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SHORT COMMUNICATION

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Downregulation of type III interferons in patients with severe COVID-19

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Abstract

Coronavirus disease 2019 (COVID-19) is globally rampant, and to curb the growing burden of this disease, in-depth knowledge about its pathophysiology is needed. This was an observational study conducted at a single center to investigate serum cytokine and chemokine levels of COVID-19 patients, based on disease severity. We included 72 consecutive COVID-19 patients admitted to our hospital from March 21 to August 31, 2020. Patients were divided into Mild-Moderate I (mild) and Moderate II-Severe (severe) groups based on the COVID-19 severity classification developed by the Ministry of Health, Labor and Welfare (MHLW) of Japan. We compared the patient characteristics as well as the serum cytokine and chemokine levels on the day of admission between the two groups. Our findings indicated that the severe group had significantly higher levels of serum fibrinogen, D-dimer, lactate dehydrogenase, C-reactive protein, ferritin, Krebs von den Lungen-6, surfactant protein (SP)-D, and SP-A than the mild group. Strikingly, the levels of interleukin (IL)-28A/interferon (IFN)-λ2 were significantly lower in the severe group than in the mild group. We believe that reduced levels of type III interferons (IFN-λs) and alterations in the levels of other cytokines and chemokines may impact the severity of the disease.

KEYWORDS

chemokine, COVID-19, cytokine, IFN-lambda, SARS-CoV-2, type III interferon

1 | INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) is globally rampant, and more than a billion people to become infected and two million deaths worldwide in less than a year since the first case was identified. However, the pathogenesis of COVID-19 is not fully understood. Because of the high risk of exposure to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), bronchoscopy is not recommended.[1] Therefore, reliable diagnostic and prognostic biomarkers are urgently required. In addition, the biomarkers are likely to lead to new therapeutic targets.

Among the several proposed biomarkers, the newly discovered type III interferons (IFNs), also known as IFN-λs, has been reported to be restrictively produced by airway epithelial cells, hepatocytes, and type 2

Abbreviations: COVID-19, coronavirus disease 2019; IFN, interferon; IL, interleukin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SP, surfactant protein; TNF-α, tumor necrosis factor-α.

myeloid dendritic cells in response to viral infection, and it plays a role in eliminating and limiting the viral load.[2,3] In the previous report, experiments using a mouse model of acute influenza A infection demonstrated that intranasal IFN-λ2/3 administration reduced the viral load.[4] Furthermore, in a study using samples of COVID-19 nasopharyngeal swabs, the expression of type III IFNs, especially IFN-λ2,3, was decreased by SARS-CoV-2 infection.[5] IFN-λs might, therefore, play a fine-tuning role in providing immunity against viruses and may contribute to the anti-cytokine storm in COVID-19.[4] However, this has not been proven by experiments with serum samples of COVID-19.

In this study, we evaluated the profiles of COVID-19 patients and their levels of serum cytokines and chemokines based on the disease severity, to discover new targets, especially type III IFNs.

2 | MATERIALS AND METHODS

This observational study was conducted at the Showa University Hospital in Japan. We included 72 consecutive COVID-19 patients admitted to the hospital between March 21 and August 31, 2020, who were naive to specific treatments for COVID-19, including remdesivir or favipiravir.[6,7] The included patients had positive polymerase chain reaction (PCR) test results for COVID-19 from pharyngeal swabs. Disease severity was determined based on the COVID-19 severity classification developed by the Ministry of Health, Labor, and Welfare (MHLW) of Japan (Table S1).[8] Based on this classification, COVID-19 can be categorized as mild ($n = 19$), moderate I ($n = 31$), moderate II ($n = 7$), and severe ($n = 15$), according to the presence or absence of symptoms, clinical status, and percutaneous oxygen saturation. We divided the patients into the Mild-Moderate I (mild) and Moderate II-Severe (severe) groups and compared their background characteristics and laboratory findings as well as serum cytokine and chemokine profiles. Blood examinations were performed on the first day of hospitalization. The following serum cytokines and chemokines were measured: IL-29/interferon (IFN)-λ1, IL-28A/IFN-λ2, IL-28B/IFN-λ3, IFN-α, IFN-β, IFN-γ, IL-6, and tumor necrosis factor (TNF)-α. The serum concentrations of cytokines and chemokines were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems). The method of ELISA was according to the manufacturer's protocol.

