Reasons For Requesting the Test in Antinuclear Antibody-Positive Patients and Final Diagnosis of Patients

Antinükleer Antikor Pozitif Hastalarda Test İsteme Nedenleri ve Hastaların Nihai Tanıları

Nimet ÖNER, Elif ÇELİKEL, Zahide EKİCİ TEKİN, Vildan GÜNGÖRER, Serkan COŞKUN, Melike Mehveş KAPLAN, Cüneyt KARAGÖL, Nilüfer TEKGÖZ, Müge SEZER, Merve Cansu POLAT, Banu ÇELİKEL ACAR

Department of Pediatric Rheumatology, University of Health Sciences, Ankara Bilkent City Hospital, Ankara, Turkey

ABSTRACT

Objective: The aim of this study was to determine the reasons for the request for antinuclear antibody (ANA) in ANA-positive patients and to determine the final diagnosis of these patients and whether they developed a rheumatologic disease.

Material and Methods: In this retrospective study, the files of 559 patients with positive ANA were reviewed. Demographic, laboratory and clinical characteristics of the patients were noted. At the end of follow-up, the final diagnosis was recorded.

Results: The study included 346 patients. 233 of the patients were female, and 113 were male. The mean age at the time of ANA positivity was 9.4±4.7 years, and the mean follow-up period was 19±5.7 months. The most common symptom was myalgia/arthralgia (21.7%). Other common reasons were urticaria, abdominal pain, thrombocytopenia, and proteinuria. Extractable nuclear antigens (ENA) panel results were negative in 170 patients (49.1%). In the ENA panel, dense fine speckled antigen 70 antibodies were most frequently positive in 135 patients (39.2%). At the end of follow-up, 234 patients had no disease. One hundred and one patients were diagnosed with non-rheumatologic diseases, and 11 patients were diagnosed with rheumatologic diseases. Eleven patients with rheumatologic diseases were girls. Rash was the most common symptom in patients with rheumatologic diseases. The positive predictive value of ANA positivity for rheumatologic disease was 3.2% and 1.7% for systemic lupus erythematosus.

Conclusion: Due to the low positive predictive value of ANA testing, patients at risk for autoimmune diseases should be identified and carefully evaluated before ANA is requested.

Key Words: Antinuclear antibody, DFS 70, Systemic lupus erythematosus

0000-0003-0403-151X : ÖNER N
0000-0003-0129-4410 : ÇELİKEL E
0000-0002-5446-667X : EKİCİ TEKİN Z
0000-0002-9838-2603 : GÜNGÖRER V
0000-0003-2568-9329 : COŞKUN S
0000-0002-8012-2774 : KAPLAN MM
0000-0002-2987-1980 : KARAGÖL C
0000-0002-2235-4489 : TEKGÖZ N
0000-0002-9254-9935 : SEZER M
0000-0003-3279-8435 : POLAT MC
0000-0002-1808-3655 : ÇELİKEL ACAR B

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by the Ethics Committee of Ankara City Hospital (04/01/2023, E2-23-3099) and followed the principles of the Helsinki Declaration.

Contribution of the Authors / Yazarların katkısı*: *ÖNER N: Constructing the hypothesis or idea of research and/or article, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in the writing of the whole or important parts of the study. ***ÇELİKEL E:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***EKİCİ TEKİN Z:*** Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study. ***GÜNGÖRER V:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in the writing of the whole or important parts of the study. ***COŞKUN S:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results. ***KAPLAN MM:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results. ***KARAGÖL C:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results. ***TEKGÖZ N:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results. ***SEZER M:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results. ***POLAT MC:*** Organizing, supervising the course of progress and taking the responsibility of the research/study, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results. ***ÇELİKEL ACAR B:*** Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the researchstudy, Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / *Atıf yazım şekli* : Öner N, Çelikel E, Ekici Tekin Z, Güngörer V, Coşkun S, et al. Reasons For Requesting the Test in Antinuclear Antibody-Positive Patients and Final Diagnosis of Patients. Turkish J Pediatr Dis 2023;17:347-353.

