

Full Length Article

Evaluation of neuronal and immunological biomarkers in post-COVID-19 condition

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ABSTRACT

Objective: To investigate evidence of central nervous system (CNS) injury in Post-COVID-19 Condition (PCC) through plasma biomarkers of neuronal damage (APP, NFL, Tau), inflammation (IL-2, IL-6, IL-10, IL-18, IFN- γ , TNF- α), and immune response (IgM, IgA, IgG) in individuals with PCC and neurological symptoms.

Methods: We analyzed 155 volunteers from a post-COVID-19 Center in Rio de Janeiro, categorized into four groups: COVID-19 control (COV; recovered without PCC), PCC without memory loss and reasoning difficulty (PCC-noML/RD), PCC with memory loss and reasoning difficulty (PCC-ML/RD), and healthy controls (HC). Serological and biomarker analyses were used to quantify inflammatory markers, immunoglobulins, and neuronal injury-related proteins.

Results: PCC groups showed significantly altered IgA and IgG levels compared to the COV group. Volunteers with PCC exhibited elevated IL-6, IL-18, IFN- γ , and TNF- α , alongside decreased IL-2, relative to COV and HC groups. Total Tau (t-Tau) concentrations were highest in the COV group and lowest in the PCC-noML/RD group, with the PCC-ML/RD group presenting a distinct intermediate profile. A β 1–40 levels differed between PCC-noML/RD and PCC-ML/RD groups. A positive correlation between TNF- α and t-Tau was observed specifically in the PCC-ML/RD group.

Interpretation: In conclusion, our findings suggest that Post-COVID-19 Condition is associated with persistent systemic inflammation and subtle alterations in neurological biomarkers. This inflammatory profile may contribute to cognitive symptoms, including memory impairment, reasoning difficulties, and hypoprosia, even in the absence of marked evidence of neuronal injury.

1. Introduction

The year 2020 was marked by a pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly infectious disease responsible for unprecedented global mortality and morbidity. With over 778 million confirmed cases and 7.1 million deaths worldwide (World Health Organization, 2026), while many COVID-19 survivors achieved full recovery, a significant subset developed persistent symptoms. The prolonged symptoms were named Post-COVID-19 Condition (PCC) by the World Health Organization (WHO) and are characterized as a multisystem symptomatic presentation emerging within three months after acute SARS-CoV-2 infection, persisting at least two

months, with no alternative diagnosis. This condition frequently impairs activities of daily living, including occupational performance and domestic responsibilities, while substantially reducing social participation capacity (World Health Organization, 2025) and has been estimated to affect 10–50% of survivors (Nalbandian et al., 2023; Taher et al., 2025). Some conditions could predispose the patients to develop PCC and have been identified as key risk factors such as obesity, diabetes, autoimmune diseases, severe acute SARS-CoV-2 infection or other pre-existing medical condition (Tsampasian et al., 2023). Vaccination has been demonstrated to have high significant protective effects, reducing the likelihood to develop severe COVID-19 and the risk of receiving a diagnosis of PCC. The effectiveness of vaccination demonstrated that

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three doses prevented PCC in 73% than one dose which prevented in 21% (Notarte et al., 2022; Byambasuren et al., 2023; Lundberg-Morris et al., 2023).

PCC is characterized by a wide array of symptoms, from pulmonary complications and systemic issues (e.g., persistent cough, fatigue, myalgia) to stress-related neurocognitive impairments. Among these, neurological sequelae constitute a major long-term burden. Symptoms such as memory impairment, lack of concentration, attention deficits, and brain fog are not only highly prevalent but can be among the most frequently reported, representing up to 70% of all self-reported symptoms in some cohorts (Huff et al., 2025). Crucially, a growing body of evidence indicates that the emergence of these sequelae is independent of confounding factors such as pre-existing self-reported neurological or psychiatric conditions and previous hospitalization (James et al., 2025).

The pathophysiologic mechanisms underlying PCC and its neurologic symptoms remain to be elucidated and several mechanisms have been considered to explain these secondary effects. These include direct viral penetration inside the brain, systemic inflammation with complement activation, microglia activation, oxidative stress, neurodegenerative processes and development of functional antibodies against G-protein coupled receptors (James et al., 2025; Wallukat et al., 2021).

Viral infections can directly affect the central nervous system by targeting neurons, astrocytes, and microglial cells. The activation of glial cells triggers a cascade of inflammatory mediators, establishing a state of neuroinflammation. This neuroinflammatory response is hypothesized to be a critical driver of PCC. Within this process, cytokines are essential for mounting an immune response against SARS-CoV-2; however, their persistent and prolonged production can induce damage to the vasculature, glial cells, and neurons (Ziff et al., 2022). A key consequence of this damage is the disruption of the blood-brain barrier (BBB). Such compromise of the BBB's integrity can permit the leakage of specific neural and neuronal proteins into the bloodstream, allowing them to be detected as potential molecular neurological markers in peripheral blood (Talkington et al., 2025).

The neuronal proteins amyloid precursor protein (APP), Tau, and neurofilament light chain (NFL) are critical for key cellular processes, including synaptic vesicle transport, neurotransmission, and structural integrity. Dysregulation of these proteins is implicated in a cascade of cerebral dysfunction. For instance, the accumulation of amyloid- β plaques, a hallmark of Alzheimer's disease (AD) and other age-related neurodegenerative disorders, arises from the altered proteolytic processing of APP. In the amyloidogenic pathway, sequential cleavage by β - and γ -secretases generates amyloid- β peptides, particularly toxic soluble oligomers that disrupt neuronal signaling and homeostasis (Van der Kant and Goldstein, 2015; Zheng and Koo, 2011). The subsequent aggregation of these peptides into insoluble plaques is a defining neuropathological feature (Zetterberg and Blennow, 2020).

Tau and NFL are neuronal cytoskeletal proteins being the principal constituents of microtubules found in the axons. Their dysregulation is increasingly recognized in association with varied neurological disorders (Yuan and Nixon, 2023).

Several studies have reported increased levels of NFL and phosphorylated Tau (p-Tau) associated with central nervous system (CNS) injury during the acute phase of both severe and mild-to-moderate COVID-19, suggesting neuroaxonal damage related to acute SARS-CoV-2 infection (Ameres et al., 2020; Cooper et al., 2020; Gutman et al., 2024). Alterations in these CNS biomarkers have also been linked to the emergence of neurological symptoms and disorders, highlighting their potential clinical value for early detection and monitoring of neuro-COVID manifestations. Such biomarkers could assist clinicians worldwide in implementing more targeted and personalized approaches. However, it remains unclear whether PCC affects plasma levels of APP, NFL, and Tau (Marwaha, 2023).

Here, we aimed to investigate whether evidence of CNS injury can be detected through plasma biomarkers reflecting neuronal (APP, NFL, and Tau), inflammatory (interleukins IL-2, IL-6, IL-10, IL-18, interferon- γ), and tumor necrosis factor- α (TNF- α), and immunological (immunoglobulins IgM, IgA, and IgG) processes in volunteers with PCC presenting neurological symptoms.

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2. Materials and methods

2.1. Experimental ethical criteria

Volunteers who sought care at the post-COVID-19 Care and Rehabilitation Center (Centro de Acolhimento e Reabilitação Pós-COVID-19, CARP) in Macaé, Rio de Janeiro, Brazil, due to persistent sequelae following COVID-19 infection were invited to participate in the study. A total of 252 volunteers sought medical assistance, and 155 agreed to participate and signed the informed consent form (Supplemental Material). The study was approved by the Research Ethics Committee of UFRJ-Macaé (CEP/UFRJ-Macaé; CAAE protocol: 57373422.8.0000.5699). Inclusion criteria comprised a confirmed diagnosis of COVID-19 and signed informed consent. Exclusion criteria included individuals under 18 years of age and pregnant women.

