EJMA

DOI: 10.14744/ejma.2022.96268
EJMA 2022;2(2):55–60

Research Article

Real World Evidence on the Availability of Anti-Nuclear Antibody (ANA) in Diagnosis of Connective Tissue Diseases; A Retropective Single Center Trial

Polat Olgun,[1] Veli Yazısız[2]

[1]Department of Medical Oncology, Near East University, Faculty of Medicine, Nicosia, Cyprus
[2]Department of Rheumatology, Akdeniz University Faculty of Medicine, Antalya, Turkey

Abstract

Objectives: Antinuclear antibodies (ANA) are autoantibodies against the cellular compartments such as the nucleus, nucleolus, nuclear membrane and cytoplasm. Determining the significance of ANA in the diagnosis of connective tissue diseases was aimed.

Methods: The test results and diagnosis of the patients who applied to Şişli Etfal Research Hospital Rheumatology outpatient clinic between June 2011 and October 2012. The sensitivity, specificity, positive and negative predictive values of IIF-ANA in the diagnosis of connective tissue diseases were also calculated.

Results: It was determined that for every 1 out of 5 patients who applied to Rheumatology Outpatient Clinic, ANA test was ordered and for one third of those patients for whom the order was made, ANA test results were positive. For titres of 1/320 and higher, the specificity and positive predictive value of ANA in the diagnosis was found to be 99% and 96% respectively. For 1/100 and higher as the titer, sensitivity was 65%, specificity was 88% and positive and negative predictive values were 64% and 88% respectively. These values showed that 1/100 titer was suitable to use in screening.

Conclusion: Positive ANA results , clinical findings and symptoms of the patient are important in diagnosis of connective tissue diseases.

Keywords: Antinuclear antibodies (ANA), connective tissue diseases, ANA titers

Cite This Article: *Olgun P, Yazısız V. Real World Evidence on the Availability of Anti-Nuclear Antibody (ANA) in Diagnosis of Connective Tissue Diseases; A Retropective Single Center Trial. EJMA 2022;2(2):55–60.*

Autoimmune diseases are a group of diseases in which immune system cells, which develop as a result of impaired immune tolerance, form an immune response against their own antigens. Although they have many common features pathophysiologically, based on clinical findings and organ involvement, they are defined in the medical literature with different names such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and systemic sclerosis (Ssc) (Table 1).[1]

Autoimmune diseases may occur in isolation or sometimes in overlapping form. Systemic or organ-specific clinical signs and symptoms of multiple autoimmune diseases can be seen in the same patient.[2]

Anti-nuclear antibodies (ANA) are a heterogeneous group of autoantibodies developed against nuclear antigens in autoimmune diseases.[3] It is a standard test in individuals with suspected rheumatic disease. It is one of the most often requested laboratory tests for screening purposes

Address for correspondence: Polat Olgun, MD. Yakin Dogu Universitesi Tip Fakultesi Tibbi Onkoloji Anabilim Dali, Lefkosa, Kibris
Phone: +90 392 675 10 00 **E-mail:** olgunpolat100@gmail.com

Submitted Date: February 02, 2022 **Accepted Date:** May 02, 2022 **Available Online Date:** April 28, 2022

Table 1. Classification of the Autoimmune Diseases

Organ-specific Autoimmune Diseases	Systemic Autoimmune Diseases
Hashimoto's Thyroiditis	Rheumatoid Arthritis
Autoimmune Hemolytic Anemia	Systemic Lupus Erythematosus
Pernicious Anemia	Sjogren's Syndrome
Addisons Disease	Scleroderma
Autoimmune Encephalitis	Polymyositis
Goodpasture Syndrome	Mixed Connective Tissue Disease
Autoimmune Thrombocytopenia	Undifferentiated Connective Tissue Disease
Insulin-Induced Diabetes Mellitus	Myasthenia Gravis
Primary Biliary Cholangitis	
Active Chronic Hepatitis	
Ulcerative Colitis	

by clinicians involved in the diagnosis and treatment of the ilness. However, there is insufficient information about its effective use in diagnosing autoimmune diseases or excluding these diseases. Even in some healthy individuals, it can be detected positive after the course of infectious and malignant diseases and the use of some drugs (Table 2).