Correspondence Address / Yazışma Adresi:

Nimet ÖNER
Department of Pediatric Rheumatology, University of Health Sciences,
Ankara Bilkent City Hospital, Ankara, Turkey
E-posta: nimetpatat@gmail.com

Received / Geliş tarihi : 16.03.2023
Accepted / Kabul tarihi : 25.04.2023
Online published : 14.06.2023
Elektronik yayın tarihi
DOI: 10.12956/tchd.1266045

ÖZ

Amaç: Bu çalışmanın amacı antinükleer antikor (ANA)-pozitif hastalarda ANA istenmesinin nedenlerini belirlemek ve bu hastaların son tanılarını ve romatolojik bir hastalık geliştirip geliştirmediklerini saptamaktır.

Gereç ve Yöntemler: Bu çalışmada ANA pozitif 559 hastanın dosyaları geriye dönük incelendi. Hastaların demografik, laboratuvar ve klinik özellikleri kaydedildi. Takip sonunda son tanıları kaydedildi.

Bulgular: Çalışmaya 346 hasta dahil edildi. Hastaların 233'ü kadın, 113'ü erkekti. ANA pozitifliği saptandığında, ortalama yaş 9.4±4.7 yıl ve ortalama takip süresi 19±5.7 aydı. En sık görülen semptom miyalji/artraljiydi (%21.7). Diğer yaygın nedenler ürtiker, karın ağrısı, trombositopeni ve proteinüriydi. Ekstrakte edilebilir nükleer antijenler (ENA) panel sonuçları 170 hastada (%49.1) negatifti. ENA panelinde en sık 135 hastada (%39.2) yoğun ince benekli antijen 70 antikorları pozitif bulundu. Takip sonunda 234 hastada hastalık yoktu. Yüz bir hastaya romatolojik olmayan hastalık, 11 hastaya ise romatolojik hastalık tanısı konuldu. Romatolojik hastalığı olan 11 hasta kızdı. Romatolojik hastalığı olan hastalarda döküntü en sık görülen semptomdu. ANA pozitifliğinin romatolojik hastalıklar için pozitif prediktif değeri %3.2 ve sistemik lupus eritematozus için %1.7'di.

Sonuç: ANA testinin pozitif prediktif değerinin düşük olması nedeniyle, otoimmün hastalıklar açısından risk altında olan hastalar ANA istenmeden önce belirlenmeli ve dikkatle değerlendirilmelidir.

Anahtar Sözcükler: Antinükleer antikor, DFS 70, Sistemik lupus eritematozus

INTRODUCTION

Anti-nuclear antibody (ANA) is a type of antibody that is found in the serum of patients with several rheumatic diseases and is directed against structures in the cell nucleus, such as DNA, histones, and centromeres (1). Although ANA was initially discovered in systemic lupus erythematosus (SLE) patients, it has also been found to be associated with many other autoimmune diseases such as systemic sclerosis, scleroderma, Sjogren's syndrome, and juvenile idiopathic arthritis (JIA) (2). ANA is a frequently used laboratory test for autoimmune disease screening, especially in patients with musculoskeletal complaints or skin symptom (3).

Anti-nuclear antibody can be detected using the enzyme-linked immunosorbent assay (ELISA) method or the immunofluorescence technique using Human Epithelial type 2 (HEp-2) cells as a substrate. The results of the test are reported in two sections: the titer of the antibodies, and the staining pattern produced by the antibodies. The titer of the antibodies is measured in dilutions, such as 1:80, 1:100, 1:320, 1:1000, or 1:3200 and a positive result is considered as a titer of 1:80 or higher. The staining pattern can be homogeneous, granular, diffuse, nucleolar, or speckled (4). Recently, a new staining pattern called 'anti-dense fine speckled antigen70' (anti-DFS70) has been described, in which the nucleoplasm is densely speckled. ANA test is commonly requested in patients suspected of having rheumatological disease. However, ANA positivity can also be found in varying frequencies in healthy individuals (5-7). A positive ANA test is not always an indicative of a rheumatological disease and further testing and a detailed clinical evaluation of the patient is needed to establish a diagnosis.