2.2. Patient screening and trial groups

Post-COVID-19 volunteers who continued to experience symptoms after the acute phase of infection were recruited between January 2022 and February 2023. All participants underwent a clinical anamnesis conducted by a physician at the CARP and completed a questionnaire including sociodemographic and health status information. The physician performed a clinical interview and administered the Mini-Cog test to assess the presence or absence of memory impairment, reasoning difficulties, and other neurological symptoms. For serological analysis, blood samples were collected and classified into four groups: COV (COVID-19 control; individuals who had COVID-19 but did not develop PCC), PCC-noML/RD (PCC without memory loss and reasoning difficulty), PCC-noML/RD (PCC with memory loss and reasoning difficulty), and healthy control (HC; SARS-CoV-2-unexposed individuals; blood samples collected in January 2020 from the Municipal Hemotherapy Service, prior to the first reported COVID-19 case in Brazil). The presence of mainly memory loss, reasoning difficulty, dyslexia, aphasia, paresis, encephalitis and convulsion were considered neurological symptoms, as identified by CARP physicians, and were used as a criterion to distinguish participants in the PCC-ML/RD group. All other symptoms were categorized into non-neurological group (Gutman et al., 2024; Alonso-Domínguez et al., 2023; Freitas et al., 2025; Grafström et al., 2025; Jimenez et al., 2026).

2.3. S-UFRJ ELISA for the detection of anti-S immunoglobulins

Blood was collected in 4 mL EDTA K3 tubes (Firstlab), and then the centrifugation at 4000 rpm (Daiki DT-5000) for 10 min at 25 °C was performed to obtain plasma, which was stored at -80 °C. The in-house S-UFRJ ELISA was assayed by immobilizing the trimeric SARS-CoV-2 spike glycoprotein on plates, as described by (Alvim et al., 2022). Volunteers' samples were added to each well and after, the secondary antibodies were used at 1:200 v/v dilution: anti-human IgM (Fc) HRP-conjugated (#MFCD00162459; Sigma), anti-human IgG (H + L) HRP-conjugated (#W4031; Promega), and anti-human IgA HRP-conjugated (#A18781; Invitrogen). Detection was performed at 450 nm using a Multiskan Sky microplate reader (Thermo Scientific). Results were expressed as optical density (OD) as described by Sousa et al. (2023).

2.4. LumitTM RBD:ACE2 interaction assays

Neutralizing antibodies were assessed using the LumitTM SARS-CoV-2 Spike RBD:ACE2 immunoassay kit (Promega), following the manufacturer's instructions. Bioluminescence was measured using a Fluoroskan FL microplate reader (Thermo Scientific) and Youden's index was used to determine optimal cutoff values (Youden, 1950).

2.5. Cytokines levels detection

The concentration of 6 cytokines (interleukin IL-2, IL-6, IL-10, IL-18, IFN- γ and TNF- α , were quantified in the plasma of volunteers by the ELISA technique using a human ELISA kit to each cytokine (#RAB0286-1KT; #RAB0306-1KT; #RAB0244-1KT; #RAB0543-1KT; #RAB0222-1KT and; #RAB0476-1KT; Sigma) according to manufacturer procedures. A standard curve was prepared for each cytokine and concentrations of cytokines were measured using a Multiskan Sky microplate reader (Thermo Scientific) at 450 nm, with output expressed in pg/mL.

2.6. Neurological markers detection

ELISA analyses were carried out according to the manufacturer's protocol using plasma of volunteers. The levels of Human Amyloid β peptide 1–40 (A β 1–40) (ELK8921;ELK Biotechnology), human phosphorylated microtubule-associated protein Tau (p-Tau) (Q9H3S4; Wuhan Fine Biotech Co., Ltd), human total microtubule-associated protein Tau (t-Tau) (AMS.E01T0024; AMSBIO) and NfL (E1310Hu;BT LAB) levels were determined using a human ELISA kit. All procedures were performed on a ThermoScientific Spectrophotometer Multiskan™ Sky (Model number 51119700) and the absorbance was read at 450 nm.

2.7. Statistical analysis

Data analysis was performed using GraphPad Prism 9 software (GraphPad Software Inc., USA) and graphs were expressed as medians and interquartile ranges (median (IQR)), frequencies, and percentages. Gaussian distribution was verified using the Kolmogorov-Smirnov test and the assay variability and distribution coefficient was also evaluated by GraphPad Prism 9. The outliers identified by the program were removed before to perform the following statistical analysis. The Mann-Whitney *U* test was then used to compare two groups, and the Kruskal-Wallis test was used to compare more than two groups, followed by Dunn's post hoc test. Differences were considered significant when $p < 0.05$. Linear regression analyses were performed to investigate the relationship between neuronal and immunological biomarkers. Variables significantly associated in the univariate analyses were entered into multivariable linear regression models. Regression coefficients (β), 95% confidence intervals (95% CI), adjusted R^2 , and p -values were calculated. Besides, Spearman rank correlation was used to compare two variables and identify the relationship between them. Past 4.03 software was used to perform the multivariate analysis (Principal Component Analysis - PCA).

3. Results

3.1. Socio demographic characteristics of volunteers with PCC

Data were obtained from volunteers' interviews and medical anamnesis, which were divided into four groups (HC, COV, PCC-noML/RD and PCC-ML/RD). A total of 155 volunteers were included in this study (Table 1). The COVID-19 group (COV) was formed mainly by women volunteers (72%) who had COVID-19 but did not show any post-COVID-19 symptoms. A total of 130 volunteers took medical assistance and presented PCC. Most of them presented memory loss and/or reasoning difficulty symptoms (PCC-ML/RD) and were women (73%) and 52% presented general symptoms (PCC-noML/RD). The mean age of all volunteers was 48.0 years and the PCC-noML/RD group is the oldest (57.6 years), followed by the PCC-ML/RD group (49.6 years). The vaccination schedule was evaluated in the volunteer groups. The COV group showed the highest percentage of vaccinated volunteers, 80% receiving three doses. In the PCC-ML/RD group 48% of volunteers did not receive any vaccine dose, while 27% received two doses and 18% received three doses while, in the PCC-noML/RD group, the rate of unvaccinated individuals was even higher (54%).

Table 1

Socio-demographic data of the patients interviewed (N = 155).

	Control without PCC (n = 25)	Total PCC (n = 130)	PCC-noNS (n = 27)	PCC-NS (n = 103)
Variables	Mean (SD)			
Age (years)	30.6 (9.9)	51.3 (15.5) ****	57.6 (14.2) ****	49.6 (16.0) ****
Men	34.4 (11.3)	52.3 (17.5)	58.1 (12.0)	49.6 (19.1)
Women	29.0 (9.2)	50.8 (14.6)	57.1 (16.3)	49.7 (14.1)
Weeks after infection	–	45.2 (38.7)	35.4 (30.6)	47.8 (40.5)
		n°/total n° (%)		
Sex				
Men	7/25 (28%)	41/130 (32%)	13/27 (48%)	28/103 (27%)
Women	18/25 (72%)	89/130 (68%)	14/27 (52%)	75/103 (73%)
Vaccination Schedule				
0 dose	0/25 (0%)	51/103 (50%)	14/26 (54%)	37/77 (48%)
1 dose	0/25 (0%)	6/103 (6%)	1/26 (4%)	5/77 (6%)
2 doses	5/25 (20%)	25/103 (24%)	4/26 (15%)	21/77 (27%)
3 doses	20/25 (80%)	21/103 (20%)	7/26 (27%)	14/77 (18%)
Comorbidities				
Neuropsychiatric	–	23/121 (19%)	5/27 (19%)	18/94 (19%)
Metabolic	–	36/121 (30%)	8/27 (30%)	28/94 (30%)
Cardiovascular	–	71/121 (59%)	24/27 (89%)	47/94 (50%)
Inflammatory/ Systemic	–	30/121 (25%)	5/27 (19%)	25/94 (27%)
Others	–	22/121 (18%)	5/27 (19%)	17/94 (18%)
Symptoms				
Memory loss	–	64/121 (53%)	0/27 (0%)	64/94 (68%)
Myalgia	–	70/121 (58%)	14/27 (52%)	56/94 (60%)
Anxiety	–	62/121 (51%)	9/27 (33%)	53/94 (56%)
Hypoprosexia	–	52/121 (43%)	1/27 (4%)	51/94 (54%)
Myoclonus	–	61/121 (50%)	11/27 (41%)	50/94 (53%)
Fatigue	–	63/121 (52%)	16/27 (59%)	47/94 (50%)
Weight change	–	50/121 (41%)	7/27 (26%)	43/94 (46%)
Reasoning difficulty	–	43/121 (36%)	0/27 (0%)	43/94 (46%)
Dyspnoea	–	49/121 (40%)	9/27 (33%)	40/94 (43%)
Alopecia	–	48/121 (40%)	9/27 (33%)	39/94 (41%)
Sleep disorder	–	42/121 (35%)	4/27 (15%)	38/94 (40%)
Humor	–	41/121 (34%)	5/27 (19%)	36/94 (38%)
Arrhythmia	–	40/121 (33%)	5/27 (19%)	35/94 (37%)
Headache	–	40/121 (33%)	6/27 (22%)	34/94 (36%)
Depression	–	36/121 (30%)	5/27 (19%)	31/94 (33%)
Ageusia	–	40/121 (33%)	10/27 (37%)	30/94 (32%)

