JEIMP
THE SCIENCE
Journal of
European Internal Medicine Professionals
J Eur Int Med Prof
2023, Volume 1

10.5281/zenodo.7562171
Original Article

The Frequency of ANA-positivity and Inflammatory Markers in COVID-19

Tugba Izci Duran[1], Melih Pamukcu[2], Sanem Kayhan[3], Ismet Battal[4], Mehmet Derya Demirag[5]
1.Denizli State Hospital, Clinic of Rheumatology, Denizli, Turkey
2.Etlik City Hospital, Clinic of Rheumatology, Ankara, Turkey
3.Etlik City Hospital, Department of Internal Medicine, Ankara, Turkey
4.Etlik City Hospital, Clinic of Infectious Diseases, Ankara, Turkey
5.Samsun University, Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Samsun, Turkey

Corresponding author: Tugba Izci Duran, MD. Sırakapılar, Selçuk Cd, 20010 Merkezefendi/Denizli/Turkey. E-mail: drtugbaizciduran@gmail.com
Cite this article: Duran TI, Pamukcu M, Kayhan S, Battal I, Demirag MD. Frequency of ANA-positivity and inflammatory markers in COVID-19. *J Eur Int Med Prof*. 2023,1:1-5.

Received: 12.12.2022, **Accepted**: 03.01.2023, **Published**: 26.01.2023

ABSTRACT

Background: Immune system activation plays an important role in pathogenesis and mortality in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The inflammatory response during the disease is caused by the innate and adaptive immune systems. Anti-nuclear antibody (ANA) positivity rate increases in SARS-CoV-2-positive patients due to adaptive immune system activation. This study aims to investigate the association between ANA-positivity rate and pulmonary symptoms, and inflammatory markers (C-reactive protein [CRP] and fibrinogen).

Material and Methods: One hundred five consecutive patients with the diagnosis of COVID-19 were included in this cross-sectional study. Participants were divided into groups according to the ANA and pulmonary symptom status. Clinical (gender, age) and biochemical (hemogram, liver function tests, kidney function tests, D-Dimer, CRP, and fibrinogen) were compared between the groups and the impact of ANA positivity on pulmonary symptoms development was assessed.

Results: Of the 105 patients, 60 of them had no pulmonary symptoms. The remaining 45 patients had at least one pulmonary symptom. ANA immunofluorescence assay (IFA) positivity rate was 19% (20/105 patients) in the study group. 60% of the ANA-positive patients were positive at 1/160, 30% at 1/320 and 10% at 1/1000 titer. ANA-IFA positivity rate was found higher among patients with pulmonary symptoms; however, the difference was not statistically significant (26.7% vs. 8/60 13.3%, respectively; p=.085). The CRP and fibrinogen levels were (6.9 vs. 3.4, p=.132, and 346.5 vs. 326, p=.183) among ANA positive and negative patients. Twelve (63.2%) patients with ANA-positivity had pulmonary symptoms, and 33 (39.3%) patients with ANA-negativity had pulmonary symptoms (p=0.058).

Conclusions: Although there is no difference between patients with or without pulmonary symptoms, ANA, which may reflect the pathogenetic role of adaptive immune dysregulation, can often be detected in patients with Coronavirus disease 2019.

Keywords: Antibodies, antinuclear, immunology, autoimmunity, COVID-19

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first detected in December 2019. The severity of this disease, which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), varies significantly in clinical ways. The disease has a broad spectrum of symptoms from asymptomatic to respiratory failure characterized by SARS. While it primarily affects the respiratory system, COVID-19 may also affect many systems that have cellular access receptors for the virus such as the cardiovascular, gastrointestinal, renal, and central nervous systems (1). COVID-19-related mortality is usually associated with coagulopathy, cytokine storm syndrome, and multi-organ failure (2). Many antiviral drugs have been tried against the disease, but no drug has proven efficacy yet (3). However, corticosteroid

therapy can significantly prevent immune-mediated lung injury and decrease mortality (4). Immune system activation plays an important role in pathogenesis and mortality in SARS-CoV-2 infection. The inflammatory response during the disease is caused by the innate and adaptive immune systems. Cytokines, which are an important part of the inflammatory process, are produced by various immune cells including innate immune system components such as macrophages, dendritic cells, natural killer cells, and components of the adaptive immune system such as T and B lymphocytes (5). Antinuclear antibody (ANA) positivity increases in SARSCoV-2-positive patients due to adaptive immune system activation (6). We aimed to investigate the effect of ANA positivity on pulmonary symptoms in addition to routine laboratory tests.

