Neutralizing type I interferon autoantibodies in Japanese patients with severe COVID-19

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Abstract

Purpose

Autoantibodies (aAbs) to type I interferons (IFNs) have been found in less than 1% of individuals under the age of 60 in the general population, with the prevalence increasing among those over 65. Neutralizing autoantibodies (naAbs) to type I IFNs have been found in at least 15% of patients with life-threatening COVID-19 pneumonia in several cohorts of primarily European descent. We aimed to evaluate the prevalence of aAbs and naAbs to IFN-α2 or IFN-ω in Japanese patients who suffered from COVID-19 as well as in the general population.

Methods

Patients who suffered from COVID-19 (n=622, aged 0–104) and an uninfected healthy control population (n=3,456, aged 20–91) were enrolled in this study. The severities of the COVID-19 patients were: critical (n=170), severe (n=235), moderate (n=112), and mild (n=105). ELISA and ISRE reporter assays were used to detect aAbs and naAbs to IFN-α2 and IFN-ω using E. coli-produced IFNs.

Results

In an uninfected general Japanese population aged 20–91, aAbs to IFNs were detected in 0.087% of individuals. By contrast, naAbs to type I IFNs (IFN-α2 and/or IFN-ω, 100 pg/mL) were detected in 10.6% of patients with critical infections, 2.6% of patients with severe infections, and 1% of patients with mild infections. The presence of naAbs to IFNs was significantly associated with critical disease (P=0.0012),

Age over 50 (P=0.0002) and male sex (P=0.137). A significant but not strong correlation between aAbs and naAbs to IFN-α2 existed (r=-0.307, p-value <0.0001) reinforced the importance of measuring naAbs in COVID-19 patients, including those of Japanese ancestry.

Conclusion

In this study, we revealed that patients with pre-existing naAbs have a much higher risk of life-threatening COVID-19 pneumonia in Japanese population.

Key words: COVID-19, Antibodies to type I IFNs, IFN-α2, IFN-ω, Neutralization assay, IFN-α2 concentration

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The clinical spectrum of COVID-19 varies in severity: approximately 80% of cases are asymptomatic or presenting mild to moderate (nonhypoxemic pneumonia) disease while 20% of cases develop severe pneumonia (15%) or critical pneumonia (5%) (1). As this virus is highly contagious and virulent, health care systems globally faced a crisis. Therefore, establishing a rapid examination system to identify the patients who are at high risk of life-threatening COVID-19 disease are desired.

To date, age of the patient remains the strongest epidemiological risk factor for life-threatening COVID-19, especially among patients over 65 years old (2-5). By contrast, other variable factors, such as male sex, cardiovascular disease, chronic obstructive pulmonary disease, chronic pulmonary disease, obesity, type 2 diabetes mellitus, and smoking are modestly associated with COVID-19 aggravation (6-8). However, there is inter-individual variability among severe cases of COVID-19 and some patients developed severe COVID-19 disease in the absence of these risk factors. Patients with inherited impairments to the innate immune system displayed rapid viral replication early in the infection followed by excessive inflammatory cytokine production that exacerbated the disease (9-14). Indeed, genetic abnormalities in TLR3, IRF7, and TLR7 that affect type I interferon (IFN) signaling have been reported in severe COVID-19 (15, 16). On the other hand, neutralizing autoantibodies (naAbs) to type I IFNs have also been identified as risk factors for life-threatening COVID-19. These naAbs predate the infection and

represent a serious risk factor in COVID-19 aggravation. The naAbs to type I IFNs have also been associated with life-threatening adverse reactions to yellow fever vaccine (YFV) (17). Bastard et al. reported that 10.2% of patients with life-threatening COVID-19 pneumonia had naAbs to type I IFNs, compared to 0.33% of healthy individuals and 0% of patients with asymptomatic/mild disease (18). Further, 20% of patients with critical COVID-19 over the age of 80 years and deceased patients of all ages had naAbs to type I IFNs (19). Importantly, the clinical impact of these naAbs is not apparent until infection with SARS-CoV-2, which makes it difficult to predict the risk of severe COVID-19 disease due to naAbs to type I IFNs.

These previous studies suggest that approximately 5% of younger patients have a risk of aggravation due to genetic abnormalities associated with type I IFNs while approximately 20% of older patients have a risk of aggravation due to naAbs to type I IFNs. While these observation have been supported by subsequent studies (20-26), further analysis are needed to clarify the pathophysiology of life-threatening COVID-19 pneumonia in individual ethnic groups that have similar genetic background and lifestyles for precise characterization of the role of antibodies to type I IFNs in COVID-19 aggravation.

Our current study aimed to determine the prevalence of neutralizing autoantibodies to type I IFNs in Japanese COVID-19 patients and their contribution to severe COVID-19 disease. In addition, we studied the prevalence of aAbs in the uninfected Japanese population to clarify the differences of the prevalence among ethnic groups.

Materials and methods

COVID-19 patients and individuals in the general population subjected to analysis

We conducted the study at Hiroshima University Hospital, Tokyo Medical and Dental University Medical Hospital, and Osaka City University Hospital. We enrolled 622 COVID-19 patients admitted to our institutes as well as 3,456 individuals from the general population which included 1,000 previously reported individuals (19) (Fig. 1A, B, Table 1). The details of the patients and the general population are described in the Supplemental materials and methods.

We assessed the severity of COVID-19 based on the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia described previously (18). “Critical” included patients who required mechanical ventilation (including intubation, high flow nasal cannula, continuous positive airway pressure and bilevel positive airway pressure, etc.), septic shock, any other organ failure and/or use of ECMO in the intensive care unit. “Severe” were defined as patients required oxygen therapy < 6 L/min because of pneumonia. The patients with mild pneumonia but no requirement for oxygen therapy were classified into “Moderate”. “Mild” were defined as patients with some mild symptoms without pneumonia.

Neutralization assay of autoantibodies (aAbs) to type I IFNs

We performed luciferase reporter assays as described previously (18). The detailed method of neutralization assay is described in the Supplemental materials and methods.

Measurement of aAbs to type I IFNs and IFN-α2 concentration

The details of ELISA and ProQuantum™ Immunoassay are described in the Supplemental materials and methods.

Statistical analysis

The detailed method of statistical analysis is described in the Supplemental materials and methods.

Results

The frequency of aAbs to type I IFNs was high in patients with critical COVID-19

We first measured aAbs to type I IFNs by ELISA in 622 Japanese COVID-19 patients aged 0–104 years, including 170 critical, 235 severe, 112 moderate, 105 mild cases. We detected aAbs to IFN-α2 or IFN-ω at the following frequencies: 5.9% critical cases, 1.7% severe cases, 0.9% moderate cases, 3.8% mild case (Fig. 1C, Table 2). In detail, 4.7% (95% CI: 2.4-9.0) of patients with critical disease had aAbs to IFN-α2, 3.5% (95% CI: 1.6-7.5) to IFN-ω, and 2.4% (95% CI: 0.9-5.9) to both IFN-α2 and IFN-ω (Table2). Among patients who had IFN-α2 or IFN-ω aAbs, there were several patients who had isolated aAb solely to IFN-α2 or IFN-ω (Table S2). The aAbs to IFN-α2 or IFN-ω were also detected in 3.8% of patients with mild disease and 0.9% of those with moderate disease. Unlike patients with critical COVID-19, none of the

patients with mild to severe disease had aAbs to both interferon subtypes (Table 2). Among patients over 50 years old, 3.6% (95% CI: 2.2-5.7) had aAbs to IFN-α2 or IFN-ω, while 1.7% (95% CI: 0.6-4.9) of patients younger than 50 years had these aAbs (Table 2). Overall, these aAbs to type I IFNs were detected more frequently in patients with critical disease and patients over 50 years old. However, isolated aAbs to IFN-α2 or IFN-ω was also detected in some of the patients with mild or moderate disease in the current study.

naAbs to type I IFNs were frequently detected in patients with critical COVID-19

aAbs which react with type I IFNs were detected by ELISA, however, their neutralizing activity could not be assessed by ELISA. We thus measured neutralizing activity against type I IFNs using the ISRE reporter assay in sera from 622 patients with COVID-19 (19). Sera were considered to have neutralizing activity if the induction of ISRE activity, which was normalized to Renilla luciferase activity, was less than 15% of the median values of healthy controls (19). These data are summarized in Tables 3 and Table S3. Strongly neutralizing naAbs, capable of neutralizing 10 ng/mL of IFN-α2 or IFN-ω, were found in 5.9% of critical cases, 2.1% of severe cases, 0.9% of moderate cases and 0% of mild cases (Fig. 2A, Table 3). In patients with critical disease, antibody prevalence was as follows: 5.9% (95% CI: 3.2-10.5) had naAbs to IFN-α2, 4.1% (95% CI: 2.0-8.3) to IFN-ω, and 4.1% (95% CI: 2.0-8.3) to both IFN-α2 and IFN-ω (Table3). On the other hand, less than 1% of patients with mild to moderate disease had naAbs to type I IFNs (Table

3). Among patients over 50 years old, 3.6% (95% CI: 2.2-5.7) had naAbs to IFN-α2, 2.2% (95% CI: 1.2-4.1) to IFN-ω, 2.2% (95% CI: 1.2-4.1) to both IFN-α2 and IFN-ω, and 3.6% (95% CI: 2.2-5.7) to IFN-α2 or IFN-ω (Table3). By contrast, none of the patients younger than 50 years old had naAbs to type I IFNs (Table 3). These results are summarized according to disease severity in Figure 2B. All patients having neutralizing activity against IFN-ω had neutralizing activity against IFN-α2. Of note, in contrast to the prevalence of aAb (TableS2), no patients had isolated naAbs to IFN-ω (TableS3, FigureS1).

Next, we analyzed serum neutralizing activity under more sensitive conditions by stimulating cells at lower concentrations (100 pg/mL) of IFN-α2 or IFN-ω. Under this condition, consistent with previous reports (19), the prevalence of naAbs was observed in 10.6% of critical cases, 2.6% of severe cases, 0.9% of moderate cases and 1.0% of mild cases (Fig. 3A, Table 3, Table S3). In detail, 7.1% (95% CI: 4.1–11.9) of critical cases had naAbs to IFN-α2, 10.0% (95% CI: 6.3–15.4) to IFN-ω, and 6.5% (95% CI: 3.7–11.2) to both IFN-α2 and IFN-ω (Table3). Only 1% or less of the patients with mild to moderate disease had these naAbs to IFN-α2 or IFN-ω (Table 3). Among patients over 50 years old, 4.5% (95% CI: 2.9-6.8) had naAbs to IFN-α2, 4.7% (95% CI: 3.1-7.1) of them to IFN-ω, 3.4% (95% CI: 2.0-5.5) to both IFN-α2 and IFN-ω, and 5.8% (95% CI: 4.0-8.4%) to IFN-α2 or IFN-ω. By contrast, none of the patients younger than 50 years old had naAbs to IFN-α2 or IFN-ω (Table 3).

Using this more sensitive condition, the percentage of the patients with naAbs to IFNs was higher than in the condition with 10ng/ml (Table3). We detected naAbs against IFN-α2 in an additional 4 patients at the

100pg/ml condition compared to the 10ng/ml condition. Among these 4 patients, 3 had critical/severe disease, and 1 patient had mild disease (Fig. 4A, Fig. S3). Regarding naAbs to IFN-ω, an additional 11 patients showed neutralizing activity only against 100 pg/mL. All 11 patients had critical/severe disease (Fig. 4B, S4). It is known that the concentration of type I IFNs in the blood of patients with acute and benign SARS-CoV-2 infections ranges from 1 to 100 pg/mL (13, 27). Moreover, it has been experimentally proven that 100 pg/mL of type I IFNs can impair SARS-CoV-2 replication in epithelial cells.(19) Therefore, a neutralization assay using 100 pg/mL of type I IFNs, which reflects physiological conditions, detected naAbs more precisely than the assay using 10 ng/mL, especially naAbs to IFN-ω.

