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Rapid Communication

The real-time reverse transcription-polymerase chain reaction threshold cycle values for severe acute respiratory syndrome coronavirus 2 predict the prognosis of coronavirus disease 2019 pneumonia

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ABSTRACT

The clinical course of coronavirus disease 2019 (COVID-19) varies from mild to critical. We retrospectively examined whether clinical and laboratory findings on admission could predict COVID-19 prognosis. Among various factors associated with COVID-19 severity, our results indicated that the real-time reverse transcription-polymerase chain reaction (RT-PCR) threshold cycle (Ct) values for severe acute respiratory syndrome coronavirus 2 were the most useful predictor of COVID-19 prognosis.

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Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) is rapidly spreading worldwide. In Japan, a total of 82,494 COVID-19 cases were confirmed with 1557 corresponding deaths as of September 30, 2020 [1]. The COVID-19 pandemic threatens to overwhelm the healthcare systems in many countries through issues such as lack of beds and ventilators. In the clinical course of COVID-19, most patients have mild or

Abbreviations: COVID-19, coronavirus disease 2019; Ct, threshold cycle; RT-PCR, real time reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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Table 1 – Patient characteristics and laboratory findings on admission.

Variables	Total (n = 19)	Non-critical (n = 12)	Critical (n = 7)	P-value
Demographic data				
Age (years)	69 ± 14	65 ± 17	75 ± 8	0.34
Sex				
Male	16 (84%)	9 (75%)	7 (100%)	
Female	3 (16%)	3 (25%)	0 (0%)	0.26
Smoking history				
Yes	13 (68%)	7 (58%)	6 (86%)	
No	6 (32%)	5 (42%)	1 (14%)	0.33
Comorbidities				
Hypertension	8 (42%)	4 (33%)	4 (57%)	0.38
Diabetes	4 (21%)	3 (25%)	1 (14%)	1
Cardiovascular disease	8 (42%)	4 (33%)	4 (57%)	0.38
Chronic liver disease	2 (11%)	0 (0%)	2 (29%)	0.12
Chronic lung disease	4 (21%)	2 (17%)	2 (29%)	0.6
Chronic kidney disease	3 (16%)	0 (0%)	3 (43%)	0.05
Cancer	5 (26%)	2 (17%)	3 (43%)	0.31
Immunocompromising conditions	3 (16%)	1 (8%)	2 (29%)	0.52
Signs and symptoms				
Fever	12 (63%)	8 (67%)	4 (57%)	1
Cough	9 (47%)	6 (50%)	3 (43%)	1
Fatigue	8 (42%)	3 (25%)	5 (71%)	1
Diarrhea	3 (16%)	2 (17%)	1 (14%)	1
Shortness of breath	13 (68%)	9 (75%)	4 (57%)	0.62
Laboratory findings				
WBC ($\times 10^9$/L)	5.1 (1.6)	5.1 (1.1)	5.3 (2.8)	0.97
Lymphocyte ($\times 10^9$/L)	0.87 (0.84)	0.80 (0.57)	1.18 (1.67)	0.77
NLR (Neutrocyte/lymphocyte ratio)	4.0 (5.2)	4.3 (4.2)	3.2 (14.2)	0.38
CRP (mg/L)	5.1 (8.5)	9.1 (9.5)	4.0 (3.9)	0.30
AST (U/L)	25 (27)	25 (23)	29 (27)	1
ALT (U/L)	20 (21)	23 (21)	18 (17)	0.65
LDH (U/L)	273 (159)	300 (192)	234 (71)	0.34
Ferritin (ng/mL)	598 (1617)	395 (1631)	700 (3694)	0.34
D-dimer (ng/mL)	2.6 (4.9)	3.2 (7.6)	1.8 (3.4)	0.25
Ct values of RT-PCR (N1)	25.9 (12.1)	30.4 (9.4)	21.2 (5.4)	0.0031**
Ct values of RT-PCR (N2)	22.5 (12.8)	28.3 (6.4)	14.6 (6.3)	0.0004**
CT scan findings				
GGO (not including consolidation)	11 (58%)	7 (58%)	4 (57%)	
GGO including consolidation	8 (42%)	5 (42%)	3 (43%)	1
Bilateral pneumonia findings	17 (89%)	11 (92%)	6 (86%)	0.53
Oxygen demand				
Ambient air	12 (63%)	7 (58%)	5 (71%)	1
Nasal cannula	7 (37%)	5 (42%)	2 (29%)	1

