Fatal outcome of Severe Fever With Thrombocytopaenia Syndrome (SFTS) and Severe and Critical COVID-19 is associated with the hyperproduction of IL-10 and IL-6 and the low production of TGF-β

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Abstract

Severe fever with thrombocytopaenia syndrome virus (SFTSV) and Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can cause the hyperproduction of inflammatory cytokines, which have pathological effects in patient including severe or fatal cytokine storms. To characterize the effect of SFTSV and SARS-CoV-2 infection on the production of cytokines in SFTS and COVID-19 patients, we performed an analysis of cytokines in SFTS and COVID-19 patients and also investigated the role of IL-10 *in vitro* studies: LPS-induced THP-1-derived macrophages, SFTSV infection of THP-1 cells, and SARS-CoV-2 infection of THP-1 cells. In this study, we found that levels of both IL-10 and IL-6 were significantly elevated, the level of TGF-β was significantly decreased and IL-10 was elevated earlier than IL-6 in severe and critical COVID-19 and fatal SFTS patients, and inhibition of IL-10 signalling decreased the production of IL-6 and elevated that of TGF-β. Therefore, the hyperproduction of IL-10 and IL-6 and the low production of TGF-β have been linked to cytokine storm-induced mortality in fatal SFTS and severe and critically ill COVID-19 patients and that IL-10 can play an important role in the host immune response to severe and critical SARS-CoV-2 and fatal SFTSV infection.

1. INTRODUCTION

Severe fever with thrombocytopaenia syndrome virus (SFTSV, officially named *Dabie bandavirus*) an emerging tick-borne virus in Asia, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and avian influenza A (H5N1) viruses can cause severe and often fatal disease that is characterized by hyperinflammation with features of cytokine storm.[1-5]

The cytokine storm is a common pathogenic characteristic, namely, imbalanced immune responses with an exaggerated inflammatory cytokine reaction, excessive activation of immune cells, and life-threatening systemic inflammatory syndromes, which can cause serious pathological changes and result in multiorgan dysfunction.[1-11]

Interleukin 6 (IL-6) is a proinflammatory cytokine with pivotal roles in inflammation and a key cytokine in cytokine syndrome-induced mortality.[5-11]

IL-10 is a regulatory cytokine with pleiotropic roles in the immune system and is known to be an important immunoregulatory cytokine.[12] However, fatal severe fever with thrombocytopaenia syndrome (SFTS) and severe and critical coronavirus disease 2019 (COVID-19) had significantly higher serum levels of IL-10 than those of patients with mild to moderate and nonfatal illness; in addition, systemic hyperproduction of both IL-6 and IL-10 can generate a cytokine storm, which contributes to their pathology and is more strongly correlated with the outcome of death.[5. 7. 9.10]

Transforming growth factor-β (TGF-β) is a regulatory cytokine with pivotal functions in the control of inflammation. SARS-CoV-2 induces a TGF-β-dominated chronic immune response in severe COVID-19 while TGF-β was downregulated in SFTS patients compared with healthy controls.[12-15]

In this study, we found that the levels of IL-6 and IL-10 were significantly higher and produced at robust levels in fatal SFTS patients and severe and critically ill COVID-19. In contrast, TGF-β was significantly lower in fatal SFTS and severe and critical COVID-19 patients than in patients with nonfatal SFTS patients and mild to moderate COVID-19. Namely, elevated levels of IL-6 and IL-10 and decreased levels of TGF-β have been linked to severe inflammation and fatality SFTS and in COVID-19 patients.

We also found that IL-10 is elevated earlier than IL-6 and TGF-β, and the blocking of IL-10 signalling using an antibody against the IL-10 receptor can reduce IL-6 production and increase TGF-β production in LPS-induced, SFTSV and SARS-CoV-2 -infected immune cells, respectively, suggesting that IL-10, IL-6 and TGF-β may contribute to disease severity in SFTS COVID-19 and patients.

