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Plasma Markers of Neurologic Injury and Inflammation in People With Self-Reported Neurologic Postacute Sequelae of SARS-CoV-2 Infection

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Abstract

Background and Objectives

The biologic mechanisms underlying neurologic postacute sequelae of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (PASC) are incompletely understood.

Methods

We measured markers of neurologic injury (glial fibrillary acidic protein [GFAP], neurofilament light chain [NfL]) and soluble markers of inflammation among a cohort of people with prior confirmed SARS-CoV-2 infection at early and late recovery after the initial illness (defined as less than and greater than 90 days, respectively). The primary clinical outcome was the presence of self-reported CNS PASC symptoms during the late recovery time point. We compared fold changes in marker values between those with and without CNS PASC symptoms using linear mixed-effects models and examined relationships between neurologic and immunologic markers using rank linear correlations.

Results

Of 121 individuals, 52 reported CNS PASC symptoms. During early recovery, those who went on to report CNS PASC symptoms had elevations in GFAP (1.3-fold higher mean ratio, 95% CI 1.04–1.63, $p = 0.02$), but not NfL (1.06-fold higher mean ratio, 95% CI 0.89–1.26, $p = 0.54$). During late recovery, neither GFAP nor NfL levels were elevated among those with CNS PASC symptoms. Although absolute levels of NfL did not differ, those who reported CNS PASC symptoms demonstrated a stronger downward trend over time in comparison with those who did not report CNS PASC symptoms ($p = 0.041$). Those who went on to report CNS PASC also exhibited elevations in interleukin 6 (48% higher during early recovery and 38% higher during late recovery), monocyte chemoattractant protein 1 (19% higher during early recovery), and tumor necrosis factor α (19% higher during early recovery and 13% higher during late recovery). GFAP and NfL correlated with levels of several immune activation markers during early recovery; these correlations were attenuated during late recovery.

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Glossary

BMI = body mass index; **COVID-19** = coronavirus disease 2019; **GFAP** = glial fibrillary acidic protein; **IFN** = interferon; **Ig** = immunoglobulin; **IL** = interleukin; **IP** = IFN- γ induced protein; **IQR** = interquartile range; **MCP-1** = monocyte chemoattractant protein 1; **NfL** = neurofilament light chain; **PASC** = postacute sequelae of SARS-CoV-2 infection; **RBD** = receptor-binding domain; **SARS-CoV-2** = severe acute respiratory syndrome coronavirus 2; **TNF** = tumor necrosis factor.

Discussion

Self-reported neurologic symptoms present approximately 4 months after SARS-CoV-2 infection are associated with elevations in markers of neurologic injury and inflammation at earlier time points. Some inflammatory pathways seem to be involved months after acute infection. Additional work will be needed to better characterize these processes and to identify interventions to prevent or treat this condition.

There is an urgent need to understand the pathophysiology that underlies the postacute sequelae of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (PASC), a set of conditions characterized by persistent symptoms in some individuals recovering from coronavirus disease 2019 (COVID-19).¹ Although a spectrum of symptoms is reported among individuals experiencing PASC, neurologic symptoms are particularly common.¹⁻³ Limited data are available on the biologic predictors and correlates of these symptoms.

Neurologic involvement during COVID-19 is common.^{4,5} Acute illness is associated with substantial immune activation^{6,7} and CNS dysfunction.^{8,9} A handful of studies have explored the measurement of serum glial fibrillary acidic protein (GFAP), an intermediate filament protein found in the cytoskeleton of CNS astrocytes,¹⁰ and neurofilament light chain (NfL), a cytoskeletal protein expressed in the axons of neurons¹¹ during acute COVID-19.¹²⁻¹⁹ In some cases, higher levels of these markers were identified in patients with neurologic symptoms during acute infection¹³⁻¹⁷; other studies have not identified such relationships.¹⁹

Recent work has shown structural brain changes in individuals with a history of COVID-19.²⁰ A high proportion of individuals experience ongoing physical or mental health symptoms in the 2–12 months after COVID-19.^{1,21-23} Although the biology is not well understood, recent work has suggested that immune activation might play a role in PASC.^{24,25} In the postacute period, inflammatory markers such as interleukin (IL)-6, tumor necrosis factor (TNF) α , and interferon (IFN)- γ -induced protein (IP)-10 have been associated with ongoing symptoms.²⁵ These markers, particularly IL-6, may correlate with long-term neuropsychiatric manifestations of COVID-19.²⁶ However, there are limited data on the relationship between biomarkers of immune activation, neurologic injury, and neurologic symptoms during the postacute period.^{17,27-29}

In a prior analysis using the broadest possible case definition of PASC (i.e., presence of any 1 of 32 COVID-19–attributed symptoms), we found that subtle differences in levels of

inflammatory markers predicted the presence of persistent symptoms >90 days after COVID-19.²⁵ In the current report, we investigated a more specific outcome measure defined by 8 self-reported neurologic symptoms. In addition to the previous measures of inflammation, we evaluated markers of neurologic injury among those with and without this more specific PASC phenotype. A better understanding of the relationships between these markers among individuals with neurologic manifestations of PASC could help in identifying therapies to prevent and/or manage this condition as the pandemic continues.

Methods

Study Participants and Procedures

Beginning in April 2020, we conducted a prospective longitudinal study of individuals who had recently recovered from confirmed SARS-CoV-2 infection (Long-term Impact of Infection with Novel Coronavirus cohort; NCT04362150).³⁰ Recruitment occurred using a combination of clinician referrals, paper and web-based advertisements, and mailings to all patients testing positive at university-affiliated sites. Most of them (78%) had not been hospitalized during the acute phase. The determination of eligibility was agnostic to the presence or absence of persistent SARS-CoV-2–attributed symptoms.

