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Original Article

Anti-Nuclear Antibody Staining Patterns in Juvenile Idiopathic Arthritis: Association of AC-1 Pattern and Elevated Titers with Uveitis

Juvenil İdiopatik Artritte Anti-Nükleer Antikor Boyanma Paternleri: AC-1 Paterni ve Yüksek Titrelerin Üveit ile İlişkisi

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ABSTRACT

Objective: This study aimed to investigate antinuclear antibody (ANA) staining patterns and titers in patients with juvenile idiopathic arthritis (JIA)-associated uveitis, idiopathic uveitis, and JIA without uveitis, in order to identify serologic profiles that may contribute to disease pathogenesis and guide clinical decision-making.

Methods: We analyzed patients with JIA and/or uveitis at our tertiary center with ANA titers ≥1/100. Patients were grouped as JIA-associated uveitis, JIA without uveitis, and idiopathic uveitis. Diagnoses followed International League of Associations for Rheumatology and standardization of uveitis nomenclature criteria. ANA testing was performed by indirect immunofluorescence on *HEp-2* cells, and patterns and titers were evaluated per International Consensus on ANA Patterns guidelines. ANA profiles were compared across patient groups and JIA subtypes.

Results: Ninety-one patients were included: 21 (23%) with idiopathic uveitis, 12 (13.1%) with JIA-associated uveitis, and 58 (63.7%) with JIA without uveitis. The AC-1 pattern was present in all uveitis patients. The most common ANA patterns in JIA were AC-1 (65.7%), AC-4/5 (21.4%), and AC-2 (10%). ANA profiles differed across JIA subtypes ($p<0.001$), with AC-1 dominant in oligoarticular JIA (74.5%) and AC-4/5 in enthesitis-related arthritis (50%).

Conclusions: Our findings show that ANA pattern differences in JIA subtypes may provide significant clues regarding disease pathogenesis and clinical prediction. In particular, the prominence of the AC-1 pattern in JIA-associated uveitis may suggest a potential biomarker for the early identification of uveitis risk, which should be further explored in larger prospective studies

Keywords: Antinuclear antibodies, uveitis, juvenile idiopathic arthritis, staining patterns, titers

ÖZ

Amaç: Bu çalışma, juvenil idiyopatik artrit (JIA) ile ilişkili üveit, idiyopatik üveit ve üveitsiz JIA hastalarında antinükleer antikor (ANA) boyama paternlerini ve titrelerini araştırarak, hastalığın patogenezine katkıda bulunabilecek ve klinik karar verme sürecine rehberlik edebilecek serolojik profilleri belirlemeyi amaçlamıştır.

Yöntemler: Üçüncü basamak merkezimizde ANA titresi ≥1/100 olan JIA ve/veya üveitli hastaları analiz ettik. Hastalar JIA ile ilişkili üveit, üveitsiz JIA ve idiyopatik üveit olarak gruplandırıldı. Tanı, Uluslararası Romatoloji Dernekleri Birliği ve üveit terminolojisinin standartlaştırılması kriterlerine göre konuldu. ANA testi, *HEp-2* hücreleri üzerinde dolaylı immünofloresan ile yapıldı ve paternler ve titreler, ANA Paternleri Uluslararası Konsensüsü kılavuzlarına göre değerlendirildi. ANA profilleri, hasta grupları ve JIA alt tipleri arasında karşılaştırıldı.

Bulgular: Doksan bir hasta çalışmaya dahil edildi: 21 (%23) idiyopatik üveit, 12 (%13,1) JIA ile ilişkili üveit ve 58 (%63,7) üveitsiz JIA tanılı idi. AC-1 paterni tüm üveit hastalarında mevcuttu. JIA'da en sık görülen ANA paternleri AC-1 (%65,7), AC-4/5 (%21,4) ve AC-2 (%10) idi. ANA profilleri JIA alt tiplerine göre farklılık gösterdi ($p<0,001$), oligoartiküler JIA'da AC-1 (%74,5) ve entezit ilişkili artritte AC-4/5 (%50) baskındı.

