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Plasma exchange therapy for the post COVID-19 condition: a phase II, double-blind, placebo-controlled, randomized trial

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The post-COVID-19 condition (PCC) is a highly debilitating and persistent postinfectious syndrome that affects millions of people worldwide and has no effective treatment. Therapeutic plasma exchange (TPE) has the potential to improve the PCC by clearing the peripheral soluble pro-inflammatory immune milieu derived from acute or persistent SARS-CoV-2 infection. In a phase II, double-blind, placebo-controlled, randomized trial, fifty subjects with PCC were randomly assigned (1:1) to receive six sessions of either TPE or a sham plasma exchange and were followed for 90 days (ClinicalTrials.gov registration: NCT05445674). The primary endpoint was safety; secondary endpoints included functional status, symptomology, quality of life, neurocognitive symptoms, and peripheral biochemistry, hematology, coagulation and inflammation parameters. Both study arms had a similarly favorable safety profile. There were no diferences between groups in any of the efficacy parameters evaluated. Whereas TPE is safe, it did not lead to any discernible improvement of the PCC in this clinical trial.

The post-COVID-19 condition (PCC), or "Long COVID", is a highly disabling syndrome that affects at least 5% of subjects who survive SARS-CoV-2 infection[1–4]. Without a targeted, effective treatment, the PCC has a poor prognosis[5–7] and negatively impacts patients' quality of life, their social bearing and their capacity to work[8–11]. It also causes a large economic burden due to expenses incurred by social, occupational, and health care[12,13]. It is thus paramount to find a treatment to cure or alleviate the PCC. Therapeutic plasma exchange (TPE) has been proposed as a one of such possible treatments[14].

By eliminating soluble substances implicated in the pathogenesis of PCC such as cytokines, autoantibodies, thrombo-inflammatory molecules, or immune complexes[9,15–19], TPE has the potential to alleviate PCC symptoms and thereby improve patients' functional status. Although it is an invasive procedure, TPE rarely causes adverse effects and, when they occur, they are usually mild and reversible[20]. Therapeutic plasma exchange has been used to treat different immune, hematological, and infectious diseases[20], including severe acute SARS-CoV-2 infection[21–23]. Thus, the risk-benefit ratio is favorable to evaluating TPE in individuals with PCC with significant functional

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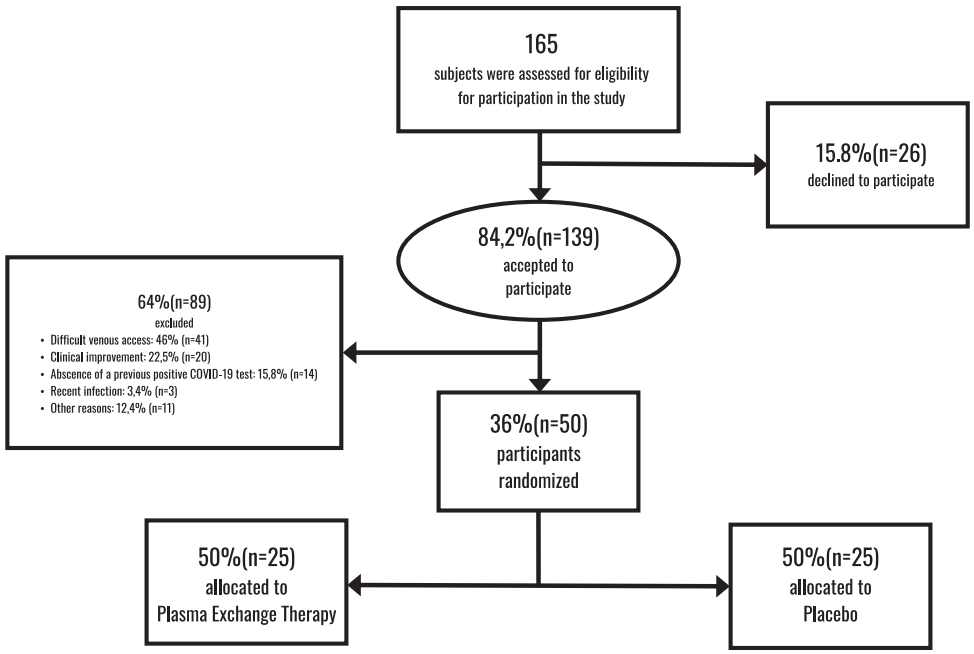

Fig. 1 | CONSORT diagram.

impairment. However, current evidence of its efficacy in the PCC is anecdotal, and clinical trials are lacking.

Here we present a phase II, double-blind, placebo-controlled, randomized trial to assess the safety and efficacy of TPE in subjects with PCC (ClinicalTrials.gov registration: NCT05445674).

Results

Study design and endpoints

Subjects aged 18 years and older, who fulfilled the WHO criteria of PCC[24] and had moderate to severe impairment in their functional status defined by grade 3 or 4 in the Post-COVID-19 Functional Status Scale (PCFS)[25], were eligible to enroll in this study. All participants had to have a microbiological confirmation of SARS-CoV-2 acute infection by nasopharyngeal SARS-CoV-2 polymerase chain reaction (PCR), transcription-mediated amplification (TMA), validated nasopharyngeal lateral flow assay rapid antigen test (RAT), SARS-CoV-2 serology against S2 or/and RBD proteins before SARS-CoV-2 vaccination, or SARS-CoV-2 serology against N protein.