A research coordinator administered a study questionnaire at early (≤ 90 days) and late (> 90 days) recovery time points after COVID-19 symptom onset. Participants were queried regarding the presence of 32 symptoms, including 8 neurologic symptoms (eTable 1, links.lww.com/NXI/A727). A symptom was recorded as present if it was reported at the time of the visit and was either new in onset since the time of SARS-CoV-2 infection or had worsened since the time of SARS-CoV-2 infection. Symptoms that preceded and were unchanged after SARS-CoV-2 infection were recorded as absent. We also collected information about demographics and medical history and retrospectively collected information on symptoms experienced during the acute phase of the illness. Blood was collected by venipuncture at each visit.

Plasma was isolated using centrifugation of heparinized blood and stored at -80°C .

Clinical Outcomes

The primary clinical outcome was CNS PASC, defined as the presence of at least 1 CNS symptom at a late recovery visit occurring >90 days from initial COVID-19 symptom onset. These symptoms included memory/concentration issues, headache, vision problems, dizziness, and balance issues. We selected these symptoms because they were believed to best reflect dysfunction of the CNS and most likely to associate with biologic processes that could be identified using the 2 primary biomarker outcomes. A secondary analysis examined any neurologic symptom, which included the following in addition to the central neurologic symptoms: problems with smell or taste, smelling an odor that is not really present, and numbness/tingling (eTable 2, links.lww.com/NXI/A727). In addition to symptom data collected at the time of the visit, we also used data collected retrospectively at the time of enrollment regarding the presence or absence of neurologic symptoms during the acute phase of infection to examine changes in biomarkers at early and late follow-up. For hospitalized participants, medical records were requested and reviewed by a study physician. Our rationale for and methods of symptom ascertainment have been described in detail elsewhere.³⁰

Biomarker Assays

Plasma was isolated using centrifugation of heparinized blood and stored at -80°C . Samples were thawed on the day of analysis, centrifuged at $10,000g$ for 5 minutes, and plated in Quanterix-supplied 96-well plates. Plasma biomarker measurements were performed using the fully automated HD-X Simoa platform at 2 time points: early recovery (median 52 days) and late recovery (median 123 days). Those performing the assays were blinded to clinical information. The primary analytes were plasma GFAP and NfL measured using the GFAP Discovery and NF-light Advantage kit assays, respectively (Quanterix). We also measured the levels of markers that have been found to be important during acute SARS-CoV-2 infection^{6,7} using multiplex (Cytokine 3-PlexA: IL-6, IL-10, TNF α) or single-plex (IFN- γ , IP-10, monocyte chemoattractant protein 1 [MCP-1]) kits. SARS-CoV-2 receptor-binding domain (RBD) immunoglobulin (Ig) G was also assayed.

Plasma was assayed according to the manufacturer's recommended 1:4 dilution for all assays except IFN- γ , which was assayed at the recommended 1:2 dilution, and SARS-CoV2 IgG, which was diluted 1:1,000. To minimize the number of freeze-thaw cycles, samples were divided up into several batch runs per assay, and analyzed in 2 Quanterix instruments simultaneously with 2 assays each, and then refrozen at -80°C . The analysis of all samples was performed in 2–3 separate batch runs in singlicate within a week period per assay, using the same kit lot for each assay. All assays were performed according to the manufacturer's instructions, and assay

performance was consistent with the manufacturer's specifications.

Statistical Analysis

We log-transformed all biomarkers to reduce the influence of outliers and to permit interpretation of fold changes. As in prior work,²⁵ to compare values at early and late time points as well as assess whether trajectories in marker values differed between those with and without PASC, we compared the ratio of the mean transformed values for each biomarker between those with and without neurologic symptoms using linear mixed-effects models with terms for PASC, time period (early vs late recovery), and their interaction. We exponentiated the coefficients to give the ratio between the untransformed biomarker values to calculate fold changes and 95% confidence intervals. We used Spearman correlations to evaluate relationships between levels of neurologic and immune markers. All p values are 2-sided. We used Stata (version 16.1; StataCorp, College Station, TX) and Prism (version 9.1.2, GraphPad Software, L.L.C., San Diego, CA).

Standard Protocol Approvals, Registrations, and Patient Consents

All participants provided written informed consent. This study was approved by the Institutional Review Board at the University of California, San Francisco.

Data Availability

The data that support the findings of this study are available from the corresponding author, M.J.P., on reasonable request.

Results

Study Participants

This study included 121 individuals with primary outcome data (Table 1), 92 of whom (76%) had a paired early recovery sample for analysis. During acute COVID-19, most (89, 74%) had been symptomatic outpatients while 27 (22%) had been hospitalized. Five (4%) reported asymptomatic SARS-CoV-2 infection. Of those hospitalized, 23 (85%) required supplemental oxygen and 3 (11%) required mechanical ventilation. SARS-CoV-2-targeted treatment was uncommon: 4 individuals (15%) received remdesivir, 1 (4%) received convalescent plasma, and 5 (19%) received steroids. No participant experienced an acute neurologic event (e.g., stroke, seizure) during their hospitalization. All samples were collected before the availability of SARS-CoV-2 vaccination.

