Multisystem Involvement in Post-Acute Sequelae of Coronavirus Disease 19

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Objective: The purpose of this study was to describe cerebrovascular, neuropathic, and autonomic features of post-acute sequelae of coronavirus disease 2019 ((COVID-19) PASC).

Methods: This retrospective study evaluated consecutive patients with chronic fatigue, brain fog, and orthostatic intolerance consistent with PASC. Controls included patients with postural tachycardia syndrome (POTS) and healthy participants. Analyzed data included surveys and autonomic (Valsalva maneuver, deep breathing, sudomotor, and tilt tests), cerebrovascular (cerebral blood flow velocity [CBFv] monitoring in middle cerebral artery), respiratory (capnography monitoring), and neuropathic (skin biopsies for assessment of small fiber neuropathy) testing and inflammatory/autoimmune markers.

Results: Nine patients with PASC were evaluated 0.8 ± 0.3 years after a mild COVID-19 infection, and were treated as home observations. Autonomic, pain, brain fog, fatigue, and dyspnea surveys were abnormal in PASC and POTS (n = 10), compared with controls (n = 15). Tilt table test reproduced the majority of PASC symptoms. Orthostatic CBFv declined in PASC ($-20.0 \pm 13.4\%$) and POTS ($-20.3 \pm 15.1\%$), compared with controls ($-3.0 \pm 7.5\%$, $p = 0.001$) and was independent of end-tidal carbon dioxide in PASC, but caused by hyperventilation in POTS. Reduced orthostatic CBFv in PASC included both subjects without (n = 6) and with (n = 3) orthostatic tachycardia. Dysautonomia was frequent (100% in both PASC and POTS) but was milder in PASC ($p = 0.002$). PASC and POTS cohorts diverged in frequency of small fiber neuropathy (89% vs 60%) but not in inflammatory markers (67% vs 70%). Supine and orthostatic hypocapnia was observed in PASC.

Interpretation: PASC following mild COVID-19 infection is associated with multisystem involvement including: (1) cerebrovascular dysregulation with persistent cerebral arteriolar vasoconstriction; (2) small fiber neuropathy and related dysautonomia; (3) respiratory dysregulation; and (4) chronic inflammation.

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Summary
Post-acute sequelae of coronavirus disease 2019 (COVID-19; PASC) is a complication of COVID-19 disease associated with chronic fatigue, brain fog and orthostatic intolerance. Pathophysiology of PASC is poorly understood.
This study evaluated patients with PASC following mild COVID-19 infection using standardized autonomic assessments, including Valsalva maneuver, deep breathing, sudomotor and tilt tests with cerebral blood flow velocity (CBFv) measurements, and skin biopsies for small fiber neuropathy (SFN).
This study showed that PASC following mild COVID-19 is associated with multisystem involvement, including cerebrovascular and respiratory dysregulation, small fiber neuropathy, and related dysautonomia and chronic inflammation.
Autonomic testing expanded for cerebrovascular measurements and skin biopsies can explain some of the PASC symptoms and could guide treatment.

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Additional supporting information can be found in the online version of this article.

Glossary

BRAF-NRS = Bristol Rheumatoid Arthritis Fatigue Numerical Rating Scales, V2 Revised
BFS = Brain Fog Scale
COVID-19 = coronavirus disease 19
CVRi = cerebrovascular resistance index
ENFD = epidermal nerve fiber density
NTSS = Neuropathy Total Symptom Score-6, SAS Survey of Autonomic Symptoms
PASC = post-acute sequelae of COVID-19
POTS = Postural Tachycardia Syndrome
QASAT = Quantitative Scale for Grading of Cardio-vascular Autonomic Reflex Tests and small fibers from skin biopsies
SARS-CoV-2 = severe acute respiratory syndrome corona-virus 2
SFN = small fiber neuropathy
SGNFD = sweat gland nerve fiber density

Coronavirus disease 19 (COVID-19) is a novel infectious disease caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), which incited a global pandemic. Post-acute sequelae of COVID-19 (PASC) is provisionally defined as persistence or worsening of symptoms for >4 weeks or new symptoms attributable to COVID-19, affects 20–60% of patients.[1] Common complaints are fatigue, dyspnea, brain fog, and orthostatic intolerance, and a variety of systemic complaints.[2]

PASC is a multisystem disorder, however, the exact pathophysiology remains poorly understood. Potential mechanisms include post-infectious inflammation, immune-mediated vascular dysfunction, thromboembolism, lung dysfunction, and nervous system dysfunction due to occult neuronal injury during SARS-CoV-2 infection.[3,4]

Our case report[5] suggested that PASC can be associated with autoimmune-mediated multisystem abnormalities. A patient with PASC developed intravenous immunoglobulin-responsive symptoms suggestive of cerebral hypoperfusion due to cerebrovascular arteriolar dysfunction, small fiber neuropathy (SFN), and dysautonomia.[6] We aimed to confirm these findings in a group of patients with PASC. Postural tachycardia syndrome (POTS) is characterized by symptoms that are similar to PASC, including chronic fatigue, orthostatic intolerance, and brain fog linked to reduced orthostatic cerebral blood flow and dysautonomia.[7–9] Patients with POTS thus provide a useful comparison group aside from healthy controls, to enable identification of pathophysiology distinct to PASC.

We hypothesize that PASC is associated with auto-immune/inflammatory mediated widespread cerebrovascular and metabolic dysregulation and neuropathic changes.

Methods

Study Design

Patient Population and Study Design. The study was approved by the Brigham and Women's Hospital (BWH) Institutional Review Board as a minimal risk study.

This was a single-center, retrospective study of consecutive patients who presented to BWH Autonomic Laboratory for evaluation of PASC. Inclusion criteria for the study were: (1) adult patients 18 years of age or older; (2) documented diagnosis of COVID-19 disease by history of positive reverse transcriptase-polymerase chain reaction (PCR) positive for SARS-CoV-2; (3) completion of comprehensive autonomic testing with skin biopsies; (4) availability of electronic health care records; (5) satisfying inclusion criteria for PASC; (6) for the POTS controls, matched by age and sex, satisfying criteria for POTS[10]; and (7) age and sex matched asymptomatic healthy controls were used from our previous University of Massachusetts (UMASS) study.[11] Testing at the UMASS was identical to the Brigham protocol[12] used in this study except electrochemical skin conductance (ESC)[13] was used instead of quantitative sudomotor axon reflex test (QSART) for sudomotor evaluations. ESC correlates with loss of sweat gland nerve fibers and therefore can be used as a proxy for sudomotor function.[13] In addition, the inflammatory markers were not available for controls.