Identifying the patients in whom ANA should be requested and its indications will increase knowledge on the rational use of laboratory tests. The aim of this study was to evaluate the reasons for requesting ANA in patients who admitted to a tertiary pediatric rheumatology clinic with ANA positivity or were found to be positive during follow-up. We also aimed to determine the final diagnosis of patients with ANA positivity and to reveal whether they developed a rheumatologic disease.

MATERIAL and METHODS

The medical records of children who were admitted to the pediatric rheumatology department with ANA positivity or who were found to be ANA positive during follow-up between January 2019 and December 2022 were retrospectively analyzed.

Inclusion-Exclusion Criteria

Patients with ANA positivity who were followed up for more than 1 year were included in the study. Patients with missing medical records, those followed up for less than 1 year, and those who had ANA positivity in another center but tested negative in our center were excluded. Also, patients who had ANA positivity detected during the course of other rheumatological diseases [juvenile idiopathic arthritis (JIA), SLE, Raynaud phenomenon] were also excluded from the study. ANA positivity with cytoplasmic and mitotic staining pattern, which is not expected in rheumatologic diseases, was excluded from the study.

Data Collection

The demographic characteristics (age, gender, age of diagnosis), family history (presence of SLE or other autoimmune disease) were recorded.

Laboratory findings including complete blood count (neutropenia, lymphopenia, anemia, thrombocytopenia), acute phase reactants [Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), liver enzymes (AST-ALT), and kidney function tests [blood urea nitrogen (BUN) and creatinine (Cr)], complement 3, complement 4, direct Coombs, and urinalysis (hematuria, proteinuria) were noted.

ANA positivity was determined using the indirect immunofluorescence method with HEp-2 cells. ANA titer was recorded as 1:100, 1:320, 1:1000, 1:3200.

According to ANA titer, positivity was defined as:

- <1:100 = Negative
- 1:100 = Weak positive
- 1:100-1:320 = (1+) positive
- 1:320- 1:1000 = (2+) positive
- 1:1000- 1:3200= (3+) positive
- >1:3200= (4+) positive

Different (between 1-3) staining patterns and titers reported in the same patient were noted separately. In terms of staining pattern on HEp-2 cells;

- ***Nuclear:*** Homogeneous, DFS, fine speckled, coarse speckled, centromere, few dots, many dots
- ***Cytoplasmic:*** Fibrillar, speckled, AMA, golgi, rods and rings
- ***Mitotic:*** Centrosome, intercellular bridge, fine filaments, mitotic chromosomes.

Other autoantibodies [Anti-dsDNA (antibodies against double-stranded DNA), extractable nuclear antigens (ENA) panel] and the final diagnosis during follow-up were recorded.

ENA panel, rRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Pm-Scl, Jo-1, CENP B, PCNA, DsDNA, nucleosome, histon, ribosomal P Protein, AMA-M2, and DFS 70 antibodies were evaluated using the immunoblotting method.

Anti-dsDNA was evaluated by both the ELISA method and an ENA panel. Anti dsDNA was <99.99 RU/ml negative and >100 positive in ELISA method.

According to the final diagnosis, patients were divided into two groups: those with and without a rheumatological disease. The patients without a rheumatological disease were further divided into two subgroups: healthy individuals and those with other diseases that caused ANA positivity. The definition of a healthy individual was the absence of any signs of disease after further investigation and follow-up.

The study was approved by the Ethics Committee of Ankara City Hospital (04/01/2023, E2-23-3099) and followed the principles of the Helsinki Declaration.

Statistical Analysis

Statistical analysis was performed using version 26 of the Statistical Package for Social Sciences (SPSS). Continuous variables were expressed as mean±standard deviation and categorical variables as n(%). The normal distribution of continuous parameters was tested using the Kolmogorov-Smirnov or Shapiro-Wilk test.

RESULTS

In this study, the medical records of 559 patients with positive ANA were reviewed. 164 patients were excluded from the study due to missing data, a follow-up period of less than 1 year, ANA positivity diagnosed in another center but found negative in our center, and cytoplasmic and mitotic staining pattern. Forty-nine patients with SLE, Raynaud phenomenon or JIA were not included in the study.