Sonuçlar: Bulgularımız, JIA alt tiplerindeki ANA patern farklılıklarının, hastalığın patogenezi ve klinik öngörü ile ilgili önemli ipuçları sağlayabileceğini göstermektedir. Özellikle, JIA ile ilişkili üveitte AC-1 paterninin öne çıkması, üveit riskinin erken tanımlanması için potansiyel bir biyomarker olarak düşünülebilir. Ancak, bu sonuçları doğrulamak ve klinik uygulamaya entegre etmek için daha büyük, prospektif çalışmalar gereklidir.

Anahtar kelimeler: Antinükleer antikorlar, üveit, juvenil idiyopatik artrit, boyama desenleri, titreler

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INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of diseases characterized by chronic arthritis of unknown cause with onset before the age of 16[1,2]. Different clinical subtypes are defined by international classification criteria and antinuclear antibody (ANA) positivity stands out as a determinant serologic marker, especially in oligoarticular JIA[3]. Chronic anterior uveitis is a significant clinical manifestation of JIA, predominantly observed in the oligoarticular subtype, and in those with ANA positivity[4]. If not promptly identified and managed, this condition can result in irreversible vision impairment, thereby constituting a major health concern for individuals with JIA[4-7].

The ANA test is a widely used method in the serologic evaluation of autoimmune diseases[8,9]. This test, which is performed on *HEp-2* cells by indirect immunofluorescence (IIF) method, defines not only autoantibody positivity and titer level, but also staining patterns determined according to the target structures of autoantibodies[10]. These patterns are mainly categorized into nuclear, cytoplasmic, and mitotic groups and provide important clues in clinical evaluation[11]. The diagnostic and prognostic value of ANA patterns has increasingly been investigated because they show characteristic associations with some autoimmune diseases[12-14]. A limited number of studies in the literature have evaluated the discrimination of specific ANA patterns in JIA and JIA-associated uveitis[15,16]. However, the clinical reflection of specific patterns underlying ANA positivity and their relationship with the development of uveitis in JIA patients has not yet been fully elucidated. Accordingly, this study investigates ANA staining patterns and titers in patients with JIA-associated uveitis, idiopathic uveitis, and JIA without uveitis, aiming to delineate distinctive serologic markers that may inform the pathogenesis and guide clinical decision-making.

MATERIALS and METHODS

Patients and Data Collection

Patients admitted to our tertiary care center between June 2022 and June 2025 with a diagnosis of JIA and/or uveitis and a positive ANA test titer of 1/100 or higher were included in the study. Exclusion criteria included those with concomitant systemic autoimmune or autoinflammatory conditions or infectious uveitis, and those without sufficient clinical and laboratory data. Patients were divided into three groups according to their clinical diagnosis: JIA-associated uveitis, JIA without uveitis, and idiopathic uveitis. Patients with JIA were classified according to the International League of Associations for Rheumatology criteria[17]. Patients with uveitis were classified according to the Standardization of Uveitis Nomenclature criteria[18]. All uveitis diagnoses were established by ophthalmologists, and patients were followed jointly by pediatric rheumatology and ophthalmology clinics. In our cohort, all patients with JIA-associated uveitis were diagnosed with uveitis either at the time of JIA diagnosis or within the first year of follow-up. Demographic variables such as age at diagnosis and sex, laboratory parameters including acute phase reactants and ANA titers and patterns, clinical features such as JIA subtype and presence or type of uveitis, and as well as treatment data, were collected from patient records. In patients with JIA, disease activity at diagnosis was assessed using the juvenile arthritis disease activity score-71 (JADAS-71).

This study was conducted in compliance with the Helsinki Declaration as well as local laws and regulations. Informed consent was obtained from the patients and their legal caregivers. The ethics committee of Istanbul Medeniyet University Göztepe Training and Research Hospital tertiary center approved our study (approval number: 2023/0919, dated: 12023).

