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Negative Conversion of Polymerase Chain Reaction and Clinical Outcomes according to the SARS-CoV-2 Variant in Critically Ill Patients with COVID-19

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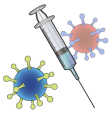
Negative conversion of polymerase chain reaction and clinical outcomes according to the SARS-CoV-2 variant in critically ill patients with COVID-19

Study design
- A single-center, retrospective, case-control study

Patients
- 259 critically ill patients with COVID-19
- 166 (64.1%) received mechanical ventilation
- 85 (32.8%) required rescue therapy
  - Extracorporeal membrane oxygenation: 28
  - Inhaled nitric oxide: 71
  - Prone position: 26

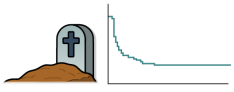
Comparisons
- Baseline characteristics
- Variants
- Medical treatments



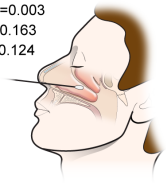
Outcomes
- Mortality
- Negative conversion of polymerase chain reaction (PCR)

RESULTS

Risk factors of survival outcome
- Charlson comorbidity index: hazard ratio 1.555, p<0.001
- Vaccination: hazard ratio 0.492, p=0.033
- Delta variant: hazard ratio 2.469, p=0.002



Risk factors of negative conversion of SARS-CoV-2 PCR
- Delta variant: hazard ratio 0.288, p=0.003
- Vaccination: hazard ratio 2.002, p=0.163
- Remdesivir: hazard ratio 0.526, p=0.124



CONCLUSIONS
The Delta variant of SARS-CoV-2 is associated with lower survival and negative viral conversion. Contrary to expectations, vaccination and remdesivir may have no effect on viral negative conversion in critically ill patients with COVID-19.

Abstract

Background: Coronavirus disease 2019 (COVID-19) is an ongoing global public health threat and different variants of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been identified. This study aimed to analyse the factors associated with negative conversion of polymerase chain reaction (PCR) and prognosis in critically ill patients according to the SARS-CoV-2 variant.

Methods: This study retrospectively analysed 259 critically ill patients with COVID-19 who were admitted to the intensive care unit of a tertiary medical center between January 2020 and May 2022. The Charlson comorbidity index (CCI) was used to evaluate comorbidity, and a negative PCR test result within 2 weeks was used to define negative PCR conversion. The cases were divided into the following three variant groups, ac-

cording to the documented variant of SARS-CoV-2 at the time of diagnosis: non-Delta (January 20, 2020–July 6, 2021), Delta (July 7, 2021– January 1, 2022), and Omicron (January 30, 2022–April 24, 2022).

Results: The mean age of the 259 patients was 67.1 years and 93 (35.9%) patients were female. Fifty (19.3%) patients were smokers, and 50 (19.3%) patients were vaccinated. The CCI (hazard ratio [HR], 1.555; p<0.001), vaccination (HR, 0.492; p=0.033), and Delta variant (HR, 2.469; p=0.002) were significant factors for in-hospital mortality. The Delta variant (odds ratio, 0.288; p=0.003) was associated with fewer negative PCR conversion; however, vaccination (p=0.163) and remdesivir (p=0.124) treatments did not.

Conclusion: The Delta variant of SARS-CoV-2 is associated with lower survival and negative PCR conversion. Contrary to expectations, vaccination and remdesivir may not affect negative PCR conversion in critically ill patients with COVID-19.

Keywords: COVID-19; Critically Ill; Negative Conversion; Survival; Variant

Introduction

Coronavirus disease 2019 (COVID-19) remains a threat to global public health and, in South Korea, cases of critically ill patients with COVID-19 pneumonia are still reported[1]. Since the initial COVID-19 outbreak occurred in late 2019 in Wuhan, Hubei Province, China, mutations of different severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, including Delta and Omicron, have been identified[2]. Vaccination and medications, such as remdesivir and dexamethasone, have been used to limit the effects of the disease[3]; however, the clinical course of the disease varies among patients. Disease severity may be related to the cytokine immune response, and the etiology of critically ill cases such as acute respiratory distress syndrome and multiorgan dysfunction syndrome has been attributed to inflammatory cytokine storm[4].

