

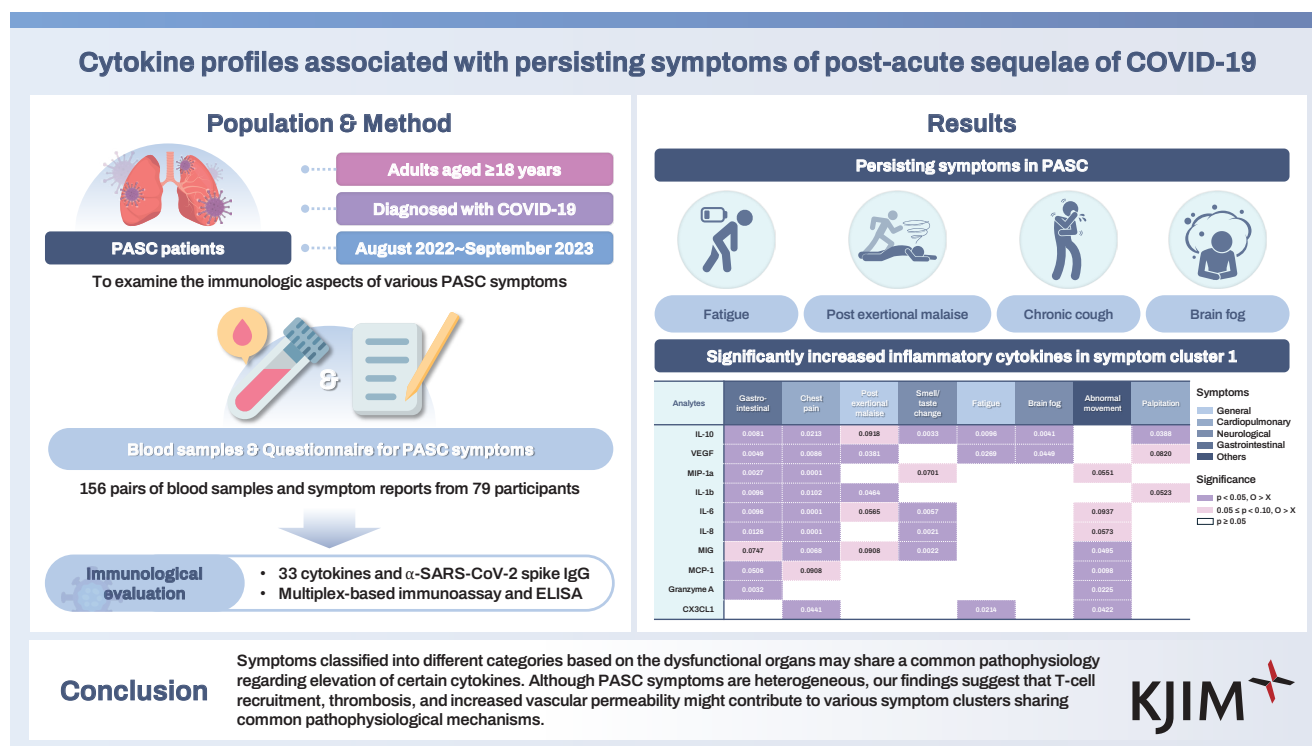


Cytokine profiles associated with persisting symptoms of post-acute sequelae of COVID-19

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Background/Aims: Post-acute sequelae of COVID-19 (PASC) are highly heterogeneous; therefore, the pathophysiological mechanisms for PASC remain unclear. In this study, we aimed to examine the immunologic aspects of various PASC symptoms.

Methods: We prospectively enrolled adults aged ≥ 18 years who were diagnosed with COVID-19 between August 2022 and September 2023. Blood samples were collected from all participants, who were interviewed using a questionnaire for PASC symptoms at least once between 1 and 6 months after the COVID-19 diagnosis. For immunological evaluation, plasma concentrations of SARS-CoV-2 spike subunit 1-specific IgG and 33 cytokines were measured using enzyme-linked immunosorbent assays and multiplex-based immunoassay, respectively.

Results: In total, 156 pairs of blood samples and symptom reports from 79 participants were eligible for analysis. The most frequent symptom was fatigue, followed by post exertional malaise, chronic cough, thirst, and brain fog. Gastrointestinal symptoms, chest pain, post exertional malaise, smell/taste change, fatigue, brain fog, abnormal movement, and palpitation were accompanied by significant increases in IL-10, VEGF, and inflammatory cytokines like MIP-1 α , IL-1 β , IL-6, IL-8, MIG, granzyme A, and CX3CL1 levels, while chronic cough, dizziness, dyspnea, and hair loss were not accompanied by significant differences in cytokine levels.

Conclusions: Symptoms classified into different categories based on the dysfunctional organs may share a common pathophysiology regarding elevation of certain cytokines. Although PASC symptoms are heterogeneous, our findings suggest that T-cell recruitment, thrombosis, and increased vascular permeability might contribute to various symptom clusters sharing common pathophysiological mechanisms.