Fifty-two individuals, of whom most were women, reported CNS symptoms at the late recovery time point (Table 2). Among them, the most commonly reported CNS symptoms were trouble concentrating (81%), headache (35%), and dizziness (35%).

Early recovery visits took place at a median of 52 (interquartile range [IQR] 38–64) days postinfection. Late recovery visits took place at a median of 123 (IQR 114–135)

Table 1 Characteristics of the Study Cohort

	All (n = 121)	CNS PASC (n = 52)	No CNS PASC (n = 69)
Demographic characteristics			
Age, y, median (IQR)	44 (37–57)	48 (38.5–57)	43 (36–55)
Sex at birth			
Male	55 (45.5)	15 (28.9)	40 (58.0)
Female	66 (54.6)	37 (71.2)	29 (42.0)
Gender			
Male	54 (44.6)	14 (26.9)	40 (58.0)
Female	65 (53.7)	36 (69.2)	29 (42.0)
Transgender	2 (1.7)	2 (3.9)	0 (0)
Race/ethnicity			
White	69 (57.0)	32 (62.8)	37 (56.1)
Hispanic/Latino	30 (24.8)	14 (27.5)	16 (24.2)
Asian	14 (11.6)	4 (7.8)	10 (15.2)
Black/African American	3 (2.5)	1 (2.0)	2 (3.0)
Pacific Islander/Native Hawaiian	1 (0.8)	0 (0)	1 (1.5)
Other/unknown	4 (3.3)	1 (2.0)	3 (4.3)
Sexual orientation			
Straight/heterosexual	85 (70.2)	35 (72.9)	50 (84.8)
Gay/lesbian/same-sex loving	17 (14.0)	9 (18.8)	8 (13.6)
Asexual	1 (0.8)	1 (2.1)	0 (0)
Questioning/unsure	3 (2.5)	3 (6.3)	0 (0)
Unknown/prefer not to answer	15 (12.4)	4 (7.8)	11 (15.9)
Highest level of education			
Grades 1–6	4 (3.3)	1 (1.9)	3 (4.4)
Grades 7–11	5 (4.1)	1 (1.9)	4 (5.8)
High school/general educational development	12 (9.9)	6 (11.5)	6 (8.7)
At least some college/associate's degree	11 (9.1)	7 (13.5)	4 (5.8)
4 y of college/bachelor's degree	54 (44.6)	22 (42.3)	32 (46.4)
At least some graduate school	35 (28.9)	15 (28.9)	20 (29.0)
Clinical characteristics			
Preexisting medical conditions			
Autoimmune disease	9 (7.4)	6 (11.5)	3 (4.4)
Cancer (treatment within 2 y before COVID-19)	3 (2.5)	2 (3.9)	1 (1.5)
Diabetes	14 (11.6)	7 (13.7)	7 (10.1)
Lung problems (asthma, COPD, or other lung disease active within 5 y before COVID-19)	23 (19.0)	12 (23.1)	11 (16.2)
BMI category			
24.9 or less	42 (34.7)	15 (28.9)	27 (39.1)
25 to 29.9	37 (30.6)	8 (15.4)	29 (42.0)

Continued

Table 1 Characteristics of the Study Cohort (continued)

	All (n = 121)	CNS PASC (n = 52)	No CNS PASC (n = 69)
30 or greater	41 (33.9)	29 (55.8)	12 (17.4)
Characteristics of acute COVID-19 illness			
Asymptomatic	5 (4.1)	1 (1.9)	4 (5.8)
Symptomatic/outpatient	89 (73.6)	38 (73.1)	51 (73.9)
Symptomatic/hospitalized	27 (22.3)	13 (25.0)	14 (20.3)

Abbreviations: BMI = body mass index; COPD = chronic obstructive pulmonary disease; COVID-19 = coronavirus disease 2019; IQR = interquartile range; PASC = postacute sequelae of SARS-CoV-2 infection; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. All values listed are number (percentage) unless otherwise specified.

days postinfection. The early recovery visit for those reporting CNS PASC symptoms occurred slightly later than for those who denied CNS PASC symptoms (60 [IQR 40–67] and 49 [IQR 37–59] days, respectively). The timing of the late recovery visit was similar between those with and without CNS PASC (123 [IQR 117–137] and 124 [IQR 113–134] days, respectively).

Levels of Neurologic and Inflammatory Markers Among Those With and Without CNS PASC Symptoms

We first compared the levels of each marker measured during early recovery between those who went on to report CNS PASC symptoms and those who did not (Figures 1–3, eTables 3 and 4, links.lww.com/NXI/A727). At the early recovery time point, those who went on to report CNS PASC had significantly higher levels of GFAP (1.3-fold higher mean ratio, 95% CI 1.04–1.63, $p = 0.02$; Figure 1A), but not NfL (1.06-fold higher mean ratio, 95% CI 0.89–1.26, $p = 0.54$; Figure 1B). Those who went on to report CNS PASC also had higher levels of cytokines IL-6 (1.48-fold higher mean ratio, 95% CI 1.12–1.96, $p = 0.006$; Figure 2A), TNF α (1.19-fold higher mean ratio, 95% CI 1.06–1.34, $p = 0.003$; Figure 2B), and the chemokine MCP-1 (1.19-fold higher mean ratio, 95% CI 1.01–1.40, $p = 0.034$; Figure 3A), compared with those who did not report CNS PASC. Trends for other markers were in a similar direction, although the differences did not achieve statistical significance.

