristics, pre-existing comorbidities, laboratory tests, severity scores, and therapeutic interventions in our hospital or the previous hospital. Ventilator-free days were calculated in the first 28 days or the days alive minus days under mechanical ventilation after admission. Laboratory data including procalcitonin (PCT), IL-6, presepsin, and hemoglobin A1c (HbA1c) were obtained. Severity of illness was evaluated according to the Sequential Organ Failure Assessment (SOFA) score and the Acute Physiology and Chronic Health Evaluation (APACHE) II score. Clinical follow-up data were collected up to February 18, 2021.

Analysis of specific biomarkers and antibody test for SARS-CoV-2

Serum IL-6 and PCT levels were assessed by the Elecsys test (Roche Diagnostics K.K., Tokyo, Japan) using the Roche cobas analyzer. Serum SARS-CoV-2 antibody levels (cutoff index) were assessed by Elecsys Anti-SARS-CoV-2 RUO (Roche Diagnostics K.K.) also using the Roche cobas analyzer. Serum presepsin levels were assessed by PATHFAST Presepsin (LSI Medience Corporation, Tokyo, Japan).

Main outcome

The primary outcome was the patient's condition at discharge. Poor outcome was defined as death or receiving a tracheostomy during the hospital stay. We performed tracheostomy for the following criteria: no improvement of the respiratory state after admission and a P/F ratio of <200 under >8 cm H_2O positive end-expiratory pressure at 7–10 days after admission. A favorable outcome was defined as patients who received planned extubation and were discharged.

The change of biomarkers was evaluated as the secondary outcome. The biomarkers are associated with severity and prognosis,[3] and knowledge of the serial change of biomarkers is important in understanding the pathophysiology of COVID-19.

Statistical analysis

Patient age and other demographic data are expressed as median ± interquartile range (IQR) or counts and percentages. Laboratory data are expressed as median with IQR. The

Table 1. Characteristics of patients with COVID-19 on admission and outcome

	Total (*N* = 97)	Poor outcome (*N* = 22)	Favorable outcome (*N* = 75)	*P*
Age, median (IQR)	68 (58–77)	75 (70–82)	66 (55–74)	0.001
Sex, *n* (%)				
Male	67 (69)	15 (68.2)	52 (69.4)	
Female	30 (31)	7 (31.8)	23 (30.6)	1.0
Body mass index (kg/m^2), median (IQR)	24.7 (22.4–27.1)	24.4 (22.4–27.6)	24.8 (22.4–27.1)	0.969
Blood groups				
A RhD positive, *n* (%)	36 (37.1)	8 (36.3)	28 (37.3)	
B RhD positive, *n* (%)	26 (26.8)	6 (27.3)	20 (26.7)	
AB RhD positive, *n* (%)	11 (11.3)	5 (22.7)	6 (8.0)	
O RhD positive, *n* (%)	24 (24.7)	3 (13.7)	21 (28.0)	0.229
Intubation, *n* (%)	97 (100)	22 (100)	75 (100)	-
P/F ratio after intubation, median (IQR)	224 (176–286)	186 (149–251)	236 (186–298)	0.019
ECMO, *n* (%)	1 (1.0)	0 (0)	1 (1.3)	-
Tracheostomy, *n* (%)	10 (10.3)	10 (47.6)	0 (0)	-
Day from the onset of first symptoms, median (IQR)	9 (6–11)	5 (3–7)	10 (8–12)	<0.001
Intensive care unit stay (day) , median (IQR)	11 (6–14)	13 (11–28)	9 (6–13)	<0.001
SOFA score, median (IQR)	5 (3–6)	5 (4–7)	5 (3–6)	0.087
Ventilator-free days (day), median (IQR)	19 (12–23)	0 (0–0)	21 (17–23)	<0.001
APACHE II score, median (IQR)	11 (8–14)	12 (11–15)	10 (7–14)	0.022
Laboratory data				
WBC (×100/μL), median (IQR)	82.2 (56.9–110.3)	92.6 (65.2–123.4)	77.2 (56.3–104.6)	0.283
CRP (mg/dL), median (IQR)	9.6 (4.8–15.2)	9.3 (4.9–12.5)	9.6 (4.7–18.2)	0.398
FDP D-dimer (μg/mL), median (IQR)	1.5 (0.9–3.2)	3.1 (1.5–15.5)	1.3 (0.9–2.2)	<0.001
PCT (ng/mL), median (IQR)	0.13 (0.07–0.31)	0.19 (0.09–0.41)	0.12 (0.06–0.3)	0.181
IL-6 (pg/mL), median (IQR)	46.5 (15.3–92.9)	64.0 (25.7–135.7)	44.3 (14.6–82.3)	0.171
Presepsin (pg/mL), median (IQR)	440 (256–713)	579 (283–939)	433 (253–686)	0.183
Lactate dehydrogenase (U/L), median (IQR)	399 (302–478)	458 (370–613)	388 (294–449)	0.008
HbA1c ≥ 6.5, *n* (%)	37 (38.5)	9 (40.9)	28 (37.3)	0.607
SARS-CoV-2 antibody (COI), median (IQR)	2.19 (0.27–9.75)	0.26 (0.09–1.51)	3.41 (0.57–10.9)	<0.001
Mortality, *n* (%)	14 (14.4)	14 (63.6)	0 (0)	<0.001
Therapeutic drug				
Favipiravir, *n* (%)	90 (92.8)	21 (95.5)	69 (92.0)	
Remdesivir, *n* (%)	4 (4.1)	0 (0)	4 (5.3)	
Tocilizumab, *n* (%)	7 (7.2)	1 (4.8)	6 (8.0)	
Methylprednisolone, *n* (%)	96 (99.0)	22 (100)	74 (98.7)	
Dexamethasone, *n* (%)	45 (46.4)	8 (36.3)	37 (49.3)	-

