Blood group O and post-COVID-19 syndrome

Sara Díaz-Salazar (1), Raquel Navas (2), Laura Sainz-Maza (2), Patricia Fierro (1), Meryam Maamar (3), Arancha Artime (4), Héctor Basterrechea (1), Benedetta Petitta (1), Stefanie Pini (5), José Manuel Olmos (6,7) Carmen Ramos (2,6), Emilio Pariente* (1,6) and José Luis Hernández (6,7)

* Corresponding author

(1) Camargo Interior - Primary Health Care Center. Servicio Cántabro de Salud. Muriedas. Cantabria. Spain.
(2) Camargo Costa - Primary Health Care Center. Servicio Cántabro de Salud. Maliaño. Cantabria. Spain.
(3) Emergency Service. Osakidetza. Servicio Vasco de Salud. Bilbao. País Vasco. Spain.
(4) El Llano - Primary Health Care Center. SESPA- Servicio Asturiano de Salud. Gijón. Asturias. Spain.
(5) Hospital at Home Department. Hospital Universitario Marqués de Valdecilla. Santander. Cantabria. Spain.
(6) Depto. de Medicina y Psiquiatría. Universidad de Cantabria. Santander. Cantabria. Spain.
(7) Servicio de Medicina Interna. Hospital Universitario Marqués de Valdecilla. Instituto de Investigación Valdecilla (IDIVAL). Santander. Cantabria. Spain.

José M. Olmos: ORCID ID: 0000-0002-7765-0891
Carmen Ramos: ORCID ID: 0000-0003-1970-1627
Emilio Pariente: ORCID ID: 0000-0003-0035-3298
José L. Hernández: ORCID ID: 0000-0002-6585-8847

CORRESPONDING AUTHOR:
Emilio Pariente Rodrigo, MD, PhD
"Camargo Interior" Primary Care Center
Associate Professor, University of Cantabria
Avda Bilbao, s/n. 39600-Muriedas, Cantabria, Spain
Tel: +34 942 254 499
Email: emilio.pariente@scsalud.es
ORCID ID: 0000-0003-0035-3298

Blood group O and post-COVID-19 syndrome

Objective

The ABO system modulates the inflammatory response, and it has been involved in SARS-CoV-2 infection. There is increasing evidence of underlying immune-inflammatory mechanisms in post-COVID-19 syndrome (PCS). Blood group O seems to protect against COVID-19 infection, but there is no data on the relationship between blood group O and PCS. This study aimed to assess this potential association.

Subjects and methods

A case-control study including subjects who had suffered from mild COVID-19 in a community setting was designed. Cases were PCS+ patients, controls were PCS- subjects and the exposure variable was blood group O. Baseline epidemiological data (age, sex, BMI, smoking, comorbidities), and clinical and laboratory parameters (inflammatory markers and IgG anti-N antibodies) 3 months after the acute episode, were obtained. Five composite indices of inflammation were built, combining the upper ranges of the distributions of inflammatory markers. Blood group and Rh factor were obtained from the patient's medical history or capillary blood samples. Bivariate and multivariate analyses were performed.

Results

One-hundred and twenty-one subjects were analyzed (56.2% women). The mean age was 45.7±16 years (range, 18-88 years). Blood group frequencies were 43.3% (group A), 7.7% (group B), 5.7% (group AB), and 43.3% (group O). Thirty-six patients were classified as PCS+ (25 women, 11 men; p=0.07). The most frequent symptom was fatigue (42.8%). There were no significant differences between PCS+ and PCS- subjects regarding age, BMI, smoking, or previous comorbidity. The prevalence of PCS was 43.2% (19/44) in the blood group O and 23.7% in non-O subjects (14/60) (p=0.036). The mean number of PCS symptoms was 0.82±1 in the blood group O and 0.43±0.9 in the non-O group (p=0.017). Regarding inflammatory markers, there were no significant differences between PCS+ and PCS- in A, B, and AB groups. In contrast, blood group O PCS+ patients had significantly higher lymphocyte count, plasma CRP, fibrinogen levels, and higher percentages of C2, C3, and C4 composite indices than PCS- subjects. The blood group O had an increased risk of developing PCS compared to non-O subjects (adjusted OR=4.20 [95%CI, 1.2-14]; p=0.023). The variables that contributed the most to the predictive model were blood group O, lymphocyte count, neutrophil count, and female sex. R^2 was 0.46, and the area under the ROC curve was 0.807 [95% CI, 0.70-0.90] (p=0.0001).

Conclusion

An increased risk of PCS associated with blood group O has been observed. Slightly, albeit significant, raised levels of fibrinogen, CRP, and lymphocyte count, not observed in the other ABO blood groups, have been demonstrated in blood group O. Further investigations are needed to confirm these results.

KEYWORDS

Blood group O. Post-COVID-19 syndrome. ABO blood group. COVID-19.

INTRODUCTION

Blood groups A, B, AB, and O configure the ABO system, the most important blood group system in humans. It is located on chromosome 9 (band 9q34.2) and is composed of proteins and oligosaccharides expressed on the surface of red blood cells. Blood groups play a relevant role in cardiovascular and oncological diseases, thromboembolic and hemorrhagic processes, as well as infectious diseases (parasitic, bacterial, and viral), acting sometimes as receptors or co-receptors that facilitate viral particles from entering the cells [1]. People with blood group O have a lower risk of diabetes mellitus, arteriosclerosis, and ischemic heart disease [2], but a higher risk of tuberculosis, cholera, and Norovirus infections [3].

Since the beginning of the SARS-CoV-2 pandemic, a higher prevalence of COVID-19 in blood group A subjects and lower susceptibility to acquiring the infection in those with blood group O was noted [4,5]. This finding has been related to the anti-A antibodies presented in blood group O individuals. These antibodies bind themselves to the A-like antigens or to the spike protein expressed on the virus envelope, blocking its interaction with the ACE-2 receptor, which is an identified gateway for different coronaviruses to infect host cells [6].

Regarding clinical outcomes, blood group A has been associated with a higher risk of hospitalization, mechanical ventilation, and death [6,7]. These adverse outcomes have been related to higher levels of soluble circulating proteins linked to vascular adhesion in people with this group [8]. The studies are discordant concerning the clinical outcomes of group O patients [7,8]. Thus, some have observed a better prognosis [9], that has been related to lower circulating levels of factor VIII and von Willebrand factor -and a subsequently reduced risk of thrombotic phenomena-, and to lower angiotensin-converting enzyme (ACE) activity [10]. However, in other studies, group O has been related to serious outcomes [11-13].

After COVID-19, a variable percentage of patients, even those with mild initial forms of the disease, report prolonged and recurrent symptoms for weeks or months. These symptoms include asthenia -the most frequent-, myalgia, dyspnea, anosmia/ageusia, autonomic dysregulation manifested as orthostatic hypotension, tachycardia, thermoregulation or gastrointestinal disturbances, attention deficit, memory loss, and cognition alterations, leading to a significant impact on the quality of life [14-16]. This condition, known as Long COVID or Post-COVID Syndrome (PCS) [17], has certain similarities with the chronic profile of other epidemic coronavirus infections, such as SARS and MERS [18].

A previous study by our group [19] has shown that in PCS, 3 months after the acute episode, there are slight but significant elevations of inflammatory markers, compared to controls who have had COVID-19 but not PCS, supporting the hypothesis of chronic low-grade inflammation underlying PCS pathogenesis [20,21]. On the other hand, the ABO system modulates endothelial function and influences the inflammatory response [8,22], although the mechanisms are not fully understood [8].

