Title: “Vascular Inflammation in Neuropsychiatric Long COVID”

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ABSTRACT (300/300)

Background: Neuropsychiatric symptoms are prominent and can have a substantial functional impact in many people with Long COVID (LC). The underlying pathophysiology of neuropsychiatric LC (N-LC) is unknown.

Methods: 28 individuals with acute COVID-19 (AC), 50 N-LC (new or worsening neuropsychiatric symptoms >3 months after COVID-19), and 29 post-COVID-19 controls with no LC (>3 months prior) were enrolled. Participants underwent cross-sectional blood testing, and the N-LC and control groups underwent neuropsychiatric testing, including verbal learning and memory, fluency, processing speed, and mental health assessments. Fourteen soluble biomarkers of vascular health were measured in plasma. ANCOVA testing with a Benjamini-Hochberg procedure was used to compare biomarkers between groups adjusting for co-morbidities.

Findings: Participants with N-LC and controls were similar demographically, while the AC group had higher rates of obesity and hypertension. Biomarkers of leukocyte adhesion to the endothelium and endothelial inflammation were elevated in N-LC compared to controls, including L-selectin, ADAMTS13, sP-selectin, and sICAM-1, whereas coagulopathy measures (D-dimer, fibrinogen) did not differ. Most biomarkers were highest in AC and lower in N-LC (AGP, CRP, haptoglobin, SAA, ADAMTS13, PF4, sP-selectin, sVCAM-1, and D-dimer). However, three biomarkers were highest in N-LC compared to AC: Fetuin (vascular calcification), L-selectin (leukocyte adhesion), and α-2 macroglobulin (endothelial adhesion). In N-LC, higher sP-selectin was strongly associated with lower fluency and verbal learning. Lower AGP was strongly associated with lower verbal memory, verbal learning, fluency, mood, and anxiety.

Interpretation: Alterations in biomarkers of vascular inflammation strongly associate with the presence of N-LC. Biomarkers of endothelial adhesion and vascular calcification are only elevated in N-LC compared to both groups, suggesting a pathophysiology distinct from the resolving effects of AC. Biomarkers related to endothelial adhesion and systemic inflammation associate with specific cognitive domains, linking vascular inflammation with brain function. Identifying abnormalities in vascular endothelial function, calcification, and remodeling may lead to therapeutic targets for N-LC.

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INTRODUCTION

Early in the pandemic it became apparent that individuals with acute COVID-19 (AC) were at an increased risk of macro- and microvascular complications leading to significant morbidity and mortality.[1] Biomarkers of endothelial and platelet dysfunction are highly elevated in individuals with AC, including increased fibrinogen, D-dimer, and von Willebrand factor.[2,3] Thromboinflammation in AC is complex and involves coagulopathy, thrombocytopathy and endotheliopathy.[4] Signs of thromboinflammation such as microvascular injury, fibrinogen leakage, microhemorrhages, and microvascular congestion have been associated with neurologic manifestations such as stroke, seizure, and encephalopathy in AC.[5]

The most common neuropsychiatric Long COVID (N-LC) symptoms include “brain fog,” cognitive dysfunction, headache, dizziness, dysautonomia, neuropathy, altered sleep, and altered mood. Individuals with N-LC demonstrate global cognitive impairment as well as domain-specific impairment in executive functioning, processing speed, attention, category fluency, memory encoding, recall, and verbal fluency.[6,7] Additional symptoms include “brain fog”, altered sleep, and peripheral neuropathy.

Preliminary studies have suggested that cerebral microvascular dysfunction may contribute to N-LC. White matter hyperintensities on brain MRI consistent with small vessel disease (SVD) have been shown in individuals with N-LC, and white matter hyperintensity load has been correlated with verbal memory deficits two months after an AC hospitalization.[8-10] Arterial spin labeling MR perfusion (ASL) reveals reduced cerebral blood flow (CBF) in the frontal, parietal, and temporal cortices in individuals with N-LC cognitive impairment compared with age and sex matched healthy controls.[11] Additionally, microvascular and endothelial dysfunction has been linked to the pathogenesis of fatigue and impaired exercise tolerance in LC.[12] A controversy in the field is whether coagulopathy and “microclots” may be playing a role .

Since the start of the pandemic, numerous studies demonstrate cognitive deficits in individuals after COVID-19. A variety of subjective and objective testing instruments have been used and, in most cases, there are no controls. Several recent studies have demonstrated objective cognitive dysfunction in N-LC on standardized neuropsychological testing, negative impacting quality of life.[13] Depending on the severity, one or more domains may be affected and typically include episodic memory, attention, working memory, processing speed, executive dysfunction, and verbal fluency.[14-17] Common co-morbid symptoms include fatigue, anxiety, and sleep disturbances. Preliminary studies have reported clinical benefits to cognitive interventions and rehabilitation.[13,18]

A recent study has found that approximately 6.9% of US adults, 8.6% of all females and 5.1% of males, have had LC, which represents almost 18 million Americans.[19] Despite the high prevalence of LC, relatively little is known about the underlying pathophysiology and the long-term health implications. LC continues to cause significant disability, especially N-LC, and treatments are urgently needed.[20] N-LC is a pressing public health issue that patients, providers, and researchers are eager to understand. A key question is whether the vascular inflammation and pathology present in some people with AC continues and contributes to the pathophysiology of N-LC.[21] We compared biomarkers of vascular inflammation among individuals with AC, N-LC, and post-COVID controls with no LC, demonstrating that endothelial dysfunction may contribute to both objective and subjective cognitive impairment in individuals with LC.

METHODS

Study Participants

This case-control study enrolled participants at Yale University in New Haven, Connecticut. Three groups were prospectively enrolled: 1. 28 individuals with acute COVID-19 (AC) cross-sectionally enrolled in the Yale IMPACT study of individuals hospitalized with AC;[22] 2. 50 individuals with N-LC (new or worsening neuropsychiatric symptoms >3 months after laboratory confirmed COVID-19); 3. 29 control participants (history of COVID-19 >3 months prior with no LC symptoms) enrolled in the longitudinal, outpatient-based COVID Mind Study at Yale. Participants with N-LC and controls were recruited from the NeuroCOVID Clinic at Yale and the community and underwent clinical assessment (surveys, medical chart review) and blood collection. Exclusion criteria for N-LC and controls included age <18, pregnancy, major neurologic illness, psychotic disorder, and anticoagulant use. Participants hospitalized with AC had no history of dementia, thrombotic event, or coagulopathy during hospitalization. A SARS-CoV-2 infection was confirmed for all participants with positive SARS-CoV-2 PCR or rapid antigen testing between March 2020 and October 2023.