WBC, white blood cell; CRP, C-reactive protein; AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase; Ct, threshold cycle; RT-PCR, reverse transcription-polymerase chain reaction; GGO, ground-glass opacity.
Data are expressed as number (percentage), mean ± standard deviation (SD), or median (interquartile range [IQR]).
P-values comparing data between critical and non-critical patients during hospitalization was calculated using Mann-Whitney *U* test and Fisher's exact test.
**$P < 0.01$.

moderate symptoms and recover spontaneously, but a certain number of patients develop severe respiratory failure. Therefore, if we can accurately predict the clinical course of COVID-19, we may provide intensive care to patients at high risk of respiratory failure, thereby avoiding medical collapse and ensuring reduced mortality. However, clinical and laboratory findings for predicting the prognosis of COVID-19 pneumonia has not been fully elucidated. Therefore, this study retrospectively examined whether clinical and laboratory findings on admission could predict prognosis.

To investigate which factors can predict the prognosis of COVID-19, we studied 19 patients with COVID-19 pneumonia confirmed through computed tomography (CT) scan, who were hospitalized in non-intensive care unit (ICU) wards at our hospital between March 24 and May 14, 2020. In our hospital, real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed in-hospital, using nasopharyngeal swab samples with the National Institute of Infectious Disease primer sets [2]. The threshold cycle (Ct) values, the number of reaction cycles required for the fluorescence signal to exceed the background levels, were

reported for two genetic markers, the N1 and N2 viral nucleocapsid protein gene regions. During their hospitalization, some patients developed critical illness including use of mechanical ventilation, transfer to ICU, and death. We retrospectively reviewed the clinical and laboratory findings and compared patients who developed critical illness with those who did not. This study was approved by the Ethics Committee of Keio University School of Medicine (No. 20200063).

Table 1 shows the summary of the clinical characteristics and laboratory findings of the 19 patients on admission. The median age was 69 years, and 16% of patients were female. Hypertension and diabetes were present in 42% and 21% of patients, respectively. Most patients had fever (63%) and dyspnea (68%). Lymphopenia, elevated neutrophil-to-lymphocyte ratio (NLR), and elevated levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, and d-dimer were noted. Sixteen of the 19 patients had Ct values for both N1 and N2, while 3 patients had Ct values only for either N1 or N2, probably due to low viral load. Every patient had ground-glass opacity, and half of the patients had consolidation on the CT scan. Most patients only needed a small amount of oxygen on admission. Fig. 1 shows the change in oxygen demand in each patient, and 7 (37%) patients developed critical illness during hospitalization (defined as death [n = 3], clinical need for mechanical ventilation [n = 5], or transfer to the ICU [n = 5]).

Next, we investigated which findings were different between the "critical illness" and "non-critical illness" groups. NLR, CRP, LDH, ferritin, d-dimer, and liver enzyme levels were not significantly different between groups, although these biomarkers have been reported to correlate with the severity of COVID-19 (Supplementary Fig. 1) [3–6]. The finding suggests that the values of these biomarkers on admission may not be useful in predicting the future clinical course of COVID-19. Furthermore, oxygen demand and imaging findings were not different between groups. In contrast, there was a significant difference in the real-time RT-PCR Ct values between groups (Table 1, Fig. 2). All the three patients who detected Ct values for either N1 or N2 only were in the non-critical group. Ct values for N1 and N2 in the critical group were 9.6 and 13.0 lower compared to those in the non-critical group, respectively (95% confidence interval: 3.8–17.9 and 6.9–17.9, respectively, Hodges-Lehmann estimator). Notably, the patients whose Ct values were <20 for both N1 and N2 developed acute respiratory distress syndrome (ARDS) rapidly and required mechanical ventilation within 7 days. In contrast, any patients whose Ct values were >30 for either N1 or N2 did not develop critical illness. Taken together, our findings indicate that the Ct values on admission were the most predictive markers of the clinical course of COVID-19 pneumonia.