The etiology of autoimmune diseases is not clearly known. It is considered that genetic, environmental, hormonal, and immunological variables all play a role.[4-7]

The presence of autoantibodies has been detected in several autoimmune disorders months or years before the disease's symptoms arise. Autoantibodies can provide information regarding a disease's specific clinical findings, severity, and course.[8]

Depending on the method utilized, the false positive rate in ANA testing enhances the procedure's sensitivity while decreasing its specificity. The immunofluorescent nuclear staining pattern and titer reporting mechanism supplied at the highest dilution is employed to lower this false positivity rate. Although the 1:40 threshold has a very sensitive value, its specificity is weak. In a study, ANA positivity was found at a rate of 13.8% in the healthy population, although there are exceptions. In general, the higher the ANA titer, the higher the probability of ANA-related disease.[9, 10]

The purpose of this study was to evaluate retrospectively the ANA test results requested from the same polyclinic during a specific time period and studied using the same method, the contribution of the obtained results to the diagnosis of autoimmune diseases, the total cost of examination, and cost analysis.

Tablo 2. Diseases that cause ANA positivity

Autoimmune diseases	Drugs	Infections	Malignancy	Other
SLE	Hydralazine	Chronic bacterial infections	Lymphomas	Silicone implant
Systemic Sclerosis (Scleroderma)	Procainamide	HIV	Malignant melanoma	Fibromyalgia
Sjogren's Syndrome	Anti-TNF drugs	HCV	Adenocarcinomas	Rheumatoid vasculitis
Dermato/polymyositis	Anti- Thyroid drugs	EBV		Primary pulmonary HT
RA	Isoniazide	CMV		Idiopathic pulmonary fibrosis
MCTD	Betablockers	Parvo B19		Vasculitides
JIA	Minocyclines	Tbc		Relatives of autoimmune patients
Raynaud's phenomenon	Chlorpromazine	Malaria		
Multiple sclerosis	Quinidine	Leprosy		Healthy individuals
ITP	Methyldopa			
Discoid lupus	Penicillamine			
Autoimmune Liver diseases				
Autoimmune Thyroid diseases				

EBV: Epstein barr virus; CMV: Cytomegalovirus; Tbc: Tuberculosis; HCV: Hepatitis C Virus; HIV: Human immunodeficiency virus; SLE: Systemic Lupus Erythematosus; RA: Rheumatoid Arthritis, MCTD: Mixed connective tissue disease, ITP; Immune thrombocytopenic purpura; JIA; Juvenile idiopathic arthritis.

Methods

All patients who applied to the Rheumatology outpatient clinic of Şişli Etfal Training and Research Hospital between June 1, 2011 and October 31, 2012 with any complaint and requested ANA test were included in the study. ANA tests, which were studied in the same laboratory and with the IIF method, were evaluated. All of the data used in the study were gathered retrospectively from file records. The most recent diagnostic criteria of SLICC[11] for SLE, ACR scleroderma committee[12] for SSc, 2010 ACR-EULAR[13] for RA, American-European Consensus Group recommendations for primary SjS,[14] and Bohan-Peter for Dermato-polymyositis[15] was used in the study.

Results

In the 18-month period between June 1, 2011 and November 31, 2012, 6535 individuals were admitted to the rheumatology outpatient clinic, with 1312 IIF-ANA requests made by 1289 (19.7 percent) of these patients. ANA was requested 2 times from 22 patients and 3 times from 1 patient. ANA was positive in 401 (30.5%) of these samples. A diagnosis of any connective tissue disease was made in 320 (25%) patients (ANA negative in 93 patients, ANA positive in 227 patients).

Among the 902 individuals who tested negative for ANA, 93 were diagnosed with a connective tissue illness. The most commonly diagnosed disease was RA (n=45), and the second most common was undifferentiated connective tissue disease (n=31). Other diagnosed disorders were primary SjS (n=4), primary Raynaud's Phenomenon (n=6), and primary APA (n=2) syndrome.

The diagnoses of 401 patients who tested positive for ANA were examined. 225 patients (56.1%) were diagnosed with any connective tissue disease. In some patients, more than one autoimmune disease was detected at the same time. SLE, RA, SSc, uBDH/MKDH, and pimer SjS were the most common diagnoses (Table 3).

The most common ANA patterns were homogeneous (33.9%), granular (30.6) and mixed (14.7) patterns. The ANA patterns detected in connective tissue diseases varied. Homogeneous pattern in SLE, granular pattern in primary SjS, and centromeric pattern in SSc was more common than other patterns.