These studies were approved by the institutional review board at Yale University (HIC numbers for HARC and IMPACT) and all participants provided either phone or written consent. De-identified data sharing is available subject to approval. This study followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

Cognitive and Symptom Assessments
The N-LC and post-COVID-19 control participants underwent cognitive testing and completed mental health questionnaires. Individuals hospitalized with AC did not undergo cognitive testing. To assess cognition, we used the Global Neuropsychological Assessment (GNA) from which we administered four subtests (Story Learning and Memory, Perceptual Comparison, Verbal fluency-category fluency [animals]). The primary outcome measure for verbal learning was the total correct on the immediate story recall trial, for verbal memory the total correct response on the delayed story recall trial, for processing speed total correct minus errors on the Perceptual Comparison test, and for fluency the total animals correctly generated. Higher scores on each of these outcomes equate to better performance. Participants also completed the General Anxiety Disorder 7 (GAD-7), a measure of anxiety symptoms, and the Patient Health Questionnaire 9 (PHQ9), a measure of depressive symptoms. The total score on the GAD-7 and PHQ-9 were used for analysis.

Laboratory Methods
Soluble biomarkers were measured in frozen plasma aliquots by analytic services at Eve Technologies (Calgary, Alberta, Canada). Two commercially available arrays of cardiovascular disease related analytes were used: the Human Cardiovascular Disease Panel 3 9-PLEX Discovery Assay ® Array (HDCVD9) and the Human Cardiovascular Disease Panel 2 6-PLEX Discovery Assay ® Array (HDSAA6). Samples were tested using multiplex bead-based ELISA for the following analytes: α1-acid glycoprotein (AGP), α-2 macroglobulin, ADAMTS13, C-reactive protein (CRP), D-dimer, Fetuin A36, fibrinogen, haptoglobin, L-selectin, platelet factor 4 (PF4), serum amyloid protein (SAP), serum amyloid A (SAA), soluble intercellular adhesion molecule-1 (sICAM-1), soluble platelet selectin (sP-Selectin), and soluble vascular cell adhesion molecule-1 (sVCAM-1) 100uL samples were run in duplicate with quality control samples run in every assay session. All biomarkers were fully detected except for seven out of upper range values for SAA in the AC group. The upper limit of detection was used.

Statistical Analysis
Kruskal-Wallis and Fisher's exact tests were used to compare demographics features between all three groups when available and between N-LC and controls for remaining features. A Benjamini-Hochberg procedure was performed for each biomarker comparing all groups. After this adjustment for multiple comparisons, all biomarkers were significantly different between the three groups and included in the analysis. The data from each biomarker was evaluated for normality. Fibrinogen, L-selectin, SAP, and ADAMS13 were all normally distributed. The remaining biomarkers were log transformed for analysis. A confounder analysis was performed and identified age, BMI, and type 2 diabetes as significant confounders. ANCOVA testing was used to compare all groups for each biomarker adjusting for age, BMI, and type 2 diabetes. A Benjamini-Hochberg procedure was applied to the resulting p-values to adjust for multiple comparisons. Then between group comparisons were made adjusting for age, BMI, and type 2 diabetes. The resulting F-values were then adjusted using the residual error and degrees of freedom from the overall model to generate an adjusted p-value for each biomarker.

For analysis of the relationships between biomarkers and cognitive and mental health endpoints, we initially conducted a series of Pearson correlations in the total sample and by group. A series of partial correlations were then conducted to examine the relationships among the total sample and each group after adjusting for relevant covariates which included age, BMI, and type 2 diabetes. A Benjamini-Hochberg procedure was applied to the resulting p-values to adjust for multiple comparisons.

RESULTS
50 N-LC and 29 post-COVID-19 controls with no LC symptoms were enrolled in The COVID Mind Study for this project. Samples from 28 participants enrolled during AC were selected for comparison analysis based on matched age and gender with the N-LC group. Fatigue (82%), poor concentration (74%), post-exertional malaise (74%), word finding difficulty (72%), poor memory (72%), and anxiety (62%) were the most frequently reported post-COVID-19 symptoms in the N-LC group (**Figure 1**). The N-LC and control groups were similar demographically, in terms of age, gender, race, vascular risk factors, and medication history (**Table 1**). The

participants with N-LC had a median age of 49 [IQR 40-62], 70% female (n=35), and 82% white (n=41), while the AC group had a median age of 49 [IQR 43-54], 61% female (n=17), and 54% white (n=15). The control participants with no LC had a median age of 41 [IQR 31-55], 72% female (n=21; p=0.99), and 69% white (n=20; p=0.21). No participant in the control group was hospitalized for AC compared to 18% in the N-LC group and 100% in the AC group. In the N-LC group, study visits were performed a median of 378 days [IQR 296—669] after AC, which is similar to 445 days [IQR 322 – 619; p=0.54] in the control group. A higher proportion of the control group were vaccinated prior to infection (control: 62%) compared to the N-LC and AC groups (N-LC: 20% v. AC: 0%; p<0.001) due to vaccine availability at the time of recruitment.

Rates of vascular risk factors were similar between the N-LC and control groups: smoking (N-LC: 4% v. control: 10%; p=0.55), hyperlipidemia (N-LC:20% v. control:7%; p=0.19), diabetes (N-LC:12% v. C:0%; p=0.08), pre-existing cardiovascular disease (N-LC:2% v. control: 0%; p=0.99), and history of alcoholism (N-LC: 4% v. control: 10%; p=0.35). The AC group did have a higher BMI (AC group: 34 v. N-LC: 29 v. control:25; p=0.008) and prevalence of hypertension (AC group: 36% v. N-LC: 22% v. control: 7%; p=0.03) compared to the N-LC and control groups. No participants in the N-LC or control group were taking an anticoagulant and there were similar rates of aspirin/antiplatelet use in these groups (N-LC: 20% v. control: 7%; p=0.19). Data on anticoagulant and antiplatelet use was not available for the AC group.

Group Differences in Vascular Biomarkers

The three groups differed across all 14 biomarkers (**Figure 1**). Each of these biomarkers has several functions in vascular and immune health. We will be classifying them according to the hypothesized role in LC. As expected, in 10 biomarkers, values were highest in the AC group and were lower in the N-LC and control groups. The acute phase proteins, AGP (p=0.0005), CRP (p<0.0001), and haptoglobin (p=0.0003), were markedly elevated in AC compared to N-LC. In AC, D-dimer (p<0.0001) was highly elevated, as expected. Biomarkers of endotheliopathy and platelet activation were markedly elevated in AC compared to N-LC, including ADAMS13 (p=0.001), PF4 (p=0.0001), sP-selectin (p<0.0001), and sVCAM-1 (p<0.0001).

Biomarkers were measured at a median of 8 days [IQR 5-9] during AC and 378 days [IQR 296 – 669] after COVID-19 in LC. sICAM-1 (endothelial adhesion) (p=0.052) and SAP (p=0.51) are similar in the AC and N-LC groups. Lastly, three biomarkers were higher in N-LC group compared to the AC group: Fetuin (p<0.0001) (vascular calcification), L-selectin (leukocyte adhesion) (p=0.002), and α-2 macroglobulin (endothelial adhesion/immune function) (p<0.0001).

Strikingly, there were several differences in biomarker levels between the N-LC and control groups. Acute phase proteins AGP (p=0.0005) and C-reactive protein (p=0.04) were elevated in N-LC compared to controls (**Figure 2**). Biomarkers involved in atherosclerosis were elevated in the N-LC group, including SAP (p=0.0005) and fetuin (p=0.001). Biomarkers of leukocyte endothelial adhesion and endothelial inflammation were elevated in N-LC compared to controls, including L-selectin (p=0.01), ADAMTS13 (p<0.0001), sP-selectin (p=0.0001), and sICAM-1 (p=0.03). Notably, there was no evidence of coagulopathy in N-LC. D-dimer (p=0.15) and fibrinogen (p=0.33) were similar in the N-LC and control groups.

