Clin Rheumatol (2017) 36:671–676
DOI 10.1007/s10067-017-3540-x

BRIEF REPORT

Remission rate is not dependent on the presence of antinuclear antibodies in juvenile idiopathic arthritis

M. Glerup[1] · **T. Herlin**[1] · **M. Twilt**[1,2]

Received: 2 November 2016 / Revised: 10 January 2017 / Accepted: 10 January 2017 / Published online: 17 January 2017

Abstract Recently, it has been hypothesized that the subcategories of the ILAR classification of juvenile idiopathic arthritis (JIA) are not homogeneous, and that the presence of antinuclear antibodies (ANA) should lead to a separate entity. Therefore, the aim of this study was to evaluate ANA positivity as a predictor of achieving remission. A retrospective single-center cohort study including all JIA patients diagnosed between January 2000 and May 2014. A minimum follow-up of 1 year was required plus the ANA status. ANA positivity was defined as at least two positive results with a titer ≥1:160. Demographic and clinical features were collected. Remission at last follow-up was defined by the Wallace criteria. A total of 625 patients met the inclusion criteria and 230 (37%) were found ANA positive. Analysis showed no difference in remission rate between ANA-positive and ANA-negative patients. Additionally, joint count at diagnosis and at last follow-up were comparable in both groups. ANA positivity was correlated to a female predominance and young age at diagnosis ($p < 0.001$). Remission rates are not different in ANA-positive patients than in ANA-negative patients. This does not support the hypothesis to possibly divide JIA patients based on their ANA status.

Keywords Antinuclear antibodies · Juvenile idiopathic arthritis · Remission

✉ M. Glerup
miagleru@rm.dk

[1] Department of Paediatrics, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark

[2] Department of Paediatrics, division of Rheumatology, Alberta Children's Hospital, University of Calgary, Calgary, AB, Canada

Introduction

Juvenile idiopathic arthritis (JIA) is the most common autoimmune condition in childhood, with a yearly incidence of 15 per 100,000 children in Nordic countries [1]. The International League of Associations for Rheumatology (ILAR) developed criteria to divide JIA into categories based on distinct clinical features and/or laboratory findings to identify homogeneous entities suitable for studies and guidance in clinic [2].

In recent years, the rationale for the ILAR criteria for JIA has been debated. It is felt that each of the subcategories still encounters heterogeneous conditions and the criteria may not be adequate for selecting homogeneous entities [3–8]. Antinuclear antibody (ANA) positivity can be found in all subtypes but is more frequent in patients with oligoarthritis and rheumatoid factor negative polyarthritis. The frequency of ANA positivity among oligoarticular patients has been reported between 61 and 75% [9, 10]. However, the antigenic specificity of the ANAs in JIA has not been elucidated, and no evidence of a role for ANAs in the pathophysiology has been demonstrated [11].

It has been hypothesized that ANA-positive patients irrespective of their underlying JIA subtype represent the same disease and it has been questioned whether patients with early onset disease and a positive ANA should be grouped as a separate category unrelated to the number of joints affected [7, 8, 12–14]. This is based on the fact that most ANA-positive patients are younger at age of onset, demonstrate a female predominance, predominantly have asymmetric arthritis, have a higher risk of developing uveitis, and show a lack of hip involvement [8]. The literature is not uniform concerning the number of affected joints during the disease course. In ANA-negative patients with polyarticular JIA Ravelli et al. found a greater cumulative number of affected joints over time [8]. On the other hand, Ma et al. reported a higher number of cumulative active joints among

ANA-positive Japanese patients within the first year of the disease [14] and Guzman et al. reported an increased risk of flare associated with ANA positivity in a larger study in the ReACCh-Out cohort [15].

In the literature, evidence to support a separation based on ANA status is contradictory and depends heavily on the specific outcomes studied.

Therefore, the aim of this study was (1) to describe the characteristics of JIA patients based on their ANA status and (2) to compare remission and inactive disease rates based on ANA status.