According to World Health Organization (WHO) guidelines, a positive result of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assays of nasal and pharyngeal swabs plays a key role in diagnosis[5]. In clinical practice, polymerase chain reaction (PCR) tests are repeated during treatment, and a series of PCR results are obtained according to the course of treatment.

A long time from symptom onset to a positive PCR test result was found to be a risk factor for delayed negative PCR conversion regardless of the severity of the disease[6]. Other studies have suggested that older age, female sex, comorbidities, and disease severity are risk factors for prolonged negative PCR conversion[7]. A previous study reported that patients with the Delta variant had lower levels of upper respiratory antiviral immunoglobulin G, which may be associated with increased infectious viral loads compared with the Alpha variant[8]. In South Korea, studies on COVID-19 outcomes in critically ill patients are lacking, and there are scarce reports on the negative conversion of PCR and prognosis according to the SARS-CoV-2 variant. This study analysed the factors affecting survival and negative conversion of PCR and clinical outcomes in critically ill patients with COVID-19, according to the SARS-CoV-2 variant.

Materials and Methods

1. Study design and patients

This was a study analysed the clinical data of 259 patients admitted with COVID-19 pneumonia and treated in the intensive care unit (ICU) of a tertiary medical center in South Korea between January 2020 and May 2022. In this study, factors of PCR negative conversion were analysed with a case-control study design, and the risk of mortality was analysed with a retrospective cohort design. According to the WHO[5], laboratory confirmation of SARS-CoV-2 was defined as a positive result of the RT-PCR assay of nasal and pharyngeal swabs. This study included all patients with a confirmed diagnosis of SARS-CoV-2 infection and hypoxic respiratory failure requiring at least a high-flow nasal cannula (HFNC) or higher levels of respiratory support, including mechanical ventilation (MV) and the clinical significance of PCR negative conversion was analysed (Figures 1, 2). Rescue therapy consisted of higher respiratory support and included extracorporeal membrane oxygenation (ECMO), inhaled nitric oxide (NO), or prone positioning of the patient.

The epidemic periods were divided into three groups based on the documented variant of SARS-CoV-2 at the time of diagnosis: the non-Delta variant group (January 20, 2020–July 6, 2021), the Delta variant group (Delta variant spread period: July 7, 2021–January 29, 2022), and the Omicron variant group (Omicron variant spread period: January 30, 2022–April 24, 2022)[9].

2. Data collection

Data were collected and analysed retrospectively. Information was obtained from electronic medical, prescription, and clinical observation records. The following data were recorded and analysed: (1) demographic characteristics, comorbidities, vaccination status, and COVID-19 diagnosis date; (2) modalities and commencement of MV and rescue therapy; (3) laboratory findings on the day of admission; (4) prescribed medications including remdesivir and steroids; (5) the clinical outcomes of in-hospital mortality or survival; and (6) results and date of repetitive COVID-19 virus PCR. The series of PCR was performed at intervals of 2 to 3 days until the patient was released from quarantine by the in-hospital infection control team during hospitalization. Survival analysis of mortality was performed based on in-hospital mortality.

In our study, the Charlson comorbidity index (CCI) was used to evaluate age and comorbidities of the participants[10,11]. Since age was included in the CCI score, CCI was used as an index for comorbidity including age in risk analysis. All patients were prescribed steroids including dexamethasone, methylprednisolone, or prednisolone. The first PCR result of the negative result within 14 days of diagnosis was used to evaluate the negative conversion of PCR. The period of PCR testing from the diagnosis to the last follow-up during hospitalization differed depending on the period according to the change in quarantine policy. Because PCR was performed at least 2 weeks after diagnosis, regardless of the period of the variant group, negative PCR within 2 weeks was evaluated as a negative PCR conversion.

3. Statistical analysis

Student's t-test was used for the descriptive analysis comparisons. For the analysis of the three groups among the variants, the Bonferroni method was used as a one-way analysis of variance. To evaluate the association between two nominal variables, a chi-square test was performed. Logistic regression analysis was

Figure 1. Flow diagram of the study population. *Negative conversion; patients with negative results within 2 weeks of repeated polymerase chain reaction tests performed after hospitalization. ICU: intensive care unit.

