Prospective Evaluation of Antinuclear Antibody Positivity in Children: Clinical Course and Diagnostic Outcomes

Çocuklarda Antinükleer Antikor Pozitifliğinin Prospektif Değerlendirmesi: Klinik Gidiş ve Tanısal Bulgular

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ABSTRACT

Background: Antinuclear antibody (ANA) testing is widely used in children with non-specific symptoms, yet its clinical relevance remains uncertain due to high positivity rates in healthy individuals. This study aimed to prospectively assess the clinical course and diagnostic outcomes of children referred for ANA positivity, to better inform follow-up strategies.

Materials and Methods: Forty-eight ANA-positive pediatric patients without a prior rheumatologic diagnosis and referred to the pediatric rheumatology clinic were prospectively followed for at least two years using a standardized protocol.

Results: Of the 48 patients included in the study, 35 (72.9%) were female. The most common referring department was general pediatrics (n=23, 47.9%), followed by pediatric hematology (n=13, 27.1%). The most frequent reason for ANA testing was joint pain (n=14, 29.2%), followed by thrombocytopenia (n=6, 12.5%) and urticaria (n=6, 12.5%). During follow-up, two patients were diagnosed with systemic lupus erythematosus and one with juvenile idiopathic arthritis. ANA titers became negative over time in 39.6% of the patients. Among those who did not receive a diagnosis, the median follow-up duration was 34 months (interquartile range: 26.5-50).

Conclusion: ANA positivity in children is often transient and clinically insignificant, and management should prioritize clinical context and symptom-guided monitoring rather than routine extensive evaluation.

Keywords: Antinuclear antibody, connective tissue diseases, lupus, pediatric rheumatology

ÖZ

Amaç: Antinükleer antikor (ANA) testi, özgül olmayan semptomları olan çocuklarda yaygın olarak kullanılmaktadır. Ancak sağlıklı bireylerde de yüksek pozitiflik oranları görülmesi nedeniyle klinik önemi belirsizliğini korumaktadır. Bu çalışmanın amacı, ANA pozitifliği nedeniyle çocuk romatoloji polikliniğine yönlendirilen hastaların klinik gidişatını ve tanı sonuçlarını prospektif olarak değerlendirmek ve takip stratejilerine daha iyi yön verebilmektir.

Gereç ve Yöntemler: Daha önce romatolojik hastalık tanısı olmayan ve ANA pozitifliği nedeniyle çocuk romatoloji polikliniğine yönlendirilmiş 48 pediatrik hasta, standart bir protokol ile en az iki yıl boyunca prospektif olarak takip edilmiştir.

Bulgular: Çalışmaya dahil edilen 48 hastanın 35'i (%72,9) kızdı. Hastaların en sık yönlendirildiği bölüm çocuk sağlığı ve hastalıkları idi (n=23, %47,9), bunu çocuk hematolojisi izledi (n=13, %27,1). ANA testi istenme nedenleri arasında en sık eklem ağrısı (n=14, %29,2), ardından trombositopeni (n=6, %12,5) ve ürtiker (n=6, %12,5) yer aldı. Takip süresince iki hastaya sistemik lupus eritematozus ve bir hastaya jüvenil idiyopatik artrit tanısı konuldu. Hastaların %39,6'sında ANA titresi zamanla negatifleşti. Tanı almayan hastalarda takip süresinin medyanı 34 ay (çeyrekler arası açıklık: 26,5-50) idi.

Sonuç: Çocuklarda ANA pozitifliği çoğu zaman geçici ve klinik olarak önemsizdir. Bu nedenle yönetimde klinik bağlam ve semptomlara dayalı izlem ön planda tutulmalı, rutin olarak kapsamlı değerlendirmelerden kaçınılmalıdır.

Anahtar Kelimeler: Antinükleer antikor, bağ dokusu hastalıkları, lupus, pediatrik romatoloji

Introduction

Antinuclear antibody (ANA) are autoantibodies directed against various nuclear antigens and are widely used as diagnostic biomarkers in connective tissue diseases, particularly systemic lupus erythematosus (SLE). The ANA test was first described in the 1940s through the identification of the lupus erythematosus cell phenomenon, in which sera from SLE patients induced nuclear changes in healthy bone marrow cells (1). Today, the gold standard for ANA detection is the indirect immunofluorescence assay (IIFA) using HEp-2 cells (2). However, clinical implementation of the test has revealed that ANA positivity is not specific to autoimmune diseases and can also be found in healthy individuals or in non-rheumatologic conditions (3,4).