Therefore, IL-10 plays an important role in the host immune response to severe and critical SARS-CoV-2 and fatal SFTSV infection, and these results demonstrated that targeting IL-10 signalling using a monoclonal antibody against the IL-10 receptor is a potential immune-based intervention against fatal SFTS and severe and critically ill COVID-19 disease.[7, 10]

2. MATERIAL and METHODS

2.1 SFTS patients

We performed a retrospective study on eligible patients with SFTS from May 2013 to April 2022. During the study period, 84 patients were confirmed to be positive for partial small (S) and large (L) segments of SFTSV RNA using real-time PCR.[16]

Of these patients, 65 confirmed patients were analysed in the present study (**Supplemental table 1**). The study was approved by the Institutional Review Board (IRB) at the Jeju National University Hospital (IRB file no. 2021-03-012) and the study design and baseline characteristics of SFTS patients are available in the**Supplemental Data 1** .

2.2 COVID-19 patients

We performed a retrospective study on eligible patients with COVID-19 from August 2020 to July 2021.

During the study period, 188 confirmed patients were admitted, and 109 of these patients were analysed in the present study (**Supplemental table 2**). The study was approved by the Institutional Review Board (IRB) at the Jeju National University Hospital (IRB file no. 2020-10-019), and the study design and baseline characteristics of COVID-19 patients are available in the**Supplemental Data 2** .

2.3 Analyses of cytokines in SFTS and COVID-19 patients

To characterize the effect of SFTSV and SARS-CoV-2 infection on the production of serum cytokines in SFTS and COVID-19 patients, Interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17A, interferon-γ (IFN-γ), and tumour necrosis factor (TNF-α) were measured using human Th1/Th2/Th17 CBA kits (BD Bioscience, San Diego, CA) according to the manufacturer's instructions, with minor modifications. Sample acquisitions were

performed with a FACS Canto II flow cytometer and analysed by FCAP Array software version 3.0 (BD Bioscience). All statistical analyses were performed using SPSS 22.0 (SPSS, an IBM Company). TGF-β was measured in the collected serum using a TGF-β-1 Human ELISA Kit (Thermo Fisher Scientific) according to the manufacturer's protocols.

To compare the mean difference between patients with fatal and nonfatal SFTS disease and between patients with severe and critical and mild to moderate COVID-19 disease, we usually used a two-sample t-test. When using this method, we checked some assumptions, such as normality, equal variance, and independence. When these assumptions were not met, we used a nonparametric two-sample t-test called the Wilcoxon-Mann-Whitney test.[7] P values$<$0.05 indicated statistical significance.

2.4 THP-1 cells, SFTSV, and SARS-CoV-2

The human monocytic cell line THP-1 (ATCC TIB-202) was used to model macrophages. THP-1 cells were cultured in RPMI-1640 medium (Gibco) supplemented with 10% foetal bovine serum (FBS) (Gibco), 1% penicillin-streptomycin (Gibco), 200 mM L-Glutamin (Gibco) and 55 mM 2-mercaptoethanol (Gibco) and kept in a humidified 5% CO_2 incubator at 37°C.

THP-1 cells were differentiated into a macrophage phenotype at a density of 2-4 $\times$ 10^5 cells/mL in 100mm Cell Culture Dish (Corning Ins.), treated with 100 ng/mL phorbol myristate acetate (PMA) (Sigma-Aldrich) for 24 hours (h), washed and suspended in culture medium without PMA.

SFTSV (GenBank accession no. MN329148-MN329150) was isolated from a Korean SFTS patient. The virus was propagated and titrated in Vero E6 cells (ATCC CRL-1586), which were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% FBS.

SARS-CoV-2 /BA.1.1 was isolated from a nasopharyngeal swab taken from a patient with COVID-19. The virus was propagated and titrated in Vero E6 cells (ATCC CRL-1586), which were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 2% FBS and 1% penicillin-streptomycin (Gibco).[17]

2.5 LPS-induced THP-1-derived macrophages to investigate the role of IL-10 and IL-6.

THP-1 cells were divided into three different treatment groups: THP-1 cells treated with LPS (2 μg/mL) (Sigma-Aldrich) as the control group; cells treated with LPS (2 μg/mL) (Sigma-Aldrich) and IL-6R polyclonal antibody (10 μg/mL) (Invitrogen); and cells treated with LPS (2 μg/mL, Sigma-Aldrich, St Louis, USA) and IL-10RA polyclonal antibody (10 μg/mL) (Invitrogen).