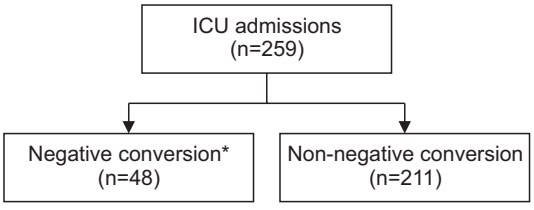
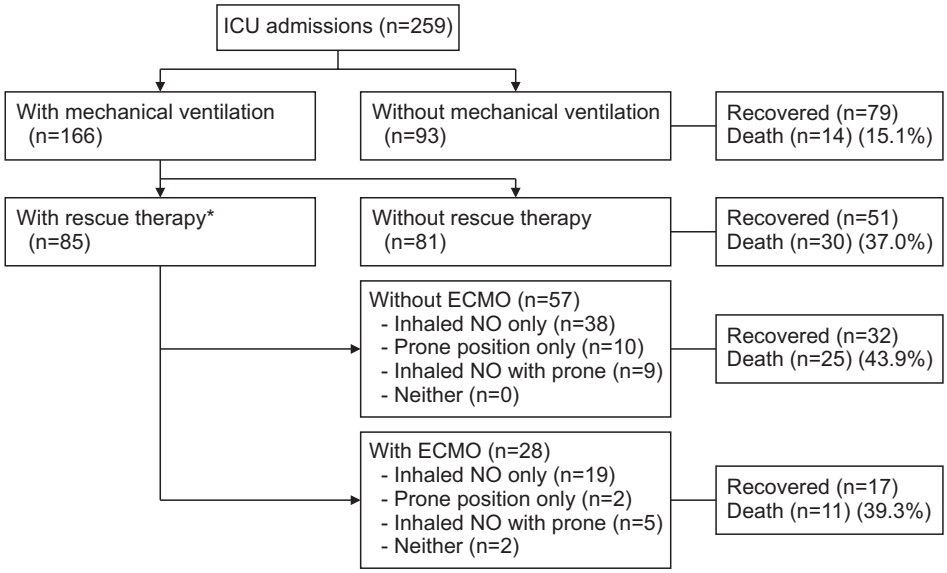


Figure 2. Treatment applied to the study population. *Rescue therapy includes extracorporeal membrane oxygenation (ECMO), inhaled nitric oxide (NO), or prone position. ICU: intensive care unit.



used to evaluate the risk factors of negative PCR conversion. The Cox proportional hazards test was used for the analysis of mortality. All variables including vaccination, remdesivir and the variants in the univariate analysis were entered into the multivariable analysis because they were judged to be factors that could affect the prognosis. Statistical significance was set at $p<0.05$. Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA) and R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

4. Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (IRB No. B-2209-779-104). The study

Table 1. Baseline characteristics of the patients

Characteristic	All patients (n=259)	Negative conversion* (n=48)	Non-negative conversion (n=211)	p-value
Age, yr	67.1±12.5	65.7±12.9	67.4±12.4	0.403
Female sex	93 (35.9)	19 (39.6)	74 (35.1)	0.556
Body mass index, kg/m^2	25.3±4.5	26.5±4.7	25.0±4.4	0.039
Smoking history				0.358
Never smoker	209 (80.7)	41 (85.4)	168 (79.6)	
Ever smoker	50 (19.3)	7 (14.6)	43 (20.4)	
CCI	2.6±1.8	2.2±1.4	2.7±1.7	0.089
Vaccination	50 (19.3)	9 (18.8)	41 (19.4)	0.891
Variant				<0.001‡
Non-Delta	100 (38.6)	31 (64.6)	69 (32.7)	
Delta	139 (53.7)	17 (35.4)	122 (57.8)	
Omicron	20 (7.7)	0 (0.0)	20 (9.5)	
Respiratory support				
High-flow nasal cannula	93 (35.9)	13 (27.1)	80 (37.9)	0.158
MV	166 (64.1)	35 (72.9)	131 (62.1)	
Rescue†	77 (29.7)	13 (27.1)	64 (30.3)	0.657
ECMO	28	8	20	
Inhaled nitric oxide	71	12	59	
Prone position	26	3	23	
Medical treatment				
Remdesivir	214 (82.6)	32 (66.7)	182 (86.3)	0.001
Steroids	259 (100.0)	48 (100.0)	211 (100.0)	
Laboratory results				
C-reactive protein, mg/dL	12.0±8.4	12.4±7.7	11.8±8.5	0.673
White blood cell, 10^9/L	10.4±6.4	9.8±4.5	10.5±6.7	0.467
Hemoglobin, mg/dL	12.7±3.0	13.0±2.0	12.7±3.2	0.498
Creatinine, mg/dL	1.3±1.6	1.0±0.9	1.3±1.8	0.182
Length of stay in the hospital, day	34.6±40.7	39.0±32.6	33.6±42.4	0.402
In-hospital mortality	80 (30.9)	8 (16.7)	72 (34.1)	0.018

