Microvascular Remodeling and Endothelial Dysfunction Across the Post-COVID-19 Spectrum: A Prospective Observational Case-Control Study

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Research Article

Keywords: Post-COVID-19 Syndrome, myalgic encephalomyelitis/chronic fatigue syndrome, endothelial dysfunction, retinal microcirculation, retinal vessel analysis, neurovascular coupling

Posted Date: February 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8702908/v1

Additional Declarations: Competing interest reported. TW, RG, IL, AR, ML, LR, BH, LS, HH, MW, and CS declare that they have no competing interests. FCO reports current research funding from the German Research Foundation (DFG; TRR418), the Hertie Foundation, the German ME/CFS Foundation, Novartis, and UCB, all unrelated to this project. She reports past fellowship support from the American Academy of Neurology and the National Multiple Sclerosis Society (until 2023), and past research funding from the German Neurological Society (DGN) and the DFG-TWAS program. FCO reports speaker honoraria from UCB and Novartis, and travel support from the Guthy-Jackson Charitable Foundation, the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), and the American Academy of Neurology. She serves as academic editor at DGNeurologie and Neurological Research & Practice, is a board member of the IMSVISUAL consortium, and co-chairs the Committee for Neuro-ophthalmology and Neuro-otology of the German Neurology Association.

Abstract

Background

Post-viral diseases, including post-COVID-19 syndrome (PCS) and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), cause substantial long-term morbidity. Persistent cardiovascular risk after acute infection highlights the need for accessible tools to quantify microvascular health.

Methods

All Eyes on PCS is a prospective, observational study investigating the retinal microcirculation using retinal vessel analysis (RVA). We compared RVA parameters in 102 PCS patients with 204 age- and sex-matched healthy controls (matched from n = 303). Secondary matched analyses included never infected controls (n = 96), recovered individuals (n = 102), PCS patients, and PCS patients fulfilling ME/CFS criteria (n = 62). Laboratory variables, circulating markers of endothelial dysfunction and inflammation were compared between cohorts and their associations with RVA parameters were examined.

Results

Compared with healthy controls, PCS patients showed reduced venular flicker-induced dilation (3.7 ± 2.2% vs. 4.5 ± 2.7%, p = 0.005, Cohen´s d: 0.32), narrow retinal arterioles (CRAE, 178.3 ± 15.5 µm vs. 183.3 ± 15.9 µm, p = 0.009, d: 0.32), and lower arteriolar-to-venular ratio (0.83 ± 0.06 vs. 0.86 ± 0.07, p = 0.004, d: 0.35). Findings persisted after adjustment for cardiovascular risk factors and remained evident in an extended secondary matched analysis across never infected, recovered, and PCS patients. ME/CFS patients showed the most pronounced alterations. PCS severity correlated with lower AVR (r = -0.21, p = 0.037) and reduced arteriolar flicker-induced dilation (r = -0.21, p = 0.039), particularly for neurocognitive symptoms. IL-6, ICAM-1 and VCAM-1 were elevated in PCS and ME/CFS and lower AVR correlated with inflammatory and iron-related markers (all adjusted p < 0.01). A combined model discriminated ME/CFS patients with good accuracy (AUC = 0.80).

Conclusions

PCS is associated with persistent endothelial dysfunction, most pronounced in ME/CFS patients and linked to symptom severity and ongoing inflammation. These findings support the potential use of RVA as a non-invasive tool for assessing and monitoring endothelial health in post-viral syndromes, with implications for cardiovascular risk stratification.

Trial Registration

The All Eyes on PCS Study has previously been registered at ClinicalTrials.gov (NCT05635552).

Background

Post-COVID-19 syndrome (PCS), also referred to as long COVID, remains a major public health challenge, frequently resulting in prolonged morbidity, work incapacity, and a substantial socioeconomic burden (1–4). Although multiple terms and definitions are used by different authorities, PCS is commonly defined as the persistence or development of new symptoms three months after the initial onset of SARS-CoV-2 infection, lasting for at least two months and not attributable to alternative diagnoses. While many individuals recover fully from acute infection, a considerable subset experience persistent, multisystem symptoms, including fatigue, cognitive impairment, and exercise intolerance, that may persist for months to years (5, 6). A subset of PCS patients fulfills diagnostic criteria for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), characterized by profound fatigue and post-exertional malaise (PEM) (7, 8).

Multiple, potentially interacting mechanisms, include viral persistence, immune dysregulation, autoimmunity, metabolic and mitochondrial dysfunction, muscle and neurovascular (NV) alterations, and persistent endothelial dysfunction (ED) (6, 9–13). While these processes contribute to marked biological heterogeneity of post-viral syndromes such as PCS and ME/CFS, they may converge on shared downstream alterations at the level of endothelial function (14–16). Subsequently, elevated blood biomarkers of ED, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), vascular endothelial growth factor (VEGF), pro-inflammatory chemokines and cytokines including C-C motif chemokine ligand-5 (CCL5), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and C-X-C motif chemokine ligand-10 (CXCL10), as well as procoagulant markers such as von Willebrand factor (vWF), D-dimers, and thrombocytes, have been reported in acute COVID-19, post-COVID-19 syndrome (PCS), and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (17–20).

SARS-CoV-2 infection confers a sustained increase in cardiovascular (CV) risk extending beyond the acute phase, even after mild infection, supporting the concept of persistent vascular injury and accelerated vascular ageing as a central long-term consequence of COVID-19 (21–23). Consequently, persistent macrovascular and microvascular dysfunction assessed by flow-mediated dilation (FMD), pulse-wave velocity (PWV), post-occlusive reactive hyperemia (POH) and optical coherence tomography angiography (OCT-A) have been reported in patients with ongoing symptoms (16, 21, 24–26).

Notably, neurocognitive symptoms such as fatigue, impaired concentration, and reduced executive function have been linked to NV involvement and disturbed cerebral perfusion in post-viral diseases, suggesting that ED may contribute to central nervous system manifestations (10, 27). In this context, the retina represents an accessible surrogate of cerebral microvascular function. The retinal microvasculature shares embryological origin, anatomical features, and autoregulatory mechanisms with the cerebral microcirculation, and retinal flicker-induced vasodilation (FID) reflects NV coupling mediated by NO-dependent endothelial signaling (28, 29).

Retinal vessel analysis (RVA), including static retinal vessel analysis (SVA) and dynamic retinal vessel analysis (DVA), provides a window into systemic microvascular health (30). SVA measures retinal vessel calibers, including the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), which reflect microvascular structural integrity, as well as the arteriolar-to-venular ratio (AVR), calculated as CRAE divided by CRVE, which reflects retinal microvascular autoregulatory function. Changes in SVA parameters are indicative of systemic vascular health and have been validated as predictors of CV outcomes in large cohort studies (31–33).

DVA non-invasively quantifies FID of retinal arterioles (aFID) and venules (vFID) as the percentage change from baseline diameter in response to flickering light stimulation. In a cohort of patients undergoing dialysis, we previously demonstrated that reduced vFID is an independent predictor of all-cause mortality (34). Retinal FID has been shown to correlate with dynamic cerebral hemodynamic changes assessed by functional MRI, highlighting RVA as a non-invasive approach to probe NV function relevant to cognition (35).

Despite growing evidence for objective biological correlates of persistent post-viral symptoms, accessible non-invasive tools to quantify microvascular dysfunction remain limited, creating a gap in clinical assessment and monitoring (6, 19). As a result, comprehensive case-control studies comparing endothelial function across never infected (NI) controls, SARS-CoV-2–recovered individuals, PCS patients, and ME/CFS patients are lacking. Consequently, it remains unclear whether the degree of ED correlates with symptom severity or inflammatory status in PCS.

The primary aim of this study was to compare RVA parameters, as a non-invasive measure of microvascular endothelial health, between patients with PCS and matched healthy controls. As secondary objectives, we compared RVA parameters across NI, recovered, PCS patients, and PCS patients fulfilling criteria for ME/CFS and examined whether retinal ED was associated with patient-reported outcome measures (PROMs) and circulating markers of ED and chronic inflammation.

Methods

Study design and cohort

This study is based on the previously published “All Eyes on PCS” protocol, a prospective, observational, single-center study designed to examine the retinal microvasculature in PCS patients (36). The study protocol was approved by the Ethics Committee of the Technical University of Munich, TUM School of Medicine and Health, Klinikum Rechts der Isar (Approval number: 2022-317-S-SR) and is registered at ClinicalTrials.gov (NCT05635552). The study was conducted in accordance with the Declaration of Helsinki. Sample size was defined a priori in the study protocol based on power calculations conducted with the institutional department of statistics and reflects all eligible participants recruited during the study period.

Between October 2022 and September 2023, 105 PCS patients meeting the WHO definition of post-COVID-19 condition (persistent symptoms ≥ 3 months after confirmed SARS-CoV-2 infection, not explained by an alternative diagnosis) were included in the study (5). Most participants (72.4%, 76/105) were recruited through social media with the help of the patient organization “lost voices”, while the remaining (27.6%, 29/105) were referred from the PCS outpatient clinic of the LMU. Three patients were excluded from the analysis: one due to insufficient evidence of SARS-CoV-2 infection, another because of a missing temporal link between infection and PCS symptom onset, and a third because PCS-related symptoms were absent at the time of recruitment. In addition, 105 SARS-CoV-2–recovered individuals without persistent symptoms were recruited as controls and examined between October 2022 and January 2025. Three individuals were excluded from the final analysis: one due to a diagnosis of myocarditis, one due to epilepsy precluding RVA, and one due to incomplete data. NI participants were selected from a cohort of 233 individuals with no history of prior SARS-CoV-2 infection. Of these, 227 were recruited before the COVID-19 pandemic as healthy control participants as part of the Citrate-Acetate Study (recruited between 2016 and 2018; ClinicalTrials.gov: NCT02745340) and the COMPLETE Study (recruited between January 2018 and July 2019; ClinicalTrials.gov: NCT03986892) (30).Six healthy patients were recruited after the COVID-19 pandemic who reported no prior SARS-CoV-2 infection. Detailed description of patient recruitment and clinical characterization can be found in the previously published study protocol (36).

Clinical assessment

A standardized 78-item questionnaire (Munich COVID History Questionnaire; MuCOV) was used to collect demographic data, pre-existing conditions, medication history, and PCS-related symptoms. All participants underwent a structured clinical assessment conducted by a physician. For participants recruited via social media, an initial screening survey assessed acute SARS-CoV-2 infection characteristics and persistent PCS-typical symptoms. For both PCS and recovered participants eligibility required documented proof of prior SARS-CoV-2 obtained at least three months before study inclusion. For PCS patients the presence of PCS-typical symptoms persisting for at least two months after acute infection. The temporal relationship between infection and symptom onset was reviewed in all cases, and alternative explanations for symptoms were systematically excluded. In cases of diagnostic uncertainty, eligibility was reviewed in a bi-weekly multidisciplinary meeting involving the Chief Investigator, Principal Investigator, and the study team, with final decisions made by majority vote. PROMs were assessed: PCS Severity (PCS Severity Score (37) and COVID-19 Yorkshire Rehabilitation Scale (C19-YRS) (38)), fatigue (Fatigue Severity Scale, FSS (39)), depression (Patient Health Questionnaire-9, PHQ-9 (40)), anxiety disorders (Generalized Anxiety Disorder-7, GAD-7 (41)), health-related quality of life (EuroQol 5-Dimensions 5-Levels questionnaire (EQ5DL (42)) and ME/CFS status using the Canadian Consensus Criteria (CCC) (43).

Laboratory values

Blood sampling and standard laboratory measurements were conducted as previously described (44), with IL-6, CCL-5, CXCL10, MCP-1, VCAM-1, ICAM-1 and VEGF quantified using the Cytometric Bead Array Flex system (BD Biosciences, San Diego, US). The measurements were performed according to the manufacturer's instructions.

Retinal vessel analysis

RVA was conducted with the Dynamic Vessel Analyzer (DVAlight; Imedos Health GmbH, Jena, Germany), while static vessel analysis (SVA) was carried out using the Static Vessel Analyzer (Imedos Health GmbH, Jena, Germany) in combination with a TRC-NW8 non-mydriatic retinal camera (Topcon, Tokyo, Japan). A detailed description of the procedure can be found in a previous publication (44).