Population-based studies have reported ANA positivity rates up to 30% in healthy individuals (5-7). These rates increase with age, are more common in females, and are usually observed at low titers (1:40, 1:80). In the pediatric population, ANA positivity has been reported in 10-15% of children (8,9). This variability contributes to uncertainty regarding the clinical relevance of incidental ANA positivity, particularly in children, and makes interpretation in the absence of systemic findings challenging.

In daily clinical practice, ANA testing is frequently requested in children presenting with non-specific complaints. Positive results often lead to referrals to pediatric rheumatology clinics, even in the absence of other abnormal findings. There is still no consensus on the optimal follow-up strategy for ANA-positive children, and reliable predictive markers for future disease development remain unclear. Most of the available evidence comes from retrospective studies, which limits the ability to draw firm conclusions about the natural course and clinical relevance of ANA positivity in children.

This study aimed to prospectively follow children referred for ANA positivity over a two-year period, to evaluate the clinical course, changes in ANA status, and the proportion of patients who developed a diagnosis of connective tissue disease. The goal was to generate evidence that might guide the clinical management of this frequently encountered patient group.

Materials and Methods

This study included 48 pediatric patients aged 0-18 years who were referred to the Pediatric Rheumatology Outpatient Clinic of İstanbul University Faculty of Medicine due to ANA positivity. Initial ANA testing for all patients was performed at multiple external laboratories prior to referral to our center. Most laboratories used IIF on HEp-2 cell substrates, while a minority employed enzyme-linked immunosorbent assays (ELISA), resulting in minor variations in assay platforms and manufacturers across centers. Because these tests were conducted externally, detailed information regarding sample handling and storage conditions was not consistently available. All referred patients with a positive ANA titer of ≥1:40 were included. Patients who had a previously established diagnosis of any rheumatologic disease were excluded from the study. All patients were followed at 6-month intervals in the pediatric rheumatology clinic.

From the initial visit and throughout the follow-up period, patients were evaluated for signs and symptoms suggestive of systemic connective tissue diseases. Additional laboratory tests were performed as clinically indicated. Each patient was assessed using a standardized evaluation form, and clinical findings and laboratory data were systematically recorded. Only patients with a minimum follow-up duration of two years were included in the final analysis.

The study recorded the initial clinical complaints that prompted ANA testing, the referring department, clinical findings at presentation, ANA titers and staining patterns, results of other autoantibody tests, and laboratory parameters. Changes in ANA titers over time, newly emerging clinical findings, and final diagnoses—if established—were documented during follow-up.

Informed consent was obtained from all participants prior to inclusion in the study. The study was approved by the Ethics Committee of İstanbul Faculty of Medicine (approval number: 223382, dated: 27.10.2020), and was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical Analysis

All data were compiled using Microsoft Excel (Microsoft Corporation, Redmond, WA) and analyzed with SPSS version 17.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the data. Categorical variables were presented as frequencies and percentages (n, %). Continuous variables were reported as mean ± standard deviation for normally distributed data, and as median with interquartile range (IQR) for non-normally distributed data. Normality of distribution was assessed using visual inspection (histograms and Q-Q plots) and the Shapiro-Wilk test.

Results

Of the 48 patients included in the study, 35 (72.9%) were female. The mean age at initial presentation was 10.22±3.08 years for the entire cohort. Among patients who did not receive a diagnosis, the median follow-up duration was 34 months (IQR: 26.5-50).

ANA testing was most commonly performed due to joint pain (n=14, 29.17%), followed by thrombocytopenia (n=6, 12.5%) and urticaria (n=6, 12.5%). Patients were most frequently referred by general pediatrics clinics (n=23, 47.92%), followed by pediatric hematology clinics (n=13, 27.08%) (Table 1).