Exclusion criteria were: (1) significant metabolic, and acid–base disarrangement and anemia that can influence autonomic and respiratory system and/or cerebral blood flow; (2) evidence of cardiac or pulmonary disease; (2) presence of disorders associated with secondary SFN or dysautonomia (diabetes, pre-diabetes, parkinsonism, Parkinson's disease, history of heavy alcohol use, B12 and/or folate deficiency, thyroid disease, celiac disease, hepatitis C, HIV infection, chemotherapy exposure and/or cancer diagnosis, history of autoimmune disease, and any comorbid conditions or use of medications that have been associated with SFN); (3) evidence of large fiber neuropathy on neurological examination (loss of tendon reflexes or loss of proprioception) or on nerve conduction studies; (4) the use of medications that may affect the autonomic nervous system during testing; and (5) inability to complete the Brigham protocol.

Definition of PASC. Fatigue, brain fog, and dyspnea are characteristic for PASC.[2] To grade fatigue, we used the Bristol Rheumatoid Arthritis Fatigue Numerical Rating

Scales (BRAF-NRS, V2 Revised),[10] a validated self-rating scale which measures fatigue dimensions: severity (range: 0–10), effect on life (range: 0–10), and coping ability (range: 0–10).

There is no validated brain fog scale (BFS). For the purpose of this study, we defined the 11-point scale from 0 (no brain fog) to 10 (severe disabling brain fog, always present). The scale is based on a brain fog scale from 0 to 100 in 10-point intervals showing brain fog in POTS correlated with fatigue.[7]

At present, there are no established criteria for PASC.[3] Therefore, we followed the current guidelines[14] and provisional definition of PASC.[15] In general, PASC is described as signs and symptoms that develop during or after an infection consistent with COVID-19, continue for weeks and are not explained by an alternative diagnosis. Furthermore, neurological disease occurring in the 6-week interval after acute infection is associated with autoimmune mechanisms.[15]

For the purpose of this study, we defined the inclusion criteria for PASC by the following: (1) presence of chronic (>4 weeks) fatigue (grade 3 or more on each of the BRAF-NRS parts), and brain fog (grade 3 or more on BFS) which developed within the 6-week interval after acute COVID-19 infection.

Patients with POTS were defined using standard criteria.[10,16]

Additional validated neurologic symptom surveys were completed at the time of autonomic testing. Sensory complaints were surveyed by the Neuropathy Total Symptom Score-6 (NTSS), which is a validated instrument for evaluation of neuropathic pain features.[17] The NTSS quantifies sensory symptoms (aching pain, allodynia, burning pain, lancinating pain, numbness, and prickling sensation). A score >0 indicate the presence of sensory symptoms, the NTSS range is 0 to 21.96. Autonomic symptoms were obtained from the Survey of Autonomic Symptoms (SAS), which is a validated instrument for evaluation of autonomic complaints. The symptoms were grouped into orthostatic, sudomotor, vasomotor, gastrointestinal, urinary, and sexual (for men) categories.[18] The SAS score ranges from 0 to 60 for men and 0 to 55 for women with the higher number representing a more severe severity of symptoms. A modified Borg scale, which is based on category-ratio scale (CR-10), with score 0 (no dyspnea) to 10 (unbearable dyspnea) was used for grading dyspnea.[19]

Definition of SFN. SFN is defined as combination of symptoms and signs.[20] The criteria for SFN were SAS or NTSS >1 and abnormal skin biopsies or abnormal sudomotor testing.[13,20]

Autonomic Testing. The Brigham protocol,[12] which consists of functional autonomic testing and skin biopsies for evaluation of small fibers, was used in this study. Autonomic testing included deep breathing, Valsalva maneuver, 10 minutes of the tilt test, and sudomotor evaluation. We described details of testing previously.[12] Electrocardiogram, continuous and intermittent blood pressure, end tidal CO_2, finger pulse oximetry, and cerebral blood flow velocity (CBFv) in the middle cerebral artery (MCA) using transcranial Doppler were recorded. Normative data were published previously.[12] For the CBFv, the lower limit for women/men are 82.2–0.45 (cm/s)*age (years)/72.09–0.38 (cm/s)*age (years).[12] In healthy subjects, orthostatic CBFv is either unchanged or may decline slightly. The normal decline in orthostatic CBFv from baseline is equal or less than 10% (first minute), 11% (fifth minute), and 15% (10th minute) of the tilt test.[12]

Brigham protocol results were graded using Quantitative Scale for Grading of Cardiovascular Autonomic Reflex Tests and Small Fibers from Skin Biopsies (QASAT).[12] QASAT is an objective instrument for grading severity of dysautonomia, small fiber neuropathy, and cerebral blood flow. Scores are calculated for each domain, including heart rate, blood pressure, cerebral blood flow, and end tidal CO_2. The score equal to 0 is normal, above 0 is abnormal with the severity proportional to the number.

CO_2 has a profound effect on CBFv. Hypocapnia/hypercapnia decreases/increases diameter of cerebral arterioles[21] which decreases/increases CBFv due to increase/decrease in cerebrovascular resistance. Generally, in a healthy cerebral vasculature, a decrease of end tidal CO_2 by 1 mmHg decreases cerebral blood flow by about 3%.[22] We separated the CO_2-induced CBFv changes as follows. First, we assessed the QASAT CBFv tilt test response score from raw CBFv (QASAT-CBFv$_{tilt}$). Second, we calculated the adjusted QASAT CBFv score (QASAT-Adjusted CBFv$_{tilt}$) from the CBFv adjusted for the effect of CO_2 by calculating the how much orthostatic drop of CBFv is due to the drop of ET-CO_2. The proportion of the CBFv changes due to the effect of CO_2 was then calculated as:

$$QASAT - CBFv_{tilt} - QASAT - Adjusted\,CBFv_{tilt}.$$

For example if the QASAT-CBFv$_{tilt}$ = 10 (100%) and QASAT-Adjusted CBFv$_{tilt}$ = 3 (30%), than 70% ([10–3]*100) of the reduced CBFv is due to effect of CO_2 and 30% is due to cerebral arteriolar vasoconstriction.

The percentage of the cerebral arteriolar dysfunction is referred as arteriolar orthostatic index (AOI).

All data were recorded using Labchart system (ADInstruments, AU), sampled at 400 Hz. The data for final statistical analyses were obtained after 10 minutes of baseline period, and at each minute of the tilt test.

The blood pressure at the level of MCA was calculated from arm pressure by adjustment for the hydrostatic gradients.[23] With average MCA-arm distance the MVA pressure was obtained by subtracting 20 from arm pressure. That reduction is ~2 mmHg for 2.5 cm of vertical displacement. Cerebral vascular resistance index (CVRi) was calculated as mean blood pressure at the level of MCA divided by mean CBFv.[6]

We also monitored the blood oxygenation by pulse oximetry from a finger.