(continued on next page)

Table 1 (continued)

Variables	Control without PCC (n = 25)	Total PCC (n = 130)	PCC-noNS (n = 27)	PCC-NS (n = 103)
	Mean (SD)			
Vertigo	–	34/121 (28%)	4/27 (15%)	30/94 (32%)
Anosmia	–	36/121 (30%)	8/27 (30%)	28/94 (30%)
Paresthesia	–	33/121 (27%)	7/27 (26%)	26/94 (28%)
Angina	–	23/121 (19%)	1/27 (4%)	22/94 (23%)
Eating disorder	–	26/121 (21%)	4/27 (15%)	22/94 (23%)
Dyslexia	–	20/121 (17%)	0/27 (0%)	20/94 (21%)
Hoarseness	–	22/121 (18%)	3/27 (11%)	19/94 (20%)
Delirium	–	15/121 (12%)	1/27 (4%)	14/94 (15%)
Dysphagia	–	15/121 (12%)	1/27 (4%)	14/94 (15%)
Aphasia	–	12/121 (10%)	0/27 (0%)	12/94 (13%)
Paresis	–	11/121 (9%)	0/27 (0%)	11/94 (12%)
Encephalitis	–	5/121 (4%)	0/27 (0%)	5/94 (5%)
Convulsion	–	3/121 (2%)	0/27 (0%)	3/94 (3%)
Thrombosis	–	4/121 (3%)	2/27 (7%)	2/94 (2%)

Symbols *, **, *** and **** denote statistically significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively) compared to the control group. Non-parametric comparisons were performed using the Kruskal-Wallis test due to the non-normal distribution of the data. Post-hoc pairwise comparisons were conducted using Dunn's test with a Bonferroni correction to control for multiple comparisons.

Some studies demonstrate that the prognosis of PCC is associated with certain comorbidities (Xie et al., 2022; Mateu et al., 2023; Cai et al., 2024). In this study, the cardiovascular comorbidity was the most reported by PCC-noML/RD (89%) and PCC-ML/RD (50%) groups. Metabolic comorbidity was reported by 30% of volunteers of all groups. The following comorbidities (neuropsychiatric and inflammatory/systemic) were reported in similar proportions. In addition, cardiovascular comorbidity was the most frequently reported condition among PCC-ML/RD volunteers (50%), compared with all other comorbidities. The PCC-ML/RD group showed a high frequency of neurological symptoms, such as memory loss (68%), reasoning difficulties (46%), and hypoprosia (54%). These symptoms were almost absent in the PCC-noML/RD group. Fatigue (52%), myalgia (58%), and anxiety (51%) were common in both Post-COVID-19 groups, but fatigue were more frequently reported by PCC-noML/RD group (59%), while convulsion (3%) and encephalitis (5%) were the least frequently reported in the PCC-ML/RD and absent in the PCC-noML/RD groups (Fig. S1A and B).

3.2. Immunoglobulin profile of volunteers with neurological symptoms of PCC

Previous research identified elevated levels of immunoglobulin in the serum of patients with PCC (Souza et al., 2023), therefore, the present study analyzes the serological response of anti-Spike SARS-CoV-2 immunoglobulins (IgM, IgA, and IgG) of the volunteers between the PCC groups. Samples with optical density (OD) values below the cut-off were considered as negative samples (Fig. 1A to D).

Data revealed that volunteers from different groups (HC, COV, PCC-noML/RD and PCC-ML/RD) were not positive for IgM (Fig. 1A), remaining below the cut-off (0.347 OD). Levels of IgA and IgG were

significantly different for the post-COVID-19 groups (PCC-noML/RD and PCC-ML/RD) compared to the COV group. While IgA levels (Fig. 1B) were higher for the PCC-ML/RD group (0.585 (0.314–0.840) OD), the IgG levels (Fig. 1C) were higher in the COV group (2.062 (1.903–2.152) OD). IgG levels remained above the cut-off (0.687 OD) in all groups except for the pre-pandemic volunteers' group (HC). Additionally, neutralizing antibody levels were positive in all analyzed samples, but no significant differences were found between groups (Fig. 1D). The HC group presented a statistically significant difference for all groups in IgA, IgG and neutralizing antibody measures.

3.3. Cytokine levels of volunteers with PCC

Recent studies have shown a persistent diffuse inflammatory cytokine profile in patients with PCC (Phetsouphanh et al., 2022; Low et al., 2023; Ganesh et al., 2024). To analyze the persistent inflammatory process in the volunteers who presented prolonged neurological symptoms, the levels of circulating pro and anti-inflammatory cytokines were measured in the plasma of COV, PCC-noML/RD and PCC-ML/RD volunteers. We quantified the plasma concentrations of the following six cytokines: IL-2, IL-6, IL-10, IL-18, IFN- γ and TNF- α (Fig. 2).

Patients with PCC (PCC-noML/RD and PCC-ML/RD) exhibited higher levels of IL-6 (65.1 (36.9–116.9) pg/mL; 54.8 (31.0–102.0) pg/mL), IL-18 (43.7 (39.5–62.9) pg/mL; 46.3 (36.5–59.9) pg/mL), IFN- γ (0.0 (0.0–8.3) pg/mL; 0.0 (0.0–2.3) pg/mL), and TNF- α (0.0 (0.0–0.0) pg/mL; 99.9 (10.9–176.6) pg/mL) compared to the COV and HC groups. Whereas, levels of IL-2 (42.3 (0.0–208.4) pg/mL; 0.0 (0.0–123.1) pg/mL) were lower in the PCC-noML/RD and PCC-ML/RD groups compared to COV and HC groups (Fig. 2A). IL-10 levels were not different between all the groups analyzed (Fig. 2C).

In a comparison between PCC groups, IL-2 showed lower levels of production in the both groups although, PCC-ML/RD showed lower results than the PCC-noML/RD group (Fig. 2A). Therefore, IL-6 and IL-18 showed high levels of production in both PCC-noML/RD and PCC-ML/RD groups compared to the COV and HC groups, the levels of IL-6 and IL-18 were not different between PCS and nPCS groups (Fig. 2B and D). TNF- α levels exhibited an increase in the PCC-ML/RD compared to the other groups. Fig. 2F presents remarkable data in which 66% of volunteers from the PCC-ML/RD group exceeded the cut-off values and showed high levels of TNF- α while none of the volunteers of the HC, COV and PCC-noML/RD groups were positive to the TNF- α .

