CLINICAL RESEARCH STUDY

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Post-Acute COVID-19 Syndrome: Prevalence of Peripheral Microvascular Endothelial Dysfunction and Associations With NT-ProBNP Dynamics

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ABSTRACT

BACKGROUND: Post-acute COVID-19 syndrome (PACS) has been linked to microvascular endothelial dysfunction as a potential underlying pathomechanism and can manifest even following a mild course of the initial infection. Prevalence of microvascular endothelial dysfunction and circulating natriuretic peptides in such PACS patients remains unknown.

METHODS: This prospective, cross-sectional cohort study enrolled 92 patients (82% females, median age 48 years) with PACS. Reactive hyperemia index (RHI) was evaluated with peripheral arterial tonometry, where <1.67 was defined as microvascular endothelial dysfunction, 1.67-2.0 as impaired function, and >2 normal endothelial function, on average 31 months after the acute infection. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels were collected at 2 different time points within over a 1-year span.

RESULTS: In total, 41% of PACS subjects had microvascular endothelial dysfunction and 20% had impaired RHI. No major differences in clinical characteristics, routine chemistry laboratory testing, or symptom burden were observed across the groups. Only subjects with microvascular endothelial dysfunction and impaired endothelial function had a significant increase in NT-proBNP levels over time, and those with larger increase in NT-proBNP had significantly lower RHI. There was a significant correlation between relative or absolute increase in NT-proBNP and RHI, which remained significant in a multivariable adjusted linear regression.

CONCLUSIONS: Peripheral microvascular endothelial dysfunction was prevalent in a symptomatic PACS population long after recovery from a mild acute infection. Increases in NT-proBNP levels were associated with microvascular endothelial dysfunction, suggesting a link between, and providing a foundation for, future studies on post viral microvascular endothelial dysfunction in PACS.

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KEYWORDS: Microvascular endothelial dysfunction; Natriuretic peptides; Post-acute COVID-19 syndrome

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INTRODUCTION

COVID-19 may cause persistent symptoms and organ damage beyond 3 months. This is considered a novel and enigmatic clinical long-term condition: post-acute COVID-19 syndrome (PACS).[1] Given the wide range of symptoms and evidence of multiorgan damage in PACS, it has been suggested that microvascular endothelial dysfunction with impaired tissue perfusion may represent a unifying pathophysiological mechanism in PACS.[2,3] We have reported that acute COVID-19 induces microvascular endothelial dysfunction in hospitalized patients, which persists up to 4 months after hospital discharge.[4] More recently, in a preliminary report, we have documented that coronary and peripheral microvascular endothelial dysfunction is common in patients with PACS-related postural orthostatic tachycardia syndrome, and that it is associated with reduced cardiac perfusion during adenosine stress.[5] Furthermore, in another recent study, we have detected substantial dysregulation of proteins in patients with PACS, mainly related to metabolism, immune response, and angiogenesis.[6]

CLINICAL SIGNIFICANCE

- Peripheral microvascular endothelial dysfunction is common among patients with post-acute COVID-19 syndrome years after a mild acute infection.
- Microvascular endothelial dysfunction is associated with increased N-terminal pro-B-type natriuretic peptide levels.
- The link between microvascular dysfunction and cardiac stress advances our understanding of the cardiovascular sequelae among patients with post-acute COVID-19 syndrome.

N-terminal pro-B type natriuretic peptide (NT-proBNP) is a sensitive biomarker of cardiac dysfunction reflecting increased myocardial wall stress and volume overload, and serves as a diagnostic and prognostic marker in heart failure.[7,8] NT-proBNP levels below established cut-off thresholds for heart failure may provide a prognostic value.[7,9] Importantly, the rise in NT-proBNP levels over time, even when remaining below the upper normal limit (<125 ng/L) serves as a prognostic marker for incident heart failure and cardiovascular disease.[9,10] NT-proBNP is frequently elevated among patients in acute COVID-19, and serves as an independent predictor of cardiovascular complications.[11] However, the role of NT-proBNP in PACS is unknown.

In this study, we aimed to assess the prevalence of peripheral microvascular endothelial dysfunction in patients with PACS and its association with levels and temporal changes in NT-proBNP as a marker of cardiovascular stress, volume overload, and cardiac remodeling.

MATERIAL AND METHODS

Study Population

This cross-sectional study is based on a cohort of prospectively enrolled patients meeting the diagnosis criteria of PACS, as defined by the World Health Organization.[12] In short, this definition includes a confirmed or probable previous severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and ongoing symptoms for at least 3 months that cannot be explained by an alternative diagnosis. All patients were recruited from the tertiary post-COVID clinic at Karolinska University Hospital, Stockholm, Sweden. All subjects included in the current cohort contracted the acute infection during the first and second wave of the pandemic (March 2020−January 2021) and did not require hospitalization. The referral criteria include at least 6 months of disabling symptoms and included being on sick leave of at least 50% of full working capacity, indicating a severe disease burden with large socioeconomic, personal, and public health consequences. NT-proBNP and other laboratory tests were evaluated at the initial outpatient visit and follow-up. Laboratory analyses below the limit of detection were set at the lowest threshold (troponin: 5 ng/L, D-dimer: 0.20 mg/L, and NT-proBNP: 11 ng/L). All patients with clinical suspicion of postural orthostatic tachycardia syndrome underwent head-up tilt test.[13] Patients were asked prospectively to be included in the cohort study UppCov, aiming to characterize the long-term consequences of PACS in a comprehensible fashion. Symptoms were retrieved from patients' electronic medical charts and compiled in Research Electronic Data Capture. The investigation was approved by the Swedish Ethical Review Authority (Approval number: 2021-03293) and conducted according to the Declaration of Helsinki.

