

Persistent venular dysfunction despite structural convergence in post-COVID-19 syndrome: a prospective six-month retinal vessel analysis follow-up

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Research Article

Keywords: Post-COVID syndrome, Long COVID, Retinal Vessel Analysis, Flicker-induced dilation, Endothelial Dysfunction, Myalgic encephalomyelitis/chronic fatigue syndrome, Longitudinal study, Microvascular disease

Posted Date: October 8th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11101115/v1>

Abstract

Background

Post-COVID-19 syndrome (PCS) has previously been associated with structural and functional alterations of the retinal microvasculature, assessed cross-sectionally by retinal vessel analysis (RVA). Whether these alterations persist, resolve, or progress over time is unknown.

Methods

In this prospective single-center study, 102 PCS patients underwent static and dynamic RVA, patient-reported outcome measures (PROMs), and blood sampling at baseline; 82 (80.4%) completed six-month follow-up. Recovered controls were not reassessed longitudinally; follow-up between-group comparisons therefore used recovered baseline values, via matched and covariate-adjusted analyses. Longitudinal change, associations with PROMs and circulating biomarkers, and differences by ME/CFS status were examined.

Results

Within the PCS cohort, central retinal arteriolar equivalent (CRAE) increased from 178.2 ± 16.0 to 184.1 ± 17.6 μm and the arteriolar-to-venular ratio (AVR) increased from 0.83 ± 0.06 to 0.86 ± 0.06 (both $p < 0.001$). Venular flicker-induced dilation (vFID) remained stable within the PCS cohort over six months ($3.66 \pm 2.25\%$ to $3.80 \pm 2.70\%$, $p = 0.821$) but stayed significantly lower than in recovered controls at follow-up (adjusted $\beta = -1.31$ percentage points, 95% CI -2.58 to -0.04 , $p = 0.045$), consistently across matched, unmatched, and 1:1-matching sensitivity analyses. Neither CRAE nor AVR differed from recovered controls at follow-up. RVA parameters showed little association with symptom burden. VCAM-1 correlated inversely with CRAE at follow-up ($\rho = -0.38$). vFID trajectories did not differ significantly by ME/CFS status.

Conclusions

Structural and functional retinal microvascular parameters followed divergent six-month trajectories in PCS. Reduced venular flicker-induced dilation persisted independently of symptom burden, supporting separate longitudinal assessment of structural and functional retinal domains in post-infectious illness.

Trial registration:

ClinicalTrials.gov, NCT05635552. Registered 30 November 2022 - Retrospectively registered, <https://clinicaltrials.gov/study/NCT05635552>

1. Introduction

Post-COVID-19 syndrome (PCS), defined by the WHO as new or persistent symptoms beyond three months after SARS-CoV-2 infection that last at least two months and cannot be explained by an alternative diagnosis [1], affects a substantial proportion of individuals after SARS-CoV-2 infection and spans nearly every organ system [2]. Global estimates suggest that tens of millions of people experienced persistent fatigue, cognitive, or respiratory symptom clusters in the first two years of the pandemic alone [3], and the risk and burden of these sequelae have continued to evolve across viral variants and vaccination eras [4]. Established risk factors include female sex, older age, and pre-existing comorbidity [5], yet the biological mechanisms driving symptom persistence remain incompletely understood, and objective, non-invasive markers of ongoing pathology are still lacking [6].

Among the mechanisms proposed to underlie PCS, persistent endothelial dysfunction has emerged as a unifying pathophysiological thread [7]. Direct viral infection of endothelial cells and diffuse endotheliitis were among the earliest described vascular abnormalities of acute COVID-19 [8], accompanied by widespread pulmonary microvascular thrombosis and aberrant angiogenesis [9]. Beyond the acute phase, circulating biomarkers of endothelial activation and sustained low-grade inflammation have repeatedly been reported in patients with ongoing post-COVID symptoms and remain associated with symptom severity up to two years after infection [10], and accelerated vascular ageing has been demonstrated even after mild COVID-19 [11], underscoring that SARS-CoV-2 infection confers cardiovascular risk extending well beyond acute illness.

The retinal microvasculature offers a uniquely accessible, non-invasive window into systemic and cerebral microvascular health, owing to its shared embryological origin and autoregulatory mechanisms with the cerebral circulation [12]. Static retinal vessel analysis (SVA) quantifies structural remodeling through the central retinal arteriolar and venular equivalents (CRAE, CRVE) and their ratio (AVR) [13], while dynamic retinal vessel analysis (DVA) captures functional, nitric-oxide-dependent neurovascular coupling through flicker light-induced dilation (FID) of arterioles and venules [12, 14]. Both parameters have been validated as independent predictors of long-term cardiovascular outcomes in large population-based and clinical cohorts, including incident stroke, myocardial infarction, and mortality over up to 16 years of follow-up [15, 16], and altered neurovascular coupling detectable by retinal imaging has been linked to cognitive impairment [17, 18], reflecting the retina's broader emerging role as a biomarker of systemic and neurodegenerative disease under the concept of oculomics [19].

Retinal microvascular alterations have also been repeatedly documented following SARS-CoV-2 infection. Optical coherence tomography angiography studies have shown reduced retinal capillary vessel density persisting for up to six months after COVID-19 pneumonia [20], a finding corroborated across a broader literature synthesized in a systematic review and meta-analysis [21]. Using retinal vessel analysis specifically, we previously demonstrated persistent structural and functional endothelial dysfunction in patients with PCS – including reduced venular FID, narrower retinal arterioles, and lower AVR compared with recovered and never-infected individuals – with the most pronounced alterations

observed in patients fulfilling Canadian Consensus Criteria for ME/CFS [22] and correlating with symptom severity and inflammatory markers [23]. However, this and comparable studies to date have been cross-sectional, leaving open the fundamental question of whether retinal microvascular dysfunction in PCS resolves, persists, or progresses over time. This information is essential to determine whether RVA reflects a transient post-infectious phenomenon or a marker of sustained, and potentially clinically consequential, microvascular injury.

To address this gap, we performed a prospective six-month follow-up assessment of RVA parameters within the same well-characterized PCS cohort in which we previously reported baseline microvascular alterations [24]. The primary aim of this study was to characterize the longitudinal trajectory of structural and functional RVA parameters within the PCS cohort and in comparison with recovered individuals. Secondary aims were to examine cross-sectional and longitudinal associations between RVA parameters and patient-reported symptom burden, to assess relationships with circulating biomarkers of endothelial activation and inflammation, and to explore differences by ME/CFS status.