Evaluation of ANA Staining Patterns and Titers

ANA testing was performed using IIF assay on *HEp-2* cell substrates with the EUROPLUS ANA mosaic (EUROIMMUN, Lübeck, Germany). *HEp-2* IIF staining patterns and titer levels were evaluated according to the International Consensus on ANA Patterns guidelines[19]. ANA staining patterns and titer levels were compared between the three patient groups. In addition, patients with JIA were divided into subgroups according to JIA subtypes and ANA staining patterns, and titer levels were compared among these subgroups.

Statistical Analysis

We performed the statistical analysis using Statistical Package for the Social Sciences (SPSS) for Windows, version 26.0 (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test was used to assess the distribution of continuous variables. While variables with a normal distribution were presented as mean ± standard deviation, distributed abnormally were presented as median (minimum-maximum). The chi-square test or Fisher's exact test was used to compare the categorical variables, which were expressed as numbers (percentages). For continuous variables, One-Way ANOVA or the Kruskal-Wallis test was used for comparisons across more than two groups, and the Mann-Whitney U test was applied for comparisons between two groups. Bonferroni

adjustment was applied in post-hoc analyses for multiple comparisons. In addition, analysis of JADAS-71 scores across ANA staining pattern groups in JIA patients was performed using One-Way ANOVA (or Kruskal-Wallis test where appropriate). A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of All Patients

A total of 91 patients were included in the study, of whom 21 (23%) had idiopathic uveitis, 12 (13.1%) had JIA-associated uveitis and 58 (63.7%) had JIA without uveitis. Gender distribution was 34% (n=31) male and 66% (n=60) female. The median age at diagnosis was 12.30 (3.50-16.50) years in the idiopathic uveitis group; 5.72 (1.50-13.50) years in the JIA-associated uveitis group; and 9.87 (1.00-16.42) years in the JIA without uveitis group. All patients with JIA-associated uveitis had chronic anterior uveitis and were classified in the oligoarticular JIA subtype. Among patients with JIA without uveitis, oligoarticular JIA was also the most common subtype (67.2%, n=39), followed by enthesitis-related arthritis (20.6%, n=12), while other subtypes were observed less frequently (Figure 1). In the idiopathic uveitis group, anterior uveitis was present in the majority of cases (85.7%, n=18), and panuveitis was seen in the remainder (14.2%, n=3). The AC-1 staining pattern was detected in all patients with JIA-associated uveitis. Among those without uveitis, AC-1 remained the most common pattern (58.6%), followed by AC-4/5 (22.4%) and AC-2 (12%). Patients with idiopathic uveitis most frequently exhibited AC-1 (52.3%), followed by AC-4/5 (28.5%) and AC-2 (19%) staining patterns (Figure 1). When the patients with idiopathic uveitis, JIA-associated uveitis, and JIA without uveitis were compared based on the presence/absence of the AC-1 pattern, the difference was statistically significant (Fisher's exact test, p=0.007).

Comparison of Clinical and Laboratory Characteristics Among All Patient Groups

The median age at diagnosis was significantly younger in the JIA-associated uveitis group compared to the other groups (p=0.006). In addition, C-reactive protein and erythrocyte sedimentation rate were significantly lower in the idiopathic uveitis group than in the other groups (p=0.012, p=0.010). There was no statistically significant difference in ANA staining patterns and titer levels between the groups (Table 1). Additionally, JADAS-71 scores demonstrated no significant variation across both ANA staining pattern groups (p=0.522) and ANA titer groups (p=0.247).

Comparative Analysis of ANA Profiles in JIA Subtypes

The three most prevalent patterns identified were AC-1 (44, 62.8%), AC-4/5 (13, 18.5%), and AC-2 (7, 10%). The AC-1 pattern was notably more common in oligoarticular JIA, while the AC-4/5 pattern was significantly more prevalent in enthesitis-related arthritis (ERA) compared to the other groups (p<0.001) (Table 2). An ANA titer of 1/320-1/1000 was significantly more frequent in oligoarticular JIA than in other groups, whereas a titer of 1/100 was more commonly observed in patients with ERA than in other groups (p<0.001) (Table 3).