Skin Biopsy. Epidermal nerve fiber density (ENFD) and sweat gland nerve fiber density (SGNFD) were obtained using established standards.[20,24] Skin samples were taken from the proximal thigh 20 cm distal to the iliac spine, and from the calf 10 cm above the lateral malleolus using a 3-mm circular punch tool. Skin samples were immunoperoxidase-stained for the axonal marker PGP 9.5. Skin processing and fiber counting was done at Therapath (New York, NY). Thresholds for normal values for proximal thigh were 8.3 fibers/mm (ENFD) and 37.8 fibers/mm (SGNFD) and 36.5 fibers/mm (SGNFD) for the calf, were established by Therapath. The age and gender adjusted ENFD normative values at the calf were also obtained from Therapath.

Grading of Small Fiber Neuropathy, Dysautonomia, and Cerebral Blood Flow. Test results were graded using the QASAT.[12] QASAT is an objective instrument for grading severity of dysautonomia, small fiber neuropathy, and cerebral blood flow. For each domain (heart rate, blood pressure, cerebral blood flow, and end tidal CO_2), the score equal to 0 is normal, above 0 is abnormal with the severity proportional to the number.
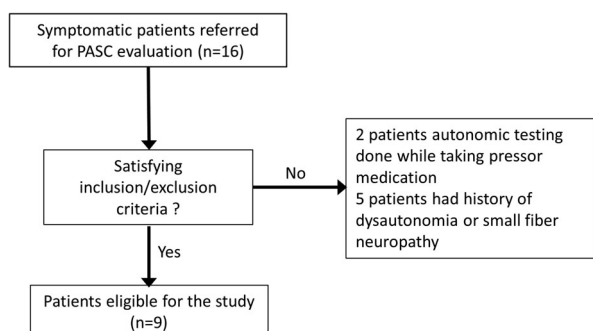

FIGURE 1: Flow chart of the study. PASC = post-acute sequelae of coronavirus disease 2019.

Inflammatory Panel. We measured the following serum inflammatory markers[25]: high resolution C-reactive protein (normative value < = 3 mg/L), tumor necrosis factor-alpha (TNF-α, < = 2.8 pg/ml), interleukin (IL) IL-6 (<7.1 pg/ml), IL-10 (<2 pg/ml), IL-1ß (<7.1 pg/ml), leptin (3.3–18.3 ng/ml), adiponectin (2.4–17.9 ug/ml), trisulfated heparin disaccharide (TS-HDS) antibody (<10,000 titers),[26] fibroblast growth factor receptor 3 (FGFR3) antibody (<3,000 titers),[26] acetylcholine receptor binding antibody (< = 0.02 nmol/L), ganglionic acetylcholine receptor antibody (< = 0.02 nmol/L),[27] neuronal VGKC antibody (< = 0.02 nmol/L), calcium channel P/Q binding antibody (<= 0.02 nmol/L), myoglobin (<= 71 ng/ml),[28] and human growth hormone (0.01–3.61 ng/mL).[28] We also evaluated norepinephrine supine (70–750 pg/ml) and standing (200–1,700 pg/ml), which is useful in the assessment of the hyperadrenergic form of POTS.[10] The TS-HDS and FGFR3 antibodies were obtained from Washington University School of Medicine (St. Louis, MO), remaining antibodies as well as TNF-α, IL-10, human growth hormone were obtained from Mayo Clinic laboratories (Rochester, MN). IL-6 was obtained at Mayo Clinic laboratories (Rochester, MN) or at BWH Clinical laboratories (Boston, MA). IL-1ß was obtained at Sunquest (Tucson, AZ). Leptin was obtained at Esoterix Endocrinology (Calabasas Hills, CA). Remaining of laboratory tests were obtained at BWH Clinical laboratories (Boston, MA) or at Quest Diagnostics (Secaucus, NJ).

Statistical Analysis

A one-way analysis of variance (ANOVA) for continuous variables and chi-squared test for categorical variables were used to determine the differences between subject's groups. A Tukey's adjusted post hoc test or *t* test (when data from healthy controls were not available) and contingency tables with Fisher's exact test were used for PASC and POTS comparisons for continuous and categorical variables, respectively. Repeated-measures ANOVA was used to evaluate the effect of time during the tilt test. Statistical analysis was obtained using R statistical (r-project.org) and tableOne python package.[29]

Results

Demographics and Symptoms

Sixteen patients with PASC were screened (Fig 1). Active pressor medication excluded 2 individuals, and a history of SFN or dysautonomia excluded 5 individuals from the study. Nine patients with PASC (age 35.8. ± 7.3 years, 9 women, all White) were age and sex-matched with 10 patients with POTS (36.3 ± 9.8, 9 women, all White) and 15 controls (40.1 ± 11.6, 13 women, all White) were included in this study (see Table 1 and

TABLE 1. Demographic Characteristics of the Study					
		Diagnosis			
		Controls	**POTS**	**PASC**		
N		**15**	**10**	**9**	**95% CI, F, and p (Controls-POTS-PASC)**	**p (POTS-PASC)**
Age, years, mean (SD)		40.1 (11.6)	36.3 (9.8)	35.8 (7.3)	33.7–41.0, F (2, 31) = 0.661, $p = 0.524$	0.993
Gender, n (%)	F	13 (86.7)	9 (90.0)	9 (100)	$p = 0.531$	0.999
	M	2 (13.3)	1 (10.0)			
BMI, kg/m^2, mean (SD)		25.3 (5.9)	23.6 (4.8)	26.4(7.3)	22.90–27.1, F (2, 31) = 0.508, $p = 0.607$	0.578
Symptom duration, years, mean (SD)		0.0 (0.0)	7.7 (4.3)	0.8 (0.3)	2.01–3.68, F (2, 31) = 35.7, $p < 0.001$	<0.001

BMI = body mass index; CI = confidence interval; F = bioavailability; PASC = post-acute sequelae of coronavirus disease 2019; POTS = postural tachycardia syndrome.

Supplementary Table S1). All patients with PASC had mild COVID-19 infection with conservative home management with one individual treated with supplemental home oxygen (see Supplementary Material Table S1). No patients with PASC included in this study received the COVID-19 vaccine and no one had a history of severe acute respiratory distress syndrome, acute kidney, or liver failure, or reported use of corticosteroids or hydroxychloroquine.

The length of time from initial documented symptoms to autonomic testing was longer in POTS compared to PASC ($p < 0.001$). No acute symptoms indicative of COVID-19 disease was exhibited up to 72 hours before and up to 5 weeks after Brigham protocol testing.