2. Methods

Study design and cohort

This study extends the “All Eyes on PCS” cohort, a prospective single-center study at the Technical University of Munich, for which recruitment, eligibility criteria, and baseline retinal findings have been reported elsewhere [24]. Briefly, adults with WHO-defined PCS and an age- and sex-comparable group of recovered individuals without persistent symptoms were enrolled between October 2022 and January 2025. A median of 190 days after their initial visit, PCS participants returned for a follow-up assessment repeating retinal vessel analysis, PROM questionnaires, and blood sampling under the same conditions as at baseline. The primary objective of the overarching study was to quantify retinal vessel responsiveness and vessel morphology using RVA in patients with PCS and to compare these parameters with those of the recovered cohort [24]; sample size was calculated accordingly. The present analysis addresses this primary objective longitudinally, at six-month follow-up within the PCS cohort. Because recovered controls were examined only once, at baseline, longitudinal comparisons are confined to the PCS group, and the between-group contrast at follow-up necessarily pairs PCS follow-up data with recovered baseline data – an approach mirrored in the companion analysis of handgrip strength within this cohort [25]. Never-infected individuals, like recovered controls, were assessed only at baseline as part of the original study design; the cross-sectional comparison between PCS and never-infected individuals has been reported previously [23]. The present analysis therefore focuses on the recovered comparator, which isolates effects specific to persistent symptoms from those attributable to SARS-CoV-2 infection per se. Ethical approval was obtained from the Technical University of Munich (Klinikum rechts der Isar; 2022-317-S-SR); the trial is registered at ClinicalTrials.gov (NCT05635552), and the study adhered to the Declaration of Helsinki with written consent from all participants.

Retinal vessel analysis

Static and dynamic retinal vessel parameters were obtained with the same imaging systems and acquisition protocol used at baseline (Static Vessel Analyzer and Dynamic Vessel Analyzer, DVAlight, both Imedos Health GmbH, Jena, Germany, with a Topcon TRC-NW8 fundus camera); full acquisition and quality-control details are given in the previous publication from the baseline cohort [23]. Arteriolar and venular caliber equivalents (CRAE, CRVE) were quantified semi-automatically with VesselMap software (Imedos Health GmbH) using the Parr-Hubbard formula, with AVR calculated as CRAE/CRVE; functional endothelial responsiveness was captured through repeated flicker-light stimulation, yielding the peak percentage dilation of arterioles and venules relative to resting diameter (aFID, vFID). RVA acquisition was performed by personnel not blinded to group assignment; all image and signal quality grading, processing, and quantitative analysis were conducted by assessors blinded to group assignment and time point, using the cumulative quality-scoring approach described previously [23], with recordings below the pre-specified threshold re-evaluated by a second observer and excluded if consensus could not be reached. Systemic blood pressure was measured directly before each RVA session at both visits.

Patient-reported outcome measures

Participants completed the PCS Severity Score [26], the COVID-19 Yorkshire Rehabilitation Scale (C19-YRS) [27], the Fatigue Severity Scale [28], the PHQ-9 for depressive symptoms [29], and the GAD-7 for anxiety [30] at both time points. ME/CFS status was ascertained using the Canadian Consensus Criteria [22].

Laboratory parameters

Venous blood was drawn and processed in parallel with the clinical assessments in an ISO-certified laboratory, following procedures established for this cohort [23]. Nineteen circulating biomarkers were analysed. Inflammatory, endothelial and coagulation markers (IL-6, IL-8, MCP-1, IP-10, RANTES/CCL5, ICAM-1, VCAM-1, VEGF, ferritin, D-dimer) were measured by routine clinical assay as described for the baseline cohort [23], D-dimer being included as a marker of coagulation activation relevant to endothelial injury; von Willebrand factor was quantified by immunoturbidimetric assay (LIAPHEN™, Hyphen Biomed), total IgG and subclasses IgG1–IgG4 by routine clinical assay, and anti-S1 IgG by enzyme-linked immunosorbent assay (LEGEND MAX™, BioLegend). Neurofilament light chain and glial fibrillary acidic protein were quantified on the Simoa® HD-X Analyzer (Quanterix) using the Neurology 2-Plex B kit; results below the manufacturer-specified quantification limits (0.8 pg/mL and 16.6 pg/mL, respectively) were excluded from analysis. Laboratory staff were blinded to clinical and imaging data.

Statistical analysis

Analyses were conducted in R (version 2026.06.0 + 242). Depending on distribution, continuous variables are summarized as mean $\pm$ SD or median (IQR), and categorical variables as counts and percentages. Values outside pre-specified physiological ranges or identified as outliers by IQR- or MAD-based rules, were flagged and retained in primary analyses, with a winsorized (1st/99th percentile) re-analysis conducted for each longitudinal comparison as a check on robustness.

Change from baseline to follow-up within the PCS group was tested using paired t-tests or, where the distribution of within-person differences departed from normality, Wilcoxon signed-rank tests; a mixed-effects regression of each parameter on study visit, with age and sex as covariates and a per-participant random intercept, served as a confirmatory check on these paired comparisons. For the principal between-group comparison – PCS follow-up values against recovered-control baseline values – patients and controls were matched 2:1 by nearest-neighbor propensity score on age and sex, with replacement, targeting the average treatment effect on the treated [31]; balance was verified via standardized mean differences, and group effects were estimated from weighted linear models with HC3-robust standard errors, adjusting caliber outcomes for mean arterial pressure and BMI in addition to age and sex, and FID outcomes for age, sex, and BMI. This matched analysis was cross-checked against an unmatched, covariate-adjusted model in the full cohort and against 1:1 matching performed without replacement.

Relationships between RVA parameters and PROMs were explored cross-sectionally and via six-month change scores using Spearman correlation, with Benjamini-Hochberg correction applied separately within each family of comparisons; whether baseline RVA values predicted follow-up PROM scores was further tested in linear models adjusted for age, sex, and the corresponding baseline PROM. Coupling between structural and functional RVA change was examined by correlating the six-month deltas of all parameter pairs. Associations with circulating biomarkers were assessed analogously, with an additional exploratory stratification by sex.

To evaluate whether RVA trajectories differed by ME/CFS status, within-group paired changes were first compared descriptively, then tested formally via a time-by-ME/CFS interaction term in the mixed-effects models described above (adjusted for age and sex), alongside adjusted cross-sectional comparisons between subgroups at each visit. The extent and pattern of missing follow-up data were characterized using Little's MCAR test and by comparing baseline characteristics of completers and non-completers separately for each RVA parameter. Two-sided $p < 0.05$ was considered significant throughout.

3. Results

Follow-up completion and data availability

Of the 102 PCS patients, both static vessel analysis (SVA; CRAE, CRVE, AVR) and dynamic vessel analysis (DVA; arteriolar and venular flicker-induced dilation, aFID/vFID) were available at both baseline and six-month follow-up (FU) in 82 patients (80.4%) for each individual parameter, with completion rates therefore comparable across all five RVA parameters; 81 patients (79.4%) had complete data for all five parameters simultaneously, and this group forms the completer column in Table 1 and Supplementary Table S2. Recovered individuals were assessed at baseline only, consistent with the cross-sectional design of the control assessment; all longitudinal and follow-up analyses are therefore restricted to the PCS cohort, and between-group comparisons at follow-up contrast PCS follow-up values with recovered baseline values, as previously applied for handgrip strength parameters in this cohort [25].

