Antinuclear antibodies in children: clinical signification and diagnosis utility

Anticorps anti-nucléaires chez l'enfant: signification clinique et intérêt diagnostique

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RÉSUMÉ

Background : La recherche des anticorps anti-nucléaires (AAN) est indiquée aussi bien chez l'adulte que chez l'enfant pour le diagnostic des connectivites et des hépatites auto- immunes. Cependant, l'interprétation d'un résultat positif peut être délicate.

Objectif : déterminer la signification clinique et l'intérêt diagnostique de la positivité des AAN chez l'enfant

Méthodes : Des patients suivis dans un service de Pédiatrie générale et présentant des AAN positifs ont été inclus (période d'étude de 2 ans). Le dépistage des AAN a été fait par immunofluorescence indirecte (IFI) sur cellules HEp-2 (BioSystems®). En cas de résultat positif (seuil de positivité : 1:80), la spécificité antigénique a été déterminée par IFI sur *Crithidia luciliea* (BioSystems®) et immunodot (Euroimmun®).

Résultats : Parmi 102 tests, 55 (53,9%) étaient positifs. Les renseignements cliniques relatifs à 38 patients (âge moyen : 9,5 ans - sex ratio : 0.72) ont été recueillis. Les signes les plus fréquents étaient les douleurs articulaires (55,3%). Le titre des AAN variait de 1:80 (39,5% des cas) à 1:1280 (2,6% des cas). Le typage était négatif dans 89,5% des cas. La majorité (42,1%) des enfants avec des AAN positifs avaient des troubles musculo-squelettiques. Les autres (57,9%) présentaient un lupus érythémateux systémique(n=2), un syndrome de chevauchement(n=1), un purpura rhumatoïde(n=2), un purpura thrombopénique idiopathique (n=1), une maladie cœliaque(n=1) ou des maladies non auto-immunes ou bien un diagnostic non confirmé (n=15).

Conclusions : La prévalence des AAN chez l'enfant parait relativement importante. En cas de probabilité pré-test faible, la valeur prédictive positive pour le diagnostic de connectivites ou d'hépatite auto-immune est faible. Cependant, selon le contexte clinique, la détection des AAN peut représenter un outil diagnostique supplémentaire pour ces maladies et/ou conduire à une surveillance clinico-biologique.

Mots Cles : anticorps anti-nucléaires ; enfant ; connectivites ; hépatites auto-immunes ; troubles musculo-squelettiques

Abstract

Background: Antinuclear antibodies (ANA) test is used to screen adults as well as children for connective tissue diseases (CTD) and autoimmune hepatitis. However, interpretation of ANA positivity can be delicate.

Aim: to determine clinical significance and diagnosis utility of ANA positivity in children.

Methods: Patients from a general pediatric department with ANA positive results were included (follow-up period of 2 years). ANA screening was performed by indirect immunofluorescence (IIF) on HEp-2 cells substrate (BioSystems®). In case of ANA positivity (cut-off: 1:80), the specificity was determined by IIF on *Crithidia luciliea* substrate (BioSystems®) and immunodot (Euroimmun®).

Results: Among 102 ANA tests, 55 (53,9%) were positive. We recorded the data of 38 patients (age average: 9,5 years - sex ratio: 0.72). The most frequent signs were join pain (55,3%). ANA titer varied between 1:80 (39,5% of cases) and 1:1280 (2,6% of cases). Typing was negative in 89,5% of cases. The majority (42,1%) of children with positive ANA test had musculoskeletal diseases. The others (57,9%) had systemic lupus erythematosus(n=2), overlap syndrome(n=1), rheumatoid purpura(n=2), idiopathic thrombocytopenic purpura(n=1), coeliac disease(n=1) or non-autoimmune diseases/no confirmed diagnosis(n=15).

Conclusions: ANA prevalence in children was relatively high. When the pretest probability is low, the positive predictive value for CTD or autoimmune hepatitis is low. However, depending on the clinical context, ANA detection can represent a supplement diagnostic tool for these diseases and/or can lead to a clinico-biological monitoring.

Keywords: antinuclear antibodies; child; connective tissue diseases; autoimmune hepatitis; musculoskeletal diseases

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INTRODUCTION

Antinuclear antibodies (ANA) are the most frequently ordered autoantibodies in clinical practice for adult patients. They represent useful markers for the diagnosis of connective tissue diseases (CTD) and autoimmune hepatitis (1). As these diseases can have a childhood onset, ANA test is used to screen children for such conditions (2). However, ANA can be found in other autoimmune diseases, cancers, infections and even in healthy individuals (3). Thus, the interpretation of ANA positivity can be delicate and some knowledge is required for appropriate clinical application. The objective of our study was to determine clinical significance and diagnosis utility of ANA positivity in children.