Keywords: Post-acute COVID-19 syndrome; Cytokines; Signs and symptoms

INTRODUCTION

The emergence of the novel coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in an unprecedented global health crisis. While the acute phase of COVID-19 primarily manifests as respiratory symptoms, a certain portion of the population experiences persistent or newly onset symptoms weeks or months after the resolution of acute infection; this condition is now termed post-acute sequelae of COVID-19 (PASC) [1]. PASC encompasses a broad range of symptoms affecting multiple organ systems, including but not limited to fatigue, dyspnea, cognitive impairment, and myalgia, significantly impacting the quality of life of recovered individuals [2,3].

Although several hypotheses, including dysregulated immune response, persistent viral reservoir, dysregulated microbiota, and endothelial abnormalities, have been proposed, the pathophysiology underlying PASC remains unclear due to the varied definitions of PASC among previous studies [4-8]. In this study, our objective was to examine the patterns of cytokine expression related to particular PASC symptoms to elucidate the underlying mechanism of PASC.

METHODS

Study cohort

We prospectively enrolled adults aged ≥ 18 years who were diagnosed with COVID-19 between August 2022 and September 2023. The visits were scheduled for 1, 3, and 6

months after the COVID-19 diagnosis. The blood sampling and questionnaire survey were conducted on the same day during the participant's visit, ensuring that the timing of symptom report corresponded with the antibody and cytokine analysis. The questionnaire survey was conducted to investigate representative symptoms, including those presented in the study by Thaweethai et al. [9]: change in sense of smell or taste, post exertional malaise (PEM), chronic cough, brain fog, thirst, chest pain, dyspnea, palpitation, fatigue, cognitive dysfunction, abnormal movement, gastrointestinal symptom, dizziness, changes in sexual desire and capacity, and hair loss. These symptoms were classified into general, cardiopulmonary, neurological, gastrointestinal, and other symptoms [1]. Patients with PASC were defined by PASC index with 12 or higher, using a scoring system established in recent studies [9,10]. This study was reviewed and approved by the Institutional Review Board (IRB) of Asan Medical Center (IRB no. 2022-1477), and written informed consent was obtained from all subjects involved in the study.

Assessment of antibody response

For immunological evaluation, the plasma concentration of SARS-CoV-2 spike subunit 1-specific IgG antibody (S1-IgG) titer was measured using an in-house developed enzyme-linked immunosorbent assay as described previously [11]. Antibody titers are represented in IU/mL as per World Health Organization international standards.

Assessment of cytokine levels

We simultaneously analyzed the plasma levels of 33 cytokines using a cytometric bead array (BD Bioscience, San

Jose, CA, USA) according to the manufacturer's instructions as described in our previous study [12]. Briefly, microspheres for capturing and detecting the following cytokines were incubated with the plasma samples: interferon (IFN)- α , IFN- γ , interleukin (IL)-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, IL-21, granzyme A, granzyme B, monokine induced by IFN- γ (MIG), IFN- γ induced protein (IP)-10, IFN-inducible T cell α chemoattractant (I-TAC), tumor necrosis factor (TNF)- α , eotaxin, soluble CD40 ligand (sCD40L), C-X3-C motif chemokine ligand 1 (CX3CL1), Fas ligand (FasL), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1 α , regulated on activation, normally T-cell expressed and secreted (RANTES), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). Data acquisition was performed using a FACS CANTO II flow cytometer, FACS Diva software (BD Biosciences), and FlowJo software (FlowJo LLC, Ashland, OR, USA).

Statistical analyses

For statistical analyses, categorical variables and continuous variables were compared using Fisher's exact test or the χ^2 test and Mann-Whitney U test, respectively. All tests of significance were two-tailed, and p values less than 0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism 9.4.1 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

A total of 156 blood samples and symptom reports from 79 participants were eligible for analysis. The baseline characteristics of participants are as follows (Table 1). The mean age was 57.9 years, and approximately 60% of participants were male. Most participants (86.1%) completed a 2-dose vaccination with the adenovirus-vectored vaccine or mRNA vaccines; 72.2% of these participants had received booster shots. Additionally, 15.6% of all participants had been previously healthy, and approximately half of the participants experienced mild COVID-19. The treatments that hospitalized individuals received are summarized in Table 1. Of this cohort, 41 individuals (51.9%) met criteria for PASC and 38 (48.1%) were categorized as non-PASC (Supplementary Ta-