Statistical analysis was performed using EZR (Saitama Medical Center, Jichi Medical University). All data are presented as median (range) or number (percentage). The Fisher's exact test and Mann–Whitney *U* test were used to measure the categorical and quantitative variables, respectively. Points that exceeded 1.5 times the upper and lower quantile range were excluded as outliers. *p*-values<0.05 were considered statistically significant.

3 | RESULTS

Patients in the severe group were significantly older. The symptoms with statistically significant differences between the two groups were dyspnea and loss of taste or smell. Patients in the severe group had significantly higher levels of lymphocyte count, fibrinogen, D-dimer, urea nitrogen, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, potassium, C-reactive protein, ferritin, Krebs von den Lungen-6, surfactant protein (SP)-D, and SP-A, along with significantly lower levels of serum hemoglobin, total protein, albumin, uric acid, and sodium when compared to that of patients in the mild group (Table 1).

Figure 1 shows the serum levels of cytokines including IFNs on Day 1. Levels of IL-28A/IFN-λ2 were significantly lower in the severe group than in the mild group ($p = 0.008$; Figure 1E). On the other hand, there was no statistically significant difference between the two groups for IFN-α ($p = 0.138$; Figure 1A), IFN-β ($p = 0.961$; Figure 1B), and IFN-γ ($p = 0.091$; Figure 1C). Moreover, IL-6 was significantly higher in the severe group than in the mild group ($p < 0.001$; Figure 1G).

4 | DISCUSSION

Strikingly, this is the first report that IL-28A/IFN-λ2 was downregulated in serum samples of severe COVID-19. In a study of respiratory syncytial virus infection of primary bronchial epithelial cell cultures, type I IFNs were not detected, whereas type III IFNs, including IFN-λ1/IL-29, were detected.[9] The severity of rhinovirus infection has also been reported to be inversely correlated with IFN-λ production in asthmatic patients.[9] These in vitro studies revealed the unique properties of type III IFNs. The expression of SARS-CoV-2 RNA was seen to decrease in primary human airway epithelial cells when pretreated with IFN-λ1.[10] Felgenhauer et al.[11] found that IFN-λ inhibited SARS-CoV-2 RNA in a dose-dependent manner, and the addition of ruxolitinib promoted the replication of SARS-CoV-2 through the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway. Interestingly, in a mouse model infected with SARS-CoV-2, pegylated human IFN-λ1 suppressed SARS-CoV-2 replication.[12] According to our results and other in vitro studies, we speculated that lower levels of IFN-λs could result in worse outcomes through a loss of antiviral and anti-inflammatory mechanisms due to make the elimination of SARS-CoV-2 challenging.

This study showed that patients with severe COVID-19 have high serum levels of IL-6. Furthermore, this study found a correlation between IFN-λ2 and IL-6 ($p = 0.027$, data not shown), similar to the previous study.[13] Several clinical trials concluded that anti-IL-6 antibody treatment was not useful in COVID-19.[14,15] It is unclear whether therapeutic intervention for IFN-λs is a treatment option to cytokine storms, including IL-6, and further research should focus on it.

This study has some limitations. First, it was an observational study with a small sample size conducted at a single center, which might have resulted in selection and confounding biases. Future studies should include a larger sample size. Second, we were unable to analyze changes in the levels of cytokines and chemokines over time. Monitoring these changes will allow us to