Patients and methods

Patients

This was a single-center, retrospective study of children diagnosed with JIA at Aarhus University Hospital, Denmark, between January 2000 and May 2014. Patients were identified through the divisional database which includes all patients seen in the rheumatology clinic. Patients were included if they were diagnosed before the age of 16 years, fulfilled the ILAR criteria for JIA [2], ANA results were available, and had a follow-up of 1 year or more. ANA positivity was detected using indirect immunofluorescence (IIF) testing with Hep-2 cells as substrate [19]. ANA positivity was defined as at least two positive results with a titer of ≥1:160, with ANA tests performed at least 3 months apart. Patients with a follow-up of less than 1 year and with only one ANA test performed were excluded.

Demographic and clinical features

Demographic and clinical data were collected from the divisional database, paper charts, and electronic medical records. Demographic features included age at diagnosis, gender, age at disease presentation, number of active joints at diagnosis, presence of rheumatoid factor, ANA status, presence of uveitis, and medication during the disease course. Features at last follow-up included age at last visit, number of active joints, current medication, and remission status.

Remission

At last follow-up, remission status was defined by the Wallace preliminary criteria [16]. Remission on medication was defined as at least 6 continuous months of inactive disease on medication. Clinical remission off medication was defined as 12 months or more of inactive disease off all anti-rheumatic (and anti-uveitis) medication [16]. Inactive disease was defined as no active arthritis; no fever, rash, serositis, splenomegaly, or generalized lymphadenopathy attributable to JIA; no active uveitis; and normal erythrocyte sedimentation rate or C-reactive protein.

Statistical analysis

Descriptive statistics were reported as absolute numbers and frequencies for categorical variables and mean and standard deviation for continuous variables. Comparison analyses between two groups were performed by a *t* test. Categorical data were compared by Pearson's Chi-square test. To determine independent factors for achieving remission, binary logistic regression analyses were performed. IBM SPSS Statistics, version 24 (IBM Corp, Armonk, NY, USA) was used for all analyses.

Results

Patients

A total of 685 patients with the diagnosis of JIA between January 2000 and May 2014 were identified in the divisional database. Two patients were excluded because of a wrong diagnosis (one with isolated uveitis, one with mixed connective tissue disease (MCTD)) and 58 patients because of insufficient data on ANA status. Six hundred twenty-five patients fulfilled ILAR criteria, had two recorded ANA tests and were followed for more than 1 year, and were included in this study. Of the included patients, 405 were female and 220 patients were male with a mean age at diagnosis of 98 months (Table 1) and mean disease duration 66.9 months (SD 36.9). The most common subtype was oligoarticular persistent JIA affecting 215 (34%) children, followed by polyarticular RF negative JIA affecting 125 (20%) children and oligoarticular extended JIA affecting 101 (16%) children. All patient demographic data can be found in Table 1.

ANA positivity was present in 230 (37%) of all JIA patients, and ANA negativity in two thirds of patients (63%). There are significant differences between the two groups with a more pronounced female predominance in the ANA-positive group ($p < 0.001$) and a difference in subtype distribution. We found a significantly higher frequency of ANA positivity in oligoarticular JIA but a low frequency of positive ANA in systemic, psoriatic and undifferentiated JIA (Table 1). In the ANA-positive group, the most frequent subtype represented was the oligoarticular and polyarticular RF-negative subtypes.

ANA-positive patients had a significantly younger age at diagnosis than ANA-negative patients (Tables 1 and 2). No significant difference in active joint count either at diagnosis or at last follow-up was observed between ANA-positive and ANA-negative patients in the total JIA cohort (Table 2). Psoriatic JIA patients showed higher number of active joints

