—Short Communication—

IgG Antibodies, SARS-CoV-2 Load, and Prognostic Indicators in Patients with Severe and Mild COVID-19 in Japan

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We assessed the association of severity of coronavirus disease 2019 (COVID-19) with acute respiratory syndrome coronavirus 2 (SARS-CoV-2) load, IgG antibody level, and prognostic indicators. Twenty-one patients hospitalized with COVID-19 were classified as having severe or mild disease on the basis of average respiratory rate during hospitalization (severe: ≥22 breaths/min; mild: <22 breaths/min). Viral load in nasopharyngeal samples, blood levels of C-reactive protein (CRP), lymphocytes, and D-dimer on admission and plasma immunoglobulin G (IgG) index on Day 7±2 after symptom onset were compared in relation to disease severity. Seven patients had severe disease and 14 had mild disease. Those with severe disease had a significantly higher IgG index (median: 3.75 vs 0.56, p=0.01) and CRP (median: 8.6 vs 1.0 mg/dL, p<0.001) and D-dimer levels (median: 1.65 vs 0.75 μg/mL; p=0.002) and a significantly lower lymphocyte count (median: 1,176 vs 666 cells/μL, p=0.005) and viral load (median: 8.7×10^6 vs 2.3×10^4 copies/mL, p=0.005). Furthermore, time from symptom onset to virus disappearance was significantly longer in severe patients (median: 24 vs 17 days, p=0.03). A high IgG index in the early phase of the disease was associated with severe disease and might serve as a prognostic indicator.
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Key words: COVID-19, SARS-CoV-2, IgG index, viral load, prognostic indicators

Introduction

In Japan, the first case of coronavirus disease 2019 (COVID-19) was reported at the end of January 2020. The disease later spread throughout the country. COVID-19 has diverse manifestations. Several hypotheses have been proposed to explain these varying manifestations, including that high viral load and prolonged virus-shedding are associated with disease severity[1], and that exaggerated cytokine responses contribute to multiple organ failure and acute respiratory distress syndrome[2]. Antibody-dependent enhancement has been hypothesized to be associated with disease severity[3]. This study examined the association of disease severity with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load, immunoglobulin G (IgG) antibody titer, and prognostic indicators in patients with COVID-19.

Materials and Methods

Patients with COVID-19 who were admitted to the Toho University Omori Medical Center, a tertiary care hospital in Tokyo, Japan, from February 1 through May 31, 2020 were eligible to participate; patients with an IgG index measured 5 to 7 days after admission were included in the study. This study was approved by the ethics committee of the Toho University School of Medicine (number: A20028-A20020-A20014-A19099). SARS-CoV-2 infection was confirmed by testing nasopharyngeal samples with a quantitative PCR test (RT-qPCR, QuantStudio 5, Thermo Fisher Scientific, Waltham, MA, USA). The cycle threshold results were calculated by using a specific calculation to the viral titer ($y = 4E+10e^{-0.649x}$, $R^2 = 0.9955$ copies/mL). Participants' blood test results and nasopharyn-

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Table 1 Clinical characteristics of the patients with confirmed COVID-19

	Severe disease[a] N=7	Mild disease[a] N=14
Age, median (interquartile range), years	71 (56-76)	47 (31-60)
Sex		
Male	4	5
Female	3	9
Comorbidities[b]	4	1
Smoking status		
Current smoker	2	1
Ex-smoker	2	2
Nonsmoker	3	11
Treatment		
Ciclesonide alone	0	4
Favipiravir alone	2	0
Ciclesonide and favipiravir	5	9
No antiviral medication	0	1
Fever	6	10
Cough	6	9
Dysosmia	1	2
Dysgeusia	1	2
Pneumonia	7	10
Days from symptom onset to diagnosis	5	3

[a] Disease severity was classified in relation to mean respiratory rate as severe (≥22 breaths/min) or mild (<22 breaths/min).
[b] Presence of comorbidities was defined as a history of chronic obstructive pulmonary disease, diabetes mellitus, or hypertension.

geal PCR test results were measured on the day of hospitalization, and IgG levels were measured 7±2 days after symptom onset. Time to virus disappearance was defined as the day when the quantitative PCR test yielded two consecutive negative test results with an interval of at least 24 hours. Disease severity was defined as mild or severe in relation to whether the average respiratory rate was greater or less than 22 breaths/minute during hospitalization, including while receiving mechanical ventilation (mild: <22 breaths/min; severe: ≥22 breaths/min). The IgG index (defined as the relative luminescence value of a sample when the calibrator sample is set to 1) was measured with an Abbott ARCHITECT i2000SR analyzer with a cut-off value for a positive result of 1.4. Statistical analysis was performed using Prism8 (GraphPad software, San Diego, CA, USA). The Mann-Whitney U test was used to assess the significance of differences between groups. P-values <0.05 were considered statistically significant.