3.4. Influence of age on cytokines levels of neurological PCC volunteers

Levels of cytokines may vary according to many factors such as pathologies, comorbidities and age. Therefore, the levels of IFN- γ and TNF- α were elevated especially in the PCC-ML/RD group as shown in Fig. 3. The Spearman's rank correlation coefficient was calculated to evaluate if age could interfere with the IFN- γ and TNF- α production in the volunteers from the different groups presented in this research. For this, the ages were categorized into three groups (<40 years old; 40–60 years old and > 60 years old) according to the main ages of all the volunteers who participated in this research. IFN- γ levels did not vary according to the different groups of age as observed in Fig. 3A,C and E. TNF- α levels did not show differences between the three groups of age in the COV and PCC-noML/RD groups. TNF- α levels were different between age groups from volunteers who presented memory loss and/or reasoning difficulty symptoms (PCC-ML/RD). Volunteers with age below 40 years old exhibited the highest TNF- α concentrations, whereas those 40–60 years old which had the lowest levels. (Fig. 3F).

3.5. Neurological markers

To investigate potential neuronal damage and BBB disruption in patients with PCC, we analyzed circulating levels of key proteins typically expressed in neural tissue. The biomarkers t-Tau, p-Tau, A β 1–40

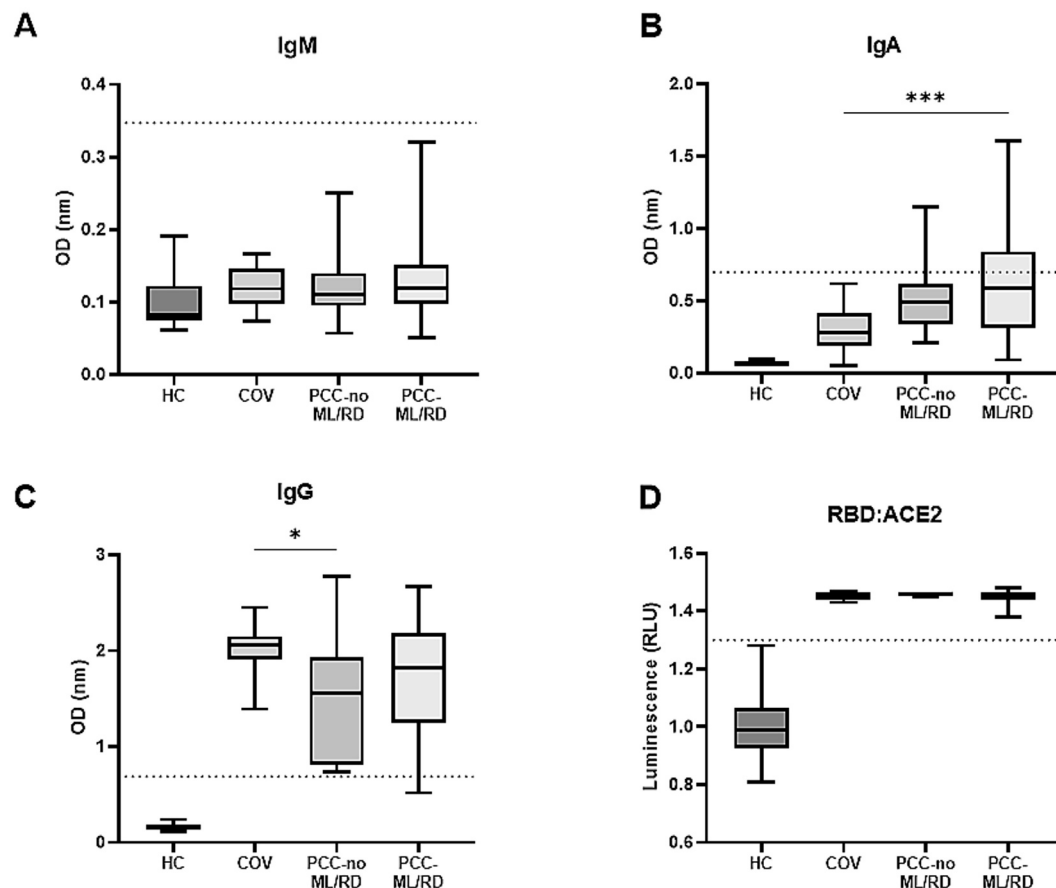


Fig. 1. Immunoglobulin production profile of the volunteers with neurological symptoms of PCC. (A-D) Comparison of immunoglobulin levels (IgM, IgA, and IgG) and neutralization capacity against SARS-CoV-2 between COV (COVID-19), PCC-noML/RD (post-COVID-19), and PCC-ML/RD (Post-COVID-19 with neurological symptoms) groups. The dotted line represents the cut-off for positive detection. $*p < 0.05$; $***p < 0.001$ indicates statistically significant differences.

and NfL were quantified and compared across study groups (Fig. 4A to D).

Tau protein is part of the microtubule-associated proteins (MAP) family. In healthy neuronal cells, Tau protein is normally found in axons and controls microtubule dynamics during neurite maturation and growth. However, in pathologies such as AD and tauopathies, hyperphosphorylation of Tau (p-Tau) affects biological and morphological functions of neurons. Therefore, the presence of t-Tau and p-Tau are used as indicators of dementia and here, in this research, the evaluation of proteins was performed in the blood to identify neuronal damage and the disruption of BBB in the patients with PCC. The t-Tau levels increased in the PCC-ML/RD group compared to the HC and PCC-noML/RD groups. Volunteers who had COVID-19 (COV) had higher levels of t-Tau in the serum samples (0.381 (0.378–0.384) ng/mL) while the PCC-noML/RD group had the lower levels (0.369 (0.368–0.374) ng/mL) and showed a significant difference between them. The PCC-ML/RD group (0.377 (0.369–0.388) ng/mL) also showed a significant difference compared to the PCC-noML/RD group. However, both PCC-noML/RD and PCC-ML/RD groups showed no statistically significant difference to the HC group (Fig. 5A). p-Tau were evaluated in the blood of HC, COV and Post-COVID-19 volunteers (PCC-noML/RD and PCC-ML/RD) and revealed similar levels in all the groups analyzed, without differences between them (Fig. 4B).

Amyloid-beta peptides (A β 1–40 and A β 1–42) are produced by the cleavage of APP in neuronal cell membranes and are considered among the main hallmarks of AD and related dementias. Thus, to evaluate the blood levels of A β 1–40, the samples were analyzed from controls (HC and COV) and Post-COVID-19 groups comparing volunteers. Measurement of A β 1–40 in the blood revealed a difference between the PCC-

noML/RD (159.1 (108.5–185.0) pg/mL) and PCC-ML/RD (199.7 (148.2–232.0) pg/mL) groups (Fig. 5C). Finally, NfL is a structural protein of neurons that present elevated levels in the blood or cerebrospinal fluid indicating potential neurological disease or trauma. The levels of blood NfL revealed a similar detection of the protein in the all groups analyzed. The levels were similar both in PCC-noML/RD and PCC-ML/RD groups and did not show statistically significant difference among all groups (Fig. 5D).

3.6. Correlation between cytokines and neural damage blood biomarkers

Inflammatory processes have been identified as playing a role in a number of neurological disorders or trauma, brain damage could release biomarkers in the blood which could be detected. Therefore, because both t-Tau and A β 1–40 and both IFN- γ and TNF- α were found in high levels in the blood of the PCC-ML/RD group, the analysis of correlation of these factors was performed.

IFN- γ did not show correlation with t-Tau in either the PCC-noML/RD or PCC-ML/RD groups (Fig. 5A and B). In addition, no correlation was observed between t-Tau and TNF- α in the PCC-noML/RD group (Fig. 5C). However, a positive correlation was found between TNF- α and t-Tau in the PCC-ML/RD group, indicating that the high levels of t-Tau and TNF- α is not random (Fig. 5D). Moreover, A β 1–40 did not show correlation with both IFN- γ or TNF- α in the PCC-noML/RD and PCC-ML/RD groups (Fig. 5E to H).