Clinical Symptoms

By definition, healthy controls were absent of chronic pain, autonomic symptoms, dyspnea, brain fog, or fatigue (Table 2). Patients with PASC and POTS reported neuropathic pain, fatigue, dyspnea, and symptoms in all surveyed autonomic domains. Several patients with PASC and POTS had a history of migraine headaches, but no one had a migraine during the testing. Autonomic complaints were not significantly different between PASC and POTS, whereas the tilt test induced dizziness and exacerbated fatigue and brain fog in both cohorts.

Autonomic Testing

Profiles of CBFv, end tidal CO_2, heart rate, and blood pressure at baseline and during the tilt test across all groups is shown in Figure 2A-F. Tables 2 to 4 summarize the test results. QASAT scores in healthy controls (parasympathetic, sympathetic, and sudomotor) were zero.

PASC had mild but widespread dysautonomia. Total QASAT score and autonomic failure score was lower in PASC compared to POTS (QASAT total score $p = 0.015$, QASAT autonomic failure score $p = 0.002$).

Parasympathetic dysfunction was observed in 4 patients (44%) with PASC and 2 POTS patients (20%). The QASAT parasympathetic score was similar to POTS.

Adrenergic dysfunction associated with abnormal blood pressure responses to the Valsalva maneuver was observed in 9 patients (100%) with PASC and 7 patients (70%) with POTS. The QASAT sympathetic score was similar between PASC and POTS. Sudomotor dysfunction was detected in 5 patients (56%) with PASC and 7 patients (70%) with POTS, the QASAT sudomotor score was similar. Each patient, in both groups, had at least one abnormal autonomic function test. Orthostatic hypotension, a marker of more advanced adrenergic failure, was not detected in any patient. Supine and orthostatic norepinephrine levels were similar in PASC and POTS.

Tilt Test

Baseline supine CBFv, heart rate, and blood pressure were similar among all three groups (Fig 2A–F, Tables 3 and 4). During the tilt test, the CBFv declined in both PASC and POTS groups compared with controls ($p < 0.001$), the decline was similar in PASC compared with POTS ($p = 0.997$). Orthostatic CBFv corrected for end-tidal CO_2 remained lower in PASC ($p < 0.001$) but was normalized in POTS ($p = 0.001$, comparison PASC vs POTS). In the PASC group, all patients had abnormally reduced orthostatic CBFv. Reduced orthostatic CBFv in PASC included both subjects without (n = 6) and with (n = 3) orthostatic tachycardia. The orthostatic CBFv decline in 6 patients with
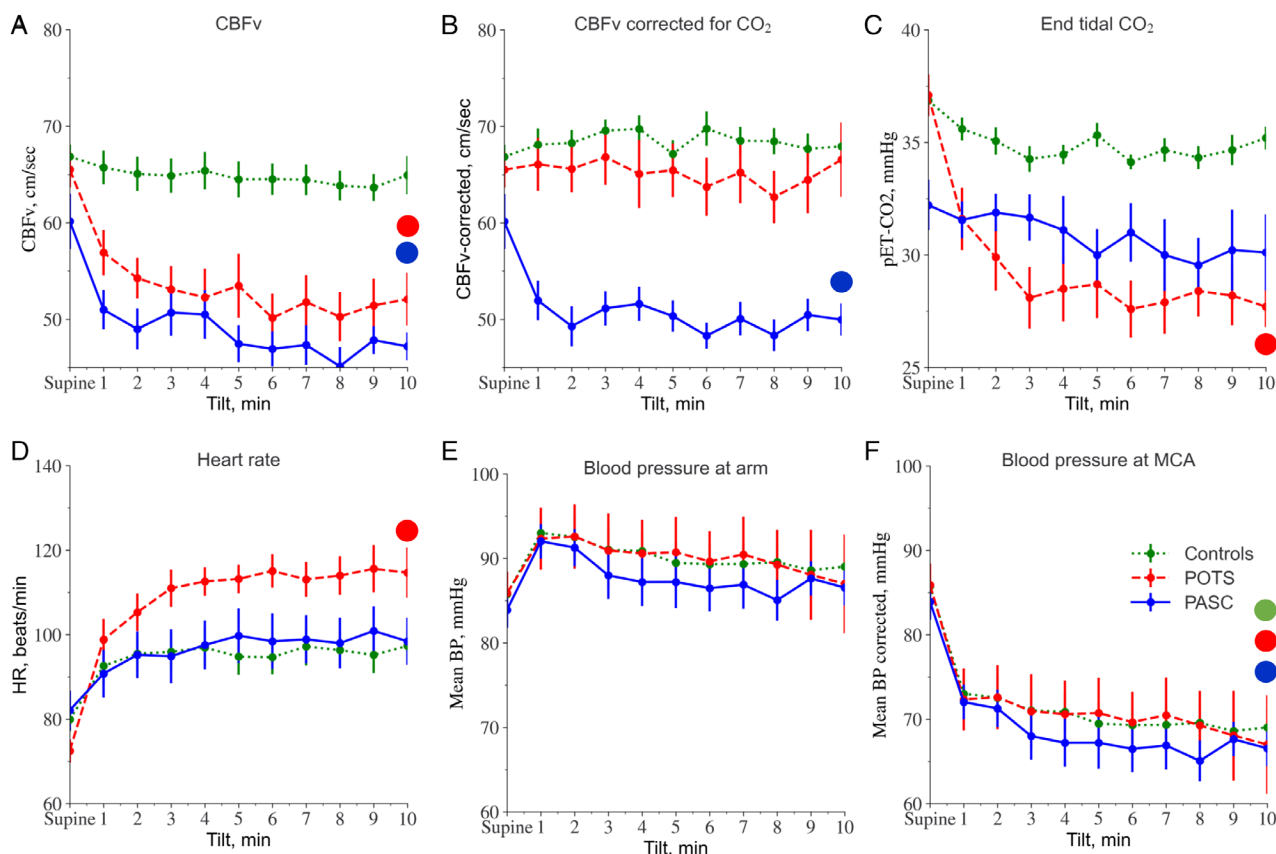
FIGURE 2: Profile of cerebral blood flow velocity (CBFv), CBFv corrected for the effect of CO_2, end tidal CO_2, heart rate (HR), and blood pressure (BP) during supine baseline and the tilt test for post-acute sequelae of coronavirus disease 2019 (PASC), postural tachycardia syndrome (POTS), and controls. Large dots indicate significance at $p = 0.001$ level for respective data obtained by repetitive measures analysis of variance (ANOVA). [Color figure can be viewed at www.annalsofneurology.org]

PASC without orthostatic tachycardia was consistent with orthostatic cerebral hypoperfusion syndrome (OCHOS)[6] and in the remaining 3 patients with PASC with orthostatic tachycardia was consistent with POTS.