Determination of Microvascular Endothelial Function

Patients who accepted participation were scheduled for a study visit at the Cardiology Research Department, Karolinska University Hospital. The patients were asked to refrain from nicotine- and caffeine-containing products for 12 hours prior to the investigation, from taking medications in the morning of the examination day, and from eating at least 6 hours or drinking clear fluids at least 2 hours prior to the visit. Microvascular endothelial function was assessed using the EndoPAT® device (Itamar-Medical, Caesarea, Israel) with fully automated postocclusion reactive hyperemia. The principle of measurement of endothelial function is based on pulse amplitude tonometry (PAT) following reactive hyperemia induced by 5-minute occlusion with supra-arterial pressure in the active fingertip (index finger). The PAT signal 60-120 seconds after reactive hyperemia is

divided by baseline PAT signal prior to cuff inflation to retrieve reactive hyperemia index (RHI) as a surrogate for peripheral microvascular endothelial function. The contralateral fingertip served as control.

Statistical Analyses

All analyses were carried out with GraphPad Prism version 7 (GraphPad Software, Boston, Mass) or R version 4.3.0. Categorical variables are expressed as frequencies and percentages, and continuous variables as medians and interquartile ranges. We assumed non-normal distribution of all datasets due to the well-known dispersed distribution of NT-proBNP. Statistical comparisons between categorical variables were analyzed using Chi-squared test. For significant differences, Fisher's exact test was used for pairwise comparisons. Continuous data were analyzed with Mann-Whitney or Wilcoxon tests, as appropriate (2 groups) or Kruskal-Wallis test with Dunn's post hoc analysis (3 groups) and correlations with Spearman correlation Test. We used multivariable linear regression (using RHI as the dependent variable) to identify correlates of NT-proBNP (and relative/absolute changes in NT-proBNP) and microvascular endothelial dysfunction. We also used multivariable adjusted [age, body mass index, sex, systolic blood pressure, diastolic blood pressure, presence of postural orthostatic tachycardia syndrome diagnosis, and days between the acute infection and NT-proBNP at visit 1 and visit 2] linear regression analyses for significant regressions. Ninety-five percent confidence intervals were calculated using the beta-coefficient plus/minus 1.96*Standard Error. All P values presented are 2-sided; $P < .05$ was considered statistically significant. The cutoff for microvascular endothelial dysfunction was set at RHI <1.67.[14] As various cutoff values have been used for microvascular endothelial dysfunction, ranging between 1.67 and 2.0,[14,15] we defined an additional group with RHI 1.67-2.0 as impaired microvascular endothelial function and >2.0 as normal microvascular endothelial function.

RESULTS

Subject Characteristics

In total, 92 consecutive patients with PACS with complete follow-up of sequential blood samples and evaluable measurements of microvascular function were recruited. Characteristics of these subjects are presented in Table 1, stratified according to RHI (1. RHI >2.0, 2. RHI 1.67-2.0, 3. RHI <1.67). The subjects were a median 48 years of age in the overall group, with the majority being female. The median time between the acute infection as reported by the study participants and examination of microvascular function was approximately 31 months, without a significant difference between the groups. NT-proBNP at the first visit and at follow-up in the post-COVID clinic were sampled, on average, 9 and 25 months, respectively, after the acute infection. Assessment of RHI was performed, on average, after 31 months. The proportion of patients suffering from cardiovascular comorbidities was, in general, low, with hypertension (17%) being the most prevalent one, and did not differ significantly between the groups. The most common medication was a beta-blocker (49%), with a slightly higher use in the microvascular endothelial dysfunction group, in many patients likely prescribed for palpitations, ranking among the most common symptoms (68%; Table 2). Apart from a tendency toward a larger proportion of females, a lower proportion of subjects suffering from depression and anxiety, and a slightly larger proportion prescribed beta-blockers in the microvascular endothelial dysfunction group, no other significant differences between the groups were observed.

The subjects had a mild acute infection, with the most common symptom being fever, followed by shortness of breath and headache (Table 3). No significant differences in acute symptomatology among subjects across the groups were observed. As indicated, a large proportion suffered from classical PACS symptoms including fatigue, chest pain, concentration deficit, and shortness of breath, during the postacute phase of the disease (Table 2). Only minor differences in symptoms in the postacute phase of the disease between the groups were observed, including differences in the prevalence of shortness of breath, impaired vision, malaise, and walking difficulties.

Prevalence of Microvascular Dysfunction

Microvascular dysfunction defined as RHI <1.67 was found in 41% (n = 38) of PACS patients, whereas 20% (n = 18) had impaired endothelial function, and only 39% (n = 36) had normal RHI (>2.0) (Figure 1A & B).