We next compared the levels of each biomarker measured during late recovery between those with and without self-reported CNS PASC at this visit (Figures 1–3, eTables 3 and 4, links.lww.com/NXI/A727). No significant differences were detected in GFAP or NfL between those with and without PASC (Figure 1, A and B). Those reporting persistent CNS PASC symptoms had persistent elevations in IL-6 (1.38-fold higher mean ratio, 95% CI 1.07–1.77, $p = 0.013$; Figure 2A) and TNF α (1.13-fold higher mean ratio, 95% CI 1.02–1.26, $p = 0.022$; Figure 2B). IFN- γ was lower (0.71-fold difference, 95% CI 0.55–0.91, $p = 0.007$; Figure 3C). Levels of SARS-CoV-2 RBD IgG did not differ between groups at either the early or late time points (Figure 3C).

Changes in Levels of Plasma Biomarkers Over Time

To examine changes in the levels of these markers between the early and late recovery time points, we used mixed models to indicate changes over time among those with and without CNS PASC symptoms (Figures 1–3, eTables 3 and 4, links.lww.com/NXI/A727). Significant differences in trends of NFL ($p = 0.041$; Figure 1B), IFN- γ ($p = 0.012$; Figure 2C), and MCP-1 ($p = 0.019$; Figure 3A) were noted between the CNS PASC and non-CNS PASC groups. As predicted from the cross-sectional analyses, consistently higher levels of IL-6 and TNF α were observed, although the trends in the levels of these markers did not differ between groups (Figure 2, A and B).

Relationships Between Neurologic and Inflammatory Markers

To examine relationships between the neurologic markers and markers of inflammation, we performed nonparametric pairwise analyses at early and late recovery time points (Figure 4). GFAP levels weakly correlated with MCP-1 ($r = 0.21$, $p = 0.02$) and IL-6 ($r = 0.18$, $p = 0.054$) at the early time point and with IL-6 at the late time point ($r = 0.19$, $p = 0.043$). NfL correlated with MCP-1 ($r = 0.41$, $p < 0.001$), IL-6 ($r = 0.23$, $p = 0.012$), IFN- γ ($r = 0.28$, $p = 0.003$), and TNF α ($r = 0.32$, $p < 0.001$) at the early time point and with MCP-1 ($r = 0.31$, $p < 0.001$) at the late time point. In addition, there was a strong correlation between NfL and SARS-CoV-2 IgG at the early time point ($r = 0.40$, $p < 0.001$).

Influence of Symptoms During Acute Infection

We did not identify significant differences in levels of markers at either recovery time point between those with and without prior CNS symptoms during acute infection (eTable 5, links.lww.com/NXI/A727). For some markers, we noted non-significant trends toward differential changes over time in groups with and without CNS symptoms during acute infection. These included NfL (more steep decline among those with acute CNS symptoms, $p = 0.066$) and anti-RBD IgG (less steep decline among those with acute CNS symptoms, $p = 0.063$).

Table 2 PASC Symptoms in Study Cohort

	All (n = 121)	CNS PASC (n = 52)	No CNS PASC (n = 69)
Total PASC symptoms			
Mean (range)	3.3 (0–18)	6.9 (1–18)	0.64 (0–7)
Median (IQR)	1 (0–6)	6.5 (4–9)	0 (0–1)
PASC, by symptom			
Trouble concentrating, trouble with your thinking, or trouble with your memory	42 (34.7)	42 (80.8)	0 (0)
Headache	18 (14.9)	18 (34.6)	0 (0)
Trouble with vision, e.g., double vision, blurry vision, or other visual issues	13 (10.7)	13 (25.0)	0 (0)
Dizziness	18 (14.9)	18 (34.6)	0 (0)
Trouble with balance or feeling unsteady	13 (10.7)	13 (25.0)	0 (0)
Trouble with taste or smell	27 (22.3)	20 (38.5)	7 (10.1)
Smelling an odor that is not actually there	8 (6.6)	7 (13.5)	1 (1.5)
Numbness, tingling, or “pins and needles” in your arms or legs	17 (14.1)	16 (30.8)	1 (1.5)
Feeling feverish	2 (1.7)	2 (3.9)	0 (0)
Measured a temperature of >100.4 °F or 38 °C	2 (1.7)	2 (3.9)	0 (0)
Chills, feeling unusually cold	2 (1.7)	2 (3.9)	0 (0)
Feeling tired or having low energy	41 (33.9)	37 (71.2)	4 (5.8)
Cough	13 (10.7)	11 (21.2)	2 (2.9)
Shortness of breath	28 (23.1)	25 (48.1)	3 (4.4)
Chest pain	18 (14.9)	13 (25.0)	5 (7.3)
Feeling your heart pound or race	15 (12.4)	13 (25.0)	2 (2.9)
Runny nose or congestion	14 (11.6)	9 (17.3)	5 (7.3)
Sore throat	7 (5.8)	6 (11.5)	1 (1.5)
Muscle aches	20 (16.5)	19 (36.5)	1 (1.5)
Loss of appetite	12 (9.9)	11 (21.2)	1 (1.5)
Nausea, gas, or indigestion	17 (14.1)	15 (28.9)	2 (2.9)
Vomiting	1 (0.8)	1 (1.9)	0 (0)
Stomach pain	6 (5.0)	3 (5.8)	3 (4.4)
Constipation	3 (2.5)	3 (5.8)	0 (0)
Diarrhea or loose bowels	9 (7.4)	8 (15.4)	1 (1.5)
New spots or a rash on your skin	10 (8.3)	9 (17.3)	1 (1.5)
Fainting spells	0 (0)	0 (0)	0 (0)
Pain in your arms, legs, or joints such as knees and hips	9 (7.4)	9 (17.3)	0 (0)
Back pain	7 (5.8)	6 (11.5)	1 (1.5)
Trouble sleeping	32 (26.5)	26 (50.0)	6 (8.7)
Menstrual cramps or other problems with your periods	2 (1.7)	2 (3.9)	0 (0)
Pain or problems during sexual intercourse	0 (0)	0 (0)	0 (0)