The prevalence of naAbs by sex was 5.5% at 100 pg/mL and 3.4% at 10 ng/mL for males and 1.1% at 100 pg/mL and 0.5% at 10 ng/mL for females (Table S4, Fig. S5). NaAbs to IFNs were significantly associated with critical disease (P=0.0152 at 10ng/ml, P=0.0012 at 100pg/ml) compared to mild disease, age over 50 (P=0.0085, P=0.0002) and male sex (P=0.0488, P=0.137) (Table 4). COVID-19 aggravation was strongly associated with naAbs among critical patients using both assay conditions (At 10 ng/mL, IFN-α2 and IFN-ω odds ratio (OR) = 9.3, IFN-α2 or IFN-ω OR =13.5. At 100 pg/mL, IFN-α2 and IFN-ω OR = 14.9, IFN-α2 or IFN-ω OR =12.7.) (Figure 2C and 3C). These data are consistent with previous reports that identified a high prevalence, 10.2-18% in patients with critical disease, of naAbs to type I IFNs (table S5) (18-26).

Comparison of the results of the neutralization assay and ELISA.

While the IFN neutralization assay is the gold standard in assessing the biological effect of aAbs and the ISRE reporter assay is a sensitive method, it is time-consuming. On the other hand, ELISA is more high-throughput with faster turnaround times. We thus compared the results of neutralizing activity against type I IFNs measured by the ISRE reporter assay with the results of aAbs to type I IFNs measured by ELISA. When the presence of naAbs to IFN-α2 was predicted by the results of aAbs to IFN-α2, the sensitivity was 50%, the specificity was 99.3%, the positive predictive value (PPV) was 66.7%, the negative predictive value (NPV) was 98.7% at 10 ng/mL (Fig. 4C), and these two detection methods had a weak negative correlation (a correlation coefficient -0.307 (95% CI: -0.376～-0.234, P value <0.0001)). For the 100 pg/mL condition, the sensitivity was 40%, the specificity was 99.3% (PPV of 66.7% and NPV of 98.0%), and these two detection methods had a weak negative correlation (a correlation coefficient -0.199 [95% CI: -0.273～-0.123, P value <0.0001]) (Fig. 4E). We thus realized that ELISA-based detection of aAbs to IFN-α2 can be an alternative method to enable testing of multiple samples, e.g., screening tests for the general population, and to evaluate antibodies to type I IFNs in sera. In contrast, for IFN-ω, ELISA failed to adequately detect the presence of naAbs to IFN-ω. Indeed, ELISA-based detection of aAbs to IFN-ω pointed out the presence of naAbs to IFN-ω (10 ng/mL condition) with a sensitivity of 10% and specificity of 98.4% (PPV of 9.1% and NPV of 98.5%) (Fig. 4D). Regarding the 100 pg/mL condition, aAbs to IFN-ω only indicated naAbs to IFN-ω with a sensitivity of 9.5% and a specificity of 98.5% (PPV of 18.2% and

NPV of 96.9%) (Fig. 4F).

Sera from COVID-19 patients with naAbs to IFN-α2 show low concentrations of IFN-α2.

We analyzed the concentration of IFN-α2 using 269 samples for which the exact time of specimen collection could be determined with the ProQuantum™ Human IFN alfa Immunoassay, which is a qPCR-based technique. The level of IFN-α2 in sera in patients with naAbs was significantly lower compared to those without naAbs. The serum IFN-α2 levels were below detection limit (<4 pg/ml) in all but one 1 patient with naAbs detected by the high sensitivity condition (Fig. 5A, B). However, there is no correlation between disease severity and the concentration of IFN-α2 (P=0.2238). We also compared the level of IFN-α2 between the samples collected from onset to day4 and those from day 5 to day 7 after onset. We found that the concentration of IFN-α2 were significantly higher in samples from onset to day 4 compared to those from day 5 to day 7 (P=0.0009) (data not shown). These results are consistent with a previous report (18).

Prevalence of aAbs to IFN-α2 in uninfected individuals from the general Japanese population.

In order to understand the risk of the general Japanese population to severe COVID-19 and other viral infections, we sought to determine the prevalence of naAbs to type I IFNs in the Japanese population by detecting aAbs to IFN-α2 via ELISA. We studied 3,456 Japanese individuals aged 20-91 years and unaffected by COVID-19. In this population, 3 individuals had aAbs to IFN-α2 (0.087% [95% CI: 0.0295-

0.255%]) (Fig. 5C). These 3 individuals consisted of an 86-year-old female, a 78-year-old male, and a 42-year-old male. These data suggest that the prevalence of aAbs, and by inference, that of naAbs, is low in the healthy general Japanese population.

Discussion

The current study investigated aAbs and naAbs to type I IFNs in 622 patients with COVID-19 before the Delta variant became predominant. This is the second largest study on the scale of the samples, also the largest study focusing on a single ethnic group, and the first in Asia. To minimize selection bias, we collected sera from COVID-19 patients from three geographically different areas (Tokyo, Osaka and Hiroshima) in Japan. The prevalence of naAbs to type I IFNs was high among patients with critical disease, elderly patients, and male COVID-19 patients. These observations were consistent with a previous study (18), providing strong evidence to support the risk of COVID-19 aggravation in individuals with naAbs to type I IFNs. The modest risk factors that are well known so far are male sex (OR = 1.457) (7), cardiovascular disease (adjusted risk = 2.6) (6), chronic pulmonary disease (OR = 1.089) (7), diabetes mellitus with chronic complications (rate ratio = 1.295) (7). Although it is impossible to compare the odds ratios directly between different cohort studies, the risk of COVID-19 aggravation among individuals with naAbs to type I IFNs was estimated to be relatively high (100 pg/mL of IFN-α2 and IFN-ω OR = 14.9, IFN-α2 or IFN-ω OR =12.7). A recent review article also described that aAbs to IFNα, IFNβ and/or IFNω are

found in about 15-20% of patients with critical COVID-19 pneumonia over 70 years old and regarded aAbs against IFNs as a major risk factor for critical COVID-19 disease (5). As shown in this study and a previous study (3, 5, 19), the prevalence of naAbs to type I IFNs increased with age, especially high in the population over age of 50. This might be one of the reasons why age is the most striking epidemiological risk factor. Consistent with this, naAbs to type I IFNs are found in 1% or less of patients with mild to moderate COVID-19. Therefore, although the presence of naAbs to type I IFNs is a strong risk factor for aggravation, not all patients with these naAbs developed severe or critical COVID19 disease (28).

Approximately 1% of the patients with naAbs to IFN-α2 and ω also have naAbs to IFN-β (19). Therefore, IFN-β therapy might be effective in severe COVID-19 cases with naAbs to type I IFNs (29-32). In addition, the removal of naAbs to type I IFNs with plasma exchange may be beneficial in the treatment of COVID-19 patients (33). Since these treatments may be effective only in the early stage of the infection (20), establishing rapid test system to evaluate naAbs to type I IFNs are necessary for appropriate therapeutic interventions. Therefore, we evaluated the utility of a rapid ELISA instead of the ISRE reporter-based neutralization assay. ELISA data correlated well with neutralization assay for aAbs and naAbs for IFN-α2 but not for IFN-ω. Indeed, a strong association exists between the severity of COVID-19 and the presence of naAbs to IFN-α2, whereas the risk of aggravation by naAbs to IFN-ω alone was not clear (19).

We thus performed a systematic study by ELISA in 3,456 individuals without COVID-19 and found that 0.087% of this population were positive for aAbs to IFN-α2. Since the examination of aAbs to IFN-α2

by ELISA predicted the presence of naAbs to IFN-α2 with sensitivities of 50% (10 ng/dL condition) and 40% (100 pg/mL condition) as shown in this study, the prevalence of naAbs to IFN-α2 was assumed to be 0.17–0.22%. This prevalence in the general population in Japan was slightly lower than that in a previous international study (0.33%) (18). The lower prevalence of naAbs in patients with critical disease in Japan compared to that in previous international study (10.6% v.s. 13.6%) can be explained by this lower prevalence of naAbs in general population.

In our study, we also found that some patients with high titer aAbs did not exhibit neutralizing activity against type I IFNs as reported elsewhere (26). This may be explained by binding of aAbs to non-neutralizing epitopes. Another explanation is that these aAbs may have neutralizing activity at concentrations lower than 100 pg/mL of stimulation. We used 10% sera in our neutralization assay, so this assay using 100 pg/mL of stimulation can detect only naAbs which neutralize 1000 pg/mL of cytokines. On the other hand, the IFN-α2 concentrations of most patients in this study were below 100 pg/mL in sera. Therefore, it is worthwhile to extend this study with neutralization conditions with lower cytokine concentrations, e.g. 10 pg/mL. Despite these limitations, this study was the first study to characterize the relationship between naAbs to type I IFNs and COVID-19 aggravation in a Japanese population and the second largest study on this theme, providing strong evidence to support the contribution of naAbs to type I IFNs to the risk of COVID-19 aggravation.

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Author information

Shohei Eto and Yoko Nukui contributed equally to this work.

Declarations

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Conflicts of Interest

The authors declare no competing interests.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability

Not applicable.

Authors' contributions

Shohei Eto, Miyuki Tsumura and Yoko Mizoguchi performed ELISA experiment, Neutralizing assay and measured IFN-a2 concentration. Shohei Eto prepared the first draft. Shintaro Nagashima and Junko Tanaka collected samples of general population before the appearance of COVID-19 and revised the draft. Yoko Nukui, Kenichi Kashimada, Keisuke Okamoto, Akifumi Endo, Kohsuke Imai, Hirokazu Kanegane, Tomohiro Morio, Yu Nakagama, Yasutoshi Kido, Hidenori Ohnishi, Masanori Ito, and Hiroki Ohge,

collected samples of patients with COVID-19 and general population after the appearance of COVID-19 and revised the draft. Paul Bastard, Jean-Laurent Casanova and Osamu Ohara analyzed and interpreted the data and revised it critically for important intellectual content. Satoshi Okada designed and supervised the study and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committees and Institutional Review Board of Hiroshima University.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Included subjects or their representatives have consented to publication of their data.