Dynamics in NT-ProBNP Levels and Microvascular Endothelial Dysfunction

Applying the established upper limit of normal (125 ng/L) for a nonacute setting,[8] median and interquartile ranges of NT-proBNP levels were within the normal range. At visits 1 and 2, 6 and 14 individuals, respectively, had NT-proBNP levels above the cut-off. There was a 21% increase in NT-proBNP levels between visit 1 at baseline and the follow-up visit (visit 2) in the overall cohort (Table 1). This increase was not significant in patients with normal RHI, but was significant in both impaired endothelial function and with microvascular endothelial dysfunction (Figure 2A-C). To further investigate possible implications of these increases, relative change in NT-proBNP was calculated and compared in the 3 groups. This revealed that the relative change in NT-proBNP was higher in both the microvascular endothelial dysfunction and the impaired RHI group compared with the normal RHI group (Figure 3A). Subjects with more than 50% increase in NT-proBNP levels in the overall cohort had lower RHI compared with the subjects with no increase or decrease (Figure 3B). Delta NT-proBNP levels (NT-proBNP level at visit 2 − visit 1) were divided into above vs below median (median = 7 ng/L) and plotted

Table 1 Clinical Characteristics of the Study Subjects Stratified Based on the Presence or Absence of Microvascular Endothelial Dysfunction

	All n = 92	Normal Endothelial Function RHI >2.0 n = 36	Impaired Endothelial Function RHI 1.67-2.0 n = 18	Microvascular Endothelial Dysfunction RHI <1.67 n = 38
Age (years)	48 (41-54)	49 (45-55)	45 (39-53)	46 (40-54)
BMI (kg/m^2)	25 (22-30)	24 (22-30)	24 (20-27)	25 (23-30)
Female sex, n (%)	75 (82)	27 (75)	14 (78)	34 (89)
Postural orthostatic tachycardia syndrome diagnosis, n (%)	25 (27)	8 (22)	8 (44)	9 (24)
Requiring hospitalization	0 (0)	0 (0)	0 (0)	0 (0)
Days between acute infection and RHI evaluation	928 (812-987)	946 (830-1006)	875 (790-977)	860 (811-981)
Days between acute infection and NT-proBNP 1	286 (233-382)	353 (254-404)	282 (194-391)	269 (228-368)
Days between acute infection and NT-proBNP 2§	757 (529-1125)	805 (595-1141)	580 (400-753)	855 (513-1147)
Days between acute infection and symptom report	291 (238-393)	335 (244-468)	330 (216-408)	284 (233-353)
SBP Visit 1 (mm Hg)	125 (120-130)	125 (120-140)	120 (120-130)	128 (120-130)
SBP Visit 2 (mm Hg)	125 (116-130)	120 (115-130)	129 (119-131)	125 (116-131)
DBP Visit 1 (mm Hg)	80 (80-84)	80 (80-84)	80 (80-88)	80 (80-85)
DBP Visit 2 (mm Hg)	80 (75-85)	80 (75-84)	81 (78-86)	80 (73-90)
RHI (arbitrary units)§§§	1.85 (1.46-2.24)	2.33 (2.15-2.67)	1.86 (1.74-1.93)**	1.43 (1.33-1.53)***,###
Laboratory analysis				
NT-proBNP Visit 1 (ng/L)	42 (24-69)	50 (27-97)	37 (13-55)	36 (24-66)
NT-proBNP Visit 2 (ng/L)	51 (29-91)	51 (27-91)	51 (24-80)	51 (32-96)
Hemoglobin (g/L)	137 (129-141)	137 (129-141)	136 (124-144)	135 (130-146)
Leukocytes (10^9/L)	6.3 (5.5-7.3)	6.3 (5.5-7.5)	6.1 (5.4-8.7)	6.6 (5.1-7.3)
Platelets (10^9/L)	268 (241-323)	259 (239-343)	268 (210-314)	277 (255-325)
CRP (mg/L)	1.0 (1.0-2.8)	1.0 (1.0-4.0)	1 (1-1)	1.0 (1.0-2.0)
Creatinine (μmol/L)	67 (58-77)	68 (58-77)	64 (56-73)	67 (60-76)
HbA1c (mmol/mol)	35 (32-36)	34 (32-36)	34 (32-35)	35 (33-38)
Hs-TnT (ng/L)	5 (5-5)	5 (5-6)	5 (5-6)	5 (5-5)
D-dimer (mg/L)	0.27 (0.20-0.40)	0.24 (0.20-0.38)	0.26 (0.20-0.36)	0.27 (0.20-0.42)
Fibrinogen (g/L)	2.9 (2.5-3.4)	2.8 (2.6-3.1)	2.8 (2.4-3.2)	3.2 (2.5-3.6)
Ferritin (μg/L)	77 (34-126)	75 (36-139)	67 (44-184)	80 (34-123)
Comorbidities, n (%)				
Depression/anxiety§§§	27 (29)	15 (42)	7 (39)	5 (13)***,#
Asthma	18 (20)	9 (25)	1 (6)	8 (21)
Hypertension	16 (17)	6 (17)	3 (17)	7 (18)
Thyroid disease	15 (16)	7 (19)	3 (17)	5 (13)
Diabetes	3 (3)	0 (0)	1 (6)	2 (5)
Atherosclerotic disease†	2 (2)	1 (3)	0 (0)	1 (3)
Chronic kidney disease	0 (0)	0 (0)	0 (0)	0 (0)
Heart failure	0 (0)	0 (0)	0 (0)	0 (0)
Medications, n (%)				
Beta-blockers§	45 (49)	14 (39)	9 (50)	22 (58)*
Inhalators	31 (34)	16 (44)	5 (28)	10 (26)
Ivabradin	29 (32)	9 (25)	8 (44)	12 (32)
Antidepressants	25 (27)	11 (31)	5 (28)	9 (24)
Statins	12 (13)	3 (8)	2 (11)	7 (18)
RAASi	9 (10)	2 (6)	3 (17)	4 (11)
Calcium channel blocker	6 (7)	2 (6)	2 (11)	2 (5)

BMI = body mass index; CRP = C-reactive protein; DBP = diastolic blood pressure; HbA1c = hemoglobin A1c; HsTnT = high-sensitivity troponin T; NT-proBNP = N-terminal pro-B type natriuretic peptide; RAASi = renin-angiotensin-aldesterone system inhibitors; RHI = reactive hyperemia index; SBP = systolic blood pressure.

