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Evaluation of Systemic Autoimmune Rheumatic Autoantibodies in post-COVID-19 Patients and Their Impact on Clinical Outcomes: A Retrospective-Cohort Study

COVID-19 Geçiren Hastalarda Sistemik Otoimmün Romatizmal Otoantikorların Değerlendirilmesi ve Bunların Klinik Sonuçlar Üzerindeki Etkisi: Retrospektif Kohort Bir Çalışma

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ABSTRACT Objective: The role of systemic autoimmunity in post-coronavirus disease-2019 (COVID-19) patients from real-world data is still unknown. The aim of our study was to evaluate the presence of rheumatic and antiphospholipid antibodies in post-COVID-19 patients and examine their frequencies and the clinical outcomes. **Material and Methods:** The data of 393 people who had COVID-19 and were followed up in our outpatient clinic were retrospectively analyzed. In our descriptive study, clinical and biochemical parameters as well as the presence of antinuclear antibodies, antiphospholipid antibodies, romatoid factor and immunoglobulin G cyclic citrullinated peptide 3rd generation were evaluated in all patients. **Results:** One hundred fifty nine of 393 (40.5%) patients had at least one autoimmune reactivity. 31 patients with 1 or more autoantibodies positivity and/or accompanying musculoskeletal symptoms after COVID-19 were referred to the rheumatology outpatient clinic. Autoimmune disease was diagnosed in 9 of these patients. The distribution of autoimmune disease diagnoses is as follows; systemic lupus erythematosus (SLE) or SLE-like syndrome was diagnosed in 3 patients, mixed connective tissue disease in 3 patients, Sjögren's syndrome in 2 patients and scleroderma in 1 patient. **Conclusion:** Although it is not possible to establish a cause-and-effect relationship due to the lack of a control group in the design of our study and the lack of serologic laboratory test results before COVID-19 in patients diagnosed with autoimmune diseases, we think it may be important that autoantibodies and acute phase reactants other than ANA and CCP were found to be higher in hospitalized patients and that various systemic autoimmune reactivities were detected in 40% of post-COVID-19 patients.

Keywords: Coronavirus disease-2019; post-acute-coronavirus disease-2019 syndrome; autoantibodies; autoimmune diseases; musculoskeletal abnormality

ÖZET Amaç: Koronavirüs hastalığı-2019 [coronavirus disease-2019 (COVID 19)] geçiren hastalarda sistemik otoimmünitenin rolü gerçek dünya verilerinde hala bilinmemektedir. Çalışmamızın amacı, COVID-19 sonrası hastalarda romatizmal ve antifosfolipid antikorların varlığını değerlendirmek ve sıklıklarını ve klinik sonuçlarını incelemekti. **Gereç ve Yöntemler:** COVID-19 geçiren ve polikliniğimizde takibe alınan 393 kişinin verileri retrospektif olarak incelendi. Tanımlayıcı çalışmamızda, tüm hastaların klinik ve biyokimyasal parametrelerinin yanı sıra antinükleer antikorlar, antifosfolipid antikorlar, romatoid faktör ve immünoglobulin G siklik sitrüline peptid [cyclic citrullinated peptide üçüncü nesil varlığı değerlendirildi. Bulgular: 393 hastanın 159'unda (%40,5) en az bir otoimmün reaktivite vardı. COVID-19 sonrası 1 veya daha fazla otoantikor pozitifliği ve/veya eşlik eden kas-iskelet sistemi semptomları olan 31 hasta romatoloji polikliniğine konsülte edildi. Bu hastaların 9'unda otoimmün hastalık teşhisi kondu. Otoimmün hastalık tanılarının dağılımı 3 hastada sistemik lupus eritematozus (SLE) veya SLE benzeri sendrom, 3 has tada karışık bağ dokusu hastalığı, 2 hastada Sjögren sendromu ve 1 hastada skleroderma şeklindeydi. **Sonuç:** Çalışmamızın tasarımında kontrol grubunun olmaması ve otoimmün hastalık tanısı almış hastalarda COVID-19 öncesi serolojik laboratuvar test sonuçlarının bulunmaması nedeniyle bir neden-sonuç ilişkisi kurmak mümkün değildir. Ancak hastanede yatan hastalarda ANA ve CCP dışındaki otoantikorların ve akut faz reaktanlarının daha yüksek bulunması ve COVID-19 sonrası hastaların %40'ında çeşitli sistemik otoimmün reaktivitelerin tespit edilmiş olmasının önemli olabileceğini düşünüyoruz.