DISCUSSION

This study compared ANA profiles among patients with JIA-associated uveitis, idiopathic uveitis, and JIA without uveitis, revealing serological differences between the groups. The AC-1 staining pattern was significantly more common in oligoarticular JIA patients, while the AC-4/5 pattern predominated in patients with ERA. In contrast, all patients with JIA-associated uveitis exhibited the AC-1 pattern. When the patients with idiopathic uveitis, JIA-associated uveitis, and JIA without uveitis, were compared according to the presence or absence of the pattern, a statistically significant difference was observed, indicating a marked enrichment of AC-1 in JIA-associated uveitis. ANA patterns in the other patient groups were more heterogeneous. This finding suggests that the AC-1 pattern may serve as a potential serological marker for uveitis risk in JIA; however, given the limited sample size, this observation should be interpreted with caution and requires validation in larger, prospective studies.

Uveitis is the most common extraarticular involvement in patients with JIA and may lead to permanent vision loss or blindness if left untreated[4]. It is critical to understand the risk of uveitis in the follow-up of JIA patients, due to its significant contribution to prognosis. ANA positivity in oligoarticular JIA is known to be a risk factor for the development of uveitis[5]. In our study, all JIA-associated uveitis patients were observed to exhibit AC-1 positivity, which is a nuclear homogenous staining pattern. This pattern is usually associated with autoantibodies against nuclear antigens such as double-stranded DNA, nucleosomes, histones, and chromatin[17]. Indeed, one study has shown that JIA-associated uveitis is associated with antihistone antibodies[20]. A proteomic analysis to identify specific novel autoantigens for JIA-associated uveitis revealed that 17 autoantigens were associated with uveitis, and five of them were against nuclear components[21]. Moreover, studies in adult rheumatoid arthritis (RA) have reported more frequent pulmonary

involvement in patients with nuclear homogeneous ANA staining, suggesting that this pattern may also be relevant to systemic organ involvement in other contexts.

The heterogeneous ANA staining patterns, we found in the idiopathic uveitis group suggest that this disease group differs immunologically from JIA-associated uveitis and that it may have more diverse autoantibody profiles, perhaps playing a secondary or limited role in the pathogenesis of the disease. Indeed, a previous study has shown that ANA positivity in pediatric patients with noninfectious uveitis is not an independent risk factor for the need for biologic therapy or the development of uveitis-related complications[22]. This finding suggests that the clinical predictive power of ANA in idiopathic uveitis may not be as strong as in JIA-associated uveitis. Additionally, the significantly lower acute phase reactant levels observed in the idiopathic uveitis group compared to the JIA groups suggest a more limited inflammatory process and further support the distinct immunological nature of these disease entities.

Previous studies have reported that the most frequently observed staining patterns in JIA are nuclear homogeneous and fine granular patterns[10,15,16].