Multivariate analysis (PCA) was performed to compare almost all biomarkers previously analyzed here. PC1 was mainly driven by IFN- γ , IL-6, TNF- α , and IL-2, whereas PC2 was primarily influenced by IgA, IgG, and IL-18, indicating that the overall variability of the dataset was

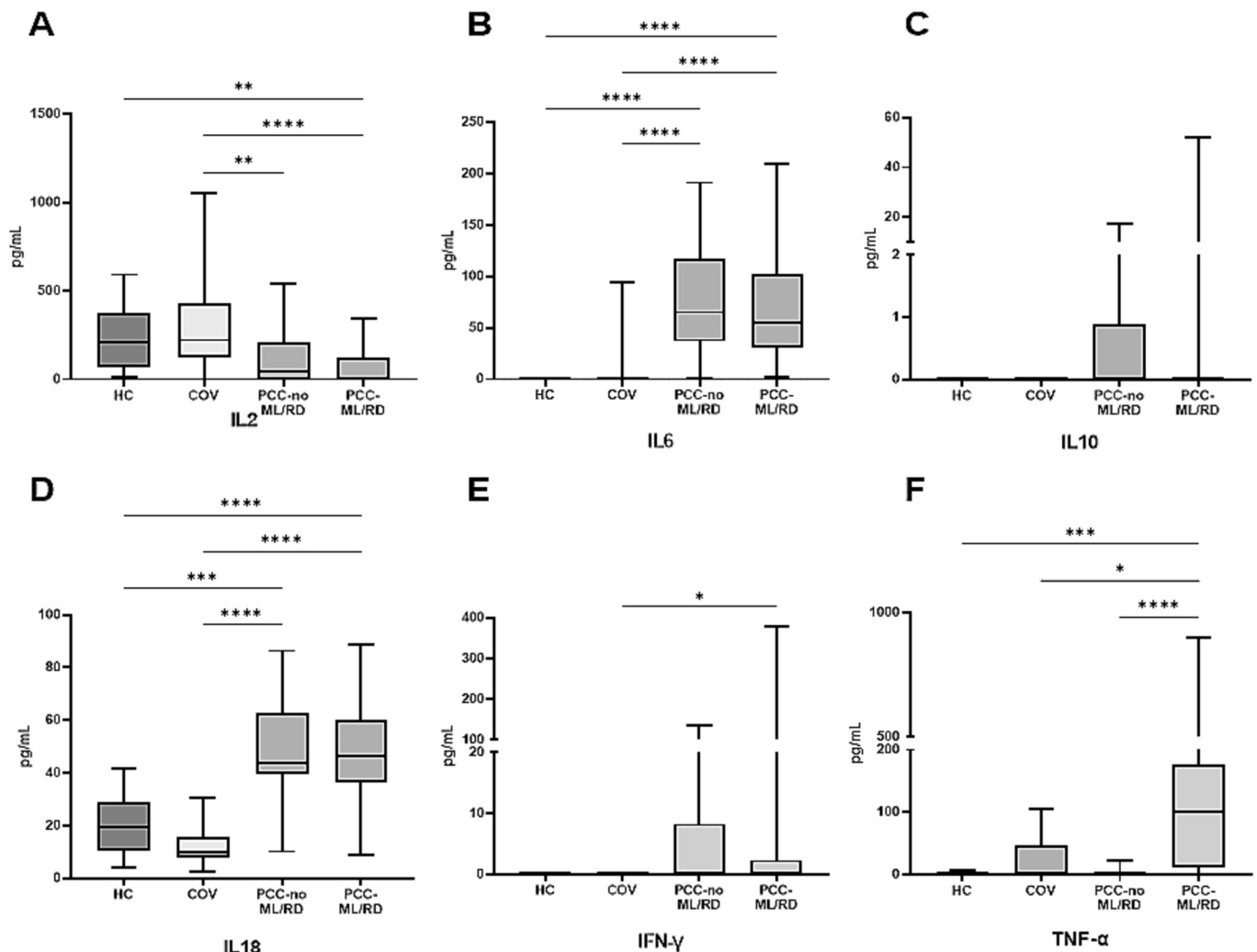


Fig. 2. Cytokine levels in the PCC patients. Comparison of cytokine levels (A) IL-2, (B) IL-6, (C) IL-10, (D) IL-18, (E) IFN-γ, and (F) TNF-α between COV (COVID-19 control), PCC-noML/RD and PCC-ML/RD (volunteers who presented neurological symptoms) groups. The dotted line denotes the cutoff value for positive levels. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ **** $p < 0.0001$ statistically significant differences.

predominantly associated with inflammatory and humoral immune markers rather than neuronal biomarkers. Cleared separation between PCC-noML/RD and PCC-ML/RD groups was not observed (Fig. S3).

4. Discussion

This work contributes to the ongoing effort to identify potential biomarkers associated with the development of PCC, providing a better understanding of its underlying pathophysiology, improving our understanding of the disease and supporting future early diagnostic strategies. Data obtained from cohort ($N = 155$) revealed that post-COVID-19 volunteers exhibit a distinct clinical and immunological profile. This profile is characterized by a higher prevalence of cardiovascular comorbidities, immune dysregulation, and subtle alterations in neurological markers.

Sociodemographic data show that both PCC groups have a higher mean age than the COV group (30.6 years). However, volunteers in the PCC-ML/RD group are younger adults (49.6 years) than those in the PCC-noML/RD group (57.6 years). The cohort is predominantly female (approximately 70%), with a post-infection period of no more than one year and more pronounced cardiovascular and metabolic comorbidities. Incomplete or absent vaccination appears to be a relevant factor, particularly in the PCC-noML/RD group.

The plasma immunoglobulin measurement corroborated the findings from our previous study (Sousa et al., 2023), which discussed that elevated IgA levels in patients with PCC may not be associated with disease severity (Santa Cruz et al., 2023), instead could be associated with the development of PCC and may be an immunological signature to predict prolonged symptoms in COVID-19 patients. Here, in this cohort, IgA levels in both PCC groups were higher than those in the control groups, especially in patients with PCC who presented memory loss and/or reasoning difficulty symptoms (0.585 (0.314–0.840) OD). Also, the multivariate analysis (Fig. S3) showed that IgG and IgA levels were relevant in the HC group, as expected due to absence of infection by SARS-CoV-2, compared to the other groups (COV, PCC-noML/RD and PCC-ML/RD).

The assessment of cytokine levels in blood plasma revealed significantly different concentrations of IL-2, IL-6, and IL-18 (Fig. 2A, B and D respectively) in the Post-COVID-19 groups compared to the control groups, suggesting possible immune dysregulation.