†Includes coronary artery disease, stroke, and peripheral artery disease.

P values are indicated for the comparison of microvascular endothelial dysfunction vs no microvascular endothelial dysfunction. Continuous variables are presented as median and interquartile ranges. Categorical variables are presented as numbers and percentages. Continuous variables were analyzed with Kruskal-Wallis test with Dunn's post hoc analysis, categorical variables with Chi-squared test across all groups, in significant comparisons, Fisher's exact test for pairwise comparisons was used.

§*P* < .05,

§§§*P* < .001 across the groups;

**P* < .05,

***P* < .01,

***P < .001 vs Normal RHI >2.0;

#*P* < .05,

###*P* < .001 vs Impaired RHI 1.67-2.0.

Table 2 Frequency and Percentage of PACS Symptoms 291 Days After Acute COVID-19

Symptom	All n = 80	Normal Endothelial Function RHI >2.0 n = 32	Impaired Endothelial Function RHI 1.67-2.0 n = 15	Microvascular Endothelial Dysfunction RHI <1.67 n = 33
Fatigue	74 (93)	27 (84)	15 (100)	32 (97)
Shortness of breath§	62 (78)	26 (81)	8 (53)	28 (85)#
Palpitations	54 (68)	25 (78)	9 (60)	20 (61)
Chest pain/pressure	52 (65)	20 (63)	10 (67)	22 (67)
Joint pain	48 (60)	19 (59)	8 (53)	21 (64)
Concentration deficit	45 (56)	17 (53)	8 (53)	20 (61)
Dizziness	42 (53)	18 (56)	9 (60)	15 (45)
Parestesia	40 (50)	16 (50)	9 (60)	15 (45)
Headache	39 (49)	13 (41)	9 (60)	17 (52)
Short-term memory deficit	36 (45)	15 (47)	7 (47)	14 (42)
Weight gain	35 (44)	12 (38)	7 (47)	16 (48)
Cough	34 (43)	12 (38)	7 (47)	15 (45)
Fever	34 (43)	16 (50)	9 (60)	9 (27)
Reduced fitness	34 (43)	13 (41)	4 (27)	17 (52)
Insomnia	28 (35)	12 (38)	6 (40)	10 (30)
Brain fog	26 (33)	10 (31)	4 (27)	12 (36)
Nausea	25 (31)	8 (25)	7 (47)	10 (30)
Post exertional malaise	23 (29)	6 (19)	4 (27)	13 (39)
Impaired vision§	21 (26)	4 (13)	7 (47)*	10 (30)
Ageusia	17 (21)	7 (22)	5 (33)	5 (15)
Anosmia	16 (20)	6 (19)	5 (33)	5 (15)
Blemishes	15 (19)	7 (22)	1 (7)	7 (21)
Muscle weakness	15 (19)	5 (16)	2 (13)	8 (24)
Weight loss	15 (19)	7 (22)	3 (20)	5 (15)
Diarrhea	14 (18)	7 (22)	1 (7)	6 (18)
Fainting	13 (16)	8 (25)	3 (20)	2 (6)
Sensitivity to impression	13 (16)	4 (13)	5 (33)	4 (12)
Tinnitus	12 (15)	6 (19)	1 (7)	5 (15)
Respiratory pain	11 (14)	4 (13)	5 (33)	2 (6)
Urticaria	11 (14)	4 (13)	3 (20)	4 (12)
Appetite defiency	10 (13)	4 (13)	2 (13)	4 (12)
Dysphagia	10 (13)	4 (13)	1 (7)	5 (15)
Worried	9 (11)	4 (13)	2 (13)	3 (9)
Depressed	7 (9)	4 (13)	1 (7)	2 (6)
Urination irregularities	7 (9)	1 (3)	3 (20)	3 (9)
Anxiety	6 (8)	3 (9)	1 (7)	2 (6)
Imbalance	6 (8)	1 (3)	0 (0)	5 (15)
Malaise§	6 (8)	0 (0)	3 (20)*	3 (9)
Opstipation	6 (8)	3 (9)	2 (13)	1 (3)
Abdominal pain	5 (6)	1 (3)	2 (13)	2 (6)
Nightmares	4 (5)	2 (6)	0 (0)	2 (6)
Walking difficulties§	4 (5)	0 (0)	0 (0)	4 (12)
Impaired hearing	3 (4)	0 (0)	1 (7)	2 (6)
Menstruation irregularities	3 (4)	2 (6)	0 (0)	1 (3)
Swollen joints	3 (4)	0 (0)	0 (0)	3 (9)
Tremor	3 (4)	0 (0)	1 (7)	2 (6)
Hair loss	2 (3)	1 (3)	1 (7)	0 (0)
Voice impairment	2 (3)	1 (3)	0 (0)	1 (3)
Mood changes	1 (1)	0 (0)	1 (7)	0 (0)
Rigor	1 (1)	1 (3)	0 (0)	0 (0)
Seizures	1 (1)	0 (0)	0 (0)	1 (3)
Slow movement	1 (1)	1 (3)	0 (0)	0 (0)
Erectile dysfunction	0	0 (0)	0 (0)	0 (0)
Infection susceptibilty	0	0 (0)	0 (0)	0 (0)
Yawnings	0	0 (0)	0 (0)	0 (0)

PACS = post-acute COVID-19 syndrome; RHI = reactive hyperemia index.

Data presented as numbers and percentage within parentheses, sorted in descending frequency order. Chi-squared test across all groups, in significant comparisons, Fisher's exact test for pairwise comparisons was used.