MATERIALS AND METHODS

Study Design and Participants

Between the 1st of August and the 30th of September 2020, the patients diagnosed with SARS-CoV-2 were evaluated in terms of eligibility for inclusion in the study. A positive result in polymerase chain reaction (PCR) analysis of a sample collected on a nasopharyngeal swab was defined as a COVID-19 case. 105 patients aged >18 years who were diagnosed with SARSCoV-2 by PCR and hospitalized were included in this cross-sectional study. Of the 105 patients, 60 had no pulmonary symptoms, and the remaining 45 patients had pulmonary symptoms (dyspnea, tachypnea, cough, pulmonary infiltrate) and 1 patient was transferred to the intensive care unit (ICU) who was ANA-negative. The symptom frequencies of the study group were presented in Table 1. The oxygen saturation of all patients without pulmonary symptoms was above 92% in room air and they were hospitalized for close follow-up. 86 patients were scanned with thorax computed tomography (CT) for the presence of pulmonary findings. While thorax CT was normal in 35 (33.3%) patients, at least one pulmonary finding was detected in 51 (48.6%) patients.

Patients diagnosed with rheumatologic, immunological disease, malignancy, end-stage heart, and renal failure, advanced liver failure, and used immunosuppressive drugs for other reasons were excluded from the study. The patients were evaluated for the presence of an ANA-related disease by anamnesis and physical examination. The previous ANA status of the participants is unknown.

Laboratory examinations

Serum urea, creatinine, complete blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), D-dimer, ferritin, CRP, fibrinogen, and lactic dehydrogenase (LDH) levels were recorded in all patients. Antibodies to nuclear antigens were detected using the HELMED ANA screen multiplex autoimmune assay. Specimens demonstrating reactivity for any nuclear antigen were additionally tested using indirect immunofluorescence on HEp-2 cells at a dilution of 1:160. The blood samples were collected on the first hospitalization day for ANA detection and the collected samples were studied later simultaneously.

Ethical approval

This study was carried out in accordance with the Declaration of Helsinki. All participants have been informed. Consent of all participants was obtained. The study was approved by the Ankara Dışkapı Training and Research Hospital ethics committee and the Ministry of Health (Date: 06.07.2020, Approval number: 91/20).

STATISTICAL ANALYSIS

Numerical data are presented as mean (standard deviation) or median (min-max) according to their suitability for normal distribution. The presentation of categorical data was presented as n (%), and the chi-square test was used for comparisons. Mann Whitney U test or Student t test was used to compare numerical data. A univariate regression analysis was performed for ANA status and pulmonary symptoms presence. If the P-value < .05, it was considered statistically significant.

RESULTS

Of 105 patients with COVID-19, the ANA-IFA positivity rate was 44 (41.9%) in the whole study group

Table 1. Demographics, clinical, and laboratory findings, and comorbidities of the patients

Demographics		**Comorbidities, n (%)**	
Age, years, mean (SD)	40.3 (14.9)	Hypertension	16 (15.2)
Sex, Male, n (%)	44 (41.9)	Chronic obstructive pulmonary disease / Asthma	9 (8.6)
Laboratory, n (%) ANA-positivity		Thyroid disease	7 (6.7)
1/160	12 (11.4)	Coronary artery disease	4 (3.8)
1/320	6 (5.7)	Diabetes mellitus	3 (2.9)
1/1000	2 (1.9)	Chronic renal failure	2 (1.9)
Symptoms, n (%)			
Asymptomatic cases	19 (18.1)	Anosmia	9 (8.6)
Fever	30 (28.6)	Diarrhea	7 (6.7)
Sore Throat	21 (20)	Cough	39 (37.1)
Headache	20 (19)	Dyspnea	23 (21.9)
Arthralgia/myalgia	28 (26.7)	Cough and/or Dyspnea	45 (42.9)

(20/105 patients). Twelve (60%) of the ANA-positive patients were positive at 1/160, six (30%) at 1/320, and two (10%) at 1/1000 titer. Demographics, clinical and laboratory findings, and comorbidities of the patients are shown in Table 1. While 28 (46.7%) of the patients without pulmonary symptoms were male, 16 (35.6%) of the patients with pulmonary symptoms were male. There was no significant difference in gender between the two groups. The median age was 39.5 (18-87) in patients without pulmonary symptoms and it was 40 (18-83) in those with pulmonary symptoms. There was no significant difference in age between the two groups. Hemoglobin (Hgb), white blood cell (WBC), neutrophil, lymphocyte, thrombocyte, urea, creatinine, AST, ALT, LDH, D-dimer, and ferritin levels were similar in both groups. The CRP and fibrinogen values were significantly higher in patients with pulmonary symptoms. The data were presented in Table 2.