The three different treatment groups were stimulated for 6, 12, 24 and 48 h, and the levels of IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ, and TNF-α were measured in the collected supernatants using human Th1/Th2/Th17 CBA kits (BD Bioscience) according to the manufacturer's instructions, with minor modifications. Sample acquisitions were performed with a FACS Canto II flow cytometer and analysed by FCAP Array software version 3.0 (BD Bioscience). TGF-β was measured in the collected supernatants using a TGF-β-1 Human ELISA Kit (Thermo Fisher Scientific) according to the manufacturer's protocols.[7]

2.6 Investigating the role of IL-10 in SFTSV infected THP-1-derived macrophages.

To investigate the role of IL-10 in SFTSV-infected THP-1 cells, THP-1 cells were infected with SFTSV at a MOI of 1, and SFTSV-infected THP-1 cells were divided into two different treatment groups: SFTSV-infected THP-1 cells were used as the control group, and SFTSV-infected THP-1 cells were treated with the IL-10RA polyclonal antibody (10 μg/ml) (Invitrogen). The two different treatment groups were incubated for 6, 12, 24 and 48 h, and the levels of IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α, and IFN-γ were measured using human Th1/Th2/Th17 CBA kits (BD Bioscience) according to the manufacturer's instructions, with minor modifications. Sample acquisitions were performed with a FACS Canto II flow cytometer and analysed by FCAP Array software version 3.0 (BD Bioscience). TGF-β was measured in the collected supernatants using a TGF-β-1 Human ELISA Kit (Thermo Fisher Scientific) according to the manufacturer's protocols.[7, 18]

3. RESULTS

3.1 Λεελς οφ σερυμ ΙΛ-6, ΙΛ-10, ανδ ΤΓΦ-β ιν ΣΦΤΣ ανδ ῞Ο῞ΙΔ-19 πατιεντς.

Among SFTS patients, serum IL-6 and IL-10 concentrations in those with fatal disease were significantly higher than those in patients with nonfatal disease, and TGF-β concentrations in the former were significantly lower than those in the latter during the initial clinical course of hospitalization (**Figure 1** and **Supplemental table 3**).

However, there were no statistically significant differences in serum levels of IL-2, IL-4, IL-17A, IFN-γ, and TNF-α between patients with fatal disease and those with nonfatal disease (**Supplemental figure 1** and **Supplemental table 3**).[7]

In COVID-19 patients, serum IL-6 and IL-10 concentrations in patients with severe and critical disease were significantly higher than those in patients with mild to moderate disease, and TGF-β concentrations in patients with severe and critical disease were significantly lower than those in patients with mild to moderate disease during the initial clinical course of hospitalization (**Figure 2** and**Supplemental table 4**).

However, similar to the results of SFTS patients, there were no statistically significant differences in plasma levels of IL-2, IL-4, IL-17A, IFN-γ, and TNF-α between patients with mild to moderate and severe/critical disease (**Supplemental figure 2** and**Supplemental table 4**).

In this study, we found that the levels of serum IL-6, IL-10, and TGF-β were significantly associated with the outcomes of patients with SFTSV and SARS-CoV-2 (**Figures 1** and**2**).[7, 9, 10, 14, 15].

3.2 Κινετιςς οφ ΙΛ-6, ΙΛ-10, ανδ ΤΓΦ-β ιν ΣΦΤΣ ανδ ῞Ο῞ΙΔ-19 πατιεντς.

We studied the kinetics of the IL-6, IL-10, and TGF-β levels in patients with nonfatal SFTS and severe COVID-19 patients, and the results showed that IL-10 is elevated earlier than IL-6 (**Tables 1** and**2**).[7] In contrast, TGF-β is decreased later (**Tables 1** and **2**).

