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Coronavirus Pandemic

Comparison of lymphocyte populations, cytokine, and autoantibody profile in patients with rheumatoid arthritis: a study of cases with COVID-19 and controls without COVID-19

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Abstract

Introduction: The SARS-CoV-2 pandemic is associated with the development of acute respiratory distress syndrome (ARDS) and post-COVID syndrome (PCS). PSC has been linked to autoimmune diseases, including rheumatoid arthritis (RA), a condition characterized by chronic joint pain driven by dysregulated immune response. This study aims to evaluate the impact of SARS-CoV-2 infection on patients with RA.

Methods: A total of 300 RA patients were included in the study, categorized into two groups: patients with a history of SARS-CoV-2 infection (n = 148) and without prior COVID-19 infection (control group; n = 152). Demographic information, comorbidities, treatments, autoantibodies, cell populations, and cytokines were assessed.

Results: A majority of the patients included in this study were female. A high percentage of patients completed the COVID-19 vaccination schedule. The mean age at RA diagnosis was 44.71 years, with most patients presenting with low disease activity. Patients with a history of SARS-CoV-2 infection reported headache, cough, and fatigue more often. A proportion of these symptoms persisted beyond 12 weeks, consistent with PCS. Autoantibody analysis revealed a high seropositivity rate in both groups, with no statistically significant differences related to SARS-CoV-2 infection. Similarly, the evaluation of immune system cell populations showed no significant variations between groups. Cytokine level analysis also demonstrated no statistically significant differences between cases and controls. However, IL-6 data were unavailable for 37% of participants.

Conclusions: Long-term follow-up did not demonstrate statistically significant alterations in the immunological profile of RA patients with SARS-CoV-2 infection. Nevertheless, further prospective studies are required to elucidate potential long-term immunological effects in this population.

Key words: arthritis; rheumatoid; COVID-19; post-acute COVID-19 syndrome; cytokines; antibodies.

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Introduction

SARS-CoV-2, the virus responsible for COVID-19, caused a global pandemic, with over 604 million reported infections [1]. As the number of survivors increases, there is a growing incidence of long-term complications, collectively referred to as Long COVID (LC). These manifestations include Post-Acute Sequelae of COVID-19 (PASC), defined as symptoms persisting between 4 and 12 weeks, and Post-COVID (PCS) (> 12 weeks), referring to symptoms lasting beyond 12 weeks. LC (> 4 weeks) affects individuals across all severity levels, including those who were asymptomatic [2]. Commonly reported manifestations include fatigue, muscle weakness, chronic rhinitis, dysgeusia, and headaches. Studies indicate that approximately 10% of recovered patients experience lingering symptoms for six months post-infection [3].

The persistence of these symptoms has led to investigations into potential underlying causes, with one hypothesis suggesting a chronic inflammatory response triggered by an imbalance in cytokine production, specifically involving TH1 and TH17 cellular responses, contributes to a sustained pro-inflammatory state [4]. This chronic inflammatory response resembles the immunopathological features of autoimmune diseases such as RA, which is characterized by systemic inflammation due to an overactive immune response. Additionally, the activation of distinct cytokine pathways perpetuates the systemic inflammatory response [5].

Concerns have emerged regarding the potential dysregulation of immune responses following SARS-CoV-2 infection, especially among individuals with underlying rheumatic diseases. Consequently, researchers have focused on understanding the long-term implications of these immune responses on disease

progression. Alterations in immune function have been linked to the activation of IL-6-dependent pathways, which contribute to cytokine storms and impact antigen presentation. Furthermore, changes in the immune profiles of patients with rheumatic diseases, including RA, have been observed [6]. For example, some reports have described increased Rheumatoid Factor seropositivity following COVID-19, with rates as high as 76% percent within 30 days post-infection [6,7].

Despite emerging evidence, there remains a gap in the literature regarding the specific long-term immune response mechanisms to SARS-CoV-2 infection in RA patients, who inherently exhibit heightened baseline inflammation. It is still unclear whether the immune system returns to its pre-infection equilibrium. To address this gap, this study aims to investigate potential disparities in the long-term immunological profiles of RA patients who have contracted COVID-19 compared to those who have not. This study, therefore, aims to enhance our understanding of the immunological changes associated with COVID-19 in this specific patient population.

Materials and methods

Study Design

A nested case-control study was conducted at a specialized autoimmune disease center in Bogotá, Colombia, between May 9, 2022, and September 15, 2022, involving a cohort of RA patients. Eligible participants were adults aged 18 to 70 years with a diagnosis of RA confirmed by ICD-10 codes M059, M060, and M069, and fulfilling the 2010 ACR/EULAR classification criteria. All patients were managed under a treat-to-target (T2T) approach, which involved rigorous disease monitoring through regular telematic and in-person assessments conducted by a multidisciplinary team. Throughout the COVID-19 pandemic, these patients were routinely screened for respiratory symptoms and COVID-19 infection status.

Case patients were defined as RA patients with laboratory-confirmed COVID-19 within the past 24 months, determined via polymerase chain reaction (PCR), antigen testing, or serological assays (with the infection timing based on each patient's last reported positive test). Control patients were RA individuals with no known history of COVID-19 infection. During the recruitment process, all participants were explicitly asked about any prior episodes of respiratory illness, including flu-like symptoms (e.g., fever, cough, shortness of breath, sore throat, anosmia), in the previous 24 months. Those reporting any such symptoms were further questioned about diagnostic testing. Only participants with no history of COVID-19-compatible symptoms and no positive test results for SARS-CoV-2 were included as controls. Exclusion criteria comprised pregnant patients and those who had experienced febrile illness within 30 days before blood sample collection.

Sample Collection

Patients who met the inclusion criteria provided written informed consent before sample collection. Venous blood samples (10 mL per patient, total of four samples per patient) were collected via intravenous puncture using a vacuum collection system.

For cell population analysis, 5 mL of peripheral blood was collected into EDTA tubes, gently inverted 6–8 times to ensure proper mixing with the anticoagulant, and transported at room temperature to the designated research laboratory. Samples were processed within 24 hours of collection; clotted samples were excluded.

For cytokine analysis, blood samples were centrifuged within 20–30 minutes after collection. The resulting serum was stored at 2–8 °C until aliquoted into five cryovials (≥400 µL each) and frozen at –20 °C. Samples were analyzed in batches once the required number was met. Remaining samples were discarded according to institutional biosafety protocols.