ANA-IFA positivity was found to be a higher frequency among patients with pulmonary symptoms, but the difference was not significant (12/45 (26.7%) vs. 8/60 (13.3%) respectively). While 12 (63.2%) ANA-positive patients had pulmonary symptoms, only 33 (39.3%) ANA-negative patients had pulmonary symptoms (p=0.058). In the univariate analysis, ANA status was not significantly associated with the presence of pulmonary symptoms (95% CI 2.64 (0.94-7.42) p=.064).

White blood cells and leukocytes were higher in patients with ANA-positivity. There was no difference in the other laboratory results in the subgroups of positive and negative ANA (Table 3).

DISCUSSION

Pneumonia is the most common serious complication of COVID-19 infection. It is characterized by fever, cough, shortness of breath, and bilateral infiltration on lung imaging. In those patients, adult respiratory distress syndrome (ARDS) usually develops after the second week. This does not only result from an uncontrolled viral replication but also an explosive immune response in the host (7). In this study, we investigated the association between ANA (an indicator of an adaptive immune response) and pulmonary symptoms in the early period of COVID-19.

There is a high prevalence of antibodies against nuclear antigens in COVID-19 patients. A recent study demonstrated that ANA was positive in 50% of patients, and 92% of 11 intensive care unit (ICU) patients (6). The patients with severe pulmonary symptoms who were followed up in the intensive care unit had a high frequency of ANA positivity (6). Similarly, 25% (16/64) of the COVID-19 patients in the study conducted by Lerma et al. had a positive result in the ANA test and 75% (12/16) of them were followed up in the ICU (8). Additionally, Lerma et al. emphasized that patients with ANA positivity (14/16, 88%) had weak reactivity and two patients with strong ANA positivity had a history of systemic lupus erythematosus (8). In our study, the frequency of ANA-positivity was lower than in those three studies. In our study, the patients had low ANA positivity since none of them required ICU. The titer was 1/160 in most of the ANA-positive patients [12/20 (60%)], which was in line with the study of Lerma et al. Although ANA is the hallmark of many autoimmune diseases, it can also be found commonly in acute illnesses including viral infection (9,10). Acute infections have been associated with ANA positivity, which does not indicate subsequent autoimmune disease but reflects transient auto reactive B and plasma cell activation (10). ANA positivity rate was 33.3% in a prospective study involving 33 consecutive patients followed by Pascolini et al. (11). During the follow-up nine of 33 patients (27.2%) needed ICU, and four of them died. Although the ANA positivity rate was higher among the patients who died, this difference was not significant (57% vs. 26.9%, respectively; p=0.10).

Table 2. Laboratory parameters in patients with COVID-19 are stratified according to the presence or absence of pulmonary symptoms.

	Patients without pulmonary symptoms	Patients with pulmonary symptoms	P value
WBC	5145 (497-12250)	5260 (950-13960)	.526
Neutrophil	2735 (1000-9850)	3250 (170-8200)	.433
Lymphocyte	1500 (530-5010)	1440 (610-3510)	.629
Thrombocyte (10^3/μL)	214.5 (94-371)	226 (64-512)	.204
Urea (mg/dL)	24.6 (5.1-60)	26 (13-57.8)	.897
Creatinine (mg/dL)	0.75 (0.46-1.91)	0.76 (0.47-1.35)	.995
AST (U/L)	19 (11-125)	19 (12-147)	.636
ALT (U/L)	19 (5-185)	20 (4-141)	.943
LDH (U/L)	179 (118-474)	200 (117-557)	.143
D-dimer	0.26 (0.17-2.21)	0.27 (0.20-2.30)	.645
Ferritin (mg/L)	95.9 (6.67-421)	107 (8.1-200)	.333
CRP (mg/L)	2.74 (1-253.2)	5.02 (0.30-278.5)	.009
Fibrinojen (mg/dL), mean (SD)	320.9 (73.8)	379.6 (100.9)	.001

