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Original Article

Antinuclear Antibody (ANA) and ANA Profile Tests in Children with Autoimmune Disorders: A Retrospective Study

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Abstract: The study objective was to determine the clinical value of positive antinuclear antibody (ANA) and ANA profile tests in children with autoimmune disorders. A retrospective chart review was carried out of all patients under 18 years of age with a positive ANA test (HEp-2 cell substrate, titre $\geqslant$1:40) and ANA profile (ELISA) referred to the paediatric rheumatology service at the authors' institution between 1992 and 1996. Of 245 children with a positive ANA test, 134 (55%) had an autoimmune disease, including juvenile rheumatoid arthritis ($n = 49$), systemic lupus erythematosus (SLE) ($n = 40$) and others ($n = 45$). The remaining 111 patients did not have identifiable autoimmune diseases. Patients with autoimmune disorders had significantly higher ANA titres of $\geqslant$1:160 ($\chi^2 = 16$, $P < 0.0001$). In addition, of the 245 patients with a positive ANA test, 86 had an ANA profile performed; this was positive in 32 and negative in 54. All 32 patients with a positive ANA profile (100%) had an autoimmune disorder, compared to 22 (41%) of 54 with a negative ANA profile who had autoimmune disorders. Of 22 SLE patients with a positive ANA profile, 16 (73%) had positive anti-dsDNA and 15 (68%) had positive anti-Sm and positive anti-RNP. A positive ANA profile correlated strongly with an ANA titre $\geqslant$1:640 ($\chi^2 = 5.7$, $P < 0.02$). The study demonstrated that only 55% of children with a positive ANA test had a definitive diagnosis of autoimmune disorder. These children tend to have higher ANA titres of $\geqslant$1:160. However, a positive ANA profile was strongly correlated with an ANA titre $\geqslant$1:640 and highly indicative of an autoimmune disorder (100%). We suggest that in order to reduce cost, an ANA profile should not be performed on all patients with positive ANA, but reserved for those with an ANA titre of $\geqslant$1:640 and/or those with a high clinical index of suspicion for autoimmune disorder, especially SLE.

Keywords: Antinuclear antibody (ANA); ANA profile; Juvenile rheumatoid arthritis (JRA); Systemic lupus erythematosus (SLE)

Introduction

The ANA test is frequently used as a screen for autoimmune disorders in children [1–4]. However, a positive ANA test result is known to occur in the absence of autoimmune disease, as the result of various infections, drug therapies and haematological disorders, and in about 5% of normal children [5–14]. Three recent studies suggest that the presence of ANA in childhood is not invariably associated with the development of an autoimmune disease [4,14,15]. However, information regarding the role of the ANA test, and especially the ANA profile, in the pediatric population is limited [1]. In recent years the ANA profile has become commonly used not only for evaluation, but also in screening for autoimmune disorders, despite its relatively high cost compared to the cost of the ANA test only.

We retrospectively reviewed the records of children with a positive ANA test and, among them, those with a positive ANA profile who were seen during a 4-year period (1992–1996) to define more clearly the significance of a positive ANA test and the indication for an ANA profile.

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Patients and Methods

We reviewed the charts of all patients under 18 years of age with a positive ANA and ANA profile tests or both who were referred to the Pediatric Rheumatology Clinic at Children's Hospital of New Orleans, Louisiana, from 1992 to 1996.

Laboratory Methods

A titre $\geqslant$1:40 was considered a positive ANA test. Antibody assays were performed on sera collected from patients early in their presentation and stored at –20 °C. The presence of ANA for all sera was confirmed concurrently in our laboratory by indirect immunofluorescence on monolayers of human larynx epidermoid carcinoma cells (HEp-2) (Kallestad Laboratories, Austin, TX) at a screening dilution of 1:40 (cost $63.00) [12]. For the ANA profile, antibodies to SS-A/Ro, SS-B/La, Sm, RNP, Scl-70, centromere and dsDNA were determined at Puckett Laboratories (Hattiesburg, MS) using an indirect non-competitive enzyme immunoassay. The presence of one or more antibodies in the panel was considered a positive ANA profile ($430.00).

Clinical Data

The following information was recorded for each patient at the first visit: sex, age, clinical symptoms, and ANA titre and ANA profile results (the presence of antibodies to ds-DNA, SS-A/Ro, SS-B/La, RNP, Sm, Scl-70 and centromere). The diagnoses of specific autoimmune disorders were based on clinical criteria defined by the American College of Rheumatology [16–17]. Patients with musculoskeletal symptoms but without evidence of a definable autoimmune disorder could potentially have fibromyalgia or joint hypermobility, or were referred to as 'non-specific musculoskeletal complaints.'

Statistical Methods

Data were analysed using the χ^2 test. A P value of <0.05 was considered statistically significant.