Therefore, we studied the correlation between IL-6, IL-10, and TGF-β in LPS-induced THP-1-derived macrophages treated with IL-6R and IL-10RA polyclonal antibodies. The results showed that the level of IL-6 was decreased and that of TGF-β increased when we treated with the IL-10RA polyclonal antibody (**Figure 3** and **Supplemental table 5**).

However, there were no significant differences between IL-6, IL-10, and TGF-β in LPS-induced THP-1-derived macrophages treated with the IL-6R polyclonal antibody (**Figure 3** and **Supplemental table 5**).

We also treated THP-1-derived macrophages infected with SFTSV and SARS-CoV-2 with the IL-10RA polyclonal antibody to determine the role of IL-10 in SFTSV and SARS-CoV-2 infection and the results were similar to those for LPS-induced THP-1-derived macrophages: the level of IL-6 was decreased and that of TGF-β increased (**Figures 4** and **5**and **Supplemental table 6 and 7**).

4. DISCUSSION

Fatal SFTS and severe and critically ill COVID-19 patients develop a pathological state termed cytokine release syndrome.[1, 3-11]

Cytokine release syndrome can be triggered by infections and is characterized by rapid and prolonged systemic elevation of inflammatory cytokines and chemokines, and IL-6 is a proinflammatory cytokine and a key cytokine in cytokine release syndrome-induced mortality.[1, 3-11]

Serum IL-10 is an important anti-inflammatory cytokine.[12] However, the serum IL-10 concentration was significantly higher in fatal SFTS, severe and critically ill COVID-19, and H5N1 patients and, like IL-6, can predict poor outcomes in SFTS and COVID-19 patients (**Figures 1** and**2**).[5, 7, 9, 10]

In this study, we found that TGF-β concentrations were significantly lower in fatal SFTS and severe and critical COVID-19 patients (**Figures 1** and **2**). Namely, the hyperproduction of IL-6 and IL-10, which is a feature of cytokine storms, and the low production of TGF-β have been linked to cytokine storm-induced mortality in fatal SFTS and severe and critically ill COVID-19 patients (**Figures 1**and **2**).

Furthermore, IL-10 was elevated earlier than IL-6, and TGF-β was decreased later than IL-10 in SFTS and COVID-19 patients (**Tables 1** and **2**).

When we blocked IL-10 signalling using an antibody against the IL-10 receptor, the production of IL-6 was decreased, and the production of TGF-β was increased (**Figures 3, 4, and 5** and**Supplemental table 5, 6,** and **7**).

IL-10 is usually known as an anti-inflammatory cytokine [12]. However, IL-10 can also be an immune-activating and proinflammatory cytokine in some autoimmune diseases, cancers, and severe and critically ill COVID-19 patients. Patients with fatal SFTS and H5N1 present with dramatically elevated serum IL-10 concentrations that correlate with disease severity.[5, 7, 9-11]

Fatal SFTS and severe and critically ill COVID-19 patients present with dramatically elevated serum levels of IL-10 and IL-6 and dramatically decreased serum levels of TGF-β that correlate with disease severity.

When we blocked the signal of IL-10 using an antibody against the IL-10 receptor, IL-6 was decreased and TGF-β was elevated in the THP-1-cell study (**Figures 3-5**).

Therefore, we suggest that IL-10 can induce the production of IL-6 and inhibit the production of TGF-β in cytokine storms and might play a pathological role in SFTS and COVID-19 disease progression and also propose that IL-10 may be a potential target for reducing SFTS and COVID-19 mortality.

5. CONCLUSION

In conclusion, our findings demonstrated that IL-10 is a potential target for the treatment of SFTSV and SARS-CoV-2-related immunopathology.

