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The Presence of Antinuclear Antibodies (ANAs) in Children with Juvenile Idiopathic Arthritis and Arthritis Related to Infection: a Single Center Experience

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Abstract

Objective - to determine the presence of antinuclear antibodies (ANAs) in children with juvenile idiopathic arthritis (JIA) and arthritis related to infection (ARI). **Methods** - Retrospective-prospective study at the Clinic for Children's Diseases Tuzla, from January 2014 to January 2020. Results - 81 children were included in the study: 26 boys and 16 girls with JIA (median age 9.9 years) and 22 boys and 17 girls with ARI (median age 9.6 years). The most common form of JIA was oligoarticular (64.2%), while reactive arthritis (ReA) was the most common form of ARI (64.1%). ANAs were positive in 7/42 (16.6%) children with JIA: 5/27 (18.5%) children with the oligoarticular and 2/10 (20%) children with polyarticular rheuma factor negatve form. Considering ARI, ANAs were positive in 12/39 (30.7%) children: 9 children with ReA (23.1%) and 3 children with postinfectious arthritis (7.6%). We did not find a significant difference in ANAs positivity between the examined groups of children ($\chi 2=1.523$; P=0.21). One child with JIA and 25.6% of children with ARI had a positive ANA profile; a statistically significant difference (P=0.002) was found. **Conclusion** - ANAs tests have little diagnostic utility in clarifying the diagnosis of JIA, and should not be used as a screening tool if JIA is the diagnosis under consideration. The persistence of ANAs was not associated with the type of arthritis, juvenile or ARI.

Key Words: Antinuclear Antibodies ▪ ANA Profile ▪ Arthritis Related to Infection ▪ Juvenile Idiopathic Arthritis.

Introduction

Antinuclear antibodies (ANAs) are a group of autoantibodies against antigenic nuclear structures, including nucleic acids, nucleosomes, phospholipids, and several nuclear and nucleolar proteins (1, 2). These antibodies are associated with numerous autoimmune diseases such as Juvenile idiopathic arthritis (JIA), Systemic lupus erythematosus, Scleroderma, Sjögren's syndrome, Mixed connective tissue disease, Polymyositis/Dermatomyositis and Rheumatoid arthritis. However, they also may be associated with other conditions such as non-autoimmune inflammatory diseases, acute and chronic infections, arthritis related to infection-ARI (septic arthritis, reactive arthritis-ReA, postinfectious arthritis), malignancies and in apparently healthy individuals (3). JIA is the most frequent chronic disease of childhood and represents a heterogeneous group of inflammatory arthropathies, which begins in a child under the age of 16 years and lasts at least 6 weeks, and is not attributable to any other causes of arthritis like septic arthritis, reactive arthritis (ReA), postinfectious arthritis or Lyme disease (4). In everyday practice, it is important to distinguish JIA from other arthritis such as

ARI, since they have similar clinical presentation which includes joint pain, warm and swollen periarticular tissues, and limited range of motion (5, 6).

One of the most common tests used in the diagnosis of JIA is represented by ANAs titers, but the specificity of these autoantibodies in JIA has not been fully determined so far, and the results must be considered in a broader clinical context (2, 7). ANAs positivity amongst the JIA subtypes is highest in patients with oligoarticular JIA (up to 70%) and is particularly more prevalent in young, female patients (8). In children with undifferentiated JIA and systemic JIA, ANAs positivity is less common, although a recent study showed that patients with systemic JIA had rising ANAs titers over time (9, 10). When the ANAs test is used as a screen in patients with non-specific clinical symptoms, such as fever, swelling and pain in the joints, myalgias, fatigue, rash or anemia, the likelihood of a positive ANAs result due to infection will increase, especially in children (3, 7).

The aim of this study was to determine the presence of ANAs in children with JIA and ARI.