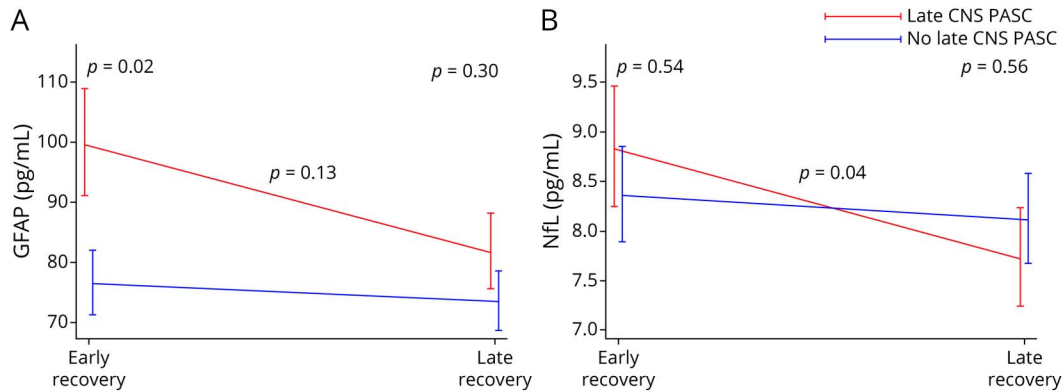
Abbreviations: IQR = interquartile range; PASC = postacute sequelae of SARS-CoV-2 infection; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2. All values listed are number (percentage) unless otherwise specified.

Sensitivity Analyses

Because of the relationship between age and levels of NfL,³¹ we performed an age-adjusted analysis which did not change

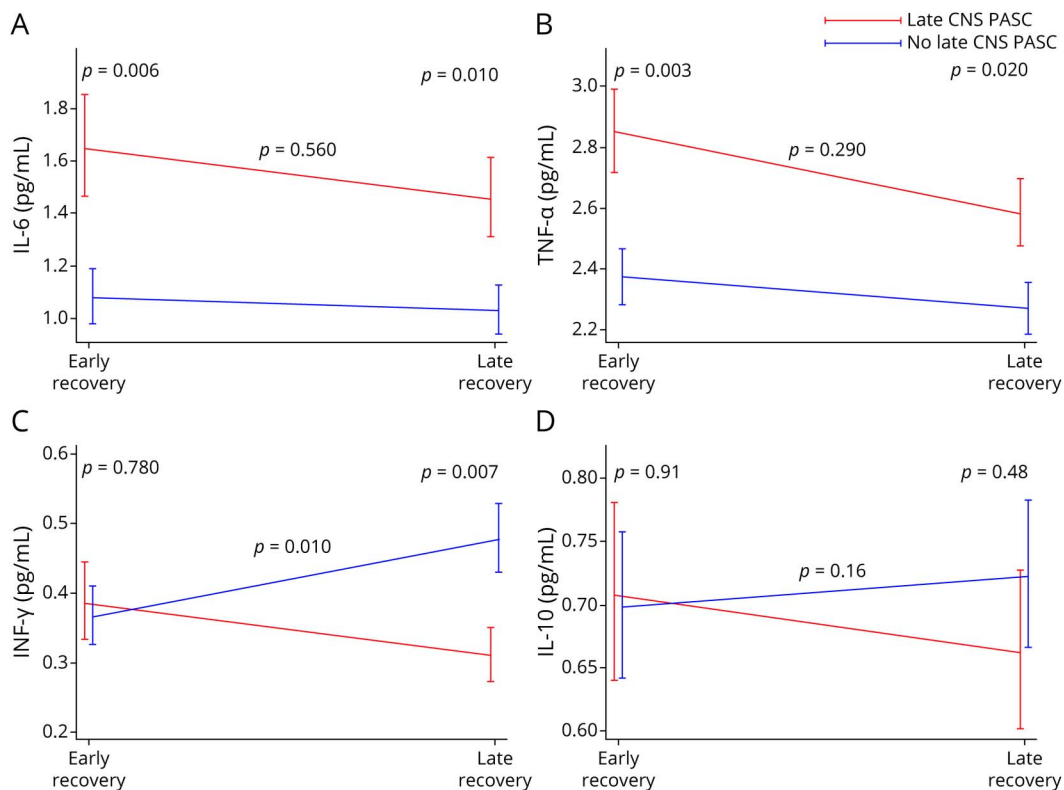
the primary results (no new relationship between NfL and PASC was identified) (eTable 6, links.lww.com/NXI/A727). We also repeated the primary analysis adjusting for age, sex,

Figure 1 Cross-Sectional Measurements and Longitudinal Trends in Neurologic Marker Levels Among Those With and Without CNS PASC