In short: All measurements were conducted in a quiet, temperature-controlled room. Blood pressure was assessed before and after RVA to minimize potential hemodynamic confounding. Pupillary dilation was achieved using topical mydriatics. Whenever feasible, the left eye was examined to ensure inter-individual comparability. The non-examined eye was occluded during measurements. For SVA, high-resolution fundus images centered on the optic disc (field angle 50°) were acquired. At least three high-quality images were obtained per participant. Retinal arteriolar and venular calibers were semi-automatically quantified using VesselMap software (Imedos Health GmbH, Jena, Germany) and summarized as CRAE and CRVE according to the Parr–Hubbard formula; the AVR was calculated as CRAE/CRVE. One measuring unit corresponds to 1 µm in Gullstrand's normal eye model. Images with insufficient quality were excluded (1 measurement in PCS 1/103; 0.9%). For DVA, participants were instructed to fixate on the internal target and vessel segments (approximately 0.5-1 mm in length) were selected roughly two optic-disc diameters from the optic disc margin, preferentially in the superior-temporal quadrant (alternatively inferior-temporal if required). Diameters of the selected retinal arteriole and venule were continuously recorded for a total of 350 s. Following a baseline period, three flicker-light cycles (20 s each) were applied, each followed by a recovery period. FID was calculated as percent change relative to baseline (aFID/vFID). RVA measurements were acquired by trained personnel involved in the clinical assessment of participants; therefore, blinding to clinical group (PCS, recovered) was not feasible during data acquisition. Importantly, all RVA data processing, quality control, and quantitative analyses were conducted by investigators blinded to clinical group assignment. To ensure high measurement quality, vessel response curves were rated using a cumulative scoring approach (scale 0–5). Recordings with a total score < 2.5 were re-evaluated by a second experienced observer and excluded if consensus could not be reached. For vFID and aFID, 3 measurements in PCS (3/103; 2.9%) had to be excluded.

Data analysis

All statistical analyses were performed using R (version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria) in RStudio and followed the "Strengthening the Reporting of Observational studies in Epidemiology" (STROBE) guidelines. Key packages included Matching, MatchIt, WeightIt, survey,

gtsummary, ggplot2, pROC, and corrplot. Analyses were conducted using complete-case data; no imputation was performed, as missingness was low and did not show systematic patterns. Normality was assessed by visual inspection of histograms and Q-Q plots and formally tested using the Shapiro-Wilk test. Normally distributed variables are presented as mean ± standard deviation (SD), and non-normally distributed variables as median with interquartile range (Q1, Q3).

For subgroup secondary matched analyses, participants were categorized into PCS, recovered, and NI groups. As recovered participants were substantially younger and showed limited age overlap with PCS, age matching was applied only for the NI reference group, whereas comparisons involving recovered were addressed using age-adjusted and overlap-weighted sensitivity analyses (ATO). PCS patients were age- and sex-matched to NI controls using nearest-neighbor matching, with balance assessed using the MatchBalance function. Due to substantial age differences between PCS and recovered cohorts, these groups were matched on sex only. Primary analyses adjusted for age and other relevant confounders using multivariable regression models. In addition, to address residual age imbalance and limited covariate overlap, we performed overlap weighting targeting the average treatment effect in the overlap population (ATO estimand) using the WeightIt package. Weighted group comparisons for RVA parameters were performed using survey-weighted t-tests via the survey package. One eye per participant was included in the analysis, such that each observation represents a single eye from a unique individual. Unweighted group comparisons were conducted using Welch's two-sample t-test or the Mann-Whitney U test for two-group comparisons and the Chi-square (χ^2) test for categorical variables. For comparisons involving more than two groups, we used one-way ANOVA when model assumptions were met; otherwise, the Kruskal-Wallis test was applied. For ANOVA, post-hoc pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test. For Kruskal-Wallis, post-hoc pairwise comparisons were performed using Dunn's test with multiplicity correction. Multivariable linear regression models were fitted to adjust for established confounders of RVA parameters (45–48). Continuous biomarkers with skewed distributions or outliers were analyzed using robust linear regression based on M-estimation. Correlation analyses were performed using Pearson's correlation for normally distributed data and Spearman's rank correlation for non-normally distributed data. For correlation analyses involving multiple biomarkers, p-values were adjusted using the Benjamini-Hochberg procedure. For primary, pre-specified group comparisons, no multiplicity correction was applied. Receiver operating characteristic (ROC) analyses were used to assess the discriminative ability of individual variables, with confidence intervals calculated using DeLong's method. To evaluate whether a combination of inflammatory, metabolic, and microvascular markers improves discrimination of the ME/CFS phenotype among SARS-CoV-2-infected individuals, a multivariable logistic regression model including transferrin, ICAM-1, neutrophils, creatine kinase, IL-6 and AVR was fitted (**Supplementary Table 1**). ME/CFS status served as the dependent variable. Predicted probabilities were used to construct ROC curves and estimate the area under the curve (AUC). Please see the Major Resources Table in the Supplemental Materials for detailed information on study resources (**Supplementary Table 2**).

Results

Cohort characteristics

Table 1 presents the baseline demographic and clinical characteristics of the study population, showing cohort comparison between PCS patients (n = 102, age: 41.9 ± 11.6 years, 75% female) and age- and sex-matched healthy controls (HC; matched out of 303 participants, n = 204, age: 42.9 ± 15.4 years, 75% female), which includes both NI individuals (n=117) and recovered individuals (n=87). No differences were observed in BMI or systolic blood pressure between groups. CV risk factors such as arterial hypertension (16% vs. 2.0%, p < 0.001) and hypercholesterolemia (22% vs. 5.4 %, p =0.002) were more prevalent in PCS patients and were therefore used for controlling for confounders when comparing RVA parameters. Further cohort characteristics with a detailed three-group comparison are presented in later sections.

Baseline characteristics	**Healthy controls** N = 204	**Post-COVID-19 Syndrome** N = 102	**p-value**
Age (years), mean (±SD)	42.9 (±15.4)	41.9 (±11.6)	0.50
Gender (female), n(%)	154 (75%)	77 (75%)	>0.9
BMI (kg/m^2), mean (±SD)	23.7 (±3.9)	24.5 (±4.8)	0.12
RR_{syst}[1](mmHg), mean (±SD)	122.2 (±17.1)	121.8 (±16.9)	0.80
Cardiovascular Risk Factors			
Arterial Hypertension, n(%)	4 (2.0%)	16 (16%)	**<0.001*** **
Hypercholesterolemia,n(%)	5 (5.4%)	22 (22%)	**0.002****
Smoking history, n(%)	29 (14%)	10 (9.8%)	0.40
Diabetes mellitus (I and II), n(%)	0 (0%)	1 (1.0%)	0.70

Functional and Structural Retinal Microvascular Alterations in PCS vs. Healthy Controls

RVA parameters revealed persistent retinal ED in PCS patients compared to HC. Venular flicker-induced dilation (vFID) was reduced in PCS patients (3.7 ± 2.2% vs. 4.5 ± 2.7%, mean difference: 0.8 %, 95%CI: 0.2 to 1.4 %, p = 0.005, Cohen´s d: 0.32), indicating ongoing endothelial dysfunction. Arteriolar flicker-induced dilation (aFID) was numerically lower in PCS patients (2.9 ± 2.3% vs. 3.2 ± 2.0%), though this difference was not statistically significant (p = 0.261) (**Fig. 1a and b**). PCS patients showed narrower retinal arterioles (CRAE, 178.3 ± 15.5 µm vs. 183.3 ± 15.9 µm, mean difference: 5.0 µm, 95%CI: 1.3 to 8.8 µm, p = 0.009, d: 0.32), while retinal venular diameters (CRVE) did not differ between groups (213.85 ± 16.13 µm vs. 214.02 ± 18.36 µm, p = 0.937). Consequently, PCS patients exhibited a lower AVR (0.83 ±

0.06 vs. 0.86 ± 0.07, mean difference: 0.023, 95%CI: 0.007 to 0.038, p = 0.004, d: 0.35) (**Fig. 1c–e**). To assess potential confounding, we adjusted for arterial hypertension and hypercholesterolemia using forest plots. After adjustment, lower vFID (Standardized [Std.] β = −0.48, 95% CI −0.77 to −0.18; p = 0.002) and CRAE (Std. β = −0.39, 95% CI −0.67 to −0.12; p = 0.006) remained associated with PCS status, whereas the association with AVR was weaker (Std. β = −0.26, 95% CI −0.55 to 0.02; p = 0.073) (**Fig. 1f**). To assess whether the association between PCS status and microvascular changes differed by sex, we included a cohort-by-sex interaction term in the linear model. No significant sex-by-cohort interactions were observed for AVR, CRAE, or vFID, suggesting that retinal ED in PCS is independent of sex (**Supplementary Table 3**).

Cohort Characteristics for PCS patients, Recovered, and Never Infected individuals

An extended three-group comparison across age- and sex- matched NI (n = 96), sex- matched recovered (n = 102), and PCS patients (n = 102) is presented in **Supplementary Table 4**. Age differed between groups (p < 0.001), with recovered individuals being younger (33.3 ± 11.4 years, 72% female) than both NI (43.0 ± 14.5 years, 76% female) and PCS patients (41.9 ± 11.6 years, 75% female). Systolic blood pressure before RVA varied between cohorts (p < 0.05), with recovered participants showing the lowest values. CV risk such as arterial hypertension was present in 16% of PCS patients compared with 4.9% of recovered and 0% of NI individuals (p < 0.001), and hypercholesterolemia was more common in PCS than in recovered (22% vs. 5.9%).

Acute SARS-CoV-2 infection characteristics showed differences: PCS patients experienced more severe acute disease (WHO progression scale; p < 0.001) and were more frequently infected with alpha or delta variants (p = 0.004). Most recovered and PCS individuals reported a single infection (61% and 70%, respectively). PCS burden remained high, with a median symptom duration of 404 days (IQR: 291 to 575), median cumulative sick leave of 199.5 days (44.0 to 377.0), and 16% reporting occupational loss (all p < 0.001 compared with recovered). PCS symptom severity was markedly elevated (36.9 ± 10.6 vs. 1.8 ± 5.9, p < 0.001), and 61% of PCS patients fulfilled ME/CFS diagnostic criteria.

Hematologic and biochemical parameters differed between groups: PCS patients showed higher neutrophil percentages (p = 0.001), lower lymphocyte percentages (p = 0.041), and higher platelet counts (p = 0.040). Iron metabolism markers indicated elevated ferritin (p = 0.008) and reduced transferrin (p < 0.001) in PCS compared with recovered individuals, while hemoglobin was higher in NI than in both post-infection groups (p < 0.001). Lipid profiles differed across cohorts (total cholesterol: p = 0.002; LDL cholesterol: p = 0.001). Total cholesterol was lower in recovered individuals, whereas LDL cholesterol was highest in PCS patients. Creatine kinase (CK) was significantly lower in PCS (p<0.001). Immunoglobulin profiles were broadly similar, except for lower IgG3 levels in PCS patients (p = 0.021). Given the younger age of recovered participants, laboratory differences were examined in age-adjusted sensitivity analyses. In unweighted linear models including all three cohorts, hemoglobin, CRP, and leukocyte counts differed significantly between PCS patients and NI individuals (hemoglobin: standardized β = 0.20, p = 0.004; CRP: standardized β = −0.41, p < 0.001; leukocytes: standardized β =

−0.14, p = 0.028), with no difference between recovered and PCS. In complementary age-balanced sensitivity analyses using overlap weighting (ATO), differences in neutrophil counts (mean difference +3.97%, CI 1.05 to 6.90; p = 0.008), transferrin (mean difference −16.57 mg/dL, CI −27.90 to −5.24; p = 0.004), and CK between PCS and recovered remained significant, while differences in lymphocytes, platelets, ferritin, lipid parameters, IgG3, and hemoglobin were attenuated (**Supplementary Table 5 and 6**).

Functional and Structural Retinal Microvascular Alterations Across PCS, Recovered, and Never Infected

Mean venular FID (vFID) differed between the three groups (p = 0.007). In post-hoc comparisons, PCS patients showed lower vFID than recovered individuals (3.7% ± 2.2 vs. 4.8% ± 3.0, p = 0.005). No differences were observed between PCS and NI (4.1% ± 2.5, p = 0.541) or between recovered and NI participants (p = 0.101). Arteriolar FID showed a trend toward lower values in PCS compared with recovered individuals (2.9% ± 2.3 vs. 3.4% ± 2.1), but this did not reach significance (**Fig. 2a and b**).