Patients and Methods

The retrospective-prospective study was conducted at the Department of Rheumatology, Immunology and Allergy of the Clinic for Children's Diseases, University Clinical Center (UCC) Tuzla, from January 2014 to January 2020. The medical records of 42 children with JIA and 39 children with arthritis ARI were analyzed. The diagnosis of JIA was made based on the diagnostic and classification criteria for JIA 2001 by International League of Associations for Rheumatology (ILAR) (11). Diagnostic criteria for ARI were a) septic arthritis: acute arthritis of any joint with confirmed presence of an infectious agent in the synovial fluid; b) ReA: arthritis of one or more joints (symmetrical or asymmetrical) with confirmed presence of an infectious agent in some other parts of the body; c) postinfectious arthritis in case of acute or recurrent arthritis of one or more joints, which appears immediately after an infection (5, 6).

Exclusion criteria included failure to fulfill the diagnostic criteria for JIA and ARI, children who received synthetic or biological disease modifying drugs in the treatment of JIA, trauma, hematological disorders, and the parents' refusal to let their child take part in the study. The following were analyzed: gender, age of children, form of JIA, form of ARI. Serology tests (enzyme linked immunosorbent assay–ELISA) were used for detection of the presence of antibodies against *Borrelia burgdorferi*, *Chlamydia* and *Mycoplasma pneumoniae* (cut off 25 IU/ml); antibody titers (nephelometry) to antistreptolysin O (ASO) and anti Deoxyribonuclease B (anti DNaseB) with the cut off 200 IU/ml. We analyzed microbiological test results of throat and nose swab, hemoculture, urine culture, stool for the presence of *Salmonellae, Shigellae*; and synovial fluid for the presence of bacteria. To determine ANAs and ANA profile, peripheral blood was obtained from all patients within their standard laboratory testing. Samples for the determination of the presence of ANAs and ANA profile were taken into BD vacutainer SST Advance tubes. Serum was extracted from 5 ml of blood by centrifuging at a speed of 3.000 rpm for 5 minutes. After serum extraction the existence of ANAs was detected by Elisa Hycor. The cut-off value of ANAs was 23 IU/ml, and the children were considered ANAs-positive if they had two positive test results performed over 3 months apart. In the case of ANAs positivity, the ANA profile was determined by immunoblotting using a commercial assay (Euroimmun AG, Lubeck, Germany).

Ethics Statement

The study protocol was approved by Ethics Committee of UCC Tuzla. Informed consent was signed by parents of all participants.

Statistical Analysis

Statistical analysis was performed using the statistical package MedCalc software (version 12.1.3.0 for Windows, MedCalc). Continuous data were

presented as median and range, while categorical data were presented as frequencies and relative frequencies (percentages). Yates $\chi 2$-test for comparison of frequencies and odds ratio (OR) with 95% confidence interval (CI) was used. The difference between the samples was considered significant if $P < 0.05$.

Results

The study included 81 children, 42 children with JIA (26 boys and 16 girls) and 39 children with ARI (22 boys and 17 girls), with the median age at the moment of diagnosis of 9.9 years (range: 2.2 to 14.3 years) and 9.6 years (range: 3.1 to 14.6 years), respectively. Eight children with arthritis met the exclusion criteria, i.e. 4 with trauma, 2 children had leukemia and 2 had recurrent multifocal osteomyelitis. The most common form was oligoarticular in the JIA group (64.2%), while ReA was the most common form in the group of children with ARI (64.1%).

The clinical characteristics and prevalence of particular forms of arthritis of 81 children included in the study are shown in Table 1.

The results of serology tests and microbiological analysis of children included in the study are shown in Table 2.

Table 1. The Clinical Characteristics and Prevalence of Particular Forms of Arthritis of 81 Children (42 with JIA* and 39 with ARI†) Included in the Study

Children's characteristics		N (%)
Gender	Male	48 (59.3)
	Female	33 (40.7)
Form of JIA*	Olygoarticular	25 (30.9)
	Olygoarticular associated uveitis	2 (2.5)
	Polyarticular Rf+‡	3 (3.7)
	Polyarticular Rf-	10 (12.3)
	sJIA§	2 (2.5)
Form of ARI†	ReA‖	25 (30.9)
	Postinfectious arthritis	11 (13.6)
	Septic arthritis	3 (3.6)

*Juvenile idiopathic arthritis; †Arthritis Related to infection; ‡Rheuma factor; §Systemic juvenile idiopathic arthritis; ‖Reactive arthritis.