The study was conducted in 346 patients. There were 233 girls and 113 boys, with a female to male ratio of 2:1. The mean age at the time of ANA positivity was 9.4±4.7 years and the mean follow-up period was 19±5.7 months. In family history, 24 patients had a first-degree relative with an autoimmune disorder, and 47 patients had a second-degree relative with an autoimmune disorder.

Table I: Reasons and rates of ANA positivity by departments

Departments	Patient n= 346, n (%)
Pediatric Rheumatology	82 (23.7)
Arthralgia/ Myalgia	64 (18.5)
Alopecia	5 (1.4)
Lymphadenopathy	5 (1.4)
Recurrent oral aphthous ulcer	4 (1.2)
Rash	4 (1.2)
Pediatric Allergy	66 (19)
Urticaria	43 (12.4)
Prolonged coughing	16 (4.6)
Rash	7 (2)
Pediatric Nephrology	50 (14.5)
Proteinuria	26 (7.5)
Hematuria	18 (5.2)
Abdominal pain	6 (1.8)
Pediatric Hematology	48 (13.9)
Thrombocytopenia	27 (7.8)
Anemia	8 (2.3)
Leukopenia	6 (1.8)
Neutropenia	5 (1.4)
Lymphadenopathy	2 (0.6)
Pediatric Gastroenterology	41 (11.8)
Abdominal pain	24 (6.9)
Stomachache	7 (2)
Elevated liver transaminase levels	7 (2)
Autoimmune hepatitis	3 (0.9)
Pediatric Neurology	25 (7.2)
Headache	11 (3.2)
Convulsion	7 (2)
Sudden loss of vision	5 (1.4)
Sudden hearing loss	2 (0.6)
Pediatrics	19 (5.5)
Arthralgia/ Myalgia	9 (2.6)
Fatigue	7 (2)
Urticaria	3 (0.9)
Dermatology	11 (3.2)
Rash	8 (2.3)
Urticaria	3 (0.9)
Other Departments	4 (1.2)
Pediatric cardiyology (Arthralgia/ Myalgia)	2 (0.6)
Pediatric endocrinology (Autoimmune thyroiditis)	1 (0.3)
Ophthalmology (Uveitis)	1 (0.3)

Table II: Patients with rheumatologic disease among patients with ANA positivity

Patient No	Sex	Age, years	Symptom	Department	Diagnosis	WBC, (10^3/µL)	Hb, (g/dL)	PLT, (10^3/µL)	ANA-1, titer (positivity)/ staining patterns	ANA 2, titer (positivity)/ staining patterns	ANA 3, titer (positivity)/ staining patterns	Anti-dsDNA, RU/ml	ENA panel	C3 g/L	C4 g/L	Direct Coombs	Urinalysis	ESR, (mm/h)	CRP, (g/L)
1	Female	16	Recurrent oral aphthous ulcer	Pediatric Rheumatology	UCTD	7.7	12.7	322	320 (++)/ homogeneous	320 (++)/ granular	1000 (+++)/ fine speckled	Negative	Negative	1.1	0.2	Negative	Normal	10	10
2	Female	8.9	Rash	Pediatric Allergy	SLE	3.3	12.8	200	3200 (++++)/ granular	-	-	143	rRNP/Sm, sm, dsDNA	0.6	0.1	2+	Normal	21	0
3	Female	10.3	Arthralgia/ Myalgia	Pediatric Rheumatology	SLE	4.2	12.1	318	3200 (++++)/ homogeneous	3200 (++++)/ granular	-	307	Nucleosome	1.5	0.3	Negative	Normal	41	50
4	Female	16.5	Rash	Dermatology	Livedoid vasculopathy	7.6	12.5	361	100 (+)/ granular	-	-	Negative	Negative	1.1	0.1	Negative	Normal	5	0
5	Female	11.5	Fatigue	Pediatric Rheumatology	AFAS	10.6	13.5	293	1000 (+++)/ fine speckled	100 (+) DFS	-	Negative	Pm-Scl, DFS70	1.3	0.3	Negative	Normal	8	0
6	Female	16.6	Rash	Pediatric Rheumatology	CL	5.7	13.1	253	100 (+)/ granular	-	-	Negative	Ro-52, Jo-1	1.1	0.2	Negative	Normal	5	0
7	Female	16.6	Rash	Pediatric Allergy	SLE	3.4	9.3	245	3200 (++++)/ homogeneous	-	-	>800	dsDNA, nucleosome, histon	0.3	0.1	2+	Proteinuria + Hematuria	60	1.7
8	Female	8.4	Rash	Pediatric Rheumatology	SLE	2.9	12.3	457	320 (++)/ homogeneous	-	-	>800	Ro-52, Scl-70, nucleosome, histon, AMA-M2	0.9	0,1	2+	Normal	28	40
9	Female	15.6	Arthralgia/ Myalgia	Pediatric Rheumatology	SLE	3.6	13.5	277	320(++)/ DFS	-	-	Negative	DFS70	0.7	0.2	Negative	Normal	6	0
10	Female	12.7	Lymphadenopathy	Pediatric Rheumatology	SLE	7.6	12.2	326	3200 (++++)/ homogeneous	3200 (++++)/ granular	-	>800	Nucleosome	0.3	0	2+	Normal	30	12
11	Female	9.3	Rash	Pediatric Rheumatology	CL	5.2	12.5	260	1000 (+++)/ DFS	-	-	Negative	DFS70	0.9	0.2	Negative	Normal	3	0