Associations between Biomarkers, Cognition, and Mental Health Symptoms

Compared to controls, the N-LC group demonstrated poorer verbal memory (.38 .13's d=0.61; p=0.02) and processing speed (Cohen's d =0.67; p=0.03); and demonstrated a trend towards lower fluency (Cohen's d= 0.47; p=0.09) compared to the control group (Supplementary Table 2). There was no difference in verbal learning between groups (Cohen's d=0.38; p=0.13). In terms of mood, the N-LC group reported higher anxiety (p=0.0004) and depression (p<0.000001) compared to the control group.

The relationship between the biomarkers and cognitive and mental health endpoints (after adjusting for confounders: age, BMI, and diabetes) was investigated to better understand the clinical relevance of the elevated biomarkers (**Figure 3**). In the total sample, higher sP-selectin (endotheliopathy) was associated with poorer fluency (p=0.00005), processing speed (p=0.0008), verbal learning (p=0.002) and verbal memory (p=0.004) as well as greater anxiety (p=0.02) and depressive symptoms (p=0.001) (**Figure 3a, c**). Higher levels of fetuin (atherosclerosis) and ADAMS13 (endotheliopathy) were both associated with greater anxiety (p's=0.01) and depressive symptoms (p's<0.05). Higher levels of fetuin were also related to poorer fluency (R=-0.27; p=0.04).

Among the N-LC group only, higher levels of sP-selectin were associated with poorer fluency (R=-0.53; p=0.002) and verbal learning (R=-0.42; p=0.02) (**Figure 3b**). **Figure 3d** demonstrates the strong relationship between fluency in both the LC group and whole cohort. Lower levels of AGP were associated with poorer verbal learning (R=0.47; p=0.009) and memory (R=0.56; p=0.001) as well as fluency (R=0.50; p=0.005). Conversely, lower levels of AGP were associated with greater anxiety (R=-0.38; p=0.04) and depressive symptoms (R=-0.41; p=0.03). Lower levels of fetuin were also associated with poorer fluency (R=-0.36; p=0.05). Interestingly, there are several notable associations in the control group. Lower processing speed was associated with higher CRP and sP-selectin. Anxiety and depression symptoms were higher in those with higher ADAMTS13 and sICAM-1.

DISCUSSION

Long COVID currently affects almost 18 million Americans and relatively little is known about the underlying pathophysiology and the long-term health implications.[19] N-LC ("brain fog," headache, neuropathy, altered sleep, altered mood) have a large impact on quality of life and functional capacity.[15] In our dedicated study aimed to reveal the pathophysiology of neurologic symptoms after COVID-19 (N-LC) we observed elevations in blood biomarkers related to systemic inflammation, leukocyte adhesion to the endothelium, endothelial dysfunction, vascular calcification, and vascular remodeling compared to people who had previously had COVID-19 but had no persistent or emergent symptoms. These findings suggest that persistent or emergent neurologic symptoms after COVID-19 are associated with abnormalities of the vascular endothelial function.

We observed two main biomarker patterns between the three groups. The most common pattern demonstrated high levels of the biomarkers in the AC group, moderate elevation in the N-LC group, and lowest in the control group. This pattern was seen in AGP, CRP, haptoglobin, SAA, SAP, ADAMTS13, sP-selectin, and sVCAM-1. AGP is intricately involved in the inflammatory cascade and regulates binding of pathogens and modulating the leukocyte attack sequence.[23] Typically it increases in response to tissue injury, cancer, or infection. CRP is an acute phase protein during the infectious/inflammatory cascade. It is a biomarker of both acute and chronic inflammation and has been directly linked to atherogenesis.[24,25] Several markers that were elevated in N-LC compared to controls are involved in vascular remodeling and atherogenesis, suggesting that N-LC symptoms may relate to vascular regulation or may heighten risk for long-term vascular sequelae. SAP is involved in cardiac remodeling and plays a key role in innate immunity and atherosclerosis.[26,27]

The N-LC group was also characterized by elevation of markers that facilitate enhanced leukocyte adhesion to the endothelium. ADAMTS13 is plasma protease that cleaves circulating von Willebrand factor (vWF) to prevent microvascular platelet clumping. vWF is a glycoprotein involved in platelet and subendothelial collagen adhesion in the vessel wall.[28] Elevated ADAMTS13 indicates ongoing platelet activation. sP-selectin promotes leukocyte adhesion to activated platelets and the endothelium.[29,30] sICAM-1 is an adhesion receptor that is highly expressed on the endothelium and regulates endothelial leukocyte adhesion and TEM. It has also been identified to play a role in immune cell effector functions, pathogen and dead cell clearance, and T-cell activation.[31]

The other pattern between biomarkers we found particularly interestingly was marked elevation in N-LC compared to AC and the control groups. Elevations in L-selectin, Fetuin, and α-2 macroglobulin in N-LC suggests a distinct pathophysiology from the pattern of resolving AC. L-selectin is an endothelial adhesion and signaling molecule that promotes early leukocyte adhesion, chemotaxis, and transendothelial migration (TEM) on the vessel wall.[32,33] Fetuin is involved in vascular calcification and also associated with atherogenesis.[34,35] α-2 macroglobulin is a broad spectrum protease inhibitor that is involved in amplifying ICAM-1 and aiding neutrophil adhesion to the vascular endothelium as well as activating the procoagulant function of factor VIII and von Willebrand glycoprotein.[36,37] It was previously shown to be low in AC and normalize with recovery as we demonstrated here.[38] Lastly, it is important to note that we found no evidence of coagulopathy (D-dimer, fibrinogen) in the N-LC group compared to controls, which aligns with the clinically observed lack of clotting pathology in individuals with N-LC.

Fetuin and two other biomarkers demonstrate key relationships to cognitive and mental health endpoints in the N-LC group and whole cohort. Elevated sP-selectin (endotheliopathy) was associated with poorer verbal learning and fluency. These are both defining clinical characteristics of LC "brain fog." Participants with N-LC describe word finding difficulty, loosing track of their thoughts while speaking, and difficulty recalling conversations and tasks. One possible explanation is that endotheliopathy affects cognitive function through small vessel inflammation and dysfunction in the brain. Interestingly, lower levels of AGP (systemic inflammation) were

associated with poorer verbal learning and memory, and fluency as well as poorer mood and anxiety. These biomarkers are clinically relevant to the N-LC syndrome. From a pathophysiology perspective, these findings align with LC two ways. First, lower concentrations of AGP stimulate mononuclear cell proliferation, particularly T-cells.[39] In LC, there is T-cell immune dysregulation with increased frequencies of CD4+ T cells poised to migrate to inflamed tissues and exhausted SARS-CoV-2-specific CD8+ T cells.[40] Second, lower levels of AGP inhibit platelet aggregation and enhance neutrophil aggregation.[39] In AC, AGP was shown to regulate SARS-CoV-2 infected neutrophil netosis.[41] Neutrophil netosis is the process of forming neutrophil extracellular traps made up of chromatin and bactericidal proteins, and it is triggered by pathogens, antibodies and immune complexes, and cytokines.[42] There is new evidence that neutrophil netosis is persistently elevated in LC.[43,44] Lower AGP levels could be a symptom of excessive platelet and neutrophil aggregation and netosis seen in LC. The link between these biomarkers and cognitive and mental health outcomes is a novel finding. We hypothesize that endotheliopathy in the brain has a significant impact on function of blood brain barrier. In future studies, we hope to directly investigate whether dysfunction of the blood brain barrier is the underlying mechanism leading to cognitive dysfunction. Additionally, using these biomarkers, we may be better able to identify a subset of participants with a specific LC phenotype to target and enroll in clinical trials. Further studies will need to further investigate this line of inquiry using a multi-modal approach.