Table 1 Demographic data of study population

	Total	ANA negative	ANA positive	Odds Ratio[a]	Chi-square	*P*
JIA (all)	625	395 (63%)	230 (37%)			
Gender	405 girls (64.8%)	224 girls (56.7%)	181 girls (78.7%)	0.355	29.851	<0.001
	220 boys (35.2%)	171 boys (43.3%)	49 boys (21.3%)			
Systemic JIA	52 (8.3%)	47 (11.9%)	5 (2.2%)	0.165	16.77	<0.001
Oligoarticular persistent	215 (34.4%)	119 (30.1%)	96 (41.7%)	1.662	8.179	0.004
Oligoarticular extended	101 (16.2%)	53 (13.4%)	48 (20.9%)	1.702	5.420	0.020
Polyarticular RF-neg	125 (20.0%)	70 (17.7%)	55 (23.9%)	1.459	3.107	0.078
Polyarticular RF-pos	21 (3.4%)	12 (3.0%)	9 (3.9%)	1.300	0.126	0.722
Psoriatic JIA	41 (6.6%)	35 (8.9%)	6 (2.6%)	0.276	8.278	0.004
ERA	38 (6.1%)	30 (7.6%)	8 (3.5%)	0.438	3.623	0.057
Undifferentiated JIA	32 (5.1%)	29 (7.4%)	3 (1.3%)	0.167	9.700	0.002
Age at diagnosis* (months)	98 (±53)	110 (±49)	77 (±53)			
Age 0–48 months	163 (26.1%)	67 (17%)	96 (42%)	3.51	45.013	<0.001
Age 49–96 months	125 (20%)	77 (19.5%	48 (21%)	1.089	0.097	0.756
Age 97–144 months	193 (30.9%)	141 (36%)	52 (23%)	0.526	11.060	0.001
Age > 144 months	144 (23%)	110 (28%)	34 (15%)	0.449	13.267	<0.001

Percentage in brackets indicate % within total, ANA negative or ANA positive, respectively

JIA juvenile idiopathic arthritis, *ANA* antinuclear antibodies, *RF* rheumatoid factor, *ERA* juvenile enthesitis-related arthritis

[a] Odds ratio: Mantel-Haenszel common odds ratio estimate

at diagnosis in the ANA-positive group ($p = 0.048$). In all other subtypes, ANA-positive and ANA-negative patients had a similar average joint count at diagnosis. At last follow-up, the average joint counts were low in all subtypes and, irrespective of their ANA status, all subtypes had comparable average joint count (Table 2).

Almost 80% of patients were in remission on or off medication at their last follow-up appointment (Table 3), which was also resembled in the low average joint count at last follow-up (0.1 ± 0.459) with 93% of patients with no active joints (joint count = 0). More than half of the patients were in remission off medication for more than 12 months at last follow-up. We found no significant difference in the remission rate on or off medication between the ANA-positive and ANA-negative patients (Table 3). ANA-positive patients show a significant high risk for the presence of uveitis (OR = 7.8, $p < 0.001$) at one point during the course of the disease, and a low probability of HLA-B27 positivity ($p = 0.046$) (Table 3). At last follow-up, 41/625 patients had 1 or more active joints, without a significant difference in ANA-status (Table 3).

The likelihood of achieving remission on medication for at least 6 months or remission off medication for more than 12 months was not related to ANA or gender (Table 4). The likelihood of achieving remission off medication (for more than 12 months) was significantly less seen in RF-positive patients, in HLA-B27-positive patients, and in patients with uveitis at any time during the disease course (Table 4).

Discussion

To our knowledge, this is the first study to systematically evaluate remission on and off medication in JIA patients based on their ANA status.

As expected, in our study, ANA positivity was found to be associated with younger age at onset, female predominance, oligoarticular subtypes, and presence of uveitis at any time during the disease course. These findings indicate the generalisability of the cohort, as it closely resembles what is found in the literature [1, 2, 17].

This study shows that remission rates are not significantly different based on ANA positivity. Recently, questions have been posed if ANA positivity should lead to a different classification, where ANA positivity weighs heavily towards certain categories [7, 8, 12–14]. Our study clearly shows that ANA positivity does not alter disease outcome per se. This is in agreement with the findings of Albers et al. [18] who reported no difference in ANA status between patients with a remitting and an unremitting course 3–5 years after

Table 2 *t* test of mean differences of "age at diagnosis" and "joint count at diagnosis" and at follow-up between patients with pos. or neg. ANA