Unless otherwise stated, values are presented as median (min-max). WBC; white blood count, AST; aspartate aminotransferase, ALT; alanine aminotransferase, LDH; Lactic dehydrogenase, CRP; C-reactive protein

It has been reported that the levels of some blood markers may be associated with the severity and mortality of COVID-19 patients (12,13). Of these clinical parameters, serum CRP is an important marker that changes significantly in severe COVID-19 patients (14). It is a non-specific, acute-phase inflammatory protein whose expression is increased in response to tissue damage, inflammation, and infection (15). Increased CRP levels in COVID-19 patients may be associated with disease severity and disease progression (13). Wang et al. showed that severe cases and 7.7% of non-severe COVID-19 patients who progressed to severe disease after hospitalization had pulmonary symptoms and significantly higher CRP concentrations compared to non-severe cases (median 43.8 vs. 12.1 mg/L) (14). Another study showed that having an elevated CRP level at baseline may be a valuable early marker in predicting the probability of disease progression in COVID-19 patients (16). It emphasized that this could help healthcare professionals to identify these patients at an early stage for early treatment. We also found that CRP levels were significantly higher in the patients with pulmonary symptoms at the beginning of COVID-19 disease. Therefore, COVID-19 patients with high CRP levels at the time of diagnosis should be closely watched in terms of the risk of progression, even if they do not have symptoms yet to meet the criteria for severe disease. Fibrinogen is a glycoprotein and a positive acute phase reactant that is produced in the liver. Fibrinogen also plays a role in fibrin formation as the last step in induced coagulation. Intravascular coagulation and even disseminated intravascular coagulation (DIC) can be present in COVID-19 patients and lead them to death. D-dimer increases and fibrinogen levels decrease when DIC develops (17). In the study of Han et al., the levels of fibrinogen and degradation products were higher in COVID-19 patients compared to healthy controls as well as higher levels of severe COVID-19 patients compared to mild patients (18). Tang et al. showed that fibrinogen and antithrombin activity levels were also significantly lower in nonsurvivors, which suggested that Conventional coagulation parameters during COVID-19 were significantly associated with prognosis (17). In the present study, fibrinogen levels were significantly higher in the patient group with pulmonary symptoms and it was considered an independent risk factor for pulmonary symptoms. Therefore, pulmonary involvement and COVID-19 progression should be considered in patients with increased fibrinogen at the time of diagnosis. Moreover, low fibrinogen levels in follow-up should be a warning for DIC development.

Limitations of the study

The present study has some limitations such as the data consists of only the patients who applied in 2 months period and the patients with mild symptoms were enrolled in the study. The study cohort involves relatively a small sample size of patients and the patients' previous ANA status is unknown. In addition, autoantibodies other than ANA were not examined and their interactions are not available in this study.

CONCLUSION

While ANA-positivity was detected in 19% of our patients, the difference between patients with and without pulmonary symptoms in terms of ANA positivity was not statistically significant. The low number of patients and/or our selection of patients with the mild disease might have caused this outcome. ANA, which may reflect a pathogenetic role of adaptive immune dysregulation, can often be detected in COVID-19 patients. The significance of ANA-positivity in the acute phase of the infection is unclear and prospective studies involving large-scale patient groups are needed.

DECLARATIONS

Declaration of conflicting interests: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Table 3. Comparison of laboratory parameters of anti-nuclear antibody positive or negative patient groups

	ANA positive patients	ANA negative patients	P
WBC	6315 (2630-12250)	5030 (497-13960)	.034
Neutrophil	3965 (830-9850)	2850 (170-8200)	.042
Lymphocyte	1570 (690-5010)	1420 (530-4210)	.308
Thrombocyte (10^3/µL)	229 (64-334)	215 (84-512)	.194
Urea (mg/dL)	24.8 (14-57.8)	26.9 (5.1-60)	.290
Creatinine (mg/dL)	0.76 (0.47-1.35)	0.74 (0.46-1.91)	.977
AST (U/L)	20.8 (12.4-147.3)	19 (11.6-144)	.263
ALT (U/L)	19 (10-185)	19.8 (4-141)	.668
LDH (U/L)	202.5 (144-479)	182 (117-557)	.157
D-dimer	0.27 (0.2-2.3)	0.25 (0.17-1.94)	.201
Ferritin (mg/L)	105.5 (19.6-1087)	101 (6.67-2000)	.524
CRP (mg/L)	6.9 (0.3-253.2)	3.4 (0.1-278.5)	.132
Fibrinojen (mg/dL)	346.5 (242-631)	326 (143-679)	.183

ANA; anti-nuclear antibody; ALT; alanine aminotransferase, AST; aspartate aminotransferase, CRP; C-reactive protein, LDH; lactic dehydrogenase, WBC; white blood count.