Limitations

In our study, differences in vascular biomarkers between the N-LC and control groups cannot be attributed to differences in demographics or vascular risk factors given the similarities between enrolled study participants in each group. However, a limitation of our study includes the fact that we did not have complete vascular risk factor and medication information on participants in the AC group. The rate of critical illness and vaccination status were different between the three groups. This related to enrollment of the AC group in 2020 and enrollment of other groups more recently. Vaccination can prevent hospitalization and development of LC. It is unclear how vaccination may affect biomarker levels in participants. Finally, limited sample sizes in all groups may mask significant differences.

Conclusions

The results of this study provide further evidence that the health and function of the vascular endothelium is associated with N-LC. Overall, vascular inflammation appears to have a declining trend between AC, N-LC, and asymptomatic post-COVID-19 participants. This may represents resolving inflammation, but not a coagulopathy. However, as biomarkers related to vascular endothelial adhesion (α-2 macroglobulin, L-selectin) and vascular calcification (fetuin) were distinctly elevated in the N-LC group, the pathophysiology of LC may be distinct from resolving inflammation. Three biomarkers (fetuin, sP-selectin, AGP) also have close associations to cognitive and mental health outcomes, which demonstrates the clinical relevance of this biomarker signature. Further studies will longitudinally investigate biomarkers of neuropsychiatric dysfunction as well as endothelial adhesion and dysfunction in individuals with N-LC. Addressing impaired endothelial leukocyte adhesion, endothelial function, vascular calcification, and vascular remodeling may be avenues towards therapeutics in LC.

Table 1. Demographics and Clinical Characteristics of Study Participants.

	Acute COVID-19 N = 28 Median [IQR] N (%)	**Neuropsychiatric Long COVID** N = 50 Median [IQR] N (%)	**Controls** N = 29 Median [IQR] N (%)	**P-Value Between 3 Groups**
Age (years)	49 [43 – 54]	49 [40 – 62]	41 [31 – 55]	0.06
Female Gender	17 (61)	35 (70)	21 (72)	0.62
BMI (kg/m^2)	34 [27 – 39]	29 [25 – 31]	25 [22 – 32]	0.008**
Race				0.10
Asian	0 (0)	0 (0)	2 (7)	
Black	8 (29)	7 (14)	4 (14)	
White	15 (54)	41 (82)	20 (69)	
Mixed/Other	0 (0)	2 (4)	3 (10)	
Ethnicity				
Hispanic	5 (18)	7 (14)	5 (17)	0.84
Hospitalized for Acute COVID-19	28 (100)	9 (18)	0 (0)	<0.0001***
Days Since Onset of Acute COVID-19	8 [5 – 9]	378 [296 – 669]	445 [322 – 619]	0.54 (2 groups)
Vaccination Status				0.0007**
None	33 (100)	9 (18)	3 (10)	
Prior to infection	0 (0)	10 (20)	18 (62)	
After infection	0 (0)	31 (62)	8 (28)	
Vascular Risk Factors				
Smoking History				0.55
Never	-	33 (66)	17 (59)	
Current	-	2 (4)	3 (10)	
Former	-	15 (30)	9 (31)	
Hypertension	10 (36)	11 (22)	2 (7)	0.03*
Diabetes	7 (25)	6 (12)	0 (0)	0.08
Hemoglobin A1c	6.6 [5.5 – 7.4]	-	-	
Obesity (BMI > 30)	17 (61)	13 (36)	8 (28)	0.03*
Hyperlipidemia	-	10 (20)	2 (7)	0.19
Cardiac Disease	3 (11)	1 (2)	0 (0)	0.11
Excessive Alcohol	-	2 (4)	3 (10)	0.35
Medications				
Aspirin/Anti-Platelet	-	10 (20)	2 (7)	0.19
Anti-Coagulant	-	0 (0)	0 (0)	-
Statin	-	8 (16)	2 (7)	0.31
Anti-Hypertensive	-	9 (18)	2 (7)	0.31
Anti-Depressant	-	16 (32)	7 (24)	0.61

Figure 1. **Neuropsychiatric Long COVID Symptoms**. Detailed frequency of Long COVID symptoms in the neuropsychiatric Long COVID group. PEM = post-exertional malaise.