Table 1

Baseline characteristics of recovered individuals and PCS patients (full baseline cohort and the subset completing six-month RVA follow-up). Continuous variables are mean $\pm$ SD; categorical variables are n (%); RVA parameters are baseline values. CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; AVR = arteriolar-to-venular ratio; FID = flicker-induced dilation; BMI = body-mass index; ME/CFS = myalgic encephalomyelitis/chronic fatigue syndrome. $\square$ Recovered vs. PCS (full cohort), Welch's t-test (continuous) or χ^2 test (categorical). Percentages are based on the number of participants with available data for each variable. Completers are patients with complete follow-up data for all five RVA parameters; baseline characteristics did not differ between completers and non-completers (Supplementary Table S2).

Variable	Recovered (n = 102)	PCS, full cohort (n = 102)	PCS, completers (n = 81)	p $\square$
Age, years	33.3 $\pm$ 11.4	41.9 $\pm$ 11.6	42.1 $\pm$ 11.4	< 0.001
Female sex, n (%)	73 (71.6%)	77 (75.5%)	60 (74.1%)	0.634
BMI, kg/m ²	23.5 $\pm$ 4.0	24.5 $\pm$ 4.8	24.9 $\pm$ 4.9	0.106
Hypertension, n (%)	5 (4.9%)	16 (15.7%)	14 (17.3%)	0.021
Hypercholesterolemia, n (%)	21 (22.1%)	46 (46.5%)	37 (45.7%)	< 0.001
ME/CFS, n (%)	0 (0.0%)	62 (61.4%)	48 (59.3%)	< 0.001
PCS Severity Score	0.0 $\pm$ 0.0	36.9 $\pm$ 10.6	37.2 $\pm$ 10.6	< 0.001
Fatigue Severity Scale	2.4 $\pm$ 1.1	5.7 $\pm$ 1.3	5.6 $\pm$ 1.3	< 0.001
CRAE (μ m)	186.07 $\pm$ 15.71	178.25 $\pm$ 15.47	177.82 $\pm$ 15.79	< 0.001
CRVE (μ m)	217.92 $\pm$ 15.47	213.85 $\pm$ 16.13	213.46 $\pm$ 16.17	0.069
AVR	0.86 $\pm$ 0.07	0.83 $\pm$ 0.06	0.83 $\pm$ 0.06	0.022
Arteriolar FID (%)	3.26 $\pm$ 2.19	2.88 $\pm$ 2.33	3.02 $\pm$ 2.48	0.241
Venular FID (%)	4.81 $\pm$ 2.98	3.69 $\pm$ 2.17	3.64 $\pm$ 2.26	0.003

Baseline characteristics did not differ meaningfully between patients with and without complete follow-up RVA data for any parameter, including CRAE (177.8 $\pm$ 15.8 vs. 179.9 $\pm$ 14.4 μ m, p = 0.567) and AVR (0.83 $\pm$ 0.06 vs. 0.84 $\pm$ 0.06, p = 0.863; Supplementary Table S2), arguing against a systematic attrition bias in the longitudinal and between-group comparisons that follow (see Table 1 for baseline characteristics by group and Supplementary Table S1 for outlier/plausibility flagging). Missingness in follow-up data was formally not consistent with a completely-random pattern (Little's MCAR test: χ^2 = 400.0, df = 236, p < 0.001, across 25 distinct missing-data patterns); longitudinal comparisons were

therefore corroborated using linear mixed-effects models under a missing-at-random assumption, in addition to the complete-case paired analyses reported below.

Longitudinal retinal microvascular changes within the PCS cohort

Over six months, structural and functional retinal parameters followed distinct and parameter-specific trajectories within the PCS cohort (Table 2, Fig. 1). Arteriolar caliber (CRAE) increased significantly from baseline to follow-up (178.2 ± 16.0 to $184.1 \pm 17.6 \mu\text{m}$; Wilcoxon $p < 0.001$), and this increase was confirmed in a linear mixed-effects model adjusting for age and sex ($\beta = 5.93 \mu\text{m}$, 95% CI 3.72 to 8.15, $p < 0.001$). AVR increased in parallel (0.83 ± 0.06 to 0.86 ± 0.06 ; paired t $p < 0.001$; LMM $\beta = 0.022$, $p < 0.001$). In contrast, venous caliber (CRVE) remained essentially unchanged (213.9 ± 16.5 to $215.2 \pm 17.2 \mu\text{m}$; $p = 0.121$; LMM $p = 0.114$).

In contrast, both functional (DVA) parameters remained stable over the same interval. Venular FID did not change significantly within the PCS cohort ($3.66 \pm 2.25\%$ at baseline to $3.80 \pm 2.70\%$ at follow-up; Wilcoxon $p = 0.821$; LMM $\beta = 0.12$ percentage points, 95% CI -0.47 to 0.72, $p = 0.680$), and arteriolar FID likewise remained unchanged ($3.01 \pm 2.46\%$ to $2.98 \pm 2.55\%$; $p = 0.902$; LMM $p = 0.896$).

Table 2

Longitudinal course of retinal vessel analysis parameters within the PCS cohort. Values are mean $\pm$ SD. Paired comparisons used paired t -tests or Wilcoxon signed-rank tests depending on the distribution of the difference score; the linear mixed-effects model (LMM) included fixed effects for time, age and sex, and a random intercept for participant. CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; AVR = arteriolar-to-venular ratio; FID = flicker-induced dilation; BL = baseline; FU = follow-up. Δ LMM β represents the estimated mean change from baseline to follow-up, adjusted for age and sex. Δ values are calculated from unrounded data and may differ marginally from the difference of the rounded baseline and follow-up means shown.

Parameter	n	Baseline	Follow-up	Δ (FU-BL)	p (paired)	LMM β (95% CI) Δ	p (LMM)
CRAE (μm)	82	178.15 ± 15.97	184.10 ± 17.62	5.95 ± 10.29	< 0.001	5.93 (3.72 to 8.15)	< 0.001
CRVE (μm)	82	213.85 ± 16.45	215.20 ± 17.15	1.35 ± 7.80	0.121	1.36 (-0.33 to 3.05)	0.114
AVR	82	0.83 ± 0.06	0.86 ± 0.06	0.02 ± 0.04	< 0.001	0.022 (0.012 to 0.032)	< 0.001
aFID (%)	82	3.01 ± 2.46	2.98 ± 2.55	-0.03 ± 2.40	0.902	0.03 (-0.47 to 0.54)	0.896
vFID (%)	82	3.66 ± 2.25	3.80 ± 2.70	0.13 ± 2.87	0.821	0.12 (-0.47 to 0.72)	0.680

Together, these findings indicate that within-PCS change over six months was confined to the arteriolar structural domain: CRAE and AVR increased significantly, while CRVE and both FID parameters remained stable. Because AVR is calculated as CRAE/CRVE, its increase over time is mechanistically linked to change in both caliber parameters, consistent with a strong positive correlation between the six-month

change in CRAE and the change in AVR ($\rho = 0.74$, $p < 0.001$, FDR $q < 0.001$; Supplementary Table S5) and a moderate positive correlation between the change in CRAE and the change in CRVE ($\rho = 0.38$, $p < 0.001$, $q = 0.003$). The six-month change in arteriolar and venular FID were themselves moderately correlated ($\rho = 0.38$, $p < 0.001$, $q = 0.003$; Supplementary Table S5), consistent with a shared functional trajectory distinct from the structural parameters. Neither caliber parameter's six-month change was associated with the change in either FID parameter (all $|p| \leq 0.19$, $q \geq 0.18$; Supplementary Table S5), indicating that the structural and functional domains changed independently of one another. This pattern is presented in full in Supplementary Table S5, and its relationship to change in patient-reported symptom burden in Supplementary Table S6.