§$P < .05$ across the groups;

*$P < .05$ vs Normal RHI >2.0;

#$P < .05$ vs impaired RHI 1.67-2.0.

Table 3 Frequency and Percentage of Symptoms During the Acute Infection as Reported by the Study Subjects

Symptom	All n = 78	Normal Endothelial Function RHI >2.0 n = 33	Impaired Endothelial Function RHI 1.67-2.0 n = 15	Microvascular Endothelial Dysfunction RHI <1.67 n = 30
Fever	55 (71)	25 (76)	10 (67)	20 (70)
Shortness of breath	32 (41)	12 (36)	9 (60)	11 (37)
Headache	26 (33)	13 (39)	4 (27)	9 (30)
Myalgia	21 (27)	9 (27)	7 (47)	5 (17)
Anosmia or parosmia	20 (26)	8 (24)	5 (33)	7 (23)
Tiredness	13 (17)	4 (12)	1 (7)	7 (23)
Sore throat	11 (14)	5 (15)	0 (0)	6 (20)
Rhinitis	9 (12)	6 (18)	0 (0)	3 (10)
Blocked nose	7 (9)	3 (9)	1 (7)	3 (10)
Diarrhea*	7 (9)	6 (18)	0 (0)	1 (3)
Nausea	6 (8)	4 (12)	0 (0)	2 (7)
Chest pain	5 (6)	1 (3)	3 (20)	1 (3)
Light sensitivity	3 (4)	0 (0)	2 (13)	1 (3)
Bruises	1 (1)	1 (3)	0 (0)	0 (0)

RHI = reactive hyperemia index.
Data presented as numbers and percentage within parenthesis, sorted in descending frequency order.
*$P < .05$ across the groups with Chi-squared test. No significant differences in pairwise analyses with Fisher's exact test were observed.

against RHI. This corroborated the finding that subjects with a larger increase in NT-proBNP between the visits is associated with lower RHI (Figure 3C). A significant correlation and linear regression between the relative change in NT-proBNP and RHI were observed (Figure 3D, Table 4). Levels of NT-proBNP at visit 1 (Figure 4A) and visit 2 (Figure 4B) did not differ among the 3 groups. There were no significant correlations or regressions between RHI and NT-proBNP at visit 1 (Figure 4D, Table 4) or visit 2 (Figure 4E, Table 4). However, a significant difference in delta NT-proBNP levels was observed between normal RHI and impaired RHI and an almost significant difference between normal RHI and microvascular endothelial dysfunction (Figure 4C). This was corroborated by a significant correlation and linear regression between RHI and delta NT-proBNP, which remained significant in a multivariable adjustment analysis (Figure 4F and Table 4)

DISCUSSION

The main finding of the current cross-sectional study is that peripheral microvascular endothelial dysfunction is common in a limited sample size among highly symptomatic patients with PACS from a single center and can be detected over 2 years after a relatively mild initial COVID-19 infection during the first wave of the pandemic. The second finding is that the presence of microvascular endothelial dysfunction is associated with increasing levels of NT-

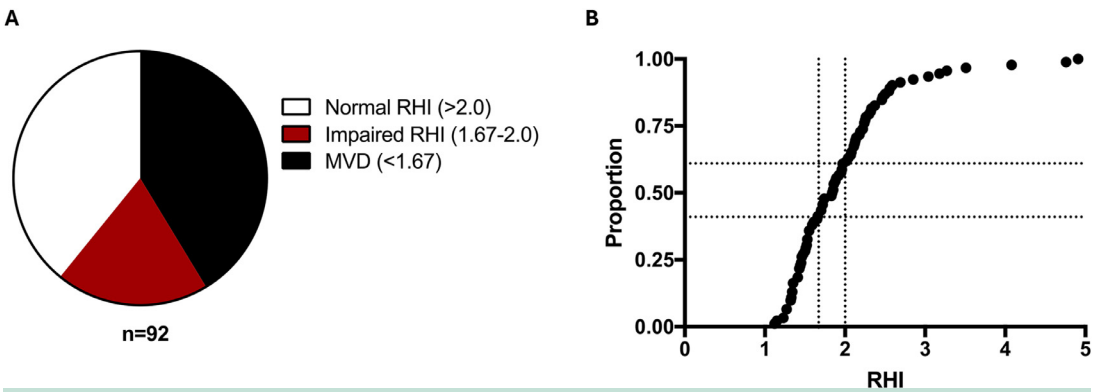

Figure 1 Prevalence of microvascular endothelial dysfunction. (**A**) Proportion of patients with post-acute COVID-19 syndrome (PACS) with normal reactive hyperemia index (RHI), borderline reduced RHI (impaired RHI), and with microvascular endothelial dysfunction (MVD) with a cutoff of RHI 1.67. (**B**) Cumulative distribution of RHI among patients with PACS. Vertical dashed lines represent cutoff for MVD (1.67) and impaired RHI (2.0), intercepted horizontally with the proportion of patients with MVD (0.41) and impaired RHI (0.61).