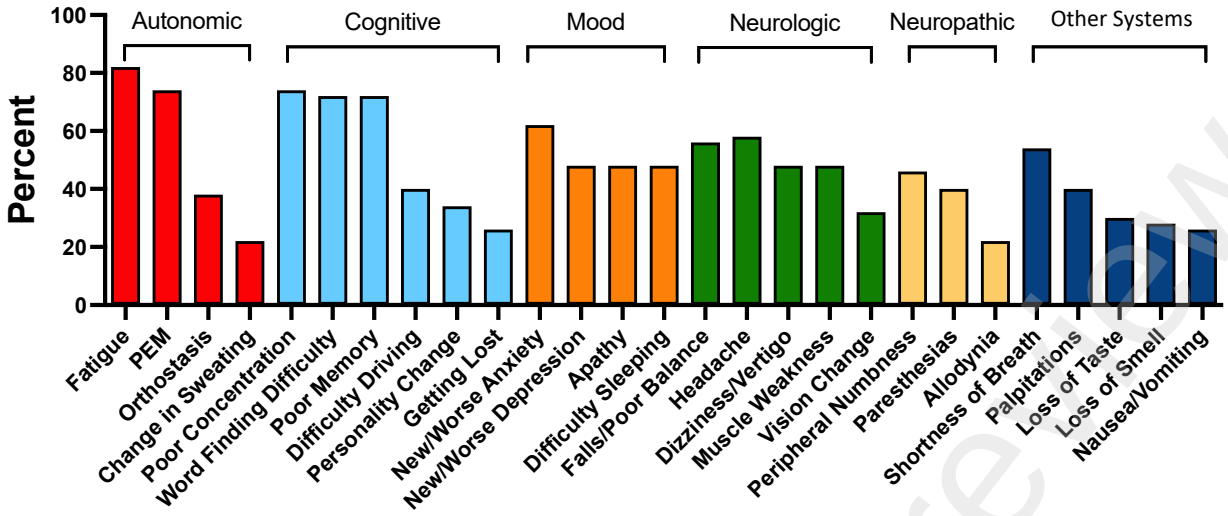
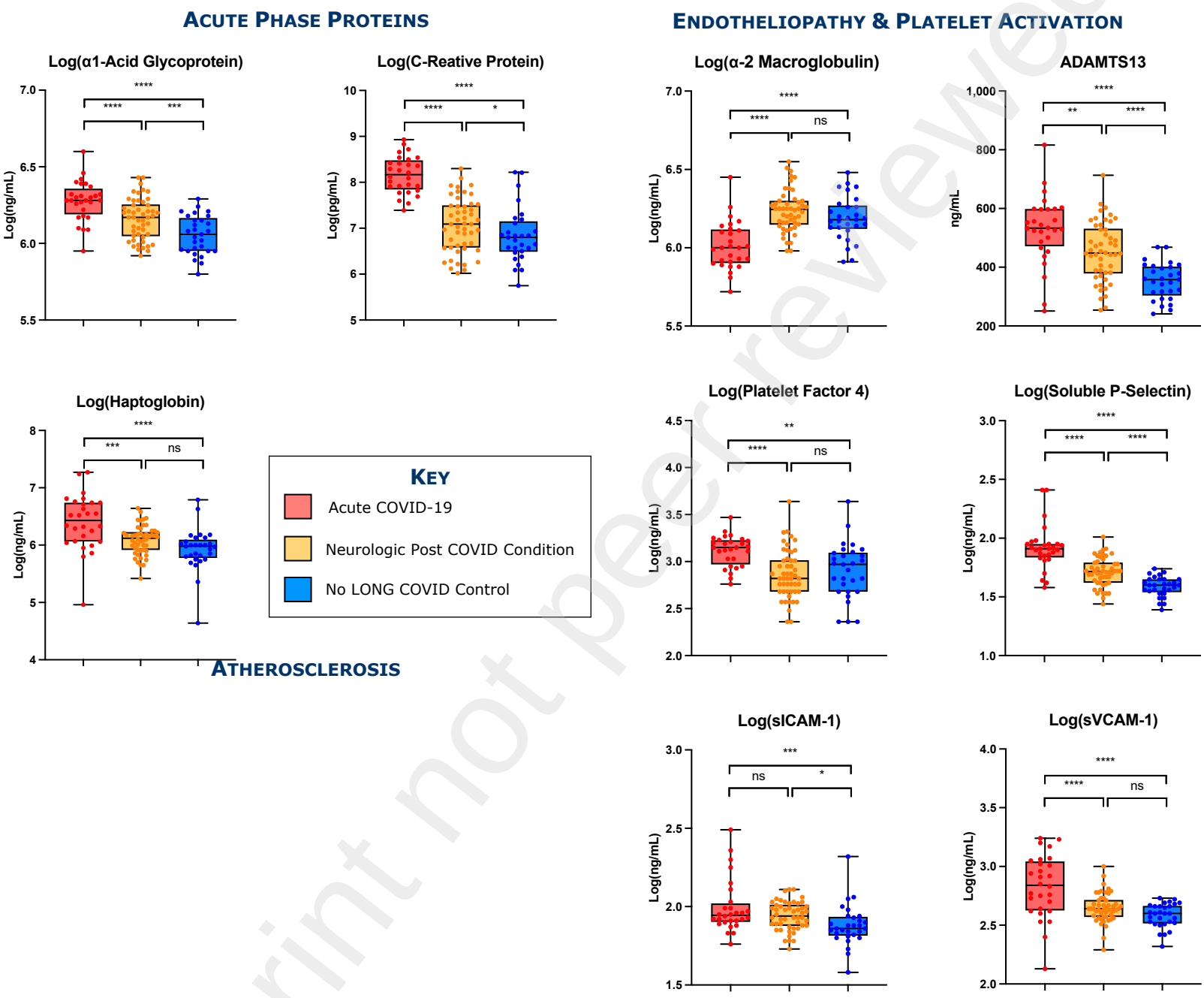
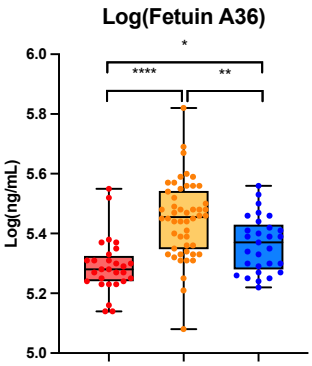
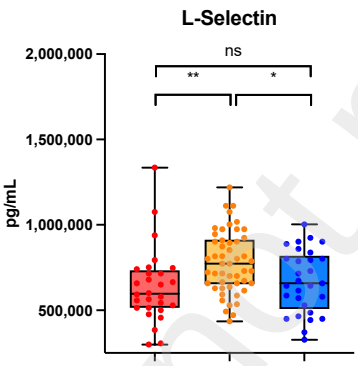
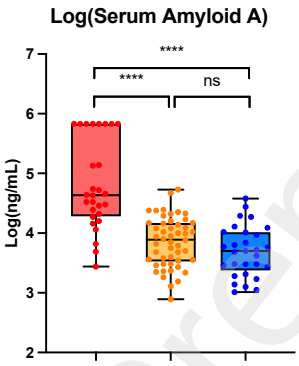


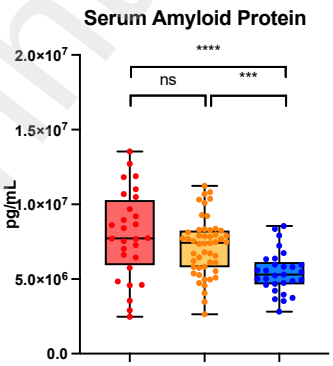
Figure 2. **Plasma Inflammatory Vascular Biomarkers**. Detailed results from the biomarkers in the serum are reported in acute COVID-19 (red; n=28), neuropsychiatric Long COVID (orange; n=50), and control group (blue; n=29). All results have been adjusted for multiple comparisons as well as age, BMI, and diabetes. Several biomarkers have numerous functions related to vascular and immune function. Here we provided a broad classification. (ns, $p>0.05$, * $p\leq0.05$, ** $p\leq0.01$, *** $p\leq0.001$, **** $p\leq0.0001$).