Evaluation of Cell Populations, Autoantibodies, and Cytokines

Evaluation of Cell Populations

To assess immune cell populations, absolute counts were obtained by multiparametric flow cytometry of peripheral blood (PB). Immunophenotyping was performed on EDTA-treated PB using the BD Multitest™ 6-Color TBNK reagent with BD Trucount™ Tubes (Cat# 337166), following the manufacturer's instructions (BD Multitest™ 6-Color TBNK IFU) [8]. Briefly, 50 µL of whole blood was incubated with the reagent for 15 minutes at room temperature in the dark, followed by red blood cell lysis using BD FACS™ Lysing Solution (1X, Cat# 349202). Samples were acquired on a BD FACS Canto II or BD FACSLyric™ flow cytometer using BD FACSCanto™ Clinical software. At least 2,500 $CD45^+$ lymphocyte events were recorded per sample.

Cytokines

Cytokine profiling was conducted using two different bead-based immunoassay kits from BD Biosciences. The BD™ Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Cytokine Kit (Cat# 560484) was

used to quantify IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A in human serum samples, following the manufacturer's protocol, BD CBA Manual [9].

Additionally, IL-12p70 was measured using the BD™ CBA Human IL-12p70 Flex Set (Cat# 558267), as per manufacturer instructions BD Flex Set Guide [10]. Serum samples were diluted 1:4 in Assay Diluent. Standards were prepared via serial dilution (1:2 to 1:256). Capture bead mixtures were assembled and incubated with samples and detection reagent for 3 hours at room temperature, protected from light. Tubes were washed and resuspended in Wash Buffer.

Table 1. Sociodemographic Variables.

Variables	Total Group 300	Controls (152)	Cases (148)	*p*
Age (Median - Interquartile Range)	59 (11)	60 (54-65)	59 (54-64)	0.8630[±]
Marital Status, n (%)				0.675
Married	110 (36.67)	51 (33.55)	59 (39.86)	
Single	86 (28.67)	44 (29.95)	42 (28.38)	
Free Union	62 (20.67)	32 (21.05)	30 (20.27)	
Divorced	24 (8)	15 (9.87)	9 (6.08)	
Widowed	18 (6)	10 (6.58)	8 (5.41)	
Socioeconomic Status n (%)				0.765
Low	188 (62.67)	94 (61.84)	94 (63.51)	
Middle/High	112 (37.33)	58 (38.16)	54 (36.49)	
Place of Origin n (%)				0.684
Urban	244 (81.33)	125 (82.24)	119 (80.41)	
Rural	56 (18.67)	27 (17.76)	29 (19.59)	
Occupation n (%)				0.325
Home	167 (55.67)	90 (59.21)	77 (52.03)	
Manual Labor	64 (21.33)	30 (19.74)	34 (22.97)	
Retired	53 (17.67)	27 (17.76)	26 (17.57)	
Intellectual Activity	16 (5.33)	5 (3.29)	11 (7.43)	
Comorbidities	171 (57)	89 (58.55)	82 (55.41)	0.582
Arterial Hypertension	82 (27.33)	39 (25.66)	43 (29.05)	0.509
Osteoporosis	67 (22.33)	41 (26.97)	26 (17.57)	0.055
Hypothyroidism	59 (19.67)	32 (21.05)	27 (18.24)	0.541
Type 2 Diabetes	35 (11.67)	16 (10.53)	19 (12.84)	0.533
Cancer	9 (3)	5 (3.29)	4 (2.70)	0.508
Acute Myocardial Infarction	7 (2.33)	1 (0.66)	6 (4.05)	0.056[ϕ]
Cardiovascular Disease	7 (2.33)	1 (0.66)	6 (4.05)	0.056[ϕ]
Heart Failure	2 (0.67)	1 (0.66)	1 (0.68)	0.744[ϕ]
Chronic Renal Insufficiency	1 (0.33)	1 (0.66)	0	0.507[ϕ]
Chronic Infections	22 (7.33)	13 (8.55)	9 (6.08)	0.412
TBC	15 (5)	9 (5.92)	6 (4.05)	0.318
Viral Hepatitis	4 (1.33)	3 (1.97)	1 (0.68)	0.321[ϕ]
Toxoplasmosis	2 (0.67)	1 (0.66)	1 (0.68)	0.744[ϕ]
Epstein Barr	1	0	1 (0.67)	0.497[ϕ]
Smoker	54 (18)	21 (13.82)	33 (22.30)	0.056
Blood Type n (%)				0.233
O	186 (62.21)	93 (61.18)	93 (62.27)	
A	77 (25.75)	44 (28.95)	33 (22.45)	
B	28 (9.36)	10 (6.58)	18 (12.24)	
AB	8 (2.68)	5 (3.29)	3 (2.04)	
RH				0.802
Positive	288 (96.32)	146 (96.05)	142 (96.60)	
Negative	11 (3.68)	6 (3.95)	5 (3.40)	
COVID Vaccine	290 (96.67)	147 (96.71)	144 (96.62)	0.966
Vaccine Dose				0.503
1	12 (4.15)	4 (2.74)	8 (5.59)	
2	77 (26.64)	41 (28.08)	36 (25.17)	
3	200 (69.20)	101 (69.18)	99 (69.23)	
Type of Vaccine for the First Dose n (%)				0.016
BNT162b2	131 (45.17)	78 (53.06)	53 (37.06)	
CoronaVac	79 (27.24)	37 (25.17)	42 (29.37)	
ChAdOx1-S	44 (15.17)	20 (13.61)	24 (16.78)	
Ad26.CoV2.S	23 (7.23)	10 (6.80)	13 (9.09)	
ARNm-1273	13 (4.48)	2 (1.36)	11 (7.69)	
Type of Booster Vaccine				0.524
BNT162b2	82 (41)	37 (36.63)	45 (45.45)	
ChAdOx1-S	41 (20.50)	22 (21.78)	19 (19.19)	
ARNm-1273	41 (20.50)	24 (23.76)	17 (17.17)	
CoronaVac	35 (17.50)	18 (17.82)	17 (17.17)	

*: chi square Pearson; [ϕ]: Fisher exact test; [±]: Mann Whitney U; TBC: Tuberculosis.

Data was acquired on the same day using a BD FACSCanto™ II flow cytometer and BD FACSDiva™ software, and analyzed using FCAP Array™ Software v3.0.1 (Soft Flow Hungary, Ltd.).