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Table 1. Characteristics of 622 patients with COVID-19 and 3,456 general population in this study

	622 patients with COVID-19			3,456 general population in this study		
Age (years)	**Total cases [n = 622](%)**	**Male [n = 439]**	**Female [n = 183]**	**Total cases [n = 3,456](%)**	**Male [n = 1,502]**	**Female [n = 1,954]**
0–9	22 (3.5%)	15	7	-	-	-
10–19	8 (1.3%)	6	2	-	-	-
20–29	31 (5.0%)	18	13	536 (15.5%)	72	464
30–39	45 (7.2%)	28	17	439 (12.7%)	164	275
40–49	69 (11.1%)	52	17	522 (15.1%)	267	255
50–59	127 (20.4%)	104	23	340 (9.8%)	174	166
60–69	112 (18.0%)	79	33	992 (28.7%)	495	497
70–79	144 (23.1%)	103	41	519 (15.0%)	267	252
80–89	51 (8.2%)	27	24	105 (3.0%)	60	45
90–	13 (2.1%)	7	6	3 (0.1%)	3	0
Severity	**Total cases [n = 622](%)**	**Male [n = 439]**	**Female [n = 183]**			
Mild	105 (16.9%)	67	38			
Moderate	112 (18.0%)	68	44			
Severe	235 (37.8%)	166	69			
Critical	170 (27.3%)	138	32			

Table 2. The prevalence of aAbs to type I IFNs in 622 patients with COVID-19 according to disease severity or age

aAbs detected by ELSIA					
Severity	No. of patients	IFN-α2	IFN-ω	IFN-α2 and -ω	IFN-α2 or -ω
Mild	105	1 (1.0%[0.2-5.2])	3 (2.9%[1.0-8.1])	0 (0.0%)	4 (3.8%[1.5-9.4])
Moderate	112	1 (0.9%[0.2-4.9])	0 (0.0%)	0 (0.0%)	1 (0.9%[0.2-4.9])
Severe	235	2 (0.9%[0.2-3.1])	2 (0.9%[0.2-3.1])	0 (0.0%)	4 (1.7%[0.7-4.3])
Critical	170	8 (4.7%[2.4-9.0])	6 (3.5%[1.6-7.5])	4 (2.4%[0.9-5.9])	10 (5.9%[3.2-10.5])
Total	627	12	11	4	19
Age (years)	No. of patients	IFN-α2	IFN-ω	IFN-α2 and -ω	IFN-α2 or -ω
0–49	175	1 (0.6%[0.1-3.2])	2 (1.1%[0.3-4.1])	0 (0.0%)	3 (1.7%[0.6-4.9])
50–	**447**	**11 (2.5%[1.4-4.4])**	**9 (2.0%[1.1-3.8])**	**4 (0.9%[0.3-2.3])**	**16 (3.6%[2.2-5.7])**
50–59	127	5 (3.9%[1.7-8.9])	4 (3.2%[1.2-7.8])	2 (1.6%[0.4-5.6])	7 (5.5%[2.7-10.9])
60–69	112	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])	0 (0.0%)	2 (1.8%[0.5-6.3])
70–	208	5 (2.4%[1.0-5.5])	4 (1.9%[0.8-4.8])	2 (1.0%[0.3-3.4])	7 (3.4%[1.6-6.8])

Table 3. The prevalence of naAbs to type I IFNs in 622 patients with COVID-19 according to disease severity and age

naAbs detected by Neutralizaiton assay									
		10 ng/mL				100 pg/mL			
Severity	No. of patients	IFN-α2	IFN-ω	IFN-α2 and -ω	IFN-α2 or -ω	IFN-α2	IFN-ω	IFN-α2 and -ω	IFN-α2 or -ω
Mild	105	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%[0.2-5.2])	0 (0.0%)	0 (0.0%)	1 (1.0%[0.2-5.2])
Moderate	112	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])
Severe	235	5 (2.1%[0.9-4.9])	2 (0.9%[0.2-3.0])	2 (0.9%[0.2-3.0])	5 (2.1%[0.9-4.9])	6 (2.6%[1.2-5.5])	3 (1.3%[0.4-3.7])	3 (1.3%[0.4-3.7])	6 (2.6%[1.2-5.5])
Critical	170	10 (5.9%[3.2-10.5])	7 (4.1%[2.0-8.3])	7 (4.1%[2.0-8.3])	10 (5.9%[3.2-10.5])	12 (7.1%[4.1-11.9])	17 (10.0%[6.3-15.4])	11 (6.5%[3.7-11.2])	18 (10.6%[6.8-16.1])
Total	622	6	0	10	16	5	6	15	26
Age (years)	No. of patients	IFN-α2	IFN-ω	IFN-α2 and -ω	IFN-α2 or -ω	IFN-α2	IFN-ω	IFN-α2 and -ω	IFN-α2 or -ω
0–49	175	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
50–	**447**	**16 (3.6% [2.2-5.7])**	**10 (2.2% [1.2-4.1])**	**10 (2.2% [1.2-4.1])**	**16 (3.6% [2.2-5.7])**	**20 (4.5% [2.9-6.8])**	**21 (4.7% [3.1-7.1])**	**15 (3.4% [2.0-5.5])**	**26 5.8% [4.0-8.4])**
50–59	127	8 (6.3%[3.2-11.9])	6 (4.7%[2.2-9.9])	6 (4.7%[2.2-9.9])	8 (6.3%[3.2-11.9])	8 (6.3%[3.2-11.9])	10 (7.9%[4.3-13.9])	7 (5.5%[2.7-10.9])	11 (8.7%[4.9-14.8])
60–69	112	2 (1.8%[0.5-6.3])	1 (0.9%[0.2-4.9])	1 (0.9%[0.2-4.9])	2 (1.8%[0.5-6.3])	5 (4.5%[1.9-10.0])	5 (4.5%[1.9-10.0])	3 (2.7%[0.9-7.6%])	7 (6.3%[3.1-12.3])
70–	208	6 (2.9%[1.3-6.1])	3 (1.4%[0.5-4.2])	3 (1.4%[0.5-4.2])	6 (2.9%[1.3-6.1])	7 (3.4%[1.6-6.8])	6 (2.9%[1.3-6.1])	5 (2.4%[1.0-5.5])	8 (3.8%[2.0-7.4])

Table 4. Comparison of patients with and without naAbs according to disease severity, age and sex

naAbs detected by neutralization assay						
	10 ng/mL			100 pg/mL		
Severity	No. of naAb positive	No. of naAb negative	p-value	No. of naAb positive	No. of naAb negative	p-value
Mild	0	105	1	1	104	1
Moderate	1	111	0.3291	1	111	0.4439
Severe	5	230	0.0152	6	229	0.0012
Critical	10	160		18	152	
Age (years)	No. of naAb positive	No. of naAb negative	p-value	No. of naAb positive	No. of naAb negative	p-value
0–49	0	175	0.0085	0	175	0.0002
50–	16	431		26	421	
Sex	No. of naAb positive	No. of naAb negative	p-value	No. of naAb positive	No. of naAb negative	p-value
Female	1	182	0.0488	2	181	0.0137
Male	15	424		24	415	

Fig. 1

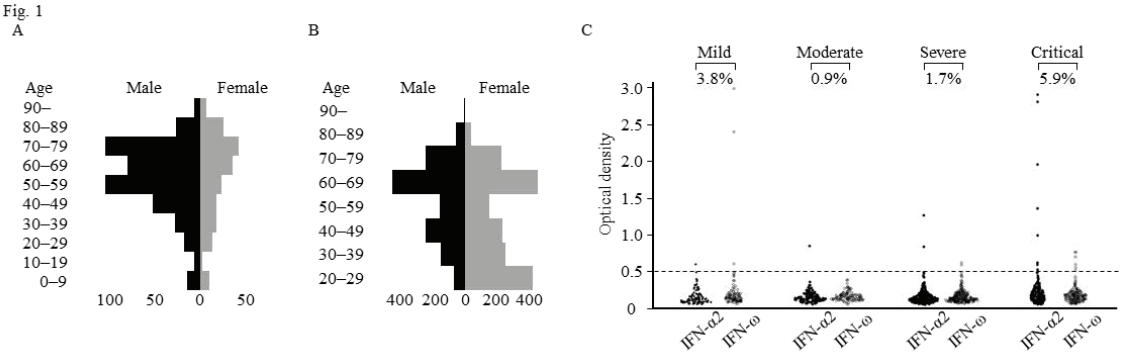

A
Age
Male
Female
90–
80–89
70–79
60–69
50–59
40–49
30–39
20–29
10–19
0–9
100 50 0 50
B
Age
Male
Female
90–
80–89
70–79
60–69
50–59
40–49
30–39
20–29
400 200 0 200 400
C
Mild
3.8%
Moderate
0.9%
Severe
1.7%
Critical
5.9%
Optical density
3.0
2.5
2.0
1.5
1.0
0.5
0
IFN-α2
IFN-ω
IFN-α2
IFN-ω
IFN-α2
IFN-ω
IFN-α2
IFN-ω

Fig. 2

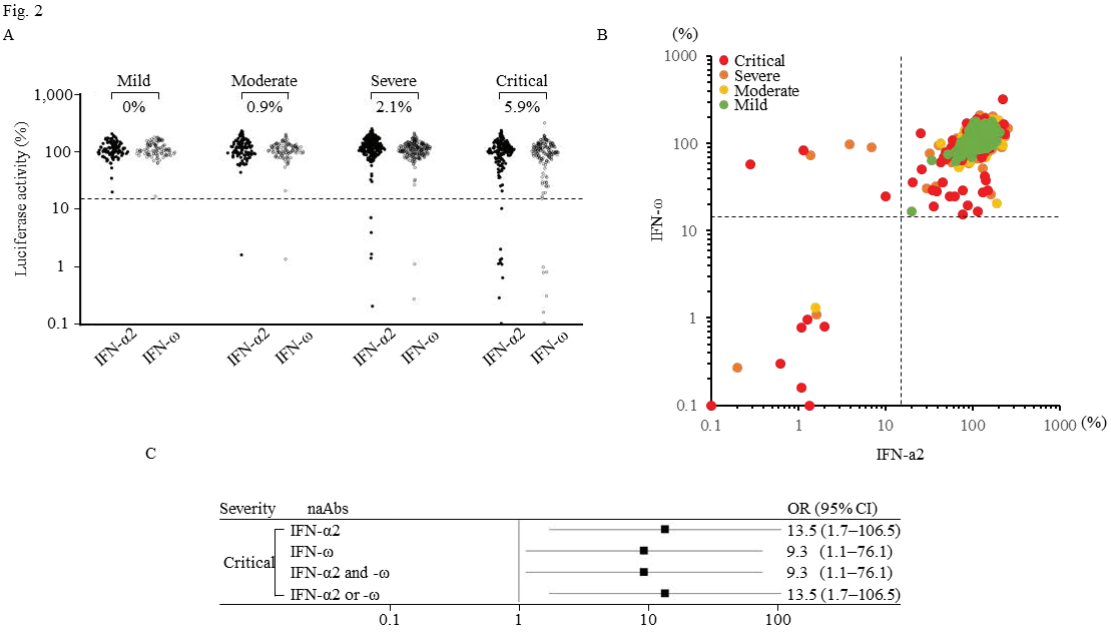

A
Mild
0%
Moderate
0.9%
Severe
2.1%
Critical
5.9%
Luciferase activity (%)
1,000
100
10
1
0.1
IFN-α2
IFN-ω
B
(%)
Critical
Severe
Moderate
Mild
IFN-ω
IFN-a2
C
Severity
naAbs
OR (95% CI)
Critical
IFN-α2
IFN-ω
IFN-α2 and -ω
IFN-α2 or -ω
13.5 (1.7–106.5)
9.3 (1.1–76.1)
9.3 (1.1–76.1)
13.5 (1.7–106.5)