Comparison with recovered controls: persistent between-group differences at follow-up

In propensity score-matched, weighted comparisons (2:1 nearest-neighbor matching with replacement, average treatment effect on the treated, robust HC3 standard errors; covariate balance in Supplementary Table S4) adjusted for age, sex, mean arterial pressure and body-mass index, venular FID remained significantly lower in PCS patients at follow-up than in recovered individuals at baseline (adjusted $\beta = -1.31$ percentage points, 95% CI -2.58 to -0.04, $p = 0.045$; Table 3, Fig. 2). This difference was consistent in direction across every sensitivity analysis performed, though modest in magnitude: an unmatched, covariate-adjusted comparison in the full cohort ($\beta = -1.12$, $p = 0.018$) and an alternative 1:1 nearest-neighbor matching without replacement ($\beta = -1.13$, $p = 0.029$) both remained significant, whereas a winsorized (1st/99th percentile) re-analysis of the within-PCS longitudinal change was not significant ($p = 0.796$; Supplementary Table S3), consistent with the absence of within-PCS change described above. Venular FID was therefore not a marker of ongoing decline, but of a deficit present at baseline that persisted, without improvement, through six-month follow-up.

Arteriolar FID showed no significant between-group difference at follow-up in any comparison (matched adjusted $\beta = -0.34$, $p = 0.500$; unmatched adjusted $\beta = -0.24$, $p = 0.560$; 1:1 matched $\beta = -0.38$, $p = 0.408$), supporting a selective, venule-specific pattern of persistently reduced function rather than a generalized neurovascular deficit.

For the structural parameters, CRAE and AVR did not differ significantly between PCS patients at follow-up and recovered individuals at baseline in any of the three analytical approaches: the matched, adjusted comparison (CRAE $\beta = 6.03$ μm , 95% CI -0.25 to 12.30, $p = 0.062$; AVR $\beta = 0.018$, $p = 0.212$), the unmatched adjusted comparison (CRAE $\beta = 2.81$, $p = 0.326$; AVR $\beta = 0.014$, $p = 0.206$), and the 1:1 matching sensitivity analysis (CRAE $\beta = -0.74$, $p = 0.799$; AVR $\beta = 0.007$, $p = 0.530$). This consistent null finding indicates that, despite the significant within-PCS increase in CRAE and AVR described above, retinal arteriolar caliber in PCS approached but did not exceed the level observed in recovered individuals. CRVE did not differ between groups at follow-up in any comparison (all $p \geq 0.33$).

Table 3

Matched, propensity score–weighted comparison between PCS patients at six-month follow-up and recovered individuals at baseline (2:1 nearest-neighbor matching with replacement, average treatment effect on the treated; HC3 robust standard errors). CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; AVR = arteriolar-to-venular ratio; FID = flicker-induced dilation; HC3 = heteroskedasticity-consistent robust standard errors. $\boxtimes$ Adjusted for age, sex, mean arterial pressure and body-mass index (CRAE, CRVE, AVR) or age, sex and body-mass index (arteriolar/venular FID). Positive β indicates higher values in PCS at follow-up relative to recovered at baseline.

Parameter	Adjusted β (95% CI) $\boxtimes$	p
CRAE (μm)	6.03 (-0.24 to 12.29)	0.062
CRVE (μm)	2.38 (-4.53 to 9.29)	0.501
AVR	0.018 (-0.010 to 0.046)	0.212
Arteriolar FID (%)	-0.34 (-1.34 to 0.65)	0.500
Venular FID (%)	-1.31 (-2.58 to -0.04)	0.045

Cross-sectional and predictive associations with symptom burden

Cross-sectional Spearman correlations between RVA parameters and patient-reported symptom measures (PCS Severity Score, C19-YRS, GAD-7, PHQ-9, FSS) were weak to modest at both baseline and follow-up (Fig. 3). At baseline, only CRVE showed a nominally significant association, with the PCS Severity Score ($\rho = 0.22$, unadjusted $p < 0.05$), which did not survive FDR correction. At follow-up, CRVE showed nominally significant positive correlations with the PCS Severity Score ($\rho = 0.30$), C19-YRS ($\rho = 0.38$), and FSS ($\rho = 0.23$; all unadjusted $p < 0.05$), indicating that wider venous caliber was cross-sectionally associated with greater symptom burden at this time point; none of these correlations survived correction for multiple testing (Benjamini-Hochberg FDR, all $q \geq 0.05$). No other RVA parameter – including CRAE, AVR, arteriolar FID or venular FID – showed a significant correlation with any PROM at either time point.

In univariable and multivariable linear regression models (adjusted for age, sex and the corresponding baseline PROM), baseline RVA parameters showed little predictive value for symptom burden at six-month follow-up (Supplementary Table S7). Of 26 predictor–outcome combinations tested, only baseline venular FID showed a nominally significant adjusted association with follow-up Fatigue Severity Scale score ($\beta = -0.120$ per percentage point, 95% CI -0.223 to -0.017, $p = 0.023$); this association did not survive FDR correction ($q = 0.565$) and should be regarded as exploratory. No other baseline RVA parameter was significantly associated with any follow-up symptom measure after adjustment. Six-

month change scores (Δ RVA) were likewise unrelated to change scores in PROMs (all $q \geq 0.66$; Supplementary Table S6).

Associations with circulating biomarkers

Among 19 circulating inflammatory, endothelial and neuroaxonal biomarkers examined in relation to RVA parameters (Supplementary Table S9), the most consistent association was an inverse correlation between VCAM-1 and CRAE, which reached significance after correction for multiple testing at follow-up ($\rho = -0.38$, $p < 0.001$, FDR $q = 0.040$) and showed a consistent direction and magnitude at baseline ($\rho = -0.33$, $p < 0.001$, $q = 0.061$), narrowly missing the pre-specified significance threshold at this earlier time point. No other biomarker–RVA or biomarker–PROM association reached significance after correction for multiple comparisons at both time points; nominally significant associations are listed in Supplementary Table S9 for completeness, and sex-stratified exploratory associations in Supplementary Table S10. Exploratory interaction analyses testing whether baseline RVA–outcome associations differed by ME/CFS status yielded several nominally significant interaction terms (e.g., baseline CRAE $\times$ ME/CFS for follow-up GAD-7, $p = 0.003$) but none survived FDR correction (all $q \geq 0.08$; Supplementary Table S8).