The real-time RT-PCR Ct values have been reported to correlate with the amount of viral RNA [7]. In addition, the association between viral load and severity of disease was suggested in severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) [8,9]. A recent study reported that the real-time RT-PCR Ct values for SARS-CoV-2 determined from sputum samples, were associated with disease severity and risk of progression of COVID-19 [10]. From these findings, Ct values, reflecting the viral load on admission, may be a useful predictor of subsequent COVID-19 prognosis.

There are some limitations to this study. First, it was a single-center study, and the number of cases was limited. Second, PCR results are often affected by variability in specimen collection. However, there was little variation in the

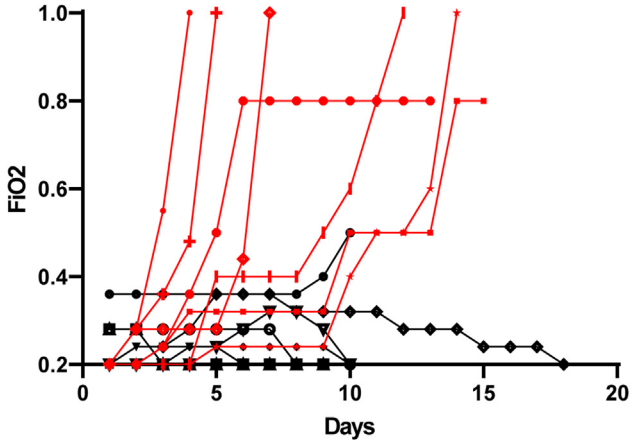

Fig. 1 – Clinical course of hospitalized patients with COVID-19 pneumonia. Clinical course of oxygen demand in 19 patients with coronavirus disease 2019 (COVID-19) pneumonia during hospitalization. Each line represents the concentration of oxygen (fraction of inspiratory oxygen, FiO2) required to treat each patient and terminates when the patient developed critical illness (death, use of mechanical ventilation, transfer to the intensive care unit [ICU]) or when severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was eliminated. Red; critical cases. Black; non-critical cases. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

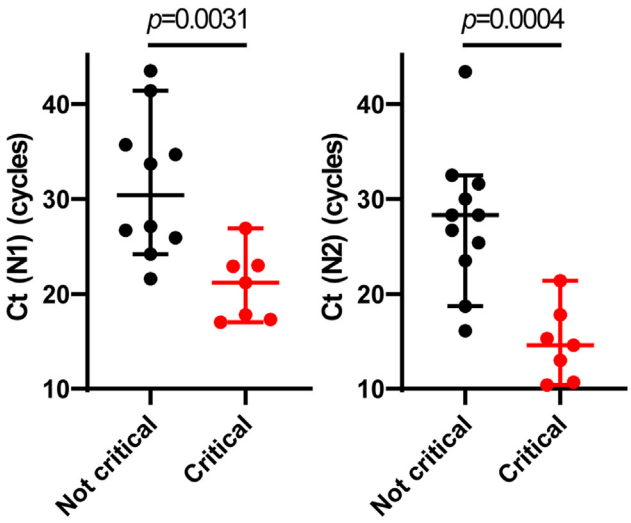

Fig. 2 – Ct values of real-time RT-PCR in patients with COVID-19 pneumonia. A comparison of threshold cycle (Ct) values of real-time reverse transcription-polymerase chain reaction (RT-PCR) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) between critical and non-critical cases. Ct (N1): n = 10 in the non-critical group, n = 7 in the critical group. Ct (N2): n = 11 in the non-critical group, n = 7 in the critical group.

results although nasopharyngeal swab collection was performed by multiple physicians in our hospital. Therefore, our results should be applicable even in a multicenter setting.

In conclusion, the clinical course of COVID-19 is wide ranging, and the use of real-time RT-PCR Ct values as a predictor of critical respiratory failure in patients with COVID-19 pneumonia may be very useful.

Novel findings

Real-time reverse transcription-polymerase chain reaction (RT-PCR) threshold cycle (Ct) values on admission are the most valuable markers to predict coronavirus disease 2019 (COVID-19) prognosis.

Conflict of Interest

Koichi Fukunaga received honoraria from Boehringer Ingelheim and AstraZeneca; other authors have no conflict of interest.

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Appendix. A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.resinv.2020.12.011.

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