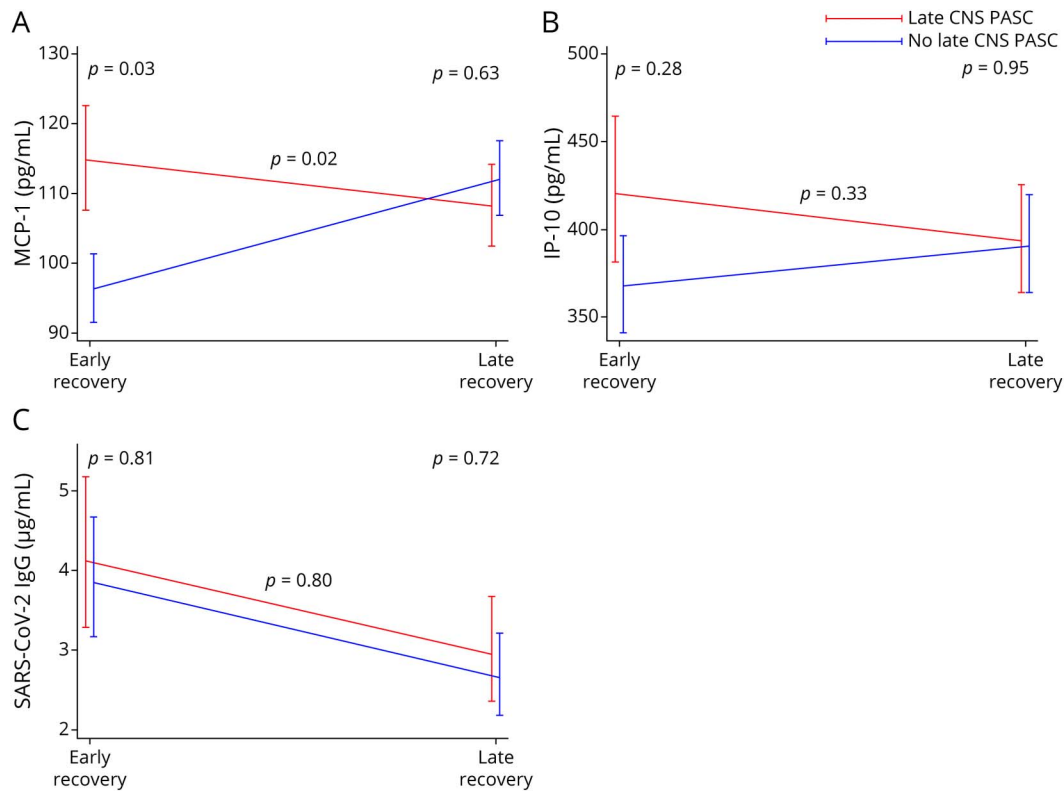
p values reflect group comparisons during early and late recovery as well as comparison of change over time between groups. Early recovery represents a median of 52 days post-SARS-CoV-2 symptom onset (or positive PCR); late recovery represents a median of 123 days post-SARS-CoV-2 symptom onset (or positive PCR). GFAP = glial fibrillary acidic protein; NfL = neurofilament light chain; PASC = postacute sequelae of SARS-CoV-2 infection; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Figure 2 Cross-Sectional Measurements and Longitudinal Trends in Cytokine Levels Among Those With and Without CNS PASC



p values reflect group comparisons during early and late recovery as well as comparison of change over time between groups. Early recovery represents a median of 52 days post-SARS-CoV-2 symptom onset (or positive PCR); late recovery represents a median of 123 days post-SARS-CoV-2 symptom onset (or positive PCR). IFN = interferon; IL = interleukin; PASC = postacute sequelae of SARS-CoV-2 infection; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TNF = tumor necrosis factor.

Figure 3 Cross-Sectional Measurements and Longitudinal Trends in Chemokine Levels and Antibodies Among Those With and Without CNS PASC



Early recovery represents a median of 52 days post-SARS-CoV-2 symptom onset (or positive PCR); late recovery represents a median of 123 days post-SARS-CoV-2 symptom onset (or positive PCR). IgG = immunoglobulin G; IP-10 = IFN- γ -induced protein 10; MCP-1 = monocyte chemoattractant protein 1; PASC = postacute sequelae of SARS-CoV-2 infection; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

and prior hospitalization status which did not change the interpretation of the results (eTable 7), although some of the relationships were slightly attenuated. Because the proportion of individuals with preexisting autoimmune disease (most commonly thyroiditis) differed between groups, we adjusted for this and the results were largely unchanged (eTable 8). The IL-6 relationship was attenuated when we additionally adjusted for body mass index (BMI) (eTable 9), but the relationships identified with GFAP and TNF α were maintained.

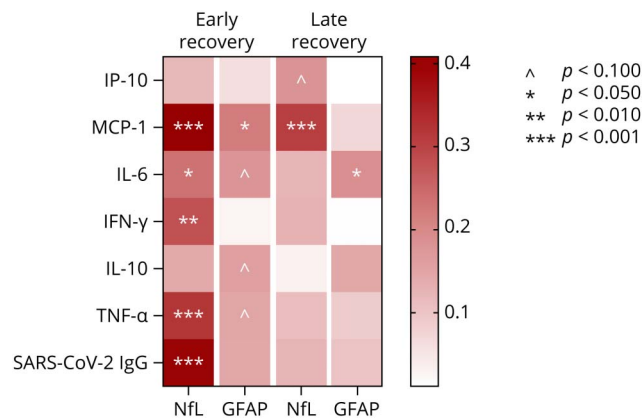
We performed a secondary analysis in which individuals reporting any symptom that could be attributed to a primary neurologic cause (including the peripheral nervous system) were compared against individuals reporting no neurologic symptoms (eTable 10, links.lww.com/NXI/A727). In this analysis, the elevation in GFAP seen at early follow-up among those reporting any neurologic PASC symptom was slightly attenuated (mean ratio 1.24, 95% CI 1.00–1.55, $p = 0.052$). Similar elevations were seen in IL-6, MCP-1, and TNF α at early follow-up among those reporting any neurologic PASC symptom. At late follow-up, those with any neurologic PASC had higher levels of IL-6 and TNF α . The difference in IFN- γ seen among those with CNS PASC in the primary analysis was no longer statistically significant.