Therefore, blockade of IL-10 signalling using monoclonal antibodies against the IL-10 receptor is a promising therapeutic for treating fatal SFTS and severe and critically ill COVID-19 patients.[7, 10]

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Figure legends

Figure 1. The serum levels of IL-6, IL-10, and TGF-β in SFTS patients with nonfatal and fatal diseases. The serum concentrations of IL-6, IL-10, and TGF-β from SFTS patients (total 65; nonfatal (n =58) and fatal (n =7)) were analysed immediately after hospital admission. Each dot shows the cytokine concentration in an individual, and the horizontal bars indicate the respective group median.

Figure 2. The serum levels of IL-6, IL-10, and TGF-β between mild to moderate patients and severe and critically ill COVID-19 patients. The serum concentrations of IL-6, IL-10, and TGF-β from COVID-19 patients (total 109; mild (n =40), moderate (n =40) and severe (n =27) and critical (n =2)) were analysed immediately after hospital admission. Each dot shows the cytokine concentration in an individual, and the horizontal bars indicate the respective group median.

Figure 3. The lipopolysaccharide (LPS)-induced IL-6 concentration in THP-1 cells is suppressed by the IL-10RA polyclonal antibody, and the LPS-induced TGF-β concentration in THP-1 cells is induced by the IL-10RA polyclonal antibody. Human monocyte THP-1 cells were treated with LPS (10 μg/mL), LPS plus IL-10RA polyclonal antibody (10 μg/mL) or LPS plus IL-6R polyclonal antibody for 6, 12, 24 and 48 h,

and the levels of IL-6 and IL-10 were measured using human Th1/Th2/Th17 CBA kits (BD Bioscience, San Diego, CA). TGF-β was measured in the collected supernatants using a TGF-β-1 Human ELISA Kit (Thermo Fisher Scientific) according to the manufacturer's protocols [10].

Figure 4. IL-6 concentration in SFTSV-infected THP-1 cells is suppressed by IL-10RA polyclonal antibody and TGF-β concentration in SFTSV-infected THP-1 cells is induced by IL-10RA polyclonal antibody. Human monocyte THP-1 cells were infected with SFTSV with IL-10RA polyclonal antibody for 6, 12, 24 and 48 h, and the levels of IL-6 and IL-10 were measured using human Th1/Th2/Th17 CBA kits (BD Bioscience, San Diego, CA). TGF-β was measured in the collected supernatants using a TGF-β-1 Human ELISA Kit (Thermo Fisher Scientific) according to the manufacturer's protocols [10].

Figure 5. IL-6 concentration in SARS-CoV-2-infected THP-1 cells is suppressed by IL-10RA polyclonal antibody and TGF-β concentration in SARS-CoV-2-infected THP-1 cells is induced by IL-10RA polyclonal antibody. Human monocyte THP-1 cells were infected with SARS-CoV-2 or SARS-CoV-2 with IL-10RA polyclonal antibody for 6, 12, 24 and 48 h, and the levels of IL-6 and IL-10 were measured using human Th1/Th2/Th17 CBA kits (BD Bioscience, San Diego, CA). TGF-β was measured in the collected supernatants using a TGF-β-1 Human ELISA Kit (Thermo Fisher Scientific) according to the manufacturer's protocols [10].

Supplemental Figure 1. The levels of IL-2, IL-4, IL-17A, IFN-γ, and TNF-α between nonfatal patients and fatal SFTS patients. The serum concentrations of IL-2, IL-4, IL-17A, IFN-γ, and TNF-α from SFTS patients were analysed immediately after hospital admission. Each dot shows the cytokine concentration in an individual, and the median with its range is presented. *The concentrations of IFN-γ and TNF-α in all fatal patients were similar to those in nonfatal patients except one fatal patient. This fatal patient had the highest concentration of IFN-γ (3258 pg/mL) and TNF-α (51.3 pg/mL). IL-10 (145 pg/mL) and IL-6 (4106 pg/mL) levels in this fatal patient were the highest, and TGF-β (51.3 pg/mL) levels were lower than those in nonfatal patients. Therefore, the IFN-γ and TNF-α data of this fatal patient were not included in the IFN-γ and TNF-α data shown here.