Participants were randomly assigned (1:1) to receive TPE (n = 25) or sham plasma exchange (placebo arm, n = 25). The TPE consisted of six sessions on days 1, 3, 8, 10, 15, and 17 (Mondays and Wednesdays of three consecutive weeks) including human serum albumin. The placebo arm also received six sessions of sham plasma exchange, which involved infusing 200 to 250 cc of sterile saline solution 0.9% on the same days and times as TPE. The medical device used for TPE and sham was the Spectra Optia® Apheresis System. This device uses continuous flow centrifugation technology to separate and collect blood components continuously during the TPE.

The primary endpoint of this trial was safety. Secondary endpoinds were efficacy, measured as the changes in functional status (Post-Covid Functional Scale, PCFS), PCC symptoms (Germans Trias' Long COVID Symptom Questionnaire), fatigue (Fatigue Severity Scale, FSS), quality of life (EuroQol-5D and adapted version of MOS-HIV), neurocognitive performance (NEU Screen [composed of the Trail Making Test (Part A and B) and the Controlled Oral Word Association Test] and semantic fluency test), subjective everyday memory complaints (Memory Failure of Everyday Questionnaire [MFE-30]), and anxiety and depression symptoms (Hospital Anxiety and Depression Scale [HADS]). Additional secondary efficacy parameters were the longitudinal evaluation of biochemistry, hematology, coagulation and inflammation blood biomarkers.

Demographics and clinical characteristics at baseline

From September 2022 to June 2023, 168 patients were screened and 50 were enrolled in the trial (Fig. 1). Both study groups were well balanced at study entry (Table 1). Study participants were predominantly women in their late fourties. In all included patients, sex coincided with gender. About half of them had an advanced education level (bachelor´s degree or equivalent). Half were on sick leave when the study began and, among those working, 25% had an adapted work schedule due to PCC symptoms. More than 80% had comorbidities predating SARS-CoV-2 infection, with allergies and dyslipidemia being the most frequent. Most subjects were first infected with the ancestral SARS-CoV-2 variant and before they received any SARS-CoV-2 vaccine dose. However, when the trial was performed, more than 75% of study participants had received at least 2 vaccine doses.

Safety

All study participants had at least one adverse event (AE) and most participants in both groups presented three or more (Table 2). No statistically significant differences were found between the groups in the characteristics of AEs. No serious AEs were described in any arm, being most of them moderate (grade 2 or lower). There was only one severe AE reported, i.e.: grade 3 increased fatigue in the TPE arm. Overall, AEs were more likely to be attributed to the study procedure in the TPE arm than in subjects receiving placebo. The most common AE reported were local symptoms at the administration site, accounting for 41 (17.4%) in the TPE arm and 37 (19.6%) in the placebo arm. Gastrointestinal disturbances were noted in 39 (16.6%) individuals receiving TPE and 28 (14.8%) receiving placebo. Musculoskeletal disorders were observed in 31 (13.1%) participants treated with TPE arm and in 13 (6.9%) receiving placebo (Table 2, Supplementary Table 1).

Efficacy

Therapeutic plasma exchange had no discernible beneficial effect on any of the efficacy parameters evaluated in this study, compared to placebo. Functional status, symptomology, quality of life, and

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Table 1 | Demographics and clinical characteristics of participants at baseline

	Therapeutic Plasma Exchange group (n = 25)	Placebo group (n = 25)
Age (years), mean (s.d.)	48.9 (8.10)	48.4 (10.54)
Female sex assigned at birth, n (%)	16 (64)	17 (68)
Education, n (%)		
Primary education (primary education or first stage of basic education)	1 (4)	3 (12)
Intermediate education (secondary education)	11 (44)	10 (40)
Advanced education (bachelor's or equivalent level)	13 (52)	12 (48)
Employment status, n (%)		
Employed	9 (36)	8 (32)
Unemployed	2 (8)	1 (4)
Sick leave	14 (56)	13 (52)
Pensioner and/or disabled	0	1 (4)
Other	0	2 (8)
Working Schedule, n (%)		
Full-time	6 (75)	5 (63)
Reduced schedule due to Long Covid	2 (25)	2 (25)
Reduced schedule due to other reason	0	1 (13)
Type of sick leave, n (%)		
Temporary	10 (71)	12 (92)
Permanent	4 (29)	1 (7.7)
Sick leave, n (%)		
Related to Long Covid	13 (93)	13 (100)
Not related to Long Covid	1 (7.1)	0 (0)
Commorbidities (previous to SARS-CoV-2 infection)		
Yes	21 (84)	21 (84)
No	4 (16)	4 (16)
Number of commorbidities		
None	4 (16)	4 (16)
1-3	9 (36)	15 (60)
4 or more	12 (48)	6 (24)
Most prevalent pathologies, n (%)		
Allergy*	6 (24)	3 (12)
Dyslipidemia	7 (28)	2 (8)
Hypothyroidism	4 (16)	3 (12)
Vitamin D deficiency	3 (12)	3 (12)
Migraine	5 (20)	1 (4)
Anxio-depressive síndrome	3 (12)	2 (8)
Insomnia	3 (12)	1 (4)
Adeinomectomy	1 (4)	3 (12)
Asthma	1 (4)	3 (12)
Anaemia	3 (12)	0
Urticaria	0	3 (12)
SARS-COV-2 infection		
Ancestral (12.03.2020 – 07.02.2021)	19 (76)	17 (68)
Alpha (08.02.2021 – 27.06.2021)	1 (4)	3 (12)
Delta (28.06.2021 – 19.12.2021)	4 (16)	3 (12)
Omicron (20.12.2021 – Present)	1 (4)	2 (8)
SARS-COV-2 vaccination	23 (92)	24 (96)
Number of SARS-COV-2 vaccine doses		
1 dose	4 (17.4)	2 (8.3)
2 doses	8 (34.8)	14 (58.3)
3 doses	9 (39.1)	6 (25)
4 doses	2 (8.7)	2 (8.3)

cognitive scores did not improve in individuals who received TPE compared to placebo, overall or in any subdimension evaluated. (Figs. 2 and 3)