Anahtar Kelimeler: Koronavirüs hastalığı-2019; post-akut koronavirüs hastalığı-2019 sendromu; otoantikorlar; otoimmün hastalıklar; kas iskelet anomalileri

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Coronavirus disease-2019 (COVID-19), caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), led to significant morbidity and mortality worldwide.[1] It is also known that the prognosis of COVID-19 remarkably varies among acutely infected patients and convalescents. Even though most patients present with mild symptoms, some convalescents can be afflicted by long-lasting symptoms such as fatigue, dyspnea, arthralgia, sleep disturbance, and myalgia. The World Health Organization Clinical Case Definition Group defined this phenomenon as the "post-COVID-19 condition". According to this definition, patients with a history of SARS-CoV-2 infection, usually 3 months from the onset, with symptoms lasting for at least 2 months which another disease process cannot explain, are diagnosed with this condition.[2] A severe inflammatory cytokine release, including, interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha, can lead to clinical presentations similar to those encountered in systemic autoimmune rheumatic diseases (SARDs) in patients with SARS-CoV-2 infection.[3] In addition, it is well documented that a viral infection can activate innate and acquired immune responses in genetically predisposed patients.[4,5] Parvovirus B19, Epstein-Barr virus (EPV), cytomegalovirus, herpesvirus-6, Human T-lymphotropic virus 1, hepatitis A and C virus, rubella virus, alphaviruses such as chikungunya or O'nyong-nyong can all trigger these responses. As such, amplification in the rate of cases of rheumatoid arthritis has been reported following viral outbreaks.[4]

COVID-19 is a relatively new virus; therefore, it is unknown if it can trigger autoimmune conditions. However, recent evidence demonstrated the ability of the COVID-19 virus to induce hyperstimulation of the immune system and the formation of autoantibodies.[5,6] In addition, the heterogeneous expression of antibodies in COVID-19 indicates the alterations in the patients' innate and adaptive immune responses. These immunological alterations range from an abnormal immune response and cytokine production to the hyperactivation of T cells.[6,7] Earlier studies reported that specific autoantibodies such as anti-cardiolipin (ACA), antiβ2-glycoprotein (a-β2GPI), and lupus anticoagulant might be associated with the thromboembolic complications occurring in some COVID-19 patients.[8,9] In the cohorts conducted with the patients with severe COVID-19, it was determined that almost half of these patients had at least one antiphospholipid antibodies (aPL).[10] Another study showed that SARS-CoV-2 could lead to autoantibody formation by activating the toll-like receptors, the complement system, and releasing neutrophil extracellular traps.[11] Borghi et al. reported that aPLs formed in COVID-19 patients differed from those detected in the anti-phospholipid syndrome (APS).[12] The persistence of these antibodies can be a harbinger of an increased predisposition to thrombosis, as in APS.[13]

The aim of our study was to evaluate the presence of rheumatic and antiphospholipid antibodies in post-COVID-19 patients and examine their frequencies and the clinical outcomes. The secondary outcome is to evaluate the relationship between post-COVID-19 symptoms related to the musculoskeletal system and the presence of autoantibodies.

MATERIAL AND METHODS

STUDY DESIGN AND POPULATION

Adult (age≥18) patients diagnosed with COVID-19 by a positive reverse transcriptase-polymerase chain reaction test result and discharged from the hospital at least 6 weeks ago constituted the target population of this study. All patients were under follow-up at the COVID-19 outpatient clinic. The study period covered the time between December 1, 2020-July 31, 2021. Patients with a previous diagnosis of autoimmune diseases, hepatit B or C infections or malignancies were excluded.

Patient data, including demographic parameters, data regarding smoking history and comorbidities, and symptoms at presentation, were retrospectively evaluated. Laboratory panels determined by the Ministry of Health were obtained from all patients. The C-reactive-protein (CRP), fibrinogen, D-dimer, international normalized ratio (INR), partial thromboplastin time (PTT), prothrombin time (PT), Immunglobulin M (IgM) rheumatoid factor (RF), IgG 3rd-generation anti-cyclic citrullinated peptide (CCP), Ig M and Ig G ACA, IgM Ig G and IgA a-

β2GPI and anti-nuclear antibody (ANA) tests were all performed during the first visit of all patients. The results of these tests were collected from the electronic patient folders. Post-COVID-19 patients with positive autoantibodies and/or aPL and/or accompanying symptoms were referred to a rheumatologist. Rheumatologists performed further tests on these patients if necessary. One of these tests was the analysis of the antibodies to extractable nuclear antigens (ENAs) anti-smith (Sm) and anti-ribonucleoprotein (RNP)/Sm, RNP70, A and C proteins, SSA-Ro52, SSB-Ro60, Scl-70, polymyositis-scleroderma, Jo-1, centromere protein-B (CENP-B), PCNA, deoxyribonucleic acid (ds)-DNA, nucleosomes, histones, ribosomal P protein, and M2. In addition, an enzyme-linked immunosorbent assay (ELISA) test was performed for quantification in cases where RF, CCP, Ig M, and Ig G ACAs and a-β2GPI were detected.