Supplemental Figure 2. The levels of IL-2, IL-4, IL-17A, IFN-γ, and TNF-α between mild to moderate patients and severe and critically ill COVID-19 patients. The serum concentrations of IL-2, IL-4, IL-17A, IFN-γ, and TNF-α from COVID-19 patients were analysed immediately after hospital admission. Each dot shows the cytokine concentration in an individual, and the median with its range is presented.

Supplemental data 1

Study design and SFTS patients

We performed a retrospective study on patients with severe fever with thrombocytopaenia syndrome at Jeju National University Hospital in Jeju Island, South Korea. This region had the highest incidence of SFTS in South Korea. The study was approved by the Institutional Review Board (IRB) at the Jeju National University Hospital (IRB file no. 2021-03-012).

Patients were admitted for confirmed SFTS during May 2013-April 2022, and these patients were eligible to participate in the study. During the study period, a total of 84 patients were confirmed to be positive for small (S) and large (L) segments of SFTSV RNA using real-time RT-PCR, based on the recommended diagnosis method in South Korea. Of these confirmed patients, 65 were analysed in the present study.

The demographic and clinical characteristics data were obtained from the electronic medical records and included the patient demographics, activity at the time of exposure, history of tick bites, presence of initial symptoms, vital signs, past medical history, CCI, multiple organ dysfunction score (MODS) during hospitalization and after 72 h of treatment (inotropes, intubation, renal replacement therapy, and plasma exchange), disposition after hospital admission, and 30-day mortality. The patients' blood samples were collected at the first visit to the hospital and then at regular intervals (every two days in the acute phase, twice a week in the recovery phase during hospitalization, and every 2 to 3 months after discharge).

Patients were divided into the severe group (SG) and nonsevere group (NSG). The SG comprised patients with three or more organ dysfunctions as defined by Sequential Organ Failure Assessment (SOFA) score including the following: for the lung a PaO2/FiO2 <400 or patients requiring mechanical ventilation; for the liver a serum bilirubin [?]1.2 mg/dL; for the kidney a serum creatinine [?]1.2 mg/dL, urine output <500 mL/day or patients requiring haemodialysis; for the cardiovascular system a mean arterial pressure <70 mmHg or patients requiring vasopressor; and for the central nervous system a Glasgow Coma Scale <15, or deceased patients (reference https://doi.org/10.3947/ic.2022.0073.).

Baseline characteristics of patients with SFTS

A total of 65 patients were included in the study. Table 2 shows the demographic and baseline clinical characteristics of the patients with SFTS. Of the patients, 37 (56.9%) were male, and the mean age was 63.7 ± 14.0 years. All patients were inhabitants of Jeju Island. The mean CCI was very low at 0.5 ± 0.8. Most patients (85%) were exposed to the virus through occupational and outdoor activities, with agricultural activities being the most common route of exposure. Some patients did not know whether they had been bitten by ticks. Most cases occurred in summer, followed by autumn. The mean time from symptoms to diagnosis of SFTS was 5.8 ± 3.3 days, and most cases were confirmed within two days of admission. The mean initial MODS was 2.7 ± 2.5, and the mean SFTS viral load was 82,115,042 ± 643,558,056. The 30-day mortality rate was 13.4%, and 30 patients (46.2%) were admitted to the intensive care unit. Initial laboratory findings revealed neutropaenia, thrombocytopaenia, increased liver enzymes, increased creatine kinase (CK), lactate dehydrogenase (LDH), and activated partial thromboplastin time (aPTT).

In the comparison of the recovered group and death group with SFTS, male patients and older ages showed a tendency for inclusion in the death group. There were no significant differences in comorbidities, exposure type, tick bite history, or seasonal occurrence in either group. However, the mortality of SFTS showed an increasing trend in the spring season. A higher SFTS viral load and MODS were associated with the death group more than with the recovered group. Vasopressor, mechanical ventilation, and CRRT were applied in the death group. There were no significant differences in the application of therapeutic plasma exchange, immunoglobulin, or anti-IL-6 antagonist between the groups. Most fatal cases were deceased within one week of admission.