AFAS: *Antiphospholipid antibody syndrome,* ***ANA:*** *Antinuclear antibody,* ***Anti-dsDNA:*** *antibodies against double-stranded DNA (RU/ml <99.99: negative ³100: positive),* ***CL:*** *Cutaneous lupus erythematosus,* ***CRP:*** *C-reactive protein,* ***C3:*** *Complement 3 [Reference range: (0.9-1.8 g/L)],* ***C4:*** *Complement 4 [Reference range: (0.1-0.4 g/L)],* ***DFS70:*** *Dense fine speckled antigen70,* ***ENA:*** *Extractable nuclear antigens,* ***ESR:*** *Erythrocyte sedimentation rate,* ***Hb:*** *hemoglobin,* ***PLT:*** *Platelet,* ***SLE:*** *Systemic lupus erythematosus,* ***UCTD:*** *Undifferentiated connective tissue disease,* ***WBC:*** *White blood cell.*

The departments that referred patients to our center were as follows: 66 patients (19%) from pediatric allergy, 50 (14.5%) from pediatric nephrology, 48 (13.9%) from pediatric hematology, 41 (11.8%) from pediatric gastroenterology, 25 (7.2%) from pediatric neurology, 19 (5.5%) from pediatrics, 11 (3.2%) from dermatology, and 4 (1.2%) from other departments (pediatric cardiology, pediatric endocrinology, ophthalmology). ANA positivity was detected in 82 patients (23.7%) in the pediatric rheumatology department.

The most common reason for ANA testing was myalgia/arthralgia (n=75, 21.7%). Other common reasons were urticaria, abdominal pain, thrombocytopenia, and proteinuria (14.2%, 8.7%, 7.8%, and 7.5% respectively). Table I summarizes the rates and reasons for requesting ANA in positive patients according to departments.

Two hundred and forty-two patients had ANA positivity with a single, 79 with 2 different, and 25 with 3 different staining patterns and titers. In terms of antibody titer, there were 274 patients with 1:100, 119 patients with 1:320, 59 patients with 1:1000, and 23 patients with 1:3200. Forty-three patients had weak positive ANA, 246 patients had 1+ positive ANA, 111 patients had 2+ positive ANA, 55 patients had 3+ positive ANA, and 20 patients had 4+ positive ANA. In terms of staining pattern, 170 patients had DFS, 129 had homogenous, 64 had granular, 56 had fine granular, 33 had nucleolar, 14 had centromeric, and 9 had speckled fine.