In most of the cases, ANA was positive at 1/100 dilution. The ANA was below 1/320 titer in approximately 3/4 of the patients. As the dilution rate increased, the number of ANA positive cases decreased at the same time. If the 1/320 dilution is used as the cut-off value, the vast majority of cases with ANA positive at the 1/320 titer or higher were diagnosed with connective tissue disease. It performed admirably, with a positive predictive value of 96 percent and a specificity of 99 percent at titers of 1/320 and above (Table 4).

Table 3. Diagnoses of ANA Positive Patients

Disease	Number of patients	%
Autoimmune Diseases		
SLE	50	12.46
RA	38	9.47
SSc	28	6.98
uCTD/MCTD	55	13.71
Primer SjS	42	10.47
PBC	3	0.74
Dermato/polymyositis	2	0.49
Primer APA	1	0.24
JIA	1	0.24
Discoid Lupus	1	0.24
Erythromelalgia	1	0.24
Morphea	1	0.24
Livedoid Vasculitis	1	0.24
Autoimmune Hepatitis	1	0.24
Seronegative Spondyloarthropathy	3	0.74
PMR	1	0.24
HCV	2	0.49
Paraneoplastic Vasculitis	1	0.24
Unknown	6	1.49
Non-Autoimmune Rheumatological Pathologies	163	40.6

* 2 PBS with other autoimmune diseases (1 Primary SjS, 1 SSc), 2 APA syndrome with SLE.

Comment

This study was conducted to investigate the clinical use of ANA in the diagnosis of connective tissue diseases by the IIF method. All patients who were admitted to the rheumatology outpatient clinic during the 18-month period and underwent ANA were included in the study. The study was conducted in a recently constructed tertiary health institution, where the majority of the patients were undiagnosed. The same rheumatology specialist evaluated all of the patients in the trial, and the test findings from the same centre were analysed. The majority of the studies in the lit-

Tablo 4. Performance of the ANA test in screening for connective tissue diseases

	Sensitivity	Specificity	PPV	NPV
ANA (+)	0.71	0.83	0.57	0.90
≥ 1/100	0.65	0.88	0.64	0.88
≥ 1/320	0.32	0.99	0.96	0.82

PPV: positive predictive value; NPV: negative predictive value

erature analysing the contribution of the ANA test to the clinic were conducted by reviewing the diagnoses of ANA-positive cases and retrospectively examining the samples admitted to the laboratory. Our study included all patients who were hospitalized to the rheumatology outpatient clinic and required ANA. In the study, polyclinic records were taken into account rather than laboratory records. Therefore, the results reflect the daily routine practice of a rheumatology outpatient clinic.

Without a doubt, ANA is one of the most requested screening tests in patients with suspected connective tissue disease. There is no published data on the frequency of ANA requests among patients admitted to a rheumatology outpatient clinic. According to the findings of our study, ANA was requested by one out of every five patients (19.7 percent) who applied to the rheumatology outpatient clinic. The rates may be low since the study was conducted in a newly established unit, there was no pre-elimination unit in patient admittance, and patients with non-inflammatory locomotor system symptoms may be referred directly. This incidence is expected to be greater in rheumatology reference centers where connective tissue illness is considered and/or more difficult patients are referred.

According to laboratory data, in a study in which ANA was analyzed in 3435 blood samples,[16] ANA was positive in 18.9% of the samples and only 8% of the samples sent by rheumatology specialists. In this study, 1/40 titer was accepted as the cut-off value for ANA positivity. In two other studies that looked at laboratory records, ANA positive rates were found to be 28 percent and 23.5 percent, respectively.[17, 18] Although we accept higher titers as positive in this study, our ANA positivity rate is higher than these studies. Our findings suggest that the required sensitivity is demonstrated while making the ANA request, and that superfluous requests are avoided.

The results of the examination of 2195 ANA results performed in 2114 patients in a report presented at a congress, in a third-level reference university hospital rheumatology unit in our country, revealed that 40% of the patients were diagnosed with a connective tissue disease.[19] Factors such as the study's location in one of the first established and reference rheumatology centers, the inclusion of patients sent specifically for the consideration of connective tissue disease, and the request for ANA from previously diagnosed patients may have all played a role in the emergence of this difference. When all ANA requests are included, the results of our study show that the ANA result helps to the diagnosis of connective tissue disease in roughly 20% of individuals.