Autoantibody detection was performed using the NOVA Lite® HEp-2 ANA kit (Ref 508100, Inova Diagnostics, a Werfen Company), based on indirect immunofluorescence on HEp-2 cells for antinuclear antibodies (ANAs) screening. Detection of anticardiolipin antibodies was conducted by enzyme-linked immunosorbent assay (ELISA) using the QUANTA Lite® ACA IgG III (Ref 708625) and ACA IgM III (Ref 708630) kits, also from Inova Diagnostics. For lupus anticoagulant screening and confirmation, we used the dilute Russell's Viper Venom Time (dRVVT) reagents LA 1 (Screening, Ref OQGP17) and LA 2 (Confirmation, Ref OQGR13) by Siemens Healthineers, following the stepwise ISTH-recommended algorithm

Clinical Data Collection

Clinical data included sociodemographic characteristics, comorbidities, and RA-specific parameters (Tables 1 and 2). Key RA-related variables

Table 2. Rheumatoid Arthritis Specific Variables.

Rheumatoid Arthritis Variables	Total Group 300 n (%)	Controls (152) n (%)	Cases (148) n (%)	*p*
Age at Diagnosis (Mean-SD) years	44.71 (10.98)	44.11 (10.92)	45.33 (11.05)	0.8449¥
Duration of Symptoms (Median-IQR) years	12 (12)	12 (8-20)	12 (6-19)	0.3726±
Erosivity	145 (48.33)	83 (54.61)	62 (41.86)	0.028*
Extra-Articular manifestations	14 (4.68)	5 (3.31)	9 (6.08)	0.195*
Pulmonary	5 (1.67)	1 (0.66)	4 (2.70)	
Cutaneous	6 (2)	1 (0.66)	5 (3.38)	
Cardiac	2 (0.67)	2 (1.32)	0	
Neurological	2 (0.67)	2 (1.32)	0	
Polyautoimmunity	44 (14.67)	22 (14.47)	22 (14.86)	0.924*
Sjögren's Syndrome	31 (10.33)	16 (10.53)	15 (10.14)	0.911*
Systemic Lupus Erythematosus	9 (3)	5 (3.29)	4 (2.70)	0.517*
Others	7 (2.33)	2 (1.32)	5 (3.38)	0.213ϕ
Systemic Sclerosis	2 (0.67)	2 (1.32)	0	0.256ϕ
Vasculitis	2 (0.67)	1 (0.66)	1 (0.68)	0.744ϕ
Antiphospholipid Syndrome	1 (0.33)	1 (0.66)	0	0.503ϕ
RF	216/294 (73.47)	109/149 (73.15)	107/145 (73.79)	0.901*
Anti CCP	210/286 (73.43)	111/147 (75)	99/139 (71.74)	0.533*
PAS				0.909*
Remission	43 (14.58)	24 (15.79)	19 (13.29)	
Low / Minimal	212 (71.86)	108 (71.05)	104 (72.73)	
Moderate	38 (12.88)	19 (12.50)	19 (13.29)	
High /Severe	2 (0.68)	1 (0.66)	1 (0.70)	
VAS Pain (Median-IQR)	2 (3)	2 (3)	2 (3)	0.775±
VAS Global (Median-IQR)	1 (2)	1 (2)	1 (2)	0.491±
HAQ				0.265*
Low	283	143 (94.08)	140 (94.59)	
Moderate	12	8 (5.26)	4 (2.70)	
Severe	5	1 (0.66)	4 (2.70)	
DAS 28				0.600*
Remission	144 (68.57)	69 (43.39)	75 (50.68)	
Low / Minimal	23 (10.95)	10 (6.58)	13 (8.78)	
Moderate	34 (16.19)	19 (12.50)	15 (10.14)	
High /Severe	99 (	54 (35.53)	45 (30.41)	
Current Treatment				
Glucocorticoids	196 (65.33)	98 (64.47)	98 (65.22)	0.751*
DMARDs	39 (13)	18 (11.84)	21 (14.19)	0.546*
NSAIDs	20 (6.67)	10 (6.58)	10 (6.76)	0.951*
Methotrexate	182 (60.67)	82 (53.95)	100 (67.57)	0.016 *
Other Analgesics	255 (85)	132 (86.84)	123 (83.11)	0.365*
DMARDs (SSZ or LEF)	170 (56.67)	95 (62.50)	75 (50.68)	0.039*
Biologics	115 (38.33)	65 (41.76)	50 (33.78)	0.110*
Anti TNF	78 (68.42)	45 (69.23)	33 (67.35)	
Anti IL 6	1 (0.88)	1 (1.54)	0	
Anti CD20	11 (9.65)	6 (9.23)	5 (10.20)	
Co-Stimulation Inhibitors	24 (21.05)	13 (20.00)	11 (22.45)	
Jak Kinase Inhibitors	9 (3)	6 (3.95)	3 (2.03)	0.264*

*: chi square pearson; ϕ: Fisher exact test; ±: Mann Whitney U; ¥: student T test; Anti TNF: Tumor necrosis factor inhibitor; Anti-CCP: Antibody Cyclic Citrullinated Peptide; DAS28: Disease Activity Score-28; DMARDs: Disease-Modifying Antirheumatic Drugs; HAQ: Health Assessment Questionnaire; IQR: Interquartile Range; LEF: Leflunomide; NSAIDs: Nonsteroidal Anti-Inflammatory Drugs; PAS: Patient Activity Scale; RF: Rheumatoid Factor; SSZ: Sulfasalazine; VAS Global: Global Visual Analog Scale; VAS pain: Pain Visual Analog Scale.

encompassed age at diagnosis, joint and extra-articular manifestations, pain assessment scales, disease activity scores, including the Patient Activity Scale (PAS) and Disease Activity Score-28 (DAS-28), as well as current treatment regimens. These data were sourced from electronic medical records and standardized collection forms, stored and managed via the Research Electronic Data Capture (REDCap) platform [11].

For patients with prior exposure to COVID-19 infection, Table 3 summarizes clinical characteristics during the acute phase. A semi-structured questionnaire was developed to capture common symptoms reported in the literature for both acute and chronic phases of COVID-19, aligning with the National Institute for Health and Care Excellence (NICE) definitions and timelines [2]. The distinction between LC (symptom persistence beyond four weeks) and PCS (symptoms persisting beyond 12 weeks) was considered.