Exploratory comparison of RVA trajectories by ME/CFS status

As 62 of 101 PCS patients with available ME/CFS status (61.4%) fulfilled Canadian Consensus Criteria for ME/CFS, we performed an exploratory comparison of RVA trajectories stratified by ME/CFS status (Fig. 4; Supplementary Tables S11–S13). Descriptively, CRAE and AVR increased within both subgroups. Neither functional parameter changed significantly within either subgroup: venular FID remained stable in both patients without ME/CFS ($3.56 \pm 2.66\%$ to $3.82 \pm 2.72\%$, Wilcoxon $p = 0.238$, $n = 33$) and those with ME/CFS ($3.73 \pm 1.96\%$ to $3.78 \pm 2.72\%$, $p = 0.441$, $n = 49$), consistent with the absence of within-PCS change in the overall cohort described above.

Formal tests of a time $\times$ ME/CFS interaction in linear mixed models did not reach significance for any RVA parameter, including venular FID (interaction $\beta = -0.18$, $p = 0.766$, FDR $q = 0.766$; Table 4), and cross-sectional comparisons between ME/CFS and non-ME/CFS patients at either time point were likewise non-significant after adjustment (all $q \geq 0.40$; Supplementary Table S13). The only nominal, uncorrected between-group difference was a lower AVR in ME/CFS patients across both time points combined (age- and sex-adjusted $\beta = -0.028$, $p = 0.031$), consistent with the lower AVR previously reported in ME/CFS relative to non-ME/CFS PCS patients in the baseline characterization of this cohort. Given the modest subgroup sizes, these findings should be regarded as hypothesis-generating rather than confirmatory, and the primary longitudinal findings reported above are not attributable to the ME/CFS subgroup alone.

Table 4

Linear mixed-effects model: time $\times$ ME/CFS interaction for retinal vessel analysis parameters, adjusted for age and sex. No interaction reached significance after FDR correction. CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; AVR = arteriolar-to-venular ratio; FID = flicker-induced dilation; ME/CFS = myalgic encephalomyelitis/chronic fatigue syndrome; FDR = false discovery rate. Full model coefficients (time and ME/CFS main effects) are provided in Supplementary Table S12.

Parameter	Interaction β	p	q (FDR)
CRAE (μm)	1.54	0.499	0.624
CRVE (μm)	-2.03	0.242	0.403
AVR	0.012	0.215	0.403
Arteriolar FID (%)	-0.45	0.390	0.651
Venular FID (%)	-0.18	0.766	0.766

4. Discussion

Principal findings

Retinal structural and functional parameters followed distinct six-month trajectories. Arteriolar caliber increased, while both functional (FID) parameters remained unchanged. vFID was the most consistently abnormal parameter across all analyses: it was reduced at baseline, remained reduced at follow-up, and this between-group difference was stable across matched, unmatched, and 1:1-matching sensitivity analyses, even though the deficit itself did not widen further within the PCS cohort. Retinal findings were largely independent of symptom burden.

A structure–function dissociation

Static and dynamic RVA parameters capture different properties of the retinal vessel wall. One is structural; the other depends on acute nitric-oxide-mediated signaling [14]. Arteriolar caliber changed over six months. Neither FID parameter did. This argues against treating RVA as a single composite readout of vascular health in PCS. Structural and functional domains appear to carry at least partially independent information and may warrant separate tracking in future work. The six-month changes in arteriolar and venular FID were moderately correlated with each other ($\rho = 0.38$, $q = 0.003$; Supplementary Table S5), consistent with a shared functional trajectory. Neither caliber parameter's change was associated with either FID parameter's change (Supplementary Table S5). This null result is more informative than the absence of change alone. It suggests the two domains did not just fail to change together – they also did not co-vary. This is consistent with independent trajectories. However, the modest sample size limits how confidently smaller correlations can be excluded. AVR is calculated as

CRAE/CRVE, so its correlation with the change in CRAE follows mathematically and is not independently informative.

vFID is a functional vasodilatory response, not a direct measurement of endothelial cells or molecules. We therefore describe it as a surrogate marker of nitric-oxide-dependent vascular signaling rather than as direct evidence of endothelial cell dysfunction; the terms are used loosely as near-synonyms elsewhere in this literature, but the distinction matters for how the present findings should be read.

We cannot offer a mechanistic explanation for the direction of the caliber change. An account based on resolution of early post-acute vasoconstriction is not tenable here, given a median time since infection exceeding 15 months at baseline [23]; whatever process underlies this change, it is not an early recovery phenomenon in this cohort. The inverse correlation between VCAM-1 and arteriolar caliber, present at both time points, is compatible with continued endothelial activation accompanying caliber change, but it cannot establish direction or rule out a shared upstream process, and should be read as hypothesis-generating rather than explanatory.

Relation to prior findings

The venular functional deficit identified in the baseline characterization of this cohort [23] appears not to be self-limiting: it persisted, unchanged, through six-month follow-up, remaining significantly below the level observed in recovered individuals. The arteriolar structural deficit did not follow the same course, instead moving toward the values seen in recovered controls. This asymmetry could not have been anticipated from cross-sectional data alone. One abnormality persisted without improvement; another attenuated. A single label such as “persistent endothelial dysfunction” may therefore be too coarse. At least two distinguishable processes, with different time courses, appear to be involved. Structural and functional RVA parameters have separately shown prognostic value for cardiovascular outcomes in other populations [15, 16], and accelerated vascular ageing after COVID-19 has been reported more broadly [11]. Whether the venular deficit observed here carries comparable prognostic weight is untested and would require outcome data beyond the scope of this study.

This uncertainty should be weighed against direct evidence that individuals with long COVID face an elevated risk of incident cardiovascular disease. In a population-based cohort of over 1.2 million individuals, those with a long COVID diagnosis had a substantially higher cumulative incidence of cardiovascular events – including cardiac arrhythmias and coronary artery disease – than matched individuals without long COVID, an effect present even among those who had not been hospitalized for acute infection [32]. This does not establish that venular FID specifically predicts these outcomes in PCS, but it does establish that the population in which this deficit was observed carries a measurably elevated cardiovascular risk, lending plausibility to further investigation of RVA parameters as candidate markers of this risk.

Retinal microvascular change and symptom burden

Retinal microvascular change was largely uncoupled from patient-reported symptom burden, both cross-sectionally and over time. A biologically demonstrable abnormality need not track with subjective disease burden, and this argues against treating RVA parameters as symptom surrogates. Why this uncoupling occurs cannot be determined from the present data. Retinal microvascular change may index a physiological process not directly perceived by patients, or the symptom measures used here may capture domains not mechanistically linked to venular endothelial function.

ME/CFS subgroup comparison

Neither the persistence of the venular deficit nor the stability of the other RVA parameters differed meaningfully by ME/CFS status: within-group trajectories were similarly flat in patients with and without comorbid ME/CFS, and the formal interaction test found no evidence of a differential trajectory for any parameter. This argues against ME/CFS status as a major driver of the retinal findings reported here, though with a non-ME/CFS subgroup of this size we cannot exclude a smaller difference than this design was powered to detect. PCS and ME/CFS are frequently managed separately despite overlapping presentation and, in this cohort, substantial diagnostic overlap; the absence of a clear RVA-based distinction between them here does not rule out such a distinction emerging in larger samples.