Table 2. Results of Serology Tests and Microbiological Analysis of 81 Children with arthritis (JIA* and ARI†) Included in the Study

Children with arthritis		JIA* N (%)	ARI† N (%)
Serology tests	*Borrelia burgdorferi*		
	IgM positive	-	5 (12.8)
	IgG positive	7 (16.7)	3 (7.7)
	Mycoplasma pneumonia		
	IgM positive	-	3 (7.7)
	IgG positive	6 (14.3)	4 (10.29)
	ASO‡ (>200 IU/ml)	9 (21.4)	9 (23.1)
	antiDNaseB§ (>200 IU/ml)	9 (21.4)	9 (23.1)
Microbiological analysis	Throat swab		
	β-*hemolytic streptococcus*	-	9 (23.1)
	Streptococcus pneumoniae	-	3 (7.7)
	Nose swab		
	Staphylococcus aureus	1 (2.4)	6 (15.4)
	Streptococcus pneumoniae	-	1 (2.6)
	Hemoculture		
	Coagulase-negative staphylococci	-	1 (2.6)
	Staphylococcus aureus	-	1 (2.6)
	Urine culture		
	Escherichia coli	1 (2.4)	3 (7.7)
	Klebsiella pneumoniae	-	1 (2.6)
	Enterococcus faecalis	1 (2.4)	1 (2.6)
	Stool		
	Salmonella species	1 (2.4)	4 (10.2)
	Synovial fluid		
	Staphylococcus aureus	-	2 (7.7)
	Coagulase-negative staphylococci	-	1 (2.6)

*Juvenile idiopathic arthritis; †Arthritis Related to Infections; ‡Antistreptolysin O; §Anti Deoxyribonuclease B.

Table 3. The Numerical Ratio of the Existence of positive ANAs tests of 81 Children with Arthritis (JIA and ARI) Included in the Study

ANAs* (IU/ml)	Children with arthritis		Total
	JIA†	ARI‡	
>23	7	12	19
≤23	35	27	62
Total	42	39	81

χ^2=1.523, P=0.21; OR = 0.45 (95%CI: 0.15-1.29). †Antinuclear antibodies; *Juvenile idiopathic arthritis; ‡Arthritis related to infection.

Nineteen of 81 (23.4%) children included in the study had positive ANAs test results; 7/42 (16.6%) of whom had JIA: 5/27 (18.5%) children with oligoarticular (two of them oligoarticular form associated uveitis), 2/10 (20%) children with polyarticular rheuma factor (Rf) negative form of JIA. Twelve of 39 (30.7%) children with ARI had positive ANAs test results, nine children with ReA and three children with postinfectious arthritis; but none of the children with septic arthritis had positive ANAs test results. We did not find significant differences in ANAs positivity between JIA or ARI group of children (χ^2=1.523, P=0.21) (Table 3).

Only one child with JIA and 10/39 (25.6%) children with ARI had a positive ANA profile, where the positivity of three antibodies was observed in the former (against centromere protein B, nucleosome, histone). The statistically significant difference (P=0.002) was found for the positivity of the ANA profile between the examined groups of children. Three children with ARI had the positivity of more than one antibody, one child of two (antibody against ribonucleoprotein, histone) and two children of three antibodies (against centromere protein B, histone, antiribosomal P protein) in the ANA profile.