Statistical Analysis

Statistical analyses followed a stepwise approach. Initially, comparisons were performed between rheumatoid arthritis (RA) patients with and without a history of COVID-19. Subsequently, analyses focused on comparing participants with and without post-COVID condition, and those with and without long COVID. Categorical variables were summarized as frequencies and percentages, while continuous variables were described using measures of central tendency and dispersion according to their distribution. The Shapiro–Wilk test was used to assess normality. Differences in immune marker levels between groups were evaluated using Pearson or Spearman correlation coefficients for continuous and ordinal variables, respectively. For comparisons involving dichotomous variables, Student's t-test or the Mann-Whitney U test was applied; for polytomous variables, the Kruskal-Wallis test was used due to the non-normal distribution of several variables. Statistical significance was set at $p < 0.05$. All analyses were conducted using STATA v.15® statistical software [12].

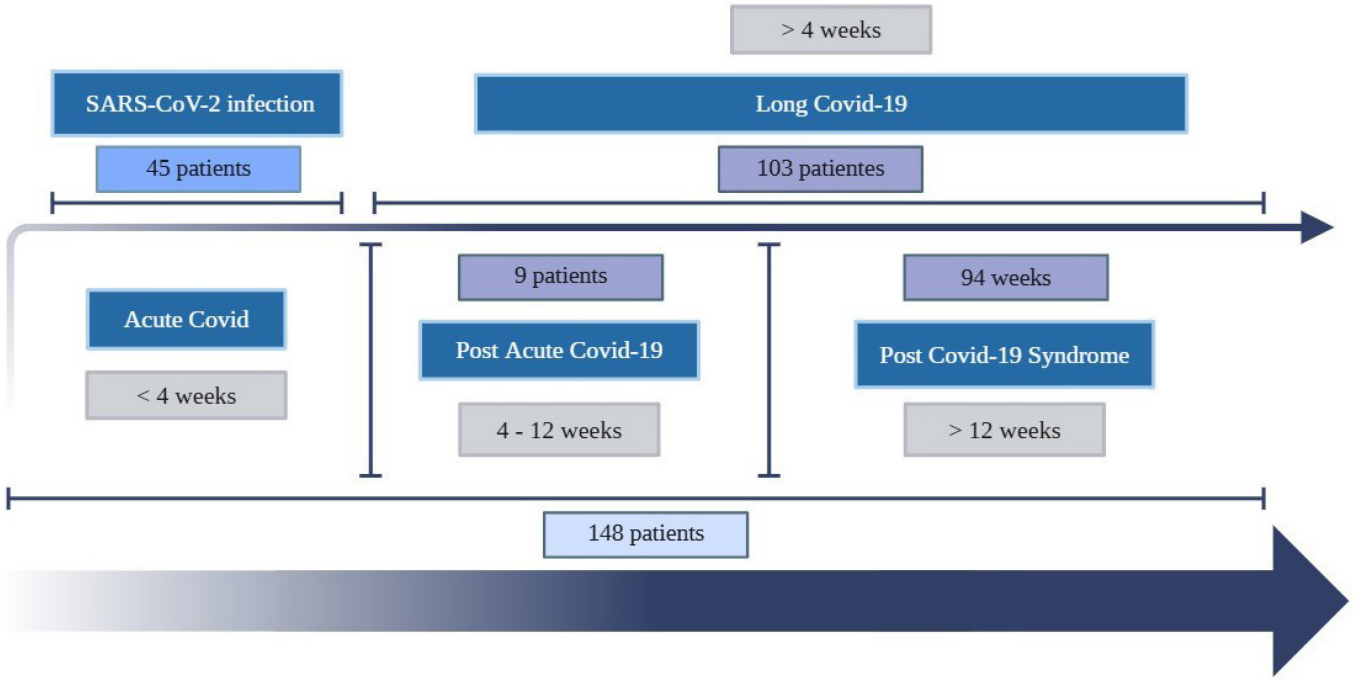
Results

Sociodemographic Characteristics and Medical History

Among the 3,000 patients in the RA cohort, 152 patients had RA without a history of COVID-19, while 148 patients had RA with a confirmed history of COVID-19. Arterial hypertension was the most frequent comorbidity, affecting 27.33% of patients (Table 1).

Most participants had received at least two doses of a COVID-19 vaccine (96.67%), with the BNT162b2 (Pfizer-BioNTech) vaccine being the most administered (45.17%). There were no significant differences in vaccination timing between patients with

Figure 1. SARS-CoV-2 infection timeline.



Chronological representation of the duration of COVID-19 infection. Classified according to the duration of symptoms and the categorization of these periods according to the NHS.

and without a history of COVID-19 (7 SD 3.47 vs. 6.9 SD 3.47, respectively; $p > 0.05$).

The mean age at RA diagnosis was 44 years, and the average disease duration was 12 years. Erosive disease was observed in 48.33% of patients, with a higher prevalence among patients without a history of COVID-19. Most patients demonstrated low disease activity, as assessed by the DAS-28 score (7 SD 3.47 vs. 6.9 SD 3.47; $p > 0.05$) (Table 2).

The most frequently prescribed RA medications were glucocorticoids (65.33%) and methotrexate (60.67%). Statistically significant differences between groups were observed for methotrexate use ($p = 0.016$) and DMARDs (SSZ or LEF) use ($p = 0.039$).

Clinical Characteristics of Patients with COVID-19

Among the 148 RA patients with a previous SARS-CoV-2 infection, 103 patients (69.59%) met the criteria for LC (> 4 weeks), including 9 patients (8.74%) classified as PASC and 94 patients (91.26%) classified as PCS (> 12 weeks) (Figure 1).

The median time since COVID-19 diagnosis was 18.5 months, with an interquartile range (IQR) of 7 months. The most common diagnostic test was PCR, used in 87.84% of cases.

During the acute phase of infection, the most frequently reported symptom was headache (54.05%). Among patients with PCS (> 12weeks), headache (63.51%), fatigue (36.49%), hair loss (29.05%), and arthralgia (23.81%) were the most prevalent symptoms (Table 3).

Autoantibody and Immune Cell Population Analysis

Despite the presence of positive results for ANAs (80%), anticardiolipin IgG (7%), and IgM (50%) in the study cohort, no significant differences were observed between patients with and without a history of COVID-19 (Table 4).

Regarding the lymphocyte population analysis, the frequencies of CD4+ and CD8+ T cells were 45.13% and 24.16%, respectively, with no significant differences between the case and control groups. Similarly, the proportions of B lymphocytes (CD19+) and natural killer (NK) cells (CD16+CD56+) were 12.4% and 12.64%, respectively, with no statistically significant variations between groups (Table 4).