ENA panel was negative in 170 patients (49.1%). rRNP/Sm antibodies in 8 patients (2.3%), Sm antibodies in 12 patients (3.5%), SS-A antibodies in 11 patients (3.2%), Ro-52 antibodies in 7 patients (2%), SS-B antibodies in 18 patients (5.2%), Scl-70 antibodies in 23 patients (6.7%), DsDNA antibodies in 22 patients (6 4%), nucleosome antibodies in 5 patients (1.5%), histone antibodies in 12 patients (3.5%), ribosomal P protein antibodies in 4 patients (1.2%), AMA-M2 antibodies in 17 patients (4.9%), and DFS 70 antibodies in 135 patients (39.2%) were positive.

Twenty-two patients tested positive for anti-dsDNA in the ENA panel, while in the ELISA test, 16 patients tested positive for anti-dsDNA.

The final diagnoses of the patients were as follows: 234 patients had no disease. One hundred and one patients were diagnosed with non-rheumatologic diseases and 11 with rheumatologic diseases. Among the rheumatological diseases, there were 6 cases of SLE, 2 cases of cutaneous lupus erythematosus, 1 case of antiphospholipid antibody syndrome, 1 case of undifferentiated connective tissue disease and 1 case of livedoid vasculopathy. All patients were female. Among patients with rheumatologic diseases, 6 had rash, 2 had arthralgia/myalgia, 1 had lymphadenopathy, 1 had fatigue, and 1 had recurrent oral aphthous ulcer. Positive ANA findings were detected in 8 patients in pediatric rheumatology, 2 patients in pediatric allergy, and 1 patient in dermatology. The mean age at the time of ANA positivity was 12.3 ± 3.6 years. Five patients were positive for anti-dsDNA. Two patients had negative ENA panel. Four patients had low C3 and 1 patient had low C4. The demographic and detailed laboratory findings of these patients are given in Table II.

The positive predictive value of ANA positivity for rheumatologic disease was 3.2% and 1.7% for SLE. Of the 82 cases with ANA positivity in the pediatric rheumatology clinic, the rate of rheumatologic disease as the final diagnosis was 9.8%, which is the highest rate among the departments where ANA positivity was detected.

DISCUSSION

Antinuclear antibody testing is used in the diagnostic evaluation of autoimmune diseases; however, it can also be positive in many other diseases and even in healthy individuals (8). In this study, rheumatologic disease was diagnosed in 11 of 346 patients with positive ANA test. All patients diagnosed with rheumatologic diseases were female and adolescents. Among the departments that requested ANA testing, the highest ANA positivity rate was found in the rheumatology department. Regardless of the final diagnosis, musculoskeletal symptoms were the most common symptoms in ANA-positive patients, while rash was in patients with a final diagnosis of rheumatologic disease.

Both autoimmune diseases and ANA positivity are more common in females (9-11). Davis et al. (9) reported that 68.1% of ANA positive patients were female. Similarly, Racoubian et al. (10) found that the rate of female patients was 1.5–2.4 times higher than that of male patients in a prevalence study of 2860 patients with ANA positivity. Haşlak et al. (11) reported that 64.2% of 358 ANA positive patients were female. In this present study, the female rate was 67.3%.

Studies on ANA positivity in children have shown that positivity is generally more common in the adolescent age group (12-14). This may be due to the fact that SLE and other autoimmune diseases are more common in this age group of patients. In our study, the mean age of patients was 9.4±4.7 years. Moreover, the mean age of the patients diagnosed with rheumatologic diseases was 12.3±3.6 years, closer to adolescence.

ANA test can be positive in autoimmune rheumatologic diseases, autoimmune liver diseases, thyroid diseases, malignancies, drug exposure and even in healthy individuals (15,16). Therefore, ANA test is requested by clinicians from different departments. In our study, ANA positivity was most commonly requested from the pediatric rheumatology department (23.7%), followed by pediatric allergy (19%), pediatric nephrology (14.5%), pediatric hematology (13.9%), pediatric gastroenterology (11.9%), and other departments (17%). The most common indication for ANA testing was musculoskeletal system symptoms (21.7%), urticaria (14.2%), abdominal pain (8.6%), thrombocytopenia

(7.8%), proteinuria (6.9%). Abeles et al. (17) and Bilginer et al. (18) also reported that the most common reason for requesting ANA was musculoskeletal symptoms.