		ANA neg (SD)	ANA pos (SD)	*t*	*p*
N		395	230		
Age at diagnosis (months)		110 ± 49	77 ± 53	7.81	< 0.001
Systemic	N	47	5	−1.482	0.209
	JC at Dx	0.81 (1.728)	3.20 (3.564)		
	JC at FU	0 (0)	0 (0)		
Oligo persistent	N	119	96	−0.841	0.401
	JC at Dx	1.39 (0.702)	1.47 (0.725)	0.710	0.478
	JC at FU	0.08 (0.372)	0.11 (0.432)		
Oligo extended	N	53	48	−1.797	0.075
	JC at Dx	1.74 (0.923)	2.10 (1.134)	−0.501	0.617
	JC at FU	0.08 (0.267)	0.10 (0.309)		
Poly RF-neg	N	70	55	1.426	0.156
	JC at Dx	7.70 (5.251)	6.44 (4.455)	0.688	0.493
	JC at FU	0.16 (0.828)	0.07 (0.424)		
Poly RF-pos	N	12	9	0.444	0.662
	JC at Dx	7.00 (7.160)	5.89 (2.472)	−0.692	0.497
	JC at FU	0.17 (0.389)	0.33 (0.707)		
Psoriatic	N	35	6	−2.037	0.048*
	JC at Dx	2.37 (2.486)	4.67 (2.944)	−1.535	0.180
	JC at FU	0.14 (0.43)	0.67 (0.816)		
Enthesitis related arthritis	N	30	8	−0.066	0.947
	JC at Dx	2.30 (3.098)	2.38 (1.302)	0.720	0.476
	JC at FU	0.17 (0.648)	0 (0)		
Undifferentiated	N	29	3	−0.381	0.706
	JC at Dx	2.52 (2.098)	3.00 (2.00)	0.317	0.753
	JC at FU	0.03 (0.186)	0 (0)		
Joint count at diagnosis		2.89 ± 3.8	3.1 ± 3.2	−0.81	0.42
Joint count at last follow-up		0.09 ± 0.48	0.12 ± 0.43	−0.623	0.534

ANA antinuclear antibodies, *RF* rheumatoid factor, *JC at Dx* joint count at diagnosis, *JC at FU* joint at follow-up, *SD* standard deviation

diagnosis. In contrast, Guzman et al. [15] found that ANA positivity was associated with increased risk of any flare after attaining inactive disease and this was only partly explained by their association with uveitis (Hazard ratio (HR) 1.16 when excluding all flares with uveitis). Notably, they define the cut-off for ANA positivity as low as 1:80 which can explain the higher ANA positivity (43.7%) compared to our cohort and may contribute to the high HR.

Table 3 Chi-square test of ANA-status and RF, HLA-B27, uveitis, and remission

	Total	ANA negative	ANA positive	Odds ratio[a]	Chi-Square	*p*
JIA (all)	625	395 (63.2%)	230 (36.8%)			
RF	23 (3.7%)	13 (3.3%)	10 (4.3%)	1.336	0.208	0.648
HLA-B27	71 (11.4%)	53 (13.4%)	18 (7.8%)	0.548	3.975	0.046
Uveitis	53 (8.5%)	11 (2.8%)	42 (18.3%)	7.799	42.887	<0.001
Active joints at last FU[b]	41 (6.6%)	22 (5.6%)	19 (8.3%)	1.527	1.307	0.253
Remission on medication >6 months	160 (25.6%)	97 (24.6%)	63 (27.4%)	1.159	0.473	0.491
Remission off medication >12 months	336 (53.8%)	223 (56.5%)	113 (49.1%)	0.745	2.850	0.091

JIA juvenile idiopathic arthritis, *ANA* antinuclear antibodies, *RF* rheumatoid factor

[a] Odds ratio: Mantel-Haenszel common odds ratio estimate

[b] Number of patients with one or more active joints at last follow-up

Table 4 Binary logistic regression analysis predicting likelihood of achieving remission on or off medication