Retinal arteriolar diameters differed across groups (p = 0.001). PCS patients had narrower arterioles compared to both recovered (178.3 ± 15.5 µm vs. 186.1 ± 15.7 µm, p = 0.001) and NI (184.1 ± 15.1 µm, p = 0.023), whereas recovered and NI did not differ (p = 0.641). Retinal venular diameters also differed between groups (p = 0.040). Recovered had slightly larger venular calibers than NI (217.9 ± 15.5 µm vs. 212.2 ± 16.9 µm, p = 0.037), while differences between recovered and PCS were not significant (p = 0.176) (**Fig. 2c and d**). AVR differed between groups (p < 0.001). PCS patients exhibited lower AVR compared with both NI (0.83 ± 0.06 vs. 0.87 ± 0.06, p < 0.001) and recovered (0.86 ± 0.07, p = 0.037). (**Fig. 2 e**). Age, arterial hypertension, BMI, and systolic blood pressure before RVA measurement were included as confounders, with hypercholesterolemia added in a sensitivity model for PCS vs. recovered. After adjustment, higher vFID remained significantly associated with recovered (Std. β = 0.25, p < 0.001). Higher CRAE remained significantly associated with NI (Std. β = 0.15, p = 0.021), whereas the association with recovered (Std. β = 0.11, p = 0.105) was attenuated. For CRVE associations were no longer significant. For AVR, higher values remained significantly associated with NI (Std. β= 0.21, p = 0.002), whereas the association with recovered was weaker (Std. β= 0.10, p = 0.167) (**Supplementary Table 7**).

In ATO analysis, vFID remained significantly reduced in PCS patients compared with recovered individuals (mean difference -1.3%, CI: -2.0 to -0.5%, p = 0.001). Retinal arteriolar narrowing also persisted, with lower CRAE in PCS (mean difference -5.6 µm, CI -10.3 to -0.8 µm, p = 0.022). In contrast, the difference in AVR was attenuated and narrowly missed statistical significance (mean difference -0.02, CI -0.04 to - 0.0003, p = 0.053), indicating a small residual effect after age balancing. CRVE and aFID did not differ between groups following age adjustment. (**Supplementary Table 8**). Sensitivity analyses including hypercholesterolemia did not alter the results (not shown).

Association of Patient-Reported Outcome Measurements with Persistent Changes of Retinal Microcirculation

PROMs, including the PCS Severity Score and the COVID-19 Yorkshire Rehabilitation Scale (C19-YRS), were associated with persistent alterations in retinal microcirculation. AVR showed a modest correlation with both PCS severity score ($r = -0.21$, $p = 0.037$) and C19-YRS scores ($r = -0.20$, $p = 0.043$) (**Fig 3 a and b**). Likewise, aFID was inversely correlated with the PCS severity score ($r = -0.21$, $p = 0.039$; **Fig. 3 d**), however not with C19-YRS score (**Fig. 3 e**). In regression analyses, models were adjusted for sex and BMI, as age and CV risk factors were not associated with PCS severity within this subgroup. In these models, lower aFID remained independently associated with PCS severity score, whereas the association between lower AVR and symptom severity was attenuated (**Fig. 3 c and f**). vFID, CRAE, and CRVE showed no significant association with PCS severity within the PCS cohort.

To further investigate the relationship between symptom burden and RVA parameters, all previously infected individuals (recovered and PCS) were stratified into tertiles based on PCS severity scores. The low symptom severity group predominantly comprised recovered (light grey), while the moderate and high severity groups were largely composed of PCS patients (dark grey). For AVR, a significant overall difference across severity levels was observed ($p = 0.017$), with post-hoc comparisons revealing significantly lower AVR values in the high vs. low severity group ($p = 0.021$). For aFID, the overall group difference was significant ($p = 0.012$), with reduced aFID observed in both the moderate vs. high ($p = 0.026$) and low vs. high ($p = 0.047$) severity comparisons (**Fig. 3 g and h**).

To further stratify which individual symptoms are most closely linked to persistent retinal ED, we compared AVR values between previously infected individuals reporting versus not reporting each of the 16 key symptoms included in the PCS severity score. AVR was significantly lower in participants reporting symptoms predominantly within the neurocognitive cluster (e.g., fatigue, concentration difficulties, headache, balance/fine motor dysfunction) and systemic/malaise-related symptoms (e.g., general malaise, hair/skin changes, gastrointestinal symptoms). In contrast, AVR did not differ significantly for symptoms primarily related to respiratory or sensory domains (e.g., chest pain, shortness of breath, cough, disturbed smell/taste) (**Table 2**).

Symptom	AVR (Symptom Absent)	AVR (Symptom Present)	Mean Difference	95% CI	p-value
Fatigue	0.856	0.833	−0.024	[0.006, 0.042]	**0.0089****
Concentration difficulties	0.857	0.831	−0.026	[0.008, 0.043]	**0.0050****
Headache	0.856	0.829	−0.026	[0.008, 0.045]	**0.0050****
Anxiety and/or sleep disturbance	0.852	0.833	−0.020	[0.001, 0.038]	**0.036***
Balance/fine motor dysfunction	0.851	0.829	−0.022	[0.003, 0.042]	**0.026***
Chest pain	0.847	0.839	−0.008	[−0.010, 0.027]	0.38
Palpitations	0.855	0.829	−0.025	[0.008, 0.043]	**0.0054****
Shortness of breath	0.849	0.831	−0.018	[−0.003, 0.040]	0.085
Cough	0.848	0.831	−0.017	[−0.012, 0.047]	0.23
Reduced physical capacity (e.g., difficulty climbing stairs)	0.855	0.835	−0.020	[0.002, 0.038]	**0.027***
Disturbed smell or taste	0.847	0.835	−0.011	[−0.018, 0.041]	0.44
ENT symptoms (e.g., sore throat, runny nose, hoarseness)	0.850	0.829	−0.021	[0.001, 0.042]	**0.041***
Hair loss / itching / skin rash	0.850	0.822	−0.028	[0.008, 0.048]	**0.0080****
Weight loss	0.846	0.836	−0.010	[−0.015, 0.034]	0.43
Gastrointestinal symptoms (e.g., nausea, diarrhea)	0.848	0.829	−0.019	[0.001, 0.037]	0.043
General malaise / chills / flu-like symptoms	0.853	0.828	−0.025	[0.006, 0.044]	**0.010***

Table 2. Mean AVR in participants who did not report (“Symptom absent”) versus did report (“Symptom present”) each of the 16 symptoms included in the PCS Symptom Severity Score. For each symptom,

mean AVR values are shown for both groups, along with the mean difference calculated as Present minus Absent, corresponding 95% confidence intervals, and p-values from group comparisons. Symptoms are listed in the order included in the PCS Symptom Severity Score and span neurological, cardiopulmonary, sensory, and systemic domains. Group comparisons were performed using Welch's two-sample t-test or the Mann-Whitney U test, as appropriate. Statistical significance is indicated as *p < 0.05; **p < 0.01; **p < 0.001.

Retinal Microvascular Alterations in ME/CFS

Persistent ED has previously been demonstrated in post-viral syndromes and in patients with ME/CFS (16). In our cohort, PCS patients fulfilling ME/CFS criteria were slightly younger (p = 0.041) and exhibited a higher overall symptom burden, as reflected by elevated C19-YRS scores (p = 0.023), PCS Severity Scores (p < 0.001), PHQ-9 scores (p = 0.008), Fatigue Severity Scale scores (p < 0.001), and reduced quality of life as measured by the EQ-5D-5L index (p = 0.010). Consistent with this, patients with ME/CFS reported more cumulative sick days (p < 0.001). CV risk factors, including hypercholesterolemia, hypertension, and diabetes, did not significantly differ between groups, nor did characteristics of the acute SARS-CoV-2 infection. Laboratory parameters were largely comparable, although CK levels were significantly lower in the ME/CFS subgroup (p = 0.009) (**Table 3**).

Variable	PCS without ME/CFS N = 39	PCS with ME/CFS N = 62	p-value
Age (years), mean (±SD)	44.8 (±11.6)	39.9 (±11.2)	**0.041***
Gender (female), n(%)	28 (72%)	49 (79%)	0.6
BMI (kg/m^2), mean (±SD)	24.4 (±4.6)	24.6 (±4.9)	0.8
RR_{syst}[1](mmHg), mean (±SD)	121.9 (±18.8)	121.7 (±15.6)	>0.9
Cardiovascular Risk Factors			
Diabetes mellitus (I and II), n(%)	1 (2.6%)	0 (0%)	>0.9
Arterial Hypertension, n(%)	5 (13%)	11 (18%)	0.7
Hypercholesterolemia, n(%)	7 (18%)	15 (24%)	0.6
Smoking history, n(%)	4 (10%)	6 (9.7%)	>0.9
PCS-related characteristics			
C19-YRS, mean (±SD)	28.3 (15.1)	35.0 (12.7)	**0.023***
PCS Severity Score, mean (±SD)	32.0 (11.7)	40.0 (8.6)	**<0.001****
PHQ9, mean (±SD)	8.9 (4.7)	11.4 (3.9)	**0.008****
GAD7, median (IQR)	4.0 (2.0, 7.0)	6.0 (3.0, 9.0)	0.2
FSS, median (IQR)	5.4 (3.7, 6.4)	6.3 (5.8, 6.7)	**<0.001****
EQ5DL-Index, median (IQR)	0.8 (0.6, 0.8)	0.5 (0.3, 0.7)	**0.010***
Duration of PCS, days, median (IQR)	345.0 (218.0, 537.0)	409.5 (316.5, 619.0)	0.15
Cumulative sick days, median (IQR)	129.0 (0.0, 291.0)	285.5 (106.0, 445.0)	**<0.001****
Acute SARS-CoV-2 infection			
Work loss during PCS, n(%)	4 (10%)	12 (20%)	0.3
Number of infections, n(%)			0.2
1	30 (77%)	40 (65%)	
2	8 (21%)	21 (34%)	
3	1 (2.6%)	0 (0%)	
4	0 (0%)	1 (1.6%)	
Severity of acute infection, n(%) (WHO progression scale)			0.6

1	1 (2.6%)	0 (0%)	
2	25 (64%)	35 (56%)	
3	12 (31%)	22 (35%)	
4	1 (2.6%)	3 (4.8%)	
5	0 (0%)	1 (1.6%)	
6	0 (0%)	1 (1.6%)	
SARS-CoV-2 variants, n(%)			0.3
alpha	1 (2.6%)	6 (9.7%)	
delta	7 (18%)	6 (9.7%)	
omicron	8 (21%)	10 (16%)	
unknown	23 (59%)	40 (65%)	
Lab parameters			
Creatine Kinase (U/L), median IQR)	91.0 (70.0, 111.0)	71.0 (54.0, 92.0)	**0.009****
Comorbidities			
Psychiatric Diagnosis preexisting, n(%)	2 (5.3%)	6 (9.7%)	0.7
Depressive diagnosis ongoing, n(%)	9 (23%)	10 (16%)	0.5

Table 3 Baseline characteristics of PCS patients stratified by ME/CFS status according to the Canadian Consensus Criteria (CCC). One patient did not complete the CCC questionnaire and was therefore excluded from the analysis. Continuous variables are reported as mean ± SD or median (IQR), depending on distribution, and categorical variables as n (%). Group comparisons were performed using Welch's two-sample t-test for normally distributed continuous variables, the Mann-Whitney U test for non-normally distributed continuous variables, and Pearson's χ^2 test for categorical variables, as appropriate. Abbreviations: BMI, body mass index; RRsyst, systolic blood pressure before retinal vessel analysis; C19-YRS, COVID-19 Yorkshire Rehabilitation Scale; PHQ-9, Patient Health Questionnaire-9; GAD-7, Generalized Anxiety Disorder-7; FSS, Fatigue Severity Scale; EQ-5D-5L, EuroQol 5-Dimension 5-Level index; ME/CFS, myalgic encephalomyelitis/chronic fatigue syndrome. Statistical significance is indicated as *p < 0.05; **p < 0.01; **p < 0.001.

We next compared RVA parameters across NI, recovered, and PCS patients with or without ME/CFS. AVR, CRAE, and vFID differed across groups (**Figure 4a - c**). In post-hoc analyses, vFID was significantly lower in PCS patients fulfilling ME/CFS criteria compared with recovered (p = 0.046), whereas no difference was observed relative to NI (p = 0.890). CRAE was likewise reduced in PCS patients with ME/CFS compared with recovered (p = 0.020), while the comparison with NI individuals did not reach

statistical significance (p = 0.263). AVR was markedly lower in PCS patients with ME/CFS than in both NI controls (p < 0.001) and recovered individuals (p = 0.010) (**Supplementary Table 9)**.