Considering the associations of the ABO system with the inflammatory response, the evidence of the relationship between the ABO system and COVID-19, and PCS as an expression of low-grade inflammation, it is worth asking whether the protective effect of group O against infection extends to the post-acute phase. Taking into account the above considerations, we aimed to assess whether blood group O is related to PCS after mild COVID-19.

SUBJECTS AND METHODS

Design

A case-control study including subjects who had suffered from mild COVID-19 in a community setting was designed. Cases were PCS+ patients, controls were PCS- subjects and the exposure variable was blood group O. The study was conducted on the general population of a semi-urban area attended by a Primary Care center in Northern Spain. The general design of the study is shown in Figure 1.

Participants

Details of participants' enrollment have been previously published [19]. Patients suffering from COVID-19 between April and September 2020 were selected. All the cases were exclusively followed in the Primary Care setting and none of them had been vaccinated against SARS-CoV-2 at the moment of inclusion. SARS-CoV-2 infection was confirmed by a positive real-time reverse transcription-polymerase chain reaction (RT-PCR) test or by the presence of anti-SARS-CoV-2 IgG, three months after COVID-19. A second inclusion criterion was a mild course of infection, according to the WHO definition [23] and characterized by fever, malaise, cough, upper respiratory symptoms, and/or less common COVID-19 manifestations, in the absence of dyspnea. The choice of outpatients with mild COVID-19 reasonably ruled out post-acute symptoms due to previous comorbidities or organ sequelae [19]. No exclusion criteria were considered.

Data collection

Three months after the acute episode (median=115 days), epidemiological variables (sex, age, body mass index -BMI, measured in kg/m^2-, smoking habit, medical history, and Charlson's comorbidity index), clinical data (number of symptoms -as a variable assimilated to 'intensity' of the condition [24]- and specific symptoms) and laboratory test (inflammation markers and anti-SARS-CoV-2 IgG antibodies) were collected. One year later, participants were interviewed once again, and their blood group and Rh factor were obtained.

The interviews were conducted by physicians from the research team, using a structured questionnaire. PCS diagnosis was established when the National Institute for Care and Excellence (NICE) criteria was met:

signs and symptoms that develop during or after an infection consistent with COVID-19, that last more than 12 weeks, and that are unexplained by an alternative diagnosis [17].

Serum inflammatory markers

Serum C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), fibrinogen and D-dimer levels, as well as neutrophil and lymphocyte counts, and neutrophil/lymphocyte ratio (NLR) were analyzed. CRP was measured in mg/dL, and the detection limit was 0.4 mg/dL. Low-grade inflammation has been defined by a serum CRP level >0.3 mg/dL and <1.0 mg/dL [25]. Normal ranges for LDH (U/L), ferritin (ng/mL), fibrinogen (mg/dL), D-dimer (ng/mL), neutrophil count, and lymphocyte count were 120-246, 22-322, 180 -500, 0-500, 1.4-7.5 ($x10^3/\mu L$), and 1.2-5 ($x10^3/\mu L$), respectively.

Blood samples were obtained from an antecubital vein using the standard venipuncture procedure in the morning after a 12-hour fast. LDH and ferritin were analyzed by spectrophotometric assay on an Atellica CH analyzer (Siemens Healthcare Diagnostics Inc, Tarrytown, NY, USA). CRP was quantified by immunonephelometric assay on an Atellica CH analyzer (Siemens Healthcare Diagnostics Inc, Tarrytown, NY, USA). Hematologic cell counts were analyzed on a DXH900 (Beckman Coulter), and fibrinogen and D-dimer on an ACL TOP 750 (Werfen). Anti-N IgG was obtained by chemiluminescence (QLIA), expressing the result as positive or negative.

Blood group samples

The blood group was determined in capillary blood when it was not available in the clinical chart. To do this, a blood sample was taken by puncturing the pad of the finger with a lancet. The visualization or not of the agglutination reaction allowed the identification of the blood group when confronting the sample with reagents of known specificity (Alden Reagents, Laboquimia, Spain)

Statistical analysis

After assessing normality with the Shapiro-Wilk test, quantitative variables were expressed as mean ± standard deviation (SD) or as median [interquartile range (IQR)]. Some variables have been expressed as dichotomous using the median or tertiles. Student's t-test and ANOVA were used as parametric tests and the median, Mann-Whitney U and Kruskal-Wallis, tests as non-parametric procedures. Categorical variables have been expressed as numbers and percentages and the chi-square test was used for their comparison. Correlation analyses have been performed using Spearman's Rho and the Phi coefficient for categorical variables. The strength of an association has been expressed as an odds ratio (OR) with its corresponding 95% confidence interval (CI). Given the small number of subjects in blood groups B and AB, they were classified as group O/non-O, group A/non-A, group B/non-B, and group AB/non-AB.

After selecting the serum inflammatory markers that showed the highest correlations with PCS, the upper ranges of their distributions were combined to develop the inflammatory indices, which were designated correlatively as C1-C5 (Table 1).

Logistic regression was performed to determine the relationship between group O and PCS, after controlling for confounding variables. The confounding variables were selected according to the literature and the principle of parsimony. The model was validated using the determination coefficient R^2 and the area under the ROC curve (AUC).

IBM SPSS 28.0 statistical package (Armonk, NY: IBM Corp) was used to perform the analyses. A *p*-value <0.05 was considered significant in all calculations.

Ethical aspects

The postulates of the Declaration of Helsinki were fulfilled. The study was approved by the Clinical Research Ethics Committee of Cantabria (Internal Code 2021.102).

RESULTS

Descriptive analysis

One-hundred and thirty-four patients with confirmed COVID-19 were included, and 13 were discarded due to moderate or severe disease (Figure 1). Thus, we finally included 121 subjects with mild COVID-19, 68 of them (56.2%) were women. The mean age was 45.7±16 years (range, 18-88 years). The frequencies of the blood groups were 43.3% (group A), 7.7% (group B), 5.7% (group AB) and 43.3% (group O). Tertiles of age distribution were <41, 41-53, >53 years and BMI, <23.8, 23.8-27.6, >27.6 Kg/m². Thirty-six subjects, 29.7% of the sample, were classified as PCS+; 25 were women (35.8%) and 11 men (20.8%) (p=0.07). The remaining 85 were classified as PCS-. The most frequent PCS symptoms were fatigue (42.8%), anosmia (40%), ageusia (22.8%), dyspnea (17.1%), myalgia (11.4%) and palpitations (11.4%).

Table 2 shows the baseline characteristics of the PCS+ and PCS- participants. There were no significant differences between both groups concerning BMI, tobacco use, or previous comorbidities.

Post-COVID syndrome

The prevalence of PCS according to the blood group was: 25% (group A) and 37.3% (non-A) (p=0.18); 25% (group B) and 32.6% (non-B) (p=0.65); 16.7% (AB group) and 33% (non-AB) (p=0.40); 43.2% (O group) 23.7% (non-O) (p=0.036); 32.1% (Rh+) and 32% (Rh-) (p=0.99).