Table 1. Baseline characteristics of participants

Characteristic	Value (n = 79)
Age (yr)	57.9 $\pm$ 13.5
Sex, male	47 (59.5)
Vaccination status before diagnosis	
Unvaccinated (0 dose)	8 (10.1)
Partially vaccinated (1 dose)	3 (3.8)
Fully vaccinated (2 doses)	11 (13.9)
Fully vaccinated + monovalent booster	45 (57.0)
Fully vaccinated + bivalent booster	12 (15.2)
Predominant variant at diagnosis	
BA.5 (since Jul 4, 2022)	22 (27.8)
BA.2.75 (since Jan 16, 2023)	21 (26.6)
XBB (since Apr 24, 2023)	36 (45.6)
Number of infections (n = 74) ^{a)}	
Once	64 (86.5)
More than twice	10 (13.5)
Underlying diseases (n = 77) ^{b)}	
Previously healthy	12 (15.6)
Diabetes mellitus	28 (36.4)
Cardiovascular disease	39 (50.6)
Pulmonary disease	11 (14.3)
Chronic liver diseases	7 (9.1)
Chronic kidney diseases	16 (20.8)
Malignancy	20 (26.0)
Transplantation	27 (35.1)
Autoimmune disease	0 (0.0)
Any mental health problems	3 (3.9)
COVID-19 NIH severity at diagnosis (n = 71) ^{c)}	
Asymptomatic/presymptomatic	7 (9.9)
Mild	33 (46.5)
Moderate	14 (19.7)
Severe	9 (12.7)
Critical	8 (11.3)
Treatments of hospitalized patients (n = 55) ^{d)}	
Supplemental oxygen therapy	33 (61.1)
Steroid	41 (75.9)
Nirmatrelvir/ritonavir	1 (1.9)
Molnupiravir	3 (5.6)
Remdesivir	52 (96.3)
Tocilizumab	5 (9.3)
Baricitinib	5 (9.3)
Tocilizumab + baricitinib	3 (5.6)

Values are presented as mean $\pm$ standard deviation or number (%). NIH, National Institutes of Health.

^{a)}The infection history before participation in this study was uncertain in five individuals.

^{b)}Data of two individuals was not available.

^{c)}Data of eight individuals was not available.

^{d)}Data of one individual was not available.

ble 1). PASC group showed a higher proportion of individuals with multiple infections (more than twice) ($p = 0.097$), chronic liver diseases ($p = 0.051$), and use of tocilizumab ($p = 0.019$).

Because a few participants did not respond to all survey questions regarding certain symptoms, only the responses for each symptom were included in the analysis. The most

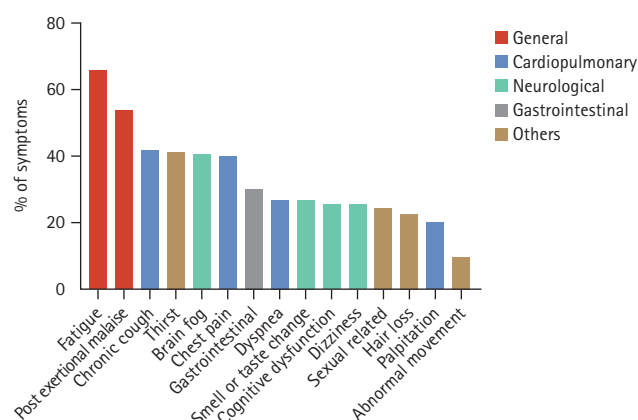


Figure 1. Frequencies of symptoms reported by participants.

frequent symptom was fatigue (65.7%), followed by PEM (53.7%), chronic cough (41.9%), thirst (41.5%), brain fog (40.3%), and chest pain (40.0%) (Fig. 1). Among 58 participants with a complete set of symptom records, 5 (8.6%) did not exhibit any symptoms. Participants who had one or more symptoms had a median of four symptoms (interquartile range 2–7). When individuals were divided into groups based on the number of symptoms and observed over time, approximately 20% of individuals still possessed more than eight symptoms even after six months post-diagnosis (Supplementary Table 2).

Based on the data collected, we analyzed whether the presence of PASC symptoms is associated with the time elapsed after the infection. The results of the χ^2 test indicate that cardiopulmonary symptoms such as PEM, chronic cough, and chest pain tended to decrease as time progressed (Supplementary Table 3, $p = 0.003$, 0.098 , and 0.061 , respectively). Except for fatigue ($p = 0.083$), we could not observe significant changes of prevalence over time for symptoms other than cardiopulmonary symptoms.

When comparing antibody and cytokine concentrations in



Figure 2. Clusters of symptoms based on cytokine expression patterns.

relation to the presence of symptoms, it was observed that the cytokine expression patterns varied for each symptom, whereas the antibody levels were not different (Fig. 2 and Supplementary Fig. 1). Among the symptoms investigated, the widest variety of cytokine levels showed significant differences in the presence of gastrointestinal symptoms. The IL-10, VEGF, IL-1 β , IL-6, IL-8, MIP-1 α , MCP-1, granzyme A, and IL-9 levels were significantly higher in participants with gastrointestinal symptoms than in those without gastrointestinal symptoms ($p < 0.05$). In contrast, there were no significant differences in cytokine levels between participants with and without chronic cough, dyspnea, dizziness, or hair loss.