According to our findings, around 40% of ANA positive patients do not have any connective tissue illness. In our study, the rate of false ANA positivity at titers of 1/320 and above was 2%. It appears that the higher the ANA titer, the higher the probability of a diagnosis of connective tissue disease. According to a study conducted by Watanabe et al.,[20] the rate of ANA positivity at 1/320 titer in healthy individuals was 1%, while the rate at titers higher than 1/320 titers was 0.3%.

According to our findings, the most common staining patterns were homogenous, granular, and mixed. Previous studies have also shown that these three patterns are the most common patterns.[17,21] Our findings support previous literature information. When the association between the patients' diagnoses and the patterns was studied, it was discovered that patients with SLE were more homogenous, while patients with primary SjS exhibited ANA positive in the granular pattern. While the mixed pattern was almost equal in all diseases, the centromeric pattern was more common in patients with SSc. Individuals who were ANA positive but had not been diagnosed with an autoimmune illness had a more granular and uniform staining pattern. The findings of the investigation in healthy people demonstrate that the ANA patterns in these patients are usually granular or homogenous at low titers.[20] Similar to the study of Karadağ et al.,[19] ANA patterns in patients without inflammatory rheumatological pathology are mostly granular and homogeneous. The findings suggest that some ANA subtypes may have evolved against nuclear antigenic determinants, inducing staining in a granular and homogenous pattern but without pathogenic characteristics. In the future, with improved technical approaches, it may be feasible to detect these specific antibodies. Furthermore, ANA titers in these patients are lower than in those with connective tissue disease. Low titer and not yet pathological (benign autoimmunity?) It is also feasible that these autoantibodies will accumulate and generate pathological characteristics (pathological autoimmunity) and clinical disease progression over time. Despite the fact that ANA is positive at high titers in some patients with SLE, SSc, DM, and primary SjS, the subtype of ANA cannot be determined despite all tests. Furthermore, an analysis of SLE patients found that ANA and other specific autoantibodies developed positive years before the diagnosis.[3] Any autoimmune illness may arise in the future in these cases, which do not meet the criteria for any connective tissue disease and have no other condition that can induce ANA positive. It may be suggested that these patients be informed and have regular check-ups.

The IIF approach had a sensitivity of 71 percent for ANA positivity for the diagnosis of a connective tissue illness, a specificity of 83 percent, and a positive predictive value of 57 percent. Sensitivity and negative predictive value declined

as the positivity limit cut-off value grew, whereas specificity and positive predictive value increased. At titers of 1/320 and above, the sensitivity of ANA positivity increases to 99%, and the positive predictive value increases to 96%. Similar to our study, a prior study,[22] which investigated IIF-ANA positivity, discovered that the sensitivity declined, the specificity and positive predictive value increased, and the negative predictive value was unaffected by the rise in titer. The screening sensitivity at 1/40 dilution was 63 percent, while the positive predictive value was found to be relatively poor, such as 33 percent. Researchers stressed that screening should begin at 1/160 dilution, which has a sensitivity of 42% and a specificity of 75% for IIF-ANA screening. Another study using the ELISA method found that the sensitivity and specificity of ANA as a screening test were 76.4 percent and 90.7 percent, respectively.[23] When the results of these two studies are compared, it is clear that the 1/100 dilution used in our study is adequate for screening ANA-related diseases, with a sensitivity of 65 percent, specificity of 88 percent, a positive predictive value of 64 percent, and a negative predictive value of 88 percent.

The advantages of this study are that it includes all patients who come to a single rheumatology subspecialty clinic for regular follow-up, that the patients are examined by the same physician, and that the disease is diagnosed using up-to-date diagnostic criteria. The study's limitations are that it was conducted retrospectively, and the number of patients was small in comparison to other research. Other drawbacks include the fact that only the IIF method can be examined and that no other methods, such as ELISA, can be verified. Examining the presence of ANA using a second approach in people who have positive IIF may improve diagnostic sensitivity and specificity.

As a result, it was concluded that 1/100 dilution using the IIF method utilizing Hep-2 cells had appropriate sensitivity and specificity to screen for connective tissue diseases in this retrospective investigation, which assessed the use of the ANA test in daily regular practice. Aside from ANA positivity, the highest positive titer and fluoroscopic patterns appear to be essential.