Results

Of the 53 patients diagnosed with COVID-19, 44 were hospitalized and 21 consented to participate in the study. **Table 1** shows participant characteristics in relation to disease severity. Median time from symptom onset to admission did not differ significantly by disease severity (severe: 5 days, mild: 3 days, p=0.699). Among patients with severe disease, median IgG index (severe: 4.07, mild: 0.04, p=0.013) and C-reactive protein (CRP) (median: 8.6 vs 1.0 mg/dL, p<0.001) and D-dimer levels (median: 1.65 vs 0.75 μg/mL; p=0.002) were significantly higher, and lymphocyte count (median: 666 vs 1,176 cells/μL, p=0.005) and viral load (median: 2.3×10^4 vs 8.7×10^6 copies/mL, p=0.005) were significantly lower, on admission (**Fig. 1**). IgG became positive within days of disease onset, but immunoglobulin M did not appear until later (data not shown). The time from symptom onset to virus disappearance was significantly longer in participants with severe disease (24 vs 17 days, p=0.03, **Fig. 1**). Most participants were treated with ciclesonide, an inhaled steroid, and two participants received systemic steroids. Seven participants were treated with favipiravir but three discontinued favipiravir treatment because of fever and liver damage. There were no deaths. All patients with severe disease required oxygen administration; one was intubated on arrival at the emergency department and four of seven patients were in poor respiratory condition (required oxygen administration up to

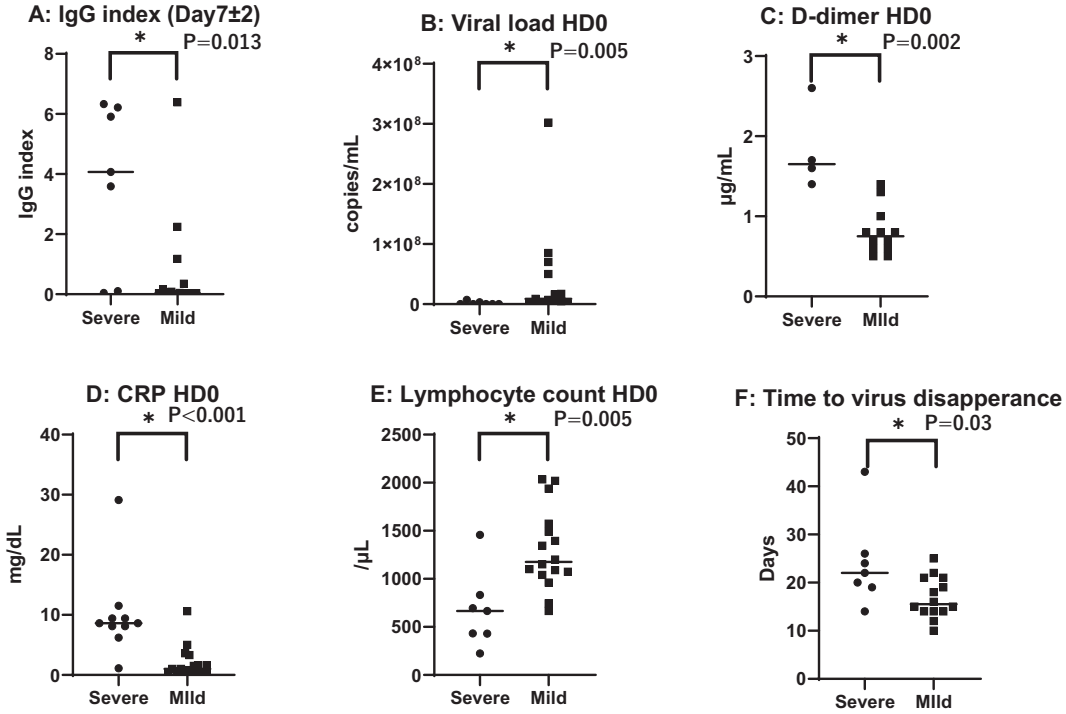

Fig. 1 Characteristics of COVID-19 in patients with mild and severe disease

A. IgG index 7±2 days after symptom onset.

B. Viral load on HD0.

C. D-dimer on HD0.

D. C-reactive protein on HD0.

E. Lymphocyte count on HD0.

F. Time to virus disappearance.

Bars indicate the median. The statistical significance of differences between groups was measured with the Mann-Whitney U test.

Abbreviations: CRP, C-reactive protein; HD0, Hospital Day 0 (day of admission); IgG, immunoglobulin G

10-15 L/min by reservoir mask, decreased PaO2/FiO_2 ratio <200 in arterial blood gas). Two of these four patients were already in poor respiratory condition when admitted to our hospital (HD0). For the other two patients, respiratory rates were less than 22 breaths/min on HD0 but worsened during hospitalization to greater than 22 breaths/min by HD3.

Discussion

Some biomarkers, including CRP, IL-6, lymphocyte, LDH, and D-dimer, were reported to be prognostic indicators[4]. Our results revealed significant differences in relation to disease severity for CRP, D-dimer, lymphocyte count, viral load, and IgG index. Nasopharyngeal viral load was reported to peak within the first week, and one study found that the mean viral load of severe cases was about 60-fold that of mild cases[5]. In contrast, we found that viral load was significantly lower among participants with severe disease.

There may be other factors that contribute to severity. In our study, IgG index was significantly higher in patients with severe disease, even early in the disease. Although antibodies are usually protective, some pathogen-specific antibodies can promote pathology, resulting in antibody-dependent enhancement[6]. In patients with recurrent dengue, the presence of higher levels of pre-existing antibodies is associated with greater disease severity[7]. SARS-CoV-2 and SARS-CoV may exhibit cross-reactivity in antibody binding to the shared spike protein, although cross-neutralization of the live viruses is rare[8]. Other studies have reported relatively early elevation of IgG but not IgM[9,10], which is consistent with our findings. In most infections, IgM is elevated before IgG. However, in SARS-CoV-2 infection, IgG and IgM seroconversion may occur simultaneously, or IgG seroconversion may precede IgM seroconversion. We found that patients with severe disease had a higher IgG index and lower viral load in the early phase of the disease. Studies

of the function of IgG antibodies might aid in vaccine development and COVID-19 prevention.

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Conflict of Interest: The authors declare no competing interests in this study.

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