METHODS

We conducted a retrospective study concerning a follow-up period of 2 years. Patients from a general pediatric department with ANA positive results were selected. The information including age, sex, clinical signs, biological results and suspected or confirmed diagnosis were collected from the medical patients' files.

ANA screening was performed by a commercial indirect immunofluorescence (IIF) test kit (BioSystems®) using human epithelial tumor cell lines, HEp-2 cells, as substrate. Detection of ANA at a dilution superior or equal to 1:80 was considered as a positive result. In case of ANA positivity, an IIF assay using *Crithidia luciliea* as substrate (BioSystems®) was used to detect anti-double strand DNA (anti-dsDNA) antibodies and immunodot (Euroimmun®) was performed for anti-extractable nuclear antigen (anti-ENA) antibodies typing. Statistical analyses were performed using SPSS software.

RESULTS

Among 102 ANA tests requested by pediatric emergencies department during the study period, 55 (53,9%) were positive. We recorded the information of 38 patients. Their ages were between 8 months and 16 years with an average of 9,5 years without a significant difference between boys and girls. The sex ratio (Male/Female) was 0.72.

As shown in table 1, the most frequent signs were join pain (55,3%) followed by fever (31,6%), digestive signs (31,6%) and biological inflammatory syndrome (31,6%).

Table 1. Clinical and biological signs of patients with ANA positive results

Clinical and biological signs	N (%)
Arthralgia or arthritis	21 (55,3)
Fever	12 (31,6)
Digestive signs (abdominal pain, transit disorders, hepatosplenomegaly)	12 (31,6)
Biological inflammatory syndrome	12 (31,6)
Mucocutaneous presentations (photosensitivity, skin rash, mucosal involvement)	7 (18,4)
Hematologic problems (thrombocytopenia, leukopenia, anemia)	6 (15,8)
Abnormal urinalysis finding (proteinuria, hematuria, renal failure)	5 (13,2)
Serositis	2 (5,3)
Neurological signs (sensation disorders, cranial nerves involvement, seizures)	2 (5,3)

ANA: antinuclear antibodies

The most frequent ANA pattern was speckled pattern (86,8%). ANA titer varied between 1:80 (39,5% of cases) and 1:1280 (2,6% of cases). ANA typing was negative in 89,5% of cases. ANA specificities were identified in 4 cases with different profiles: anti-SSB, anti- Ro52, anti-Jo1 and anti-Ribosome associated with anti-Mitochondria.

The majority (42,1%) of children with positive ANA test had musculoskeletal diseases (acute articular rheumatism or juvenile idiopathic arthritis). The diagnosis of systemic lupus erythematosus was made in 2 cases, overlap syndrome between autoimmune hepatitis and primary sclerosing cholangitis in one case, rheumatoid purpura in 2 cases, idiopathic thrombocytopenic purpura in one case and coeliac disease in one case. The remaining 15 patients had no autoimmune disease or no confirmed diagnosis. Table 2 shows ANA test results according to disease category.

Table 2 : *A*NA test results according to disease category

	MSD	SLE	OS	RP	ITP	CD	other	Total
ANA pattern								
speckled	15	2	0	2	1	1	12	33
homogeneous	0	0	0	0	0	0	1	1
nucleolar	1	0	1	0	0	0	2	4
ANA titer								
1:80	5	0	0	2	0	1	7	15
1:160	4	1	0	0	0	0	5	10
1:320	2	0	1	0	1	0	5	9
1:640	1	1	0	0	0	0	1	3
1:1280	0	0	0	0	0	0	1	1
ANA typing								
negative	16	1	0	1	1	1	14	34
positive	0	1(Rib+Mi)	1(Jo1)	1(SSB)	0	0	1(Ro52)	4

ANA: antinuclear antibodies; MSD: musculoskeletal diseases; SLE: systemic lupus erythematosus; RP: rheumatoid purpura; ITP: idiopathic thrombocytopenic purpura; OS: overlap syndrome between autoimmune hepatitis and primary sclerosing cholangitis; CD: coeliac disease

DISCUSSION AND CONCLUSION

Different studies were interested to investigate ANA prevalence in children. From a technical point of view, variation of results may be explained by variation in the screening technique used (IIF, immunoblotting, enzyme immuno assay, rat liver, mouse kidney or HEp- 2 cells substrate) and the positivity threshold applied (1:40 to 1:160) (4 , 5). In our study, we used IIF on HEp-2 cells which is the "Gold standard" technique in ANA screening (5) and the considered cut-off was 1:80. More important is the studied population. Many studies focused on ANA prevalence in healthy children (Table 3) while others focused on children with different autoimmune diseases which diagnosis is not based on ANA detection (Table 4).