The mean number of symptoms of PCS was 0.44±0.9 (group A) and 0.71±0.9 (non-A) (p=0.17); 0.50±0.9 (group B) and 0.60±1 (non-B) (p=0.77); 0.33±0.8 (AB group) and 0.61±1 (non-AB) (p=0.50); 0.82±1 (O

group) and 0.43±0.9 (non-O) (p=0.017); 0.59±0.9 (Rh+) and 0.60±1 (Rh-) (p=0.98). The symptoms analyzed were anosmia, dyspnea, palpitations, asthenia, telogen effluvium, ageusia, headache, leukonychia, myalgia, concentration difficulties, rhinitis, and cough. They were evaluated according to their presence in O and non-O subjects, with myalgia presenting a significant difference between both groups (Table 3). When analyzing these patients, it has been observed that both group O subjects and patients with myalgia have presented a high frequency of positives in the C2 composite index (64.7% of group O vs 22.7% of non-O, p=0.007; 100% of subjects with myalgia vs 46.2% of subjects without myalgia, p=0.044). Regarding biomarkers, fibrinogen levels in patients with and without myalgia were 510±82 mg/dL and 394.6±87, respectively (p=0.013).

Group O, fatigue, and serum CRP have shown to be related. In group O individuals, fatigue and raised serum CRP showed a positive correlation (Phi=0.372; p=0.022), while in non-O, fatigue and serum CRP were not correlated (Phi=0.03; p=0.80). In group A patients with fatigue, raised CRP was registered in 20%. By contrast, in group O patients with fatigue, the percentage was 37.5% (p=0.021)

Anti-SARS-CoV-2 IgG antibodies

Ninety-eight persons (81%) had anti-SARS-CoV-2 IgG. IgG+ subjects had a mean age of 47.3±15 years, and IgG- subjects, 38.8±18 (p=0.030). IgG+ subjects presented 0.61±1 symptoms compared to 0.17±0.4 symptoms in IgG- subjects (p=0.044). No differences were observed between IgG+ and IgG- subjects regarding sex distribution, Charlson index, tobacco use, or serum inflammatory marker levels. Some 91.4% of subjects with PCS had IgG antibodies, compared to 76.5% of subjects without PCS (p=0.058).

The prevalence of anti-N IgG+ was 77.3% in the blood group O and 90% in the non-O group (p=0.076). In the remaining groups, these figures were 88.9% (group A), 100% (group B), 83.3% (group AB), 84.8% (Rh+) and 84.0% (Rh-).

Inflammatory markers and composite indices

Table 4 shows correlation analyses between the 8 inflammatory markers and the number of symptoms of PCS. In blood group O, significant correlations were observed with lymphocyte count (r=0.334; p=0.025), fibrinogen level (r=0.404; p=0.009) and serum CRP (Phi=0.465; p=0.013). Rh+ people showed significant direct correlations of the number of symptoms with the count of neutrophils (Rho=0.247; p=0.029) and lymphocytes (Rho=0.253; p=0.026).

The performance of biomarkers and composite indices of inflammation (C1 to C5), concerning PCS in each blood group, was also analyzed. Groups A, B, AB, and Rh- subjects did not present any significant difference between PCS+ and PCS-. Conversely, significant differences were observed in blood group O, in which PCS+ patients had a lymphocyte count, serum CRP and fibrinogen levels, and percentages of positivity in

C2, C3, and C4 indices, significantly higher than PCS- subjects (Table 5 and Figure 2). Compared to the Rh+ subjects without PCS, those Rh+ with PCS had higher percentage of serum CRP in the low-grade inflammation range (28.6% vs. 8.5%; p=0.031), and higher percentage of subjects meeting the C1 (76 % vs. 50%; p=0.030), C3 (82.6% vs. 54%; p=0.019) and C4 criteria (69.6 vs. 44%; p=0.042).

Multivariate analysis

Table 6 shows the crude and adjusted OR (aOR) associated with the different blood groups and the Rh factor. Although in the crude analysis, groups B, AB, and Rh- showed significant ORs, the association was canceled when adjusting for age and sex. However, the OR for the blood group O remains significant. Additional adjustments for different clinical and laboratory confounders did not virtually modify this result. Blood group O subjects had a 4-fold increased risk of PCS, compared to the non-O group. Specifically, OR was 4.20 [95% CI, 1.2-14] (p=0.023), after adjusting for age, sex, BMI, tobacco use, comorbidity, Rh factor, neutrophil count, lymphocyte count, serum fibrinogen and CRP levels, hypertension, DM, and DLP. Nagelkerke's R^2 value was 0.46, and the AUC was 0.807 [95% CI 0.70-0.90] (p=0.0001) (Figure 3).

DISCUSSION

To our knowledge, this is the first study that found an independent association between blood group O and PCS. The relationships of blood group O with the PCS, inflammatory markers, fatigue, and myalgia are discussed below.

Blood group O, PCS, and inflammation

Compared to non-O subjects, group O had a significantly higher prevalence of PCS and a higher number of PCS symptoms. In these patients (group O and PCS+), 3 findings would simultaneously point to underlying low-grade inflammation: (i) slight but significant increase of the lymphocyte count, and serum fibrinogen and CRP levels, (ii) significant correlations of these same markers with the number of symptoms, and (iii) a higher percentage of individuals meeting the criteria of the composite indices C2, C3 and C4.

These results are in line with the current knowledge since there is increasing evidence of an excessive and dysregulated inflammatory/immune activation underlying PCS pathogenesis [14,26]. However, the question of why PCS has been related to inflammatory markers in subjects with blood group O, and not in other groups of the ABO system, does not have a simple answer.

A genomic study by Naitza *et al*. has linked blood group O with increased levels of interleukin (IL) 6 (IL-6) [27]. IL-6 is a multifunctional proinflammatory cytokine involved in a wide range of immunomodulatory processes. It also has a relevant role in acute and chronic inflammation [28], activating the adhesion of lymphocytes and endothelial cells and influencing the magnitude of the inflammatory response [8]. In

addition, it stimulates the synthesis of fibrinogen and CRP and regulates the recruitment of neutrophils and their subsequent controlled elimination [28-30]. Once the acute phase of COVID-19 is finished, IL-6 levels remain elevated in a variable percentage of patients, between 3.9% and 32.2% according to a recent review [14].

In our study, the subjects with blood group O and PCS+ presented significantly elevated levels of fibrinogen and lymphocytes, and a CRP in the range of low-grade inflammation. Based on what is known, it can be speculated that some group O subjects, with basal raised levels of IL-6, after acute COVID-19 maintained persistently high levels of IL-6, which could have promoted a prolonged inflammatory response through the synthesis of CRP and fibrinogen, among others.

Blood group O, fatigue, and CRP in range of low-grade inflammation

The persistent fatigue was the most self-reported symptom by participants, in line with the published papers [31]. A systematic review has shown that the pooled proportion of subjects with fatigue 12 or more weeks after the COVID-19 diagnosis was 0.32 (95% CI 0.27-0.37; p<0.001; n=25268) [14].

The exact mechanisms of chronic fatigue remain unraveled, but there is consensus in accepting 4 models: the oxidative stress and mitochondrial dysfunction, the hypothalamus-pituitary-adrenal axis, genetics and the inflammation [32]. Inflammation causes fatigue, and higher levels of circulating pro-inflammatory cytokines (tumor necrosis factor (TNF)-α, IL-1β, IL-6) and CRP than controls, have been noted in chronic fatigue [33,34]. In the same way, post-COVID fatigue has shown to be related to inflammatory markers [35-37]. The relation between fatigue and group O in COVID-19 has also been reported [38].

The results corroborate these findings. We found a significant association between fatigue, CRP in range of low-grade inflammation, and group O, not observed in the other blood groups.

Of note, in our previous work, gender showed to be a relevant variable in the relationship between fatigue and CRP, being significant only in men [19]. The correlation between fatigue and high CRP has been positive and quite similar in both subsamples (Phi=0.383; p=0.012, in men, and Phi=0.372; p=0.022 in group O).