At the onset of the trial, all subjects had moderate to severe functional decline, defined by a PCFS score of 3-4. An improvement in the PCFS score to grades 1-2 was seen at day 45 in 6 patients (24%) in the TPE arm and in 7 patients (28%) receiving placebo. Of note, at day 90, 10 (40%) participants in the placebo arm but only 6 (24%) in the TPE arm had improved to a PCFS score 1-2 (Fig. 2a).

No longitudinal changes nor differences between groups were found in the Germans Trias' Long COVID Symptom Questionnaire (Fig. 2b), the fatigue severity scale (Fig. 2c), quality of life (Fig. 2d and Supplementary Table 3), cognitive performance (Fig. 3a, b), subjective everyday memory complaints (Fig. 3c), and anxiety and depression symptoms (Fig. 3d).

Laboratory outcomes

As Fig. 4 shows, there were no longitudinal differences nor differences between study arms in any of the analytical parameters evaluated on days 8, 15, 22, 45, and 90, including biochemistry, hematology and inflammatory and coagulation parameters (Suppplentary table 4). Additionally, there were no longitudinal differences in complement (CH50, C3, C4) or IL6 levels, performed on days 0 and 22 (Supplementary Table 5). However, a reduction in cholesterol and immunoglobulin levels was observed in the TPE arm at day 22 (Supplementary Table 5) relative to day 0. Two participants (8%) in the placebo arm tested positive for antinuclear antibodies (ANAs) at day 0, and 3 patients (12%) at day 22. In the TPE arm, 10 patients (40%) were ANA-positive at day 0. By day 22, 4 of these patients were ANA-negative, 3 showed a reduction in ANA titers, and 3 exhibited no change in their titers (see Supplementary Table 6).

Discussion

The PCC is a complex, long-lasting and highly disabling disease, which has no specific treatment to date. Plasmapheresis has been proposed as a potential treatment for PCC due to its ability to remove inflammatory substances from the blood. This is the first randomized clinical trial evaluating therapeutic plasma exchange in subjects with PCC. Our findings demonstrate that, although plasma exchange is safe, unfortunately it is not effective in improving symptoms, functional status, quality of life, or neurocognitive impairment in subjects with moderate to severe PCC.

In accordance with previous studies[26], TPE was a safe procedure also in subjects with PCC. No cases of severe anemia, substantial electrolyte imbalances, or serious procedural symptoms were reported. Of note, in our study oral calcium and magnesium supplements were given before the procedure in subjects with higher risk of hypocalcemia or hypomagnesemia. Although plasmapheresis reduces immunoglobulin M levels following one or two consecutive procedures and immunoglobulin G levels after several exchanges[27] we did not observe a higher

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Table 2 | Analysis of Adverse Events

	Therapeutic Plasma Exchange group	Placebo group
Number of Total Adverse Events	236	189
Number of Adverse Events per patient, n (%)		
0	0	0
1	0	2 (8)
2	0	1 (4)
3 or more	25 (100)	22 (88)
Adverse Event grade, n (%)		
Mild	0	5 (20)
Moderate	24 (96)	20 (80)
Severe	1 (4)	0
Serious Adverse Event	0	0
Relationship with the study*, n (%)		
Yes	24 (96)	16 (64)
Relationship with albumin*, n (%)		
Unrelated	15 (60)	20 (80)
Unlikely	2 (8)	0
Possible	3 (12)	3 (12)
Likely	5 (20)	2 (8)
Relationship with plasmapheresis*, n (%)		
Unrelated	5 (20)	13 (52)
Unlikely	0	0
Possible	6 (24)	6 (24)
Likely	14 (56)	6 (24)
Relationship with other study procedure*, n (%)		
Unrelated	10 (40)	12 (48)
Unlikely	3 (12)	0
Possible	5 (20)	2 (8)
Likely	1 (4)	5 (20)
Definitely	6 (24)	6 (24)
Adverse events reported (HLGT classification)		
Anaemias nonhaemolytic and marrow depression	2 (0.9)	0
Cardiac disorders	5 (2.1)	3 (1.6)
Ear and labyrinth disorders	0	1 (0.5)
Eye disorders NEC	1 (0.4)	1 (0.5)
Gastrointestinal disorders	39 (16.6)	28 (14.8)
General disorders and administration site conditions	41 (17.4)	37 (19.6)
Hepatobiliary disorders	1 (0.4)	0
Allergic conditions	0	1 (0.5)
Infections and infestations	14 (5.9)	17 (9)
Injury, poisoning and procedural complications	1 (0.4)	3 (1.6)
Investigations	4 (1.7)	3 (1.6)
Metabolism and nutrition disorders	5 (2.1)	4 (2.1)
Musculoskeletal and connective tissue disorders	31 (13.1)	13 (6.9)
Nervous system disorders	45 (19.1)	31 (16.4)
Psychiatric disorders	12 (5.1)	6 (3.2)
Renal and urinary disorders	2 (0.9)	2 (1.1)
Reproductive system and breast disorders	0	1 (0.5)
Respiratory, thoracic and mediastinal disorders	11 (4.7)	13 (6.9)
Skin and subcutaneous tissue disorders	5 (2.1)	7 (3.7)
Surgical and medical procedures	1 (0.4)	1 (0.5)
Vascular disorders	12 (5.1)	12 (6.4)

frequency of infections in the TPE arm. It is thus unlikely that TPE might require immunoglobulin replacement in subjects with PCC. However, this study was small and TPE was not efficacious.