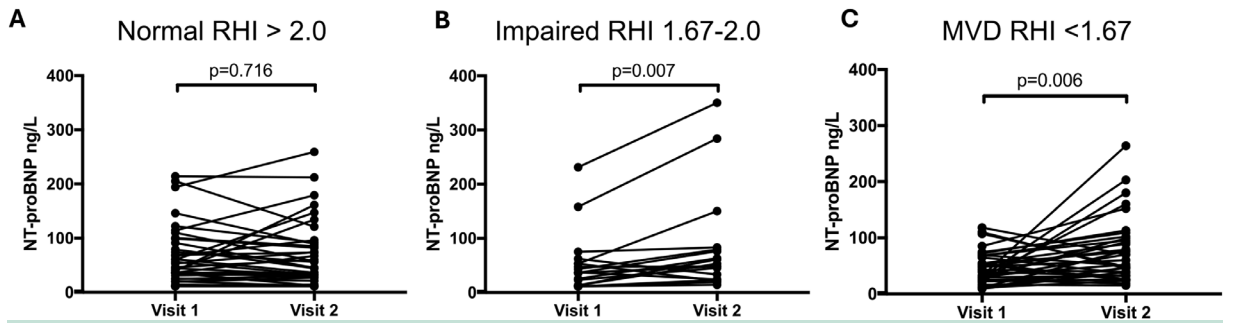

Figure 2 Change in N-terminal pro−B-type natriuretic peptide (NT-proBNP) across different categories of microvascular function. NT-proBNP levels at baseline (Visit 1) and at follow-up (Visit 2) in post-acute COVID-19 syndrome (PACS) patients with (**A**) normal RHI, (**B**) impaired RHI, and (**C**) microvascular endothelial dysfunction (MVD). Median NT-proBNP at Visit 1 and Visit 2 were: (**A**) 49.5 and 50.5, (**B**) 36.5 and 50.5, and (C) 35.5 and 51.0, respectively. *P* values are indicated. Statistical analyses with Wilcoxon test.

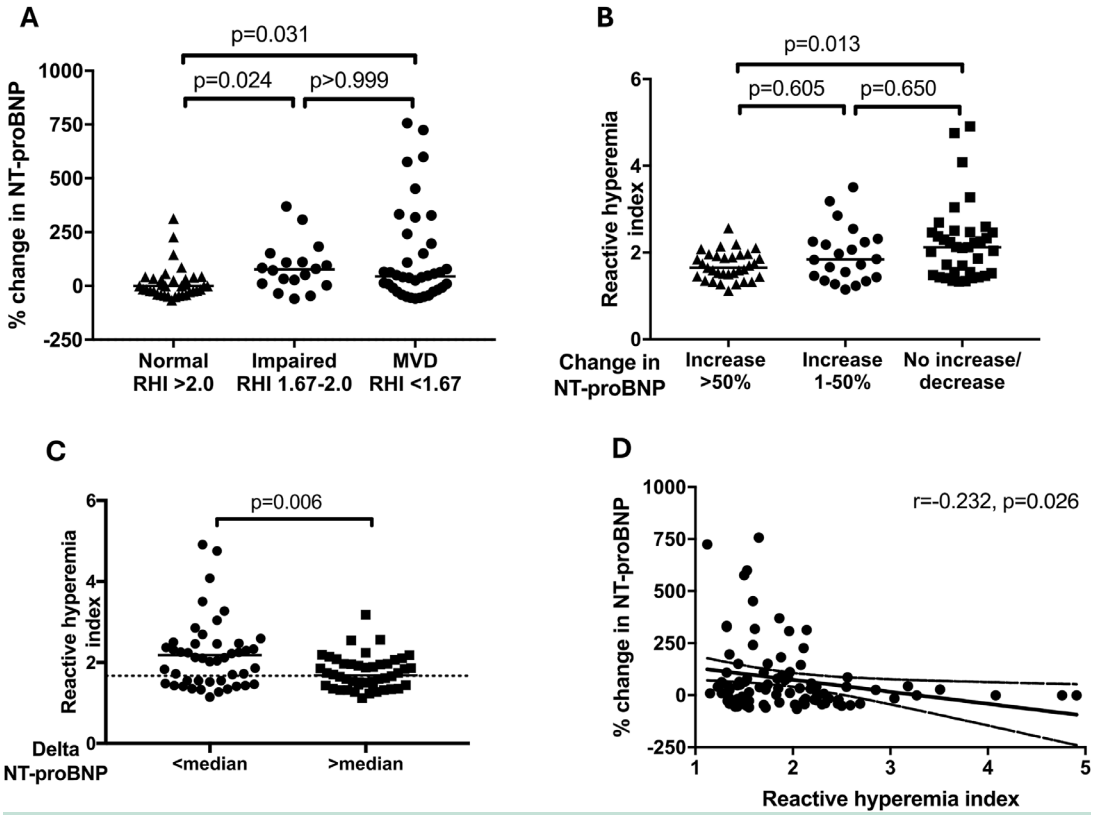

Figure 3 Relative changes in N-terminal pro−B-type natriuretic peptide (NT-proBNP) and its association to microvascular endothelial function. (**A**) Percentage change in NT-proBNP levels between visits across the different groups based on reactive hyperemia index (RHI). (**B**) RHI according to the degree of change NT-proBNP levels divided into >50% increase (n = 33), 1%-50% increase (n = 22), and no increase or decrease (n = 37). (**C**) RHI among patients divided into below and above delta NT-proBNP levels between the visits. Median NT-proBNP = 7 ng/L (n = 46 in each group). (**D**) Correlation between percentage change in NT-proBNP levels and RHI. Statistical analyses with Kruskal-Wallis test with Dunn's multiple comparison test (**A and B**), Mann-Whitney test (**C**), or Spearman correlation test (**D**). *P* values and correlation coefficient are indicated.