Finally, because we have previously found that PASC is associated with elevations in certain markers, we performed an analysis comparing those with CNS PASC with those reporting no symptoms of any kind during late recovery (eTable 11, links.lww.com/NXI/A727). The interpretation of the primary results was again unchanged, although some of the findings were attenuated in this comparison against a smaller group.

Discussion

A large proportion of individuals with PASC experience symptoms that may be attributed to nervous system dysfunction,^{1-3,32} but the pathophysiologic processes underlying such symptoms remain poorly understood. We investigated the associations between self-reported neurologic symptoms and plasma biomarkers of neurologic injury and systemic inflammation during early and late recovery periods after laboratory-confirmed SARS-CoV-2 infection. We found that those reporting CNS PASC symptoms approximately 4 months after initial infection had earlier elevations in several biomarkers, including GFAP, IL-6, and TNF α , suggesting that the acute infection resulted in direct CNS tissue injury and systemic inflammation, both of which might conceivably be causally related to the development of CNS PASC symptoms.

Figure 4 Relationships Between Biomarkers of Neurologic Injury and Systemic Inflammation in the Full Cohort



Data reflect nonparametric correlations between markers at early and late recovery time points. Early recovery represents a median of 52 days post-SARS-CoV-2 symptom onset (or positive PCR); late recovery represents a median of 123 days post-SARS-CoV-2 symptom onset (or positive PCR). GFAP = glial fibrillary acidic protein; IFN = interferon; IgG = immunoglobulin G; IL = interleukin; IP-10 = IFN-γ-induced protein 10; MCP-1 = monocyte chemoattractant protein 1; NfL = neurofilament light chain; PASC = post-acute sequelae of SARS-CoV-2 infection; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TNF = tumor necrosis factor.

Elevations in IL-6 and TNFα persisted through approximately 4 months of recovery. Replication of these findings in larger and more diverse cohorts may be a first step toward identifying interventions for their prevention and/or management.

We identified elevations in GFAP during early recovery that were associated with later CNS PASC. Although we did not observe elevations in NfL at either time point, we did identify a more precipitous decline among those reporting CNS PASC. Together, these observations lend support to the possibility of early injury that might resolve while clinical symptoms persist. Based on autopsy studies, acute SARS-CoV-2 can access the CNS.³³ A correlation between severity of COVID-19 and levels of NfL and GFAP in the acute phase has been observed,¹³⁻¹⁷ and a recent study showed structural brain changes in those with prior COVID-19.²⁰ However, studies of the trajectories of NfL and GFAP levels during the weeks after symptom onset have been inconsistent.^{19,29,34} Our findings are in line with studies that identified a correlation between NfL and/or GFAP with severity of reported neurologic symptoms during the acute phase, but found no association between the biomarker levels and persistence of neurologic symptoms after 6 months.^{27,28} Further work exploring the dynamics of these markers in cohorts with measurements performed during the period of acute illness, as well as efforts to identify downstream markers which may persist and be identified during later recovery, will be informative.

This analysis builds on several observations we and others have made suggesting that markers of systemic inflammation may be important in driving PASC.^{24,25} Although we

previously observed associations between such markers and broadly defined PASC (i.e., any 1 of 32 COVID-19–attributed symptoms), it is notable that the strength of these associations was more pronounced in the current analysis using a more specific PASC outcome (i.e., any 1 of 5 CNS PASC symptoms). At the same time, the fact that those reporting CNS PASC symptoms had a greater number of PASC symptoms overall makes it challenging to disentangle a more specific CNS PASC phenotype from more severe PASC in general. It suggests that CNS PASC might reflect 1 extreme on a spectrum of illness. Further work to compare those with distinct phenotypic clusters of symptoms, if they exist, could further elucidate the biology.

Although the purpose of this analysis was to explore the inflammatory pathways related to CNS PASC and not to define clinical factors associated with this condition, we performed several adjusted analyses to assess how these clinical factors could be involved with the inflammatory pathways. Adjustment for age, sex, and hospitalization status, 3 factors most strongly associated with PASC in large epidemiologic studies,² did not affect the interpretation of the results. Adjustment for the history of autoimmune disease, which has been variably suggested to be involved in PASC,^{35,36} also did not have an effect. Further adjustment for BMI seemed to attenuate the relationship between PASC and IL-6, but it is unclear whether it is appropriate to classify BMI as a confounder or mediator in the relationship between IL-6 and BMI based on preliminary evidence that adipose cells could be involved in the pathogenesis of SARS-CoV-2 infection.³⁷ Further work to understand the relationship between clinical factors, inflammatory pathways, and PASC will be needed to better define the biology of this condition.