Supine CVRi was similar across all groups. Orthostatic CVRi was elevated in PASC. AOI was higher in patients with PASC compared with patients with POTS. Overall, the PASC cohort had orthostatic heart rate responses similar to controls ($p = 0.088$). Orthostatic heart rate increase was more pronounced in POTS compared to PASC ($p < 0.001$). Orthostatic blood pressure was normal and QASAT adrenergic orthostatic tilt test score was equal in all studied groups ($p = 0.973$).

End Tidal CO_2
Baseline supine end tidal CO_2 was reduced in the PASC group as compared to the POTS group ($p = 0.001$) and controls ($p = 0.001$). During the tilt test, the end tidal CO_2 was stable in the controls, declined in the patients with POTS and remained low but stable in the patients with PASC. The end tidal CO_2 was similar at the end of the 10 minutes of the tilt test in the PASC and POTS groups ($p = 0.329$). The oxygen saturation was normal (>96%) in all patients throughout testing.

Skin Biopsies
Skin biopsies were normal in healthy controls. Seven patients (78%) with PASC were diagnosed with SFN on skin biopsy. One patient with normal skin biopsy had abnormal sudomotor testing consistent with SFN, thus, a total of 8 patients with PASC (89%) had SFN. ENFD was abnormal in 5 patients (56%); and SGNFD was abnormal in 5 patients (63%). One patient with normal ENFD had abnormal SGNFD. In comparison, 5 of the patients with (50%) POTS had biopsy-confirmed SFN. ENFD was abnormal in 5 patients (50%), SGNFD and ENFD were abnormal in 2 patients (20%).

One patient with POTS declined the skin biopsy but had abnormal sudomotor testing consistent with SFN. Frequency of SFN diagnosed by skin biopsy was similar

TABLE 2. Surveys Results

N		Controls 15	Diagnosis POTS 10	PASC 9	95% CI, F and *p* PASC-POTS-Controls	*p* PASC-Controls	PASC-POTS
Pain symptoms, n (%)	No	15 (100.0)			<0.001	<0.001	0.999
	Yes		10 (100.0)	9 (100)			
NTSS score, range 0–21.96, mean (SD)		0.0 (0.0)	10.9 (5.4)	10.0 (5.1)	5.58–8.36, F (2, 31) = 30.53, *p* < 0.001	<0.001	0.703
Autonomic symptoms, n (%)	No	15 (100.0)			<0.001	<0.001	0.999
	Yes		10 (100.0)	9 (100.0)			
SAS score, range 0–60 men, 0–55 women, mean (SD)		0.0 (0.0)	24.9 (5.8)	25.9 (11.2)	14.60–19.26, F (2, 31) = 63.6, *p* < 0.001	<0.001	0.942
Orthostatic symptoms, n (%)	No	15 (100.0)			<0.001	<0.001	0.999
	Yes		10 (100.0)	9 (100)			
Orthostatic survey, range 0–5, mean (SD)		0.0 (0.0)	3.9 (1.0)	3.9 (1.1)	2.32–2.87, F (2, 31) = 110.8, *p* < 001	<0.001	0.999
Sudomotor symptoms, n (%)	No	15 (100.0)		9 (100)	<0.001	<0.001	0.999
	Yes		10 (100.0)				
Sudomotor survey, range 0–20, mean (SD)		0.0 (0.0)	7.1 (2.8)	8.0 (5.0)	3.98–6.09, F (2, 31) = 27.8, *p* < 0.001	<0.001	0.783
Vasomotor symptoms, n (%)	No	15 (100.0)	1 (10.0)	1 (11.1)	<0.001	<0.001	0.999
	Yes		9 (90.0)	8 (88.9)			
Vasomotor survey, range 0–10, mean (SD)		0.0 (0.0)	5.0 (3.0)	5.2 (3.7)	2.52–4.30, F (2, 31) = 17.7, *p* < 0.001	<0.001	0.979
Gastrointestinal symptoms, n (%)	No	15 (100.0)			<0.001	<0.001	0.999
	Yes		10 (100.0)	9 (100)			
Gastrointestinal survey, range 0–15, mean (SD)		0.0 (0.0)	7.4 (3.1)	7.4 (3.6)	4.01–5.83, F (2, 31) = 38.4, *p* < 0.001	<0.001	0.999
Urinary symptoms, n (%)	No	15 (100.0)	7 (70.0)	4 (44.4)	<0.001	0.003	0.370
	Yes		3 (30.0)	5 (55.6)			
Urinary survey, range 0–5, mean (SD)		0.0 (0.0)	1.0 (1.8)	1.3 (1.5)	0.34–1.21, F (2, 31) = 3.96, *p* < 0.001	0.037	0.823
Sexual symptoms, men, n (%)	No	1 (100.0)			<0.001	0.999	0.999
	Yes		1 (100.0)				
Sexual survey, range 0–5, men, mean (SD)		0.0 (nan)	5.0 (nan)		NA	NA	0.999
Brain fog, n (%)	No	15 (100.0)			<0.001	<0.001	0.999
	Yes		10 (100.0)	9 (100)			
Brain fog score, mean (SD)		0.0 (0.0)	5.3 (1.4)	6.2 (2.4)	3.31–4.36, F (2, 31) = 66.08, *p* < 0.001	<0.001	0.363
Fatigue, n (%)	No	15 (100.0)			<0.001	<0.001	0.999
	Yes		10 (100.0)	9 (100.0)			
Total fatigue score, mean (SD)		0.0 (0.0)	20.9 (4.0)	21.3 (4.0)	13.02–15.14, F (2, 31) = 213.7, *p* < 0.001	<0.001	0.945
Dyspnea, n (%)	No	15 (100.0)			<0.001	<0.001	0.999
	Yes		10 (100.0)	9 (100)			
Dyspnea score, mean (SD)		0.0 (0.0)	4.5 (2.2)	5.4 (1.0)	2.84–3.78, F (2, 31) = 61.56, *p* < 0.001	<0.001	0.270
Dizziness during the tilt test, n (%)	No	14 (100.0)			<0.001	<0.001	0.999
	Yes		8 (100.0)	9 (100)			

CI = confidence interval; F = bioavailability; NA = not applicable; NTSS = Neuropathy Total Symptom Score; PASC = post-acute sequelae of coronavirus disease 2019; POTS = postural tachycardia syndrome.