APACHE II, Acute Physiology and Chronic Health Evaluation II; COI, cut off index; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; ECMO, extracorporeal membrane oxygenation; FDP, fibrin degradation product; HbA1c, hemoglobin A1c; IL-6, interleukin-6; IQR, interquartile range; P/F ratio, ratio of arterial oxygen concentration to the fraction of inspired oxygen; PCT, procalcitonin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.
Poor outcome was defined as death or receiving a tracheostomy during hospitalization, and favorable outcome was defined as discharge after extubation.
Ventilator-free days; the first 28 days or the days alive minus days under mechanical ventilation after admission.

Wilcoxon signed-rank test and Fisher exact test were used to compare patients' data between poor outcome and favorable outcome. The factors associated with poor outcome were evaluated by multivariable logistic regression analysis, and the adjusted odds ratio (aOR) and 95% confidence interval (CI) were calculated. In the logistic regression model, we included age, day from the onset of first symptoms to admission, P/F ratio after intubation on admission, and APACHE II

score on admission as the variables. A P value <0.05 was considered to be statistically significant. Statistical analyses were conducted with JMP Pro 15.0 for Windows (SAS Institute Inc., Cary, NC, USA). This manuscript was written based on the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement to assess the reporting of cohort and cross-sectional studies.[5]

RESULTS

Patient characteristics

THIS STUDY COMPRISED 97 patients with COVID-19 pneumonia requiring mechanical ventilation. The number of patients with poor outcome was 22 and those with favorable outcome was 75 (Fig. 1). Among them, 67 were men and 30 were women. The mortality rate was 14.4%. The median (IQR) of the body mass index, P/F ratio after intubation on admission, day from the onset of first symptoms to admission for mechanical ventilation, length of intensive care unit stay, and SOFA and APACHE II scores on admission were 24.7 (22.4–27.1) kg/m^2, 224 (176–286), 9 (6–11) days, 11 (6–14) days, and 5 (3–6) and 11 (8–14), respectively (Table 1).

Difference between patients with poor and favorable outcome

The age (median [IQR]) of patients with a poor outcome was higher than that of those with a favorable outcome (76 [70–82] versus 66 [55–74] years). A small number of O RhD-positive patients were detected among those with poor outcome. Days from the onset of first symptoms to admission for mechanical ventilation were significantly shorter in patients with poor outcome (5 [3–7] versus 10 [8–12] days). The P/F ratio after intubation on admission was lower in patients with poor outcome (186 [149–251] versus 236 [180–296]). Other differences including severity scores and laboratory data are shown in Table 1.

Factors associated with poor outcome

We assessed the factors associated with poor outcome using a multivariable logistic regression analysis in a model that included age, day from the onset of first symptoms to admission for mechanical ventilation, P/F ratio after intubation on admission, and APACHE II score on admission as the variables. Age (aOR 1.11; 95% CI 1.03–1.20) and day from the onset of first symptoms (aOR 0.69; 95% CI 0.56–0.85) were significantly associated with poor outcome (Table 2).