IL-2 is a key cytokine regulating lymphocyte proliferation and activation, including that of Natural Killer (NK) cells. During viral infections, it also participates in the differentiation of Th2 and Treg lymphocytes (Muñoz-Carrillo et al., 2018). This pleiotropic cytokine has a dose-dependent effect: high concentrations preferentially activate pro-inflammatory cells like NK cells, while low doses activate Treg cells,

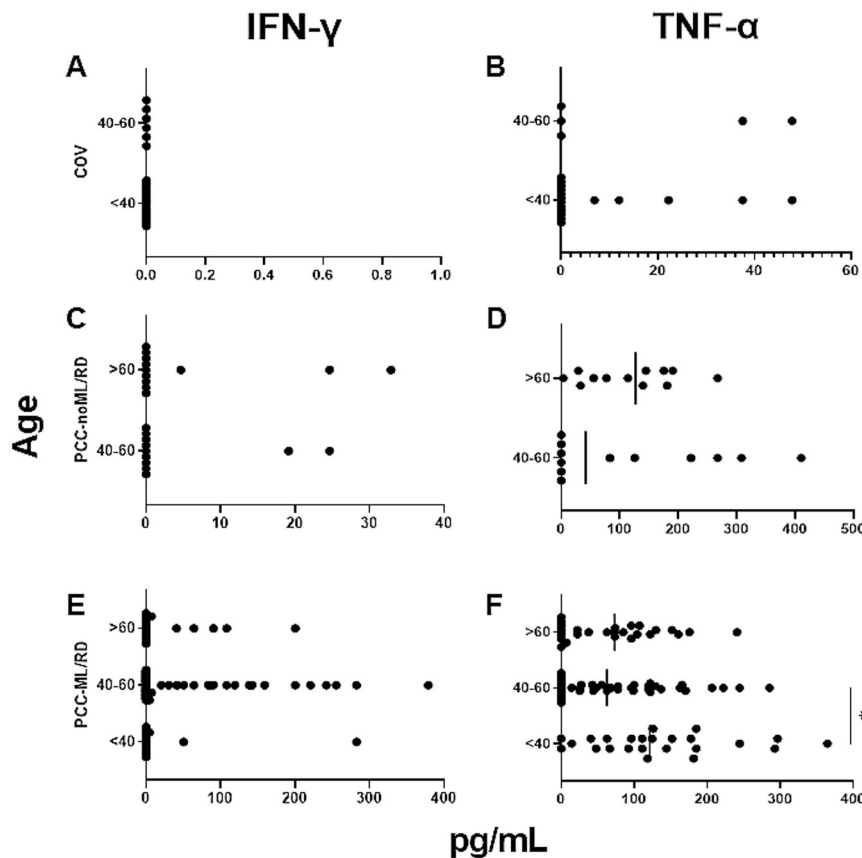


Fig. 3. Cytokine concentrations (pg/mL) across different age groups (<40 years old; 40–60 years old and > 60 years old). A and B: Control groups without PCC (COV) for IFN- γ and TNF- α . C and D: Post-COVID-19 patients with general symptoms (PCC-noML/RD). E and F: Post-COVID-19 patients with neurological symptoms (PCC-ML/RD). * $p < 0.05$ indicates statistically significant differences between the groups of ages.

which can function as immune brakes (Su et al., 2024). In both PCC groups, we observed low levels of IL-2 compared to the COV and HC groups. This finding may suggest its negative influence (down-regulation) on the dysregulated immune response reported in numerous PCC cases (Savchenko et al., 2023; Yin et al., 2024).

IL-6 exhibits both pro- and anti-inflammatory properties, whereas IL-18 is associated with pro-inflammatory processes and is strongly linked to inflammasome activation. Although these cytokines are described as central components of systemic inflammation in acute COVID-19 (Freitas et al., 2025), they also play a relevant role in the development of long-term neurological complications (Low et al., 2023; Anjum and Hussain, 2024). Levels of these cytokines were elevated in the PCC-noML/RD and PCC-ML/RD groups and were significantly different from the control groups. Combined with the reported symptoms, this may indicate that patients with PCC suffer from a degree of chronic inflammation, including within the CNS.

Furthermore, evidence suggests that elevated IL-18 levels in the chronic phase of COVID-19 may act as a signal of the increased risk of cardiovascular disease (Andrejčinová et al., 2024), which is a significant concern for patients with pre-existing cardiovascular comorbidities (Fig. S1B). Fortunately, our study did not document the emergence or worsening of cardiovascular complications in the interviewed volunteer's cohort. It is noteworthy that in our cohort, levels of certain cytokines remained elevated for periods of up to one year (the highest average of 11 months in the PCC-ML/RD group; Table 1) post-infection compared to control groups. This sustained elevation contrasts with findings from other studies (Frontera et al., 2024; Maciel et al., 2024; Phetsouphanh et al., 2024).

In this study, the PCC-ML/RD group showed the highest TNF- α levels (99.9 (10.9–176.6) pg/mL), with a statistically significant difference compared to all other groups (HC, COV, and PCC-noML/RD) (Fig. 2F). TNF- α is a key mediator of the cytokine storm, driving acute COVID-19 severity through endothelial activation, apoptosis, and BBB disruption, thereby facilitating viral neuroinvasion and immune cell infiltration into the CNS (Raza et al., 2024). While persistently high peripheral TNF- α levels characterized infections with early variants, this pattern appears attenuated in Omicron, even in patients with neurological symptoms (Ganesh et al., 2024). Importantly, elevated TNF- α has been inversely associated with executive function performance in post-COVID-19 cohorts, supporting the view that persistent systemic inflammation contributes to cognitive decline and “brain fog” (Varenaya et al., 2025). Taken together, these findings suggest a shift from peripheral inflammation toward neuroinflammation as the dominant pathophysiological driver of PCC, potentially contributing to the memory impairment and reasoning difficulties that were found as the main symptoms of volunteers from the PCC-ML/RD group.

Elevated IFN- γ , a key marker of COVID-19 severity, is linked to coagulopathies and endothelial dysfunction (Paranga et al., 2024). In our cohort, the PCC-ML/RD group exhibited the highest IFN- γ levels, significantly differing from the recovered (COV) group (Fig. 2E). This aligns with evidence that PCC is characterized by a persistent, antigen-independent IFN- γ production from CD8⁺ T cells, indicating chronic immune activation (Krishna et al., 2024) and distinguishing patients with persistent symptoms from those who fully recover. A dysfunctional IFN- γ response appears critical: while inadequate in acute critical cases, its abnormal persistence in PCC is associated with failure to control the

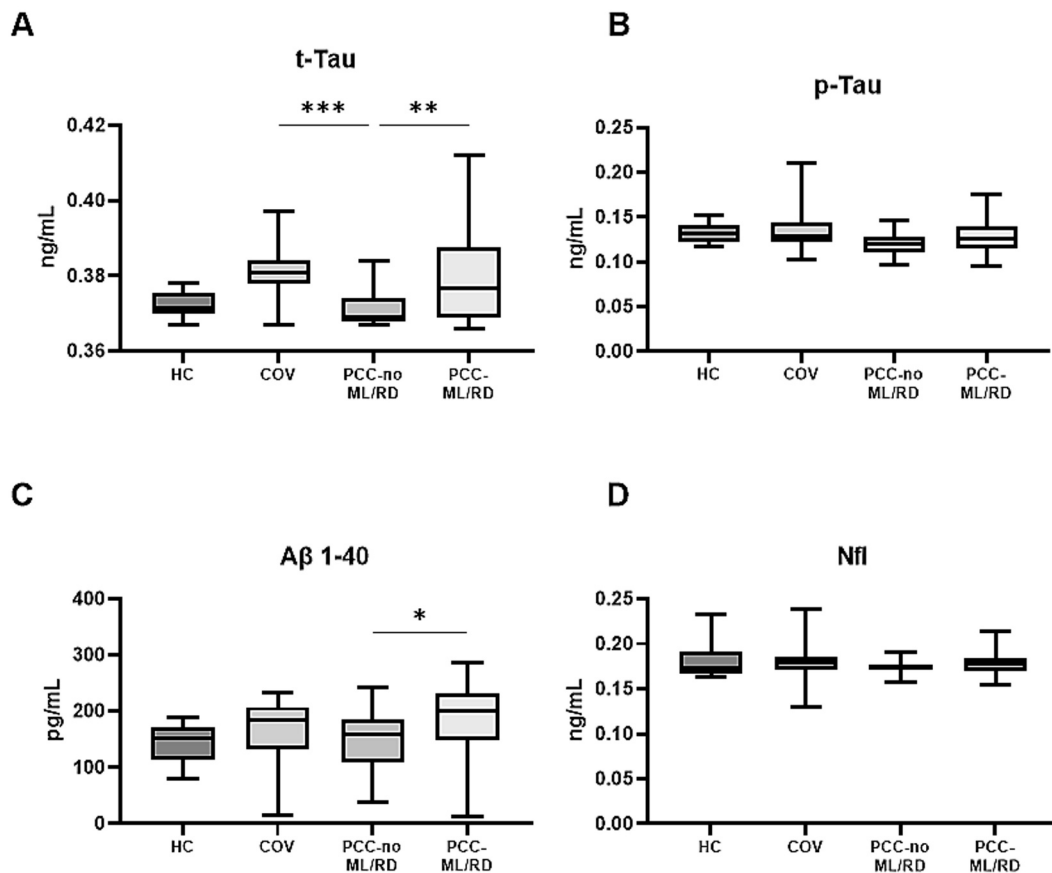


Fig. 4. Groups: HC (healthy control), COV (COVID-19 without PCC), PCC-noML/RD (PCC without memory loss and reasoning difficulty), PCC-ML/RD (PCC with memory loss and reasoning difficulty). (A) Total Tau (t-Tau). (B) Phosphorylated Tau (p-Tau). (C) β-Amyloid (Aβ 1–40). (D) Neurofilament light chain (NfL). Values represent medians and interquartile ranges. Significant differences were assessed by Kruskal-Wallis test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), followed by Dunn's post hoc test.