At presentation, the most common positive clinical findings were arthralgia (19 patients, 39.58%), recurrent aphthous stomatitis (11 patients, 22.92%), and non-specific rash (10 patients, 20.83%). The most frequently reported ANA titer in the cohort was 1:640 (12 patients, 25.0%), although ANA patterns showed considerable variability. Detailed data regarding clinical findings, ANA titers, and patterns are presented in Table 2.

During follow-up, ANA became negative in 19 patients (39.58%). Baseline positivity of other autoantibodies was evaluated, and detected as follows: anti-dsDNA in 4 patients, anti-Sm in 1 patient, antiphospholipid antibodies in 2 patients, and anti-SSA and anti-SSB each in 1 patient (Table 2). Among these patients, the positivity for anti-Sm and anti-SSA/SSB antibodies spontaneously regressed. Of the 4 patients positive for anti-dsDNA, one was diagnosed with SLE, one with juvenile idiopathic arthritis (JIA), and spontaneous regression was observed in the other two. Both patients, positive for antiphospholipid antibodies, were diagnosed with SLE.

Table 1. Demographic characteristics, family history, referral sources, and initial reasons for ANA testing in the study cohort	
Demographic characteristics	
Female, n (%)	35 (72.9)
Age at presentation (years), mean ± SD	10.22±3.08
Duration of follow-up (months), median (IQR: 25-75)	34 (26.5-50)
Referring clinic	**Frequency (n, %)**
General pediatrics	23 (47.92)
Pediatric hematology	13 (27.08)
Pediatric allergy and immunology	4 (8.33)
Dermatology	3 (6.25)
Pediatric nephrology	2 (4.17)
Pediatric infectious diseases	1 (2.08)
Pediatric gastroenterology	1 (2.08)
Pediatric neurology	1 (2.08)
Reason for initial ANA testing	**Frequency (n, %)**
Joint pain	14 (29.17)
Thrombocytopenia	6 (12.5)
Urticaria	6 (12.5)
Recurrent oral aphthae	3 (6.25)
Neutropenia	2 (4.17)
Abdominal pain	2 (4.17)
Malar rash	2 (4.17)
Other reasons*	
Positive history of rheumatic diseases in first degree relatives	**Frequency (n, %)**
Total	14 (29.17)
RA	6 (12.5)
SLE	3 (6.25)
FMF	2 (4.17)
Sarcoidosis	2 (4.17)
Vasculitis	1 (2.08)
*Other reasons include dry eyes, pancytopenia, proteinuria, screening due to family history, hypertension, recurrent diarrhea, menometrorrhagia, ecchymosis, hair loss, thrombocytosis, and evaluation for multiple sclerosis (each 1 case, 2.08%). ANA: Antinuclear antibody, SD: Standard deviation, IQR: Interquartile range, RA: Rheumatoid arthritis, SLE: Systemic lupus erythematosus, FMF: Familial Mediterranean fever	

Table 2. Summary of positive clinical findings, ANA titers and patterns at baseline, ANA negativization during follow-up, and presence of other autoantibodies	
Positive clinical findings at presentation	**Frequency (n, %)**
Arthralgia	19 (39.58)
Rash (non-specific)	10 (20.83)
Recurrent aphthous stomatitis	11 (22.92)
Thrombocytopenia	5 (10.42)
Leukopenia	2 (4.17)
Photosensitivity	2 (4.17)
Alopecia	1 (2.08)
Malar rash	1 (2.08)
Raynaud's phenomenon	1 (2.08)
Dry eyes	1 (2.08)
Elevated erythrocyte sedimentation rate	1 (2.08)
ANA titer at baseline	
1:40	5 (10.42)
1:80	4 (8.33)
1:160	10 (20.83)
1:320	9 (18.75)
1:640	12 (25.0)
1:1280 and above	7 (14.58)
Titer not known*	1 (2.08)
ANA pattern at baseline	
Homogenous	10 (20.83)
Speckled	11 (22.92)
Dense fine specked (DFS-70)	4 (8.33)
Nucleolar	2 (4.17)
Granular	1 (2.08)
Other (nuclear membrane, dots)	2 (4.17)
Pattern not known*	18 (37.5)
ANA became negative during follow-up	19 (39.58)
Presence of other autoantibodies at baseline	
Anti-dsDNA	4 (8.33)
Anti-Sm	1 (2.08)
Antiphospholipid antibodies	2 (4.17)
Anti-SSA/anti-SSB	1 (2.08)
*Missing values are due to incomplete clinical documentation at baseline. ANA: Anti-nuclear antibodies, Anti-dsDNA: Anti-double-stranded DNA, Anti-Sm: Anti-Smith antibodies, Anti-SSA: Anti-Sjögren's syndrome-related antigen A, Anti-SSB: Anti-Sjögren's syndrome-related antigen B	