Fig. 3

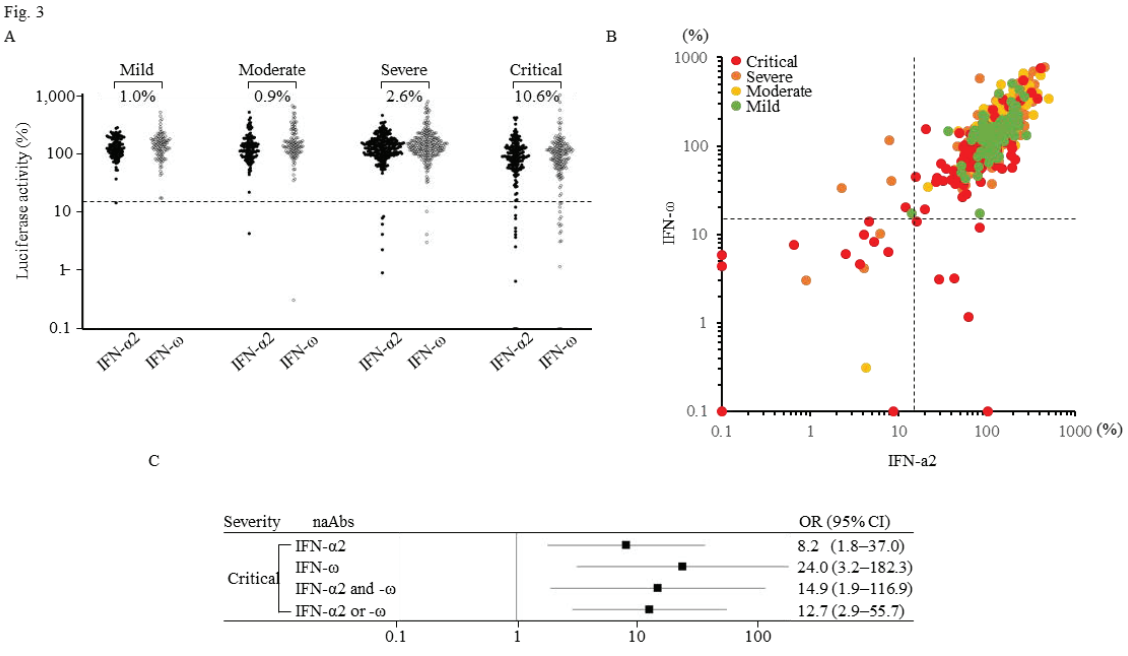

A
Mild
1.0%
Moderate
0.9%
Severe
2.6%
Critical
10.6%
Luciferase activity (%)
1,000
100
10
1
0.1
IFN-α2
IFN-ω
B
(%)
Critical
Severe
Moderate
Mild
IFN-ω
IFN-a2
1000
100
10
1
0.1
C
Severity
naAbs
OR (95% CI)
Critical
IFN-α2
IFN-ω
IFN-α2 and -ω
IFN-α2 or -ω
8.2 (1.8–37.0)
24.0 (3.2–182.3)
14.9 (1.9–116.9)
12.7 (2.9–55.7)
0.1
1
10
100

Fig. 4

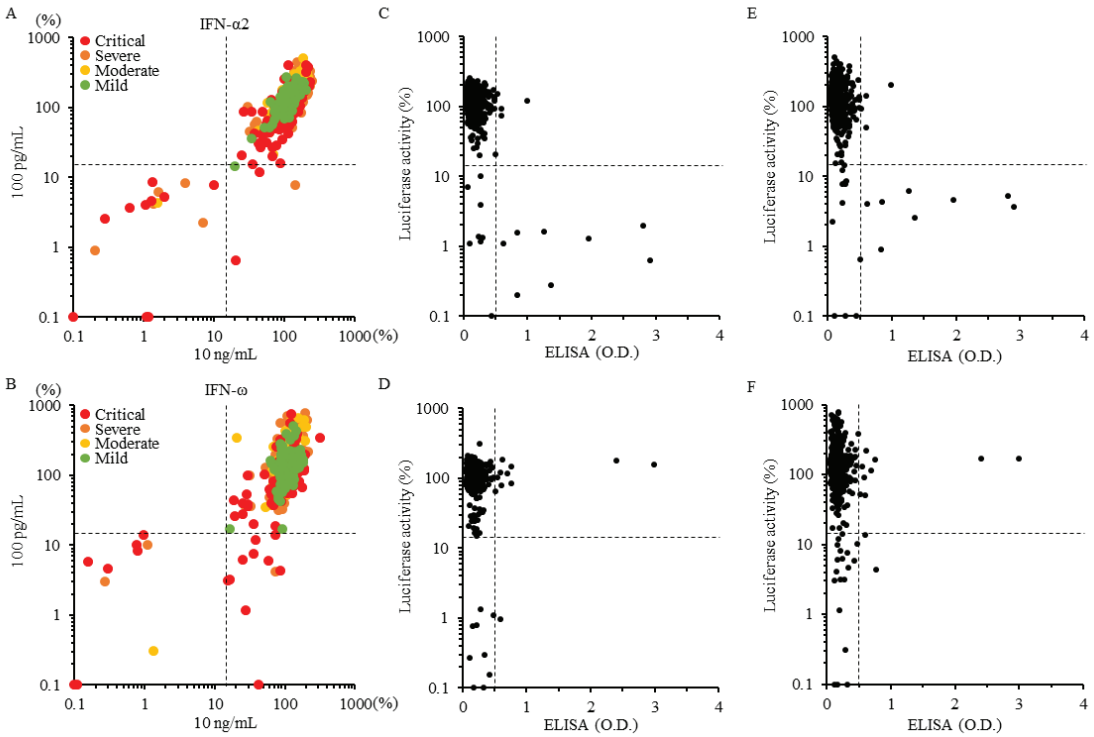

A
IFN-α2
(%)
Critical
Severe
Moderate
Mild
100 pg/mL
10 ng/mL
1000(%)
B
IFN-ω
C
D
E
F
Luciferase activity (%)
ELISA (O.D.)

Fig. 5

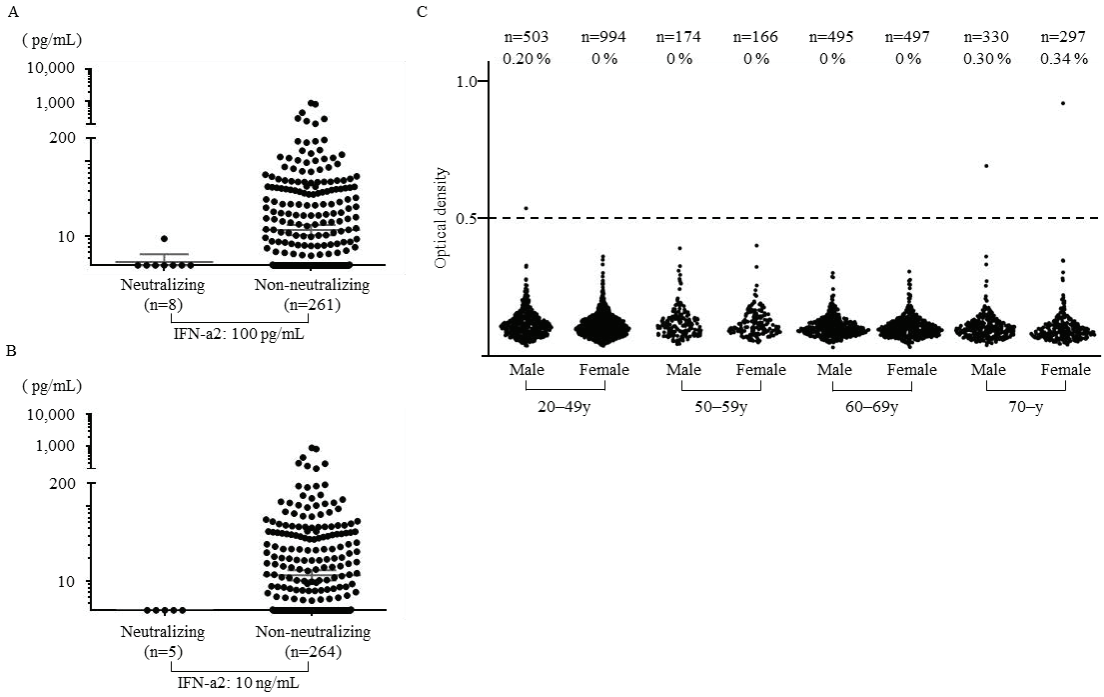

A
(pg/mL)
10,000
1,000
200
10
Neutralizing
(n=8)
Non-neutralizing
(n=261)
IFN-a2: 100 pg/mL
B
(pg/mL)
10,000
1,000
200
10
Neutralizing
(n=5)
Non-neutralizing
(n=264)
IFN-a2: 10 ng/mL
C
n=503 n=994 n=174 n=166 n=495 n=497 n=330 n=297
0.20% 0% 0% 0% 0% 0% 0.30% 0.34%
1.0
0.5
Optical density
Male Female Male Female Male Female Male Female
20–49y
50–59y
60–69y
70–y

Figure legends

Figure 1

Characteristics of 622 patients with COVID-19 and 3,456 individuals from the general population. **A** Age and sex distribution of patients with COVID-19 (n=622). The median age of the COVID-19 patients was 61 years (IQR: 46-73 years); 70.2% were males, and 29.8% were females. **B** Age and sex distribution of individuals from the general population (n=3,456). The median age of subjects from the general population was 56 years (IQR: 37-67 years); 43.5% were males, and 56.5% were females. **C** The prevalence of aAbs to type I IFNs of patients with COVID-19 according to its severity. aAbs to IFNs were detected by ELISA in 622 patients with COVID-19 including 170 critical, 235 severe, 112 moderate, and 105 mild infections. The cutoff value of ELISA was 0.5 (O.D.).

Figure 2

naAbs to type I IFNs were detected by the neutralization assay in 622 patients with COVID-19 at a cytokine concentration of 10 ng/mL. **A** Dot plot of the neutralization assay stimulated by 10 ng/mL of type I IFNs. The samples showing less than 15% of luciferase activity were defined as having neutralization activity. The prevalence of naAbs was high in patients with critical COVID-19. **B** Neutralizing activity against type I IFNs was compared between IFN-α2 and IFN-ω stimulated by 10 ng/mL. All patients having neutralizing activity against IFN-ω had neutralizing activity against IFN-α2. **C** The odds ratio (OR) associated with COVID-19 aggravation among patients in critical disease compared to mild/moderate disease.

Figure 3

naAbs to type I IFNs detected by the neutralization assay in 622 patients with COVID-19 at a cytokine concentration of 100 pg/mL. **A** Dot plot of the neutralization assay stimulated by 100 pg/mL of type I IFNs. The samples showing less than 15% of luciferase activity were defined as having neutralization activity. The prevalence of naAbs was high in patients with critical COVID-19. **B** Neutralizing activity against type I IFNs was compared between IFN-α2 and IFN-ω stimulated by 100 pg/mL. **C** The odds ratio (OR) associated with COVID-19 aggravation among patients in critical disease compared to mild/moderate disease.

Figure 4

Comparison of the results of the neutralization assay and ELISA. **A, B** Neutralizing activity against type I IFNs was compared between type I IFN concentrations of 100 pg/mL and 10 ng/mL stimulated by IFN-α2 (A) or IFN-ω (B). **C-F** aAbs to type I IFNs by ELISA were compared with naAbs by the neutralization

assay at concentrations of 10 ng/mL IFN-α2 (C), 10 ng/mL IFN-ω (D), 100 pg/mL IFN-α2 (E), and 100 pg/mL IFN-ω (F). The cutoff value of ELISA was 0.5 (O.D.). In neutralization assay, samples showing less than 15% of luciferase activity were defined as having neutralization activity.

Figure 5

IFN-α2 concentration of patients with COVID-19 and prevalence of aAbs to IFN-α2 in 3,456 individuals in the general population. The IFN-α2 concentration in most of the patients with naAbs to IFN-α2 and/or IFN-ω was below the limit of quantification (<4 pg/mL). **A** Patients with naAbs to 100 pg/mL of IFN-α2 and/or IFN-ω (n=8) and patients without naAbs (n=261) were compared. **B** Patients with naAbs to 10 ng/mL of IFN-α2 and/or IFN-ω (n=5) and patients without naAbs (n=264) were compared. **C** aAbs to IFN-α2 in the general population were detected using ELISA. The prevalence of aAbs were calculated according to age and sex.

