Occurrence of antiperinuclear, antikeratin, and anti-RA 33 antibodies in juvenile chronic arthritis

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Abstract

Objectives—Antiperinuclear factor (APF), antikeratin antibodies (AKA), and anti-RA 33 antibodies are currently considered to be good markers for the diagnosis of adult rheumatoid arthritis with or without rheumatoid factor (RF). The prevalence of these markers was retrospectively reviewed in children with juvenile chronic arthritis (JCA) to determine whether they were associated with specific features.

Methods—One hundred and twenty-four patients with JCA participated in this study. Controls included 28 patients with juvenile systemic lupus erythematosus and 21 healthy children. Antiperinuclear factor and AKA were determined by indirect immunofluorescence on buccal mucosal cells and oesophagus sections respectively. Anti-RA 33 antibodies were detected using a Western blot technique on HeLa cell nuclear extract.

Results—Antiperinuclear factor was virtually absent in all the tested subgroups and anti-RA 33 antibodies were detected only in a subset of patients with RF positive polyarticular onset. Antikeratin antibodies were found in 27% of all children with JCA and in 42% of those with RF negative polyarticular onset. These results were statistically significant compared with healthy controls, but the presence of AKA was not specific to any patient subgroup. Moreover, in contrast with previous studies in adult RA, no relation was found between the presence of AKA and disease severity or activity.

Conclusion—These data suggest that APF, AKA, and anti-RA 33 antibodies are not useful for the diagnosis or classification of JCA.

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Detection of autoantibodies contributes to the diagnosis of many inflammatory rheumatic diseases. Rheumatoid factor (RF) in rheumatoid arthritis (RA) and antinuclear antibodies (ANA) in systemic lupus erythematosus (SLE) are included in the diagnostic criteria for these disorders.[1][2] Antiperinuclear factor (APF), antikeratin antibodies (AKA), and anti-RA 33 antibodies are currently considered to be good markers for adult RA, with or without RF, but little information is available on their occurrence in juvenile chronic arthritis (JCA).

Antiperinuclear factors are IgG antibodies directed against keratohyaline granules surrounding the nuclei of human buccal mucosal cells.[3][4] The exact biochemical nature of these antigens remains unknown, however. The presence of APF has been reported in the serum of 48–92% of patients with RA[5-7] and of 8·6–76% of seronegative patients with RA.[5-11] Although their occurrence in various other rheumatic disorders[10-12] and in patients infected with the Epstein-Barr virus has also been reported,[13] the specificity of APF for RA has been estimated at more than 90%.[7][9][11][14]

Antikeratin antibodies are complement fixing IgG antibodies directed against the stratum corneum of rat oesophagus squamous cells.[15][16] Evidence suggests that the keratin antigen shares some homology with the C terminal portion of the heterogeneous nuclear RNP protein A1.[17] These antibodies have been detected in 36–69% of patients with RA[5][11][15][16][18-22] and in 5–47% of seronegative patients with RA.[5][11][16][20][23] Antikeratin antibodies were shown to be highly specific for RA when detected in synovial fluid.[24] They were found in the serum of 0–8% of healthy controls and patients with other rheumatic disorders.[15][19-21][23][24] Higher percentages were also reported in subjects with progressive systemic sclerosis[22][25] and in patients with ankylosing spondylitis.[22]

More recently, anti-RA 33, an IgG antibody directed against a nuclear protein antigen, with a molecular weight of 33 kilodaltons and which seems be identical to the A2 protein of the heterogeneous nuclear ribonucleoprotein (hnRNP),[26] was detected in approximately one third of patients with RA[27] and in 27–45% of seronegative RA serum samples.[11][27]

Juvenile chronic arthritis presents as a variety of clinical patterns characterised by the absence of the usual serological markers, with the exception of RF positive JCA and pauciarticular onset JCA positive for ANA. The occurrence of APF in JCA serum samples has already been reported.[28] Serum APF was significantly associated with JCA only in patients with RF positive and RF negative polyarticular onset, however. Furthermore, these data are at variance with a study in which APF was nearly lacking in serum samples from patients with JCA.[29] Antikeratin antibodies have been detected in only one of 28 children with JCA included in a study of the occurrence of AKA in various rheumatic disorders.[21] Antibodies to RA 33 have not been studied in JCA to date.