Table 4 Association Between RHI and NT-proBNP at Visit 1, NT-proBNP at Visit 2, Relative Change in NT-proBNP, and Delta NT-proBNP Between Visits 1 and 2

Parameters	Unadjusted		Multivariable Adjusted*	
	β-coefficient† (95% CI)	*P* Value	β-coefficient† (95% CI)	*P* Value
NT-proBNP visit 1 (ng/L)	0.095 (−0.010−0.300)	.368	–	–
NT-proBNP visit 2 (ng/L)	−0.099 (−0.204−0.107)	.349	–	–
% Change in NT-proBNP	−0.242 (−0.344 to −0.041)	.020	−0.217 (−0.325 to −0.005)	.049
Delta NT-proBNP	−0.207 (−0.311 to −0.005)	.047	−0.254 (−0.377 to −0.012)	.043

CI = confidence interval; NT-proBNP = N-terminal pro-B-type natriuretic peptide; RHI = reactive hyperemia index.
*Adjusted for age, body mass index, sex, systolic blood pressure, diastolic blood pressure, presence of postural orthostatic tachycardia syndrome diagnosis and days between the acute infection, and NT-proBNP 1 and 2.
†Standardized regression coefficient.

proBNP over time. This hypothesis-generating data, in a limited sample size, provides support to the involvement of the microvasculature in the pathophysiology of PACS and possible hemodynamic stress and cardiac remodeling.

Approximately 41% of the PACS subjects included in the study had microvascular dysfunction, underscoring a potential impact of COVID-19 on peripheral microvascular function several years after the acute infection. Previous studies of ours, and others, report that only a small minority of female subjects with comparable age have microvascular dysfunction.[5,16,17] These findings align with previous evidence provided by us and others,[5,18-22] suggesting a key role for endothelial dysfunction in the pathophysiology of PACS, and add further support to the view of PACS being a pan-vascular disease. Several previous studies have shown that coronary or peripheral microvascular endothelial dysfunction is implicated in the pathogenesis of COVID-19 after the recovery from the acute phase.[19-22] However, all these studies recruited patients with a shorter time between the acute infection and time of examination, and not all studies complied strictly to the World Health Organization criteria for PACS. Although limited in sample size, the strengths of our cohort is that participants had a long follow-up and a previous low burden of cardiovascular comorbidities, without major differences in comorbidities or medications.

The observation into NT-proBNP dynamics provides a potential link to our understanding of PACS and the interplay between microvascular endothelial dysfunction and biomarkers of cardiac stress. The slightly greater increase in NT-proBNP in PACS patients with microvascular endothelial dysfunction raises concerns about potential cardiac

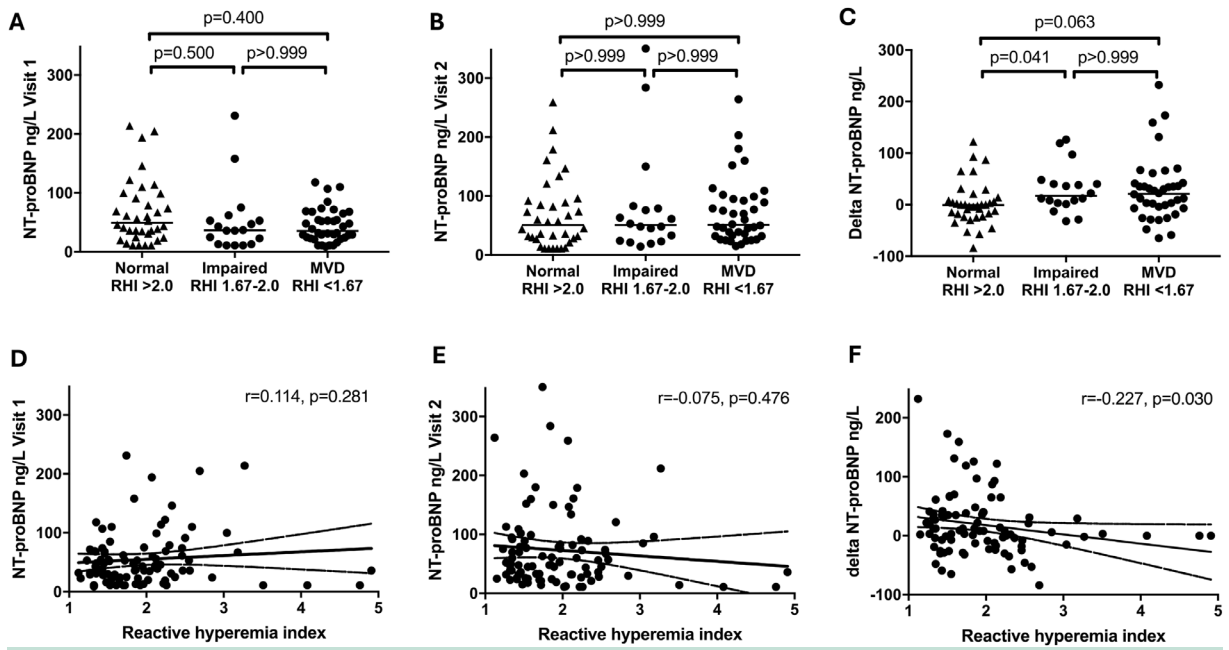

Figure 4 N-terminal pro−B-type natriuretic peptide (NT-proBNP) levels in different groups and correlation to reactive hyperemia index (RHI). NT-proBNP levels across different groups at (**A**) Visit 1, (**B**) Visit 2, or (**C**) absolute change between visits (Visit 2 − Visit 1). Correlation of NT-proBNP levels and RHI at (**D**) Visit 1, (**E**) Visit 2, or (**F**) absolute change between visits. *P* values and correlation coeffcients are indicated. Linear fit curves and 95% confidence interval are presented. Mann-Whitney test (A, B, and C) and median or Spearman correlation Test (**D, E, and F**) were used.