TABLE 3. Testing Results

N		Controls 15	Diagnosis POTS 10	PASC 9	95% CI, F, and *p* PASC-POTS-controls	*p* PASC-controls	*p* PASC-POTS
Inflammatory markers, number, mean (SD)			0.8 (0.6)	1.3 (0.8)	NA	NA	0.578
Inflammatory markers, presence, n (%)	No		3 (30.0)	3 (33.3)	NA	NA	0.999
	Yes		7 (70.0)	6 (66.7)			
Norepinephrine, supine pg/ml, mean (SD)		355.7 (123.6)	556.9 (245.2)	371.0 (161.4)	336.6–519.1, F (2, 18) = 2.53, *p* < 0.107	0.989	0.196
Norepinephrine, standing pg/ml, mean (SD)		435.8 (131.2)	723.2 (272.1)	501.0 (262.9)	438.1–668.5, F (1, 2) = 3.03, *p* < 0.075	0.893	0.240
ENFD, n (%)	Abnormal		5 (55.6)	5 (55.6)	*p* = 0.001	0.001	0.999
	Normal	15 (100.0)	4 (44.4)	4 (44.4)			
ENFD-proximal, fibers/mm, mean (SD)		14.7 (4.0)	12.1 (7.1)	9.3 (4.1)	10.20–13.90, F (2, 30) = 3.23, *p* < 0.053	0.006	0.491
ENFD-distal, fibers/mm, mean (SD)		10.4 (2.8)	7.4 (5.7)	5.9 (5.1)	6.31–9.51, F (2, 31) = 3,62, *p* < 0.048	0.032	0.737
SGNFD, n (%)	Abnormal		2 (25.0)	5 (62.5)	*p* = 0.004	0.002	0.315
	Normal	14 (100.0)	6 (75.0)	3 (37.5)			
SGNFD-proximal, fibers/mm, mean (SD)		54.7 (10.2)	64.3 (13.9)	35.6 (23.1)	44.61–58.44, F (2, 19) = 5.09, *p* < 0.017	0.072	0.013
SGNFD-distal, fibers/mm, mean (SD)		50.5 (10.0)	49.7 (36.0)	45.3 (22.2)	39.73–57.3, F (2, 25) = 0.156, *p* = 0.856	0.849	0.922
ESC (sudomotor), n (%)	Abnormal		7 (70.0)	5 (55.6)	NA	NA	0.650
	Normal		3 (30.0)	4 (44.4)			
ESC-feet, ug/kg, mean (SD)			1.2 (0.3)	1.2 (0.3)	1.09–1.36, F (2, 19) = 0.264, *p* = 0.770		0.879
Baseline mean CBFv, cm/s, mean (SD)		66.8 (5.1)	65.5 (6.4)	60.1 (9.6)	61.70–66.63, F (2, 31) = 2.755, *p* = 0.079	0.070	0.225
Mean CBFv @ 10 min of the tilt test, cm/sec, mean (SD)		64.9 (8.2)	52.1 (9.6)	47.2 (4.8)	51.90–57.58, F (2, 31) = 16.28, *p* < 0.001	<0.001	0.382
Mean CBFv drop @ 10 min of the tilt test, %, mean (SD)		−3.0 (7.5)	−20.3 (15.1)	−20.0 (13.4)	−18.64 to −10.20, F (2, 31) = 8.88, *p* < 0.001	0.005	0.997
Mean CBFv @ 10 min of the tilt test corrected for pET-CO2, cm/s, mean (SD)		67.9 (6.9)	66.6 (13.5)	50.0 (5.6)	58.23–64.73, F (2, 31) = 12.28, *p* < 0.001	<0.001	0.001
Baseline pET-CO2, mmHg, mean (SD)		36.9 (1.4)	37.1 (3.2)	32.2 (3.8)	34.41–36.38, F (2, 31) = 9.77, *p* < 0.001	0.001	0.001
pET-CO2, @ 10 min of the tilt test, mmHg, mean (SD)		35.2 (2.1)	27.7 (3.1)	30.1 (5.7)	29.70–32.30, F (2, 31) = 13.94, *p* < 0.001	0.001	0.329
AOI, %, mean (SD)		100 (0.0)	23.0 (26.7)	86.7 (21.5)	63.4–76.3, F (2, 31) = 57.51, *p* < 0.001	0.101	<0.001
CVRi supine, mmHg/cm/sec, mean (SD)		1.3 (0.2)	1.3 (0.2)	1.4 (0.3)	1.27–1.43, F (2, 31) = 1.164, *p* = 326	0.302	0.540
CVRi @ 10th min of tilt test, mmHg/cm/sec, mean (SD)		1.1 (0.2)	1.4 (0.6)	1.4 (0.2)	1.16–1.56, F (2, 31) = 2.935, *p* = 0.068	0.001	0.930

AOI = arteriolar orthostatic index; CBFv = cerebral blood flow velocity; CI = confidence interval; CVRi = cerebrovascular resistance index; ENFD = epidermal nerve fiber density; ESC = electrochemical skin conductance; F = bioavailability; NA = not applicable; NTSS = Neuropathy Total Symptom Score; PASC = post-acute sequelae of coronavirus disease 2019; POTS = postural tachycardia syndrome; SGNFD = sweat gland nerve fiber density.

TABLE 4. QASAT Grading Results

N		Controls 15	POTS 10	PASC 9	95% CI, F, and p PASC-POTS-controls	p PASC-controls	p PASC-POTS
QASAT-Sympathetic sudomotor score (ESC), range 0–6, mean (SD)		0.0 (0.0)	1.4 (1.6)	1.3 (1.4)	0.05–1.77, F (2, 18) = 0.799, p = 0.465	NA	0.994
QASAT-Parasympathetic cardiovagal score (deep breathing), range 0–3, mean (SD)		0.0 (0.0)	0.2 (0.4)	0.4 (0.5)	0.09–0.34, F (2, 31) = 4.53, p = 0.018	0.014	0.297
QASAT-Increased heart rate response to tilt test, range 0–10, mean (SD)		0.0 (0.0)	8.5 (1.4)	1.6 (2.4)	2.84–3.86, F (2, 31) = 112.7, p < 0.001	0.088	<0.001
QASAT-Sympathetic adrenergic score (Valsalva maneuver), range 0–3, mean (SD)		0.0 (0.0)	1.6 (1.2)	1.7 (0.7)	0.82–1.35, F (2, 31) = 21.11, p < 0.001	<0.001	0.978
QASAT-Sympathetic adrenergic score (tilt test), range 0–10, mean (SD)		0.0 (0.0)	0.4 (1.3)	0.9 (2.7)	−0.11 to -0.97, F (2, 31) = 0.973, p = 0.389	0.358	0.764
QASAT-Cerebral blood flow response to tilt test score, range 0–10, mean (SD)		0.0 (0.0)	7.6 (1.8)	6.7 (2.4)	4.19–5.32, F (2, 31) = 16.28, p < 0.001	<0.001	0.382
QASAT-Cerebral blood flow, total score, range 0–11, mean (SD)		0.0 (0.0)	8.1 (1.7)	7.4 (2.1)	4.67–5.69, F (2, 31) = 125.4, p < 0.001	<0.001	0.582
QASAT-pET-CO2, score, range 0–10, mean (SD)		0.0 (0.0)	6.6 (3.2)	0.9 (2.0)	1.78–3.21, F (2, 31) = 35.41, p < 0.001	0.547	<0.001
QASAT-Autonomic failure, n (%)	No	15 (100.0)			<0.001	<0.001	0.999
	Yes		10 (100.0)	9 (100)			
QASAT-Autonomic failure score, range 0–24, mean (SD)		0.0 (0.0)	12.6 (3.9)	7.1 (4.6)	5.44–7.70, F (2, 31) = 49.48, p < 0.001	<0.001	0.002
QASAT-ENFD, range 0–8, mean (SD)		0.0 (0.0)	2.6 (3.2)	2.4 (2.7)	0.88–2.45, F (2, 31) = 5.628, p = 0.008	0.028	0.993
QASAT-SGNFD, range 0–8, mean (SD)		0.0 (0.0)	1.0 (1.9)	2.2 (2.8)	0.43–1.74, F (2, 27) = 4.52, p = 0.020	0.015	0.318
QASAT-Total score, range 0–54, mean (SD)		0.0 (0.0)	30.5 (4.6)	20.7 (9.7)	15.07–19.04, F (2, 31) = 98.96, p < 0.001	<0.001	0.015