The aim of this study was to determine the occurrence of these three autoantibodies in a

large cohort of patients with JCA, across the spectrum of onset patterns, and to look for associations between these antibodies and specific clinical features. Furthermore, because APF and AKA have been associated with increased disease severity[7] [8] [16] [18] [20] [23] and extra-articular features[12] [18] [21] [24] in patients with RA, we also sought to determine whether these antibodies were of use for predicting the course of articular manifestations or the development of selected extra-articular features in patients with JCA.

Patients and methods

PATIENTS

Serum samples from 124 patients with JCA fulfilling the EULAR/WHO criteria[30] were analysed retrospectively. Nearly all the children were followed up regularly at the 'Enfants-Malades' Hospital in Paris by one of us (AMP). Patients with JCA were classified according to the mode of onset: systemic in 24 cases; early pauciarticular in 51 cases; RF negative polyarticular in 24 cases, RF positive polyarticular in seven cases; and juvenile spondylarthropathy in 18 cases. This latter subgroup was defined according to criteria proposed in 1990.[31] Sex ratio, mean (SE) age at onset, and mean (SE) disease duration at the time of the study are shown in table 1 for the different subtypes.

The course of the articular disease, occurrence of specific systemic features and of acute or chronic uveitis, and treatment during follow up were abstracted from clinical charts. Among patients with pauciarticular onset JCA, those with persistent pauciarticular disease were defined as having a cumulative total of four or fewer affected joints over the entire period of follow up, whereas those who subsequently converted to a polyarticular course were defined as having a cumulative total of more than five affected joints. Chronic uveitis was defined as the presence of typical signs on slit lamp examination (performed two to three times a year in all patients with pauciarticular onset JCA during follow up). Physical examination with particular attention to the presence of active inflammatory osteo-articular features was performed at the time of serum collection. Erythrocyte sedimentation rate (ESR) was also measured in 109 of the 124 patients.

Control patients included 21 children without rheumatic disease nor inflammatory disorders and 28 children with SLE.

ANTIPERINUCLEAR ANTIBODY ASSAY

This assay was carried out as previously described by Youinou *et al.*[9] Briefly, human buccal mucosal cells were scraped with a wooden tongue depressor from the inner side of both cheeks of a healthy volunteer. They were washed three times in phosphate buffered saline (PBS), pH 7·4, and resuspended in PBS containing colimycin and sodium azide. The cells were then transferred dropwise to multispot slides, roughly 10 000 cells/well. After drying with a fan, the slides were used as the substrate. Serum samples were initially diluted 1:40 and applied to the slide for 90 minutes in a moist chamber. Slides were then washed three times with PBS, air dried, and incubated for 30 minutes with fluorescent antihuman IgG (Cappel Products, Flobio, Coubevoie, France) diluted to 1:80. The slides were then washed twice with PBS and once with distilled water, air dried, mounted with glycerol, and studied with a fluorescence microscope. The fluorescence of perinuclear homogeneous spheres in at least 10% of examined cells was considered to indicate a positive test for antiperinuclear antibodies. If positive at this dilution, serum samples were further diluted at 1:80.

ANTIKERATIN ANTIBODY ASSAY

Unfixed cryostat sections (2 µm thick) of the distal third of rat oesophagus were used as antigen targets for the detection of AKA using an indirect immunofluorescent technique. Cryostat sections were transferred to slides and dried by silica gel granules (Prolabo, Paris, France). Slides were kept at −30°C until the assays (for a few weeks). Slides were again dried with silica gel before the assay. Patient serum samples diluted to 1:20 in PBS were incubated on the slides for 30 minutes. The slides were rinsed twice for 10 minutes in PBS, air dried, and incubated for 30 minutes with a fluorescein labelled antihuman IgG (Institut Pasteur, Paris, France) diluted to 1:80. Slides were then rinsed twice for 10 minutes in PBS, air dried, mounted with glycerol, and studied with a fluorescence microscope. Only serum samples showing laminar fluorescent staining of the superficial layer of the oesophageal epithelium were considered positive. If positive at this dilution, serum samples were further diluted until the last positive titre was determined. The sensitivity and specificity of this assay for seropositive RA have been shown to be 63 and 96% respectively. Sensitivity for seronegative RA was 47·6%.[11]