Disclosures

Ethics Committee Approval: The study was approved by The University of Health Sciences, Şişli etfal and Research Hospital Ethics Committee.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – P.O., V.Y.; Design – P.O., V.Y.; Supervision – P.O., V.Y.; Materials – P.O., V.Y.; Data collection and/or processing – P.O.; Analysis and/or interpretation – V.Y.; Literature search – P.O., V.Y.; Writing – P.O.; Critical review – P.O., V.Y.

References

1. Selmi C. Autoimmunity in 2011. Clin Rev Allergy Immunol 2012;43:194–206. [CrossRef]
2. Mosca M, Tani C, Carli L, Bombardieri S. Undifferentiated CTD: a wide spectrum of autoimmune diseases. Best Pract Res Clin Rheumatol 2012;26:73–7. [CrossRef]
3. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003;349:1526–33. [CrossRef]
4. Bulut V. Otoimmün hastalıkların patojenezi. Turkiye Klinikleri J Immunol Rheumatol-Special Topics 2011;4:1–10.
5. Canpınar H. Otoimmünitede çevresel ve diğer faktörlerin rolü. Turkiye Klinikleri J Immunol Rheumatol-Special Topics 2011;4:59–64.
6. Budak F. Otoimmünitede enfeksiyon etkenlerinin rolü. Turkiye Klinikleri J Immunol Rheumatol-Special Topics 2011;4:43–8.
7. Kayhan B. Otoimmünitede genetik duyarlılık. Turkiye Klinikleri J Immunol Rheumatol-Special Topics 2011;4:35–42
8. Esen BA, İnanç M. Antinükleer ve antifosfolipid antikorların klinik kullanımı. Türkiye Klinikleri J Int Med Sci 2006;2:26–33
9. Tan EM, Feltkamp TE, Smolen JS. Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum 1997;40:1601–11.
10. Satoh M, Chan EK, Ho LA. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 2012;64:2319–27. [CrossRef]
11. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012;64:2677–86.
12. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
13. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569–81. [CrossRef]
14. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al; European Study Group on Classification Criteria for Sjögren's Syndrome. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554–8. [CrossRef]
15. Bohan A, Peter JB. Polymyositis and dermatomyositis. N Engl J Med 1975;292:344–47. [CrossRef]
16. Minz RW, Kumar Y, Anand S, Singh S, Bamberi P, Verma S, et al. Antinuclear antibody positive autoimmune disorders in North India: an appraisal. Rheumatol Int 2012;32:2883–8. [CrossRef]

17. Roberts-Thomson PJ, Nikoloutsopoulos T, Cox S, Walker JG, Gordon TP. Antinuclear antibody testing in a regional immunopathology laboratory. Immunol Cell Biol 2003;81:409–12.
18. Peene I, Meheus L, Veys EM, De Keyser F. Detection and identification of antinuclear antibodies (ANA) in a large and consecutive cohort of serum samples referred for ANA testing. Ann Rheum Dis 2001;60:1131–6. [CrossRef]
19. Karadağ Ö, Kılıç L, Aydın N. Teknolojik gelişmeler sonrası bağ dokusu hastalıklarında ANA'nın yeri değişti mi? RAED Journal 2012;4:79–80
20. Watanabe A, Kodera M, Sugiura K, Usuda T, Tan EM, Takasaki Y, et al. Anti-DFS70 antibodies in 597 healthy hospital workers. Arthritis Rheum 2004;50:892–900. [CrossRef]
21. Wang KY, Yang YH, Chuang YH, Chan PJ, Yu HH, Lee JH, et al. The initial manifestations and final diagnosis of patients with high and low titers of antinuclear antibodies after 6 months of follow-up. J Microbiol Immunol Infect 2011;44:222–8.
22. Malleson PN, Sailer M, Mackinnon MJ. Usefulness of antinuclear antibody testing to screen for rheumatic diseases. Arch Dis Child 1997;77:299-304. [CrossRef]
23. López-Hoyos M, Rodríguez-Valverde V, Martinez-Taboada V. Performance of antinuclear antibody connective tissue disease screen. Ann N Y Acad Sci 2007;1109:322–29. [CrossRef]