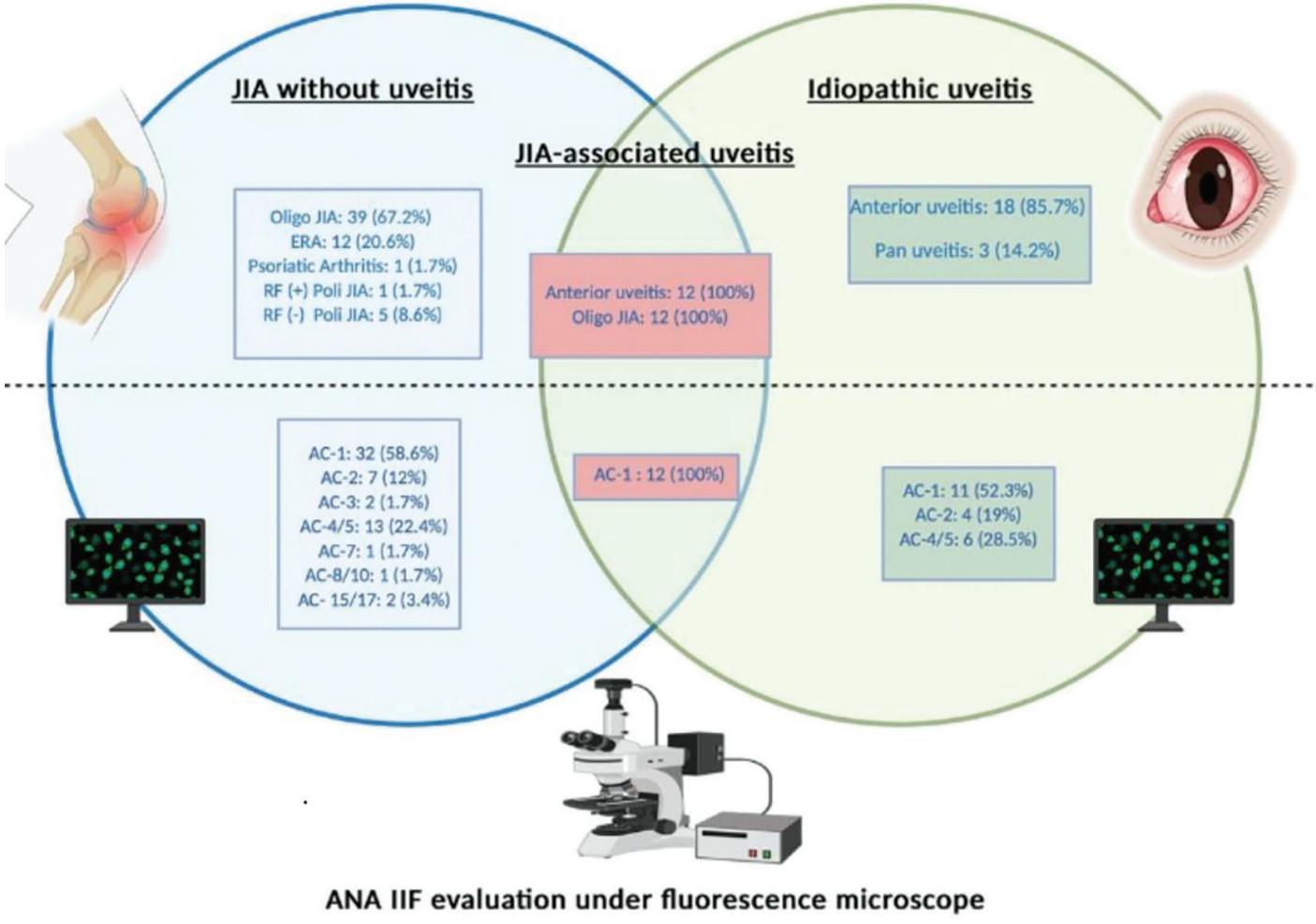

Figure 1. Distribution of antinuclear antibody (ANA) staining patterns and clinical subtypes among patients with juvenile idiopathic arthritis (JIA) with and without uveitis, and idiopathic uveitis. The Venn diagram illustrates the overlap and distinct features of JIA-associated uveitis. All patients in the JIA-associated uveitis group had anterior uveitis and were classified as oligoarticular JIA. ANA patterns were evaluated by indirect immunofluorescence using *HEp-2* cells and classified according to the International Consensus on ANA Patterns nomenclature. AC-1 pattern was detected in 100% of JIA-associated uveitis cases. In contrast, AC-4/5 pattern was more frequent in idiopathic uveitis and JIA without uveitis, particularly in patients with enthesitis-related arthritis (ERA).