The ANAs were analyzed using an indirect immunofluorescence assay on Hep-2 cells (Cobas6000 e601, Roche, Germany). Positive results were considered from dilution 1/80. In cases with ANA positivity, anti-SSA/Ro, anti-SSB/La, RNP, and Sm antibodies were further analyzed by a commercial ELISA. All the assay kits were obtained from Roche Elecys Cobas E, Germany. The ACA IgM, IgG, and aβ2GPI IgG, IgM, and IgA levels were measured by ELISA (Aesku Diagnostics, GmbH&Co. KG Mikroforum Ring 2ys 5523Roche Elecs4 Wendelsheim, Germany).

This study was done in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participants for the use of their data for study purposes. (Written consents themselves have been held by the corresponding author herself). The study was approved by our institutional ethical review board (University of Health Dışkapı Yıldırım Beyazıt Training and Research Hospital, date/no: June 14, 2021/113-20).

STATISTICAL ANALYSIS

All statistical analyses were performed using the IBM SPSS Statistics 11.5 (Chicago, IL, USA) software version. Descriptive parameters, including demographic data, were given as frequencies, percentages, and means±standard deviations (SD). Quantitative variables were expressed as means±SDs and medians [minimum-maximum], and categorical variables as numbers (n) and percentages (%). The chi-square test was implemented to compare categorical variables. Differences of variables predicted as risk factors in groups with and without hospitalization were evaluated with the continuity corrected chi-square, and Fisher's exact chi-square test, whichever is appropriate were given.

RESULTS

After applying the inclusion criteria and excluding 23 patients with a previous autoimmune disease diagnosis, 393 post-COVID-19 patients were included in this study. Sociodemographic characteristics, and hospitalization information of these patients were shown in Table 1. While 266 of all patients had pneumonia, 4 had a pulmonary embolism, 2 had acute renal failure, 1 deep vein thrombosis, and 1 cerebrovascular accident during the acute COVID-19.

TABLE 1: Demographic characteristics of 393 patients at baseline

Variables		
Sex, n (%)	Male	200 (50.9)
	Female	193 (49.1)
Age	$\bar{X}$±SD	53.32±13.68
	Median (minimum-maximum)	55.00 (18-87)
Post-COVID-19 period (month)	$\bar{X}$±SD	4.58±2.57
	Median (minimum-maximum)	4.00 (1-15)
Post-COVID-19 period, n (%)	1-3 months	149 (37.9)
	4-6 months	168 (42.7)
	7-15 months	76 (19.4)
Smoking, n (%)	No	364 (92.6)
	Yes	29 (7.4)
Hospitalization, n (%)	No	127 (32.3)
	Yes	266 (67.7)
Intensive care unit, n (%)	No	294 (74.8)
	Yes	99 (25.2)
Pneumonia, n (%)	No	127 (32.3)
	Yes	266 (67.7)
Other complications, n (%)	No	385 (98.0)
	Yes	8 (2.0)

SD: Standard deviation; COVID-19: Coronavirus disease-2019

The most common comorbidities were hypertension (36.6%), diabetes mellitus (25.2%), hyperlipidemia (17.8%), hypothyroidism (13.2%), and coronary artery disease (10.4%).

In the questionnaires that investigated symptoms related to all systems, the symptoms associated with the musculoskeletal system of 381 patients with complete data were evaluated. During the initial evaluation in our outpatient clinic, 232 (60.8%) patients had fatigue, 179 (46.9%) patients had joint pain, 62 (16.2%) had morning stiffness, 21 (5.5%) had joint swelling, 11 (2.8%) had redness and warmth in the joints, 142 (37.2%) had muscle pain, and 100 (26.2%) had muscle weakness. The patients did not have these complaints before acquiring the COVID-19 infection.

Most patients' CRP, fibrinogen, INR, aPTT, and PT levels were within the normal range.

We examined the presence of autoimmune antibodies in the sera of the patients to evaluate the long-lasting autoimmune responses triggered by the SARS-CoV-2 infection (Table 2). Overall, 159 patients (40.5%) had at least 1 autoimmune reactivity. While 135 (34.4%) patients had one autoimmune reactivity, 19 (4.8%) had 2, 4 (1%) had 3, and 1 (0.3%) had 4 autoimmune reactivities. Among these patients, ANA and RF positivity were detected in 10 patients, ANA and a-β2GPI IgG positivity were present in 3 patients, while ACA IgM and a-β2GPI IgG positivity were encountered in 2 patients. ANA and a-β2GPI IgM positivity, ANA and ACA GPI IgM positivity, RF and a-β2GPI IgG positivity, and ANA and CCP positivity were detected in one patient each. One patient had positive ANA, RF, and CCP, while another had positive ANA, RF, and a-β2GPI IgG. In addition, 1 patient had positive ACA IgM, ACA IgG, and a-β2GPI IgG; another was positive regarding ANA, RF, ACA IgG, and a-β2GPI IgG.