remodeling and a possible subclinical heart failure phenotype in development. Importantly, the inverse association of delta NT-proBNP, as well as the relative change in NT-proBNP and RHI, remained significant, even after adjusting for possible confounders. Unexpectedly, there were no differences in baseline NT-proBNP levels across the groups, and all 3 groups had comparable NT-proBNP levels at visit 2, which suggests that dynamics rather than absolute levels of NT-proBNP are associated with peripheral microvascular function. It should be acknowledged that the increase in NT-proBNP levels among patients with microvascular dysfunction was slight and within the subdiagnostic range. Although statistically significant, it can further not be excluded that the dynamics in NT-proBNP are spontaneous, due to biological variation and changes or adherence to medication in this limited sample size. However, it is known from previous studies that increases (>25%) in NT-proBNP levels in the subdiagnostic range predict incident heart failure and cardiovascular disease,[9,10] and this increase was only observed among patients with impaired endothelial function or microvascular endothelial dysfunction. Furthermore, if anything, the slightly higher use of beta-blockers in the microvascular endothelial dysfunction groups should counteract the increase in NT-proBNP levels. Consequently, although the majority of the patients had NT-proBNP levels below the upper normal limit cut-off for chronic heart failure, the slight increase observed in the groups with reduced RHI may be clinically meaningful, which should be validated in a larger cohort of patients and with longer follow-up. As low RHI has been shown to independently predict cardiovascular events among patients with other cardiovascular risk factors[23,24] and is associated with coronary endothelial dysfunction,[25] it is tempting to speculate that such a link also exists among patients with PACS.

The mechanisms underlying microvascular endothelial dysfunction in PACS is likely multifactorial, with involvement of complex mechanisms. We and others have shown that PACS is associated with a strong up-regulation of pro-inflammatory cytokines and alterations of coagulation factors.[6,26] Whether some of these changes are important mediators of microvascular endothelial dysfunction is unknown. Additional mechanisms may involve attenuation of nitric oxide bioavailability and increased production of free radicals. Alternative explanations might include the role of dysautonomia and central nervous system involvement as potential mediators of microvascular dysfunction. It is well established that PACS is associated with autonomic dysfunction.[27] Although the prevalence of postural orthostatic tachycardia syndrome was not significantly different among the groups here, this does not exclude the possible involvement of autonomic dysfunction on microvascular endothelial dysfunction in our material. This subclinical autonomic dysfunction certainly requires attention in upcoming investigations.

Several therapeutic strategies may be discussed in the light of our findings. In a pilot study, we have recently shown that enhanced external counterpulsation alleviates physical and psychological symptoms, and is associated with improved physical capacity in patients with PACS and concomitant coronary microvascular endothelial dysfunction.[28] This approach might be applied on a larger scale among patients with PACS and microvascular endothelial dysfunction. As outlined above, microvascular endothelial dysfunction is closely linked to pro-inflammation, and anti-inflammatory therapies might serve as potential future treatment options.

This study should be regarded as hypothesis generating, and is associated with several limitations that should be acknowledged. First, the single-center design at a tertiary post-COVID-19 clinic, with a high symptoms burden, may limit the generalizability of the findings, especially for less symptomatic patients. Second, the small sample size may reduce the statistical power to detect significant associations, generalize the results, and investigate associations with clinical markers. Third, the cross-sectional design of our study inherently limits the ability to make causal inferences. Specifically, we did not collect data on microvascular function prior to the acute COVID-19 infection. Consequently, it is unclear whether the observed microvascular endothelial dysfunction developed as a result of PACS or if it was a pre-existing condition that predisposed individuals to PACS after contracting acute COVID-19. This ambiguity raises the possibility that microvascular dysfunction might be a risk factor for developing PACS, rather than a consequence of the syndrome. Fourth, symptoms were retrieved from patients' electronic medical charts, which might underestimate the symptom burden. Fifth, all subjects were recruited during the first waves of the pandemic. Hence, any extrapolation to other variants of the virus and after COVID-19 vaccination should be made cautiously. Last, the lack of a healthy control group limits the interpretation of the impact of microvascular endothelial dysfunction in the PACS population. Nevertheless, we believe that due to the novelty of the PACS syndrome, the tremendous knowledge gap of the pathophysiology and long-term clinical outcomes, the results with the observed association of NT-proBNP and peripheral microvascular function are important and may lay the foundation for future trials. These include establishing and elucidating the specific pathophysiological mechanisms linking microvascular dysfunction to cardiac remodeling in PACS patients, which is essential for developing improved risk stratification tools and targeted interventions. Additionally, longitudinal studies monitoring NT-proBNP levels and cardiac outcomes including cardiac imaging parameters in larger cohorts with a longer follow-up of PACS patients could provide further validation of the utility of microvascular endothelial function as a prognostic marker in PACS. As RHI has been shown to independently predict cardiovascular events among patients with other cardiovascular risk factors[23,24] and is associated with coronary endothelial function,[25] it is tempting to speculate that such a link also exists among patients with PACS.

In conclusion, our hypothesis-generating study demonstrates that peripheral microvascular endothelial dysfunction is prevalent in highly symptomatic PACS patients, detectable over 2 years after a mild initial COVID-19 infection. We found that microvascular dysfunction is associated with increasing NT-proBNP levels over time, providing a potential involvement of the microvasculature in PACS pathophysiology and potential cardiac remodeling. These findings support the hypothesis that PACS is a pan-vascular disease, with microvascular endothelial dysfunction playing a central role.

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