TABLE 1 Clinical characteristics of COVID-19

	Mild group (n = 50)	Severe group (n = 22)	p
Age, years	40 (29.7–58)	61 (56.7–69)	<0.001
Sex, male/female, *n*	30/20	18/4	0.103
BMI, kg/m^2	23.1 (19.1–26.7)	24.3 (21.9–28.2)	0.192
Need oxygen or SpO_2 < 90% on admission			
Symptoms, *n* (%)			
Fever	41	21	0.161
Sore throat	9	3	0.744
Nasal discharge	4	0	0.305
Cough	23	8	0.606
Fatigue	10	8	0.152
Dyspnea	6	16	<0.001
Diarrhea	2	2	0.581
Loss of taste or smell	23	2	<0.001
Percutaneous oxygen saturation/ clinical status			
$SpO_2 \geqq 96\%$	19	0	–
$93\% < SpO_2 < 96\%$ or Pneumonia+	31	0	–
$93\% \leqq SpO_2$ or need oxygen supplementation	0	7	–
Admission ICU or need mechanical ventilation	0	15	–
Blood examinations			
White blood cell, /μl	5000 (4300–6225)	6150 (4175–8500)	0.240
Neutrophil count, /μl	3340 (2690–4435)	4340 (2820–6362.5)	0.066
Lymphocyte count, /μl	1170 (855–1602)	835 (592.5–1055)	0.002
Hemoglobin, g/dl	14.9 (13.3–15.7)	13.9 (11.7–14.9)	0.045
Platelet count, ×10^4/μl	22.3 (19.1–24.9)	21.7 (15–26.5)	0.328
Fibrinogen, mg/dl	384 (308.2–543.2)	582 (526–744)	<0.001
D-dimer, ng/ml	0.72 (0.55–1.02)	2.53 (1.41–4.68)	<0.001
Total protein, g/dl	7.1 (6.7–7.5)	6.7 (5.8–6.9)	<0.001
Albumin, g/dl	4.2 (3.6–4.4)	3.0 (2.6–3.5)	<0.001
Urea nitrogen, mg/dl	11.1 (9.4–13.0)	14.3 (11.1–21.6)	0.002
Creatinine, mg/dl	0.7 (0.55–0.85)	0.78 (0.64–0.92)	0.082
Uric acid, mg/dl	5.1 (4.2–5.9)	3.9 (2.7–5.4)	0.010
Aspartate aminotransferase, U/L	27 (20.7–41)	52 (30–78.2)	<0.001
Alanine aminotransferase, U/L	26 (15.7–42.2)	38.5 (30–70.5)	0.032
Lactate dehydrogenase, U/L	188.5 (156.2–250)	462 (296.7–659.7)	<0.001
Alkaline phosphatase, U/L	70 (57–93.2)	69.5 (52.7–124)	0.931
γ-glutamyl transpeptidase, U/L	36 (18–82)	51 (32–165)	0.098
Creatine kinase, U/L	79.5 (42.5–129.5)	70.5 (54–234.5)	0.398

(Continues)

TABLE 1 (Continued)

	Mild group (n = 50)	Severe group (n = 22)	p
Sodium, mEq/L	139.2 (137.3–140.6)	136.1 (134.1–138.7)	0.004
Potassium, mEq/L	3.9 (3.6–4.1)	4.1 (3.8–4.9)	0.013
C-reactive protein, mg/dl	0.6 (0.2–2.5)	10.1 (6.2–16.1)	<0.001
Ferritin, ng/ml	207 (101–490)	1953 (677–2802)	<0.001
Krebs von den Lungen-6, U/ml	211 (157–309)	318 (214–519)	0.011
SP-D, ng/ml	32.1 (22.5–61.8)	116.2 (34.8–195.1)	0.001
SP-A, ng/ml	30.8 (22.5–40.9)	72.3 (50.4–85.8)	<0.001

Abbreviations: BMI, body mass index; SP, surfactant protein.