TABLE 2: Autoimmune serological markers and antibody positivity

Post-COVID-19 patients (n=(n=393)	
ANAs (all patterns)	117 (29.8)
1/160-1/320	96 (24.4)
1/320-1/1280	12 (3.1)
1/1280-1/5120	5 (1.3)
1/5120-1/10240	4 (1.0)
Nucleolar	28 (7.1)
Speckled	58 (14.7)
Homogeneous	24 (6.1)
Cytoplasmic	5 (1.3)
Other	2 (0.6)
Anticardiolipin Ig G	8 (2.0)
Anticardiolipin Ig M	6 (1.5)
A-β2GPI Ig G	6 (1.5)
A-β2GPI Ig M	13 (3.3)
A-β2GPI Ig A	11 (2.8)
Anti-CCP3	3 (0.8)
RF	36 (9.2)
D-Dimer	56 (14.2)
Fibrinogen	56 (14.2)

ANAs Anti-nuclear antibody; Ig G: Immunoglobulin G; A-β2GPI: Anti β2glycoprotein; CCP3: 3rd-generation anti-cyclic citrullinated peptide; RF: Rheumatoid Factor

Thirty-one patients with autoantibody positivity and/or newly emergent musculoskeletal symptoms after COVID-19 were referred to the rheumatology outpatient clinic (Table 3). Our analysis revealed that further tests were performed in 27 of these patients, and 9 were diagnosed with autoimmune disease. Among these patients, 3 were diagnosed with systemic lupus erythematosus (SLE) or SLE-like syndrome, 3 with mixed connective tissue disease, 2 with Sjögren's syndrome, and 1 with scleroderma (progressive systemic sclerosis).

381 patients with complete data on musculoskeletal symptoms and laboratory results were evaluated. When the relationship between the musculoskeletal symptoms and the presence of some autoimmune antibodies and acute phase reactants was examined, no statistically significant difference was found (Table 4).

Statistically significant differences were detected when musculoskeletal symptoms and the same laboratory results were compared between hospitalized and outpatient patient groups (Table 5). All symptoms except muscle weakness and tingling in hands and feet were found to be higher in hospitalized patients. Autoantibodies and acute phase reactants other than ANA and CCP were found to be higher in hospitalized patients.

DISCUSSION

This descriptive study aimed to evaluate the presence of rheumatic and antiphospholipid antibodies in post-

TABLE 3: Some clinical and laboratory characteristics of 31 patients for whom rheumatology consultation was requested