Among the followed patients, 3 (6.25%) were diagnosed with a rheumatologic disease during the follow-up period, of these, 2 were diagnosed with SLE and 1 with JIA.

Patient 1: An 8-year-old female patient initially presented with arthralgia and was found to have a positive ANA at a titer of 1:1280 (pattern unknown). Baseline evaluation revealed elevated erythrocyte sedimentation rate, and positive anti-dsDNA antibodies, while other autoantibodies were negative. During follow-up, the patient developed arthritis by the third month and anti-dsDNA antibodies subsequently became negative. No additional autoantibody positivity or clinical features consistent with SLE emerged. The patient

has been followed for 66 months with a persistent diagnosis of rheumatoid factor (RF)-negative polyarticular JIA and no clinical or serological evidence of SLE.

Patient 2: A 16-year-old girl was evaluated for livedo reticularis on the lower extremities and demonstrated a homogeneous ANA pattern at a 1:320 titer. Baseline clinical features included arthralgia, livedo reticularis, and Raynaud's phenomenon. Laboratory findings showed positivity for anti-dsDNA and antiphospholipid antibodies, alongside decreased complement C4 levels. Based on these findings, a diagnosis of SLE was established.

Patient 3: A 10-year-old girl, previously followed in pediatric hematology for chronic immune thrombocytopenic purpura, was referred after detection of ANA positivity at a titer of 1:40 (pattern unknown). Baseline laboratory results revealed thrombocytopenia, ANA positivity, and anticardiolipin antibody positivity, without other clinical or laboratory abnormalities. At three-month follow-up, new symptoms of fatigue prompted repeat testing, which showed a marked increase in ANA titer to 1:1000 with centromere and diffuse fine speckled patterns, positivity for anti-centromere and anti-Sm antibodies, decreased C4 levels, and positivity for ribosomal P antibodies. The patient was diagnosed with SLE.

Discussion

In this prospective study, we followed children referred to a pediatric rheumatology clinic due to positive ANA tests, aiming to explore the clinical significance and evolution of ANA positivity over time. Our findings indicate that the most common reason for ANA testing was non-specific symptoms, particularly joint pain, and the majority of referrals came from general pediatrics clinics. Notably, ANA positivity reverted to negative in a significant proportion (39.58%) of the patients during follow-up, and only three patients developed a diagnosis of a rheumatologic disease (two with SLE and one with JIA).

The clinical significance of isolated ANA positivity in children has long been debated with existing studies—mostly retrospective—reporting variable diagnostic outcomes. Aygun et al. (10) retrospectively analyzed 409 ANA-positive children and found that joint pain was the most common presenting symptom, with 15% later diagnosed with systemic autoimmune disease. Similarly, Wang et al. (11) described joint pain, rash, and recurrent fever as leading complaints among ANA-positive adults. These findings align with our cohort, where joint manifestations were also the most frequent reason for referral. However, the rate of confirmed rheumatologic disease in our study was notably lower.

In contrast, Perilloux et al. (12) reported a much higher diagnostic rate, with 55% of children receiving a rheumatologic diagnosis—most commonly JIA and SLE. Likewise, McGhee et al. (13) evaluated 110 children referred for ANA positivity and identified 10 cases of SLE, one of mixed connective tissue disease, and 18 of juvenile rheumatoid arthritis. Nearly half of the remaining children had non-specific musculoskeletal pain. Importantly, their study showed that ANA titer did not distinguish JIA from benign musculoskeletal conditions, but very high titers (≥1:1080) were strongly predictive of SLE, with a reported positive predictive value of 1.0 for such titers (13).