Blood group O, myalgia, and the possible role of fibrinogen

Subjects with blood group O had a significant association with post-COVID myalgia, a relationship that remained significant after adjusting for gender. A potential role of fibrinogen can be suggested as a pathophysiological marker. Thus, as previously said, blood group O has been associated with raised levels of IL-6, which is an important regulator of fibrinogen synthesis [39]. Secondly, apart from being involved in atherogenesis, thrombogenesis, and inflammation [40], fibrinogen is closely related to chronic pain [41,42]. In this context, fibrinogen has shown high sensitivity to changes in the activity of polymyalgia rheumatica

and, therefore, it is used as a marker of the disease [43]. Along with other proteins, it has also been involved in the intensity of pain in fibromyalgia [44]. Finally, a proteomics study [45] has shown that at least 2 months after acute COVID-19, raised levels of inflammatory molecules, such as α2-antiplasmin, chains of fibrinogen, and serum amyloid A (SAA) were present in the blood serum of subjects with PCS. Taking into account all these considerations, the role of fibrinogen in the relationship between blood group O and PCS myalgia is biologically plausible.

Blood group O and prevalence of IgG+ antibodies

Hyperimmune plasma from convalescent COVID-19 donors has been a therapeutic option since the beginning of the pandemic. This has opened the option to assess a potential relationship between antibody titers and the ABO system, although the results of these studies have been controversial. Thus, on a sample of 232 plasma donors, Hayes *et al.* [46] observed that blood group O donors had lower titers than the other groups. Prevalences of high titers were 60%, 58%, 65%, and 35% for A, B, AB, and O, respectively. In the same line, Bloch *et al.* [47] analyzed 202 donors and reported that in blood group B there was a significantly higher number of subjects with high titers of neutralizing antibodies when compared with blood group O. Also, Madariaga *et al.* [48] that donors with blood group AB had higher levels of anti-spike and anti-receptor-binding domain (RBD) antibodies than subjects with blood group O.

However, other studies have not confirmed these observations. Žiberna *et al.* [49] analyzed plasma samples from 3185 convalescent donors with 3 serological tests and a neutralizing antibody test. No difference between ABO groups with any of the tests was found. Similar results have been reported in other studies [50-51]. According to these authors, the discrepancies in the results could be explained by the lack of representation of all ethnicities, the heterogeneity of the donors, the severity of the disease, the associated comorbidity, the small sample sizes in the studies carried out at the beginning of the pandemic, and the different sensitivity of the serological tests [50-52].

It is known that high SARS-CoV-2 antibody titers are associated with male gender, older age, and severe acute COVID-19 [47,48], while tobacco use is associated with lower titers [53] and that there is a rapid loss of anti-RBD antibodies in mild COVID-19 [54].

In the same way, in our study, a higher mean age (p=0.03) was observed in IgG+ subjects. However, the mean age of the study participants was 45.7±16 years, and all had suffered from mild COVID-19 disease. These two factors could explain the relatively low prevalence of IgG antibodies in our population. Noteworthy, in IgG+ patients we found a higher number of PCS symptoms (p=0.044), as reported in other studies [55]. Persistently high antibody titers, due to over-activation of the immune system, have been suggested as a risk factor for PCS [55,56].

Potential implications of the results

As previously commented, it can be speculated the sequence Group O / basal raised levels of IL-6 / persistently high levels of IL-6 after acute COVID-19 / synthesis of CRP and fibrinogen and inflammatory response, clinically expressed as fatigue, myalgia, and the other PCS symptoms.

In our opinion, this sequence could explain, at least in part, the results of the study: (i) the higher prevalence of PCS and myalgia, and the higher number of symptoms observed in group O compared to non-O, (ii) within the group O, the significant correlations between the number of symptoms and CRP, lymphocyte count and fibrinogen level, (iii) significant percentage of C2 composite index (defined by high ranges of CRP or fibrinogen) presented by subjects with myalgia and by group O subjects, (iv) the group O as a risk factor for PCS, with an adjusted OR of 4.20 (1.2-14), and (v) the differences between PCS+ and PCS- subjects observed in group O, but not in the other blood types.

Blood O group has been associated with a 4.2-fold increased risk of prevalent PCS compared to non-O subjects, after adjusting for confounders. Group O, lymphocyte and neutrophil counts, and female sex, were the variables that contributed the most in the predictive PCS model. The R^2 value (0.46) indicates a good fit and a moderate to strong effect size [57], and the AUC of 0.807, an adequate discriminatory ability of the model [58].

The implications of these results deserve a brief comment. In our study, participants with blood group A, with a higher risk of acquiring the disease and developing a worse clinical outcome, according to previous reports, did not show any association with PCS. In contrast, blood group O could play a dual role in SARS-CoV-2 infection; as a protector factor against SARS-CoV-2 infection and, unexpectedly, according to our results, as a consistent risk factor for developing PCS.

Even with the protection of group O subjects from contracting COVID-19, it may imply a high prevalence of PCS in geographic areas with a predominance of this blood group. In the clinical setting, it may be helpful for PCS risk stratification in acute COVID-19. In addition, it can be useful for the diagnosis of a patient with a clinical suspicion of PCS in the post-acute phase. PCS is a challenge for clinicians, as there are no well-defined criteria to define this condition, patient-reported symptoms can be complex, changing and fluctuating, and complementary tests may be of little diagnostic value [59]

This study has some limitations. Firstly, its design allows defining associations, but it does not establish causality. Secondly, it is a single-center study, on a Caucasian population, and the results may not be extrapolated to other populations. The frequencies of the ABO groups in the studied population are a confounding factor since geographical variations constitute potential bias [6]. In our case, the blood group proportions have been very similar to those in Spain (43.3% group A, 43.3% group O, and 13.4% groups B+AB). The main strength in this study is the well-characterized sample and the control group with subjects that came from the same population as the cases, and did not differ from them in any characteristics except

for the PCS. Biomarkers have shown slight elevations, without exceeding the upper limit of normality (Figure 2), in a similar way to our previous study [19]. Besides, the use of composite indices of inflammation has increased the ability to detect parameters with values in the upper ranges of their distribution.

Conclusion

We have found an increased risk of prevalent PCS associated with blood group O. Slightly, albeit significant, raised levels of fibrinogen, CRP, and lymphocyte count, not observed in the other ABO blood groups, have been demonstrated in blood group O. According to our results, group O could be part of the immunological link between acute COVID-19 and PCS. Further research in large populations from different geographical areas and ethnicities is needed to confirm these results and gain more knowledge about PCS pathogenesis.

REFERENCES

[1] Abegaz SB. Human ABO Blood Groups and Their Associations with Different Diseases. Biomed Res Int. 2021 Jan 23;2021:6629060.

[2] Amirzadegan A, Salarifar M, Sadeghian S, Davoodi G, Darabian C, Goodarzynejad H. Correlation between ABO blood groups, major risk factors, and coronary artery disease. Int J Cardiol. 2006 Jun 16;110(2):256-8.

[3] Yamamoto F, Cid E, Yamamoto M, Blancher A. ABO research in the modern era of genomics. Transfus Med Rev. 2012 Apr;26(2):103-18.

[4] Wu Y, Feng Z, Li P, Yu Q. Relationship between ABO blood group distribution and clinical characteristics in patients with COVID-19. Clin Chim Acta. 2020 Oct;509:220-223.