Clinical implications

Because structural and functional domains changed independently, a single summary RVA parameter would have missed the pattern reported here. Venular FID was the most consistent finding across sensitivity analyses and, being non-invasive and quick to acquire, is a reasonable candidate for repeated tracking; its weak relationship with symptom burden means it would supplement rather than replace patient-reported outcome measures, not substitute for them. A minimal clinically important difference for vFID has not been established in PCS or in comparable post-viral populations, so the statistical robustness of the present finding should not be read as a statement about its clinical magnitude.

Limitations

Recovered controls were assessed only at baseline and were not reassessed at six months; between-group comparisons at follow-up therefore necessarily contrast PCS follow-up data against recovered baseline data rather than a contemporaneous measurement, so temporal trends, measurement drift, seasonal effects, or changes in the recovered group over the same interval cannot be excluded. This was an observational study and cannot establish causal relationships between the findings reported here. Because PCS patients started below the recovered reference level and moved toward it without exceeding it, regression to the mean is a plausible statistical contributor to the observed caliber increase; with no repeat measurement in the recovered group, a parallel drift in that group over the same interval also cannot be excluded. Six months may be too short an interval to determine whether either trajectory is stable, reversible, or clinically consequential; longer follow-up would be needed to address this. Roughly one in five PCS patients did not complete follow-up RVA assessment, though completers and non-completers did not differ on the baseline characteristics examined. The ME/CFS comparison

was likely underpowered, and biomarker and sex-stratified analyses were exploratory given the number of comparisons performed. This is a single-center cohort, and replication elsewhere is needed.

Conclusion

Structural and functional retinal microvascular parameters did not change in parallel over six months in this PCS cohort, indicating that these domains should be assessed separately rather than as a single composite measure. Venular endothelial function was the most consistent abnormality identified, yet its limited relationship with symptom burden suggests it captures a dimension of post-viral vascular change not reflected in patient-reported outcomes or in objective muscle function [25]. Whether this dissociation extends to related post-infectious conditions, including ME/CFS, and what it implies for long-term cardiovascular risk, remain open questions for future work.

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 (Klinikum rechts der Isar; approval number 2022-317-S-SR). The study was conducted in accordance with the Declaration of Helsinki and all participants provided written informed consent prior to inclusion.

Consent for publication

Not applicable.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This study is sponsored by the Bavarian State Ministry of Science and Art, as part of its Special Funding Program for the Scientific Coronavirus Research Projects of the Technical University of Munich School of Medicine at the University Medical Center “Rechts der Isar” (Funding Identification Number H.4001.1.7–53/7). Furthermore, this study received Funding from the VADYS-ME Research Program.

Author Contributions

This study was conceptualized and designed by T. W. and C. S. The statistical analysis was conducted by T.W. and M.W. Laboratory measurements were conducted by A.R. and I.L. The manuscript was drafted by M.W. All authors reviewed the manuscript.

Acknowledgments

We want to thank all the study participants for their active participation, which made this study possible.

Availability of Data and Materials

The data underlying this manuscript will be shared upon reasonable request to the corresponding author. All shared data will be fully anonymized/de-identified to ensure participant privacy in accordance with institutional and GDPR regulations.

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Figures

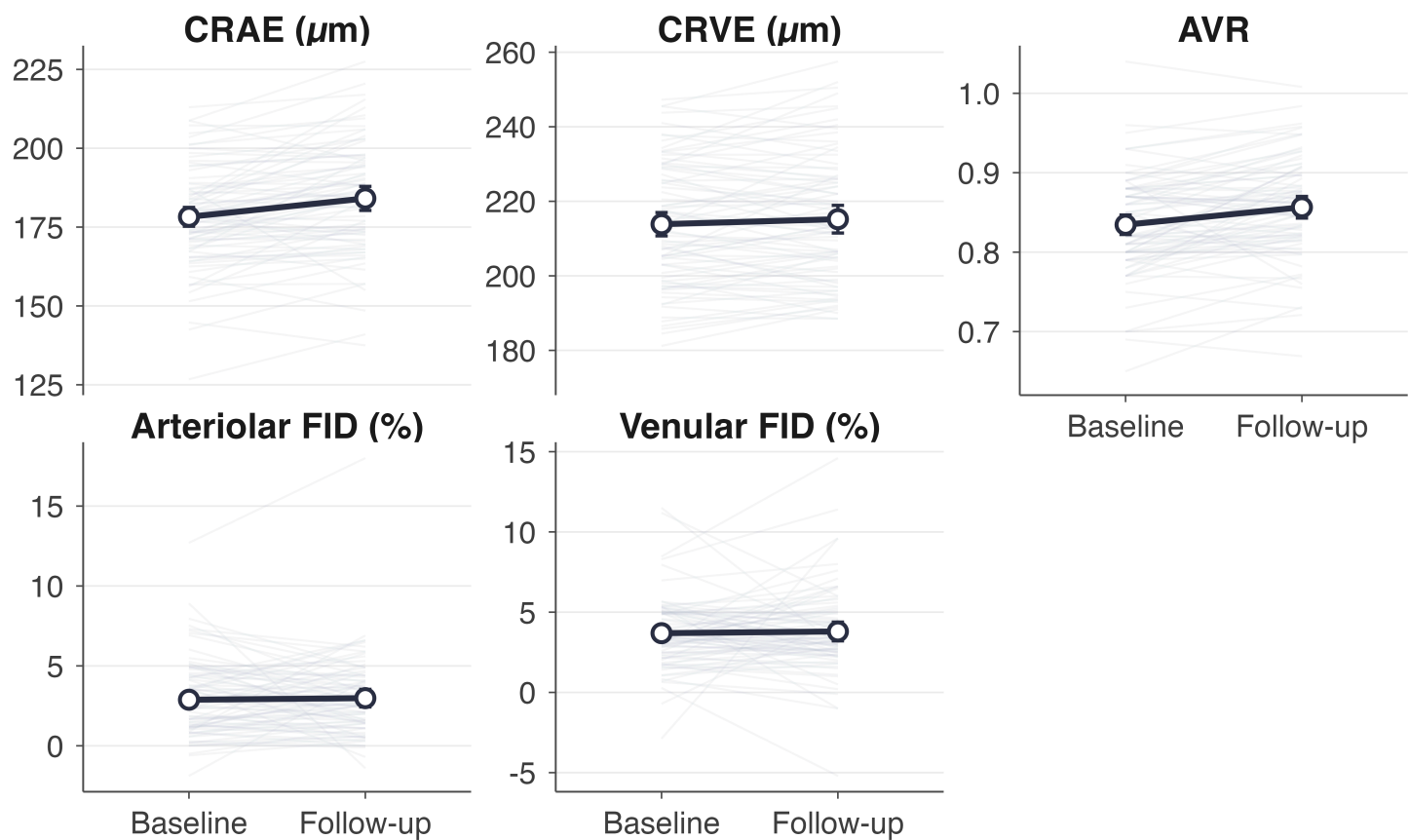


Figure 1

Individual longitudinal trajectories (thin grey lines) and estimated group means with 95% confidence intervals (dark blue) for retinal vessel analysis parameters between baseline and six-month follow-up in patients with post-COVID-19 syndrome (PCS). CRAE and AVR increase over time, while CRVE and both FID parameters remain stable. PCS = post-COVID-19 syndrome; CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; AVR = arteriolar-to-venular ratio; FID = flicker-induced dilation.