ANTI-RA 33 ANTIBODY ASSAY

Nuclear extract was prepared as previously described[27] and subjected to 12% sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure described by Laemmli.[32] Immunological detection of RA 33 antibodies was performed after transfer to nitrocellulose[33] (one hour at 2·5 mA) using serum samples diluted 25-fold in incubation buffer (PBS containing

Table 1 Sex ratio, age at disease onset, and disease duration at the time of the study in the various subgroups of juvenile chronic arthritis (JCA). Age and disease duration are given as arithmetic mean (SE)

Diagnosis	Sex ratio (F/M)	Age at disease onset (years)	Disease duration (years)
Systemic onset JCA (n = 24)	12/12	4·96 (0·85)	4·84 (0·74)
Pauciarticular onset JCA (n = 51)	43/8	3·14 (0·33)	4·21 (0·50)
Polyarticular onset JCA, RF positive (n = 7)	6/1	8·36 (0·86)	7·49 (2·69)
Polyarticular onset JCA, RF negative (n = 24)	17/7	7·59 (0·84)	5·93 (1·31)
Juvenile spondylarthropathy (n = 18)	4/14	11·05 (0·57)	4·82 (1·03)

Abbreviations: F = female; M = male; RF = rheumatoid factor.

3% non-fat dried milk with and without 0·01% Triton-X100). Nitrocellulose strips were incubated with serum samples for 120 minutes with constant shaking. Bound autoantibodies were detected using phosphatase conjugated goat antihuman IgG antibodies (Cappel products) in PBS–3% non-fat dried milk–Triton 0·01%.

OTHER SEROLOGICAL ASSAYS

Antinuclear antibodies were determined before and at the time of the study by indirect immunofluorescence on rat liver sections. The test was considered as positive at a dilution of 1:20. Rheumatoid factor was evaluated at least twice by the Singer Plotz latex test during the follow up of all children with polyarticular onset JCA.

STATISTICAL ANALYSIS

Fisher's exact test for two or five groups was used to compare percentages. Statistical analysis was performed with Statxact (Cytel Software, Cambridge, MA, USA). Quantitative data (age of onset, disease duration, ESR values) were tested using unpaired t tests; two tailed p values <0.05 were considered significant.

Results

The presence of AKA, anti-RA 33 antibodies, and APF in 124 children with different onset patterns of JCA, 28 patients with juvenile SLE, and 21 healthy controls are shown in table 2. Antikeratin antibodies were detected in 27% of

all patients with JCA. This proportion was significantly greater than that in healthy controls ($p<0.05$). Antikeratin antibodies were found in 42% of patients with RF negative polyarticular onset JCA. This proportion was significantly higher than that in healthy controls. The proportions of AKA positive patients were not significantly different in the five JCA subgroups (5 × 2 tailed Fisher's exact test, however). Anti-RA 33 antibodies were found in 11% of all patients with JCA. This proportion was not significantly different from that in controls. The occurrence of these antibodies varied significantly across the five JCA subgroups ($p<0.001$); the subset of patients with RF positive polyarticular onset JCA had a significantly higher percentage of anti-RA 33 positive serum samples than healthy controls ($p<0.01$). Specificity of RA 33 antibodies in healthy controls was 100% (95% confidence interval, 84 to 100%). Anti-RA 33 antibodies were, however, also found in 21% of the children with SLE. Antiperinuclear antibodies were nearly always absent in the different groups of subjects.

To look for associations between AKA and specific clinical outcomes, we compared children positive and negative for AKA with children with RF negative polyarticular onset JCA. Age at disease onset and disease duration were identical in the two groups. As shown in table 3, no difference was found for disease activity during the study, as defined by the number of patients with active synovitis and increased ESR levels. The number of patients receiving disease modifying antirheumatic drugs was identical in the two groups. Systemic and ocular manifestations were absent in the two groups. Functional outcome could not be assessed as none of these children had a functional score higher than 2, as defined by the Steinbrocker's functional index.[34] The presence of AKA was not associated with specific clinical features in early onset pauciarticular JCA. Conversion to polyarticular disease and occurrence of chronic uveitis were not influenced by the status of AKA.