Values are presented as mean±standard deviation or number (%).
*Negative conversion; patients with negative results within 2 weeks of repeated polymerase chain reaction tests performed after hospitalization; †Rescue therapy; since two or more types of rescue therapy were applied together to the patients, the frequency of each was indicated separately; ‡Chi-square test was performed between non-Delta and Delta.
CCI: Charlson comorbidity index; MV: mechanical ventilation; ECMO: extracorporeal membrane oxygenation.

conformed to the Declaration of Helsinki (revised edition, 2013). The requirement for informed consent was waived owing to the retrospective nature of the study.

Results

1. Baseline characteristics

The mean±standard deviation patients age was 67.1±12.5 years. Ninety-three patients (35.9%) were female and 50 (19.3%) were smokers. Fifty (19.3%) patients were identified as vaccinated against COVID-19. Regarding the SARS-CoV-2 variant, there were 100 (38.6%) cases of variant non-Delta, 139 (53.7%) cases of variant Delta, and 20 (7.7%) cases of variant Omicron (Table 1). For the variants, CCI of Omicron was higher than non-Delta and Delta variant (non-Delta vs. Delta vs. Omicron, 2.61 vs. 2.26 vs. 4.65), but there was no difference in the application of rescue therapy (Supplementary Table S1). MV was applied to 166 (64.1%) patients, and 93 (35.9%) patients recovered only with HFNC without MV. Rescue therapy was applied to 77 (29.7%), ECMO was used in 28 (10.8%) patients, inhaled NO was administered to 71 (27.4%) patients, and prone positioning was applied in 26 (10.0%) patients. In some cases, two or more types of rescue therapy were applied together to the patient (Figure 2). All participants were prescribed steroids during hospitalization, and 214 (82.6%) patients were prescribed remdesivir. The prescription of remdesivir differed according to the baseline renal function (p=0.023) and variant (p<0.001) (Supplementary Table S2). The average length of hospital stay was 34.6±40.7 days. Comparing the negative conversion within 2 weeks and non-negative conversion, age, sex, smoking history, vaccination, and CCI did not show statistical difference (Table 1). Negative conversion group had high body mass index (BMI) (p=0.039) and in the non-negative conversion, Delta variant was significantly higher than non-Delta (p<0.001) (Table 1). There was no difference in the application of MV and rescue therapy, but administration of remdesivir (p=0.001) was rather higher in the non-conversion group (Table 1).

2. Survival outcomes

In chi-square test, non-negative conversion showed higher mortality than negative conversion (Table 1). In univariate analysis, CCI, BMI, negative PCR conversion, and Delta and Omicron variants were significant factors for survival. In multivariable analysis, CCI (hazard ratio [HR], 1.555; p<0.001), vaccination (HR, 0.492; p=0.033), and Delta variant (HR, 2.469; p=0.002) were still found to be significant factors (Table 2).

3. PCR negative conversion within 2 weeks

In the univariate analysis, negative PCR results within 2 weeks of diagnosis were associated with BMI, remdesivir, Delta, and Omicron variants. However, in the multivariate analysis, only the Delta variant (odds ratio, 0.288; p=0.003) was associated with fewer cases of negative conversion within 2 weeks, but not vaccination (p=0.163) or remdesivir (p=0.124) (Table 3). We calculated the period from the date of diagnosis to the last PCR test and found that non-Delta periods was longer than Delta (p=0.003) and Omicron (p=0.031), while there was no difference between the Delta and Omicron periods (p=1.000) (Supplementary Table S3).