In our cohort, most patients who received a diagnosis did so within the early months of follow-up. Among the two children diagnosed with SLE, one fulfilled classification criteria at baseline with multiple clinical and serological features, while the other initially presented with ANA positivity and lupus-suggestive symptoms, eventually meeting full criteria during follow-up. This illustrates the evolving nature of autoimmune diseases, where diagnostic features may develop gradually over time. Conversely, the patient with arthralgia and ANA positivity was ultimately diagnosed with RF-negative polyarticular JIA—highlighting that ANA positivity alone is insufficient for diagnosing SLE, and must be interpreted in clinical context.

These discrepancies across studies likely stem from differences in referral patterns, patient selection, ANA titers, laboratory methods (e.g., IIFA vs. ELISA), and follow-up duration. Moreover, the retrospective nature of many studies introduces potential selection bias, as patients with concerning features are more likely to be followed, while others with mild or non-specific symptoms may not undergo further evaluation. This limits the generalizability of retrospective findings and may either inflate or underestimate the true predictive value of ANA positivity in pediatric populations.

Our findings—where ANA reverted in nearly 40% of patients and only 6.25% received a definitive rheumatologic diagnosis—reinforce that ANA positivity in children is often transient and of limited clinical relevance. Similarly, a large study in adult patients found that the overall positive predictive value of ANA for systemic autoimmune diseases was only 8.8%, increasing with higher titers (11.6% at 1:160 and 26.9% at 1:640) (14).

Similarly, Myckatyn and Russell (15) observed that after a mean follow-up of 5.4 years, only 3 of 53 ANA-positive adults developed connective tissue disease (CTD), despite the majority remaining persistently ANA-positive. Another long-term follow-up study by Wijeyesinghe and Russell (16) reported that although 78% of patients remained ANA-

positive after 11.5 years, only 5 out of 62 (8.06%) developed CTD. These findings support the notion that ANA testing, although sensitive, lacks specificity and should not be used in isolation to screen for autoimmune disease.

Study Limitations

This study has several limitations. First, although all patients were referred for ANA positivity, the initial ANA testing was performed in various external laboratories prior to referral. Therefore, ANA testing is not standardized across the cohort. Variations in test sensitivity, cutoff thresholds, and pattern reporting may have influenced which patients were referred and how results were interpreted. It is possible that a result considered positive in one laboratory might have been negative in another, potentially affecting which patients were referred and, consequently, the overall composition of the study population. This non-uniformity of ANA testing represents a limitation that impacts both the interpretation of individual results and the generalizability of our conclusions. Nevertheless, our prospective data reinforce that incidental ANA positivity in children, particularly in the absence of high titers or specific clinical signs, does not necessitate immediate extensive evaluation or referral, supporting a symptom-guided and cautious clinical approach. Second, the relatively small number of patients who developed definitive rheumatologic diagnoses limits the statistical power to identify predictive factors for disease progression. Despite these limitations, the study's prospective design and structured follow-up protocol provide valuable insight into the clinical trajectory of ANA-positive children in real-world settings.

Conclusion

In conclusion, these findings reinforce the notion that incidental ANA positivity in children, especially in the absence of specific clinical signs or high titers, should not prompt immediate extensive evaluation or referral. However, in the presence of accompanying symptoms, strong family history, or high-titer ANA, close clinical monitoring remains warranted. Our study provides valuable prospective evidence on the natural course of ANA positivity in children, supporting a cautious and symptom-guided approach to management.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of İstanbul Faculty of Medicine (approval number: 223382, dated: 27.10.2020), and was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Informed Consent: Informed consent was obtained from all participants prior to inclusion in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.K.K., A.D.K., S.D.A., N.A.A., Concept: G.K.K., N.A.A., Design: G.K.K., N.A.A., Data Collection or Processing: G.K.K., A.D.K., S.D.A., N.A.A., Analysis or Interpretation: G.K.K., A.D.K., S.D.A., N.A.A., Literature Search: G.K.K., A.D.K., S.D.A., N.A.A., Writing: G.K.K., A.D.K., S.D.A., N.A.A.

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