Discussion

In our study, 16.6% of children with JIA had positive ANAs findings, of which the largest number was noted in girls with oligoarticular form of arthritis at a young age, 18.5% (median 3.7 age). These findings were expected considering that ANAs positivity amongst the JIA subtypes is highest in children with the oligoarticular form of JIA (up to 70%), particularly more prevalent in young females (10, 12). Also, other researchers reported lower frequency of ANAs positive in children with JIA. Glerup et al. (8), Kwon et al. (9) and Lee et al. (13) found positive ANAs, which was most common in younger female patients with the oligoarticular form, in 37%, 28% and 33% of children, respectively. On the other hand, Raveli et al. (7) observed the high ANAs positivity in children with JIA (74.2%), 42.6% of whom had persistent oligoarthritis, 18.7% had extended oligoarthritis and 12.9% had Rf negative polyarthritis. Borchers et al. (14) found ANAs positivity in the Caucasian population, 38%-85% in pauci/oligoarthritis, 30%-50% in polyarthritis, and up to 17% in systemic JIA. The fact that the ANAs test is a non-specific test of autoantibody reactivity against nuclear antigens and that it does not clearly associate with differences in prognosis in any subtype of JIA, might be the possible reason for a low rate of positive ANAs findings in children with JIA. The monospecific immunoassays such as ELISA can lead to inaccurate (false-negative) screening results for antinuclear antibodies. This is possible primarily because of the limited number of purified or recombined antigens that are part of the assay (15). Although the positive ANAs test is not essential in the diagnosis of JIA, its prognostic utility for the risk of developing JIA-associated uveitis is clear, specifically in children with oligo- or poly-articular JIA, at younger age with asymmetric patterns of arthritis (16, 17). In our study, 2/7 positive ANAs findings in children with JIA were observed in girls with the oligoarticular form of JIA with uveitis. We noticed ANAs positivity in 30.7% of children with ARI, and 25.6% of those children had positivity in the ANA profile. This result was expected, since ANAs can be nonspecifically elevated due to infections and other causes of immune activation, which may result in the transient positivity of ANAs or a false positive result (3, 4, 10). The results of the previously conducted study by Neuer et al. (18) indicated that autoantibodies against the non-histone nucleosomal protein high mobility

group protein (HMG) 17 were detected in a high percentage of ANAs positive children with the oligoarticular form of JIA. The authors mapped the structures of HMG-17 antigens and defined proline and lysine-rich octapeptides as major epitopes recognizing more than 70% of ANAs-positive JIA children who had antibodies against HMG-17 protein. The sequence comparison showed significant homology between the autoimmune epitopes of HMG-17 and the antigens of certain infectious organisms, arguing in favor of the possible existence of molecular mimicry as a potentially significant factor in the etiology of JIA. This also highlighted the importance of determining ANAs by methods that involve the use of complete cells as antigens to detect their existence. Our results were somewhat different, we found a significant number of positive ANAs in children with JIA, 7/42 (16.6%), but only one child showed positivity in the ANA profile (antibody against cenp B, nucleosome and histone). Profiling of the autoantigen repertoire in patients with JIA is expected to greatly enhance the understanding of the molecular and cellular mechanisms that lead to a loss of immune tolerance and autoantibody production. In their study Stoll et al. (19) used a microarray approach to test sera of patients with oligoarticular JIA for reactivity to over 100 autoantigens. These authors suggested that autoantibodies directed against more specific autoantigens may be better biomarkers than ANAs with respect to JIA prognosis. ANAs positivity is the feature of many autoimmune diseases with the prevalence between 20% and 27% depending on the disease and type of autoantibodies, however, it may also be present in acute and chronic infections as well as in various types of ARI (4, 8, 16). In accordance with this, Ma et al. (20) in their study pointed out that the ANAs test demonstrated no ability to discriminate children with JIA from children presenting with other musculoskeletal complaints. Similarly, our study found no statistically significant differences with regard to the positivity of ANAs between the examined groups of children.

The Limitations of the Study

The major limitation of the study was a small sample size. Including a larger number of children with various JIA forms would enable further research on the prevalence of autoantibodies in the ANA profile.

Conclusion

In everyday practice, it is very important to distinguish JIA from other types of arthritis with the similar clinical presentation such as ARI. ANAs tests have low diagnostic utility in clarifying the diagnosis of JIA, and should not be used as a screening tool if JIA is the diagnosis under consideration. The persistence of ANAs was not associated with the type of arthritis, juvenile or ARI. Positive ANAs status does, however, increase the risk of uveitis and thus its use in clinical practice is primarily focused on predicting the ophthalmologic complications of JIA.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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