Table 2. Univariable and multivariable Cox regression model for the factors of mortality

Characteristic	Univariable analysis			Multivariable analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Female sex	0.966	0.612–1.524	0.881	0.939	0.561–1.572	0.812
CCI	1.483	1.329–1.655	<0.001	1.555	1.328–1.820	<0.001
Ever smoker	1.434	0.856–2.403	0.171	1.009	0.546–1.867	0.976
BMI, kg/m^2	0.895	0.845–0.947	<0.001	0.961	0.961–0.904	0.203
Vaccination	0.915	0.503–1.666	0.772	0.492	0.257–0.943	0.033
Remdesivir	1.075	0.550–2.103	0.832	0.750	0.396–1.421	0.378
Negative PCR conversion	0.390	0.188–0.810	0.012	0.577	0.271–1.227	0.153
Variant						
Delta	1.943	1.178–3.204	0.010	2.469	1.368–4.457	0.002
Omicron	3.780	1.879–7.602	<0.001	1.029	0.441–2.400	0.947

HR: hazard ratio; CI: confidence interval; CCI: Charlson comorbidity index; BMI: body mass index; PCR: polymerase chain reaction.

Table 3. Logistic regression model for negative conversion within 2 weeks from diagnosis

Characteristic	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value	OR	95% CI	p-value
Female sex	1.213	0.630–2.296	0.557	1.214	0.585–2.522	0.602
CCI	0.835	0.677–1.028	0.089	0.845	0.645–1.107	0.222
Ever smoker	0.667	0.259–1.509	0.361	0.704	0.266–1.863	0.479
BMI, kg/m^2	1.072	1.002–1.148	0.042	1.036	0.955–1.124	0.394
Vaccination	0.946	0.403–2.034	0.891	2.002	0.754–5.314	0.163
Remdesivir	0.319	0.156–0.661	0.002	0.526	0.232–1.192	0.124
Variant						
Delta	0.315	0.161–0.600	<0.001	0.288	0.126–0.657	0.003
Omicron	0.054	0.000–0.414	0.001	0.000	0.000–0.000	0.998

OR: odds ratio; CI: confidence interval; CCI: Charlson comorbidity index; BMI: body mass index.

Discussion

Our hospital specializes in emerging infectious diseases and admits critically ill patients identified by the Korea Centers for Disease Control and Prevention (KCDC) and requiring hospitalization. There may be differences in the number of cases and severity of the disease according to the date of diagnosis and variant. However, there were no differences in the severity of the disease by variant based on rescue therapy in the ICU. The Omicron variant appeared to be associated with poor survival in univariate analysis, but this may be due to the high CCI of the patients. Our study showed that the Delta variant was a significant risk factor for mortality and fewer cases of PCR negative conversion within 2 weeks in critically ill patients with COVID-19 infection.

The academic reason for our study, which analysed how the clinical course could vary depending on the variant, is the characteristics of the Delta variant mentioned in previous literature. The Delta variant B1.617.2 COVID-19 has 23 mutations compared with the first identified Alpha variant[12]. Twelve of these mutations were in the spike protein, which allowed attachment to the host cell. The more mutations in the spike protein, the harder it is to identify and eradicate the virus[13]. The most notable mutations are the L452R and P681R spike protein mutations. One study suggested that the structure of the SARS-CoV-2 spike was bound to the angiotensin-converting enzyme 2 (ACE2) receptor found in many host cells. Therefore, it may help evade vaccine-stimulated antibodies from binding to the spike protein[13-15]. And a previous clinical study suggested that vaccine efficacy against severe COVID-19 may have fallen since the Delta variant. The efficacy of mRNA vaccines after 20 weeks and against newer variants remains to be established[16]. These characteristics of Delta variant mentioned above, it was consistent with our study that the Delta variant itself may be associated with the fewer cases of negative PCR conversion and poor survival outcomes.