With the exception of one patient with polyarticular seropositive onset, dilution titres of serum samples positive for AKA ranged from 1:20 to 1:160 in patients with JCA, whereas these values are generally higher in adult RA, reaching 1:1000 in some instances. Dilution titres found in our study were compared with those in 14 RA serum samples positive for AKA assessed at the same time. Dilution titres in the RA serum samples positive for AKA were significantly higher than those found in our patients with JCA (data not shown). These results are in accordance with previous reports showing that high dilution titres of AKA are pathognomonic for adult RA.[16 20]

Results of tests for ANA performed before and during the study in patients with JCA and in healthy controls are shown in table 4. As previously reported,[35] these antibodies were found in significantly higher percentages of the pauciarticular and seropositive polyarticular

Table 2 Presence of antiperinuclear factors, antikeratin antibodies, and anti-RA 33 antibodies in children with juvenile chronic arthritis (JCA), juvenile systemic lupus erythematosus and healthy controls. Results given are number positive (%)

Diagnosis	No (%) positive APF	No (%) positive AKA	No (%) positive anti-RA 33
Juvenile chronic arthritis			
Systemic onset (n = 24)	0 (0)	3 (13)	2 (8)
Pauciarticular onset (n = 51)	0 (0)	12 (24)	1 (2)
Polyarticular onset, seropositive (n = 7)	1 (14)	2 (29)	4 (57)*
Polyarticular onset, seronegative (n = 24)	0 (0)	10 (42)†	2 (8)
Juvenile spondylarthropathy (n = 18)	1 (6)	6 (33)	3 (17)
Total (n = 124)	2 (1·6)	33 (27)‡	14 (11)
Juvenile systemic lupus erythematosus (n = 28)	0 (0)	5 (18)	6 (21)
Healthy controls (n = 21)	0 (0)	1 (5)	0 (0)

Abbreviations: APF = antiperinuclear factor; AKA = antikeratin antibodies. Statistical analysis by Fisher's exact test.
*$p<0.05$ v controls; comparisons of percentages between JCA subgroups showed significant differences.
†$p<0.05$ v controls; but comparisons of percentages between JCA subgroups showed no significant differences.
‡$p<0.05$ v controls.

Table 3 Comparison of patients with rheumatoid factor negative polyarticular onset juvenile chronic arthritis positive (AKA+) and negative (AKA–) for antikeratin antibodies. p Values were not significant in any instance

	AKA+ (n = 10)	AKA– (n = 14)
Mean (SD) age at onset (years)	7·72 (1·52)	7·48 (0·97)
Mean (SD) disease duration (years)	4·58 (1·7)	7·65 (1·9)
Mean (SD) ESR*	34·2 (7·9)	25·6 (4·7)
No (%) patients with active synovitis*	7/10 (70)	11/14 (79)
No (%) patients receiving DMARDs*	3/10 (30)	5/14 (36)

Abbreviations: ESR = erythrocyte sedimentation rate; DMARDs = disease modifying antirheumatic drugs. Statistical analysis by Fisher's exact test and unpaired t test.
*At time of study.

Table 4 Presence of antinuclear antibodies (ANA) in children with juvenile arthritis before and during the study

Diagnosis	No (%) ANA positive	
	Before study	During study
Juvenile chronic arthritis		
Systemic onset (n = 24)	1 (4)	0 (0)
Pauciarticular onset (n = 51)	45 (88)	36 (71)*
Polyarticular onset, RF positive (n = 7)	2 (29)	4 (57)*
Polyarticular onset, RF negative (n = 24)	2 (8)	2 (8)
Juvenile spondylarthropathy (n = 18)	0 (0)	1 (6)
Total (n = 124)	49 (40)	43 (35)*
Healthy controls (n = 21)		0 (0)

*$p<0.01$ v healthy controls by Fisher's exact test.