To determine whether an altered retinal microcirculation is specifically associated with ME/CFS, we performed multivariable regression analyses adjusting for age, sex, BMI, systolic blood pressure before RVA, and arterial hypertension. Using PCS with ME/CFS as the reference group, AVR was significantly higher in all other cohorts: NI (Std. β = 0.32, p < 0.001), PCS without ME/CFS (Std. β = 0.14, p = 0.049), and recovered individuals (Std. β = 0.17, p = 0.028). CRAE did not differ significantly after adjustment, although a trend toward wider arterioles was observed in NI individuals (Std. β = 0.14, p = 0.063) and recovered (Std. β = 0.11, p = 0.156). Recovered individuals showed significantly higher vFID compared to PCS with ME/CFS (Std. β = 0.23, p = 0.004), whereas differences between the remaining groups did not reach significance (**Supplementary Table 10**).

Finally, we performed receiver operating characteristic (ROC) analyses to identify biomarkers discriminating non-ME/CFS individuals (recovered and PCS without ME/CFS) from patients fulfilling ME/CFS criteria. Across inflammatory, iron metabolism, lipid, hematologic, and RVA parameters, 13 markers showed discriminatory performance (**Supplementary Table11)**. From an initial panel of 13 candidate variables, six markers (CK, IL-6, ICAM-1, transferrin, AVR, neutrophils) were selected for combined ROC analysis based on individual discriminatory performance, biological plausibility, and representation of distinct pathophysiological domains (**Figure 4 d**). This integrative approach resulted in improved discrimination between ME/CFS and non-ME/CFS individuals (AUC= 0.80; 95% CI: 0.73-0.87) (**Figure 4 e**). Together, these findings indicate that PCS patients with ME/CFS exhibit the most pronounced retinal microvascular alterations, particularly reduced AVR and that these changes persist even after adjustment for relevant CV risk factors.

Biomarkers of ED and Inflammation in Recovered, PCS and ME/CFS Patients and their Association with RVA parameters

Circulating biomarker levels were compared between recovered and PCS patients, to study potential ongoing endothelial activation and chronic inflammation. IL-6 levels were higher in PCS patients compared with recovered individuals (median 1.32 pg/mL vs. 0.92 pg/mL; p = 0.028). MCP-1 showed a non-significant trend toward higher levels in PCS (median 24.2 pg/mL vs. 18.5 pg/mL; p = 0.063). CC-Chemokine-Ligand-5 (CCL-5) did not differ between groups (p = 0.492), nor did CXCL-10 (p = 0.985) (**Figure 5 a - c, f**). Among ED markers, ICAM-1 levels were elevated in PCS compared with recovered participants (mean 3832 ± 636 pg/mL vs. 3505 ± 683 pg/mL, p <0.001). VCAM-1 was also increased in PCS (median 8073 pg/mL vs. 5899 pg/mL; p <0.001) VEGF concentrations did not differ between groups (median 6.56 pg/mL [PCS] vs. 7.68 pg/mL [recovered]; p = 0.339) (**Figure 5 d, e and g**). After adjustment for age, the associations of higher ICAM-1 (β = −358.9 pg/mL, p < 0.001) and VCAM-1 (β = −2319.6 pg/mL, p < 0.001) with PCS remained significant. In contrast, the associations with IL-6 (β = −0.24 pg/mL, p = 0.14) and MCP-1 (β = −3.52 pg/mL, p = 0.11) were attenuated (**Supplementary Table 11**). We additionally compared laboratory variables and circulating biomarkers between non-ME/CFS (recovered

and PCS) with ME/CFS patients after correction for age. As shown in PCS patients, markers of ED (VCAM-1, ICAM-1) and inflammation (IL-6) were associated with ME/CFS status (**Figure 5 h**).

We next asked whether circulating biomarkers were linked to retinal microvascular alterations within the post-infectious spectrum itself. We therefore examined the associations between circulating biomarkers and RVA parameters in all PCS patients (**Figure 5 i**). AVR showed significant inverse correlations with CRP ($\rho = -0.27$, $p = 0.007$), IL-6 ($\rho = -0.27$, $p = 0.006$), transferrin ($\rho = -0.34$, $p < 0.001$), and erythrocyte count ($\rho = -0.21$, $p = 0.037$), while positive correlations were observed with IgG3 ($\rho = 0.23$, $p = 0.020$), serum iron ($\rho = 0.20$, $p = 0.045$), and transferrin saturation ($\rho = 0.28$, $p = 0.0055$). Additionally, there was a correlation for lower CRAE with higher CRP ($\rho = -0.20$, $p = 0.045$) and higher VCAM-1 ($\rho = -0.33$, $p < 0.001$), and an inverse correlation between CRVE and VCAM-1 ($\rho = -0.20$, $p=0.047$). After correction for multiple testing, the correlations between AVR and IL-6, CRP, transferrin, and transferrin saturation and the correlation between CRAE and VCAM-1 remained statistically significant (**Supplementary Figure 12**).

Discussion

This observational case-control study is the first to perform an in-depth assessment of retinal microvascular alterations using RVA as a surrogate of persistent ED across a large, well-characterized cohort of NI individuals, recovered participants, and patients with PCS. We demonstrate that PCS patients exhibit markedly reduced venular FID, narrower retinal arterioles and a lower AVR compared with age- and sex-matched healthy controls. Higher symptom burden, particularly neurocognitive symptoms, was associated with microvascular remodeling, as indicated by a lower AVR. These findings replicate and expand upon our previously published pilot study, in which comparable reductions were observed in a smaller PCS cohort compared to NI (44).

A key unresolved question was whether ED resolves during recovery or persists in association with post-viral symptomatology. Our subgroup analysis demonstrated lower vFID in PCS patients compared with recovered. These differences were independent of CV risk factors and remained evident after strict age-weighted analyses, supporting an association between persistent ED and ongoing post-viral syndromes. Reduced vFID provides functional evidence of impaired NV coupling and diminished endothelial-dependent vasodilatation. Retinal FID largely depends on NO release and glia cell-regulated NV coupling (49). In individuals recovering from an acute infection, especially in those with pre-existing CV risk or severe acute illness longitudinal studies demonstrate persistent ED, by means of impaired endothelial glycocalyx, reduced NO bioavailability and vascular function (24, 50–53). In contrast, recovered patients without relevant comorbidities generally show only minimal or transient ED, with biomarker levels returning toward baseline over time (54–58). Our findings suggest that patients with PCS fail to adequately restore endothelial integrity, potentially due to unresolved chronic inflammation, as reflected by persistently elevated IL-6, acute-phase proteins, and endothelial activation markers such as ICAM-1 and VCAM-1 in our cohort, which is consistent with previous reports. (7, 59, 60).

Studies in PCS report gliosis and blood-brain barrier (BBB) disruption, and sustained neuroinflammation has been linked to persistent cognitive and affective symptoms in these patients (61, 62). Additionally, the potent vasoconstrictor endothelin-1 (ET-1), which induces neuronal apoptosis and retinal ganglion cell loss, has been shown to be elevated in PCS patients and to correlate with ongoing symptom burden. Together, these findings support NV unit dysfunction in PCS and provide a plausible explanation for the observed reduction in vFID (24, 63, 64).

Regarding SVA parameters, we observed lower AVR and CRAE in PCS compared with both recovered and NI individuals, indicating persistent microvascular remodeling and impaired retinal autoregulation. Retinal calibers depend on age, diabetes, BMI, and hypertension (45). Although the association between lower AVR and PCS was attenuated after adjustment for confounders and may partly reflect the higher CV risk profile of PCS patients compared with recovered, the parallel associations of both AVR and arteriolar FID with clinical severity suggest that retinal ED is not solely attributable to baseline risk factors but is also linked to symptom burden.

Individuals with predominantly ongoing neurocognitive symptoms showed a lower AVR than recovered individuals or PCS patients without these symptoms. Given the established associations of lower AVR and arteriolar narrowing with cerebral small vessel disease, neuroinflammation, and cognitive impairment, reduced AVR and CRAE observed in PCS provide evidence of persistent microvascular injury affecting NV integrity and offer a plausible mechanistic link to ongoing neurocognitive symptoms (65–68). Altered SVA parameters in general have been consistently linked to CV risk in landmark studies. Independently, epidemiological data demonstrate increased long-term CV risk after acute COVID-19 infection (21, 22, 31, 32). In this context, our observation of narrow retinal arterioles and lower AVR in PCS indicates sustained microvascular remodeling beyond the acute phase of infection. Given the median disease duration of 13 months in our cohort, the persistence of these microvascular alterations suggests that RVA may provide a non-invasive window into sustained microvascular injury and potentially increased CV risk in patients with PCS.

Early recognition of the clinical and biological overlap between ME/CFS and PCS has shifted research toward a unified post-infectious disease framework (7, 15). Accordingly, 61% of PCS patients in our cohort met ME/CFS criteria and showed markedly greater symptom burden and functional impairment. Across all previously infected individuals, we observed a continuous gradient of retinal ED, with progressively lower vFID, CRAE, and AVR values with increasing clinical severity, and the most pronounced impairments in PCS patients fulfilling ME/CFS criteria.

After age adjustment, IL-6, ICAM-1, and VCAM-1 remained elevated in ME/CFS and showed a similar pattern in PCS more broadly, indicating a shared inflammatory–endothelial activation axis across the post-infectious spectrum, with ME/CFS representing a clinically more severe or biologically enriched phenotype(19, 63, 69). AVR alone provided modest discrimination between ME/CFS and non-ME/CFS patients, however its combination with routine laboratory parameters substantially improved accuracy reaching an AUC of 0.80 comparable to a previously published diagnostic cell-based blood test for

ME/CFS (70, 71). Lower AVR was correlated with higher IL-6 and CRP, linking retinal microvascular remodeling to ongoing systemic inflammation. Both markers have been implicated in persistent neurocognitive symptoms in PCS, and IL-6-mediated neuroinflammation, including BBB disruption and impaired NV coupling, provides a plausible mechanistic link to reduced AVR(72–76). Narrower retinal arterioles were additionally associated with higher VCAM-1 levels, further supporting an inflammation-driven ED phenotype. Beyond inflammatory signaling, IL-6 is a central mediator of hepcidin induction and inflammation-driven alterations in iron homeostasis (77, 78). Consistently, IL-6 correlated inversely with iron-related parameters, and AVR was associated with transferrin and transferrin saturation, suggesting that retinal microvascular alterations in PCS co-occur with an inflammatory–iron axis rather than isolated iron deficiency. Similar patterns of iron dysregulation and inflammatory stress erythropoiesis have been reported following COVID-19 and linked to long-term PCS outcomes(14, 77).

From a clinical and translational perspective, the non-invasive, repeatable, and relatively low-cost nature of RVA makes it well suited for longitudinal assessment in patients with persistent post-viral symptoms (79, 80). RVA may complement symptom-based evaluation and routine laboratory testing by providing an objective, microvascular readout, particularly in patients with prominent neurocognitive impairment or ME/CFS features.

Limitations

Several limitations warrant consideration. First, this is a cross-sectional, single-center cohort study; therefore, the observed associations, while biologically plausible, should not be interpreted as causal. The present findings are exploratory and hypothesis-generating and require validation in larger, independent cohorts. Second, despite adjustment for potential confounders, the recovered cohort was significantly younger than the PCS group, which may have contributed to differences in retinal microvascular parameters. However, altered RVA measures were also observed when PCS patients were compared with the NI cohort, which was older and had a higher burden of comorbidities, supporting the robustness of the findings. Finally, the cross-sectional design precludes conclusions regarding whether PCS patients with more severe symptoms and adverse retinal microvascular phenotypes are at increased CV risk. Prospective longitudinal studies integrating RVA with adjudicated CV outcomes are required to determine whether retinal microvascular measures can identify a high-risk PCS subpopulation that may benefit from intensified CV monitoring and preventive strategies.

Conclusion

This study demonstrates that patients with PCS exhibit persistent functional and structural alterations of the retinal microvasculature, indicating ongoing endothelial and NV dysfunction. Retinal ED is most pronounced in patients with higher symptom burden and in those fulfilling ME/CFS criteria, and is accompanied by sustained inflammatory and endothelial activation, as reflected by elevated IL-6, ICAM-1, and VCAM-1. The observed associations between retinal microvascular parameters, systemic inflammation, and iron homeostasis suggest that microvascular remodeling in PCS reflects a broader,

inflammation-driven vascular phenotype rather than isolated CV risk. Together, these findings support the utility of RVA as a non-invasive window into post-infectious ED and highlight its potential role in biological stratification and risk assessment in patients with persistent symptoms. Prospective longitudinal studies are warranted to determine the prognostic value of retinal microvascular measures and their relevance for long-term CV outcomes in this population.