Different plasmapheresis techniques have been suggested as potential treatments for PCC[14,28–30]. Unfortunately, we did not find any evidence of efficacy of TPE in any of the clinical or analytical parameters evaluated. Several factors may have influenced our results.

One is the time elapsed from the acute SARS-CoV-2 infection to the plasmapheresis. In previous case reports showing improvement with plasmapheresis, the time elapsed from acute SARS-CoV-2 infection was shorter, ranging from 2 to 7 months[28,29]. In our study, the average time was two years in both groups. Over time, the concentrations of lipids, autoantibodies, cytokines or other molecules that could be removed by plasmapheresis may likely decrease.

Another factor is the specific method used for plasmapheresis. Plasmapheresis is frequently utilized to treat several immunological disorders[31,32]. It involves different techniques, although Therapeutic Plasma Exchange is the most commonly utilized method and was the one used here. Therapeutic Plasma Exchange uses centrifugation to extract autoantibodies, immune complexes, cryoglobulins, toxins, lipids, and other substances linked to immunological-mediated diseases from plasma. Subsequently, the patient's plasma is restored using fresh frozen plasma or albumin[31]. Other plasmapheresis methods include double filtration plasmapheresis (DFPP), immunoadsorption (IA) or selective plasma exchange (SPE). Double filtration plasmapheresis is a modification of the conventional TPE method, employing dual layers of filters to selectively remove specific substances, including immune complexes and high-molecular-weight elements[33]. Immunoadsorption, suggested as a potential treatment for PCC in recent studies[34], specifically targets and eliminates certain molecules, including antibodies, by utilizing adsorbent materials[32] and SPE employs a more targeted approach by removing only certain plasma components instead of the entire volume[27]. The objective of TPE in patients with PCC is to remove large amounts of pathogenic substances, such as pro-inflammatory compounds, and substitute them with albumin[14]. In our study, the amount of plasma removed per single TPE procedure varied according to the volumes of plasma exchanged. Usually, 1 to 1.5 plasma volume was exchanged because larger volumes do not add much benefit, but increase the risk of side effects. The removal of the pathogenic substances could also depend on its distribution between the intravascular and extravascular compartment[35]. For example, a notable reduction in IgM antibodies can be attained following one or two consecutive procedures, as they are mainly found in the intravascular area. On the other hand, IgG antibodies, present in both intravascular and extravascular compartments, may have needed several exchanges to reduce total body storage.

A third factor that could have potentially affected the efficacy of our TPE strategy is a mismatch between the speed of production and clearance of the target pathogenic substances and the number and frequency of TPE sessions (Mondays and Wednesdays of three consecutive weeks, in our study). Although we cannot rule out this possibility, the absolute lack of benefit in any of the intermediate timepoints evaluated, makes us feel skeptical that a different frequency, speed or duration of treatments might have elicited more positive results.

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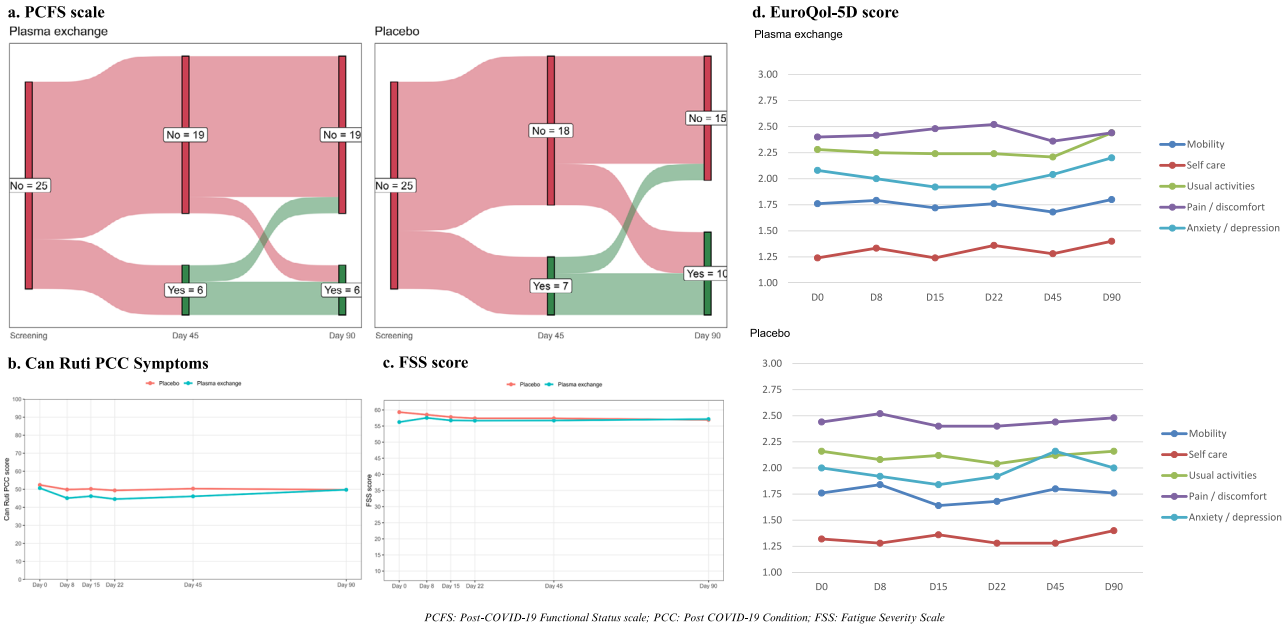