Fig. 1

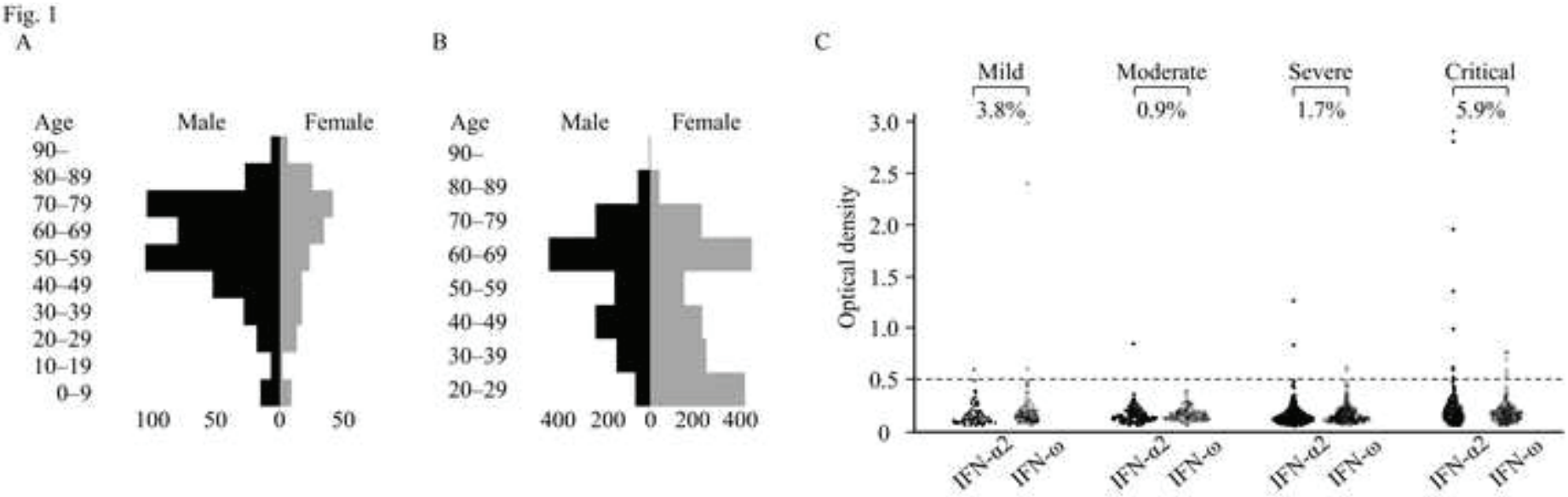


Fig. 2

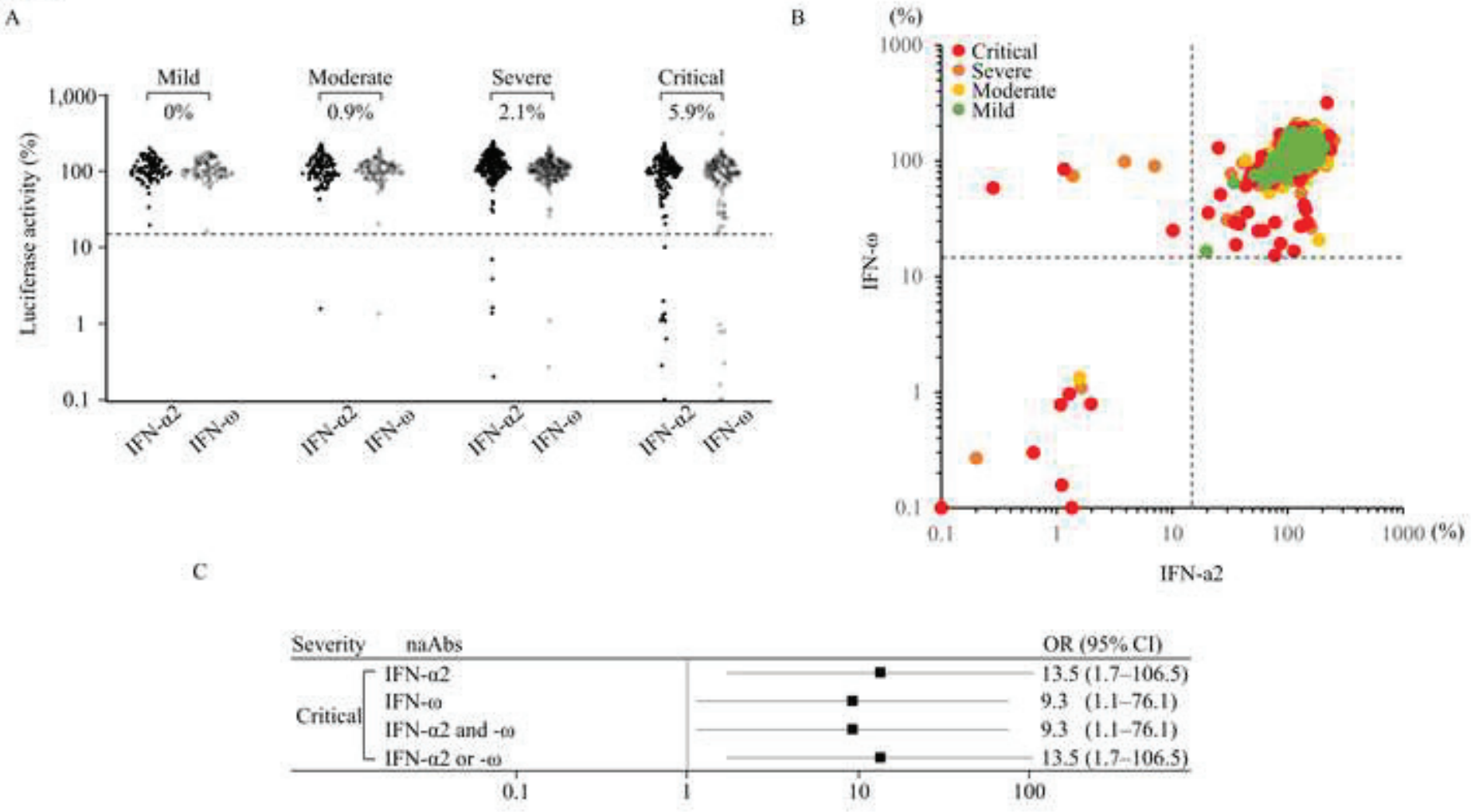


Fig. 3

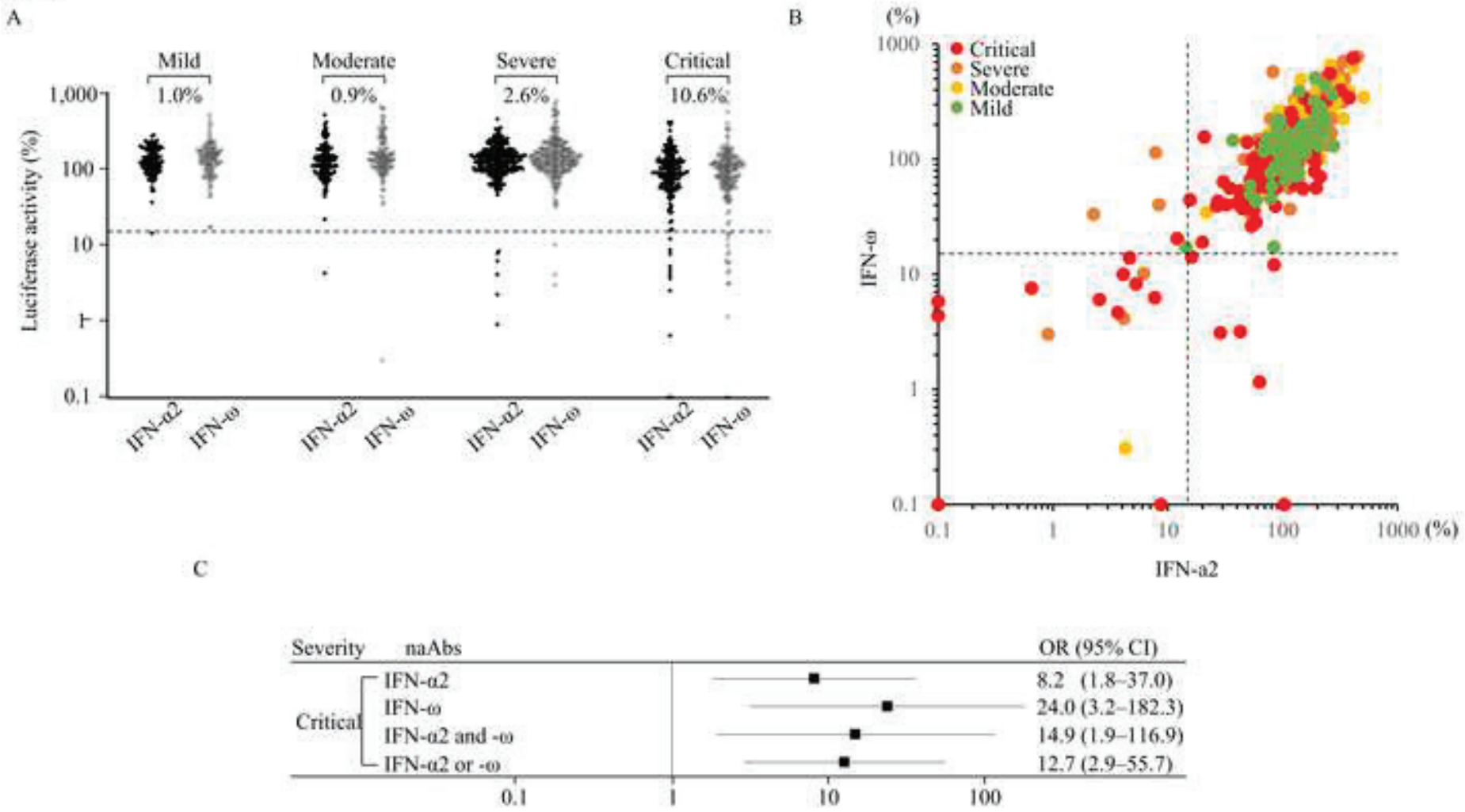


Fig. 4

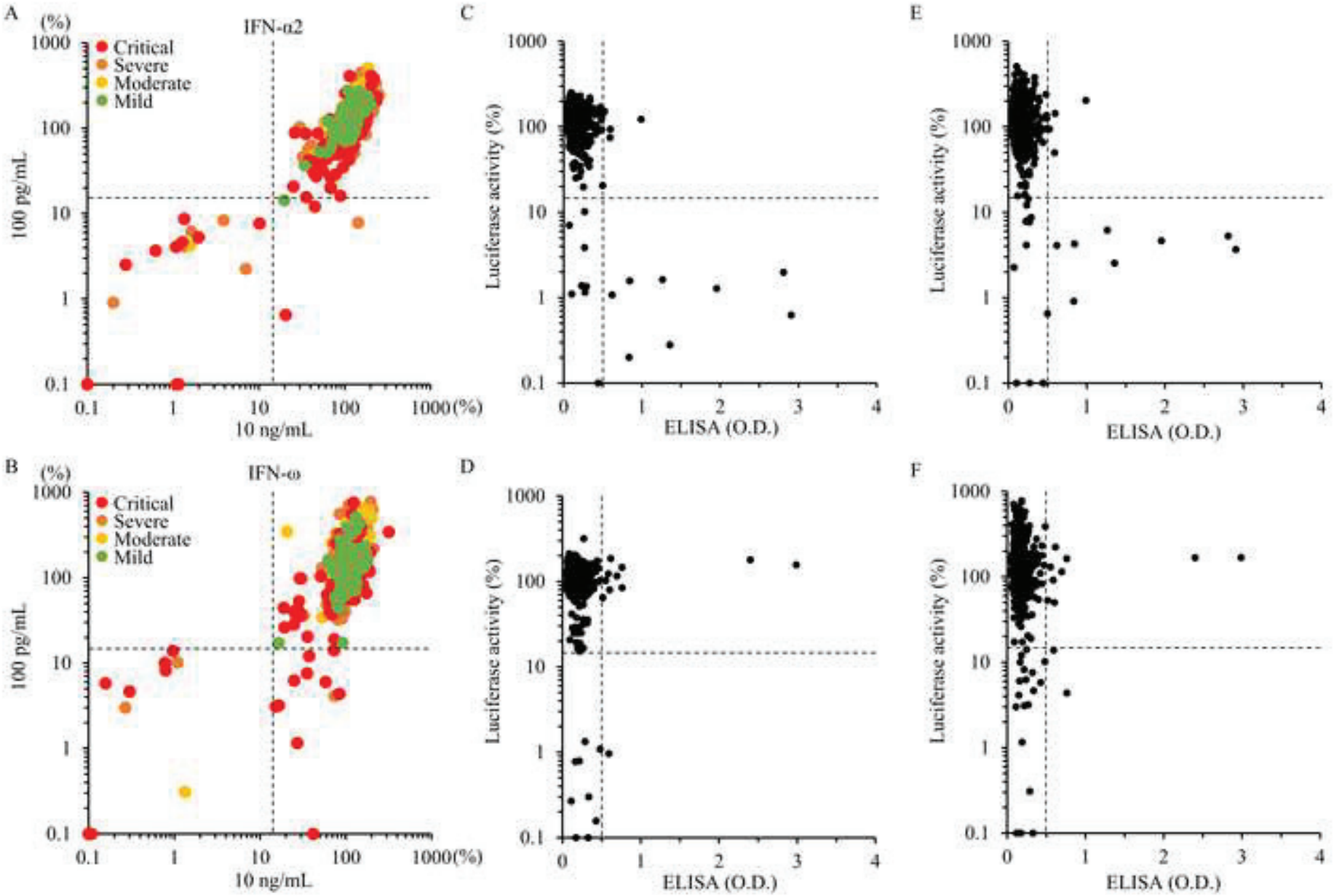
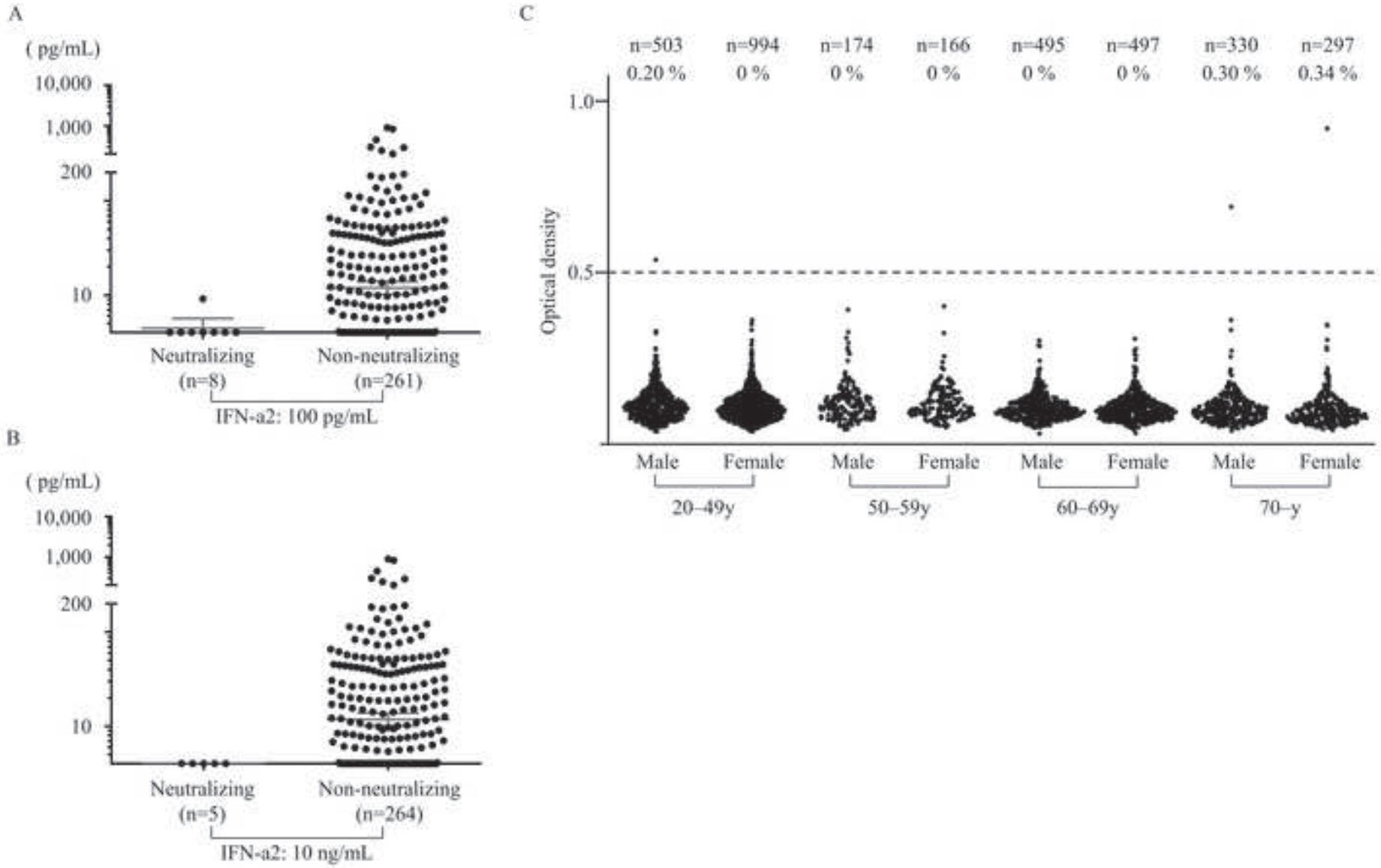


Fig. 5



Electronic Supplementary Materials

Neutralizing type I interferon autoantibodies in Japanese patients with severe COVID-19

Authors

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Supplemental Figures 1, 2, 3, 4, 5

Supplemental materials and methods

Table S1 Characteristics of general population before the appearance of COVID-19 and after the appearance of COVID-19

	Before the appearance of COVID-19			After the appearance of COVID-19		
Age (years)	Total cases [n = 2,069]	Male [n = 1,127]	Female [n = 942]	Total cases [n = 1,387]	Male [n = 375]	Female [n = 1,012]
20–29	1	0	1	535	72	463
30–39	47	20	27	392	144	248
40–49	239	166	73	283	101	182
50–59	183	127	56	157	47	110
60–69	972	484	488	20	11	9
70–79	519	267	252	0	0	0
80–89	105	60	45	0	0	0
90–	3	3	0	0	0	0

Table S2 aAbs to type I IFNs in 622 patients with COVID-19

aAbs detected by ELSIA		
Severity	IFN-α2 only	IFN-ω only
Mild	1	3
Moderate	1	0
Severe	2	2
Critical	4	2
Total	8	7
Age (years)	IFN-α2 only	IFN-ω only
0–49	1	2
50–	**7**	**5**
50–59	3	2
60–69	1	1
70–	3	2

Table S3 naAbs to type I IFNs in 622 patients with COVID-19

naAbs detected by Neutralizaiton assay				
	10 ng/mL		100 pg/mL	
Severity	IFN-α2 only	IFN-ω only	IFN-α2 only	IFN-ω only
Mild	0	0	1	0
Moderate	0	0	0	0
Severe	3	0	3	0
Critical	3	0	1	6
Total	6	0	5	6
Age (years)	IFN-α2 only	IFN-ω only	IFN-α2 only	IFN-ω only
0–49	0	0	0	0
50-	**6**	**0**	**5**	**6**
50–59	2	0	1	3
60–69	1	0	2	2
70–	3	0	2	1

Table S4 The prevalence of naAbs to type I IFNs detected by the neutralization assay in 440 male and 187 female patients with COVID-19

	naAbs detected by Neutralizaiton assay							
		10 ng/mL				100 pg/mL		
Male	Severity	IFN-α2 only	IFN-ω only	IFN-α2 and -ω	% of positive cases	IFN-α2 only	IFN-ω only	IFN-α2 and -ω	% of positive cases