Abbreviations

aFID
Arteriolar flicker–induced dilation
ANOVA
Analysis of variance
ATO
Average treatment effect in the overlap population
AVR
Arteriolar–to–venular ratio
BBB
Blood–brain barrier
BMI
Body mass index
CCL
5–CC–Chemokine–Ligand–5
CCC
Canadian Consensus Criteria
CI
Confidence interval
CK
Creatine kinase
CRAE
Central retinal arteriolar equivalent
CRP
C–reactive protein
CRVE
Central retinal venular equivalent
CV
Cardiovascular
C19
YRS–COVID–19 Yorkshire Rehabilitation Scale
CXCL10 (IP
10)–C–X–C motif chemokine 10 (Interferon gamma–induced protein–10)

DVA
Dynamic retinal vessel analysis
ED
Endothelial dysfunction
EQ
5D–5L–EuroQol five–dimension five–level questionnaire
FID
Flicker–induced dilation
FMD
Flow–mediated dilation
FSS
Fatigue Severity Scale
GAD
7–Generalized Anxiety Disorder–7
HC
Healthy controls
HIF
Hypoxia–inducible factor
ICAM
1–Intercellular adhesion molecule–1
ICTRP
International Clinical Trials Registry Platform
IgG
Immunoglobulin G
IL
6–Interleukin–6
IQR
Interquartile range
ME/CFS
Myalgic encephalomyelitis/chronic fatigue syndrome
MCP
1–Monocyte chemoattractant protein–1
NI
Never infected
NO
Nitric oxide
NV
Neurovascular
OCT
A–Optical coherence tomography angiography

PCS
Post–COVID–19 syndrome
PEM
Post–exertional malaise
PHQ
9–Patient Health Questionnaire–9
POH
Post–occlusive reactive hyperemia
PROMs
Patient–reported outcome measures
PWV
Pulse–wave velocity
ROC
Receiver operating characteristic
RVA
Retinal vessel analysis
SD
Standard deviation
Std.β
Standardized beta coefficient
SVA
Static retinal vessel analysis
VCAM
1–Vascular cell adhesion molecule–1
VEGF
Vascular endothelial growth factor
vFID
Venular flicker–induced dilation
WHO
World Health Organization

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Technical University of Munich, School of Medicine and Health, Klinikum rechts der Isar (Approval number: 2022-317-S-SR) and the Ethics Committee of North-western and Central Switzerland (EKNZ 2017 – 01451). All participants provided written informed consent prior to participation. The study was registered at ClinicalTrials.gov (NCT05635552).

Consent for publication

Not applicable.

Competing Interests

TW, RG, IL, AR, ML, LR, BH, LS, HH, MW, and CS declare that they have no competing interests.FCO reports current research funding from the German Research Foundation (DFG; TRR418), the Hertie Foundation, the German ME/CFS Foundation, Novartis, and UCB, all unrelated to this project. She reports past fellowship support from the American Academy of Neurology and the National Multiple Sclerosis Society (until 2023), and past research funding from the German Neurological Society (DGN) and the DFG-TWAS program. FCO reports speaker honoraria from UCB and Novartis, and travel support from the Guthy-Jackson Charitable Foundation, the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), and the American Academy of Neurology. She serves as academic editor at DGNeurologie and Neurological Research & Practice, is a board member of the IMSVISUAL consortium, and co-chairs the Committee for Neuro-ophthalmology and Neuro-otology of the German Neurology Association.

Funding

This work was supported by the Clinical Scientist Program of the TUM School of Medicine and Health (TW). The study is part of the VADYS-ME project (to CS and FCO), which is funded by the German Federal Ministry of Education and Research (BMBF). The funders had no role in the study design; data collection, analysis, or interpretation; manuscript preparation; or the decision to publish.

Author Contribution

CS and TW conceived the study and led the project. MW contributed to data interpretation and drafting of the manuscript. ML, IL, and AR contributed to biosample processing, laboratory analyses, and interpretation of the data. RG contributed to study development and manuscript preparation. BH supported statistical analyses and figure preparation. HH and LS contributed data from the healthy controls and provided expertise in retinal vessel analysis. FCO and LR provided neuro-ophthalmological and retinal vessel analysis expertise. All authors contributed to manuscript revision, approved the final version, and agree to be accountable for all aspects of the work.

Acknowledgement

We thank all participants for taking part in this study. We also thank the doctoral students involved in study conduct and data collection, in particular A. Woehnle, V. Kessler, and J. Negele.

Data Availability

De-identified participant-level data supporting the findings of this study are not publicly available due to ethical and data protection restrictions. Data are available from the corresponding authors upon reasonable request and subject to approval of a data access agreement and the relevant ethics requirements.

References

1. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo P, Cuapio A et al. More Than 50 Long-Term Effects of COVID-19: A Systematic Review and Meta-Analysis. Res Sq. 2021.
2. Lai CC, Hsu CK, Yen MY, Lee PI, Ko WC, Hsueh PR, Long COVID. An inevitable sequela of SARS-CoV-2 infection. J Microbiol Immunol Infect. 2023;56(1):1–9.
3. Fernandez-de-Las-Peñas C, Notarte KI, Macasaet R, Velasco JV, Catahay JA, Ver AT, et al. Persistence of post-COVID symptoms in the general population two years after SARS-CoV-2 infection: A systematic review and meta-analysis. J Infect. 2024;88(2):77–88.
4. Scott A, Ansari W, Chambers R, Reimbaeva M, Mikolajczyk T, Benigno M, et al. Substantial health and economic burden of COVID-19 during the year after acute illness among US adults not at high risk of severe COVID-19. BMC Med. 2024;22(1):47.
5. Soriano JB, Murthy S, Marshall JC, Relan P, Diaz JV. A clinical case definition of post-COVID-19 condition by a Delphi consensus. Lancet Infect Dis. 2022;22(4):e102–7.
6. Greenhalgh T, Sivan M, Perlowski A, Nikolich J. Long COVID: a clinical update. Lancet. 2024;404(10453):707–24.
7. Kedor C, Freitag H, Meyer-Arndt L, Wittke K, Hanitsch LG, Zoller T, et al. A prospective observational study of post-COVID-19 chronic fatigue syndrome following the first pandemic wave in Germany and biomarkers associated with symptom severity. Nat Commun. 2022;13(1):5104.
8. Vernon SD, Zheng T, Do H, Marconi VC, Jason LA, Singer NG, et al. Incidence and Prevalence of Post-COVID-19 Myalgic Encephalomyelitis: A Report from the Observational RECOVER-Adult Study. J Gen Intern Med. 2025;40(5):1085–94.
9. Østergaard L. SARS CoV-2 related microvascular damage and symptoms during and after COVID-19: Consequences of capillary transit-time changes, tissue hypoxia and inflammation. Physiol Rep. 2021;9(3):e14726.
10. Owens CD, Pinto CB, Szarvas Z, Muranyi M, da, Pinaffi-Langley C, Peterfi AC et al. A,. COVID-19 Exacerbates Neurovascular Uncoupling and Contributes to Endothelial Dysfunction in Patients with Mild Cognitive Impairment. Biomolecules. 2024;14(12).
11. Xu SW, Ilyas I, Weng JP. Endothelial dysfunction in COVID-19: an overview of evidence, biomarkers, mechanisms and potential therapies. Acta Pharmacol Sin. 2023;44(4):695–709.

12. Wu X, Xiang M, Jing H, Wang C, Novakovic VA, Shi J. Damage to endothelial barriers and its contribution to long COVID. Angiogenesis. 2024;27(1):5–22.
13. Bonaventura A, Vecchié A, Dagna L, Martinod K, Dixon DL, Van Tassell BW, et al. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. Nat Rev Immunol. 2021;21(5):319–29.
14. Gupta G, Buonsenso D, Wood J, Mohandas S, Warburton D. Mechanistic Insights Into Long Covid: Viral Persistence, Immune Dysregulation, and Multi-Organ Dysfunction. Compr Physiol. 2025;15(3):e70019.
15. Choutka J, Jansari V, Hornig M, Iwasaki A. Unexplained post-acute infection syndromes. Nat Med. 2022;28(5):911–23.
16. McLaughlin M, Sanal-Hayes NEM, Hayes LD, Berry EC, Sculthorpe NF. People with Long COVID and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Exhibit Similarly Impaired Vascular Function. Am J Med. 2025;138(3):560–6.
17. Fan BE, Wong SW, Sum CLL, Lim GH, Leung BP, Tan CW, et al. Hypercoagulability, endotheliopathy, and inflammation approximating 1 year after recovery: Assessing the long-term outcomes in COVID-19 patients. Am J Hematol. 2022;97(7):915–23.
18. Chioh FW, Fong SW, Young BE, Wu KX, Siau A, Krishnan S et al. Convalescent COVID-19 patients are susceptible to endothelial dysfunction due to persistent immune activation. Elife. 2021;10.
19. Maksoud R, Magawa C, Eaton-Fitch N, Thapaliya K, Marshall-Gradisnik S. Biomarkers for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): a systematic review. BMC Med. 2023;21(1):189.
20. Domingo JC, Battistini F, Cordobilla B, Zaragozá MC, Sanmartin-Sentañes R, Alegre-Martin J, et al. Association of circulating biomarkers with illness severity measures differentiates myalgic encephalomyelitis/chronic fatigue syndrome and post-COVID-19 condition: a prospective pilot cohort study. J Transl Med. 2024;22(1):343.
21. Bruno RM, Badhwar S, Abid L, Agharazii M, Anastasio F, Bellien J et al. Accelerated vascular ageing after COVID-19 infection: the CARTESIAN study. Eur Heart J. 2025.
22. Battistoni A, Volpe M, Morisco C, Piccinocchi G, Piccinocchi R, Fini M, et al. Persistent increase of cardiovascular and cerebrovascular events in COVID-19 patients: a 3-year population-based analysis. Cardiovasc Res. 2024;120(6):623–9.
23. Xie Y, Xu E, Bowe B, Al-Aly Z. Long-term cardiovascular outcomes of COVID-19. Nat Med. 2022;28(3):583–90.
24. Haffke M, Freitag H, Rudolf G, Seifert M, Doehner W, Scherbakov N, et al. Endothelial dysfunction and altered endothelial biomarkers in patients with post-COVID-19 syndrome and chronic fatigue syndrome (ME/CFS). J Translational Med. 2022;20(1):138.
25. Ståhlberg M, Fischer K, Tahhan M, Zhao A, Fedorowski A, Runold M, et al. Post-Acute COVID-19 Syndrome: Prevalence of Peripheral Microvascular Endothelial Dysfunction and Associations With NT-ProBNP Dynamics. Am J Med. 2025;138(6):1019–28.

26. Mavraganis G, Dimopoulou MA, Delialis D, Bampatsias D, Patras R, Sianis A, et al. Clinical implications of vascular dysfunction in acute and convalescent COVID-19: A systematic review. Eur J Clin Invest. 2022;52(11):e13859.
27. Hosp JA, Reisert M, Dressing A, Götz V, Kellner E, Mast H, et al. Cerebral microstructural alterations in Post-COVID-condition are related to cognitive impairment, olfactory dysfunction and fatigue. Nat Commun. 2024;15(1):4256.
28. Shah W, Gong Y, Qiao X, Lu Y, Ding Y, Zhang Z, et al. Exploring Endothelial Cell Dysfunction's Impact on the Brain-Retina Microenvironment Connection: Molecular Mechanisms and Implications. Mol Neurobiol. 2025;62(6):7484–505.
29. Hanssen H, Streese L, Vilser W. Retinal vessel diameters and function in cardiovascular risk and disease. Prog Retin Eye Res. 2022;91:101095.
30. Gunthner R, Lorenz G, Braunisch MC, Angermann S, Matschkal J, Hausinger R, et al. Endothelial dysfunction in retinal vessels of hemodialysis patients compared to healthy controls. Sci Rep. 2024;14(1):13948.
31. Seidelmann SB, Claggett B, Bravo PE, Gupta A, Farhad H, Klein BE, et al. Retinal Vessel Calibers in Predicting Long-Term Cardiovascular Outcomes: The Atherosclerosis Risk in Communities Study. Circulation. 2016;134(18):1328–38.
32. Yatsuya H, Folsom AR, Wong TY, Klein R, Klein BE, Sharrett AR, et al. Retinal microvascular abnormalities and risk of lacunar stroke: Atherosclerosis Risk in Communities Study. Stroke. 2010;41(7):1349–55.
33. Takayanagi Y, Takai Y, Kaidzu S, Tanito M. Association between Systemic Antioxidant Capacity and Retinal Vessel Diameters in Patients with Primary-Open Angle Glaucoma. Life (Basel). 2020;10(12).
34. Günthner R, Streese L, Angermann S, Lorenz G, Braunisch MC, Matschkal J, et al. Mortality prediction of retinal vessel diameters and function in a long-term follow-up of haemodialysis patients. Cardiovascular Res. 2022;118(16):3239–49.
35. van Dinther M, Voorter PHM, Schram MT, Berendschot T, Houben A, Webers CAB, et al. Retinal microvascular function is associated with the cerebral microcirculation as determined by intravoxel incoherent motion MRI. J Neurol Sci. 2022;440:120359.
36. Kuchler T, Hausinger R, Braunisch MC, Günthner R, Wicklein R, Knier B et al. All eyes on PCS: analysis of the retinal microvasculature in patients with post-COVID syndrome-study protocol of a 1 year prospective case-control study. Eur Arch Psychiatry Clin Neurosci. 2023.
37. Bahmer T, Borzikowsky C, Lieb W, Horn A, Krist L, Fricke J, et al. Severity, predictors and clinical correlates of Post-COVID syndrome (PCS) in Germany: A prospective, multi-centre, population-based cohort study. EClinicalMedicine. 2022;51:101549.
38. O'Connor RJ, Preston N, Parkin A, Makower S, Ross D, Gee J, et al. The COVID-19 Yorkshire Rehabilitation Scale (C19-YRS): Application and psychometric analysis in a post-COVID-19 syndrome cohort. J Med Virol. 2022;94(3):1027–34.