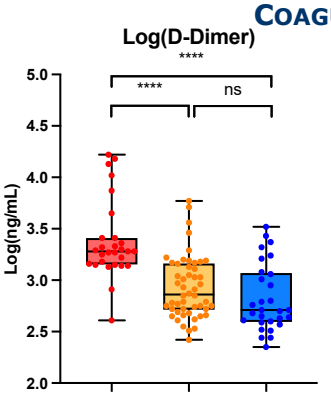








COAGULOPATHY



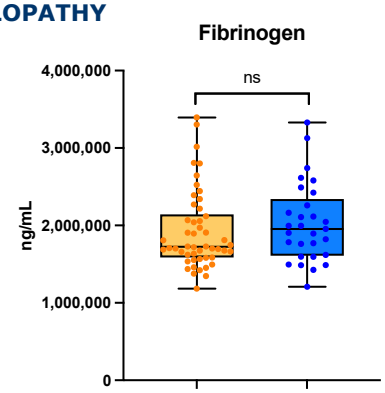
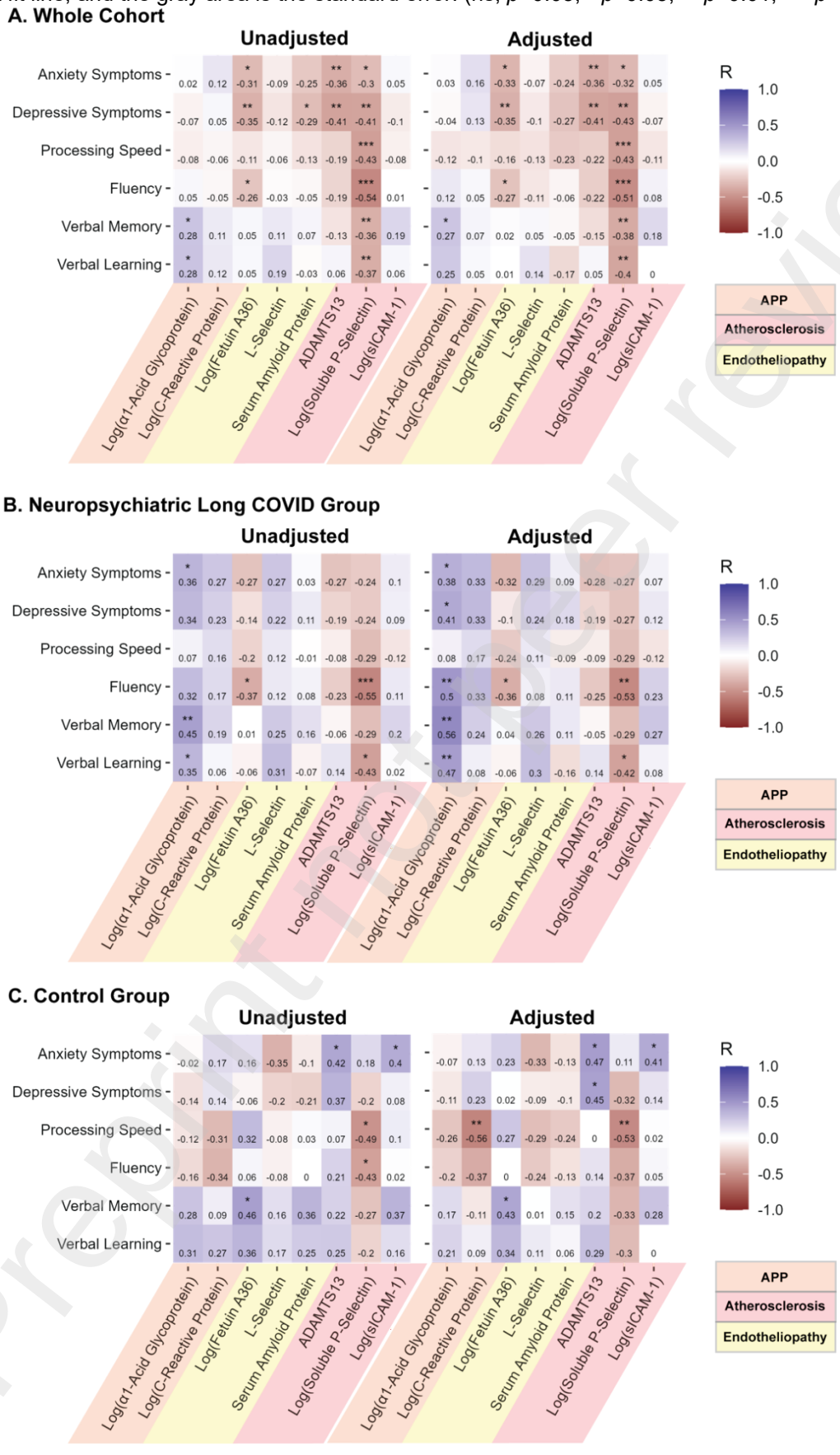
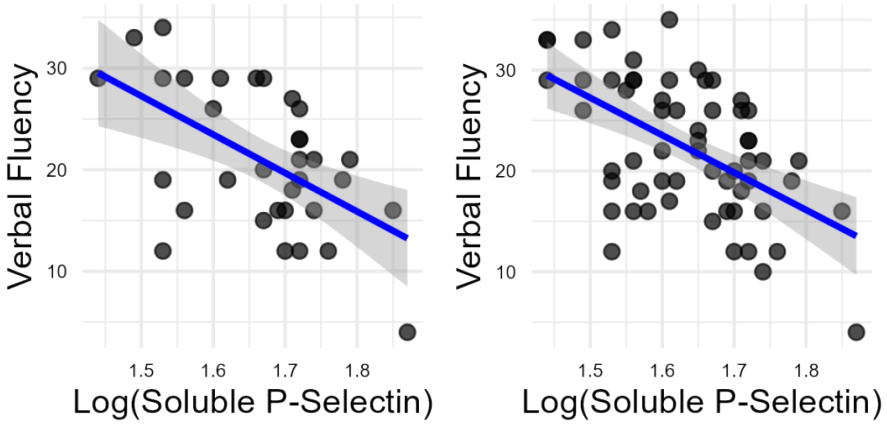


Figure 3. Associations between Vascular Biomarker and neuropsychiatric outcomes. Results are presented unadjusted and adjusted for age, BMI, and diabetes. Detailed results from cognitive and mental health assessments are reported in A) Whole cohort (n=62); B) Neuropsychiatric Long COVID (n=33); C) No Long COVID control group (n=29); D) Relationship between sP-selectin and fluency in neuropsychiatric Long COVID (left) and the whole cohort. The blue line is the best fit line, and the gray area is the standard error. (ns, $p>0.05$, * $p\leq0.05$, ** $p\leq0.01$, *** $p\leq0.001$, **** $p\leq0.0001$).



D.



Supplemental Table 1. **Complete Vascular Biomarker Results**. This table includes all group comparisons overall and between all 3 groups. The overall p-value has been corrected for multiple comparisons with False Discovery Rate (FDR), q < 0.05. (ns, $p>0.05$, * $p\leq0.05$, ** $p\leq0.01$, *** $p\leq0.001$, **** $p\leq0.0001$).