We categorized the investigated symptoms into three clusters based on cytokine expression patterns. Symptom cluster 1, including gastrointestinal symptoms, chest pain, PEM, change in sense of smell/taste, fatigue, brain fog, abnormal movement, and palpitation, primarily exhibited significantly increased levels of IL-10, VEGF, and various inflammatory cytokines like MIP-1 α , IL-1 β , IL-6, IL-8, MIG, granzyme A, and CX3CL1. Additionally, the expression of other cytokines was either significantly increased or decreased. A consistent increase in IL-10 levels was observed in participants who experienced gastrointestinal symptom, chest pain, change in sense of smell/taste, brain fog, fatigue, or palpitation (Supplementary Fig. 2). An increased VEGF level was commonly observed within the cytokine profile among participants who experienced gastrointestinal symptom, chest pain, PEM, fatigue, or brain fog. Besides these cytokines, IL-1 β , IL-6, IL-8, MIG, sCD40L, and CX3CL1 showed common expression patterns across different symptom profiles. In addition, the FasL, I-TAC, and IFN- α levels in participants with PEM or fatigue were significantly higher than in participants without these symptoms ($p = 0.003$, 0.0003 , and < 0.0001 , respectively).

Symptom cluster 2 (thirst, cognitive dysfunction, and changes in sexual desire and capacity) did not show significant differences in the levels of inflammatory cytokines that were elevated during symptom presentation in symptom cluster 1. Nevertheless, symptoms in cluster 2 were accompanied by differences in the expression levels of cytokines rather than IL-10, VEGF, and inflammatory cytokines. In contrast, symptom cluster 3 (chronic cough, dizziness, dyspnea, and hair loss) did not demonstrate significant differences in cytokine levels compared to the levels in the absence of these symptoms.

DISCUSSION

Cytokine secretion is one of the initial innate immune responses crucial for acute inflammation. Elevated levels of proinflammatory cytokines in circulation can result in systemic inflammatory symptoms and subsequent damage to secondary organs. Thus, revealing cytokine profiles is important to gain insight into the pathophysiological aspects of COVID-19 [13]. Furthermore, it has been reported that certain cytokines remain significant even after recovery from SARS-CoV-2 infection [4,14-18]. Given the impact of PASC on quality of life [19,20], it is important not only to consider the number of symptoms, but also understand which symptoms persist over time and the mechanisms underlying their persistence. In our current study, we analyzed the frequencies and evaluated the cytokine profiles of individuals with certain symptoms of PASC to better understand of the mechanism of PASC. We observed that a few symptoms emerged prominently at earlier time points and subsequently diminished, whereas others remained persistent even six months after diagnosis. This pattern of symptom occurrence seems similar to that reported in the previous studies [19-21]. In addition, our data showed that even when symptoms were classified within the same category based on the dysfunctional organs, e.g., neurological or cardiopulmonary symptoms, the patterns of cytokine expression varied markedly. Moreover, our data suggests that symptoms belonging to different categories may share a common pathophysiology in terms of the elevation of certain cytokines. To our best knowledge, few studies have clustered PASC symptoms based on cytokine profiles. While Wang et al. [22] identified clinical phenotypes using multi-omics analysis, their approach differs from ours. However, cytokine-based clustering has been applied in other conditions [23-26]. The previous studies clustered patients with similar characteristics by cytokine levels, revealing differences in prognosis despite similar clinical characteristics. This suggests that diseases with heterogeneity, it is thought to be meaningful to cluster symptoms and patients based on cytokine profiles, even among patients with similar symptoms. In the case of brain fog (symptom cluster 1), it is often considered synonymous with cognitive dysfunction (symptom cluster 2), but they are not entirely identical symptoms. Both symptoms have been thought to be associated with neuroinflammation [27-29]. Moreover, in our recent work in another independent neurological PASC cohort (not yet published) suggests

that brain fog appears to be more closely associated with PEM or fatigue, whereas cognitive dysfunction may represent an independent entity linked to neuronal damage and impaired recovery, accompanied by morphologic changes in magnetic resonance imaging. Alternatively, brain fog frequently accompanied with fatigue and high levels of blood cytokine levels in symptom cluster 1 (fatigue, PEM, and brain fog), as shown in this study, may suggest that these symptoms might be more related with systemic inflammation. Taken together, although the mechanism underlying the pathogenesis of brain fog and cognitive dysfunction have yet to be fully established, these symptoms, while similar in appearance, may arise from distinct mechanisms. Therefore, our clustering of various PASC symptoms based on cytokine characteristics instead of traditional organ-specific symptom classification may provide further insight into the heterogeneity of PASC pathophysiology.