Contributions: All authors have contributed equally to the development of the manuscript. All authors read and approved the final manuscript.
Acknowledgments: Not applicable.
Funding: The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1.Krishnan A, Hamilton JP, Alqahtani SA, A Woreta T. A narrative review of coronavirus disease 2019 (COVID-19): clinical, epidemiological characteristics, and systemic manifestations. Intern Emerg Med. 2021;16(4):815-830. doi:10.1007/s11739-020-02616-5
2.Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet.2020;395(10229):1033-1034. doi:10.1016/S0140-6736(20)30628-0
3.Hanna R, Dalvi S, Sălăgean T, Pop ID, Bordea IR, Benedicenti S. Understanding COVID-19 Pandemic: Molecular Mechanisms and Potential Therapeutic Strategies. An Evidence-Based Review. J Inflamm Res. 2021;14:13-56. Published 2021 Jan 7. doi:10.2147/JIR.S282213
4.Mahase E. Covid-19: Hydrocortisone can be used as alternative to dexamethasone, review finds. BMJ. 2020;370:m3472. Published 2020 Sep 4. doi:10.1136/bmj.m3472
5.Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. Front Immunol. 2020;11:1446. Published 2020 Jun 16. doi:10.3389/fimmu.2020.01446
6.Zhou Y, Han T, Chen J, et al. Clinical and Autoimmune Characteristics of Severe and Critical
Cases of COVID-19. Clin Transl Sci. 2020;13(6):1077-1086. doi:10.1111/cts.12805
7.Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China [published correction appears in JAMA. 2021 Mar 16;325(11):1113]. JAMA. 2020;323(11):1061-1069. doi:10.1001/jama.2020.1585
8.Lerma LA, Chaudhary A, Bryan A, Morishima C, Wener MH, Fink SL. Prevalence of autoantibody responses in acute coronavirus disease 2019 (COVID-19). J Transl Autoimmun. 2020;3:100073. doi:10.1016/j.jtauto.2020.100073Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol. 1989;44:93-151. doi:10.1016/s0065-2776(08)60641-0
9.Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol. 1989;44:93-151. doi:10.1016/ s0065-2776(08)60641-0
10.Litwin CM, Binder SR. ANA testing in the presence of acute and chronic infections. J Immunoassay Immunochem. 2016;37(5):439-452. doi:10.1080/15”321819.2016.1174136
11.Pascolini S, Vannini A, Deleonardi G, et al. COVID-19 and Immunological Dysregulation: Can Autoantibodies be Useful?. Clin Transl Sci. 2021;14(2):502-508. doi:10.1111/cts.12908
12.Tian W, Jiang W, Yao J, et al. Predictors of mortality in hospitalized COVID-19 patients: A systematic review and meta-analysis. J Med Virol. 2020;92(10):1875-1883. doi:10.1002/jmv.26050
13.Herold T, Jurinovic V, Arnreich C, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J Allergy Clin Immunol. 2020;146(1):128-136.e4. doi:10.1016/j. jaci.2020.05.008
14.Wang G, Wu C, Zhang Q, et al. C-Reactive Protein Level May Predict the Risk of COVID-19 Aggravation. Open Forum Infect Dis. 2020;7(5):ofaa153. Published 2020 Apr 29. doi:10.1093/ofid/ofaa153
15.Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation [published correction appears in N Engl J Med 1999 Apr 29;340(17):1376]. N Engl J Med. 1999;340(6):448-454. doi:10.1056/NEJM199902113400607
16.Ali N. Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. J Med Virol. 2020;92(11):2409-2411. doi:10.1002/jmv.26097
17.Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(4):844-847. doi:10.1111/ jth.14768
18.Han H, Yang L, Liu R, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med. 2020;58(7):11161120. doi:10.1515/cclm-2020-0188