Results

The study included 245 patients with a positive ANA test (165 girls, 80 boys), ranging in age from 3 months to 18 years. Of these, 134 (55%) had a recognised autoimmune disorder and 111 (45%) did not (Table 1). The most common diagnosis was juvenile rheumatoid arthritis (JRA), found in 49 of the 134 patients (37%), followed by systemic lupus erythematosus (SLE) in 40 (30%); 14 (10%) had idiopathic thrombocytopenic purpura (Table 2).

Of the 111 patients who did not have autoimmune disease, 17 (15%) had fibromyalgia, 9 (8%) joint hypermobility and 16 (14%) 'non-specific musculoskeletal complaints'. Another 26 (23%) had a positive ANA test in association with infection, mainly viral, and 29 (26%) had other conditions (Table 3). The patients in the group with autoimmune disorders were found to have significantly higher ANA titres ($\geqslant$1:160) than those with non-autoimmune aetiologies, who had lower ANA titres ($\leqslant$1:80) (χ^2 = 16, $P<0.0001$; Table 4).

Table 1. Demographic characteristics of the study population (n = 245)

Features	Autoimmune n = 134	Non-autoimmune n = 111
Mean age at testing	10.5 years	9.3 years
Sex		
Male	34 (25%)	46 (41%)
Female	100 (75%)	65 (59%)
Ethnicity		
African-American	77 (57%)	55 (49.5%)
Caucasian	50 (37%)	55 (49.5%)
Asian/Hispanic	7 (6%)	1 (1%)

Table 2. Autoimmune disorders diagnosed among 134 children with a positive ANA test

Diagnosis	Number of patients (%)
Juvenile rheumatoid arthritis	49 (37)
Systemic lupus erythematosus	40 (30)
Idiopathic thrombocytopenic purpura	14 (10)
Juvenile dermatomyositis	6 (4)
Henoch–Schönlein purpura	5 (4)
Kawasaki disease	4 (3)
Insulin-dependent diabetes mellitus	4 (3)
Mixed connective tissue disease	3 (2)
Sarcoidosis	2 (2)
Rheumatic fever	2 (2)
Autoimmune haemolytic anaemia	2 (2)
Sjögren's syndrome	1 (1)
Scleroderma	1 (1)
Crohn's disease	1 (1)
Total	134 (100)

Table 3. Diagnoses of non-autoimmune disorders among 111 children with a positive ANA test

Diagnosis	Number of patients (%)
Infection	26 (23)
Fibromyalgia	17 (15)
Non-specific musculoskeletal complaints	16 (14)
Hypermobility	9 (8)
Drugs	7 (6)
Leukaemia	6 (5)
Renal disease	6 (5)
Abnormal urinalysis	4 (4)
Asthma	3 (3)
Sickle cell disease	2 (2)
Lymphoma	1 (1)
Abnormal coagulation profile	1 (1)
No diagnoses made	13 (12)
Total	111 (100)

Table 4. ANA titres in 245 patients with a positive ANA test

	No. with ANA titre $\leqslant$1:80	No. with ANA titre $\geqslant$1:160
Autoimmune disorders (134 patients)	45	89
Non-autoimmune disorders (111 patients)	67	44
Total	112	133

($\chi^2 = 16$, $P < 0.001$)

Table 5. Autoimmune disorders diagnosed in children with positive and negative ANA profiles

Diagnosis (total no. of patients)	With (+) ANA profile $n = 32$ (%)	With (–) ANA profile $n = 54$ (%)
SLE (28)	22 (69)	6 (11)
JRA (11)	3 (9)	8 (15)
MCTD (2)	2 (6)	0
ITP (2)	2 (6)	0
HSP (2)	0	2 (4)
Juvenile dermatomyositis (1)	1 (3)	0
IDDM (2)	1 (3)	1 (2)
Scleroderma (1)	1 (3)	0
Others (37)	0	37 (68)
Total (86)	32 (100)	54 (100)

Of the 245 children with a positive ANA test, 86 also had an ANA profile performed. Of these, 32 had positive profiles and 54 were negative. All 32 (100%) patients with a positive profile and 22 (41%) of the 54 with a negative profile had an autoimmune disorder (Table 5). Furthermore, a positive ANA profile correlated significantly with an ANA titre $\geqslant$1:640 ($\chi^2 = 5.7$, $P < 0.02$). The ANA profile also correlated significantly with autoimmune diseases, particularly SLE ($\chi^2 = 13.6$, $P < 0.001$). Of the 22 SLE patients with a positive ANA profile, 16 (73%) had positive anti-dsDNA and 15 (68%) had positive anti-Sm and positive anti-RNP; 12 (55%) had positive SS-A/Ro and six (27%) had positive SS-B/La.