	Remission ON medication >6 months		Remission OFF medication >12 months	
	P	Exp(B) = Odds ratio (95% CI)	*p*	Exp(B) = odds ratio (95% CI)
Gender	0.744	0.935 (0.624–1.401)	0.208	1.255 (0.882–1.787)
ANA	0.884	1.031 (0.687–1.546)	0.399	0.858 (0.601–1.225)
RF	0.041	2.461 (1.037–5.841)	0.001	0.122 (0.035–0.418)
HLA-B27	0.006	2.117 (1.244–3.603)	0.012	0.511 (0.303–0.862)
Uveitis	0.009	2.280 (1.234–4.213)	0.012	0.458 (0.248–0.844)

ANA antinuclear antibodies, *RF* rheumatoid factor

Compared to ANA-positive patients, Ravelli et al. [7, 8] found that the cumulative number of affected joints during the first 6–24 months of disease was higher in ANA-negative patients irrespective of being categorized as oligo- or polyarticular JIA. This made Ravelli et al. conclude that ANA-positive patients should be classified as a separate category [7, 8]. In contrast, our study reports no significant difference in joint counts in ANA-positive and ANA-negative patients; both at diagnosis and after more than 5 years on average at last follow-up. The latter remained present even with all JIA subcategories represented, while Ravelli et al. [7, 8] did not include all JIA subtypes, but excluded systemic, enthesitis-related, and RF-positive polyarticular JIA.

Further, remission rates on and off medication according to the Wallace preliminary criteria were not significantly different, but very comparable in ANA-positive and ANA-negative patients. This again would not support the hypothesis that ANA positivity alone should be a separate category.

There are some limitations to this study. This is a retrospective study and this design could potentially lead to bias. However, the population studied includes all patients seen in one single tertiary center in Denmark, with a relatively homogeneous ethnical Danish population. This is still the largest study completed to date evaluating outcome based on ANA positivity, and analysis provides a clear picture of the remission rates overall and in both groups. Notably, ANA testing was performed by indirect immunofluorescence (IIF) assays on HEp-2 cells [19, 20] since testing for ANA using an enzyme-linked immunosorbent assay (ELISA) has shown limited value in patients with JIA [21]. However, the IIF test has been criticized for its variability among studies [6] which may be explained by the use of the test among different ethnical groups also known for differences in distribution of JIA subcategories [22]. All ANA tests in this study were performed with at least 3 months' interval, by the same method, and in the same lab during the years of investigation and as in previous studies [7, 8] the minimum cut-off titer for ANA positivity used here was set at 1:160 in order to lower the risk of false-positive results.

In conclusion, this study shows there is no difference in the number of active joint count at diagnosis and at follow-up or in remission rate based on ANA positivity in children with JIA. The ANA-positive patients are more frequently girls, have a younger onset age, and have associated uveitis, but these factors, however, do not differ their remission rates in this cohort. Our data do not support the hypothesis for a possible separate JIA category based on ANA positivity.

Compliance with ethical standards

Disclosures None.

References

1. Berntson L, Andersson Gare B, Fasth A, Herlin T, Kristinsson J, Lahdenne P et al (2003) Incidence of juvenile idiopathic arthritis in the Nordic countries. A population based study with special reference to the validity of the ILAR and EULAR criteria. J Rheumatol 30(10):2275–2282
2. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J et al (2004) International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 31(2):390–392
3. Ferrell EG, Ponder LA, Minor LS, Angeles-Han ST, Kennedy CW, Rouster-Stevens KA et al (2014) Limitations in the classification of childhood-onset rheumatoid arthritis. J Rheumatol 41(3):547–553
4. Martini A (2003) Are the number of joints involved or the presence of psoriasis still useful tools to identify homogeneous disease entities in juvenile idiopathic arthritis? J Rheumatol 30(9):1900–1903
5. Martini A (2012) It is time to rethink juvenile idiopathic arthritis classification and nomenclature. Ann Rheum Dis 71(9):1437–1439
6. Nordal E, Songstad NT, Rygg M (2011) Difficulties in defining antinuclear antibody-positive patients as a separate category in the