PCFS: Post-COVID-19 Functional Status scale; PCC: Post COVID-19 Condition; FSS: Fatigue Severity Scale

Fig. 2 | Evolution of the mean score of the PCFS, Can Ruti PCC Symptoms Scale, FSS and EuroQol-5D score in the two groups over time. a Post-COVID-19 Functional Status scale at screening, day 45 and day 90 after the intervention in the Plasma Exchange and placebo arms. "No" indicates no improvement and "Yes" denotes an improvement. **b** Evolution of Post COVID-19 Condition symptoms (PCC symptoms) throughtout the study of the Plasma Exchange (Turquoise) and Placebo arms (red). The plot depicts the total score obtained in the German Trias' Long COVID symptom questionnaire at day 0, 8, 15, 22, 45 and 90 of the study. **c** Evolution of the Fatigue throught the study in both groups. The plot depicts the total score of the Fatigue Severity Scale obtained at days 0, 8, 15, 22, 45 and 90 of the study. **d** Evolution of the quality of life of participants in both groups. The plot shows the total EuroQol-5D score obtained at days 0, 8, 15, 22, 45 and 90 of the study, exploring different areas: Mobility (dark blue), Self care (orange), Usual activities (green), Pain/discomfort (light blue) and Anxiety/depression (purple).

Finally, the potential inclusion in the study of subjects with different PCC subphenotypes, could have theoretically influenced our results. However, such phenotypes, as well as their precise underlying pathogenesis, remain to be defined[5,8,9,36,37]. Our randomization process allowed an adequate balance of the pre-treatment demographic chatracteristics, comorbidities and other potential confounders. Actually, the symptom profiles of both arms were comparable and did not suggest any subsyndromic unbalance. In this context, we adopted a patient-centered approach by enrolling participants based on a comprehensive assessment of their clinical symptoms and quality of life, as assessed by the PCFS scale. This methodology was intended to reflect the multifaceted nature of PCC, acknowledging that the condition can manifest in a range of different symptoms. Future studies should ideally aim to incorporate more objective and standardized biomarkers[38] to improve the precision of patient inclusion criteria, including complement system alterations, erythrocyte agglutination and specific autoantibody profiles. Finessing the phenotypic profile is crucial for more targeted interventions. The TPE, in contrast, exerts broader, simultaneous effects on many soluble substances in peripheral blood. In our opinion, the lack of efficacy of TPE likely underscores that the key pathogenic events for PCC either occur at very early stages following SARS-CoV-2 infection, and were therefore missed in this study, or are a matter of localized tissular interactions not necessarily reflected in the plasma compartment.

This study has limitations. The most obvious one is its small sample size, which does not allow to fully rule out an effect of TPE on PCC. However, our results are enough to suggest that, if such effect exists, it size its likely to be small and, unless we are ble to identify better treatment candidates, the efficiency of pursuing a larger study of this invasive procedure is low. Second, as discussed before, our findings cannot be generalized to all plasmapheresis approaches. Third, all subjects were recruited from a single site, which introduces biases linked to the characteristics of patients seen in this center and limits generalizability. Conversely, the study's single-center approach facilitated concentrated patient management and ensured homogeneity in the implementation of methods and protocols. Most importantly, it facilitated the implementation and maintainance of double-blinding, despite the logistical and technological hurdles of preparing and administering the placebo during sham plasma exchange. Double blinding is crucial in PCC trials to control for potentially large placebo effects when using qualitative endpoints and to properly assess the toxicity of study interventions, and further enhances the validity and reliability of our findings.