39. Krupp LB, Alvarez LA, LaRocca NG, Scheinberg LC. Fatigue in multiple sclerosis. Arch Neurol. 1988;45(4):435–7.
40. Costantini L, Pasquarella C, Odone A, Colucci ME, Costanza A, Serafini G, et al. Screening for depression in primary care with Patient Health Questionnaire-9 (PHQ-9): A systematic review. J Affect Disord. 2021;279:473–83.
41. Spitzer RL, Kroenke K, Williams JB, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. Arch Intern Med. 2006;166(10):1092–7.
42. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727–36.
43. Carruthers BM, Jain AK, De Meirleir KL, Peterson DL, Klimas NG, Lerner AM, et al. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. J Chronic Fatigue Syndrome. 2003;11(1):7–115.
44. Kuchler T, Günthner R, Ribeiro A, Hausinger R, Streese L, Wöhnl A, et al. Correction: Persistent endothelial dysfunction in post-COVID-19 syndrome and its associations with symptom severity and chronic inflammation. Angiogenesis. 2023;26(4):565.
45. Nagel E, Vilser W, Lanzl I. Age, blood pressure, and vessel diameter as factors influencing the arterial retinal flicker response. Invest Ophthalmol Vis Sci. 2004;45(5):1486–92.
46. Kotliar KE, Lanzl IM, Schmidt-Trucksäss A, Sitnikova D, Ali M, Blume K, et al. Dynamic retinal vessel response to flicker in obesity: A methodological approach. Microvasc Res. 2011;81(1):123–8.
47. Ponto KA, Werner DJ, Wiedemer L, Laubert-Reh D, Schuster AK, Nickels S, et al. Retinal vessel metrics: normative data and their use in systemic hypertension: results from the Gutenberg Health Study. J Hypertens. 2017;35(8):1635–45.
48. Yuen VL, Zhang XJ, Ling X, Zhang Y, Kam KW, Chen LJ, et al. Effects of firsthand tobacco smoking on retinal vessel caliber: a systematic review and meta-analysis. Graefes Arch Clin Exp Ophthalmol. 2024;262(5):1397–407.
49. Hanssen H, Streese L, Vilser W. Retinal vessel diameters and function in cardiovascular risk and disease. Prog Retin Eye Res. 2022;91:101095.
50. Lambadiari V, Mitrakou A, Kountouri A, Thymis J, Katogiannis K, Korakas E, et al. Association of COVID-19 with impaired endothelial glycocalyx, vascular function and myocardial deformation 4 months after infection. Eur J Heart Fail. 2021;23(11):1916–26.
51. Dominic P, Ahmad J, Bhandari R, Pardue S, Solorzano J, Jaisingh K, et al. Decreased availability of nitric oxide and hydrogen sulfide is a hallmark of COVID-19. Redox Biol. 2021;43:101982.
52. Lip S, Tran TQB, Hanna R, Nichol S, Guzik TJ, Delles C, et al. Long-term effects of SARS-CoV-2 infection on blood vessels and blood pressure - LOCHINVAR. J Hypertens. 2025;43(6):1057–65.
53. Charfeddine S, Ibn Hadj Amor H, Jdidi J, Torjmen S, Kraiem S, Hammami R et al. Long COVID 19 Syndrome: Is It Related to Microcirculation and Endothelial Dysfunction? Insights From TUN-EndCOV Study. Front Cardiovasc Med. 2021;8.

54. Kozłowski P, Śmiarowski M, Przyborska W, Zemlik K, Małecka-Giełdowska M, Leszczyńska A, et al. Mild-to-Moderate COVID-19 Convalescents May Present Pro-Longed Endothelium Injury. J Clin Med. 2022;11:21.
55. Oikonomou E, Souvaliotis N, Lampsas S, Siasos G, Poulakou G, Theofilis P, et al. Endothelial dysfunction in acute and long standing COVID-19: A prospective cohort study. Vascul Pharmacol. 2022;144:106975.
56. Ambrosino P, Sanduzzi Zamparelli S, Mosella M, Formisano R, Molino A, Spedicato GA, et al. Clinical assessment of endothelial function in convalescent COVID-19 patients: a meta-analysis with meta-regressions. Ann Med. 2022;54(1):3233–48.
57. Zanoli L, Gaudio A, Mikhailidis DP, Katsiki N, Castellino N, Lo Cicero L, et al. Vascular Dysfunction of COVID-19 Is Partially Reverted in the Long-Term. Circul Res. 2022;130(9):1276–85.
58. Zhang C, Cheng S, Chen H, Yang J, Chen Y. New findings on retinal microvascular changes in patients with primary COVID-19 infection: a longitudinal study. Front Immunol. 2024;15:1404785.
59. Greene C, Connolly R, Brennan D, Laffan A, O'Keeffe E, Zaporojan L, et al. Author Correction: Blood-brain barrier disruption and sustained systemic inflammation in individuals with long COVID-associated cognitive impairment. Nat Neurosci. 2024;27(5):1019.
60. Kedor C, Freitag H, Meyer-Arndt L, Wittke K, Hanitsch LG, Zoller T, et al. A prospective observational study of post-COVID-19 chronic fatigue syndrome following the first pandemic wave in Germany and biomarkers associated with symptom severity. Nat Commun. 2022;13(1):5104.
61. Braga J, Lepra M, Kish SJ, Rusjan PM, Nasser Z, Verhoeff N, et al. Neuroinflammation After COVID-19 With Persistent Depressive and Cognitive Symptoms. JAMA Psychiatry. 2023;80(8):787–95.
62. Greene C, Connolly R, Brennan D, Laffan A, O'Keeffe E, Zaporojan L, et al. Blood-brain barrier disruption and sustained systemic inflammation in individuals with long COVID-associated cognitive impairment. Nat Neurosci. 2024;27(3):421–32.
63. Jacobs LMC, Wintjens M, Nagy M, Willems L, Ten Cate H, Spronk HMH, et al. Biomarkers of sustained systemic inflammation and microvascular dysfunction associated with post-COVID-19 condition symptoms at 24 months after SARS-CoV-2-infection. Front Immunol. 2023;14:1182182.
64. Prasanna G, Krishnamoorthy R, Yorio T. Endothelin, astrocytes and glaucoma. Exp Eye Res. 2011;93(2):170–7.
65. de Jong FJ, Schrijvers EM, Ikram MK, Koudstaal PJ, de Jong PT, Hofman A, et al. Retinal vascular caliber and risk of dementia: the Rotterdam study. Neurology. 2011;76(9):816–21.
66. Arnould L, Soumaré A, D'Aoust T, Lima Rebouças SC, Bordes C, Crivello F, et al. Retinal Microvascular Correlates of Cerebral Small Vessel Disease in Older Age. Neurology. 2025;105(9):e214251.
67. Jiang K, Power MC, Bandeen-Roche K, Griswold ME, Albert MS, Knopman DS, et al. Midlife Retinal Microvascular Signs and Late-Life Neuroimaging Features of Cerebral Small Vessel Disease in the ARIC Study. Neurology. 2025;105(4):e213919.
68. Luyten LJ, Dockx Y, Madhloum N, Sleurs H, Gerrits N, Janssen BG, et al. Association of Retinal Microvascular Characteristics With Short-term Memory Performance in Children Aged 4 to 5 Years.

JAMA Netw Open. 2020;3(7):e2011537.

69. Russell L, Broderick G, Taylor R, Fernandes H, Harvey J, Barnes Z, et al. Illness progression in chronic fatigue syndrome: a shifting immune baseline. BMC Immunol. 2016;17:3.
70. Missailidis D, Sanislav O, Allan CY, Annesley SJ, Fisher PR. Cell-Based Blood Biomarkers for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Int J Mol Sci. 2020;21(3).
71. Hunter E, Alshaker H, Bundock O, Weston C, Bautista S, Gebregzabhar A, et al. Development and validation of blood-based diagnostic biomarkers for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) using EpiSwitch(®) 3-dimensional genomic regulatory immuno-genetic profiling. J Transl Med. 2025;23(1):1048.
72. Ferrando SJ, Lynch ST, Dornbush R, Groenendaal E, Klepacz L, Shahar S, et al. Associations of elevated pro-inflammatory cytokines Interleukin-6, C-reactive protein and tumor necrosis factor alpha with neuropsychiatric symptoms of post-acute sequelae of COVID-19 (PASC). J Psychiatr Res. 2025;190:128–36.
73. Nuber-Champier A, Breville G, Voruz P, Jacot de Alcântara I, Cionca A, Allali G, et al. Systemic cytokines related to memory function 6–9 months and 12–15 months after SARS-CoV-2 infection. Sci Rep. 2024;14(1):22660.
74. Tilikete C, Zamali I, Meddeb Z, Kharroubi G, Marzouki S, Dhaouadi T, et al. Exploring the landscape of symptom-specific inflammatory cytokines in post-COVID syndrome patients. BMC Infect Dis. 2024;24(1):1337.
75. Ogando NS, Elaish M, Mahabadi HM, Langdon KD, Das SK, Joseph JT et al. Immunometabolism perturbations in post-COVID-19 condition: interleukin-6 and monoamine oxidase interactions drive neuropsychiatric syndromes. Brain, Behavior, and Immunity. 2025;129:690–708.
76. García-Juárez M, Camacho-Morales A. Defining the Role of Anti- and Pro-inflammatory Outcomes of Interleukin-6 in Mental Health. Neuroscience. 2022;492:32–46.
77. Hanson AL, Mulè MP, Ruffieux H, Mescia F, Bergamaschi L, Pelly VS, et al. Iron dysregulation and inflammatory stress erythropoiesis associates with long-term outcome of COVID-19. Nat Immunol. 2024;25(3):471–82.
78. Lefebvre T, Boutten A, Raulet-Bussian C, Raynor A, Manceau H, Puy H, et al. Evaluation of iron metabolism in hospitalized COVID-19 patients. Clin Chim Acta. 2023;548:117509.
79. Evans PC, Rainger GE, Mason JC, Guzik TJ, Osto E, Stamataki Z, et al. Endothelial dysfunction in COVID-19: a position paper of the ESC Working Group for Atherosclerosis and Vascular Biology, and the ESC Council of Basic Cardiovascular Science. Cardiovasc Res. 2020;116(14):2177–84.
80. Alexander Y, Osto E, Schmidt-Trucksäss A, Shechter M, Trifunovic D, Duncker DJ, et al. Endothelial function in cardiovascular medicine: a consensus paper of the European Society of Cardiology Working Groups on Atherosclerosis and Vascular Biology, Aorta and Peripheral Vascular Diseases, Coronary Pathophysiology and Microcirculation, and Thrombosis. Cardiovascular Res. 2020;117(1):29–42.