Dysregulation of IL-6 and TNFα is potentially deleterious in inflammatory disease states, related to systemic and localized tissue inflammation and endothelial dysfunction. The reason for elevations in levels of these markers among those with CNS PASC is not clear. One possibility is that they represent residual inflammation from the period of acute infection that is slower to resolve among those with PASC. However, the identification of persistent differences months after infection suggests other possibilities such as a delayed return to immunologic homeostasis related to a persistent immune response caused by an ongoing pathophysiologic process or processes (e.g., persistent antigenic stimulation,³⁸ microvascular dysfunction,³⁹ and autoimmunity⁴⁰). Although the source of these inflammatory markers is unknown, both IL-6 and TNFα can be produced by CNS cell types as well as by peripheral immune cells and both cytokines been implicated in CNS pathology.^{41,42} Similarly, MCP-1 is a chemokine expressed by macrophages and microglia, and elevations have been implicated in other neurocognitive conditions.⁴³ Further investigation into the source of IL-6, TNFα, and MCP-1 production among those with CNS PASC symptoms, which may include coinvestigation of the peripheral blood and CSF

compartment, will be needed to provide clues to the pathophysiology underlying this condition.

Interestingly, levels of these biomarkers during early and late recovery did not differ between those with and without self-reported neurologic symptoms during acute infection. This suggests that residual neurologic injury in those with neurosymptomatic acute COVID-19, at least among study populations comprised primarily of outpatients, is not the most important causal factor. Although it is likely that the severity of acute infection is 1 contributor to the development of PASC, the determination of who will have ongoing elevations in markers of neurologic injury and/or who will go on to experience CNS PASC during late recovery seems to be more complex than identifying those who report neurologic symptoms during the acute phase of illness. The lack of differences in levels of GFAP and NfL at late recovery is evidence against a large degree of ongoing neurologic injury at this late time point, despite the persistence of symptoms.

Although the exact pathogenesis of neurologic complications from SARS-CoV-2 is yet unclear, several hypotheses have been proposed. These include direct viral infection, systemic inflammation, compartmentalized neuroinflammation, and sequelae of thrombotic injury. Viral tropism for human astrocytes has been demonstrated *in vivo*,⁴⁴ postmortem brain samples from patients with COVID-19 have shown preferential infection of astrocytes,⁴⁵ and a case-control study of brain samples uncovered altered gene expression in some astrocytes.⁴⁶ Astrocyte dysfunction, as reflected in increased plasma GFAP observed here, could relate to the emerging cognitive PASC complaints, which can encapsulate attention and working memory deficits. Direct invasion of neurons has been suggested based on their expression of ACE-2 receptor, but SARS-CoV-2 viral particles have been only rarely demonstrated in several neuropathologic autopsy studies and studies of CSF during acute infection.^{47,48} In addition, there is evidence of multifocal inflammatory infiltrates consisting of lymphocytes as well as activated innate immune cells in autopsy tissue.³³ Further investigation of PASC will require in-depth exploration of what is occurring in the CNS compartment during both acute and recovery time periods.

Our analysis has several important limitations. First, although recruitment was agnostic to the presence of persistent symptoms, the cohort is a convenience sample that is unlikely to be representative of the general population of individuals recovering from COVID-19 or experiencing PASC. For example, our study population may be enriched for people with more severe symptoms because such individuals may be more motivated to participate in research. If that were the case, the associations we identified may hold only for certain subpopulations of individuals experiencing PASC. Even so, the current understanding of PASC is so limited that we believe the identification of these biological associations remains highly informative. Second, we relied on self-report to ascertain the presence of symptoms, and many symptoms are not clearly

attributable to a neurologic cause. This risks misattribution of symptoms to neurologic causes and therefore misclassification of individuals as having CNS PASC. However, as of the time of this analysis, the World Health Organization criteria for diagnosis of PASC are reliant on self-report and do not require objective testing. Although the definition is not optimal, we believe that our case definition appropriately captures this condition as it is currently understood. Studies that do include objective measurements (which may include detailed neurologic history and examination, neurocognitive and neuropsychiatric testing, and/or neuroimaging) are likely to be more informative and are urgently needed. Third, it is difficult to disentangle neurologic symptoms from other non-neurologic symptoms which might co-occur, and it is possible that differences in these markers are driven by more severe PASC in general rather than neurologic symptoms specifically. Although we collected data related to comorbidities known to be important in both acute COVID-19 and PASC (e.g., diabetes, lung disease, obesity), we did not obtain complete medical and psychiatric histories as part of the study and we did not have available psychiatric symptom data for this analysis; affective symptoms may also co-occur and be inter-related.²⁹ Fourth, we measured a limited set of biomarkers, and there are likely to be others that are important in the pathophysiology of this condition. Our measurements were all taken in blood, and although there are established relationships between blood and CSF measurements of these markers in other disease conditions, these have yet to be established for COVID-19 and were not seen in at least 1 study.¹⁹ Furthermore, although they are of mechanistic interest regardless of their cells of origin, the markers we measured are not CNS-specific and may be generated peripherally. For this reason, more detailed studies that include CSF analyses will be critical. Fifth, for reasons described in the statistical methods literature,^{49,50} we elected to eschew adjustments for multiple comparisons because the goal of this study was to identify promising biomarkers of a poorly understood condition; we instead opted to report our analyses comprehensively without selective reporting of statistically significant findings. Finally, prepandemic specimens were not available from these volunteers, and it is therefore possible that elevations in markers of interest among those with CNS PASC preceded SARS-CoV-2 infection. Regardless, we believe that the observations made here provide important preliminary clues as to potentially important biological pathways to inform more detailed neurologic evaluations and potential therapeutic studies.

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Disclosure

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Appendix (continued)

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Continued

Appendix (continued)

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Appendix (continued)

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