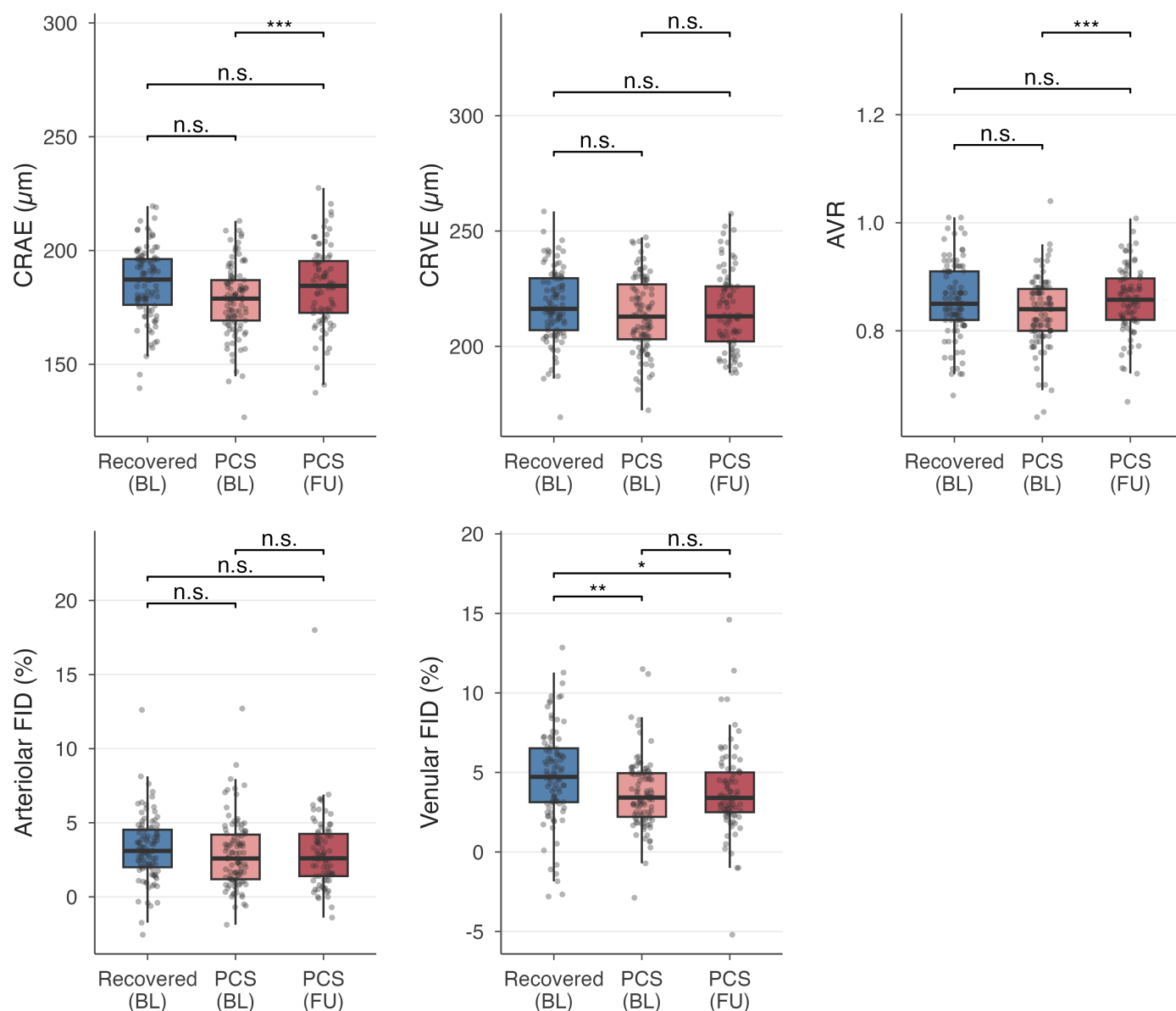


Figure 2

Retinal vessel analysis parameters in recovered individuals at baseline (BL), PCS patients at baseline, and PCS patients at six-month follow-up (FU); points and boxplots show the full, unmatched cohort. Boxplots indicate median, interquartile range and individual data points. Between-group comparisons (Recovered BL vs. PCS BL; Recovered BL vs. PCS FU) were derived from covariate-adjusted linear models (age, sex, mean arterial pressure and body-mass index for caliber parameters; age, sex and body-mass index for FID parameters) with HC3 robust standard errors, fitted to the full cohort; the within-PCS comparison (BL vs. FU) was derived from a paired t-test. An equivalent figure based on the propensity score-matched analysis is provided in Supplementary Figure S1. n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; AVR = arteriolar-to-venular ratio; FID = flicker-induced dilation; HC3 = heteroskedasticity-consistent robust standard errors.