virus and perpetuation of systemic and neuroinflammation (Ghaffarpour et al., 2025). Furthermore, IFN- γ likely interacts with other pathophysiological mechanisms, such as autoimmunity, microclot formation, and gut dysbiosis, to drive core PCC symptoms including fatigue, cognitive impairment, and dysautonomia (Peluso and Deeks, 2024).

The synergy between TNF- α and IFN- γ during acute SARS-CoV-2 infection drives exacerbated inflammation, culminating in PANoptosis, an inflammatory cell death pathway combining features of apoptosis, necroptosis, and pyroptosis (Karki et al., 2021). This cascade promotes tissue destruction, release of damage-associated molecular patterns (DAMPs), and amplification of the cytokine storm, worsening encephalopathy and endothelial dysfunction (Ng et al., 2025).

Even in non-critical cases, survivors may retain an “inflammatory scar,” characterized by persistent monocyte and T-cell activation, vascular dysfunction, and reactive microgliosis, sustained by subclinical levels of TNF- α , IFN- γ , and IL-6 (Alonso-Domínguez et al., 2023; Freitas et al., 2025). These mechanisms underlie core PCC symptoms such as chronic fatigue, cognitive impairment, persistent dyspnea, and autonomic dysfunction, as reported across multiple cohorts (Yin et al., 2024; Anjum and Hussain, 2024). Patients at highest risk include those previously hospitalized with moderate to severe disease, particularly individuals with elevated cytokine levels during the first month post-infection (Alonso-Domínguez et al., 2023). Thus, the TNF- α /IFN- γ interplay not only explains acute severity via PANoptosis but also provides a plausible link to chronic inflammation in a subset of survivors, underpinning the clinical phenotype of PCC.

We also observed a relationship between TNF- α and age groups in the PCC-ML/RD cohort, where levels of this cytokine were higher in the

younger subgroup (Fig. 3F). Our study did not identify a specific explanation for this finding. However, based on research by Choudhury et al. (2025) and Queiroz et al. (2022), it is evident that young and middle-aged adults are disproportionately affected by persistent neurological and cognitive symptoms following COVID-19, exhibiting greater impairment in quality of life and executive function. Furthermore, Queiroz et al. (2022) demonstrated that during the acute phase of the disease, younger patients (between 21 and 40 years old) had significantly elevated TNF- α levels, suggesting a more intense inflammatory response in this age group. This may be explained by the hypothesis that younger individuals mount a more robust inflammatory response to COVID-19, potentially leading to a higher burden of PCC symptoms. Nevertheless, further studies integrating these three variables (younger population, elevated TNF- α , and association with neurological symptoms of PCC) are warranted.

The elevation of t-Tau observed in PCC patients with memory loss and/or reasoning difficulty symptoms (Fig. 4A) may be associated with neuronal cytoskeletal stress resulting from persistent inflammation, although it could also reflect adaptive processes related to synaptic remodeling and neuronal plasticity. Cytokines such as IL-6 and IL-18 have been reported to activate JAK/STAT signaling and kinase cascades, including GSK-3 β and CDK5, which may contribute to Tau phosphorylation, microtubule destabilization, and the subsequent release of Tau into the extracellular space (Yin et al., 2024; Fekete et al., 2025). However, the absence of a corresponding increase in p-Tau (Fig. 4B) suggests that the observed elevation in t-Tau may not necessarily indicate a classical tauopathy. Instead, it may be compatible with increased Tau turnover associated with physiological processes of

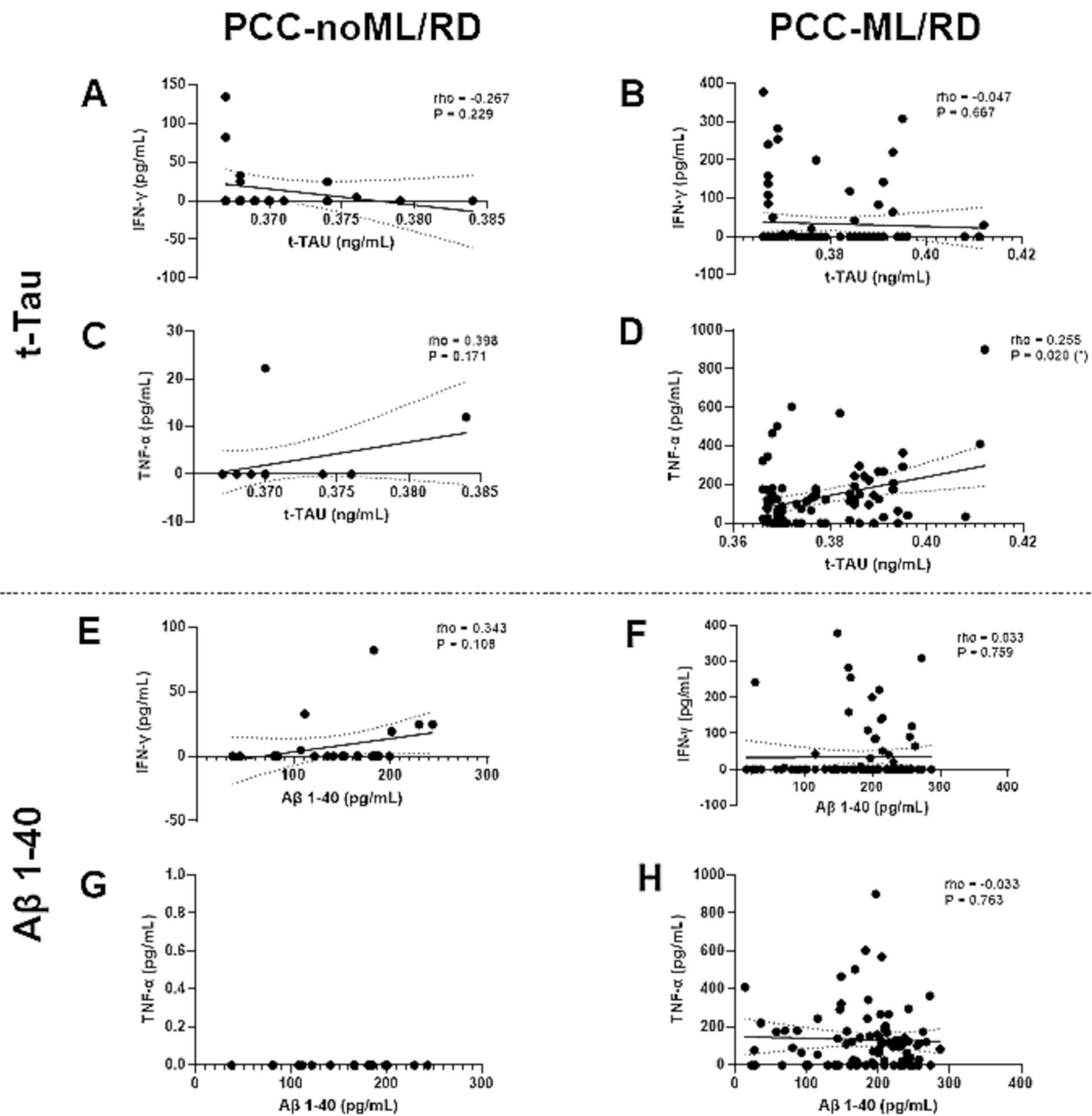


Fig. 5. Linear relationship and Spearman correlation between the cytokines IFN- γ and TNF- α and the neurological markers t-Tau and A β 1-40 in the PCC-noML/RD (PCC without memory loss and reasoning difficulty) and PCC-ML/RD (PCC with memory loss and reasoning difficulty) groups.