Table 1. Comparison of clinical and laboratory characteristics among all patient groups.				
	Idiopathic uveitis group (n=21)	**JIA-associated uveitis group (n=12)**	**JIA without uveitis group (n=58)**	**p**
Male gender (n,%)	9 (42.9%)	1 (8.3%)	21 (36.2%)	0.112
Age at diagnosis (year) (median/min-max)	12.30 (3.50-16.50)	5.72 (1.50-13.50)	9.87 (1.00-16.42)	**0.006**
ANA titers				0.168
1/100	7 (33.3%)	0 (0%)	16 (27.6%)	
1/100-1/320	8 (38.1%)	6 (50%)	21 (36.2%)	
1/320-1/1000	4 (19.0%)	4 (33.3%)	14 (24.1%)	
1/1000-1/3200	2 (9.5%)	2 (16.7%)	7 (12.1%)	
>1/3200	0 (0%)	0 (0%)	0 (0%)	
ANA patterns				0.298
AC-1	11 (52.4%)	12 (100%)	34 (58.6%)	
AC-2	4 (19.0%)	0 (0%)	7 (12.1%)	
AC-3	0 (0%)	0 (0%)	1 (1.7%)	
AC-4/5	7 (33.3%)	2 (16.7%)	13 (22.4%)	
AC-7	0 (0%)	0 (0%)	1 (1.7%)	
AC-8/10	0 (0%)	0 (0%)	1 (1.7%)	
AC-15/17	0 (0%)	0 (0%)	2 (3.4%)	
Acute phase reactants at diagnosis				
CRP (mg/L) [median (min-max)]	2 (0.2-27)	4 (0.1-21)	8.06 (0.13-146)	**0.012**
ESR (mm/h) [median (min-max)]	10 (2-53)	22 (3-42)	29.5 (2-120)	**0.010**

AC: Anti-cell, AC-1: Nuclear homogeneous pattern, AC-2: Nuclear dense fine speckled pattern, AC-3: nuclear centromere pattern, AC-4/5: Nuclear fine speckled/coarse speckled patterns, AC-7: Nuclear few dots pattern, AC-8/10: Homogeneous nucleolar/punctate nucleolar; AC-15/17: Cytoplasmic fibrillar linear/cytoplasmic fibrillar segmental, ANA: Antinuclear antibody, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate,JIA: Juvenile idiopathic arthritis

Table 2. Comparison of anti-nuclear staining patterns among Juvenile idiopathic arthritis subgroups.						
	JIA subgroups (n,%)					
	Oligo JIA (51, 56%)	**ERA (12, 13.2%)**	**Psoriatic arthritis (1, 1.1%)**	**RF (+) poliJIA (1, 1.1%)**	**RF (-) poliJIA (5, 5.5%)**	***p**
ANA staining pattern						**<0.001**
AC-1	38 (74.5%)	2 (16.7%)	1 (100%)	0 (0%)	3 (60%)	
AC-2	4 (7.8%)	2 (16.7%)	0 (0%)	1 (100%)	0 (0%)	
AC-3	2 (3.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
AC-4/5	6 (11.7%)	6 (50%)	0 (0%)	0 (0%)	1 (20%)	
AC-7	1 (1.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
AC-8/10	0 (0%)	1 (8.3%)	0 (0%)	0 (0%)	0 (0%)	
AC-15/17	0 (0%)	1 (8.3%)	0 (0%)	0 (0%)	1 (20%)	

*The AC-1 pattern was significantly more frequent in oligoarticular JIA than in other subtypes. The AC-4/5 pattern was significantly more frequent in ERA than in other subtypes.

AC: Anti-cell, AC-1: Nuclear homogeneous pattern, AC-2: Nuclear dense fine speckled pattern, AC-3: Nuclear centromere pattern, AC-4/5: Nuclear fine speckled/coarse speckled patterns, AC-7: Nuclear few dots pattern, AC-8/10: Homogeneous nucleolar/punctate nucleolar, AC-15/17: Cytoplasmic fibrillar linear/cytoplasmic fibrillar segmental, ANA: Antinuclear antibody, ERA: Enthesitis-related arthritis, RF: Rheumatoid factor, JIA: Juvenile idiopathic arthritis