Patient no	Sex/Age	Comorbidities	Anti-SARS-Cov-2 Ig G	Post-COVID-19 musculoskeletal symptoms	CRP	Fibrinogen	D-dimer	Autoantibodies	ENA Panel
1	M/42	Diabetes mellitus, Sleep apnea	POS	Myalgia	7.78	4.18	0.44	ANA 1/160 fibrillar, -β2GPI Ig G, a-β2GP Ig A	(–)
2	F/43	Hypertension, Asthma	POS	None	2.86	3.31	0.48	ANA 1/160 speckled cytoplasmic, RF:162, CCP>200	(–)
3	M/51	Hypertension, Coronary artery disease Congestive heart failure, hyperlipidemia	POS	Arthralgia	2.24	4.24	1.12	RF:76.3	
4	F/59	Diabetes mellitus, Hypertension, hyperlipidemia	POS	Arthralgia	1.32	2.81	0.2	a-β2GPI Ig A	
5	F/71	Hypertension	POS	Arthralgia, morning stiffness, swelling	3.73	3.2	0.24	ANA 1/320 nucleolar	(–)
6	M/57	Diabetes mellitus	POS	None	1	2.38	0.2	ANA 1/160 speckled cytoplasmic, a-β2GPI Ig A	(–)
7	F/56	None	POS	Arthralgia	0.89	2.56	0.2	ANA 1/5120 homogeneous	(–)
8	M/68	Diabetes mellitus, hypertension	POS	None	18.9	3.97	2.65	RF:57.8	(–)
9	M/59	Hyperlipidemia	POS	Myalgia	1.56	3.51	0.27	ANA 1/1280 nucleolar	(–)
10	F/44	None	POS	Myalgia, muscle weakness	1.15	2.94	0.25	ANA 1/1280 nuclear centromeric	CENP-B (4.6)
11	F/20	None	POS	Myalgia, muscle weakness	0.24	2.32	0.22	ANA 1/1280 speckled	Jo-1 (1.2)
12	F/57	Diabetes Mellitus, hypertension	POS	None	0.67	3.51	<0.2	RF:47, a-β2GPI Ig G, a-β2GPI Ig A	
13	F/44	None	POS	Arthralgia, Morning stiffness, Swelling, myalgia, Muscle weakness	3.65	3.97	0.31	ANA 1/160 speckled	(–)
14	M/74	Coronary artery disease	POS	None	6.75	4.93	0.43	ANA 1/160 homogeneous, RF:68.8	(–)
15	F/45	Hypothyroidism	POS	Arthralgia, morning stiffness, myalgia	1.62	2.80	<0.2	ANA 1/160 speckled	Anti-Jo-1 (Immunoblot)
16	F/84	Diabetes mellitus, Hypertension, Coronary artery disease hyperlipidemia	POS	Arthralgia, muscle weakness	0.47	3.39	0.9	a-β2GPI Ig A	
17	F/59	Chronic renal failure	NEG	Arthralgia, morning stiffness	0.7	2.89	0.25	ANA 1/160 homogeneous	(–)
18	M/55	None	POS	Arthralgia, morning stiffness, myalgia	2.88	2.53	<0.2	RF:162.2	(–)
19	F/60	Hypothyroidism	POS	Arthralgia, myalgia	6.07	4.04	<0.2	ANA 1/320 nucleolar	(–)
20	F/55	Hypertension, hyperlipidemia	POS	Arthralgia, morning stiffness, swelling, and other signs of arthritis	1.03	2.93	<0.2	ANA 1/5120 nuclear centromeric	CENP-B (7.6)
21	M/56	Hypertension, congestive heart failure	POS	Arthralgia	2.48	2.42	0.22	ANA 1/160 nucleolar RF:30.9	Anti-SSA, Anti-SSB (Immunoblot)
22	F/70	Hypertension, hypothyroidism	POS	Arthralgia	7.33	3.86	0.34	ANA 1/5120 homogeneous, a-β2GPI Ig A	(–)
23	F/46	Hypothyroidism	POS	None	0.74	2.51	0.2	ANA 1/5120 nuclear centromeric, RF: 14.9	CENP-B (9.7)
24	M/52	Diabetes mellitus, hypertension, hyperlipidemia	POS	Myalgia, muscle weakness	5	3.48	0.36	ANA 1/160 speckled, ACA (Ig M), a-β2GPI Ig A	(–)
25	F/48	Hypertension, coronary artery disease, hypothyroidism, hyperlipidemia	POS	Arthralgia	2.84	3.14	0.32	a-β2GPI Ig M, ACA Ig M	
26	F/72	Hypertension, hyperlipidemia	POS	Arthralgia, Morning stiffness, Myalgia, muscle weakness	0.42	2.58	<0.2	ACA IgG	
27	F/67	Hypertension, Coronary artery disease, Congestive heart failure, hyperlipidemia	POS	Arthralgia, Myalgia	1.96	2.89	0.78	ANA 1/1280 homogeneous, Anti-dsDNA (>300) a-β2GPI Ig A	Nucleosomes (3.0)
28	F/71	Diabetes mellitus	POS	Myalgia, muscle weakness	3.56	4.64	0.38	ANA 1/160 nucleolar, ACA IgM, a-β2GPI IgM, a-β2GPI Ig A	(–)
29	F/57	Hyperthyroidism	POS	None	1.01	3.6	<0.2	ANA 1/160 speckled, a-β2GPI Ig G, a-β2GPI IgA	RNP/Sm (1.2)
30	F/61	Hypertension, coronary artery disease, congestive heart failure, hypothyroidism	POS	Arthralgia	29.94	2.66	19	ANA 1/320 nucleolar, RF:112, a-β2GPI Ig M, a-β2GPI Ig A, ACA Ig G	SsB (La) (1.9)
31	F/31	None	POS	Arthralgia	2.11	3.94	0.44	ANA 1/160 dense fine speckled	(–)

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2; Ig G: Immunoglobulin G; CRP: C-reactive-protein; ENA: Extractable nuclear antigen antibodies; POS: Positive; ANA: Anti-nuclear antibody; RF: Rheumatoid factor; CCP: Cyclic citrullinated peptide; a-β2GPI: Antiβ2-glycoprotein; CENP-B: Centromere protein-B; NEG: Negative; ACA: Anti-cardiolipin antibodies; Sm: Anti-smith; SsB: Sjögrens antibody

TABLE 4: Evaluation of the relationship between the presence or absence of some autoimmune antibodies (ANA, CCP, RF, aPL), and acute phase reactants (D-Dimer, fibrinogen, CRP) and the musculoskeletal symptoms in 381 patients

		Autoimmune-antibodies				
		Negative		Positive		
Musculo-skeletal symptoms		n	%	n	%	p value
Fatigue (Weakness)	No	80	39.0	69	39.2	0.971[a]
	Yes	125	61.0	107	60.8	
Joint pain	No	110	53.7	92	52.3	0.787[a]
	Yes	95	46.3	84	47.7	
Morning stiffness in joints	No	167	81.9	151	85.8	0.301[a]
	Yes	37	18.1	25	14.2	
Swelling in joints	No	196	95.6	164	93.2	0.301[a]
	Yes	9	4.4	12	6.8	
Redness and warmth in the joints	No	196	95.6	174	98.9	0.059[a]
	Yes	9	4.4	2	1.1	
Muscle pain	No	129	62.9	110	62.5	0.932a
	Yes	76	37.1	66	37.5	
Muscle weakness	No	157	76.6	122	69.3	0.110[a]
	Yes	48	23.4	54	30.7	
Tingling in hands and feet	No	157	76.6	124	70.5	0.175[a]
	Yes	48	23.4	52	29.5	