	Mild	0	0	0	0.0%	1	0	0	1.5%
	Moderate	0	0	1	1.5%	0	0	1	1.5%
	Severe	3	0	2	3.0%	3	0	3	3.6%
	Critical	2	0	7	6.5%	1	5	10	11.6%
	Total	5	0	10	3.4%	5	5	14	5.5%
Female	Severity	IFN-α2 only	IFN-ω only	IFN-α2 and -ω	% of positive cases	IFN-α2 only	IFN-ω only	IFN-α2 and -ω	% of positive cases
	Mild	0	0	0	0.0%	0	0	0	0.0%
	Moderate	0	0	0	0.0%	0	0	0	0.0%
	Severe	0	0	0	0.0%	0	0	0	0.0%
	Critical	1	0	0	3.1%	0	1	1	6.3%
	Total	1	0	0	0.5%	0	1	1	1.1%

Table S5 Summary of reported articles of antibodies to type I IFNs

Authors	Detection of autoantibody		Neutralizing assay of Autoantibody			Stage at the time of sample	Region / Country
	aAb	Prevalence of autoantibody	naAb	COVID-19 patients	Healthy individuals		
P. Bastard, et al.	Type I IFNs*	Critical: 13.7 % (135/987)	IFN-α2, -ω	Critical: 10.2 % (101/987), Aymptomatic/mild: 0 % (0/663)	0.33%(4/1227)	Acute stage	CHGE project (international)
J. Troya, et al.	-	-	IFN-α2, -ω	Critical and Severe; 10.6 % (5/47), Asymptomatic: 0 % (0/118)	-	Acute stage	Madrid, Spain
SE. Vazquez, et al.	IFN-α2	3%(4/116)	IFN-α2, -ω	1.5%(2/116)	-	Convalescent stage	USA
D. Goncalves, et al.	IFN-α2	Critical: 25%(21/84), Mild: 0%(0/10)	IFN-α2	Critical: 18%(15/84)	-	Acute stage	Lyon, France
EY. Wang, et al.	Type I IFNs**	5.2% of 197 inpatients	-	-	-	Acute stage	New Haven, USA
R. Koning, et al.	IFN-α2, -ω	17%(35/210)	IFN-α2, -ω	2.9%(6/210). ※All of patients with neutralizing auto-Abs required ICU admission.	-	Acute stage	Amsterdam, Netherland
M.G.P. Wijst, et al.	IFN-α2	Critical: 19%(5/26), Severe: 6%(6/102), Moderate: 0%(0/156)	IFN-α2	Critical: 19 % (5/26), severe: 5 % (5/	-	Acute stage	San Francisco, USA
P. Bastard, et al.	-	-	IFN-α2, -ω	Critical: 13.6 % (489/3595) (including 21 % of patients >80y), Deceased: 18 % of 1124 deceased patients, Severe: 6.5 % (34/522)	<70 years: 1%, 70～80 years: 2.3%, >80 years: 6.3% of individuals	Acute stage	CHGE project (international)
A.C. Grenier, et al.	IFN-α2, -ω	Critical: 77%(107/139)	IFN-α2, -ω	Critical 7.9%(11/139) Deceaced: 21%(6/29)	-	Acute stage	France