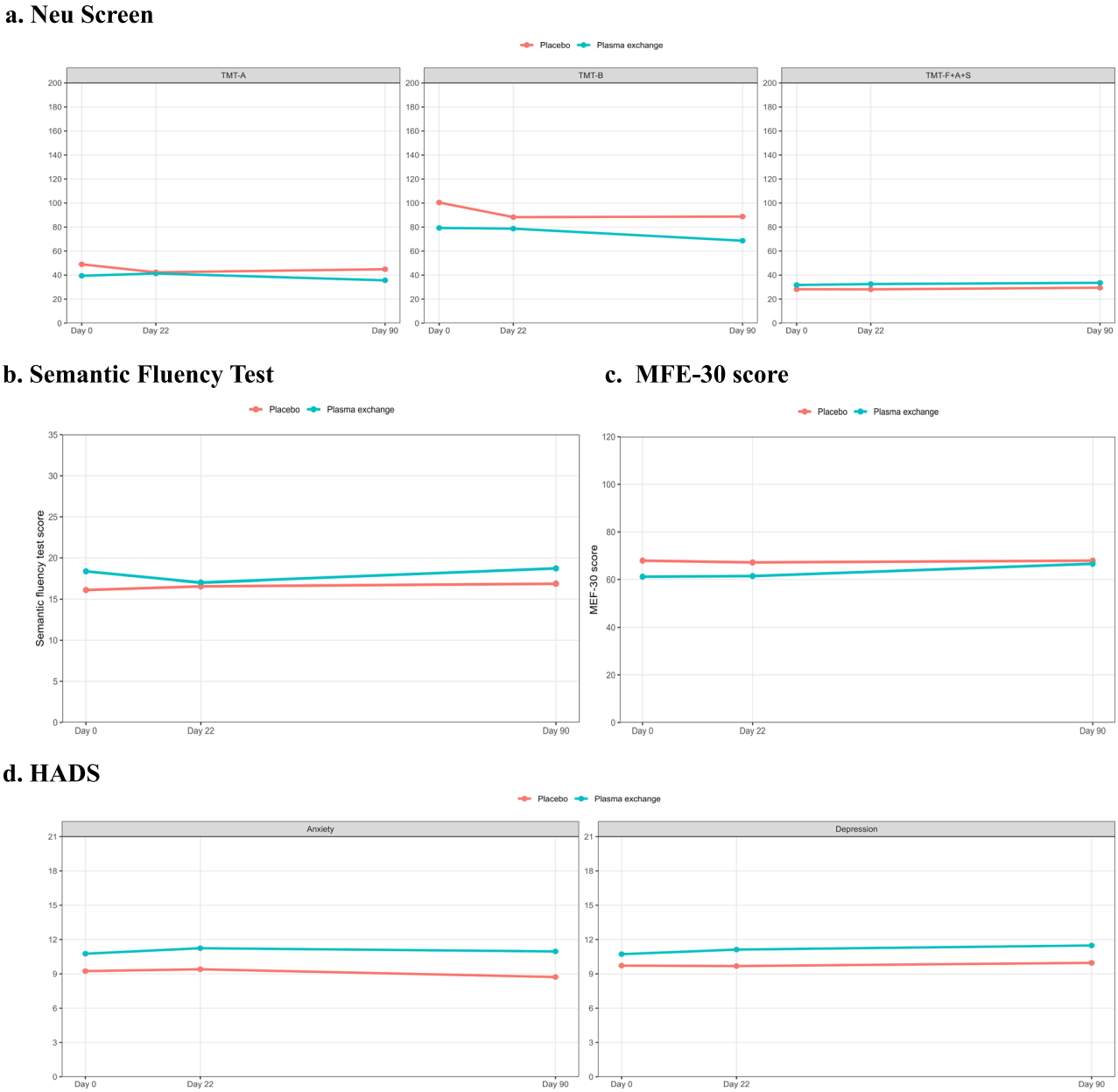
In conclusion, therapeutic plasma exchange is safe, but unlikely to improve the PCC. Long COVID remains an extraordinarily complex disease and public health challenge with no specific treatment to date.

Methods

Study design and eligibility criteria

This is a phase II, placebo-controlled, double-blind randomized clinical trial enrolling patients with PCC at the Hospital Germans Trias i Pujol, in Badalona, Catalonia, Spain (*ClinicalTrials.gov registration: NCT05445674, study preregistration date: July 6, 2022*). The study protocol and all amendments were approved by Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) and the Ethical Review Board of the Germans Trias i Pujol University Hospital (PR(AG) 191/2022). A detailed description of the protocol is in the supplementary information. This study was conducted in accordance with the Declaration of Helsinki and the international standards of good clinical practice. All study participants provided written informed consent and did not receive any compensation. The first and the last patient were enrolled in the study on September 29, 2022, and March 3, 2023, respectively. The data lock was June 15, 2023.

Individuals aged 18 years old or older, of either sex assigned at birth and self reported gender, with a diagnosis of PCC according to the WHO criteria[24] and who had tested positive for SARS-CoV-2 infection at least 90 days before the study were eligible to enroll in the study. Confirmation of SARS-CoV-2 infection was conducted through a



MFE-30: Memory Failures in Everyday questionnaire; HADS: Hospital Anxiety and Depression Score

Fig. 3 | Neuropsychological assessment. A battery of neurocognitive tests were perfomed at day 0, 22 and 90 of the study for participants of Plasma Exchange (Turquoise) and placebo arms (red) to evaluate cognitive status, including Neu Screen (**a**) Semantic Fluency test (**b**) Memory Failures in Everyday questionnaire (**c**) and Hospital Anxiety and Depression Score (**d**).

nucleic acid test (Polymerase chain reaction [PCR] or Transcription-Mediated Amplification [TMA]), a validated rapid antigen test, or SARS-CoV-2 serology before SARS-CoV-2 vaccination. They should also be experiencing symptoms that hindered their daily activities, graded as 3 or 4 on the post-COVID-19 Functional Status scale (PCFS)[25], and have a suitable peripheral venous catheter access. Female candidates of childbearing potential were required to use a highly effective form of birth control, such as abstinence, hormonal contraception, intrauterine device (IUD), or anatomical sterility.

Exclusion criteria were SARS-CoV-2 infection diagnosed during the previous 90 days, receiving a SARS-CoV-2 vaccine dose within the previous 30 days, having no significant limitations in performing all usual duties/activities (i.e., grades 0, 1, or 2 in the PCFS scale[25]), having medical conditions for which 250 mL of intravenous fluid was considered dangerous (e.g., decompensated heart failure or renal failure with fluid overload), being pregnant or breastfeeding, undergoing or planning current hospital admission for any cause during the study follow-up, participating in any other clinical trial until day 90 of follow-up, lacking the ability to consent and/or comply with study requirements in the investigator's opinion, and having contraindications for TPE (such as non-availability of an adequate peripheral venous catheter, hemodynamic instability, septicemia, known allergy to fresh frozen plasma or replacement colloid/albumin, or known allergy to heparin).