The initial levels of neutropaenia and thrombocytopaenia could not distinguish whether SFTS patients would be included in the recovered group or death group. However, liver enzymes, aPTT, CK and LDH were significantly different between the two groups.

Supplemental data 2

Study design and COVID-19 patients

We performed a retrospective study on patients with COVID-19 at Jeju National University Hospital in Jeju Island, South Korea. This hospital was designated the national negative pressure isolation ward responsible for treating patients with high-risk COVID-19 in South Korea. The study was approved by the Institutional Review Board (IRB) at the Jeju National University Hospital (IRB file no. 2020-10-019).

Patients admitted from August 2020 to July 2021 and who had confirmed SARS-CoV-2 infection, were eligible to participate in the study. During the study period, 430 cases were confirmed to be positive for SARS-CoV-2 using real-time reverse transcriptase polymerase chain reaction (RT-PCR) on Jeju Island. Of these confirmed patients, 188 patients were admitted to our hospital, and 109 were analysed in the present study (Table 1).

Demographic and clinical characteristic data included the patients' demographics (age, sex, and body mass index), past medical history and comorbidity index score (CCI), source of exposure risk to SARS-CoV-2, symptom presentation, type of oxygen therapy, hospital treatment (inotropes, extracorporeal membrane oxygenation), duration of hospitalization, and mortality. Laboratory variables confirmed in the Laboratory Department of Jeju National University Hospital were retrospectively obtained from the registry database.

The severity of illness was classified into the following five groups according to the National Institute of Health's criteria based on the patient's worst condition during hospitalization: asymptomatic, mild, moderate, severe, and critical [Reference: National Institutes of Health (NIH). Clinical spectrum of SARS-CoV-2 infection. Available at: https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/. Accessed 25 June, 2022].

Baseline characteristics of COVID-19 patients

A total of 109 patients were included in the study. Table 1 shows the demographic and baseline characteristics of the patients according to the severity of COVID-19. Of all patients, 40 (32.5%) had mild disease, 40 (17.5%) had moderate disease, 27 (45%) had severe disease, and 2 (5%) had critical disease. The mean age of the patients was 51.1 ± 19.9 years, and 53 (48.6%) were male. No patients were vaccinated because COVID-19 vaccination was not introduced in South Korea during the study period. The mean body mass index (BMI) of the patients was 24.7 kg/m^2. The mean Charlson Comorbidity Index (CCI) was as low as 1.8 ± 1.8. The median time from symptom onset to SARS-CoV-2 RT-PCR testing was 1 day, and the mean SARS-CoV-2 RT-PCR cycle threshold (Ct) value was 22.0 +- 6.8. The type of patient oxygen supplementation received by patients in the study was as follows: 82 (75.2%) received no oxygen supplementation, 21 (19.4%) received low-flow nasal cannula therapy and a facial mask with a reservoir, 4 (3.7%) received high-flow nasal cannula therapy, and 1 (0.9%) received invasive mechanical ventilation. Hydroxychloroquine was administered in 1 (0.9%), lopinavir/ritonavir in 21 (19.4%), remdesivir in 18 (16.7%), and corticosteroids in 28 (25.9%) patients. Total case fatalities were 0.9%, and the mean number of admission days was 12.5 +- 5.2. In the comparison of the mild/moderate and severe and critical groups, an older age, a higher mean BMI, and a higher CCI were associated with the severe/critical group rather than with the mild/moderate group. There was no significant differences in the mean SARS-CoV-2 RT-PCR Ct value between the mild/moderate and severe/critical groups. High flow and invasive mechanical ventilation, lopinavir/ritonavir, remdesivir, and steroids were administered in the severe/critical group compared with the mid/moderate group. There were no significant differences in the laboratory results between the groups. However, C-Reactive Protein (CRP) was higher in the severe/critical group than in the mild/moderate group. Comparisons of laboratory findings between the groups are presented in Table 1. Only fatal patients were included in the severe/critical group.

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