synaptic remodeling, consistent with the roles of Tau in neurogenesis, microtubule stabilization, and nuclear protection described by Guo et al. (2017). Likewise, the stable NfL levels may argue against extensive axonal degeneration, although they do not exclude more subtle neuronal dysfunction. Together, these findings raise the possibility that the inflammatory milieu observed in PCC may influence neuronal function without necessarily reflecting irreversible neurodegeneration. Nevertheless, this interpretation should be considered with caution, as the concomitant elevation of pro-inflammatory cytokines (IL-6, IL-18, IFN- γ , and TNF- α) has also been associated with pathological Tau phosphorylation, endothelial dysfunction, and passive Tau release into the circulation. Therefore, the present findings do not allow a clear distinction between adaptive neuronal remodeling and inflammation-related neuronal injury as the primary mechanism underlying the increase in circulating t-Tau. Future longitudinal studies incorporating serial biomarker measurements, individual p-Tau/t-Tau ratio analyses,

correlations with markers of neuronal plasticity (e.g., BDNF, DCX, and synaptophysin), and paired plasma-CSF assessments will be important to clarify the biological significance of these observations.

The increased A β 1-40 levels in the PCC-ML/RD group (Fig. 4C), in contrast with the lack of significant variation across other groups, point to a possible amyloid-vascular metabolic disturbance. A β 1-40 is more closely associated with cerebral amyloid angiopathy and endothelial dysfunction than A β 1-42. Therefore, its elevation may reflect heightened BBB permeability or peripheral overproduction driven by chronic inflammation and platelet activation (Fekete et al., 2025; Tang et al., 2024). The synergistic action of TNF- α and IFN- γ , demonstrated in endothelial models (Karki et al., 2021; Ng et al., 2025), can trigger endothelial apoptosis and tight-junction rupture, facilitating the translocation of neuronal proteins and A β peptides into the circulation. Moreover, IL-6 can upregulate β -secretase (BACE-1) activity, enhancing A β 1-40 generation from APP cleavage (Paranga et al., 2024).

Collectively, these mechanisms suggest that increased A β 1–40 in the PCC-ML/RD group may reflect persistent microvascular injury, neurovascular inflammation, and impaired glymphatic clearance, consistent with proteomic and clinical findings reported in literature (Freitas et al., 2025; Peluso and Deeks, 2024).

The lack of statistically significant differences in NfL (Fig. 4D) across groups indicates that the predominant neurological process in PCC may not involve diffuse axonal degeneration, but rather synaptic dysfunction and high-grade glial responsiveness or systemic inflammation. NfL is a sensitive biomarker of structural axonal injury but less responsive to isolated synaptic or cytoskeletal perturbations (Plantone et al., 2024). Therefore, stable NfL levels are consistent with the observed pattern of elevated t-Tau and A β 1–40 and unchanged p-Tau, suggesting neuronal stress without overt axonal loss (Xu et al., 2022). Additionally, NfL kinetics may obscure group differences if sampling occurred at heterogeneous post-infection intervals, since NfL peaks during the subacute phase and gradually normalizes over time (Al-Aly et al., 2024). Taken together, these findings suggest that persistent systemic inflammation may be a key contributor to the pathophysiology of long COVID, whereas the stable NfL levels provide limited evidence for ongoing widespread neurodegeneration. These observations underscore the importance of longitudinal biomarker monitoring to better define disease trajectories and inform the development of early therapeutic and neuroprotective strategies.

The results showed that the PCC-ML/RD group exhibited higher levels of A β 1–40 and t-Tau than the PCC-noML/RD group, despite being younger, whereas p-Tau and NfL levels remained comparable across groups. This pattern may be consistent with alterations in neurovascular and synaptic function rather than widespread axonal degeneration. In addition, a significant positive correlation was observed between TNF- α and t-Tau (Fig. 5D), suggesting an association between systemic inflammation and circulating Tau levels. This relationship underscores the role of pro-inflammatory cytokines in microtubule destabilization and endothelial permeability, mechanisms previously described in neuroinflammation models and recently confirmed in COVID-19 and PCC (Fekete et al., 2025; Tang et al., 2024). Although increases in A β and Tau are classically linked to aging and chronic neurodegenerative processes, recent evidence suggests that SARS-CoV-2 infection can induce similar alterations in younger brains via inflammatory and endothelial-mediated mechanisms. For instance, Duff et al. (2025) reported a sustained reduction in the A β 42: A β 40 ratio and an increase in p-Tau181 following infection, correlating with endothelial dysfunction and mild cognitive deficits, while Miller et al. (2025) showed that the viral Spike protein can induce A β aggregation in human neural organoids. In this context, the observed elevation of IL-6, IL-18, TNF- α , and IFN- γ , accompanied by reduced IL-2, supports the presence of a chronic and dysregulated inflammatory state that may promote the extracellular release of A β 1–40 and t-Tau.

Additionally, a negative, albeit non-significant, correlation between A β 1–40 and age was identified (Fig. S2), suggesting that higher levels of this protein in younger PCC-ML/RD individuals do not result from senescence but rather from reactive inflammatory-vascular responses. This pattern contrasts with the typical age-related amyloid accumulation and supports the hypothesis that viral infection precipitates an early inflammatory-amyloidogenic phenotype. Similarly, Villareal et al. (2024) reported persistent microglial activation and neuroimmune dysregulation in Alzheimer's patients post-COVID-19, phenomena that may also transiently occur in young brains under inflammatory stress. Thus, the concurrent elevation of t-Tau and A β 1–40 in younger PCC-ML/RD individuals, coupled with increased TNF- α , may reflect an adaptive neurovascular and glial response, characterized by a possible transient release of structural proteins during synaptic repair processes.

An important limitation of this study is that volunteers were stratified according to the presence or absence of memory loss and/or reasoning difficulty, rather than by a comprehensive neurological phenotyping. Although these cognitive symptoms were selected because

they were the most prevalent persistent neurological complaints identified in our previous clinical characterization of this cohort (Sousa et al., 2023), other neurologically relevant manifestations were also present in both groups and may have influenced circulating biomarker levels. Therefore, the observed biomarker differences should be interpreted specifically in relation to the presence of memory loss and/or reasoning difficulty, rather than as biomarkers distinguishing neurological from non-neurological PCC. Future studies incorporating standardized neurological and neuropsychological assessments will be important to further refine these associations.

In conclusion, our findings suggest that post-COVID-19 condition is associated with persistent systemic inflammation accompanied by subtle alterations in selected neurological biomarkers. This persistent inflammatory state may contribute to the neurological symptoms observed in the PCC-ML/RD group, including memory impairment, reasoning difficulties, and hypoprosia, without necessarily being associated with pronounced changes in circulating neurological biomarkers.

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CRediT authorship contribution statement

Graziele de Souza Fonseca: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Raphael de Mello Carpes:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Marcela Espindola Palmeira Pereira:** Methodology, Investigation. **Cintia Monteiro de Barros:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Clinical trial registration

Nothing to declare.

Permission to reproduce material from other sources

Nothing to declare.

Declaration of competing interest

The authors declare there is no conflict of interests.

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Data availability

Data will be made available on request.

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