Among the analytes evaluated in this study, S1-IgG level was not related to the presence of any symptom, whereas cytokines showed diverse profiles for each symptom. Participants with gastrointestinal symptoms exhibited the widest variety of alterations compared to those without symptoms, and there were no significant differences in certain symptoms.

Symptoms of the same category based on traditional organ-specific symptom classification had different cytokine profiles, whereas symptoms of different categories based on the involved organs showed certain common cytokine expression patterns. As shown in Fig. 2, IL-10 and VEGF levels were commonly increased in more than one-third of all symptoms. IL-1 β , IL-6, IL-8, MIG, sCD40L, and CX3CL1 showed certain common patterns. The shared expression patterns of certain cytokines observed in symptom cluster 1 suggest that although these symptoms have been classified differently, they might have certain shared pathophysiological mechanisms in patients with PASC, as mentioned above about brain fog and cognitive dysfunction.

Previous studies have reported that the IL-10 level was increased in participants with PASC [14,17], whereas Queiroz et al. [18] reported diminished IL-10 levels in participants with PASC. The varying definitions of PASC across studies might contribute to this difference. In our previous study, patients with severe COVID-19 showed higher IL-10 levels than patients with mild COVID-19 [12]. Considering the results of the previous and current studies, IL-10 plays a proinflammatory role in both the acute and recovery phases of

COVID-19 and seems to be related to the manifestation of PASC symptoms [30-32].

Consistent with previous studies, we observed that VEGF and sCD40L levels were increased in participants with PASC symptoms [33,34]. VEGF is a growth factor for vascular endothelial cells known as vascular permeability factor, and sCD40L is a costimulatory molecule involved in the activation of T cells, B cells, and platelets. Excessive VEGF and sCD40L expression can cause vascular damage. Endothelial damage is considered to be an important mechanism in acute COVID-19 and PASC [35-37]. Recently, it was reported that VEGF and sCD40L secreted from activated platelets interact with CX3CR1+CCR5+ monocytes and further contribute to the progression of vessel damage [34]. The elevated levels of CX3CL1 and RANTES, which are ligands of CX3CR1 and CCR5, respectively, were observed in this study. Thus, vessel damage caused by VEGF, sCD40L, CX3CL1, and RANTES might be a potential target for the treatment of PASC.

When comparing baseline characteristics, there was no statistically significant differences between the two groups in terms of age, sex, vaccination status, or viral variant. We found that PASC group had a trend toward being a higher proportion of individuals with multiple infections, which is consistent with the previous study reporting that long COVID was more prevalent when the subjects had reinfections [38]. However, there was no significant difference in the severity of the initial infection between the two groups. Previous prospective studies that followed patients from the acute phase or 6 months post-infection have reported a correlation between the severity of the initial disease and the development of long-term complications [39,40]. In contrast, the recently published long COVID definition states that PASC can occur after any severity of infection [41]. While the risk of PASC is somewhat higher in patients with severe infection, PASC can develop in patients with mild infections and due to the greater number of mild cases, they represent the majority of PASC patients [42]. In addition, our study enrolled patients with COVID, more focused on PASC: patients with PASC who had even mild COVID were more followed in this study than those without PASC who had mild COVID. Therefore, some cautious interpretation is needed for the interpretation of our data because of this kind of bias. Taken together, further longitudinal studies are needed to better understand the relationship between infection severity and the development of PASC.

Our study has certain limitations. First, since the partici-

pants enrolled in this study were mostly infected with the omicron variant of SARS-CoV-2, the risk and severity of PASC might be different from those infected with wild-type SARS-CoV-2 or variants before omicron. Previous studies have reported that people infected with the wild-type or delta variant had a higher risk and relative rate of post-acute symptoms [43,44]. Second, we did not examine the cells that serve as the source of cytokines. Recent studies have reported that monocytes and SARS-CoV-2-specific functional T cells are important in PASC; inflammation caused by the persistence of viral proteins in monocytes and interactions between cytokines and monocyte surface receptors, including Fcγ receptor, CX3CR1, and CCR5, are considered to play a major role [45-47]. In addition, the persistent presence of viral antigens could lead to the continuous activation of SARS-CoV-2-specific T cells [48,49]. Altogether, examining the association of cytokine profiles in our study with previously reported cellular candidates could enhance our understanding of PASC pathophysiology. Finally, we observed the different cytokine profiles by symptoms, however, it is challenging to determine whether this expression is the cause or a consequence of PASC. Further investigation is needed to provide more definitive insights.

In conclusion, we showed that even after recovery from the SARS-CoV-2 infection, a few degrees of inflammation were maintained. Our data suggest that symptoms classified into different organ-specific categories may share a common pathophysiology in terms of the elevation of certain cytokines. Although PASC symptoms are heterogeneous, our findings suggest that T-cell recruitment, thrombosis, and increased vascular permeability might contribute to the various symptom clusters sharing common pathophysiological mechanisms.