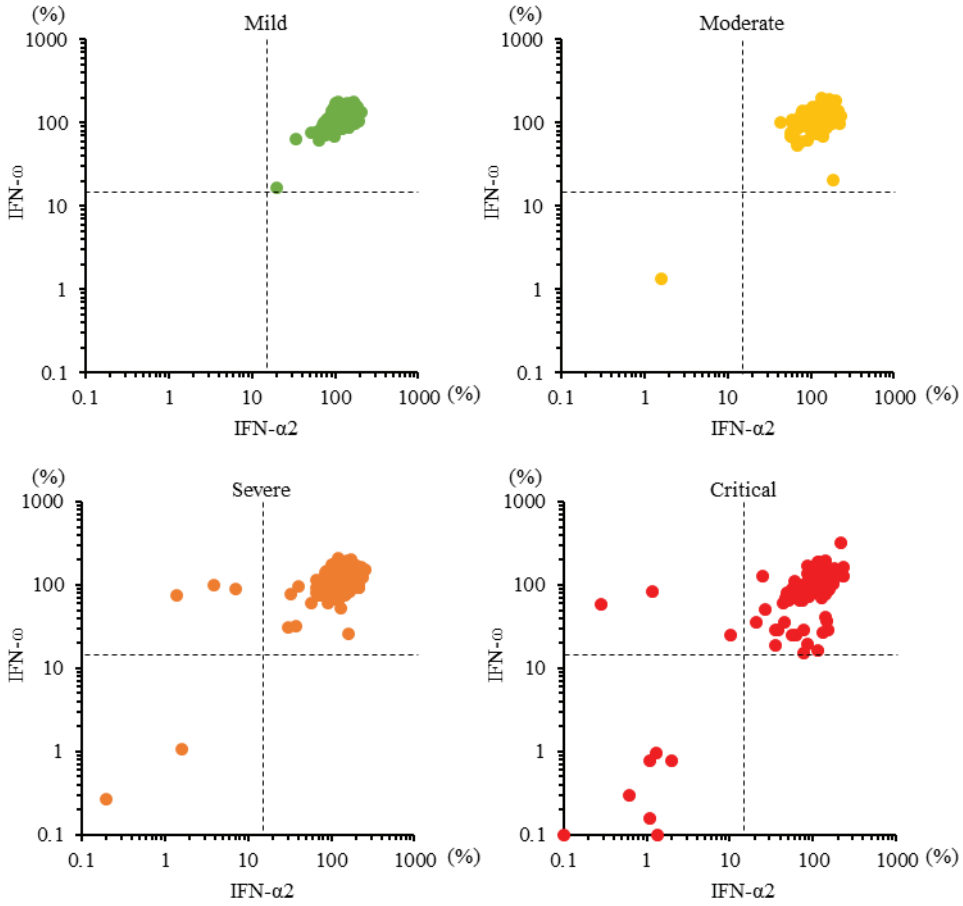

Figure S1

naAbs to type I IFNs in 622 patients with COVID-19 at a cytokine concentration of 10 ng/mL. Luciferase activity (%) against 10 ng/mL IFN-α2 or IFN-ω in patients with COVID-19 according to its severity (n=622). The cutoff value of Luciferase activity (%) was 15%.

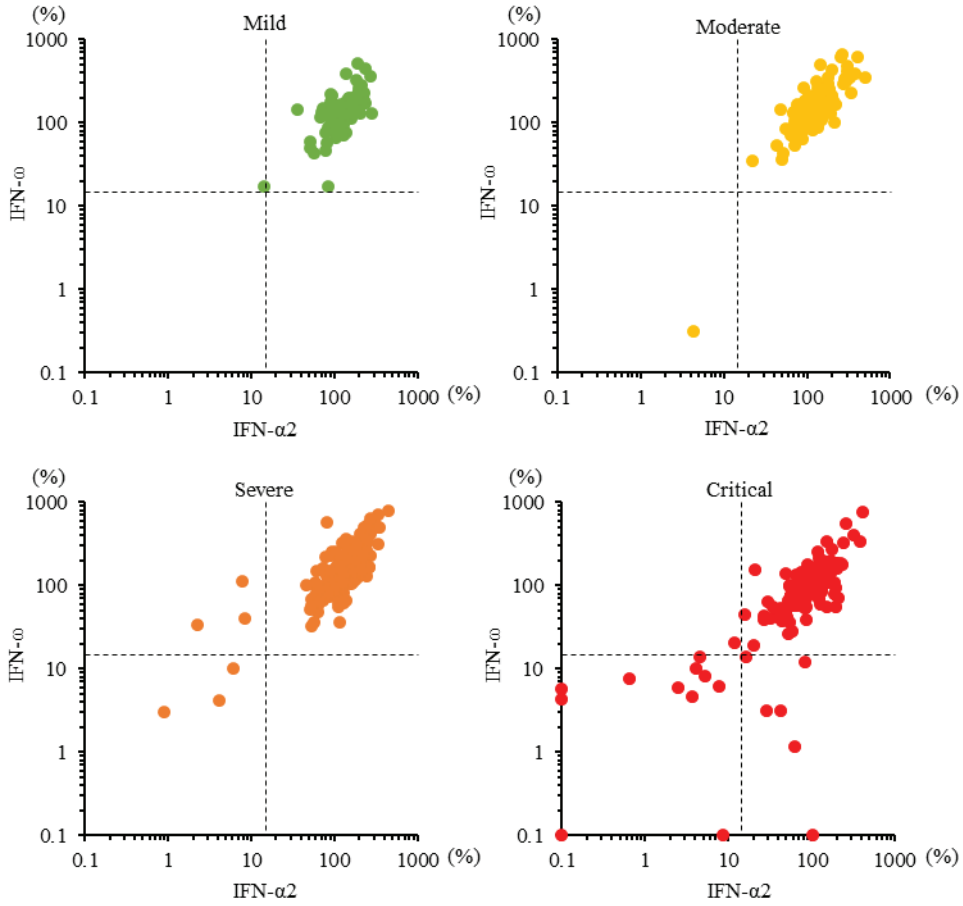

Figure S2

naAbs to type I IFNs in 622 patients with COVID-19 at a cytokine concentration of 100 pg/mL. Luciferase activity (%) against 100 pg/mL IFN-α2 or IFN-ω in patients with COVID-19 according to its severity (n=622). The cutoff value of Luciferase activity (%) was 15%.

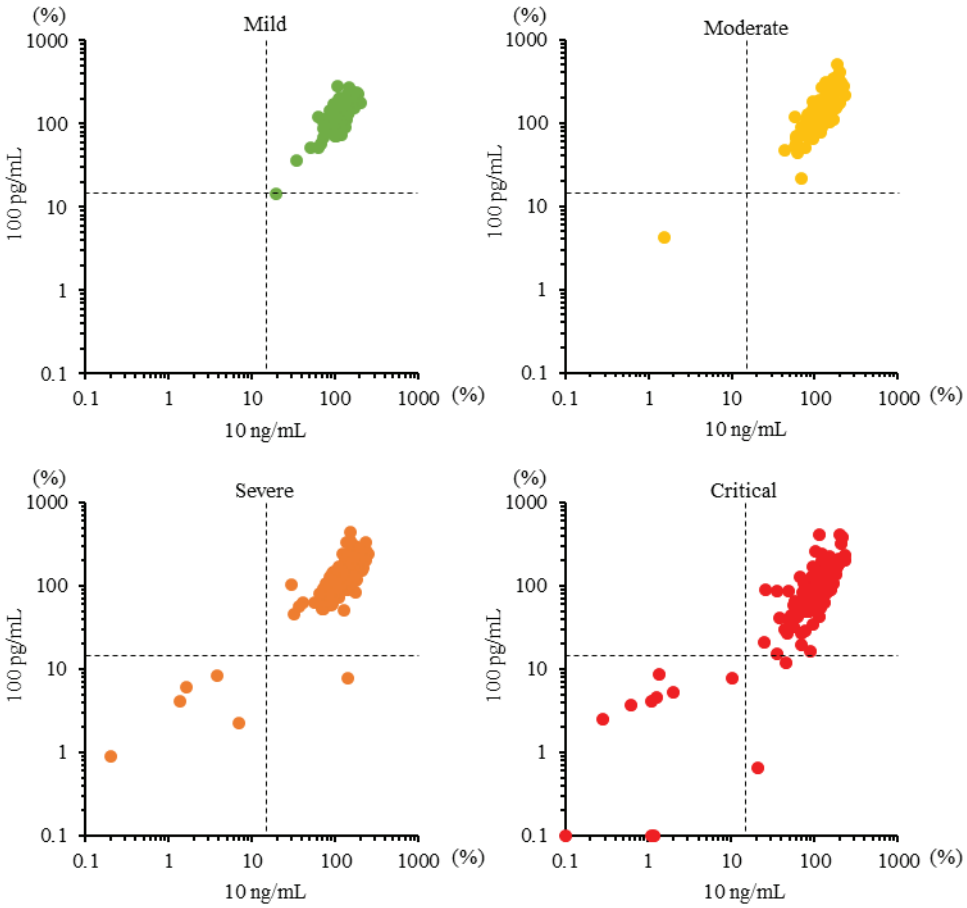

Figure S3

naAbs to IFN-α2 in 622 patients with COVID-19. Luciferase activity (%) against IFN-α2 in patients with COVID-19 according to its severity (n=622). The cutoff value of Luciferase activity (%) was 15%. Activity levels of 10 ng/mL and 100 pg/mL were compared.

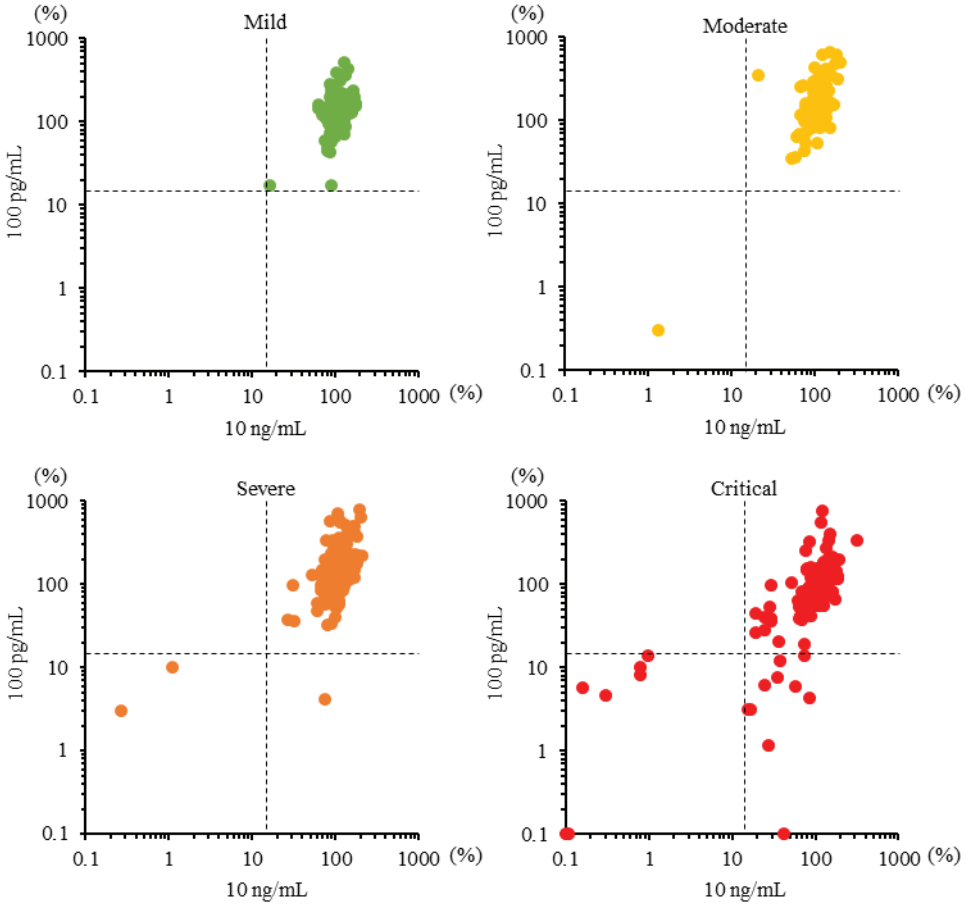

Figure S4

naAbs to IFN-ω in 622 patients with COVID-19. Luciferase activity (%) against IFN-ω in patients with COVID-19 according to its severity (n=622). The cutoff value of Luciferase activity (%) was 15%. Activity levels of 10 ng/mL and 100 pg/mL were compared.