Table 2. The factors associated with the poor outcome

	aOR	95% CI	*P*
Age	1.11	(1.03–1.20)	0.002
Day from the onset of first symptoms	0.69	(0.56–0.85)	<0.001
P/F ratio after intubation on admission	0.99	(0.98–1.00)	0.228
APACHE II score on admission	0.99	(0.82–1.20)	0.926

aOR, adjusted odds ratio; APACHE II, Acute Physiology and Chronic Health Evaluation II; CI, confidence interval; P/F ratio, ratio of arterial oxygen concentration to the fraction of inspired oxygen.

Serial changes of biomarkers, SOFA scores, and value of SARS-CoV-2 antibody with COVID-19

Several biomarkers such as IL-6, presepsin, and C-reactive protein (CRP) were decreased in the acute phase after admission. Thereafter, these markers increased again on days 5–7 in patients with poor outcome. Among them, presepsin levels showed a difference between patients with poor outcome and those with favorable outcome. Although the PCT level was normal in both groups and showed no significant difference in the acute phase, it was increased slightly in patients with poor outcome after day 7. The SOFA score decreased in the acute phase after admission in patients with favorable outcome but did not decrease in those with poor outcome. Serum SARS-CoV-2 antibody levels were already increased on admission in patients with favorable outcome (Table 1; Fig. 2).

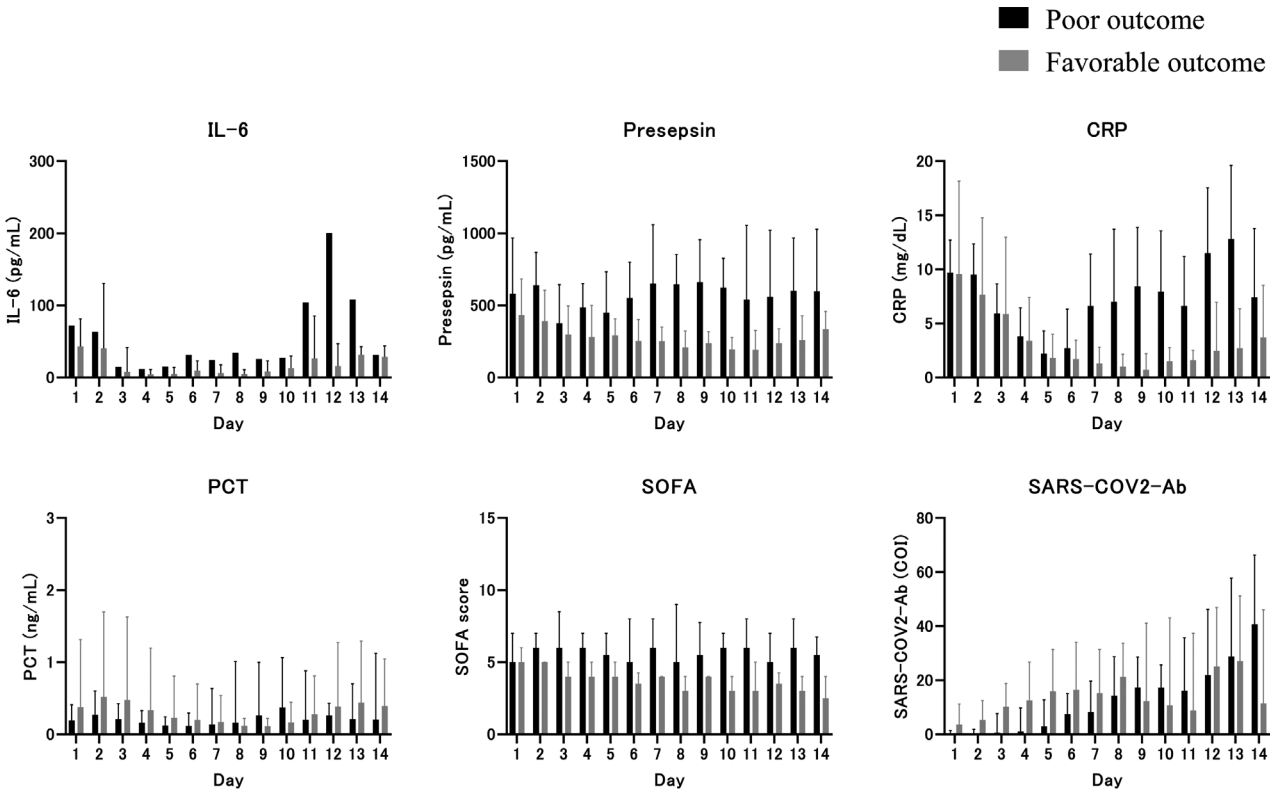
DISCUSSION

IN THIS STUDY, we evaluated the serial change of inflammatory markers and factors of prognosis in patients with COVID-19 requiring mechanical ventilation. Three important clinical results were detected. First, the day from the onset of first symptoms to start of ventilatory management in patients with poor outcome was significantly shorter than that in patients with favorable outcome. Second, lower levels of serum SARS-CoV-2 antibody on admission were detected in patients with poor outcome. Third, biomarkers such as presepsin, CRP, and IL-6 increased again on days 5–7 in patients with poor outcome compared with those with a favorable outcome.