KEY MESSAGE

1. Some PASC symptoms are associated with persistent inflammation.
2. The variation in cytokine profiles across different symptoms suggests the involvement of different pathophysiological mechanisms.
3. Symptoms exhibiting similar cytokine profiles are likely to share common pathophysiology.

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Conflicts of interest

The authors disclose no conflicts.

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Supplementary Table 1. Baseline characteristics of participants with and without PASC

Characteristic	Participant (n = 79)	PASC (n = 41)	Non-PASC (n = 38)	p value
Age (yr)	57.9 ± 13.5	59.4 ± 13.9	56.2 ± 13.0	0.295
Sex, male	47 (59.5)	26 (63.4)	21 (55.3)	0.500
Vaccination status before diagnosis				
Unvaccinated (0 dose)	8 (10.1)	2 (4.9)	6 (15.8)	0.145
Partially vaccinated (1 dose)	3 (3.8)	2 (4.9)	1 (2.6)	> 0.999
Fully vaccinated (2 doses)	11 (13.9)	5 (12.2)	6 (15.8)	0.750
Fully vaccinated + monovalent booster	45 (57.0)	26 (63.4)	19 (50.0)	0.262
Fully vaccinated + bivalent booster	12 (15.2)	6 (14.6)	6 (15.8)	> 0.999
Predominant variant at diagnosis				
BA.5 (since Jul 4, 2022)	22 (27.8)	12 (29.3)	10 (26.3)	0.806
BA.2.75 (since Jan 16, 2023)	21 (26.6)	8 (19.5)	13 (34.2)	0.203
XBB (since Apr 24, 2023)	36 (45.6)	21 (51.2)	15 (39.5)	0.368
Number of infections ^{a)}				
Once	64 (86.5)	32 (80.0)	32 (94.1)	0.097
More than twice	10 (13.5)	8 (20.0)	2 (5.9)	
Underlying diseases ^{b)}				
Previously healthy	12 (15.6)	6 (15.0)	6 (16.2)	> 0.999
Diabetes mellitus	28 (36.4)	13 (32.5)	15 (40.5)	0.487
Cardiovascular disease	39 (50.6)	20 (50.0)	19 (51.4)	> 0.999
Pulmonary disease	11 (14.3)	8 (20.0)	3 (8.1)	0.196
Chronic liver diseases	7 (9.1)	1 (2.5)	6 (16.2)	0.051
Chronic kidney diseases	16 (20.8)	7 (17.5)	9 (24.3)	0.577
Malignancy	20 (26.0)	12 (30.0)	8 (21.6)	0.445
Transplantation	27 (35.1)	13 (32.5)	14 (37.8)	0.641
Autoimmune disease	0 (0.0)	0 (0.0)	0 (0.0)	
Any mental health problems	3 (3.9)	3 (7.5)	0 (0.0)	0.241
COVID-19 NIH severity at diagnosis ^{c)}				
Asymptomatic/presymptomatic	7 (9.9)	3 (8.1)	4 (11.8)	0.703
Mild	33 (46.5)	18 (48.6)	15 (44.1)	0.813
Moderate	14 (19.7)	8 (21.6)	6 (17.6)	0.770
Severe	9 (12.7)	4 (10.8)	5 (14.7)	0.729
Critical	8 (11.3)	4 (10.8)	4 (11.8)	> 0.999
Hospitalized patients' treatments ^{d)}				
Supplemental oxygen therapy	33 (61.1)	13 (50.0)	20 (69.0)	0.178
Steroid	41 (75.9)	18 (69.2)	23 (79.3)	0.537
Nirmatrelvir/ritonavir	1 (1.9)	1 (3.8)	0 (0.0)	0.473
Molnupiravir	3 (5.6)	2 (7.7)	1 (3.4)	0.598
Remdesivir	52 (96.3)	24 (92.3)	28 (96.6)	0.598
Tocilizumab	5 (9.3)	5 (19.2)	0 (0.0)	0.019
Baricitinib	5 (9.3)	1 (3.8)	4 (13.8)	0.355
Tocilizumab + baricitinib	3 (5.6)	1 (3.8)	2 (6.9)	> 0.999

Values are presented as mean ± standard deviation or number (%).

PASC, post-acute sequelae of COVID-19; NIH, National Institutes of Health.

^{a)}The infection history prior to participation in current study is uncertain in 5 individuals.

^{b)}Data not available in 1 individual per group.

^{c)}Data not available in 4 individuals per group.

^{d)}Data not available in 1 individual in PASC group.