The titer and staining pattern should be taken into consideration in the evaluation of ANA positivity. For instance, a patient's ANA titer of 1:80 or higher is a mandatory criterion for the diagnosis of SLE. In healthy individuals, positivity at a titer of 1:40 is detected in 31.7% of the population, whereas at a titer of 1:320 this rate decreases to 3.3% (19). Wener et al. (13) reported that approximately 10% of healthy individuals were ANA positive when samples were tested at a dilution of 1:80, increasing to 20% when samples were tested at a dilution of 1:40. The higher the titer of ANA, the less likely it is to occur in healthy individuals. Kang et al. (12) tested 94,153 patients for ANA between 2010 and 2019, of which 14.4% were positive. 4.7% of ANA-positive patients were diagnosed with autoimmune rheumatological disease. This rate increases to 15.6% when ANA positivity is evaluated at a titer of 1:320.

Abeles et al. (17) found the positive predictive value of ANA test results to be 2.1% for lupus and 9.1% for any ANA-related rheumatologic disease in 232 patients.

Staining patterns also show clinical significance like titer. The most common staining pattern observed in healthy individuals is DFS pattern (5). The most commonly associated pattern with autoimmune diseases is homogenous, nucleolar pattern (8). All staining patterns and the conditions/diseases associated with ANA positivity can be accessed from the website https://anapatterns.org (20). This website offers multiple language options.

Karakeçe et al. (14) reported that nuclear pattern was observed in 425 of 755 ANA positive patients and the distribution of fine granular, coarse granular, homogeneous and nuclear membrane patterns were 69.4%, 14.1%, 15.1% and 1.4%, respectively. There is an association between some autoimmune diseases and antibodies in the ENA panel, such as anti-SS-A/Ro and anti-SS-B/La with SLE and Sjogren's syndrome; anti-Scl-70 and anti-CENP-B with scleroderma; anti-Jo-1 with polymyositis/dermatomyositis; anti-RNP with mixed connective tissue disease (21,22). In our study, 49.1% of all patients had a negative ENA panel, and the most common ENA antibody was DFS 70 antibody at 39.2%. The most common antibody found in the ENA panel of 6 patients with SLE was nucleosome at 66.7%.

This study has some limitations. The main limitation is its single center and retrospective design. Secondly, the follow-up period may need to be longer for the diagnosis of rheumatologic disease. However, emphasizing that the ANA test should be interpreted by considering the titer and staining pattern and revealing the ANA positivity rate in rheumatologic diseases are the strengths of our study.

In conclusion, this study demonstrated that the positive predictive value of ANA testing is low. The presence of rheumatologic disease should be carefully evaluated in adolescent girls with ANA positivity. The most common symptom in ANA positive patients with a final diagnosis of rheumatologic disease was rash. Multicenter studies including larger numbers of patients are needed to reflect population-based data.