Cytokine Analysis

A total of eight cytokines associated with acute and chronic inflammatory processes were analyzed: IL-17, IL-12p70, IFN-γ, IL-10, IL-6, IL-4, IL-2, and TNF.

Due to sample limitations, some cytokine measurements were not possible, although sample loss remained below 20% for most cytokines, except for IL-6, which had a 36% sample loss. However, no statistically significant differences were found between cases and controls for any of the cytokines ($p > 0.05$) (Table 5).

Table 3. COVID Variables.

COVID Variables	Cases (148) n (%)
Acute COVID (Median-IQR)	18.5 (7)
Type of Confirmation Test	
PCR	130 (87.84)
Antigen	16 (10.81)
Antibodies	2 (1.35)
Rheumatoid Arthritis treatment	
Glucocorticoids	92 (62.16)
Antimalarials	16 (10.81)
NSAIDs	8 (5.41)
Methotrexate	66 (44.59)
DMARDs (SSZ or LEF)	103 (69.59)
Biologics DMARDs	42 (28.38)
Anti-TNF alpha	27 (64.29)
Anti-IL-6	3 (7.14)
Anti-CD20	4 (9.52)
Co-Stimulation Inhibitors	8 (19.05)
COVID Management	
Ambulatory (Home)	129 (87.16)
Hospitalization	19 (12.84)
Hospitalization days (Mean-SD)	11 (8)
ICU	1 (0.67)
COVID 19 Pharmacological Treatment	
Antibiotics	38 (25.68)
Glucocorticoids	29 (19.59)
Anticoagulants	12 (8.11)
Ivermectin	10 (6.76)
Acute Symptoms	
Headache	80 (54.05)
Cough	80 (54.05)
Fatigue	79 (53.38)
Ageusia/Anosmia	79 (53.38)
Shortness of Breath	61 (41.22)
Arthralgia	60 (40.54)
Back Pain	43 (29.05)
Diarrhea	38 (25.68)
Chest Pain	34 (22.97)
Depression	13 (8.78)
Alopecia (Hair Loss)	2 (1.35)
Memory Alterations	2 (1.35)
Long COVID (> 4 weeks)	103 (69.59)

Post-COVID Syndrome (>12 weeks)	94 (63.51) n (%)	Duration Months Median (IQR)
Fatigue	54 (57.4)	11.05 (5.21)
Alopecia (Hair Loss)	43 (45.7)	8.23 (4.05)
Arthralgia	35 (37.2)	11 (5.03)
Memory Alterations	30 (31.9)	13.13 (4.24)
Ageusia/Anosmia	22 (23.4)	6.90 (4.96)
Headache	18 (19.1)	13.22 (4.58)
Cough	17 (11.49)	1 (1)
Shortness of Breath	9 (18)	2 (1)
Depression	7 (7.4)	8.71 (3.98)
Back Pain	6 (6.34)	11.66 (4.63)
Chest Pain	4 (4.2)	6 (5.65)
Diarrhea	3 (3.2)	12 (2)

Anti TNF: Tumor necrosis factor inhibitor; ICU: Intensive Care Unit; IQR: Interquartile Range; LEF: Leflunomide; NSAIDs: Nonsteroidal Anti-Inflammatory Drugs; PCR: Polymerase Chain Reaction; SD: Standard Deviation; SSZ: Sulfasalazine.

Table 4. Laboratory Tests.

Laboratories	Total Group 300 (n %)	Controls (152) (n %)	Cases (148) (n %)	$p^{\pm}$
ANAs	240 (80)	126 (82.89)	114 (77.03)	0.204*
Pattern				0.459*
Homogeneous AC-1£	66 (27.50)	39 (30.95)	27 (23.68)	
Speckled AC4,5	171 (71.25)	85 (67.46)	86 (75.44)	
Nucleolar AC8-10	1 (0.42)	1 (0.79)	0	
Mixed or others	2 (0.83)	1 (0.79)	2 (0.88)	
Current Titer				0.413*
160	46 (19.17)	23 (18.25)	23 (20.18)	
320	97 (40.42)	47 (37.30)	50 (43.86)	
640	58 (24.17)	37 (29.37)	21 (18.42)	
1280	33 (13.75)	16 (12.70)	17 (14.91)	
2560	6 (2.50)	3 (2.38)	3 (2.63)	
aCL IgG	21 (7)	12 (7.89)	9 (6.08)	0.538*
aCL IgM	150 (50)	77 (50.66)	73 (49.32)	0.817*
Cellular Populations (Median-IQR)				
Number of Lymphocyte Events	2532.5 (426.5)	2518 (281)	2584 (500)	0.0505
Quantity of Events	1283 (561)	1290.5 (537)	1275 (596.5)	0.8277
CD3 + %	72.3 (11.935)	71.975 (11.835)	72.775 (11.39)	0.3653
CD3 +	1472.92 (705.48)	1407.065 (714.735)	1509.095 (732.88)	0.5359
CD3 + CD8 + %	24.16 (11.93)	23.45 (12.22)	24.775 (11.68)	0.0734
CD3 + CD8 +	479.625 (380.385)	463.64 (360.18)	490.615 (370.565)	0.2911
CD3 + CD4 + %	45.13 (12.59)	45.775 (12.485)	43.995 (13.27)	0.3371
CD3 + CD4 +	877.37 (494.885)	877.15 (447.235)	877.37 (542.665)	0.9226
CD3 + CD4 + CD8 + %	0.76 (0.8)	0.768 (0.81)	0.745 (0.805)	0.5065
CD3 + CD4 + CD8 +	15.305 (17.07)	15.15 (18.48)	15.42 (14.29)	0.6337
CD16 + CD56 + %	12.64 (9)	12.93 (8.135)	12.4 (9.325)	0.8909
CD16 + CD56 +	262.73 (191.115)	262.73 (206.965)	264.215 (178.305)	0.5711
CD19 + %	12.405 (8.86)	12.445 (9.25)	12.305 (8.455)	0.239
CD19 +	240.115 (214.795)	236.46 (225.975)	241.645 (206.25)	0.6303
CD45 +	2013.985 (1024.93)	2023.355 (846.635)	2007.985 (1072.565)	0.4568
4/8 Ratio	1.915 (1.345)	1.98 (1.305)	1.84 (1.295)	0.133
Cytokines levels (Median-IQR)				
IL-12p70 (300)	15.64 (20.545)	15.645 (19.465)	15.59 (21.535)	0.9771
IL17 (287)	29.43 (22.15)	28.63 (21.96)	29.71 (21.15)	0.5843
IFN-γ (291)	4.57 (7.15)	3.65 (7.17)	4.96 (7.205)	0.3964
TNF (290)	13.03 (14.95)	12.48 (15.43)	13.65 (15.2)	0.6691
IL-10 (283)	5.79 (5.62)	5.52 (5.42)	5.85 (5.39)	0.2888
IL-4 (287)	4.58 (5.34)	4.62 (5.48)	4.5 (5.155)	0.7072
IL-2 (288)	8.045 (5.26)	7.57 (6.61)	8.26 (4.09)	0.643