[a]chi-square test

COVID-19 patients and examine their frequencies and the clinical outcomes. One of the critical findings of our study was that in SARS-CoV-2 infected patients, elevated autoantibody levels, persisted for months. It was determined that the elevated levels of ANA detected in the post-COVID period are not associated with the severity of the infection during the acute phase.

Several studies investigated the latent autoimmunity in the acute phase of COVID-19.[5-7,14-17] These studies showed that patients with ANAs, RF, IgM a-β2GPI antibodies, and those with high levels of CRP, ferritin, D-Dimer, and IL-6 have deleterious outcomes. However, only a few studies analyzed the serum levels of these potential prognostic biomarkers after completion of five or more weeks following recovery.[1,18-21]

In a study conducted in Germany, it was showed that ANA titer was higher than 1:160 in 43.6% of the patients at 12 months post-COVID-19 symptom onset.[18] Our study found ANA positivity (i.e., ANA titer≥1:160) to be 29.8%. Lingel and coworkers compared the RF and CCP antibody levels between healthy unexposed donors, mild COVID-19 convalescents, and those who had acute severe COVID-19 patients.[1] This analysis revealed that anti-CCP antibodies remained elevated in convalescents even after 8 months post-infection. In our patient group, only 1 of the 3 individuals with elevated CCP required further examination, but this patient was not diagnosed with an autoimmune disease at the end of this evaluation.

Some studies previously reported that SLE, romatoid artrit (RA), reactive arthritis, or other autoimmune diseases might develop in the post-COVID period.[3,5,7,22] However, Derksen et al. noted that there was no cause-effect relationship between COVID-19 and new-onset RA.[23] In contrast, Roongta et al. presented a patient diagnosed with new-onset RA after a COVID-19 infection.[24] This patient, known to be seronegative before COVID-19, became seropositive in the post-COVID-19 period. The authors suggested that COVID-19 infection triggered autoimmunity in this patient.

In our study, the pre-COVID-19 serological status of the patients is unknown. Three of the 9 patients diagnosed with an autoimmune disease did not have any musculoskeletal complaints in the post-COVID-19 period. Only in these 3 patients was the diagnosis made by further investigations performed due to the detection of seropositivity during routine investigations. Considering that the other 6 patients had no musculoskeletal complaints before COVID-19, previous serological values should be known to associate the current picture with COVID-19.

The disappearance time of the autoantibodies following COVID-19 infection is unclear.[22] As per the current literature regarding autoantibodies formed following other viral infections, these antibodies are short-lived and eventually fade. This phenomenon can also be true in the case of COVID-19. Therefore, patients are unlikely to develop strong positive autoantibodies in response to acute SARS-CoV-2 infection. In line with this hypothesis, Sapkota et al. suggested that the presence of strongly positive autoantibodies was associated with SARDs.[22]

Several hypotheses have been suggested to clarify the molecular foundation of immune dysregula-

TABLE 5: Comparison of the musculoskeletal symptoms and the some laboratory results between hospitalized and outpatient patient groups

		Disease severity in the acute phase				
		Outpatient		Inpatient		
Variables		**n**	**%**	**n**	**%**	**p value**
Weakness	No	38	30.2	111	43.5	0.012[a]
	Yes	88	69.8	144	56.5	
Joint pain	No	55	43.7	147	57.6	0.010[a]
	Yes	71	56.3	108	42.4	
Morning stiffness	No	95	76.0	223	87.5	0.005[a]
	Yes	30	24.0	32	12.5	
Swelling in joints	No	113	89.7	247	96.9	0.004[a]
	Yes	13	10.3	8	3.1	
Redness and warmth in the joints	No	117	92.9	253	99.2	0.001[b]
	Yes	9	7.1	2	0.8	
Muscle pain	No	63	50.0	176	69.0	<0.001[a]
	Yes	63	50.0	79	31.0	
Muscle weakness	No	94	74.6	185	72.5	0.670[a]
	Yes	32	25.4	70	27.5	
Tingling in hands and feet	No	91	72.2	190	74.5	0.633[a]
	Yes	35	27.8	65	25.5	
CRP	Negative	110	87.3	191	74.9	0.005[a]
	Positive	16	12.7	64	25.1	
ANA	Negative	89	70.6	174	68.2	0.634[a]
	Positive	37	29.4	81	31.8	
RF	Negative	121	96.0	223	87.5	0.008[a]
	Positive	5	4.0	32	12.5	
CCP	Negative	124	98.4	254	99.6	0.255[b]
	Positive	2	1.6	1	0.4	
Antiphospholipid antibodies	Negative	114	90.5	205	80.4	0.012[a]
	Positive	12	9.5	50	19.6	
Fibrinogen	Negative	117	92.9	215	84.3	0.019[a]
	Positive	9	7.1	40	15.7	
D-Dimer	Negative	119	94.4	208	81.6	0.001[a]
	Positive	7	5.6	47	18.4	