REFERENCES

1. Irure-Ventura J, López-Hoyos M. The Past, Present, and Future in Antinuclear Antibodies (ANA). Diagnostics 2022;12:647.
2. Bossuyt X, De Langhe E, Borghi MO, Meroni PL. Understanding and interpreting antinuclear antibody tests in systemic rheumatic diseases. Nat Rev Rheumatol 2020;16:715-26.
3. Conrad K, Shoenfeld Y, Fritzler MJ. Precision health: A pragmatic approach to understanding and addressing key factors in autoimmune diseases. Autoimmun Rev 2020;19:102508.
4. Xiao Y, Lin Y, Zhang Y, Wang J, Zeng Y. Factors associated with the antinuclear antibody (ANA) titer of systemic autoimmune rheumatic diseases in ANA-positive patients after treatment: a retrospective study. Clin Exp Med 2022;22:439-46.
5. Mahler M, Andrade LE, Casiano CA, Malyavantham K, Fritzler MJ. Anti-DFS70 antibodies: an update on our current understanding and their clinical usefulness. Expert Rev Clin Immunol 2019;15:241-50.
6. Carbone T, Pafundi V, Tramontano G, Gilio M, Padula MC, Padula AA, et al. Prevalence and serological profile of anti-DFS70 positive subjects from a routine ANA cohort. Sci Rep 2019;9:2177.
7. Hayashi N, Uto K, Imanishi A, Sugiyama D, Morinobu A, Saegusa J. Prevalence of anti-dense fine speckled 70 antibodies in healthy individuals and patients with antinuclear antibody-associated autoimmune rheumatic diseases in Japan. Medicine (Baltimore) 2021;100:e24556.
8. Sur LM, Floca E, Sur DG, Colceriu MC, Samasca G, Sur G. Antinuclear Antibodies: Marker of Diagnosis and Evolution in Autoimmune Diseases. Lab med 2018;49:e62–e73.
9. Davis LA, Goldstein B, Tran V, Keniston A, Yazdany J, Hirsh J, et al. Applying Choosing Wisely: Antinuclear Antibody (ANA) and Sub-Serology Testing in a Safety Net Hospital System. Open Rheumatol J 2015;9:82-7.
10. Racoubian E, Zubaid RM, Shareef MA, Almawi WY. Prevalence of antinuclear antibodies in healthy Lebanese subjects, 2008-2015: a cross-sectional study involving 10,814 subjects. Rheumatol Int 2016;36:1231-6.
11. Haslak F, Yildiz M, Altun I, Yilmaz G, Adrovic A, Sahin S, et al. Anti-nuclear antibody testing in children: How much is really necessary?. Pediatr Int 2021;63:1020-5.
12. Kang SH, Seo YI, Lee MH, Kim HA. Diagnostic Value of Anti-Nuclear Antibodies: Results From Korean University-Affiliated Hospitals. J Korean Med Sci 2022;37:e159.
13. Wener MH, Fink SL, Morishima C, Chaudhary A, Hutchinson K. Anti-Nuclear Antibody Quantitation: Calibration and Harmonization Adjustment via Population Interrogation. J Appl Lab Med 2022;7:46-56.
14. Karakeçe E, Atasoy AR, Çakmak G, Tekeoglu I, Harman H, Çiftci IH. Bir üniversite hastanesinde antinükleer antikor pozitiflikleri. Turk J Immunol 2014;2:5-8.
15. van Beers JJBC, Koek GH, Damoiseaux JGMC. The Role of Autoantibodies in the Diagnosis of Autoimmune Liver Disease:

Lessons Learned from Clinical Practice. J Appl Lab Med 2022;7:259-67.

16. Grygiel-Górniak B, Rogacka N, Puszczewicz M. Antinuclear antibodies in healthy people and non-rheumatic diseases - diagnostic and clinical implications. Reumatologia 2018;56:243-8.
17. Abeles AM, Abeles M. The clinical utility of a positive antinuclear antibody test result. Am J Med 2013;126:342-8.
18. Batu ED, Sönmez HE, Bilginer Y. Characteristics of Patients Referred to the Pediatric Rheumatology
19. Polyclinic with Anti-Nuclear Antibody (ANA) Positivity. Turkish J Pediatr Dis 2018;13:13-8.
20. Pashnina IA, Krivolapova IM, Fedotkina TV, Ryabkova VA, Chereahneva MV, Churilov LP, et al. Antinuclear Autoantibodies in Health: Autoimmunity Is Not a Synonym of Autoimmune Disease. Antibodies (Basel) 2021;10:9.
21. https://anapatterns.org (Date of access: 10.02.2023
22. Didier K, Bolko L, Giusti D, et al. Autoantibodies Associated With Connective Tissue Diseases: What Meaning for Clinicians?. Front Immunol 2018;9:541.
23. Yeo AL, Leech M, Ojaimi S, Morand E. Utility of repeat extractable nuclear antigen antibody testing: a retrospective audit. Rheumatology (Oxford) 2023;62:1248-53.