Laboratory test in the population of cases and controls. £According to the International Consensus on Antinuclear Antibody Patterns (ICAP). *: Chi-square Pearson ±: Mann-Whitney U; ACL IgG: Anticardiolipin antibodies IgG; aACL IgM: Anticardiolipin antibodies IgM; ANAs: Antinuclear antibodies; IFN: Interferon; IQR: Interquartile Range; TNF: Tumor necrosis factor.

Additionally, a subgroup analysis was conducted on patients who had contracted COVID-19 within one year before cytokine assessment, but no significant differences were detected.

Furthermore, stratified analyses were performed to compare patients with LC (> 4 weeks) and PCS (> 12weeks) with those who did not develop persistent symptoms. No statistically significant differences were observed in cytokine profiles between these groups (Supplementary Tables 1,2,3).

Discussion

This study presents a cohort of RA patients managed under a strict Treat-to-Target (T2T) strategy at a specialized center in Colombia during the later stages of the COVID-19 pandemic. It also provides a comparative analysis of the immune profiles of patients with and without a history of COVID-19, representing a novel approach for this population. Understanding the potential immune system disruptions in RA patients may offer insights into post-SARS-CoV-2 infection complications, a phenomenon previously documented in populations with intact immune systems [13–16].

The median age of RA patients in this study aligns with findings from previous studies [17,18], with a higher prevalence of female patients, as is characteristic of the disease. The sociodemographic and clinical characteristics of the study population closely resemble those described in previous RA cohorts [19]. The presence of joint erosivity, a marker of RA progression, was observed among patients. Notably, patients with a history of COVID-19 exhibited lower levels of erosive disease, despite its established role as an indicator of disease severity. Although this finding may suggest an association between COVID-19 susceptibility and RA progression, the cross-sectional nature of the study

Table 5. Comparison of Cellular Populations and Cytokines in subgroups related to the duration of COVID.

Variable	Cases (148) (Median-IQR)	Post-covid (> 12 weeks) (94) (Median-IQR)	Non-post-covid (54) (Median-IQR)	$p^{\pm}$
Lymph Events	2584 (500)	2581.5 (455)	2584.5 (566)	0.7803
Bead Events	1275 (596.5)	1284 (596)	71.67 (10.58)	0.4303
CD3 + %	72.775 (11.39)	73.09 (12.34)	71.83 (10.58)	0.5502*
CD3 +	1509.095 (732.88)	1472.92 (687.5)	1554.315 (922.59)	0.4187
CD3 + CD8 + %	24.775 (11.68)	25.89 (12.2)	23.76 (10.2)	0.1955*
CD3 + CD8 +	490.615 (370.565)	510.585 (365.22)	473.925 (432.49)	0.6642
CD3 + CD4 + %	43.995 (13.27)	43 (13.82)	46.375 (11.51)	0.1921*
CD3 + CD4 +	877.37 (542.665)	840.075 (541.48)	1011.26 (647.28)	0.1597
CD3 + CD4 + CD8 + %	0.745 (0.805)	0.745 (0.81)	0.74 (0.63)	0.6584*
CD3 + CD4 + CD8 +	15.42 (14.29)	15.52 (15.04)	15.305 (12.8)	0.9857
CD16 + CD56 + %	12.4 (9.325)	13.515 (9.66)	12.29 (8.95)	0.7349*
CD16 + CD56 +	264.215 (178.305)	268.23 (203.79)	247.655 (151.98)	0.9365
CD19 + %	12.305 (8.455)	11.425 (8.51)	12.85 (7.29)	0.1769*
CD19 +	241.645 (206.25)	231.215 (215.05)	242.705 (215.66)	0.263
CD45 +	2007.985 (1072.565)	1965.565 (997.2)	2186.09 (1123.31)	0.2985
4/8 Ratio	1.84 (1.295)	1.66 (1.24)	2.035 (1.33)	0.076
ANAS	114 (77.03)	68 (59.65)	46 (40.35)	0.0747
aCL IgG	9 (6.08)	6 (66.67)	3 (3.33)	0.2456
aCL IgM	73 (49.32)	43 (58.90)	30 (41.10)	0.0666
IL-12p70 (148)	15.59 (21.535)	15.73 (20.56)	15.315 (23.43)	0.8918
IL17 (141)	29.71 (21.15)	31.54 (20.2)	26.875 (24.2)	0.8158
IFN-γ (145)	4.96 (7.21)	5.25 (6.82)	4.87 (7.84)	0.6096
TNF (143)	13.65 (15.2)	14.97 (18.2)	11.87 (9.84)	0.0535
IL-10 (137)	5.85 (5.39)	6.24 (5.25)	5.13 (5.29)	0.3015
IL-4 (140)	4.5 (5.155)	4.58 (5.06)	4.5 (5.44)	0.9378
IL-2 (141)	8.26 (4.09)	8.04 (3.71)	8.77 (5.335)	0.5383

IL-6 levels are not shown due to 37% data loss; No statistically significant differences were found between the groups ($p < 0.05$). ±: Mann-Whitney. *: chi-square Pearson; ACL IgG: Anticardiolipin antibodies IgG IFN: Interferon; IQR: Interquartile Range; TNF: Tumor necrosis factor.

prevents definitive conclusions, highlighting the need for future investigations into this potential link.

Most patients exhibited low disease activity or remission, consistent with reports from Latin American studies [20–22]. Given that disease activity influences prognosis [23], this factor may have contributed to the low prevalence of hospitalization and ICU admission in this cohort.

Regarding RA treatment regimens at the time of COVID-19 infection, the most frequently used therapeutic category was non-conventional synthetic disease-modifying antirheumatic drugs (DMARDs), including sulfasalazine (SSZ) and leflunomide (LFN), consistent with findings from previous studies [20,24,25]. The use of glucocorticoids was notably high in this cohort, exceeding rates reported in Latin American and European studies [26,27].