Supplementary Table 2. Number of PASC symptoms according to time point

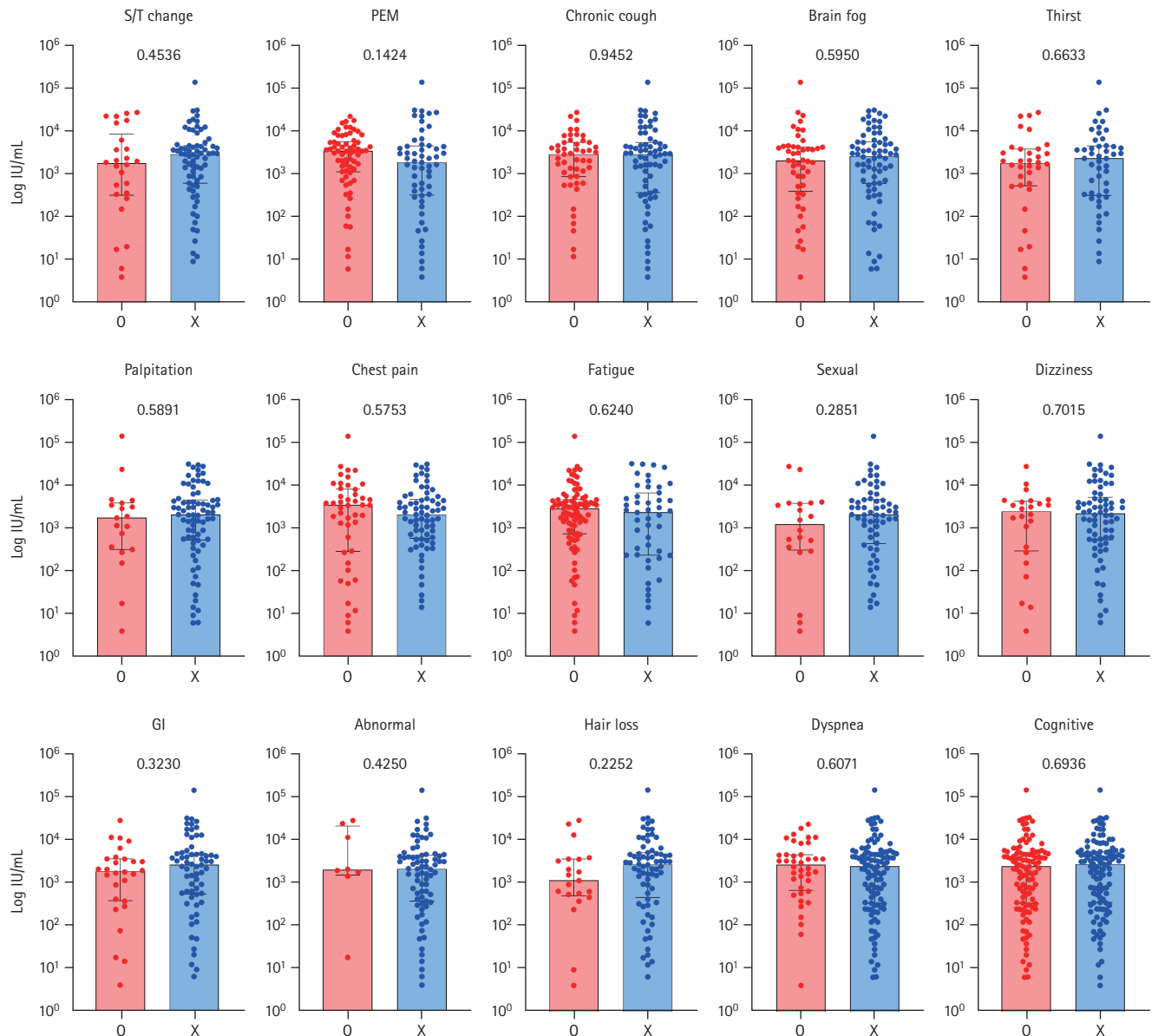
Time point	Symptom n	Record
1 mo (n = 25)	0	1 (4.0)
	1–7	19 (76.0)
	8–15	5 (20.0)
3 mo (n = 24)	0	1 (4.2)
	1–7	19 (79.2)
	8–15	4 (16.7)
6 mo (n = 30)	0	3 (10.0)
	1–7	21 (70.0)
	8–15	6 (20.0)

Values are presented as number (%).
PASC, post-acute sequelae of COVID-19.