Participants and procedures

Study participants were identified in the study records by a unique participant identification number which served as a unique identifier for each participant throughout the trial. This patient ID consisted of a

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Fig. 4 | Analytical parameters evolution post Plasma Exchange versus Placebo. A battery of analytical paramenters were perfomed at day 0, 8, 15, 22, 45 and 90 of the study in Plasma Exchange (Turquoise) and placebo arms (red), including biochemistry, hematology and inflammatory and coagulation parameters.

sequential number provided to each participant at the inclusion/baseline visit, which was not linked to any o his or her personal information. After eligibility was confirmed, randomization was carried out at the hospital's Blood and Tissue bank during the inclusion/baseline visit. Subjects who met the inclusion and exclusion criteria were randomly assigned to one of the study's two arms using a computer system and given an identification code. Each researcher maintained a patient registry, and data were stored in accordance with European and Spanish data protection regulations.

Fifty eligible candidates were randomly assigned to receive either TPE or a placebo in a 1:1 ratio. Both plasma exchange and sham plasma exchange treatments were carried out by antecubital venous access, with continuous or intermittent cell separators (Spectra Optia Apheresis System). The TPE was performed with 5% albumin as the replacement fluid. The typical schedule prescribed was an exchange of 1 volemia. The volume of plasma exchange or albumin for this study was determined based on current scientific evidence and technical recommendations from other diseases under the assumption that they might share similar pathophysiological traits to Long COVID[32] (Plasma volume (Vp)=Total blood volume×(1−Hematocrit)/Hematocrit). The total blood volume was estimated based on the patient's body weight, height and sex. The hematocrit, which represents the fraction of blood volume occupied by red blood cells, was obtained from pre-procedure laboratory analysis. The blood was split into cells and plasma; the cells were mixed with reconstituted 5% human serum albumin and reinfused into the patient with normal saline. None of the patients received conventional fresh-frozen plasma as a replacement fluid (1/3 of the exchanged plasma volume) as no baseline coagulopathies were detected (PT < 50%). The Blood and Tissues Bank at Hospital Universitari Germans Trias i Pujol provided the fresh-frozen plasma for use. For sham plasma exchange procedures, only one infusion of 200 to 250 ml of sterile saline solution 0.9% was performed during the time established for the procedure in the same TPE room. Albumin was not necessary for patients in the sham plasma exchange arm.

Participants in the TPE arm performed six sessions of TPE using human serum albumin on days 1, 3, 8, 10, 15, and 17. The placebo arm got six sessions of sham plasma exchange, which involved infusing 200 to 250 cc of sterile saline solution 0.9% on the same days and time that would have been allotted for TPE, using an intravenous line as done with the rest of the participants. Throughout the treatment period, as

well as after the final TPE/placebo sessions and during follow-up visits, all participants underwent clinical and safety assessments, and blood samples were collected. Subsequent follow-up visits were conducted on days 22, 45, and 90.

The medical device used for TPE was The Spectra Optia® Apheresis System. It uses continuous flow centrifugation technology to separate and collect blood components continuously during the plasmapheresis. This technology ensures a high yield of targeted blood components, such as plasma or platelets, while reducing the risk of hemolysis or celular damage.

Both the investigator and the participant were blinded to the study treatment. To ensure participant blinding, a curtain separated the apheresis apparatus from the patient, and the external infusion lines were always covered with a black, opaque plastic tube. All procedures were carried out while participants were resting and listening to music via headphones.

Variables and data sources

This project utilized the REDCap (Research Electronic Data Capture) tool, which is hosted at Hospital Germans Trias i Pujol. During the baseline visit, we collected the participants' basic information, such as age, gender, and other relevant demographic data, as well as microbiological findings. We also reported any pre-existing medical illnesses that existed at the time of acute COVID-19 symptoms. Additionally, we gathered information about the specific characteristics of their acute COVID-19 episode, such as the date of onset, the type of diagnostic test used, the need for hospitalization, any events during their hospital stay (for example, admission to the intensive care unit or the need of mechanical ventilation), the treatment received, and any diagnostic imaging tests performed. Data on the SARS-COV-2 variant infecting each subject were inferred from the dominant circulating variant in Catalonia (Spain) at the time of infection according to GISAID. Furthermore, we collected data about the participants' employment status at both the baseline and the final visit. This included information regarding their present job, employment type (full-time, part-time, self-employed or unemployed), and any changes in employment status since the onset of acute COVID-19 symptoms.

Interviews with study participants were conducted using structured questionnaires evaluating symptoms (Germans Trias' Long COVID Symptom Questionnaire, a thorough assessment instrument with a total score out of 100 points, with higher symptom load with higher scores), functional status (Post-Covid Functional Status scale, PCFS[25]), fatigue (Fatigue Severity Scale, FSS)[39], quality of life (EuroQol-5D and adapted version of MOS-HIV[40]), and neurocognitive status (NEU Screen[41], semantic fluency test, the Memory Failures in Everyday [MFE-30] questionnaire[42], and the Hospital Anxiety and Depression Score [HADS[43,44]]) (see Supplementary tables 7–10).