Among patients with a history of COVID-19, the median time from diagnosis to data collection was 18.5 months. The majority of these patients received outpatient treatment at home, at a higher rate than reported in global studies. This may reflect the impact of the T2T strategy, which involves rigorous, multidisciplinary monitoring, potentially leading to early detection and intervention.

The most frequently reported symptoms during the acute phase of SARS-CoV-2 infection were headache, cough, and fatigue, findings that align with studies in non-RA populations [28,29].

Post-COVID Syndrome PCS (> 12weeks) in RA Patients

The prevalence of PCS in this cohort was 63.51%, surpassing the 50.9% prevalence reported by Moreno Pérez [30] in non-RA populations. However, variations in the prevalence of PCS lasting more than 12 weeks and symptom duration have been documented across studies, largely due to differences in follow-up duration and PCS (> 12weeks) definitions [28,31–33].

Despite these discrepancies, most literature on the general population highlights musculoskeletal symptoms as a predominant feature of PCS (> 12weeks), significantly impacting quality of life. Notably, research on PCS (> 12weeks) in RA patients remains limited, with most studies focusing on new-onset autoimmune diseases and RA following SARS-CoV-2 infection [34,35].

In patients with autoimmune rheumatic diseases, such as Sjögren's syndrome, Zerón [36] reported a 29% prevalence of PCS (> 12weeks), a rate lower than that observed in the present study. However, the symptom profile closely resembled that observed in this RA cohort.

Autoantibodies and Immune Profiles

Stjepanovic *et al.* [37] investigated autoantibodies,

such as anticardiolipin antibodies (aCL), as potential COVID-19 severity markers. Subsequent studies in non-autoimmune populations suggested that the persistence of certain autoantibodies may be associated with ongoing PCS (> 12weeks) or serve as predictive markers of latent autoimmunity [38].

In the current study, a considerable proportion of RA patients, regardless of COVID-19 history, tested positive for ANAs and aCL IgM, with no significant differences between groups. This suggests the potential presence of latent polyautoimmunity, particularly involving antiphospholipid syndrome antibodies. Unlike findings by Anaya [28], the serological profile appeared consistent regardless of COVID-19 status. Anaya, in a prospective study in a Colombian population, documented a slight increase in 1:80 ANA titers compared to pre-pandemic controls, underscoring the difficulty in comparing COVID-19-positive and negative populations due to the absence of autoimmune disease history in many pre-pandemic controls.

Lymphocyte and Cytokine Analysis

The assessment of T, B, and NK lymphocyte populations showed no significant differences between cases and controls, suggesting that SARS-CoV-2 infection did not induce long-term immune alterations in this cohort. This contrasts with previous studies reporting dynamic changes in CD4+, CD8+, and NK cells over follow-up periods of 12 to 24 weeks, with functional exhaustion persisting up to 16 weeks post-infection. However, these studies tracked immune dynamics within the same individuals, whereas the present study employed a cross-sectional case-control design.

Comparative analysis of evaluated cytokines showed no significant differences between cases and controls. Further subgroup comparisons between no COVID vs. LC (> 4 weeks), no COVID vs. PCS (> 12weeks), and LC (> 4 weeks) vs. No LC revealed no statistically significant alterations in long-term immune profiles of RA patients post-COVID-19. In contrast, global prospective studies in healthy individuals have documented elevated levels of IL-6, TNF, IFN-γ, and IL-2 following SARS-CoV-2 infection [39]. However, these differences were not statistically significant. Similar trends have been described in other cohorts, where low-grade elevations of pro-inflammatory cytokines have been linked to persistent symptoms after infection. These findings suggest that subtle immunological changes may persist beyond the acute phase and could be biologically relevant. The lack of statistically significant results in the present study may also be related to the limited statistical power of our study; larger sample sizes may be required to detect these differences more robustly. Further longitudinal studies are needed to explore these patterns in greater detail and to better understand their clinical implications.

Previous studies conducted 24-week follow-ups, with frequent cytokine measurements from infection onset, whereas the present study conducted assessments beyond 12 months. Additionally, these studies focused on immunocompetent individuals, making direct comparisons challenging. Although IL-6 levels were of particular interest, the 37% data loss prevented meaningful analysis, limiting interpretation. Given that IL-6 plays a central role in both COVID-19 and RA pathogenesis, its absence from statistical analysis may have influenced findings. This loss may also impact the prompt and effective regulation of systemic cytokine levels, as the study population was managed under a Treat-to-Target (T2T) strategy with strict disease control using various medications that modulate the immune system.

Furthermore, upon conducting a direct comparison within the subset of case patients divided into those exhibiting either PASC or PCS (> 12weeks) symptoms and those who were asymptomatic, a similar distribution was observed between the two groups, with no statistically significant variances. These results suggest a return to baseline immunological equilibrium in the long term following an infection, irrespective of symptom presentation or subgroup classification. Nevertheless, the discussion on this topic remains open due to the potential immunological mechanisms and pathological interactions between COVID-19 and RA. The uncontrolled immune activation and cytokine response seen in COVID-19 bear resemblance to the immune inflammation observed in RA, albeit with potential variations that could manifest over time [40].

It is important to consider the limitations of this study. Due to its retrospective observational design, only a snapshot of the patients' immune system dynamics was captured, lacking the ability to showcase temporal changes inherent in immune responses. This may explain why the dynamic shifts documented in the literature were not evident during the early and intermediate phases of the disease. Additionally, memory bias may have influenced the self-reported variables related to COVID-19, despite the use of structured surveys administered by trained staff. A significant portion of this information could not be verified through contemporaneous clinical records due to the lack of a centralized healthcare system in

Colombia capable of integrating data across different institutions. Furthermore, although cytokine data were largely available, a general sample loss of less than 20% was observed during analysis, with IL-6 values missing in approximately 37% of participants, potentially limiting the interpretation of this specific marker.

As the study was conducted under real-life conditions, both the biological and clinical aspects of the disease at presentation were examined. Consequently, the decision was made to retrospectively gather COVID-19 test results. Additionally, conducting screenings for asymptomatic COVID-19 cases among all patients visiting the center was deemed financially prohibitive and was not within the scope of national protocols at that time. Moreover, due to the real-life nature of the study, no kinetic analysis was carried out to assess temporal variations from the onset of infection. Lastly, considering that the humoral and cellular protective responses typically do not extend beyond one year and the evaluation period in this study spanned up to 24 months, we aimed to investigate the possibility of changes, especially since clinical symptoms persisted in the described subgroups even after one year. However, no significant differences were observed.