	Acute COVID-19 N=28			**Neuropsychiatric Long COVID** N=50			**No Long COVID Control** N=29								
	Median	Quartile 1	Quartile 3	Median	Quartile 1	Quartile 3	Median	Quartile 1	Quartile 3	Overall P-Value		N-LC vs Controls P-Value		N-LC vs Acute P-Value	
a-2 Macroglobulin	1,001,295	801,219	1,301,925	1,742,400	1,409,950	1,977,275	1,513,700	1,351,300	1,805,900	0.0000	****	0.1344	ns	0.0000	****
AGP	1,914,050	1,617,325	2,149,450	1,484,800	1,132,300	1,790,400	1,124,650	886,355	1,405,075	0.0000	****	0.0005	***	0.0001	****
CRP	147,390,000	76,652,500	274,410,000	12,306,500	3,929,625	30,898,500	5,882,300	3,128,100	9,523,175	0.0000	****	0.0446	*	0.0000	****
Fetuin A36	189,142	173,775	208,482	283,256	224,859	341,091	230,007	192,167	260,028	0.0000	****	0.0012	**	0.0000	****
Fibrinogen	-	-	-	1,723,500	1,594,800	2,105,850	1,928,650	1,612,550	2,299,900	-	-	0.3286	ns	-	-
Haptoglobin	2,734,350	1,155,775	5,498,775	1,324,850	832,471	1,650,850	961,743	612,410	1,201,375	0.0000	****	0.0546	ns	0.0003	***
L-Selectin	596,697	514,247	721,467	772,704	657,702	907,725	650,522	517,830	801,486	0.0032	**	0.0139	*	0.0020	**
PF4	1,406	1,033	1,674	666	483	1,020	820	483	1,181	0.0002	***	0.5149	ns	0.0001	****
SAP	7,719,050	6,268,650	9,884,438	7,430,550	5,807,025	8,215,275	5,268,300	4,652,100	5,952,375	0.0000	****	0.0005	***	0.1136	ns
ADAMTS13	533.27	481.41	597.33	447.68	380.94	528.39	357.80	315.49	400.25	0.0000	****	0.0000	****	0.0011	**
D-Dimer	1,898.70	1,445.90	2,564.22	725.21	520.39	1,420.96	511.38	398.91	1,149.70	0.0000	****	0.1495	ns	0.0000	****
SAA	30,380.52	15,783.39	45,538.88	7,772.79	3,513.98	14,627.23	5,011.64	2,765.83	10,205.20	0.0000	****	0.1980	ns	0.0000	****
sICAM-1	87.38	80.45	99.81	87.17	75.40	101.67	73.16	65.84	84.37	0.0016	**	0.0278	*	0.0516	ns
sP-Selectin	82.10	70.30	88.72	52.04	42.40	61.70	39.36	35.17	44.71	0.0000	****	0.0001	****	0.0000	****
sVCAM-1	685.72	430.93	1,064.20	433.36	375.81	511.57	399.15	329.60	461.11	0.0000	****	0.0886	ns	0.0000	****

Supplemental Table 2. **Group differences in cognitive and menta health**. A detailed table of testing results in neuropsychiatric Long COVID (n=33) and control groups (n=29). Higher scores for verbal and memory, fluency, and processing speed suggest better performance. Higher scores for depression and anxiety equate to greater symptoms.

	Neuropsychiatric Long COVID N = 33 Median [IQR]	**No Long COVID Control N = 29** Median [IQR]	**Cohen's D [CI]**	**p-value**
Verbal Learning	15 [10 – 19]	17 [14 – 20]	0.38 [***]	0.13
Verbal Memory	7.1 [5.0 – 10.0]	9.0 [7.0 – 11.0]	0.61 [***]	0.02*
Fluency	21 [17 – 28]	24 [20 – 30]	0.47 [***]	0.09
Processing Speed	28 [22 – 36]	34 [28 – 40]	0.67 [***]	0.03*
Depressive Symptoms	12 [7 – 18]	3.1 [1.0 – 5.0]	1.71 [***]	<0.000001****
Anxiety Symptoms	9.9 [5.0 – 15.0]	3.9 [1.0 – 6.0]	1.11 [***]	0.0003***

REFERENCES:

1. Siddiqi HK, Libby P, Ridker PM. COVID-19 - A vascular disease. *Trends Cardiovasc Med*. 2021;31(1):1-5. doi:10.1016/j.tcm.2020.10.005
2. Goshua G, Pine AB, Meizlish ML, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *The Lancet Haematology*. 2020;7(8):e575-e582. doi:10.1016/S2352-3026(20)30216-7
3. Goshua G, Butt A, Lee AI. Immunothrombosis: a COVID-19 concerto. *British Journal of Haematology*. 2021;194(3):491-493.
4. Gu SX, Tyagi T, Jain K, et al. Thrombocytopathy and endotheliopathy: crucial contributors to COVID-19 thromboinflammation. *Nature Reviews Cardiology*. 2021/03/01 2021;18(3):194-209. doi:10.1038/s41569-020-00469-1
5. Lee M-H, Perl DP, Nair G, et al. Microvascular Injury in the Brains of Patients with Covid-19. *New England Journal of Medicine*. 2020;384(5):481-483. doi:10.1056/NEJMc2033369
6. Becker JH, Lin JJ, Doernberg M, et al. Assessment of Cognitive Function in Patients After COVID-19 Infection. *JAMA Network Open*. 2021;4(10):e2130645-e2130645. doi:10.1001/jamanetworkopen.2021.30645
7. Daroische R, Hemminghyth MS, Eilertsen TH, Breitve MH, Chwiszczuk LJ. Cognitive Impairment After COVID-19—A Review on Objective Test Data. Review. *Frontiers in Neurology*. 2021-July-29 2021;12doi:10.3389/fneur.2021.699582
8. Huang S, Zhou Z, Yang D, et al. Persistent white matter changes in recovered COVID-19 patients at the 1-year follow-up. *Brain*. 2021;145(5):1830-1838. doi:10.1093/brain/awab435
9. Chen B, Chen C, Zheng J, Li R, Xu J. Insights Into Neuroimaging Findings of Patients With Coronavirus Disease 2019 Presenting With Neurological Manifestations. *Front Neurol*. 2020;11:593520. doi:10.3389/fneur.2020.593520
10. Cecchetti G, Agosta F, Canu E, et al. Cognitive, EEG, and MRI features of COVID-19 survivors: a 10-month study. *J Neurol*. Jul 2022;269(7):3400-3412. doi:10.1007/s00415-022-11047-5
11. Ajčević M, Iscra K, Furlanis G, et al. Cerebral hypoperfusion in post-COVID-19 cognitively impaired subjects revealed by arterial spin labeling MRI. *Sci Rep*. Apr 10 2023;13(1):5808. doi:10.1038/s41598-023-32275-3
12. Prasannan N, Heightman M, Hillman T, et al. Impaired exercise capacity in post-COVID-19 syndrome: the role of VWF-ADAMTS13 axis. *Blood Adv*. Jul 12 2022;6(13):4041-4048. doi:10.1182/bloodadvances.2021006944
13. Braga LW, Oliveira SB, Moreira AS, et al. Long COVID neuropsychological follow-up: Is cognitive rehabilitation relevant? *NeuroRehabilitation*. 2023;53(4):517-534. doi:10.3233/nre-230212
14. Serrano Del Pueblo VM, Serrano-Heras G, Romero Sánchez CM, et al. Brain and cognitive changes in patients with long COVID compared with infection-recovered control subjects. *Brain*. Apr 2 2024;doi:10.1093/brain/awae101
15. Braga LW, Oliveira SB, Moreira AS, et al. Neuropsychological manifestations of long COVID in hospitalized and non-hospitalized Brazilian Patients. *NeuroRehabilitation*. 2022;50(4):391-400. doi:10.3233/nre-228020
16. Calabria M, García-Sánchez C, Grunden N, et al. Post-COVID-19 fatigue: the contribution of cognitive and neuropsychiatric symptoms. *J Neurol*. Aug 2022;269(8):3990-3999. doi:10.1007/s00415-022-11141-8
17. Nicotra A, Masserini F, Calcaterra F, et al. What do we mean by long COVID? A scoping review of the cognitive sequelae of SARS-CoV-2 infection. *Eur J Neurol*. Dec 2023;30(12):3968-3978. doi:10.1111/ene.16027
18. Contreras-Briceño F, Herrera S, Vega-Adauy J, et al. Circulating Vascular Cell Adhesion Molecule-1 (sVCAM-1) Is Associated With Left Atrial Remodeling in Long-Distance Runners. *Front Cardiovasc Med*. 2021;8:737285. doi:10.3389/fcvm.2021.737285