[5] Latz CA, DeCarlo C, Boitano L, Png CYM, Patell R, Conrad MF, et al. Blood type and outcomes in patients with COVID-19. Ann Hematol. 2020 Sep;99(9):2113-2118.

[6] Shibeeb S, Khan A. ABO blood group association and COVID-19. COVID-19 susceptibility and severity: a review. Hematol Transfus Cell Ther. 2022 Jan-Mar;44(1):70-75.

[7] Goel R, Bloch EM, Pirenne F, Al-Riyami AZ, Crowe E, Dau L, et al.; ISBT COVID-19 Working Group. ABO blood group and COVID-19: a review on behalf of the ISBT COVID-19 Working Group. Vox Sang. 2021 Sep;116(8):849-861.

[8] Pendu JL, Breiman A, Rocher J, Dion M, Ruvoën-Clouet N. ABO Blood Types and COVID-19: Spurious, Anecdotal, or Truly Important Relationships? A Reasoned Review of Available Data. Viruses. 2021 Jan 22;13(2):160.

[9] Hoiland RL, Fergusson NA, Mitra AR, Griesdale DEG, Devine DV, Stukas S, et al. The association of ABO blood group with indices of disease severity and multiorgan dysfunction in COVID-19. Blood Adv. 2020 Oct 27;4(20):4981-4989.

[10] Gassó P, Ritter MA, Mas S, Lafuente A. Influence of ABO genotype and phenotype on angiotensin-converting enzyme plasma activity. J Renin Angiotensin Aldosterone Syst. 2014 Dec;15(4):580-4.

[11] Zietz M, Zucker J, Tatonetti NP. Associations between blood type and COVID-19 infection, intubation, and death. Nat Commun. 2020 Nov 13;11(1):5761.

[12] Kabrah SM, Abuzerr SS, Baghdadi MA, Kabrah AM, Flemban AF, Bahwerth FS, et al. Susceptibility of ABO blood group to COVID-19 infections: clinico-hematological, radiological, and complications analysis. Medicine (Baltimore). 2021 Dec 30;100(52):e28334.

[13] Kumar G, Nanchal R, Hererra M, Sakhuja A, Patel D, Meersman M, et al. Does ABO Blood Groups Affect Outcomes in Hospitalized COVID-19 Patients? J Hematol. 2021 Jun;10(3):98-105.

[14] Ceban F, Ling S, Lui LMW, Lee Y, Gill H, Teopiz KM, et al. Fatigue and cognitive impairment in Post-COVID-19 Syndrome: A systematic review and meta-analysis. Brain Behav Immun. 2021 Dec 29;101:93-135.

[15] Meeting the challenge of long COVID. Nat Med [Internet]. December 2020;26(12):1803-1803.

[16] Brodin P. Immune determinants of COVID-19 disease presentation and severity. Nat Med. 2021;27:28-33.

[17] NICE guideline [NG188]: Managing the long term effects of COVID-19.

[18] Amenta EM, Spallone A, Rodriguez-Barradas MC, El Sahly HM, Atmar RL, Kulkarni PA. Postacute COVID-19: An Overview and Approach to Classification. Open Forum Infect Dis. 2020;7:ofaa509.

[19] Maamar M, Artime A, Pariente E, Fierro P, Ruiz Y, Gutiérrez S, et al. Post-COVID-19 syndrome, low-grade inflammation and inflammatory markers: a cross-sectional study. Curr Med Res Opin. 2022. https://doi.org/10.1080/03007995.2022.2042991.

[20] Tserel L, Jõgi P, Naaber P, Maslovskaja J, Häling A, Salumets A, et al. Long-Term Elevated Inflammatory Protein Levels in Asymptomatic SARS-CoV-2 Infected Individuals. Front Immunol. 2021 Sep 17;12:709759.

[21] Torjesen I. Covid-19: Middle aged women face greater risk of debilitating long term symptoms. BMJ. 2021;372:n829.

[22] Van Alsten SC, Aversa JG, Santo L, Camargo MC, Kemp T, Liu J, et al. Association between ABO and Duffy blood types and circulating chemokines and cytokines. Genes Immun. 2021 Jul;22(3):161-171.

[23] World Health Organization (WHO) Publications: Clinical management of COVID-19: living guidance (2nd version) 25/January/2021.

[24] Sudre CH, Murray B, Varsavsky T, Graham MS, Penfold RS, Bowyer RC, et al. Attributes and predictors of long COVID. Nat Med. 2021 Apr;27(4):626-631.

[25] Dinh KM, Kaspersen KA, Mikkelsen S, et al. Low grade inflammation is negatively associated with physical Health-Related Quality of Life in healthy individuals: Results from The Danish Blood Donor Study (DBDS). PLoS One. 2019;14(3):e0214468.

[26] Pasini E, Corsetti G, Romano C, Scarabelli TM, Chen-Scarabelli C, Saravolatz L, Dioguardi FS. Serum Metabolic Profile in Patients With Long-Covid (PASC) Syndrome: Clinical Implications. Front Med (Lausanne). 2021 Jul 22;8:714426.

[27] Naitza S, Porcu E, Steri M, Taub DD, Mulas A, Xiao X, et al. A genome-wide association scan on the levels of markers of inflammation in Sardinians reveals associations that underpin its complex regulation. PLoS Genet. 2012 Jan;8(1):e1002480.

[28] Lin H, Joehanes R, Pilling LC, Dupuis J, Lunetta KL, Ying SX, et al. Whole blood gene expression and interleukin-6 levels. Genomics. 2014 Dec;104(6 Pt B):490-5.

[29] Vardam TD, Zhou L, Appenheimer MM, Chen Q, Wang WC, Baumann H, Evans SS. Regulation of a lymphocyte-endothelial-IL-6 trans-signaling axis by fever-range thermal stress: hot spot of immune surveillance. Cytokine. 2007 Jul;39(1):84-96.

[30] Fielding CA, McLoughlin RM, McLeod L, Colmont CS, Najdovska M, Grail D, Ernst M, Jones SA, Topley N, Jenkins BJ. IL-6 regulates neutrophil trafficking during acute inflammation via STAT3. J Immunol. 2008 Aug 1;181(3):2189-95.

[31] Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo PA, Cuapio A, et al. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. Sci Rep. 2021 Aug 9;11(1):16144.

[32] Norheim KB, Jonsson G, Omdal R. Biological mechanisms of chronic fatigue. Rheumatology (Oxford). 2011 Jun;50(6):1009-18.

[33] Groven N, Fors EA, Reitan SK. Patients with Fibromyalgia and Chronic Fatigue Syndrome show increased hsCRP compared to healthy controls. Brain Behav Immun. 2019 Oct;81:172-177.

[34] Matura LA, Malone S, Jaime-Lara R, Riegel B. A Systematic Review of Biological Mechanisms of Fatigue in Chronic Illness. Biol Res Nurs. 2018 Jul;20(4):410-421.

[35] Mandal S, Barnett J, Brill SE, Brown JS, Denneny EK, Hare SS, et al. 'Long-COVID': a cross-sectional study of persisting symptoms, biomarkers and imaging abnormalities following hospitalisation for COVID-19. Thorax. 2021 Apr;76(4):396-398.

[36] Evans RA, McAuley H, Harrison EM, Shikotra A, Singapuri A, Sereno M, et al.; PHOSP-COVID Collaborative Group. Physical, cognitive, and mental health impacts of COVID-19 after hospitalisation (PHOSP-COVID): a UK multicentre, prospective cohort study. Lancet Respir Med. 2021 Nov;9(11):1275-1287.