Table 3. Comparison of anti-nuclear titers among juvenile idiopathic arthritis subgroups.						
	JIA subgroups (n,%)					
	Oligo JIA (51, 56%)	ERA (12, 13.2%)	Psoriatic arthritis (1, 1.1%)	RF (+) poliJIA (1, 1.1%)	RF (-) poliJIA (5, 5.5%)	*p
ANA titers						**<0.001**
1/100	5 (9.8%)	10 (83.3%)	0 (0%)	1 (100%)	0 (0%)	
1/100-1/320	22 (43.1%)	2 (16.6%)	0 (0%)	0 (0%)	3 (60%)	
1/320-1/1000	17 (33.3%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	
1000-1/3200	7 (13.7%)	0 (0%)	1 (100%)	0 (0%)	1 (20%)	

*ANA titer of "1/320-1/1000" was significantly more frequent in oligoarticular JIA than in other groups. A titer of 1/100 was more frequently observed in patients with ERA than in other groups.

ANA: Antinuclear antibody, ERA: Enthesitis-related arthritis, RF: Rheumatoid factor, JIA: Juvenile idiopathic arthritis

Consistently, the most common staining patterns in our JIA patients were AC-1 and AC-4/5. While AC-1 was the most common staining pattern in our patients with oligoarticular JIA, AC-4/5 was the most common one in those with ERA. This finding aligns with the results reported by Sener et al.[15], who also identified similar AC-1 positivity rates in oligoarticular JIA, although they noted AC-4/5 as the most common pattern. However, the AC-4/5 pattern was also the most frequent in their ERA patients, consistent with our observations. As is well established, human leukocyte antigens-B27 rather than ANA positivity is typically expected in ERA, in line with its immunogenetic background. In addition, ANA titer distribution showed a significant difference between JIA subtypes in our study, with medium and high titers predominating in oligoarticular JIA and low titers in the ERA subtype. The nuclear homogeneous staining pattern has been reported to be a risk factor for RA[23]. In contrast, we observed a high variability of titers and staining patterns of ANA among our JIA patients. While adult RA exhibits a homogeneous disease profile with symmetric polyarthritis, JIA is a clinically and immunologically heterogeneous disease with several subtypes[24,25]. Thus, we considered that our results may reflect the immunologic heterogeneity of JIA; and different autoantibody profiles may be at the forefront according to subtypes.

Study Limitations

The main limitations of our study are its retrospective design, the limited number of patients, and the fact that ANA testing was only evaluated at the time of diagnosis and not repeated during periods of disease remission or exacerbation. This may have resulted in missing serologic changes related to disease activity. However, the main strength of the study is it included not only patients with JIA but also patients with idiopathic uveitis, allowing comparison of serologic patterns and thus contributing to a better understanding of the pathogenesis of JIA and idiopathic uveitis. In addition, this is the second study in a limited number of studies, in which ANA staining patterns were evaluated in detail with AC codes, which is an important contribution to the literature.

CONCLUSION

Our findings show that ANA pattern differences in JIA subtypes may provide significant clues regarding disease pathogenesis and clinical prediction. In particular, the prominence of the AC-1 pattern in JIA-associated uveitis may suggest a potential biomarker for early identification of uveitis risk; however, this possibility requires confirmation in larger prospective studies before integration into clinical practice.

Ethics

Ethics Committee Approval: The ethics committee of Istanbul Medeniyet University Göztepe Training and Research Hospital tertiary center approved our study (approval number: 2023/0919, date: 13.12.2023).

Informed Consent: Informed consents were obtained from the patients and their legal caregivers.

Footnotes

Author Contributions

Concept: L.K., F.E., Ö.T., F.K., E.K., Z.A., E.N.D., H.K.D., M.Ö.B., F.H., K.Ö., Design: L.K., F.E., K.Ç., Ö.T., F.K., E.K., E.N.D., M.Ö.B., F.H., K.Ö., Data Collection and/or Processing: L.K., K.Ç., Ö.T., E.K., Z.A., H.K.D., F.H., K.Ö., Analysis and/or Interpretation: L.K., K.Ç., Ö.T., F.K., Z.A., H.K.D., M.Ö.B., Literature Search: L.K., Ö.T., F.K., Z.A., H.K.D., H.K.D., F.H., K.Ö., Writing: L.K., F.H., K.Ö.

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