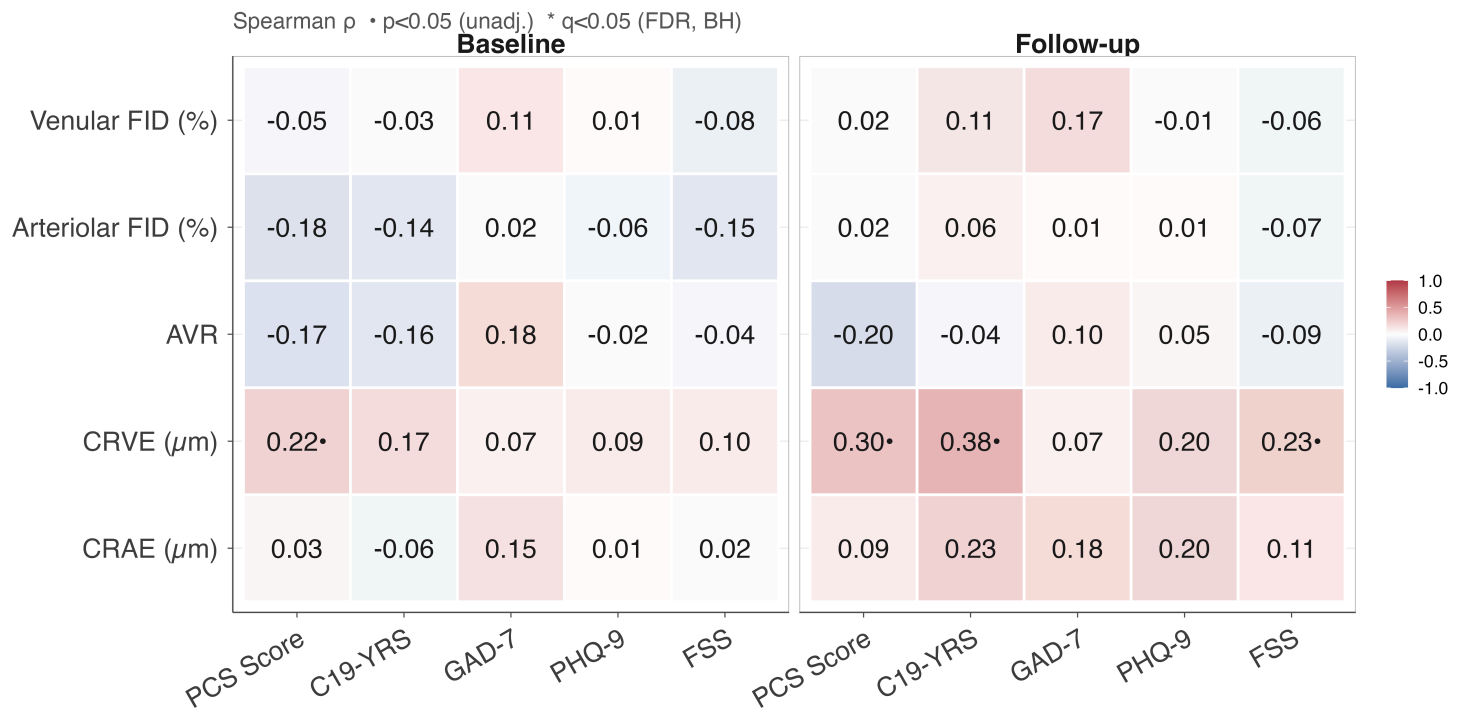


Figure 3

Cross-sectional Spearman correlations between retinal vessel analysis parameters and patient-reported outcome measures at baseline and follow-up in the PCS cohort. Numbers indicate Spearman's ρ ; dots denote nominal significance (unadjusted $p < 0.05$), asterisks denote FDR-corrected significance ($q < 0.05$, Benjamini-Hochberg). PCS Score = PCS Severity Score; C19-YRS = COVID-19 Yorkshire Rehabilitation Scale; GAD-7 = Generalized Anxiety Disorder-7; PHQ-9 = Patient Health Questionnaire-9; FSS = Fatigue Severity Scale; CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; AVR = arteriolar-to-venular ratio; FID = flicker-induced dilation; FDR = false discovery rate.

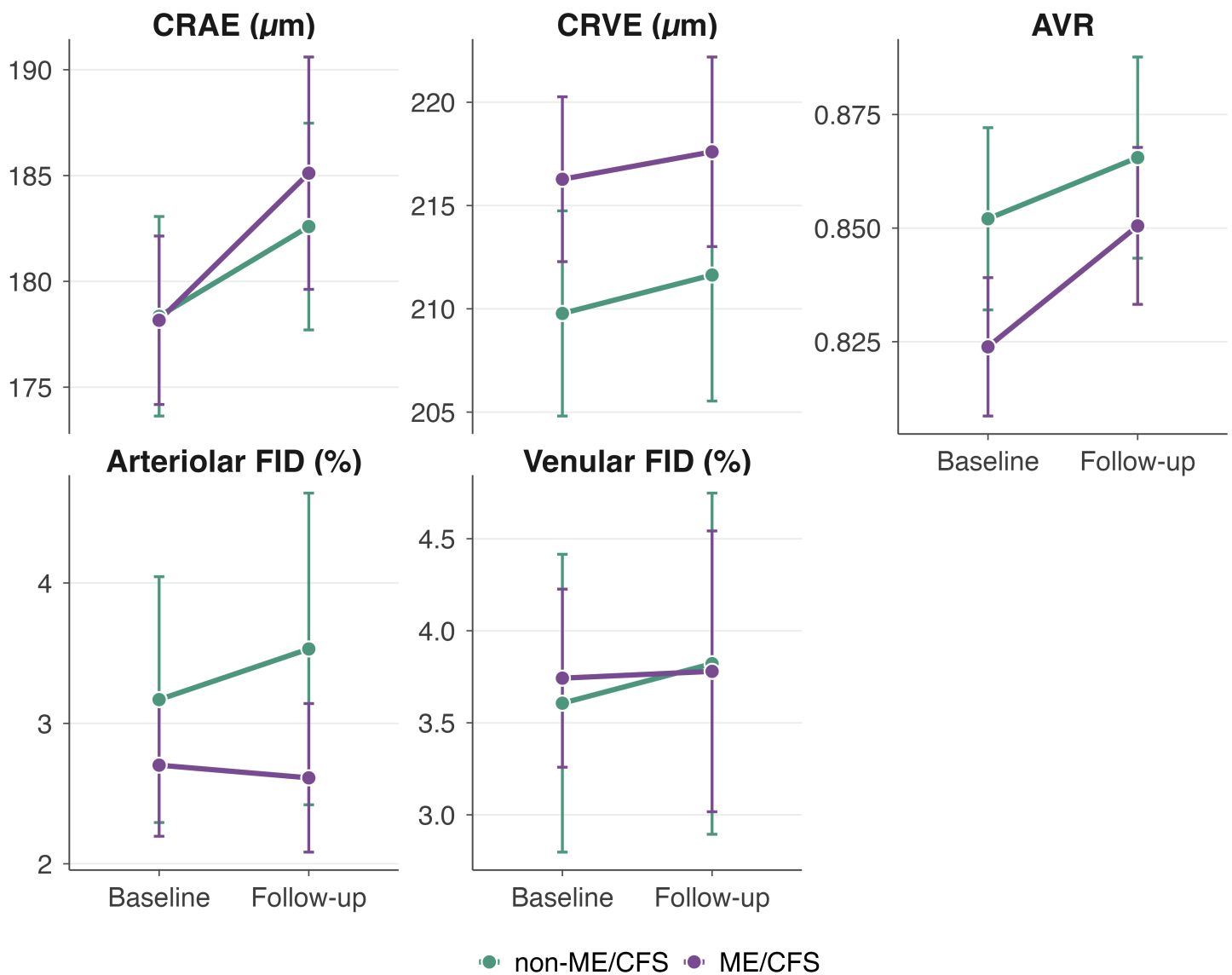


Figure 4

Retinal vessel analysis parameters between baseline and six-month follow-up, stratified by ME/CFS status (Canadian Consensus Criteria) within the PCS cohort. Points and error bars show estimated means $\pm$ 95% CI. Non-ME/CFS, n = 39 at baseline; ME/CFS, n = 62 at baseline (subgroup n per parameter and time point in Supplementary Table S11). CRAE = central retinal arteriolar equivalent; CRVE = central retinal venular equivalent; AVR = arteriolar-to-venular ratio; FID = flicker-induced dilation; ME/CFS = myalgic encephalomyelitis/chronic fatigue syndrome.

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