Discussion

The significance of a positive ANA test in adult patients has been well established. However, reports on ANA tests, and especially ANA profiles in the paediatric population are few [1,4,14]. Also, in recent years the ANA profile has become widely used not only for evaluation but also in screening for rheumatic disorders, despite the significant cost ($430.00) compared to the ANA test alone ($63.00).

Chudwin et al. [1], using indirect immunofluorescence with rat kidney and stomach as a substrate found that the majority of children with a positive ANA test, even of low titre, have a clinically apparent autoimmune disease. They described 138 patients referred to a paediatric rheumatology clinic with a positive ANA test, of whom 127 (92%) had an autoimmune or rheumatic disorder. The remaining 8% had positive ANA tests from other causes, such as viral syndromes, leukaemia and immunoglobulin deficiency. However, two recent studies in children using the same biological technique as in our patients (indirect immunofluorescence with HEp-2 cells as a substrate) suggest that a positive ANA test in childhood is not invariably associated with the development of a rheumatic disease [4,14]. Cabral et al. [14] found that 78% of their referred patients in a tertiary care setting who had positive ANA tests had definable rheumatic or autoimmune diseases, whereas 22% did not. They suggested that in the absence of clinical signs a positive ANA test was not helpful in diagnosing rheumatic diseases, and recommended that the ANA test be performed only in children for whom physical evidence suggests inflammatory or rheumatic disease. Most of the 22% who had no definable rheumatic disease were found to have joint hypermobility. In a paediatric rheumatology outpatient clinical setting at a tertiary care centre Deane et al. [4] found that 64% of referred patients with a positive ANA test had a clinically apparent autoimmune disease. Of the remaining 36%, 9% were lost to follow-up and 27% continued to be free of rheumatic disease over a mean of 37 months. Non-specific musculoskeletal complaints and hypermobility accounted for the majority of presentations. The authors suggested that a vast majority of children who have a positive ANA test but no autoimmune condition at initial presentation have a very low risk of developing one and carry an excellent prognosis.

Our study revealed that of 245 children with a positive ANA test, only 55% had specific autoimmune diseases. The most common autoimmune disorder was JRA (37%), followed by SLE (30%). Interestingly, 45% of our patients with a positive ANA test did not have an autoimmune disease. One-third of these patients were found to have fibromyalgia, joint hypermobility and non-specific musculoskeletal complaints. Infections, presumably viral in aetiology, accounted for almost one-quarter of the positive tests. Patients with autoimmune disorders were found to have a significantly higher ANA titre ($\geqslant$1:160) than those with a non-autoimmune aetiology. Accordingly, we believe that patients with a high ANA titre ($\geqslant$1:160) should undergo further evaluation for autoimmune disorders, including referral to a paediatric rheumatologist. They should also be monitored for the future development of a rheumatic disease.

The present study also showed an excellent correlation between a positive ANA profile and the presence of an autoimmune disorder. All 32 patients (100%) with a positive ANA profile were found to have such a disease. Furthermore, a positive ANA profile correlated significantly with an ANA titre of $\geqslant$1:640, and with the presence of an autoimmune disorder, particularly SLE. However, only 41% of children with a negative ANA profile had autoimmune diseases. In addition, in order to reduce cost we suggest that among ANA-positive

children an ANA profile be performed for those with a titre of $\geqslant$1:640 and/or those with a high clinical index of suspicion for SLE. This study was a retrospective chart review and the authors concur with the possibility of referral bias, and that the above data should be interpreted cautiously.

The autoantibodies of the ANA profile have been found in conjunction with many autoimmune diseases. Antibodies to SS-A/Ro have been found in approximately 60%–95% of patients with Sjögren's syndrome and only 25%–40% of SLE patients [18]. Antibodies to Sm are considered a highly specific marker for SLE and are reported in 30%–40% of these patients [19]. Anti-RNP antibodies are noted in 40%–50% of SLE patients, but are also seen in patients with scleroderma, rheumatoid arthritis, discoid lupus and Sjögren's syndrome. A very high titre of anti-RNP antibodies alone is highly characteristic of mixed connective tissue disease [20]. Patients with scleroderma tend to have antibodies to Scl-70, whereas anticentromere is noted with limited scleroderma (CREST syndrome) [18,19]. Our data in children with SLE show a higher frequency of positivity to Sm and RNP antibodies than is reported in the literature. However, the frequencies of the antibodies to ds-DNA (73%), SS-A/Ro (55%) and SS-B/La (27%) in our SLE patients were found to be similar to those reported by others [18,19].

Conclusion

Our study showed that only slightly over half of the subjects with positive ANA tests had autoimmune diseases, and that all patients with a positive ANA profile were found to have such a disease. We believe that the ANA test should remain the mainstay for rheumatic disease screening and, in order to reduce cost, an ANA profile should be performed in those with an ANA titre of $\geqslant$1:640 and/or a high index of suspicion for autoimmune diseases, especially SLE.

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