[a]chi-square test; [b]Fisher exact test. CRP: C-reactive-protein; ANA: Anti-nuclear antibody; RF: Rheumatoid factor; CCP: cyclic citrullinated peptide

tion associated with COVID-19. These include viral proteins inducing molecular mimicry, the widespread expression of the SARS-CoV-2 receptor angiotensin converting enzyme 2 (ACE2) leading to systemic effects and multiorgan involvement, immune cell activation through bystander mechanisms, the release of autoantigens from virus-induced tissue damage, superantigen-driven activation of lymphocytes, and the spreading of epitopes.[25,26] The entry and replication of SARS-Co-V-2 in host cell are facilitated by the interaction between ACE2 receptor, present on respiratory epithelial cells, and the receptor- binding domain of viral Spike glikoprotein. Then, antigen-presenting cells, including dendritic cells, macrophages, and B cells, can endocytose viral particles and present peptide antigens toCD4+ T cells via MHC class II. Activated CD4+ T cells, acting as key mediators, participate in the secretion of pro-inflammatory cytokines, macrophage activation, priming of CD8+ T cells, stimulation of B cells for antibody production, and targeting of SARS-Co-V-2. The antibody often interacts misleadingly with the host surface proteins in a phenomenon called "Molecular mimicry". Several studies have suggested

that molecular mimicry may play an important role in autoimmunity generation in COVID-19.[27] In addition, a variety of host factors such as age, comorbidities and genetic factors may also contribute.

Approximately 4% of the global population has at least one of the 80 autoimmune diseases.[1] It is known that the initial immunological signs of rheumatic diseases, including induction of autoantibodies, can be detected several years before the emergence of the first clinical symptom. In addition, it is widely accepted that viral infection can be a trigger of these diseases.[1] In our study, it is impossible to conclude that SARS-CoV-2 infection triggered autoantibody positivity and led to the diagnosis of SARDs since the pre-COVID-19 serological status of 9 patients was unknown. There is a possibility that these antibodies were detected incidentally due to further examination of all patients. In one of the 31 patients for whom rheumatology consultation was requested, the RF level was 162 mg/dl while the CCP level was higher than 200. However, no rheumatic disease diagnosis was made since there were no clinical symptoms and remarkable physical examination findings. Another patient who occasionally complained of diffuse joint and muscle pain and recurrent oral ulcers before being diagnosed with COVID-19 reported that she did not seek medical advice regarding this complaint. This patient had laboratory results including an ANA titer of 1/1280, anti-dsDNA higher than 300, and positivity for anti-ENA antibodies. She was diagnosed with SLE in our outpatient clinic in the 8th month post-COVID-19.

In our study cohort, it was found that 232 (60.8%) patients had fatigue, and 179 (46.9%) patients had joint pain. Fatigue is the most common and characteristic symptom (present in 60-70%) of post-COVID-19 syndrome patients defined in the literature.[28-30] Our finding is similar to previous results. In a meta-analysis examining 54 studies, the prevalence of joint pain was found to be between 2-65% within a time frame varying from 4 weeks to 12 months after acute SARS-CoV-2 infection. Considering that our patient cohort comprises individuals between 6 weeks and 12 months post-COVID-19, it can be asserted that the observed rate of 46.9% for joint pain aligns closely with the findings of this meta-analysis.[31]

While a definitive cause-and-effect relationship cannot be established, the absence of a positive correlation between musculoskeletal system symptoms and the presence of autoimmune antibodies, coupled with the higher incidence of symptoms and autoantibodies in hospitalized patients, suggests that SARS-CoV-2 may have distinct pathophysiogical effects on various systems. It suggests that rather than directly causing a rheumatological or autoimmune disease, the virus may trigger autoimmune rheumatological conditions in individuals predisposed to such conditions, either genetically or in conjunction with other environmental factors.