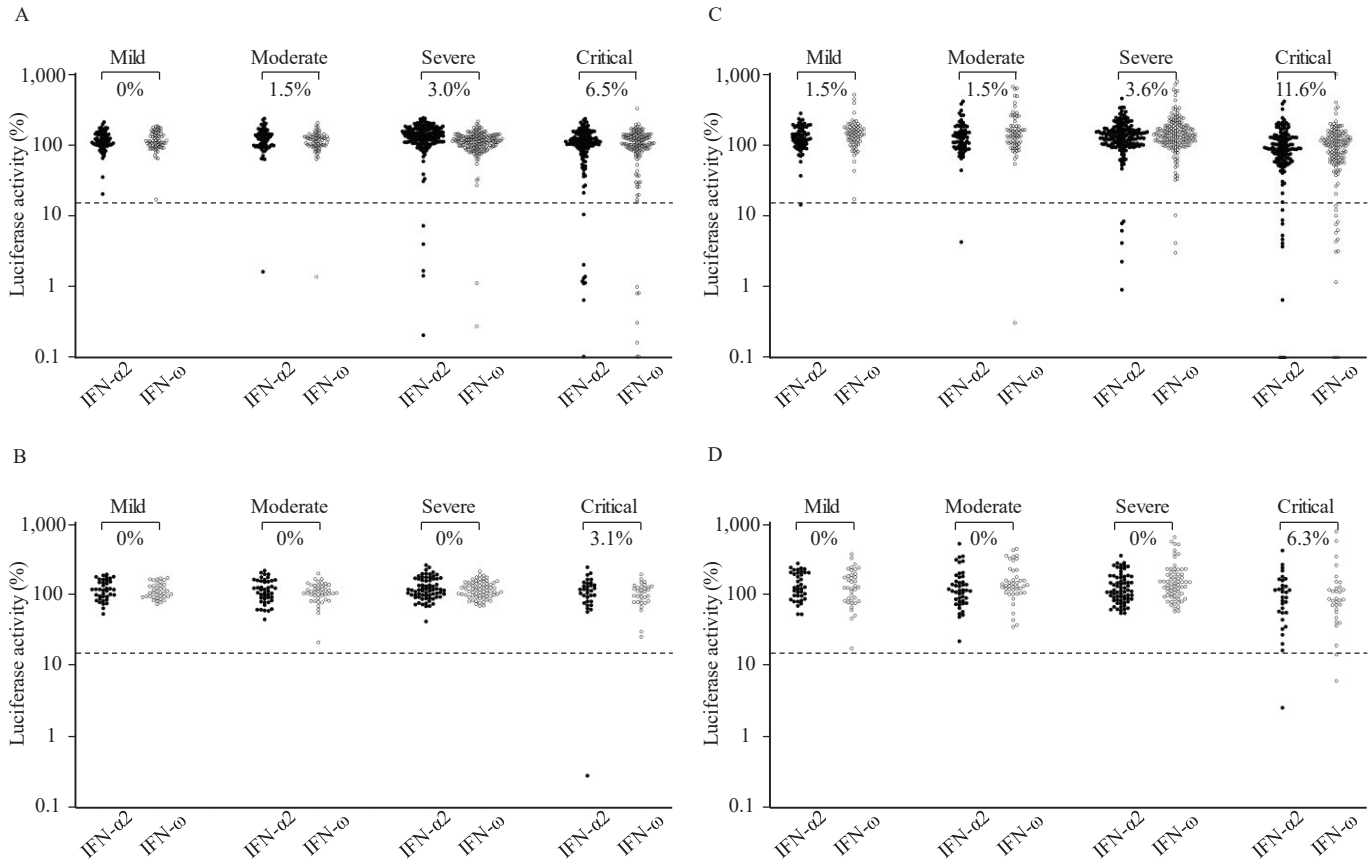

Figure S5

naAbs to type I IFNs in 622 patients with COVID-19 classified by sex and cytokine concentration. Luciferase activity (%) against IFN-α2 or IFN-ω in patients with COVID-19 according to its severity (n=622). 138 critical, 166 severe, 68 moderate, and 67 mild infections in male patients. 32 critical, 69 severe, 44 moderate, and 38 mild infections in female patients. The cutoff value of Luciferase activity (%) was 15%. **A** The neutralization assay to 10 ng/mL of type I IFNs in males. **B** The neutralization assay against 10 ng/mL of type I IFNs in females. **C** The neutralization assay against 100 pg/mL of type I IFNs in males. **D** The neutralization assay to 100 pg/mL of type I IFNs in females.

Supplemental materials and methods

COVID-19 patients and individuals in the general population subjected to analysis

We conducted the study at Hiroshima University Hospital, Tokyo Medical and Dental University Medical Hospital, and Osaka City University Hospital. 622 patients with COVID-19 admitted to our institutes and 3,456 individuals from the general population, which included 1,000 previously reported individuals, were enrolled in this study (Table1).[1] The general population consisted of 2,069 people with annual medical check-ups from April 2017 through March 2018 (before the appearance of SARS-CoV-2) and 1,387 medical staff without a history of COVID-19 infection after the appearance of SARS-CoV-2 (Table S1). The median age of the COVID-19 patients was 61 years (IQR: 46-73 years); 70.2% were males, and 29.8% were females (Fig 1A, Table 1). The median age of the general population was 56 years (IQR: 37-67 years); 43.5% were males, and 56.5% were females (Fig 1B, Table 1, E1). All subjects were recruited according to ethics codes approved by the local institutional review boards. The diagnosis of COVID-19 was made by direct detection of SARS-CoV-2 RNA by nucleic acid amplification tests. These COVID-19 samples were collected by August 2021 before the appearance of the Delta variant.

Neutralization assay of autoantibodies (aAbs) to type I IFNs

We performed luciferase reporter assays as described previously.[2] HEK293T cells were seeded on 96-well plates at a cell density of 4.0×10^4 cells/well in 100 μL media and incubated them overnight at 37°C. After 16 hours when cells were 70-80% confluent, we transfected a luciferase reporter plasmid vector containing the firefly luciferase gene driven by the promoter of the interferon-stimulated response element (ISRE) in the pGL4.45 backbone with a control reporter plasmid vector pRL-SV40 for normalization into HEK293T cells using X-tremeGene 9 transfection reagent (Roche Diagnostics, Basel, Switzerland) and incubated them for 24 hours. We added 10% serum/plasma collected from individuals which were diluted with Dulbecco's Modified Eagle medium, (DMEM) (Thermo Fisher Scientific) containing 2% HyClone™ fetal bovine serum (GE Healthcare

Life Sciences, IL, USA). We stimulated cells with rhIFN-α2 for 8 hours or rhIFN-ω for 12 hours at cytokine concentrations of 10 ng/mL or 100 pg/mL, respectively, at 37°C. Finally, we lysed the cells and measured the luciferase levels with a Dual-Luciferase Reporter assay system (Promega, WI, USA) using an EnSpire plate reader. We evaluated neutralizing activity of aAbs as follows: Firefly luciferase activity values were normalized against Renilla luciferase activity values. These values were then normalized against the median induction level for non-neutralizing samples of healthy controls tested on the same day. Luciferase activity (%) was calculated by following equation: Luciferase activity (%) = Pf/Pr ÷ Cf/Cr ×100. (Pf = Firefly luciferase activity of a patient, Pf = Firefly luciferase activity of a patient, Cf = Firefly luciferase activity of the median values for healthy controls, Cr = Renilla luciferase activity of the median values for healthy controls). Based on the result from previous report[1], samples were considered to have neutralizing activity if the Luciferase activity was below 15%.

Enzyme-linked immunosorbent assay (ELISA): Detection of aAbs to type I IFNs

We performed ELISA as described previously.[2] We coated 96-well ELISA plates (F96 MaxiSorp Nunc-Immuno Plate; Thermo Fisher Scientific, MA, USA) overnight at 4 ℃ with 1 μg/mL rhIFN-α2 (Human IFN-a2a research grade, Miltenyi Biotec, CA, USA) at 100 μL/well or 1 μg/mL rhIFN-ω (human IFN-ω, eBioscience, CA, USA) at 100 μL/well. We washed the plates with PBS three times and blocked the plates with blocking medium (PBS with 5% nonfat milk powder) for 1 hour at room temperature on an agitator. Then, we washed plates with PBS containing 0.005% Tween once? and added 100 μL of 1/50 plasma dilutions (High Performance ELISA buffer, MA, USA) for 2 hours at room temperature on an agitator. Next, we washed the plates with PBS containing 0.005% Tween, then added 2 μg/mL secondary antibody (goat anti-human IgG IgA IgM (Fc specific) conjugated with horseradish peroxidase, Nordic MUbio, Susteren, Netherlands) at 100 μL/well and incubated the plates for 1 hour at room temperature on an agitator while protected from light. Finally, we washed the plates

with PBS containing 0.005% Tween, added 100 μL/well substrate (KPL SureBlue™ TMB Microwell Peroxidase Substrate, MA, USA), kept the plates on an agitator for 5 minutes, then added the same amount of 1.8 M H_2SO_4, and measured the optical density (450 nm/630 nm) with an EnSpire® plate reader (PerkinElmer, MA, USA).

We used a machine (Wellwash™ Microplate Washer, Thermo Fisher Scientific) when we washed the plates. We set the cutoff value as 0.5 (O.D.) based on the result from previous report[2] and neutralization assay in the current study. We performed neutralization assay in samples with more than 0.3 OD among 2,069 samples (17 samples) from general population with ELISA. All samples with more than 0.5 OD had neutralizing activity while all samples with less than 0.5 OD did not have neutralizing activity. This is why we set the cut-off value as 0.5 in our experiment.

Measurement of IFN-α2 concentration

We tested the serum IFN-α2 concentration with the ProQuantum™ Human IFN alfa Immunoassay Kit (Invitrogen, MA, USA) according to its technical guide. Briefly, we diluted samples 10-fold with assay dilution buffer, then mixed 5 μL of diluted samples with the same amount of antibody-conjugate mixture and incubated them for 1 hour at room temperature. After incubation, we added 40 μL of qPCR mixture to each sample and measured them with a StepOnePlus Real-Time PCR System (Applied Biosystems, MA, USA) and analyzed them with StepOne™ Software. Finally, we multiplied the measured IFN-α2 value by 10 to return to the in vivo concentration.

Statistical analysis

As statistical analysis, comparisons of categorical variables were performed using Fisher's exact test. Two-sided p values less than 0.05 were considered statistically significant. Odds ratio for the effect of naAbs to type I IFNs on critical or severe COVID-19 were calculated with 95% Confidence Interval from a 2-by-2 table using mild/moderate patients as controls. The

nonparametric Kruskal-Wallis test was applied to compare IFN-α2 concentrations between patients with naAbs and without nAbs to IFN-α2 and/or IFN-ω on. Statistical analyses were performed using JMP software (SAS Institute, NC, USA).

References

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2. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 2020; 370.