19. Fang Z, Ahrnsbrak R, Rekito A. Evidence Mounts That About 7% of US Adults Have Had Long COVID. *JAMA*. 2024;332(1):5-6. doi:10.1001/jama.2024.11370
20. Tak CR. The health impact of long COVID: a cross-sectional examination of health-related quality of life, disability, and health status among individuals with self-reported post-acute sequelae of SARS CoV-2 infection at various points of recovery. *Journal of Patient-Reported Outcomes*. 2023;7(1):31.
21. Xie Y, Xu E, Bowe B, Al-Aly Z. Long-term cardiovascular outcomes of COVID-19. *Nature Medicine*. 2022/02/07 2022;doi:10.1038/s41591-022-01689-3
22. Network IMPAACT. Updated 2020. 2024. https://www.impaactnetwork.org/
23. Ceciliani F, Lecchi C. The Immune Functions of α(1) Acid Glycoprotein. *Curr Protein Pept Sci*. 2019;20(6):505-524. doi:10.2174/1389203720666190405101138
24. Luan YY, Yao YM. The Clinical Significance and Potential Role of C-Reactive Protein in Chronic Inflammatory and Neurodegenerative Diseases. *Front Immunol*. 2018;9:1302. doi:10.3389/fimmu.2018.01302
25. Amezcua-Castillo E, González-Pacheco H, Sáenz-San Martín A, et al. C-Reactive Protein: The Quintessential Marker of Systemic Inflammation in Coronary Artery Disease-Advancing toward Precision Medicine. *Biomedicines*. Sep 2 2023;11(9)doi:10.3390/biomedicines11092444
26. Song Z, Cai L, Guo L, Tsukamoto Y, Yutani C, Li X-A. Accumulation and expression of serum amyloid P component in human atherosclerotic lesions. *Atherosclerosis*. 2010/07/01/ 2010;211(1):90-95. doi:https://doi.org/10.1016/j.atherosclerosis.2010.01.046
27. Jenny NS, Arnold AM, Kuller LH, Tracy RP, Psaty BM. Serum amyloid P and cardiovascular disease in older men and women: results from the Cardiovascular Health Study. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27(2):352-358.
28. Lenting PJ, Christophe OD, Denis CV. von Willebrand factor biosynthesis, secretion, and clearance: connecting the far ends. *Blood*. Mar 26 2015;125(13):2019-28. doi:10.1182/blood-2014-06-528406
29. Ferroni P, Martini F, Riondino S, et al. Soluble P-selectin as a marker of in vivo platelet activation. *Clinica Chimica Acta*. 2009/01/01/ 2009;399(1):88-91. doi:https://doi.org/10.1016/j.cca.2008.09.018
30. Gross PL. Soluble P-selectin is the smoke, not the fire. *Blood*. 2017;130(2):101-102. doi:10.1182/blood-2017-05-786319
31. Bui TM, Wiesolek HL, Sumagin R. ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J Leukoc Biol*. Sep 2020;108(3):787-799. doi:10.1002/jlb.2mr0220-549r
32. Ivetic A, Hoskins Green HL, Hart SJ. L-selectin: A Major Regulator of Leukocyte Adhesion, Migration and Signaling. *Front Immunol*. 2019;10:1068. doi:10.3389/fimmu.2019.01068
33. Grailer JJ, Kodera M, Steeber DA. L-selectin: role in regulating homeostasis and cutaneous inflammation. *J Dermatol Sci*. Dec 2009;56(3):141-7. doi:10.1016/j.jdermsci.2009.10.001
34. Laughlin Gail A, Cummins Kevin M, Wassel Christina L, Daniels Lori B, Ix Joachim H. The Association of Fetuin-A With Cardiovascular Disease Mortality in Older Community-Dwelling Adults. *Journal of the American College of Cardiology*. 2012/05/08 2012;59(19):1688-1696. doi:10.1016/j.jacc.2012.01.038
35. Chekol Abebe E, Tilahun Muche Z, Behaile TMA, et al. The structure, biosynthesis, and biological roles of fetuin-A: A review. *Front Cell Dev Biol*. 2022;10:945287. doi:10.3389/fcell.2022.945287
36. Vandooren J, Itoh Y. Alpha-2-Macroglobulin in Inflammation, Immunity and Infections. *Front Immunol*. 2021;12:803244. doi:10.3389/fimmu.2021.803244

37. Switzer ME, Gordon HJ, McKee PA. Proteolytic activity of alpha 2-macroglobulin-enzyme complexes toward human factor VIII/von Willebrand factor. *Biochemistry*. Mar 15 1983;22(6):1437-44. doi:10.1021/bi00275a018
38. Akbasheva OE, Mitrofanova DK, Spirina LV, et al. Alpha-2 macroglobulin activity in SARS-CoV-2 induced infection and in the post-COVID-19 period. *Biomed Khim*. Sep 2023;69(4):240-247. doi:10.18097/pbmc20236904240
39. Hochepied T, Berger FG, Baumann H, Libert C. α1-Acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties. *Cytokine & Growth Factor Reviews*. 2003/02/01/ 2003;14(1):25-34. doi:https://doi.org/10.1016/S1359-6101(02)00054-0
40. Yin K, Peluso MJ, Luo X, et al. Long COVID manifests with T cell dysregulation, inflammation and an uncoordinated adaptive immune response to SARS-CoV-2. *Nature Immunology*. 2024/02/01 2024;25(2):218-225. doi:10.1038/s41590-023-01724-6
41. Mestriner F, Campos LC, Franco Francisco D, et al. Abstract 13776: Alpha 1-Acid Glycoprotein is Involved With Neutrophil Nets in COVID-19. *Circulation*. 2022/11/08 2022;146(Suppl_1):A13776-A13776. doi:10.1161/circ.146.suppl_1.13776
42. Vorobjeva NV, Chernyak BV. NETosis: Molecular Mechanisms, Role in Physiology and Pathology. *Biochemistry (Mosc)*. Oct 2020;85(10):1178-1190. doi:10.1134/s0006297920100065
43. Krinsky N, Sizikov S, Nissim S, et al. NETosis induction reflects COVID-19 severity and long COVID: insights from a 2-center patient cohort study in Israel. *J Thromb Haemost*. Sep 2023;21(9):2569-2584. doi:10.1016/j.jtha.2023.02.033
44. Shafqat A, Omer MH, Albalkhi I, et al. Neutrophil extracellular traps and long COVID. *Front Immunol*. 2023;14:1254310. doi:10.3389/fimmu.2023.1254310