In a retrospective cohort study aiming to examine the risk and frequency of autoimmune and autoinflammatory connective tissue diseases after COVID-19 infection, a comparison was made by taking those who did not have COVID-19 as a control group. The risk of alopecia areata, alopecia totalis, antineutrophil cytoplasmic antibody-associated vasculitis, Crohn's disease, and sarcoidosis was found to be higher in the group who had COVID-19. In the same study, the risk of alopecia totalis, psoriasis, vitiligo, vasculitis, Crohn's disease, ulcerative colitis, rheumatoid arthritis, adult-onset Still disease, Sjögren's syndrome, ankylosing spondylitis, and sarcoidosis was found to be associated with the severity of COVID-19 in the acute phase.[19]

Since the onset of the pandemic, numerous researchers have documented isolated instances of adults suffering from different post-COVID-19 autoimmune disorders. However, much like the visible part of an iceberg, the full range of autoimmune diseases, their frequency, and the likelihood of their development in those with COVID-19 versus those without, remain unclear. The absence of data from extensive cohorts has been a major obstacle in gaining a precise understanding of these issues. Two important studies have recently been published in the literature to fill this gap. In one these studies, Chang et al. used the TriNetX network [The US Collaborative Network from 48 global healthcare organizations (HCOs) in the TriNetX Research Network], which maintains the largest global COVID-19 dataset, and identified a study population of over 5.9 million adults from 48 global health care organizations.

Propensity score matching was used to generate 2 cohorts (COVID-19 and non-COVID-19) of 887,455 individuals each to identify the incidence of autoimmune conditions during the study period (1 January 2020-31 December 2021). As SARS-CoV-2 vaccination could be a potential confounding factor, only unvaccinated individuals were included in the analyses. The incidence of autoimmune conditions at 6 months follow-up was significantly higher in the COVID-19 cohort than in the non-COVID-19 group. The unique aspect of the autoimmune diseases after exposure to SARS-CoV-2, as compared with other previously known viral pathogens (such as coxsackie type 1, COVID-19 and EPV), is the spectrum of conditions seen.[20]

A comparable study by Tesch et al, assessed a group of 640,701 individuals who had not been vaccinated and had polymerase chain reaction-confirmed COVID-19 in 2020, in relation to the risk of developing autoimmune diseases. The researchers found a 42.6% increased chance of developing an autoimmune condition 3-15 months post-infection, compared to a control group of 1,560,357 individuals who were matched by age, sex, and preexisting autoimmune conditions. The highest rate ratios of incidence were observed in cases of vasculitis, which are uncommon autoimmune disorders. The findings also highlight that for individuals with existing autoimmune conditions, COVID-19 heightened the likelihood of developing another autoimmune disorder by 23%. Due to the retrospective cohort study design, these 2 studies cannot establish a direct causal relationship between SARS-CoV-2 and the onset of autoimmune diseases. However, the temporal connection with a history of COVID-19 provides strong and credible evidence suggesting that SARS-CoV-2 infection is associated with a significantly elevated risk of developing a range of new autoimmune diseases following the acute phase of infection.[21]

The current study has some limitations that must be considered while evaluating its findings. First, the pre-COVID-19 serological data of the patients were not available. Second, the symptomatic patients might have shown more willingness than the others to participate in this study. As a confounder, this difference might have caused a selection bias. Third, the study had a single-center, unblinded, and nonrandomized design. This design impacts the generalizability of the study results.

Despite these limitations, our study also has strong aspects. This study represents the first of its kind in Türkiye and is among the limited number of studies in the existing literature that systematically document cases of post-COVID-19 syndrome in patients diagnosed with autoimmune diseases through clinical and laboratory evaluation.

CONCLUSION

Although it is not possible to establish a cause-and-effect relationship due to the lack of a control group in the design of our study and the lack of serologic laboratory test results before COVID-19 in patients diagnosed with autoimmune diseases, we think it may be important that autoantibodies and acute phase reactants other than ANA and CCP were found to be higher in hospitalized patients and that various systemic autoimmune reactivities were detected in 40% of post- COVID-19 patients. We would like to draw attention to the issue of planning prospective studies in case of possible similar infection outbreaks in the future in order to obtain more precise information.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Canan Emiroğlu, Murat Dicle, Süleyman Görpelioğlu, Cenk Aypak;* ***Design:*** *Canan Emiroğlu, Murat Dicle, Süleyman Görpelioğlu, Cenk Aypak;* ***Control/Supervision:*** *Canan*

Emiroğlu, Murat Dicle, Süleyman Görpelioğlu, Cenk Aypak; ***Data Collection and/or Processing:*** *Canan Emiroğlu, Murat Dicle;* ***Analysis and/or Interpretation:*** *Canan Emiroğlu, Murat Dicle, Süleyman Görpelioğlu, Cenk Aypak;* ***Literature Review:*** *Canan Emiroğlu, Murat Dicle, Süleyman Görpelioğlu, Cenk Aypak;* ***Writing the Article:*** *Canan Emiroğlu;* ***Critical Review:*** *Canan Emiroğlu, Murat Dicle, Süleyman Görpelioğlu, Cenk Aypak;* ***References and Fundings:*** *Canan Emiroğlu, Murat Dicle, Süleyman Görpelioğlu, Cenk Aypak;* ***Materials:*** *Canan Emiroğlu, Murat Dicle, Süleyman Görpelioğlu, Cenk Aypak.*

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