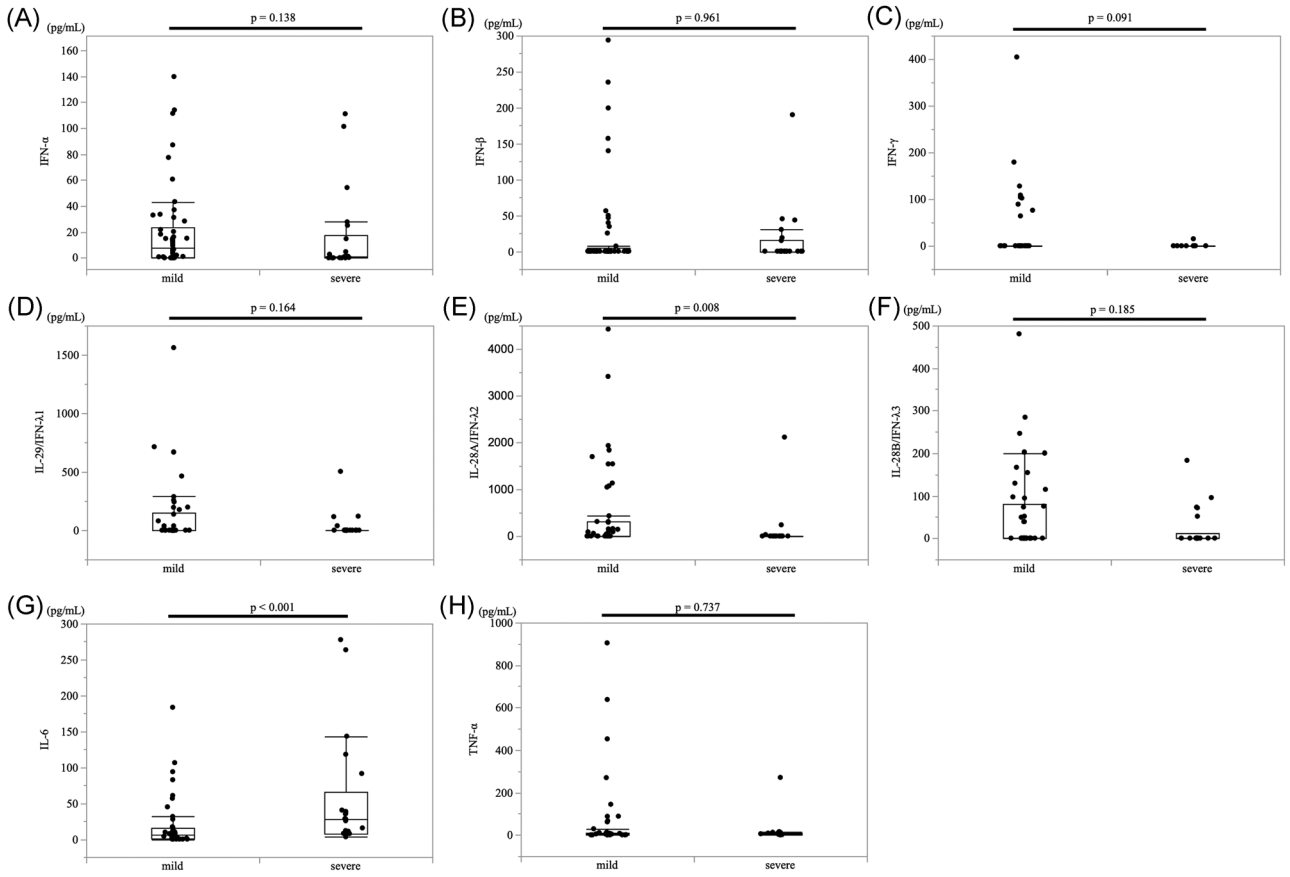

FIGURE 1 Serum levels of cytokines in the mild and severe group A comparison between serum levels of cytokines including interferons by ELISA in the mild group (n = 50) and the severe group (n = 22) based on the COVID-19 severity classification developed by the Ministry of Health, Labor and Welfare of Japan. Data are shown as median (range). Significance was calculated between two groups (Mann–Whitney U test)

understand the pathogenesis of the disease in greater detail. Third, we did not compare the cytokine levels in COVID-19 patients with the health controls. The past basic research showed that IFN-λ2 expression was observed in SARS-CoV infection compared to control but not in SARS-CoV-2 infection.[16] This result suggests that IFN-λ2 may be induced in COVID-19 patients only to the same extent as in healthy controls, but we believe that further studies are needed. Fourth, since the data were all from Japanese patients, the differences in the COVID-19 biology between different races were not apparent. Based on this

study, we are now planning a new study to overcome these limitations.

In summary, levels of IL-28A/IFN-λ2 were lower in patients with severe COVID-19. Downregulation of type III IFN-λs may help in predicting the severity of COVID-19, elucidating its pathogenesis, and developing specific therapies.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

This study was performed in accordance with the Declaration of Helsinki and was approved by the Showa University Ethics Committee (Approval number: 3176). The authors obtained informed consent from all patients.

AUTHOR CONTRIBUTIONS

Yosuke Fukuda, Tetsuya Homma, and Hideki Inoue conceived the original idea for this study. Chisato Onitsuka, Hitoshi Ikeda, Yuiko Goto, Yoko Sato, Tomoyuki Kimura, Kuniaki Hirai, and Shin Ohta acquired clinical samples and data. Yosuke Fukuda and Tetsuya Homma drafted the manuscript. Sojiro Kusumoto, Mayumi Yamamoto, Shintaro Suzuki, Akihiko Tanaka, and Hironori Sagara revised the manuscript. All authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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