[37] Skala M, Svoboda M, Kopecky M, Kocova E, Hyrsl M, Homolac M, et al. Heterogeneity of post-COVID impairment: interim analysis of a prospective study from Czechia. Virol J. 2021 Apr 12;18(1):73.

[38] Majeed KR, Al-Fahad D, Jalood HH, Hantosh HA, Ali MK, Sakthivel S, *et al.* RhD blood type significantly influences susceptibility to contract COVID-19 among a study population in Iraq [version 1; peer review: 1 approved]. F1000Research 2021, 10:38 (https://doi.org/10.12688/f1000research.27777.1).

[39] Carty CL, Heagerty P, Heckbert SR, Jarvik GP, Lange LA, Cushman M, Tracy RP, Reiner AP. Interaction between fibrinogen and IL-6 genetic variants and associations with cardiovascular disease risk in the Cardiovascular Health Study. Ann Hum Genet. 2010 Jan;74(1):1-10.

[40] Luyendyk JP, Schoenecker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. Blood. 2019;133(6):511-520.

[41] Sibille KT, Steingrímsdóttir ÓA, Fillingim RB, Stubhaug A, Schirmer H, Chen H, et al. Investigating the Burden of Chronic Pain: An Inflammatory and Metabolic Composite. Pain Res Manag. 2016;2016:7657329.

[42] Burns JW, Quartana PJ, Bruehl S, Janssen I, Dugan SA, Appelhans B, et al. Chronic pain, body mass index and cardiovascular disease risk factors: tests of moderation, unique and shared relationships in the Study of Women's Health Across the Nation (SWAN). J Behav Med. 2015 Apr;38(2):372-83.

[43] McCarthy EM, MacMullan PA, Al-Mudhaffer S, Madigan A, Donnelly S, McCarthy CJ, et al. Plasma fibrinogen along with patient-reported outcome measures enhances management of polymyalgia rheumatica: a prospective study. J Rheumatol. 2014 May;41(5):931-7.

[44] Wåhlen K, Ernberg M, Kosek E, Mannerkorpi K, Gerdle B, Ghafouri B, et al. Significant correlation between plasma proteome profile and pain intensity, sensitivity, and psychological distress in women with fibromyalgia. Sci Rep. 2020;10(1):12508.

[45] Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J, et al. Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. Cardiovasc Diabetol. 2021 Aug 23;20(1):172.

[46] Hayes C, Rubenstein W, Gibb D, Klapper E, Tanaka J, Pepkowitz S. Blood group O convalescent plasma donations have significantly lower levels of SARS-CoV-2 IgG antibodies compared to blood group A donations. Transfusion. 2021 Aug;61(8):2245-2249.

[47] Bloch EM, Patel EU, Marshall C, Littlefield K, Goel R, Grossman BJ, et al. ABO blood group and SARS-CoV-2 antibody response in a convalescent donor population. Vox Sang. 2021 Aug;116(7):766-773.

[48] Madariaga MLL, Guthmiller JJ, Schrantz S, Jansen MO, Christensen C, Kumar M, et al. Clinical predictors of donor antibody titre and correlation with recipient antibody response in a COVID-19 convalescent plasma clinical trial. J Intern Med. 2021 Apr;289(4):559-573.

[49] Žiberna K, Jež M, Jazbec K, Mali P, Potokar UR, Rožman P. ABO blood group does not influence the level of anti-SARS-CoV-2 antibodies in convalescent plasma donors. Transfusion. 2022 Jan 18. doi: 10.1111/trf.16808. Epub ahead of print. PMID: 35041206.

[50] Grujić J, Bujandrić N, Budakov-Obradović Z, Dolinaj V, Bogdan D, Savić N, et al. Demographic and Clinical Factors Associated with Reactivity of Anti-SARS-CoV-2 Antibodies in Serbian Convalescent Plasma Donors. Int J Environ Res Public Health. 2021 Dec 21;19(1):42.

[51] Hirani R, Hoad V, Gosbell IB, Irving DO. Absence of correlation between ABO Rh(D) blood group and neutralizing antibody titers in SARS-CoV-2 convalescent plasma donors. Transfusion. 2022 Feb;62(2):292-297. doi: 10.1111/trf.16781. Epub 2021 Dec 22. PMID: 34936102.

[52] Kutsuna S, Asai Y, Matsunaga A. Loss of Anti-SARS-CoV-2 Antibodies in Mild Covid-19. N Engl J Med. 2020 Oct 22;383(17):1695-1696.

[53] Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. N Engl J Med. 2020 Oct 29;383(18):1724-1734.

[54] Ibarrondo FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C, Hausner MA, Ferbas KG, Tobin NH, Aldrovandi GM, Yang OO. Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild Covid-19. N Engl J Med. 2020 Sep 10;383(11):1085-1087.

[55] Peghin M, Palese A, Venturini M, De Martino M, Gerussi V, Graziano E, et al. Post-COVID-19 symptoms 6 months after acute infection among hospitalized and non-hospitalized patients. Clin Microbiol Infect. 2021 Oct;27(10):1507-1513.

[56] Anaya JM, Rojas M, Salinas ML, Rodríguez Y, Roa G, Lozano M, et al. Post-COVID syndrome. A case series and comprehensive review. Autoimmun Rev. 2021 Nov;20(11):102947.

[57] Ferguson CJ. An effect size primer: A guide for clinicians and researchers. Prof Psychol Res Pr. 2009;40(5):532-538.

[58] Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol. 2010;5(9):1315-1316.

[59] Deer RR, Rock MA, Vasilevsky N, Carmody L, Rando H, Anzalone AJ, et al. Characterizing Long COVID: Deep Phenotype of a Complex Condition. EBioMedicine. 2021 Dec;74:103722. doi: 10.1016/j.ebiom.2021.103722. Epub 2021 Nov 25. PMID: 34839263; PMCID: PMC8613500.

Table 1: Composite indices of inflammation

COMPOSITE INDEX	DEFINITION
C1	[Neutrophil count ≥3.10* (x10³/µL)] or [NL Ratio ≥1.86**]
C2	[CRP >0.3 and <1.0 mg/dL] or [Fibrinogen ≥421** mg/dL]
C3	[Neutrophil count ≥3.10* (x10³/µL)] or [Fibrinogen ≥421** mg/dL]
C4	[NL Ratio ≥1.86**] or [Fibrinogen ≥421** mg/dL]
C5	[CRP >0.3 and <1.0 mg/dL] or [Neutrophil count ≥3.40** (x10³/µL)]

CRP: C-reactive protein; NL Ratio: Neutrophil / Lymphocyte Ratio
**Values above the median; **Values in the third tertile*