classification of juvenile idiopathic arthritis: comment on the article by Ravelli et al. Arthritis Rheum 63(9):2835 author reply -6
7. Ravelli A, Varnier GC, Oliveira S, Castell E, Arguedas O, Magnani A et al (2011) Antinuclear antibody-positive patients should be grouped as a separate category in the classification of juvenile idiopathic arthritis. Arthritis Rheum 63(1):267–275
8. Ravelli A, Felici E, Magni-Manzoni S, Pistorio A, Novarini C, Bozzola E et al (2005) Patients with antinuclear antibody-positive juvenile idiopathic arthritis constitute a homogeneous subgroup irrespective of the course of joint disease. Arthritis Rheum 52(3): 826–832
9. Guillaume S, Prieur AM, Coste J, Job-Deslandre C (2000) Long-term outcome and prognosis in oligoarticular-onset juvenile idiopathic arthritis. Arthritis Rheum 43(8):1858–1865
10. Al-Matar MJ, Petty RE, Tucker LB, Malleson PN, Schroeder ML, Cabral DA (2002) The early pattern of joint involvement predicts disease progression in children with oligoarticular (pauciarticular) juvenile rheumatoid arthritis. Arthritis Rheum 46(10):2708–2715
11. Burlingame RW, Rubin RL, Rosenberg AM (1993) Autoantibodies to chromatin components in juvenile rheumatoid arthritis. Arthritis Rheum 36(6):836–841
12. Felici E, Novarini C, Magni-Manzoni S, Pistorio A, Magnani A, Bozzola E et al (2005) Course of joint disease in patients with antinuclear antibody-positive juvenile idiopathic arthritis. J Rheumatol 32(9):1805–1810
13. van den Broek T, Hoppenreijs E, Meerding J, Scholman R, Otten HG, Swart JF et al (2015) Cytokine profiling at disease onset: support for classification of young antinuclear antibody-positive patients as a separate category of juvenile idiopathic arthritis. Ann Rheum Dis 74(2):470–472
14. Ma X, Xin L, Sun J, Liu Z (2016) Antinuclear antibody-positive cohort constitutes homogeneous entity in juvenile idiopathic arthritis. Modern Rheumatol 26(1):75–79
15. Guzman J, Oen K, Huber AM, Watanabe Duffy K, Boire G, Shiff N et al (2016) The risk and nature of flares in juvenile idiopathic arthritis: results from the ReACCh-Out cohort. Ann Rheum Dis 75(6):1092–1098
16. Wallace CA, Ruperto N, Giannini E (2004) Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis. J Rheumatol 31(11):2290–2294
17. Nordal EB, Zak M, Aalto K, Berntson L, Fasth A, Herlin T et al (2012) Validity and predictive ability of the juvenile arthritis disease activity score based on CRP versus ESR in a Nordic population-based setting. Ann Rheum Dis 71(7):1122–1127
18. Albers HM, Brinkman DM, Kamphuis SS, van Suijlekom-Smit LW, van Rossum MA, Hoppenreijs EP et al (2010) Clinical course and prognostic value of disease activity in the first two years in different subtypes of juvenile idiopathic arthritis. Arthritis Care Res 62(2):204–212
19. Osborn TG, Patel NJ, Moore TI, Zuckner J (1984) Use of the HEp-2 cell substrate in the detection of antinuclear antibodies in juvenile rheumatoid arthritis. Arthritis Rheum 27(11):1286–1289
20. Mahler M, Fritzler MJ (2012) The clinical significance of the dense fine speckled immunofluorescence pattern on HEp-2 cells for the diagnosis of systemic autoimmune diseases. Clin Dev Immunol 2012:494356
21. Nordal EB, Songstad NT, Berntson L, Moen T, Straume B, Rygg M (2009) Biomarkers of chronic uveitis in juvenile idiopathic arthritis: predictive value of antihistone antibodies and antinuclear antibodies. J Rheumatol 36(8):1737–1743
22. Saurenmann RK, Rose JB, Tyrrell P, Feldman BM, Laxer RM, Schneider R et al (2007) Epidemiology of juvenile idiopathic arthritis in a multiethnic cohort: ethnicity as a risk factor. Arthritis Rheum 56(6):1974–1984