Factors indicating high risk in severe COVID-19 were age; comorbidity including chronic obstructive pulmonary

disease; history of smoking; and elevated levels of IL-6, D-dimer, lactate dehydrogenase, PCT, and high hypersensitive troponin I.[6–11] In our study, the day from the onset of first symptoms to admission (start of mechanical ventilation) was detected as an independent factor of poor outcome. This result suggests that rapid progressive deterioration of a patient's condition from disease onset might be related to a weakened host immune system and can lead to death. To our knowledge, this is the first report of this factor to be an indicator of poor outcome of COVID-19, and analysis of this factor could be used as a simple method to identify poor prognosis without the need for any clinical examination. Our result showed that serum SARS-CoV-2 antibody levels had not increased yet on admission in patients with poor outcome, and this may support that a rapid progressive deterioration from onset is a factor of poor outcome. A previous study reported that the time from illness onset to antiviral treatment was one of the major risk factors for COVID-19 prognosis,[12] and thus, antiviral treatment in the early stage might prevent increased disease severity, and knowledge of the more precise characteristics of these patients may help us to develop a novel treatment strategy.

IL-6, CRP, and PCT are associated with severity and mortality in patients with severe COVID-19,[3,13–15] and in this



Number of patients on each day

	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Poor outcome	22	22	22	22	21	21	21	20	19	17	15	13	12	10
Favorable outcome	75	75	75	74	70	64	50	45	35	32	28	20	17	10

Fig. 2. Serial changes of several biomarkers, SOFA scores, and the value of anti-SARS-CoV-2 antibody compared between patients with poor and favorable outcomes. Data are presented as median and IQR. Ab, antibody; COI, Cut Off Index; CRP, C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOFA, Sequential Organ Failure Assessment.

study, we evaluated serial changes of these inflammatory biomarkers. IL-6, CRP, PCT, and presepsin showed differences in trend at days 5–7 between patients with poor and favorable outcome. The steroid dose was gradually decreased every 3 days in our treatment protocol, and thus, the lack of anti-inflammatory agents and response might be associated with increasing values of inflammatory markers. Although dexamethasone was reported effective in many patients hospitalized with COVID-19,[16] when focusing on patients with severe COVID-19, the evidence for doses, durations, and steroids to use remains inadequate.[17] We administered pulse steroid therapy to almost all patients in this study, and the intensive care unit mortality of 14.4% was better than that of previous reports.[18,19] Pulse steroid therapy might be advantageous as one of the anti-inflammatory treatments.

The presepsin values in this study increased, and the presepsin levels in 44.3% of patients with COVID-19 rose above the cutoff value of 500 pg/mL. Presepsin is a soluble CD14 subtype with a truncated N terminal and is reported to be a novel biomarker in sepsis. Presepsin is not only useful in the diagnosis of sepsis but might also be predictive of disease severity and mortality.[20] Only a small number of studies have reported a relationship between presepsin and COVID-19.[21–23] The complication of slight bacterial infection on admission or activation of unknown confounding signaling and other mechanisms might be associated with the elevation of presepsin levels.

We acknowledge several limitations of our study. First, the single-center design and short study duration resulted in a relatively small sample size, which may have influenced the precision of our findings. Second, we cannot evaluate the laboratory data from the onset of the disease to admission to our hospital. Third, as this is an observational study, there may be unknown confounding factors. Further research is needed to clarify and resolve these limitations.

CONCLUSIONS

AMONG PATIENTS WITH severe COVID-19 requiring mechanical ventilation, the day from the onset of first symptoms to admission for mechanical ventilation was an independent prognostic factor of poor outcome. The present results may contribute to better understanding of the mechanism of progression in severe COVID-19 and may help in the development of therapeutic strategies.

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DISCLOSURE

APPROVAL OF THE research protocol: This study was approved by the Institutional Review Board for Clinical Research of Higashiosaka Medical Center (approval no.: 02-0546-A).

Informed Consent: We applied an opt-out method on the web site to obtain patient consent.

Registry and the Registration No. of the Study/Trial: N/A.

Animal Studies: N/A.

Conflict of Interest: Authors declare no Conflict of Interests for this article.

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