Table 2: Clinical characteristics of cases and controls

	CASES (PCS+) (n=36)	**CONTROLS (PCS-) (n=85)**	***P***
Females; n(%)	25 (35.8)	43 (64.2)	0.07
Males; n(%)	11 (20.8)	42 (79.2)	
Age (years); mean ± SD	46.7 ± 14	45.1 ± 17	0.62
Age: tertile 1; n(%)	9 (25.7)	32 (38.6)	0.48
Age: tertile 2; n(%)	16 (45.7)	26 (31.3)	
Age: tertile 3; n(%)	10 (28.6)	25 (30.1)	
BMI; mean ± SD	24.7 ± 4	25.6 ± 3	0.28
Obesity; n(%)	3 (8.6)	12 (15.2)	0.33
Tobacco; n(%)	11 (31.4)	32 (39)	0.43
Charlson comorbidity index; mean ± SD	0.20 ± 0.4	0.48 ± 0.8	0.08*
CCI=0; n(%)	28 (80)	56 (68.3)	0.15
CCI=1; n(%)	7(20)	24 (29.3)	
CCI=2+; n(%)	-	2 (2.4)	
Blood group A; n(%)	11 (33.3)	33 (47.1)	0.09
Blood group B; n(%)	2 (6.1)	6 (8.6)	
Blood group AB; n(%)	1 (3)	5 (7.1)	
Blood group O; n(%)	19 (57.6)	26 (37.1)	
Rh+; n(%)	25 (75.8)	53 (75.7)	0.95
Rh-; n(%)	8 (24.2)	16 (24.3)	
Immunosuppression; n(%)	1 (2.9)	2 (2.4)	0.89
Chronic kidney disease; n(%)	-	2 (2.4)	0.95
Cerebrovascular disease; n(%)	-	1 (1.2)	0.98
Hypertension; n(%)	4 (11.4)	17 (20.7)	0.23
Diabetes mellitus; n(%)	1 (2.9)	5 (6.1)	0.60
Dyslipidemia; n(%)	12 (34.3)	26 (31.7)	0.78
Ischemic heart disease; n(%)	2 (5.7)	4 (4.9)	0.85
Asthma; n(%)	5 (14.3)	6 (7.3)	0.30

PCS+: With post-COVID syndrome; PCS-: Without post-COVID syndrome.
SD: standard deviation; BMI: Body mass index
**Mann-Whitney U-test*

Table 3: Clinical characteristics and variables related to PCS in group O and non-O subjects

	GROUP O (n=44)	**NON-O (n=60)**	***p****
Females; n(%)	26 (40.6)	38 (59.4)	0.66
Males; n(%)	18 (45)	22 (55)	
Age (years); mean ± SD	46.9 ± 16	47.3 ± 16	0.90
Age: tertile 1; n(%)	15 (46.9)	17 (53.1)	0.79
Age: tertile 2; n(%)	14 (38.9)	22 (61.1)	
Age: tertile 3; n(%)	14 (41.2)	20 (58.8)	
BMI; mean ± SD	25.1 ± 3	25.5 ± 4	0.66
Obesity; n(%)	5 (11.6)	8 (14.5)	0.67
Tobacco; n(%)	12 (27.9)	26 (44.8)	0.08
Charlson comorbidity index; mean ± SD	0.42 ± 0.8	0.43 ± 0.9	0.92*
Post-COVID-19 syndrome (+); n(%)	19 (43.2)	14 (23.7)	0.036
Number of symptoms; mean ± SD	0.82 ± 1	0.43 ± 0.9	0.017*
Anosmia; n(%)	5 (11.4)	7 (11.7)	0.96
Dyspnea; n(%)	2 (4.8)	4 (6.7)	0.64
Palpitations; n(%)	1 (2.3)	3 (5)	0.47
Fatigue; n(%)	9 (20.5)	6 (10)	0.13
Telogen effluvium; n(%)	2 (4.5)	1 (1.7)	0.57
Ageusia; n(%)	5 (11.4)	3 (5)	0.27
Headache; n(%)	2 (4.5)	1 (1.7)	0.57
Leukonychia; n(%)	1 (2.3)	-	0.42
Myalgia; n(%)	4 (9.1)	-	0.030
Concentration difficulties; n(%)	3 (6.8)	-	0.07
Rhinitis; n(%)	1 (2.3)	-	0.42
Cough; n(%)	1 (2.3)	-	0.42
Anti-SARS-CoV-2 IgG(+); n(%)	34 (77.3)	54 (90)	0.07

** Mann-Whitney U-test*

Table 4: Correlation analyses between inflammatory markers and the number of symptoms in PCS

	GROUP A		GROUP B		GROUP AB		GROUP O	
	r	*p*	r	*p*	r	*p*	R	*p*
Neutrophil count (x10³/µL)	-0.050	0.75	0.632	0.12	-0.266	0.61	0.194	0.20
Lymphocyte count (x10³/µL)	-0.025	0.87	0.001	0.99	0.664	0.15	0.343	**0.023**
Neutrophil/Lymphocyte Ratio	0.004	0.97	0.316	0.49	-0.665	0.15	0.126	0.41
Ferritin (ng/mL)	-0.058	0.71	-0.239	0.60	-0.665	0.15	-0.059	0.71
Lactate dehydrogenase (UI/L)	0.251	0.10	-0.474	0.28	-0.393	0.44	-0.241	0.13
Fibrinogen (mg/dL)	0.067	0.68	0.158	0.73	-0.393	0.44	0.384	**0.014**
D-dimer (ng/mL)	-0.082	0.63	0.001	0.98	0.131	0.80	0.266	0.10
CRP (mg/dL) ǂ	0.068	0.91	-	-	-	-	0.462	**0.017**

CRP: C-reactive protein
ǂ Correlation analysis between plasma CRP levels (<0.4 mg/dL and 0.4-0.9 mg/dL) and the number of symptoms of the PCS (expressed as 0, 1, 2 or more) was assessed with Phi coefficient.

Table 5: Inflammation markers and composite indices of inflammation in PCS, in relation to ABO system groups.

	GROUP A (n=42)			GROUP B (n=7)			GROUP AB (n=6)			GROUP O (n=45)		
	PCS+ (n=11)	PCS- (n=31)	*p*	PCS+ (n=2)	PCS- (n=5)	*p*	PCS+ (n=1)	PCS- (n=5)	*p*	PCS+ (n=19)	PCS- (n=26)	*p*
Neutrophil count (x10³/µL)	3.1±1.1	3.1±1.1	0.99	3.9±06	2.9±0.6	0.13	1.8	2.2±1.2	0.78	3.2±1.2	3±1	0.66
Lymphocyte count (x10³/µL)	1.8±0.5	1.9±0.7	0.60	2.1±0.2	2.1±0.7	0.98	2.2	1.5±0.2	0.06	2.1±0.8	1.5±1	**0.033**
Neutrophil/Lymphocyte Ratio	1.7±0.7	1.6±0.5	0.67	1.8±0.4	1.5±0.7	0.58	0.8	1.9±0.6	0.18	1.7±0.5	1.6±0.7	0.76
Ferritin (ng/mL)	46 [122]	50.5 [99]	0.64	50.5	81.5 [81]	0.57	-	185 [337]	0.33	67 [124]	57 [104]	0.98
Lactate dehydrogenase (UI/L)	189±2.3	169±29	0.05	144±66	187±20	0.19	157	193 [45]	0.51	172.4±27	190±35	0.08
Fibrinogen (mg/dL)	410±77	406±11	0.91	406±66	419±74	0.83	310	383±69	0.38	439±24	383±71	**0.024**
D-dimer (ng/mL)	199 [478]	286 [230]	0.61	308 [-]	399 [233]	0.99	-	242 [462]	0.99	319 [407]	201 [356]	0.44
CRP >0.3 and <1.0 mg/dL; n(%)	2 (28.6)	5 (71.4)	0.95	-	-	-	-	-	-	5 (31)	-	**0.008**
C1 index (+); n(%)	8 (72.3)	16 (51.6)	0.30	2 (100)	2 (40)	0.42	-	3 (60)	0.95	15 (78.9)	14 (53.8)	0.08
C2 index (+); n(%)	3 (37.5)	9 (30)	0.68	1 (50)	3 (60)	0.95	-	1 (20)	0.99	11 (64.7)	5 (22.7)	**0.007**
C3 index (+); n(%)	6 (66.7)	16 (53.3)	0.70	2 (100)	4 (80)	0.98	-	2 (40)	0.98	17 (89.5)	13 (52)	**0.008**
C4 index (+); n(%)	6 (66.7)	15 (50)	0.46	1 (50)	3 (60)	0.96	-	4 (80)	0.33	14 (77.8)	8 (32)	**0.003**
C5 index (+); n(%)	3 (33.3)	13 (44.8)	0.70	2 (100)	1 (25)	0.48	-	1 (20)	0.99	10 (55.6)	7 (28)	0.06

PCS+: With post-COVID-19 syndrome; PCS-: Without post-COVID-19 syndrome; CRP: C-reactive protein
Contrast test for quantitative variables: Student's t-test (except for ferritin and D-dimer: Mann-Whitney U).