CI = confidence interval; ENFD = epidermal nerve fiber density; ESC = electrochemical skin conductance; F = bioavailability; PASC = post-acute sequelae of coronavirus disease 2019; POTS = postural tachycardia syndrome; QASAT = Quantitative Scale for Grading of Cardiovascular Autonomic Reflex Tests and small fibers from skin biopsies; SGNFD = sweat gland nerve fiber density.

between PASC and POTS ($p = 0.999$ [ENFD] and $p = 0.315$ [SGNFD]).

Inflammatory Markers
Inflammatory markers were common but heterogeneous (Tables 3 and 5). In the patients with PASC, 6 (67%) individuals showed elevation in at least one inflammatory marker, compared to 7 (70%) of patients with POTS. The TS-HDS antibody, found in 4 patients (44%) with PASC and one (10%) in a patient with POTS, was the most commonly elevated marker.

TABLE 5. Inflammatory Markers for Each Subject. Only Abnormal Markers are Shown		
#	Diagnosis	Inflammatory markers
1	PASC	TS-HDS: 11000
2	PASC	TS-HDS: 22000, FGFR3: 10000
3	PASC	
4	PASC	CRP: 12.74 mg/L
5	PASC	TS-HDS: 29000, TNF-alpha: 3.2 pg/ml
6	PASC	TS-HDS: 14000, IL-10, TNF-alpha: 2.9 pg/ml, growth hormone: 8.71 ng/ml
7	PASC	
8	PASC	
9	PASC	FGFR3: 5200
1	POTS	
2	POTS	CRP: 6.1 mg/L
3	POTS	
4	POTS	TS-HDS: 22000
5	POTS	Adiponectin 19.4 ug/ml, TNF-alpha: 3.9 pg/ml
6	POTS	Ganglionic acetylcholine receptor antibody: 0.09 nmol/L
7	POTS	CRP: 9.2 mg/L
8	POTS	
9	POTS	FGFR3: 18000
10	POTS	Acetylcholine receptor binding antibody: 0.41 nmol/L

FGFR = fibroblast growth factor receptor; PASC = post-acute sequelae of coronavirus disease 2019; POTS = postural tachycardia syndrome; TS-HDS = trisulfated heparin disaccharide.

Discussion

This study showed widespread multisystem dysregulation affecting cerebrovascular, peripheral neural/autonomic, respiratory systems, and evidence of low-grade inflammation in PASC.

Cerebral Blood Flow Dysregulation

Orthostatic CBFv was abnormally reduced in PASC. The degree of CBFv reduction (~20%) is clinically significant and similar to POTS, where it has been linked to cerebral hypoperfusion.[8] Although the reduced CBFv was observed in both patients with PASC and patients with POTS, there is etiological difference.

The CBFv decline in PASC is largely independent of hypocapnia, whereas in POTS it is mainly driven by hypocapnia. This CBFv decline occurs with normal blood pressure (eg, without orthostatic hypotension), in both conditions. Cerebral blood flow primarily depends on blood pressure and CO_2. Hypocapnia decreases diameter of cerebral arterioles[21] with corresponding changes in vascular resistance and blood flow. In healthy subjects, the end tidal CO_2 changes only slightly during orthostasis. In upright posture, gravity alters blood flow causing increased pressure in the feet, and decreased pressure in the brain (~16 mmHg; see Fig 2F), forming hydrostatic gradients.[23] A normal response to the tilt test is cerebral arteriolar vasodilatation, which maintains relatively constant cerebral blood flow.

In PASC, orthostatic CBFv decline is independent of CO_2 because the orthostatic end tidal CO_2 did not decline. Therefore, reduction of orthostatic CBFv is likely due to failure to actively dilate cerebral arterioles to counteract reduced orthostatic blood pressure in the brain vasculature. The CBFv passively followed pressure drop in the MCA due to a downstream vascular resistance as cerebral arterioles remained relatively constant. This pattern is consistent with OCHOS.[6] In contrast to PASC, orthostatic CBFv decline in POTS is induced mainly by hypocapnic hyperventilation,[8,9] the mechanisms also observed in this study.

The cause of vascular dysregulation in PASC is unclear. Cerebral vasodilatory responses are primary determined by myogenic and metabolic factors and to a lesser extent by autonomic innervation.[30] Therefore, numerous factors affect cerebrovascular regulation. Low-grade vascular inflammation may play a role given the association of low-grade inflammation with greater arterial stiffness mediated by multiple elements in the vascular wall.[31] Reduced cerebral blood flow, increased cerebrovascular resistance, inflammation, and damage of the vascular bed may interfere with brain tissue oxygenation and has been linked to cognitive deficit.[32]

Clinically, PASC resembles myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) in many respects, including women's preponderance, symptoms overlap, and postviral sequale.[33] Patients with ME/CFS have vascular dysregulation associated with cerebral hypoperfusion and orthostatic intolerance, findings which are similar to our PASC cohort. Hence, both PASC and ME/CFS can follow a common postviral trajectory associated with endothelial dysfunction. Endotheliitis caused by infection or inflammation of endothelial cells around the brainstem has been already considered as a driver for PASC.[34]

Small Fiber Neuropathy, Dysautonomia, and Pain

Direct evidence of SFN on skin biopsy was found in 78% of PASC cases. Our findings are consistent with reported loss

of small fibers in patients with PASC using corneal confocal microscopy.[35] Sudomotor dysfunction was reported in 26% of patients who recovered from COVID-19,[36] which is less than in our study (56%). The difference may be due to differences in the patients' selection and normative values. SFN can be an underlying cause of dysautonomia, which could explain the multi-organ symptoms. Our study detected frequent dysautonomia in PASC affecting several autonomic systems (eg, parasympathetic, sympathetic adrenergic and sudomotor), in most cases, confirming a recent report.[37] Dysautonomia was mild in both patients with PASC and patients with POTS, as evidenced by lack of orthostatic hypotension.