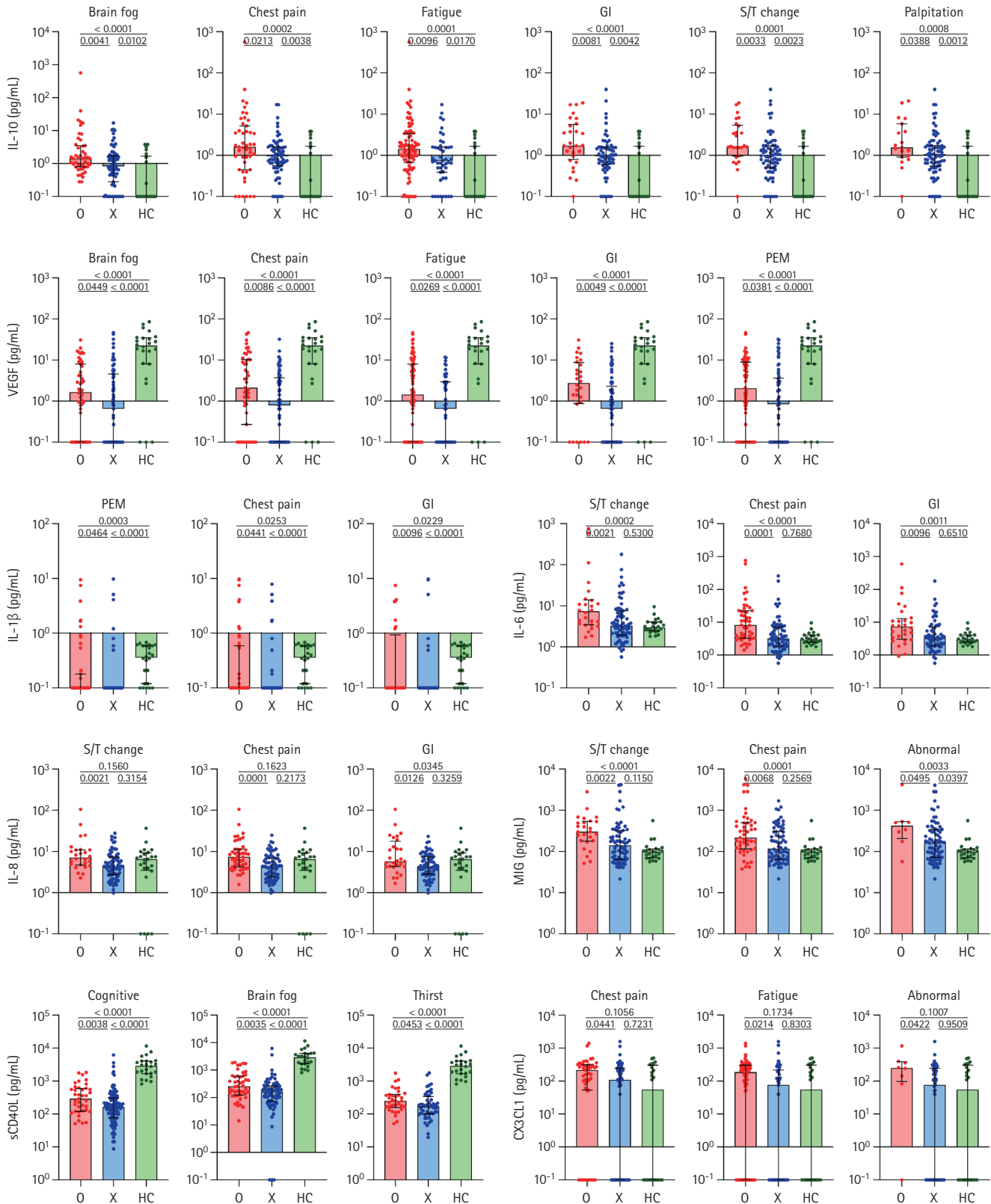
Supplementary Table 3. Symptom frequencies at 1, 3, 6 months after diagnosis

Symptom	1-mo	3-mo	6-mo	<i>p</i> value
Fatigue	71.7	70.0	50.0	0.083
Post-exertional malaise	65.3	59.5	28.1	0.003
Chronic cough	54.6	34.2	34.4	0.098
Chest pain	52.2	34.2	29.0	0.061
Thirst	42.3	48.0	35.5	0.636
Brain fog	38.3	41.0	42.4	0.928
Dizziness	31.3	22.6	22.6	0.659
Dyspnea	30.9	25.5	23.1	0.404
Smell/taste change	27.3	25.0	28.1	0.958
Cognitive dysfunction	24.6	26.8	25.6	0.964
Changes of sexual capacity	23.1	32.0	19.4	0.539
Palpitation	21.2	19.4	19.4	0.977
Hair loss	19.4	23.3	25.0	0.860
Gastrointestinal	16.1	43.3	31.3	0.068
Abnormal movement	3.9	11.5	12.9	0.475

Values are presented as percentage only.



Supplementary Figure 1. Comparison of antibody levels between participants with and without symptoms. S/T change, change in sense of smell or taste; PEM, post exertional malaise; Sexual, changes in sexual desire and capacity; GI, gastrointestinal symptoms; Abnormal, abnormal movement; Cognitive, cognitive dysfunction. Red, participants with symptoms; Blue, participants without symptoms.



Supplementary Figure 2. Comparison of cytokine levels between participants with and without symptoms. GI, gastrointestinal symptom; S/T change, change in sense of smell or taste; PEM, post exertional malaise; Abnormal, abnormal movement; Cognitive, cognitive dysfunction. Red, participants with symptoms; Blue, participants without symptoms; Green, healthy control.