Such studies highlight the lack of specificity of ANA positivity and raise the question of clinical significance and diagnosis utility of these autoantibodies. In our study, ANA prevalence was 53,9%. It should be mentioned that the studied population was heterogenous as it included children who visited Pediatric emergencies department for various symptoms.

Table 3. Reported results of ANA prevalence in healthy children

Study	Population age	Technique	ANA cut-off	ANA prevalence
Somers *et al.,* 2017 (Mexico) (18)	9–17 years			15,8%
Sperotto *et al.,* 2014 (Italy) (17)	8–13 years	indirect immunofluorescence assay on HEp-2 cells	1:80	12,3%
Wananukul *et al.,* 2005 (Thailand) (19)	5-15 years			6%
Hilario et al., 2004 (Brazil) (20)	6 months-20 years			12,6%

ANA: antinuclear antibodies

Table 4. ANA prevalence in children with different autoimmune diseases

Study	Disease	Population	ANA prevalence
Pratt *et al.,* 2005 (21)	Acute ITP	1,4-12 years	7,6%
	Chronic ITP	6-16 years	22,2%
Segni *et al.,* 2014 (22)	Autoimmune thyroid disease	12.1 ± 4.86 years	71%
Bigi *et al.,* 2014 (23)	Acquired demyelinating diseases of the central nervous system	-	9,2%

ANA: antinuclear antibodies; ITP: Immune Thrombocytopenic Purpura

The interpretation of ANA positivity should take in consideration the pattern, the titer, the antigen specificity and above all the clinical context (1). In fact, the positive predictive value for CTD of an ANA positive test is low when the pretest likelihood of CTD is low (6). Nevertheless, it is possible that some patients with positive ANA develop clinical symptoms of CTD that declares itself subsequently. A prospective study conducted by Wijeyesinghe U *et al.* (7) reported the development of CTD in 8% of positive ANA patients after a 10-year follow-up. The various patterns of nuclear fluorescence correlate with certain diseases but are not specific (8). ANA titer has a controversial importance. Some studies concluded that patients with higher ANA titers are more susceptible to autoimmune diseases (9). However, it is also reported that high ANA titers, in the absence of a clinical suspicion have a low positive predictive value (4%) for developing CTD in the upcoming 3 years (10). Concerning ANA specificities, some of them are considered disease or

manifestation specific (3); but, in a number of cases, the diagnosis of CTD can be retained even with negative ANA typing (11). In our study, the diagnosis of systemic lupus erythematosus, a CTD which is diagnosed in childhood in 10-20% of patients (12), was made for 2 patients: one with a low ANA titer and a negative typing, the other with a high ANA titer and a positive typing, showing the importance of clinical presentation.

Regarding autoimmune hepatitis, ANA were the first autoantibodies to be clearly associated with the disease and several nuclear molecular targets have been recognized including single or double-stranded DNA, tRNA, SSA-Ro, SmRNPs, laminins A and C, cyclin A or histones (13). ANA detection not only assists the diagnosis (represents one of the scoring systems criteria) but also helps to identify the type of autoimmune hepatitis (14). ANA characterize type 1 autoimmune hepatitis but can also be detected in overlap syndromes with autoimmune cholangiopathy (15) as it was the case for one of our patients.

Apart from ANA related diseases (CTD and autoimmune hepatitis), we detected ANA in cases of rheumatoid purpura, idiopathic thrombocytopenic purpura and coeliac disease but the majority of children with positive ANA included in our study (42,1%) was classified as musculoskeletal diseases. One of these diseases is juvenile idiopathic arthritis. Campanilho- Marques R *et al.* (16) conducted a systematic review analysis of the literature on the prognostic value of ANA on juvenile idiopathic arthritis and reported that the presence of ANA seems to be a risk factor for ocular involvement. Another study reported an ANA prevalence of 13,4% in a cohort of children with chronic non inflammatory musculoskeletal pain at baseline and showed an increased frequency of ANA positivity across puberty (from 13.4% to 44.8%) (17). This increased frequency was independent from the persistence of symptoms showing the absence of a significant association between ANA positivity and non- inflammatory musculoskeletal pain and supporting the hypothesis that sex hormones involved in puberty modulate immunity (17).

The relatively high prevalence of ANA in children visiting a pediatric department (55 ANA positive test among 102 requests) in our study contrasting with a low number of patients with ANA related diseases (CTD or autoimmune hepatitis) (3/38) can be attributable to unnecessary testing in patients with non-specific complaints and a low pretest probability. As for adults, ANA are not specific for

these diseases and moreover can arise across puberty. However, depending on the clinical context, ANA detection can represent a supplement criterion to retain a diagnosis of CTD or autoimmune hepatitis and thus to give the appropriate therapy. It can also lead to a clinico-biological monitoring to assess the right value of ANA positivity.

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