A variety of pain symptoms were reported in PASC.[2,3] All our patients with PASC suffered from chronic pain, however, in most cases, the pain was moderate. Here, we observed damage of small sensory fibers on skin biopsies in the majority of patients with PASC, a loss which can be an etiological source of pain.[20]

The origin of SFN and related dysautonomia in PASC remains to be clarified. In general, COVID-19 may cause SFN and related dysautonomia and sensory complaints de novo, or can exacerbate or unmask the underlying condition.[37]

Although the testing in our patients with PASC corresponds to one timepoint, the abnormal findings could be the result of COVID-19 as history of symptoms and signs indicative of SFN or dysautonomia were absent and no patient complained of brain fog and fatigue before the onset of the COVID-19.

Hypocapnic Hyperventilation

Patients with PASC reported chronic dyspnea and had lower supine baseline end tidal CO_2 compared with both patients with POTS and controls suggesting the presence of hyperventilation-induced hypocapnia. Orthostatic hypocapnia typically accompanying POTS,[8] was also noted in our PASC cohort. Chronic hyperventilation, however, can be detrimental to brain functioning because it increases pH and alters excitability of the cell membranes.[38,39]

Persistent respiratory symptoms have been reported in patients with PASC and exercise hyperventilation has been described as a major limiting factor in COVID-19 survivors.[4] The origin of hyperventilation is unknown. The respiratory symptoms and hyperventilation in PASC can be due to pathophysiology of organ damage during the initial phase of the disease or it is part of PASC, or both. In our cohort, no patient had significant metabolic or acid–base disarrangement, oxygen desaturation, or cardiorespiratory disease that may explain hypocapnia. We did not perform comprehensive pulmonary testing, however, it was shown that, in patients with PASC, respiratory complaints cannot be accounted for by impairment of pulmonary function alone.[4] We speculate that the abnormal ventilation control in PASC is compensatory and may stem from cerebral hypoperfusion by either stimulating automatic ventilator control or suppressing the inhibitory systems.

Inflammatory Markers

Elevated inflammatory markers were identified in most of PASC cases in this study, suggesting that chronic inflammation due to autoimmunity may play a role in our findings. The most common was the TS-HDS antibody, present in 4 patients (44%) with PASC but only in one patient (10%) with POTS. TS-HDS is an abundant disaccharide component of heparin sulfate found at peripheral nerve sites.[26] Anti-TS-HDS is associated with thickened basal lamina and deposition of the terminal C5b-C9-lytic complex on endoneural capillaries. Anti-TS-HDS were found previously in a subset of patients with SFN and dysautonomia.[26,40] Capillary pathology associated with TS-HDS points to immune mediated vasculitis as an underlying substrate of SFN and dysautonomia in patients with anti-TS-HDS. Nevertheless, TS-HDS antibodies were detected only in 44% of patients with PASC, and therefore its significance has to be confirmed in a larger cohort.

We observed multiple autoantibodies in PASC. We also found heterogeneous, although different antibodies in the POTS group which is consistent with the literature.[41] The reason for the mixed pattern of detectable autoantibodies for PASC and POTS syndrome is unclear. It is possible that the presence of autoantibodies represents overall immune system dysfunction, susceptibility to autoimmunity, or it is a proxy for chronic low-grade inflammation. However, it is inherently difficult to detect chronic low grade inflammation as no reliable chronic inflammatory markers exist.[25] However, the preponderance of women in PASC, also observed in other studies,[42] is consistent with underlying autoimmune mechanisms, perhaps due to the immunomodulatory and vasodilatory effect of estrogen.[43] False positive results need to be considered particularly for antibodies with low titers.[44] Recent autopsy studies of hospitalized patients with fatal COVID-19 showed evidence of immune-mediated inflammation of muscles[45,46] and peripheral nerve tissue[46] with abnormal expression of a type 1 interferon-inducible protein compared to hospitalized cohorts. Whereas an activated innate immune system response can be an underlying mechanism of SFN, none of our patients with PASC had evidence of myopathy on clinical examination; or had a muscle or nerve biopsy to determine the levels of inflammatory infiltrate. Creatine kinase was also normal in all 7 patients with PASC with available laboratory testing. The degree to which dysautonomia is observed in patients with PASC is a result

of impaired inflammatory response and remains to be fully defined.

Furthermore, 78% of patients with PASC had a history of anxiety/depression before COVID-19, therefore neuropsychiatric vulnerability for PASC may exits.

Limitations

The number of patients in each group was relatively small, although the groups were well matched. Thus, generalization of results to other patients with PASC will need to be confirmed. PASC remains a poorly defined clinical syndrome, and more studies are needed to understand its pathophysiology. This was a retrospective study and subject to referral and selection biases. We evaluated the CBFv as a proxy for cerebral blood flow, as well as for cerebral perfusion. This approach is based on the assumption that the diameter of middle cerebral artery is constant during insonation. Orthostasis does not change the middle cerebral artery diameter[47] implying that CBFv is a good substitute for cerebral blood flow measurements. We used nonspecific immune markers. Future studies may utilize more sensitive and specific inflammatory markers induced by SARS-CoV-2 virus. We did not perform cardiovascular magnetic resonance (CMR) studies to assess myocarditis that has been reported in PASC and may cause tachycardia and chest pain.[48] CMR should be performed when cardiovascular autonomic testing does not lead to diagnosis or an electrocardiogram (ECG) shows typical changes,[49] which was not seen in our cases. We also did not perform full pulmonary assessment in our PASC cohort.

Conclusions

This study detected multisystem abnormalities in cerebrovascular, respiratory, neuronal, and autoimmune systems in PASC. We suspect that low grade inflammation of small vessels plays a role. Larger prospective studies are needed to confirm our findings.

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Author Contributions

P.N., D.S., and D.F. contributed to the conception and design of the study. P.N., S.S.M., H.A.S., D.S., S.P.M., D.F., W.J.M., and D.M.P. contributed to the acquisition and analysis of data. P.N., S.S.M., H.A.S., D.S., S.P.M., D.F., W.J.M., and D.M.P. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

The authors declared no conflict of interest.

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