Table 6: Odds ratios associated with ABO groups and Rh factor, with PCS as outcome variable

	Crude analysis			Sex- and age-adjusted			Adjusted by sex, age, and additional adjustments*		
	OR	**CI 95%**	***p***	**OR**	**CI 95%**	***p***	**OR**	**CI 95%**	***p***
Group A	1.68	0.9 – 2.8	0.053	-	-	-	-	-	-
Group B	2.06	1.3 – 3.1	0.001	0.79	0.2 – 2.8	0.72	-	-	-
Group AB	2.03	1.3 – 3.1	0.001	0.84	0.2 – 2.9	0.78	-	-	-
Group O	3.21	1.7 – 5.8	0.0001	2.49	1.08 – 5.7	0.031	4.20	1.2 – 14	0.023
Rh+	2.12	0.9 – 4.9	0.079	-	-	-	-	-	-
Rh-	2.12	1.3 – 3.4	0.002	1.01	0.3 – 2.5	0.97	-	-	-

CI: Confidence interval
** Body mass index, tobacco use, Charlson index, Rh factor, neutrophil count, lymphocyte count, fibrinogen level, serum C-reactive protein, hypertension, diabetes mellitus and dyslipidemia.*

Figure 1: Flow chart and study design

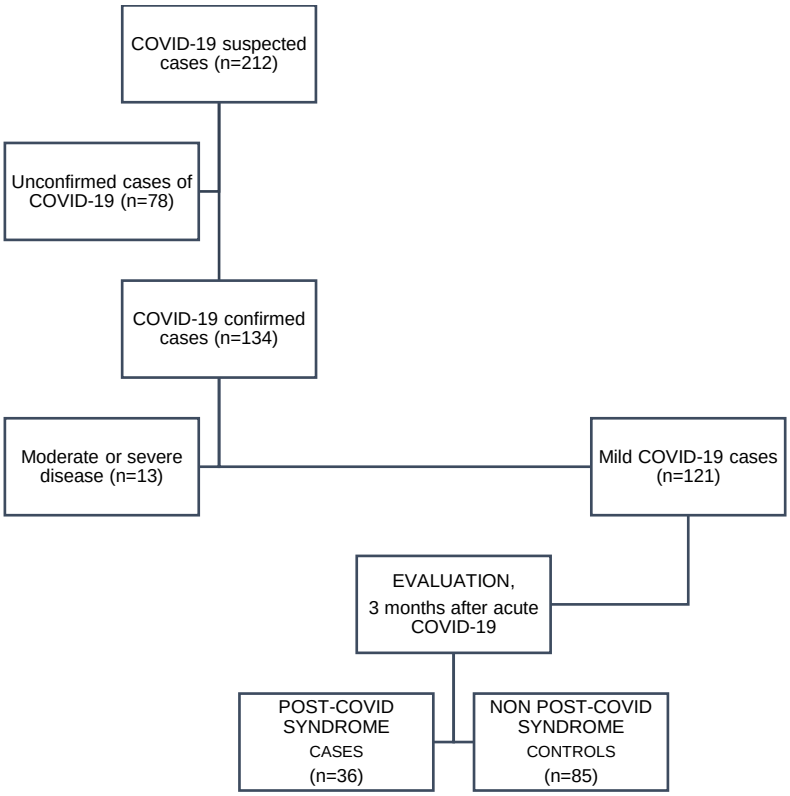
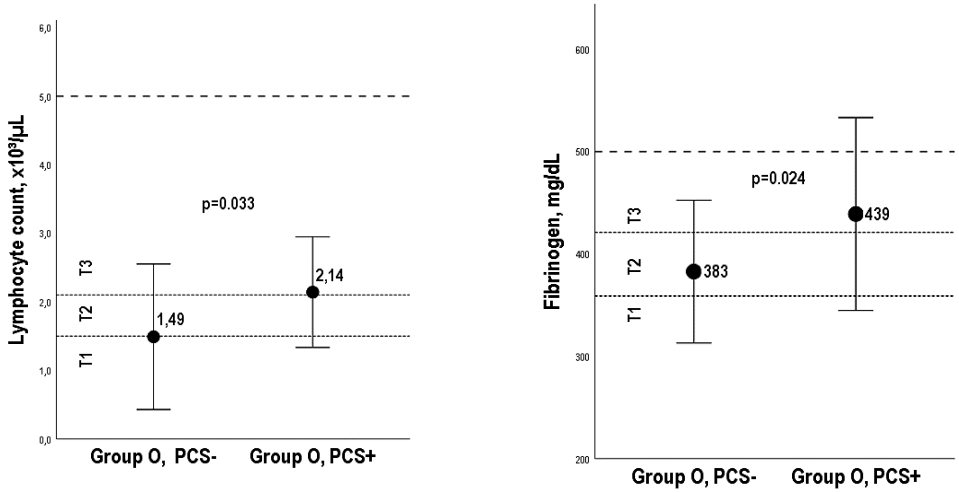
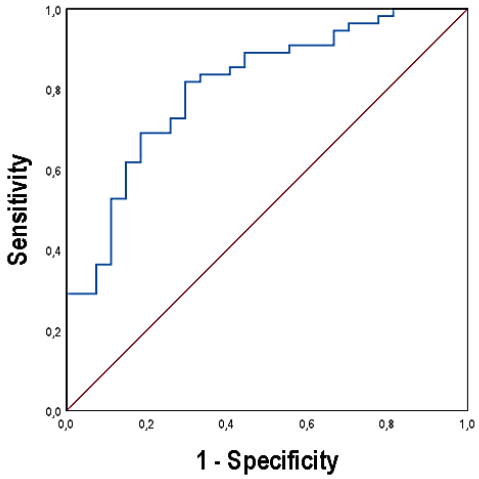


Figure 2: Lymphocyte count and plasma fibrinogen in group O patients with and without PCS



Mean plot with error bars representing ± 1 standard deviation.
PCS-: Without post-COVID syndrome; PCS+: With post-COVID syndrome.
T1, T2, T3: Tertiles (Fibrinogen: <359, 359-421, >421 mg/dL; lymphocyte count: <1.5, 1.5-2.1, >2.1 x10³/µL).
Dashed lines at 5.0 x10³/µL and at 500 mg/dL represent the upper limit of the normal range.

Figure 3: ROC curve associated with the logistic regression model, with group O as the independent variable and PCS as the outcome variable.



Area under the curve (AUC) = 0.807 (95% CI 0.70-0.90); p=0.0001

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	4-5
		(*b*) For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5-6
		(*b*) Describe any methods used to examine subgroups and interactions	5-6
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how matching of cases and controls was addressed	
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	24
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	6-7
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	23
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	26
Discussion			
Key results	18	Summarise key results with reference to study objectives	8-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

*Give information separately for cases and controls.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.