These interviews were conducted to evaluate the existence of persistent symptoms as well as the progression of their symptoms on days 0, 8, 15, 22, 45, and 90, with the goal of determining whether 2 or 4 cycles may provide a similar effect as 6. Extensive lab analysis profiles were also performed at the same days 0, 8, 15, 22, 45, and 90, including peripheral blood leukocyte and lymphocyte count, serum C-Reactive Protein (CRP) levels, coagulation tests (prothrombin time -PT- and activated partial thromboplastin time -aPTT-), and microangiopathy serum parameters (LDH, triglycerides, ferritin, D-Dimer) upon inclusion, pre and post intervention. Additional analyses were performed on days 0 and 22, including measurements of interleukin-6, lipid profile (total cholesterol, Low-Density Lipoprotein, cholesterol, triglycerides), total immunoglobulins (IgA, IgG, IgM, autoantibodies (antinuclear antibodies, antimitochondrial antibodies, anti-smooth muscle antibodies, anti-parietal cell antibodies, anti-liver kidney microsomal antibodies) and complement levels (CH50, C3, C4). To assess whether the procedure had a molecular impact, an analysis of immunoglobulins (IgA, IgM, IgG) and lipid profile (total cholesterol, Low-Density Lipoprotein, cholesterol and triglycerides) in apheresis plasma collected after the first procedure in the TPE group was also performed (Suplementary table 11).

Safety objectives included assessment of differences between groups in severity, incidence and frequency of adverse events (AEs). In accordance with the International Conference on Harmonization's criteria, an adverse event (AE) was defined as any adverse medical event that occurred either before or after the investigational technique was performed. The attending physician at the location determined the degree of AEs and classified them as mild if they did not interfere with regular activities, moderate if they limited normal activities, or severe if they made the patient unable to perform normal activities. Any unfavorable medical side effect that, at any dose, resulted in death, was life-threatening, required hospitalization or prolongation of hospitalization, caused persistent or significant disability or incapacity, resulted in a congenital anomaly or birth defect, or was suspected of transmitting any infectious agent through medication was classified as a Serious Adverse Event (SAE). The relationship of the different AEs to TPE, albumin or any other study-related procedure was determined based on the investigator's criteria.

The data was processed in accordance with current data protection legislation (LOPD, The Organic Law 3/2018 of 5 December on the Protection of Personal Data and the Guarantee of Digital Rights, Complementary to the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016, on the protection of natural persons with regard to the processing of personal data and the free movement of such data).

Statistical analysis

Given the pilot exploratory nature of this study and the main safety endpoint, no formal sample size calculation was performed to establish the number of patients to be recruited. A sample size of 25 subjects per group was stabilished as the minimum necessary to analyse the difference in the proportion of subjects with functional disability 3 or 4 by the end of the study. Categorical data were presented as frequencies and percentages, and continuous variables were expressed as means with standard deviations (SD) or medians with interquartile ranges (IQR) depending on the distribution. Numeric endpoints were described using tables and graphical representations to evaluate their evolution over time. Categorical endpoints were depicted using sankey plots to visualize the progression of each category over time. A safety analysis was also conducted, evaluating AEs per-event basis. Due to the exploratory aim of the study no statistical test were conducted with exception of the principal study outcome. Chi-square test was used to compare the PCFS scale score at 45 days and at 90 days. All statistical analyses, tables and plots were performed using R software version 4.3.0 or higher, and the most commonly used packages were gtsummary, ggplot2 and ggsankey.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The study data have been deposited in the EU CT Register database under the access code 2022-000641-33. The raw and personal data are protected and not available due to applicable data privacy legislation: The processing of the data are subjected to current legislation as regards data protection (LOPD, The Organic Law 3/2018 of 5 December on the Protection of Personal Data and the Guarantee of Digital Rights complementary to the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016, on the protection of natural persons with regard to the processing of personal data and on the free movement of such data). The processed data are available in the EU CT Register. Additionally, the data generated in this study are

provided in the supplementary information, which include the study protocol and statistical analysis. The data used in this study are available in the manuscript, and the supplementary information and the study results are available in the EU CT Register database under the access code [https://www.clinicaltrialsregister.eu/ctr-search/trial/2022-000641-33/results].

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Author contributions

LM and RP conceived the study. S.E.C., C.L., G.L., .C.r.L., J.R.S., G.D., A.G., J.C., A.A., C.F., C.M.I., B.Q., E.M.C., A.S., I.G.P., A.C., A.S.J., E.A., J.A.M.M., A.P., C.R.F., R.C., C.E., P.T.,J.P., B.C., M.M. and L.M. collected and curated the data. C.T. and Jo.C. performed the statistical analyses. L.M. and S.E.C. wrote the first draft of the manuscript; all authors critically revised and approved the final version of the manuscript.

Competing interests

LM has received honoraria as speaker from Astra-Zeneca, Gilead, Shionogui and Pfizer, and has participated in advisory boards for Gilead and MSD. CL has received support for attending meetings from Gilead. AG has received grants from Grifols, honoraria for lectures or presentations from Astra-Zeneca, Gilead, and Pfizer and has participated on DSMB or advisory boards for Gilead and MSD. RP hasparticipated in advisory boards for Pfizer, Gilead, MSD, GSK, Atea, Lilly, Roche, Astra-Zeneca, ViiV Healthcare and Theratechnologies, has participated in lectures and seminars funded by Gilead, Pfizer,GSK and AstraZeneca, and has received research funds awarded to his institution from Gilead, Pfizer and MSD. MM has received honoraria for participating in lectures and seminars funded by Gilead, not related to this project.

Additional information

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