Of the negative PCR conversion within 2 weeks, the vaccination and remdesivir did not show a relation but the Delta variant affected less conversion. A previous study suggested that remdesivir had little or no effect on hospitalized patients with COVID-19, as indicated by survival, initiation of ventilation, and duration of hospitalization[17]. Another study also suggested that remdesivir may not be beneficial in shortening the time to negative COVID-19 PCR, and the change in the PCR cycle threshold (Ct) value between the first and second PCR test after beginning remdesivir had also no difference (p=0.516)[18]. In critically ill patients, the effect of the cytokine storm is as important as the viral infection itself[4]. In our study, it was analysed that the less negative PCR conversion in remdesivir treated cases, even though the properties of drug that was antiviral agent. Because the treatment recommendation were not fully established at non-Delta periods including remdesivir, so remdesivir prescription was low in the non-Delta variant. The results of our study, there were fewer negative PCR conversion in the Delta variant in which remdesivir was prescribed more. This finding supported our hypothesis that the Delta variant itself may be a factor involved in the process of negative PCR conversion.

The COVID-19 PCR test was conducted not only for diagnosis but also for infection control. A previous study reported that the transmission of the virus required not the fragments identified by PCR, but com-

plete live viruses[19] with infectious potential declining after day 8 even with ongoing high viral loads[19]. The relationship between viral load and outcome is controversial, with evidence suggesting that viral load is associated with poor prognosis[20,21], although evidence is conflicting[22]. A further study suggested that during a PCR positive period, a second negative PCR result was associated with a lower probability of developing severe COVID-19 compared to a positive result[23]. In our study, the Delta variant was associated with fewer cases of negative PCR conversion, which may be associated with poor outcomes in critically ill COVID-19 patients.

This study has some limitations. First, the study was a retrospective observational study at a single center, and the patients were distributed according to the KCDC criteria, and the number and severity of admitted patients may differ for each variant. Therefore, multicentre studies are required to evaluate the impact of factors related to survival, including variants, vaccination, and remdesivir use. Second, because there was no genetic analysis of the SARS-CoV-2 variant, the classification of patients was based on the dominant SARS-CoV-2 variant at the time of diagnosis and this may be incorrect. However, according to previously published research, we considered that variants such as Alpha and Epsilon appeared during the period in which COVID-19 spread across South Korea; and that the Delta and Omicron variants accounted for the majority of cases during the Delta variant and Omicron spread periods, respectively[9]. Third, there may be limitations in analysing the impact of vaccination because of a lack of information on the type of vaccine, whether additional vaccinations, and the date of the last vaccination before the diagnosis of COVID-19. Fourth, the PCR Ct value could not be analysed routinely in our center, and there was a limitation in the quantitative analysis of virus titer. So instead of the Ct value, we inevitably used the PCR negative result within 2 weeks from diagnosis to evaluate the virus negative PCR conversion.

Notwithstanding these limitations, our study is one of the few studies conducted on critically ill patients with COVID-19 that evaluated the factors involved in COVID-19 PCR negative conversion and prognosis according to the variants observed in Korean patients. This study showed that comorbidity status, non-vaccinated status, and variants of the Delta were factors increasing the mortality risk in these patients; however, vaccination and remdesivir use were not related to virus negative conversion, and the Delta variant itself may be a factor accounting for the poor rates of negative conversion. Future research should focus on well-designed studies of clinical outcomes analysed with a quantitative titer of COVID-19, on variants according to genetic analysis, and medical treatments including vaccination.

In conclusion, the Delta variant of SARS-CoV-2 is associated with lower survival and negative viral conversion. Contrary to expectations, vaccination and remdesivir may have no effect on viral negative PCR conversion in critically ill patients with COVID-19.

Authors' Contributions

Conceptualization: Kim TH, Song MJ, Lim SY, Lee YJ, Cho YJ. Methodology: Ji E, Lee YJ, Cho YJ. Formal analysis: Kim TH, Ji E, Song MJ. Data curation: Kim TH. Investigation: Song MJ, Lim SY, Lee YJ. Writing - original draft preparation: Kim TH. Writing - review and editing: Kim TH, Ji E, Song MJ, Lim SY, Lee YJ, Cho YJ. Approval of final manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Supplementary Material

Supplementary material can be found in the journal homepage (http://www.e-trd.org).

Supplementary Table S1. Comorbidities and application of rescue therapy according to the variant.

Supplementary Table S2. Remdesivir prescriptions and baseline creatinine levels.

Supplementary Table S3. Period of tracking the COVID-19 PCR test from the date of diagnosis.

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