Figures

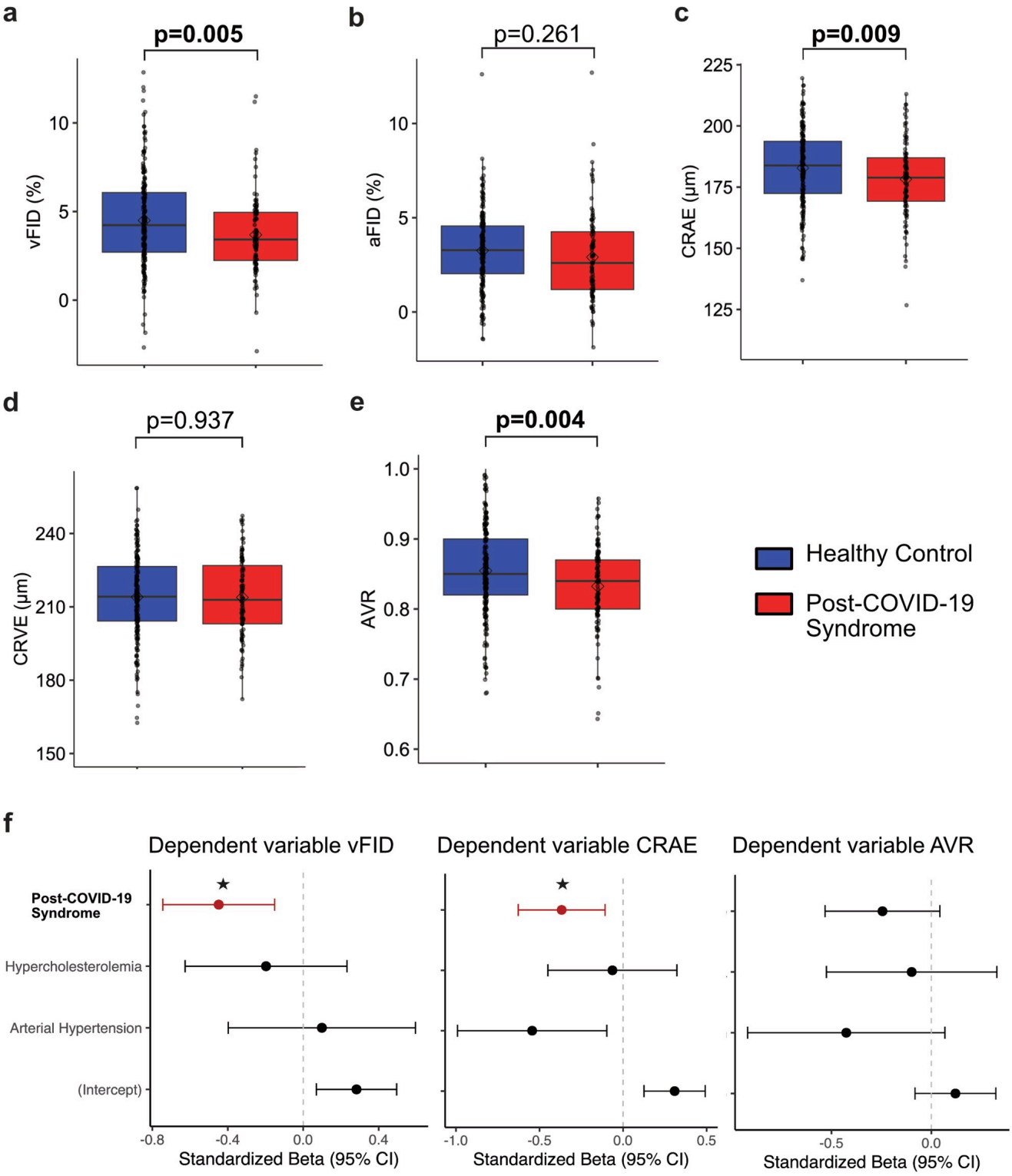

Figure 1

Retinal microvascular parameters in PCS patients and HC. Box plots show dynamic retinal vessel analysis (DVA) parameters: venular flicker-induced dilation (vFID; PCS n = 100, HC n = 195; a) and arteriolar flicker-induced dilation (aFID; PCS n = 100, HC n = 195; b), and static retinal vessel analysis

(SVA) parameters: central retinal venular equivalent (CRVE; c), central retinal arteriolar equivalent (CRAE; d), and arteriolar-to-venular ratio (AVR; PCS n = 102, HC n = 199; e) in age- and sex-matched PCS patients (red) and healthy controls (blue). Box plots indicate the median (horizontal line) and mean (black dot). Each data point represents one eye from one individual (one eye analyzed per participant). Group comparisons were performed using the Mann-Whitney U test for non-normally distributed variables and Welch's two-sample t-test for normally distributed variables. Forest plots (f) display standardized β coefficients with 95% confidence intervals from multivariable linear regression models assessing the independent association of PCS status with vFID, CRAE, and AVR, adjusted for arterial hypertension and hypercholesterolemia. Statistical significance is indicated as *p < 0.05.

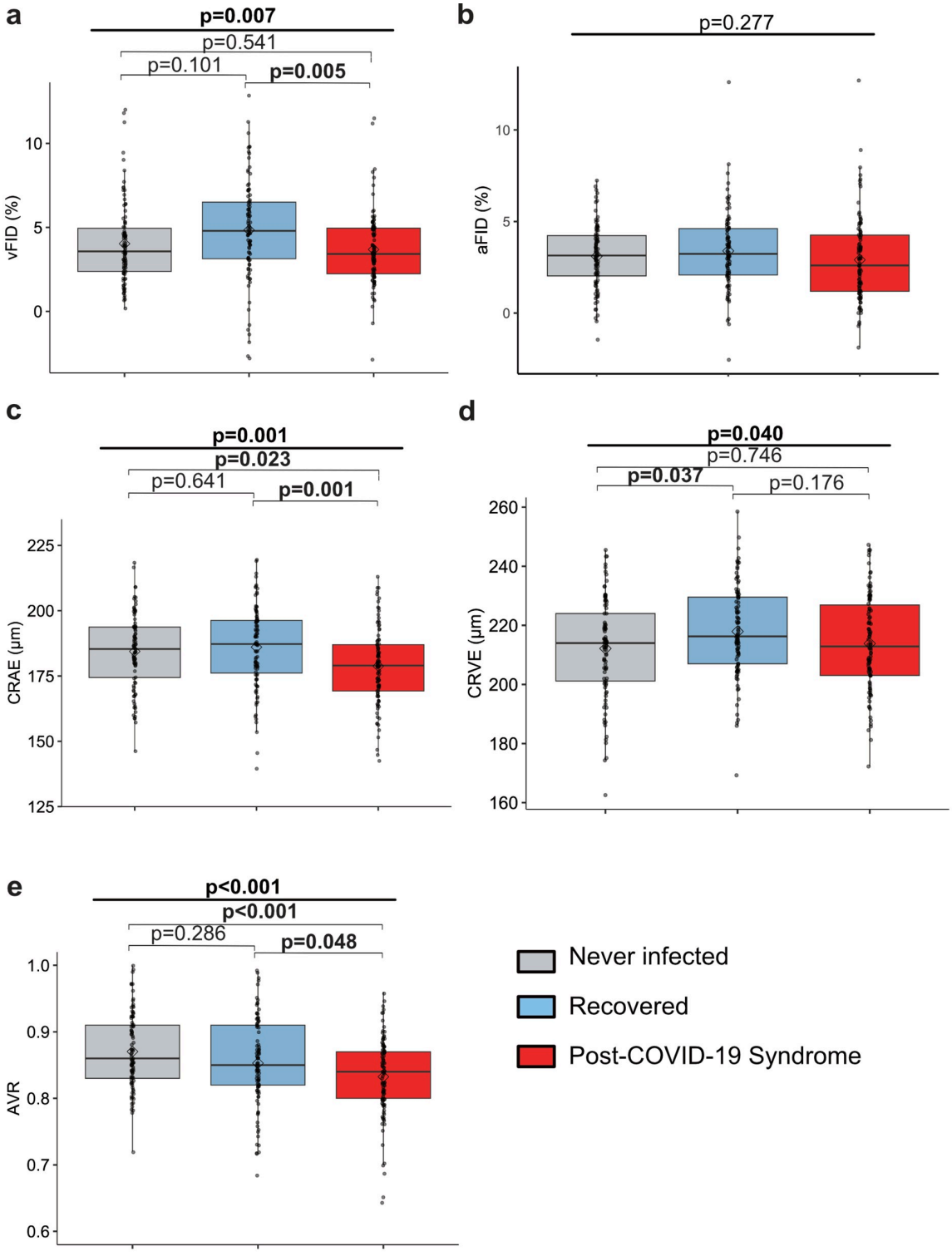

Figure 2

Retinal microvascular parameters across never infected, recovered, and PCS cohorts. Box plots show DVA parameters vFID and aFID (never infected n = 95; recovered n = 92; PCS n = 100; a, b), and SVA parameters CRAE (c), CRVE (d), and AVR (never infected n = 94; recovered n = 99; PCS n = 102; e). Groups are visualized as never infected (grey), recovered (light blue), and PCS (red). Box plots indicate the median (horizontal line) and mean (black dot). Each data point represents one eye from one

individual (one eye analyzed per participant). Group comparisons were performed using one-way ANOVA for normally distributed variables or the Kruskal-Wallis test for non-normally distributed variables. Post-hoc pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test for ANOVA models and Dunn's test with multiplicity correction for Kruskal-Wallis models.

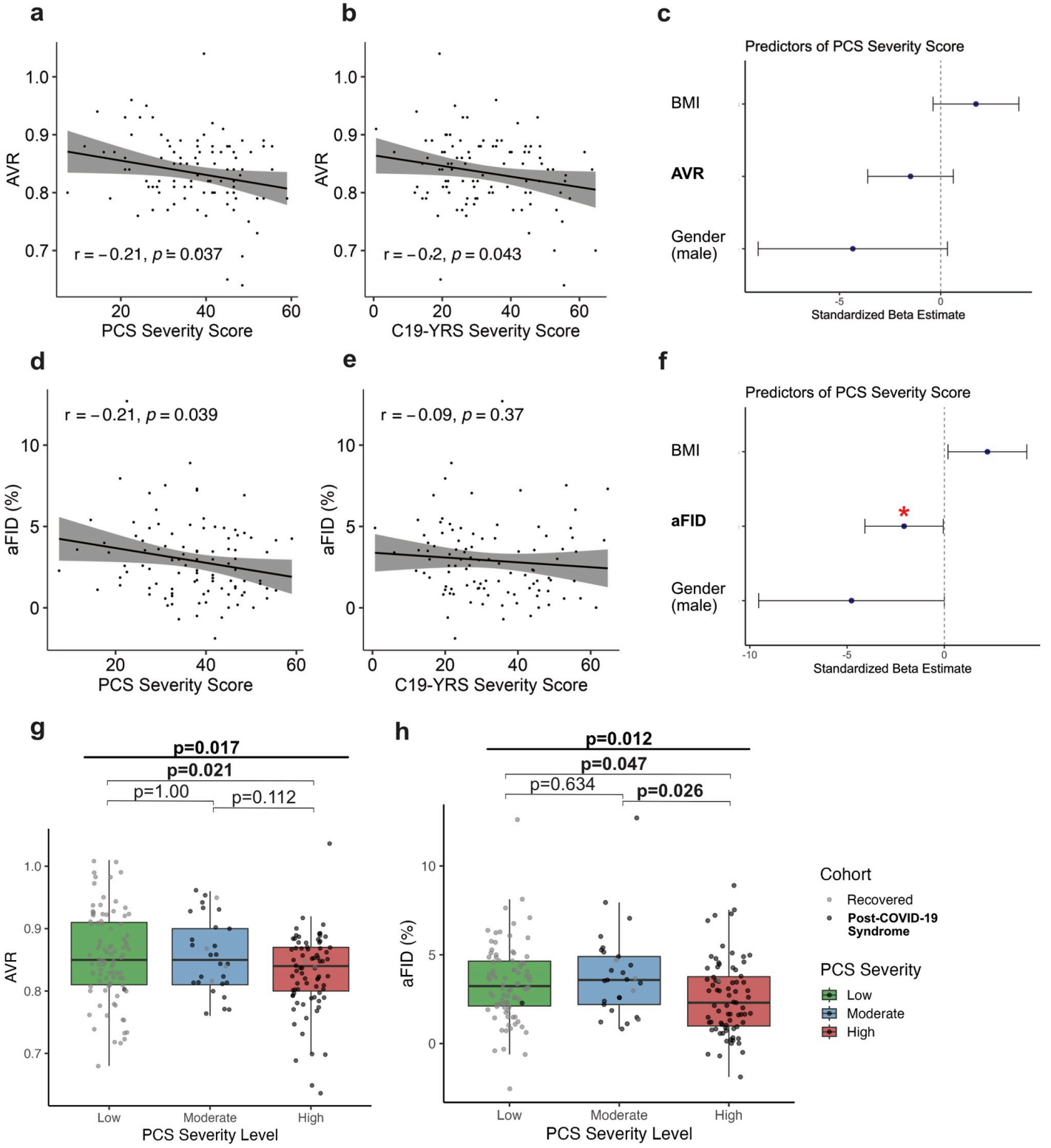

Figure 3

Associations between RVA parameters and PCS symptom burden. **(a–b, d–e)** Pearson correlations between RVA parameters and symptom scores in PCS patients. Panels **a–b** show correlations between AVR and the PCS Severity Score (**a**) and C19-YRS (**b**). Panels **d–e** show corresponding correlations for aFID with the PCS Severity Score (**d**) and C19-YRS (**e**). Points represent individual patients; the solid line indicates the linear regression fit and the shaded band the 95% confidence interval. Pearson correlation coefficients (r) and p-values are shown in each panel.