Despite its limitations, this study provides a comprehensive overview of RA patients' immune profiles post-COVID-19 in a specialized rheumatology center. These findings lay the groundwork for future prospective longitudinal studies, which are essential to understanding the long-term immunological evolution in this population.

Ethical considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Hospital Human Research Ethics Committee in Bogotá, Colombia (0733-2021), on December 17th, 2021.

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Authors contributions

Julián Arias-Aponte: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing - Original Draft Preparation, Writing - Review & Editing, Supervision. Gabriel E. Acelas-Gonzalez: Formal Analysis, Investigation, Writing - Original Draft Preparation, Writing - Review & Editing, Supervision. Rafael Parra-Medina: Visualization, Investigation, Writing - Review & Editing. María Lorcy Monsalve-Córdoba: Formal Analysis, Data Curation, Investigation, Validation, Methodology, Software. Adriana Rojas-Villarraga: Formal Analysis, Investigation, Writing - Original Draft Preparation, Writing - Review & Editing, Supervision, Data Curation. Paula Daniela Nieto-Zambrano: Writing - Review & Editing, Formal Analysis, Visualization, Methodology. Maria Camila Cortés-Osma: Formal Analysis, Investigation, Resources. Hector Fabio Restrepo-Guerrero: Formal Analysis, Data Curation, Investigation, Validation, Methodology, Software. Laura Villarreal: Investigation, Writing - Original Draft Preparation, Writing - Review & Editing. Pedro Santos-Moreno: Conceptualization, Resources, Methodology, Supervision. Arley Gómez-López: Writing - Original Draft Preparation, Writing - Review & Editing, Project Administration, Funding Acquisition.

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Conflict of interest

No conflict of interest is declared.

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Annex – Supplementary Items

Supplementary Table 1. Comparison of Cellular Populations and Cytokines in Patients with Long COVID and Non-Long COVID.

Variable	Long covid (> 4weeks) (103) (Median-IQR)	No long covid (> 12 weeks) (45) (Median-IQR)	$p^{\pm}$
Lymph Events	2586 (584)	2578 (456)	0.8774
Bead Events	1285 (652)	1235 (461)	0.3905
CD3 + Percent	1235 (461)	71.83 (10.58)	0.6496
CD3 +	1471.01 (738.19)	1615.35 (786.72)	0.3141
CD3 + CD8 + %	25.5 (12.92)	23.74 (9.08)	0.1582
CD3 + CD8 +	495.54 (377.82)	495.54 (377.82)	0.8922
CD3 + CD4 + %	43.14 (13.43)	45.24 (11.51)	0.3294
CD3 + CD4 +	868.22 (541.48)	1003.99 (659.17)	0.1857
CD3 + CD4 + CD8 + %	0.77 (0.81)	0.68 (0.58)	0.4211
CD3 + CD4 + CD8 +	15.47 (15)	15.33 (13.07)	0.8414
CD16 + CD56 + %	13.41 (9.66)	12.21 (8.45)	0.5679
CD16 + CD56 +	267.66 (203.79)	248.04 (124.42)	0.9485
CD19 + %	11.88 (7.79)	12.68 (8.04)	0.2398
CD19 +	226.77 (215.99)	243.42 (267.28)	0.3101
CD45 +	1966.75 (1062.61)	2212.93 (1100.49)	0.2648
4/8 Ratio	1.67 (1.27)	2.01 (1.26)	0.1884
ANAs	75 (65.79)	39 (34.21)	0.065
aCL IgG	7 (77.78)	2 (22.22)	0.582
aCL IgM	46 (63.01)	27 (36.99)	0.086
IL-12p70 (148)	14.85 (21.05)	16.72 (16.72)	0.7627
IL17 (141)	31.53 (20.65)	25.42 (25.42)	0.7319
IFN-γ (145)	4.96 (7.32)	4.92 (7.205)	0.9415
TNF (143)	14.68 (14.68)	11.46 (10.19)	0.0984
IL-10 (137)	6.12 (5.4)	5.05 (4.72)	0.3666
IL-4 (140)	4.54 (5.06)	4.5 (6.82)	0.9675
IL-2 (141)	8.14 (3.91)	8.72 (5.27)	0.9269

$^{\pm}$: Mann-Whitney. ANAS. ANAs: Antinuclear antibodies. IQR: Interquartile Range; TNF: Tumor necrosis factor.

Supplementary Table 2. Comparison of Cytokines in Patients with No Covid and Post-Covid.

Variable	No covid (152) (Median-IQR)	Post-Covid (> 12weeks) (94) (Median-IQR)	$p^{\pm}$
IL12p70 (300)	15.645 (19.465)	15.73 (20.56)	0.952
IL17 (287)	28.63 (21.96)	31.54 (20.20)	0.553
IFN γ (291)	3.65 (7.17)	5.37 (6.69)	0.281
TNF (290)	12.48 (15.43)	14.97 (18.20)	0.187
IL10 (283)	5.52 (5.42)	6.24 (5.25)	0.156
IL4 (287)	4.62 (5.48)	4.58 (5.06)	0.721
IL2 (288)	7.57 (6.61)	8.04 (3.71)	0.761

±: Mann-Whitney. IQR: Interquartile Range; TNF: Tumor necrosis factor.

Supplementary Table 3. Comparison of Cytokines in Patients with No Covid and Long COVID.

Variable	No covid (152) (Median-IQR)	Long Covid (> 4weeks) (94) (Median-IQR)	$p^{\pm}$
IL12p70 (300)	15.645 (19.465)	14.85 (21.05)	0.8915
IL17 (287)	28.63 (21.96)	31.53 (41.29)	0.5124
IFN γ (291)	3.65 (7.17)	5.05 (8.40)	0.4219
TNF (290)	12.48 (15.43)	14.68 (25.01)	0.2713
IL10 (283)	5.52 (5.42)	6.12 (5.40)	0.1937
IL4 (287)	4.62 (5.48)	4.54 (6.96)	0.7678
IL2 (288)	7.57 (6.61)	8.14 (10.14)	0.6109

±: Mann-Whitney. IQR: Interquartile Range; TNF: Tumor necrosis factor.