(c, f) Forest plots display standardized β-coefficients with 95% confidence intervals from multivariable regression models assessing the association of AVR (**c**) or aFID (**f**) with the PCS Severity Score after adjustment for BMI and gender. Statistical significance is indicated as *p < 0.05.

(g–h) Box plots show the distribution of AVR (**g**) and aFID (**h**) across three symptom severity groups in PCS patients (black) and recovered individuals (grey). The PCS Severity Score was categorized into low (green), moderate (blue), and high (red) severity using fixed cutoffs (<10, 10–30, >30; colors as indicated). Box plots indicate the median (horizontal line) and individual observations. Group comparisons were performed using one-way ANOVA for normally distributed variables. Post-hoc comparisons were conducted using Tukey's HSD for ANOVA models. Each data point represents one eye from one individual (one eye analyzed per participant).

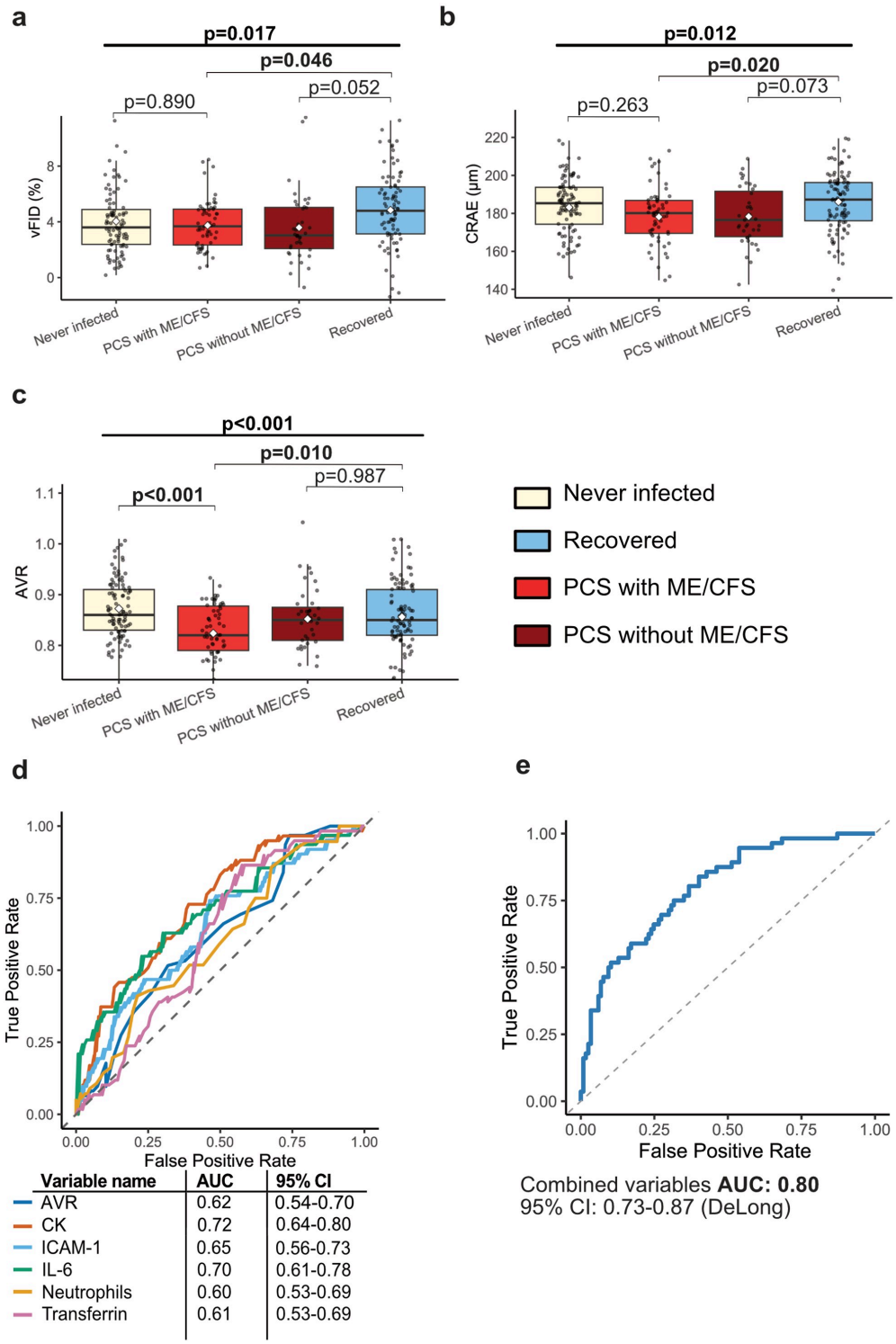

Figure 4

Retinal microvascular parameters and biomarker-based discrimination of ME/CFS. (a–c) Box plots of the DVA parameter vFID (a) and SVA parameters CRAE (b) and AVR (c) across four cohorts: never infected (n = 96, cornsilk), PCS with ME/CFS (n = 62, red), PCS without ME/CFS (n = 39, dark red), and recovered individuals (n = 102, light blue). Box plots indicate the median (horizontal line) and mean (black dot). Group comparisons were performed using one-way ANOVA for normally distributed variables or the

Kruskal-Wallis test for non-normally distributed variables, with post-hoc testing using Tukey's honestly significant difference (HSD) test for ANOVA models or Dunn's test with multiplicity correction for Kruskal-Wallis models. Adjusted p-values are shown above group comparisons. Each data point represents one eye from one individual (one eye analyzed per participant).

(d) Receiver operating characteristic (ROC) curves for individual biomarkers discriminating PCS patients fulfilling ME/CFS criteria from recovered individuals and PCS patients without ME/CFS. Variables include AVR (dark blue), creatine kinase (CK; red), C-reactive protein (CRP; light blue), interleukin-6 (IL-6; green), low-density lipoprotein cholesterol (LDL; orange), and transferrin (purple) (colors as indicated).

(e) ROC curve showing discrimination between ME/CFS patients and the combined comparator group (recovered individuals and PCS patients without ME/CFS) using a multivariable model integrating AVR, CK, ICAM-1, IL-6, transferrin, and neutrophils. Area under the curve (AUC) with 95% confidence interval (CI) was calculated using DeLong's method.

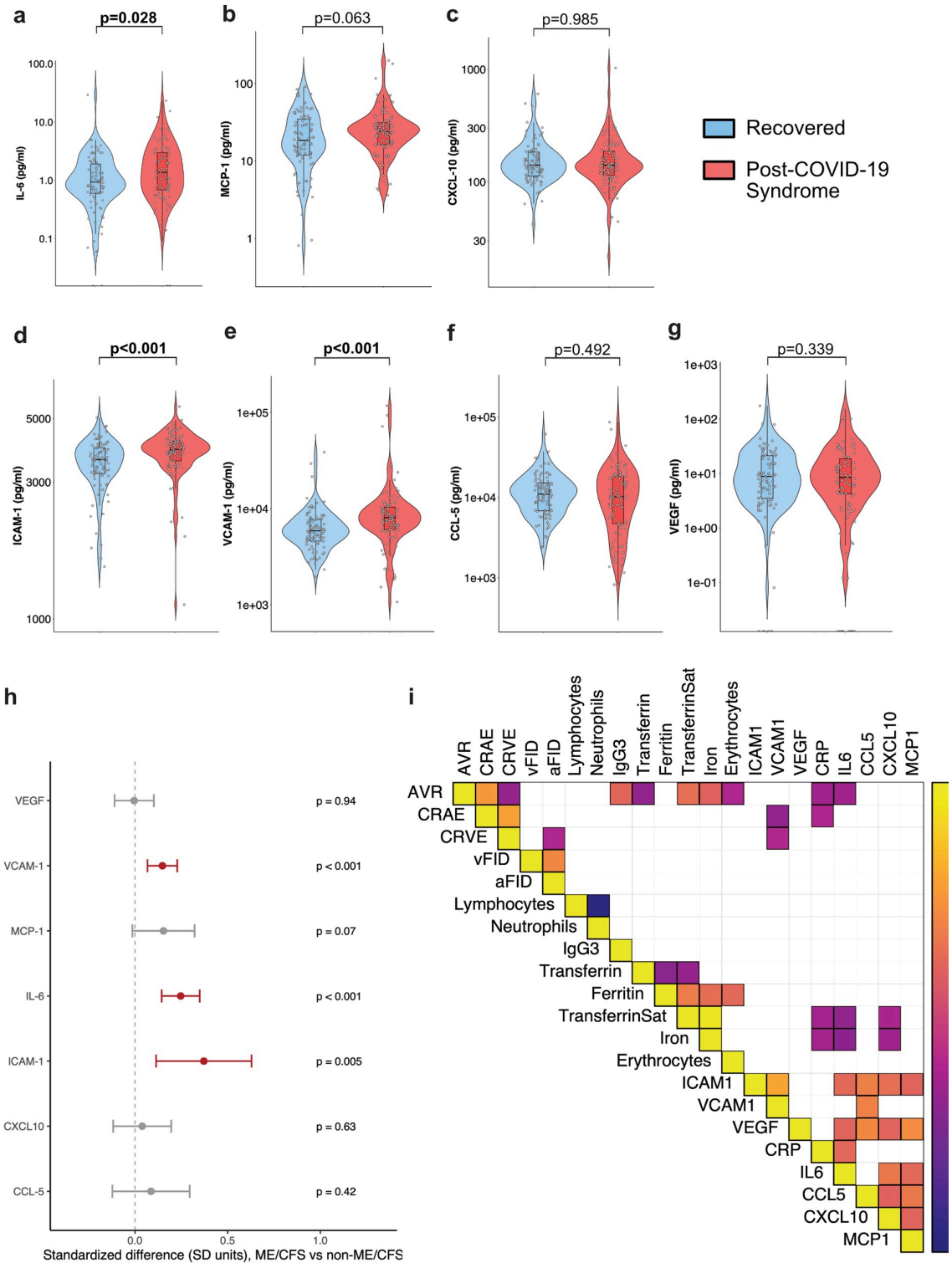

Figure 5

Circulating inflammatory and endothelial dysfunction markers in PCS and their associations with retinal microvascular parameters. (a–g) Violin plots illustrate circulating levels of inflammatory markers (IL-6, MCP-1, CXCL-10, CCL-5) and endothelial dysfunction markers (ICAM-1, VCAM-1, VEGF) in recovered individuals (light blue) and patients with PCS (red). Individual data points represent single participants. Box plots indicate the median (black line) and interquartile range (IQR). Group comparisons were

performed using the Wilcoxon rank-sum test for non-normally distributed variables and Welch's two-sample t-test for normally distributed variables, as appropriate. IL-6, CXCL-10, MCP-1, ICAM-1, VCAM-1, CCL-5, and VEGF were measured in n = 90 recovered individuals and n = 100 PCS patients.

(h) Forest plot illustrating age-adjusted associations between ME/CFS status and seven circulating biomarkers. Effect sizes are presented as standardized differences (SD units) with 95% confidence intervals derived from regression models adjusted for age. Statistically significant associations are highlighted in red (as indicated, $p<0.05$).

(i) Correlation heatmap showing significant Spearman correlations ($p < 0.05$) between selected laboratory parameters and retinal vessel analysis (RVA) parameters AVR, CRAE, CRVE, vFID, and aFID in PCS patients. Spearman's ρ is color-coded from +1 (yellow, positive correlation) to −1 (blue, negative correlation).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTablesandFiguresMicrovascularRemodeling.pdf