Table 5 Comparison between antinuclear antibodies (ANA) and antikeratin antibodies (AKA) or anti-RA 33 antibodies in serum samples of patients with juvenile chronic arthritis (JCA). Results given are numbers of positive (+) or negative (−) serum samples

Diagnosis	ANA	AKA		Anti-RA 33	
		+	−	+	−
Systemic onset (n = 24)	+	0	0	0	0
	−	3	21	2	22
Early onset pauciarticular (n = 51)	+	8	28	0	36
	−	4	11	1	14
Polyarticular onset, seronegative (n = 24)	+	1	1	0	2
	−	9	13	2	20
Polyarticular onset, seropositive (n = 7)	+	1	3	2	2
	−	1	2	2	1
Juvenile spondylarthropathy (n = 18)	+	0	1	0	1
	−	7	10	3	14
Total JCA (n = 124)	+	10	33	2	41
	−	24	57	10	71

Statistical analysis by Fisher's exact test shows no correlations between ANA and AKA or anti-RA 33 in patients with JCA (p values ranged from 0.2 to 1).

onset subgroups than in controls ($p<0.01$ and $p<0.0001$ respectively). Antinuclear antibody status was independent of the results of tests for other antibodies (table 5). Among the 45 patients with pauciarticular onset JCA positive for ANA, 41 (91%) had a homogeneous fluorescent pattern. Antinuclear antibodies were present in all the patients with chronic uveitis and in 79% of those without ocular complications ($p<0.06$). Native anti-DNA antibodies were not detected in any of the JCA serum samples.

Discussion

Consistent with a previous report,[29] APF was rarely detected in the different JCA subgroups included in this study. This result is, however, at variance with that of Nesher et al,[28] who found these antibodies in up to 37% of children with polyarticular onset JCA. This percentage was even higher in RF positive JCA (83%).

The numerous hypotheses which may explain this difference include inadequate sensitivity of our assay; methodological differences involving serum dilution and criteria for positive results; and ethnic differences between study populations.

The sensitivity and specificity of our assay have been assessed in RA and in other adult rheumatic disorders. The occurrence of APF was found in 56% of RF positive (73 serum samples) and 33% of RF negative (21 serum samples) patients with RA, with a specificity of 93% (unpublished data). These results are in accordance with those published elsewhere. A major cause of variability in APF results is the variation of substrate antigen between different

donors.[36] This problem probably had no influence in our assay as we used a single donor.

Nesher et al found that APF was significantly detected only in undiluted serum or at a dilution of 1:5.[28] As in previous studies,[9] serum samples were diluted to 1:40 and 1:80 in our assay. To determine whether this difference might explain the variation in APF results, we tested 30 APF negative JCA serum samples at dilutions ranging from 1:2 to 1:20. Antiperinuclear antibodies were detected at dilution 1:2 in only two patients, who belonged to different JCA subgroups (data not shown).

Most centres have their own criteria for positive APF results. We and others[9] consider that the test is positive when 10% or more of the cells show the typical perinuclear fluorescence. This threshold of positivity has been shown to increase specificity to 97% for the diagnosis of RA, without appreciably reducing sensitivity.[9] For others, one or a few typical cells are sufficient for a positive result. Previous studies in JCA have not provided details on this point.

It has been shown that the sensitivity of the APF test for the diagnosis of adult RA can be increased by pretreating cells with the detergent Triton-X100.[37] This procedure reduces specificity, however. We therefore did not use Triton-X100 in our assay. Furthermore, as found by Von Essen et al,[29] Triton-X100 did not increase the sensitivity of APF for the diagnosis of JCA.

Nesher et al reported that the prevalence of APF varies across populations.[38] Our patients were ethnically diverse and, consequently, it is unlikely that our results were due to a selection of a particular ethnic group.

The presence of AKA was significant only in a subset of patients with RF negative polyarticular onset JCA compared with healthy controls. The sensitivity of AKA was, however, rather poor, not exceeding 50%. In addition, AKA could not be considered specific for this subset of JCA as the percentages of serum samples positive for AKA did not differ significantly across JCA subgroups. In contrast with previous reports in adult RA,[16 18 20 21 23] AKA status was not related to disease activity or severity. The value of our result is, however, limited by the fact that our patients were not followed up longitudinally.

Anti-RA 33 antibodies have been previously reported as strongly specific for the diagnosis of RA.[27] In one study, however, anti-RA 33 antibodies were also detected in patients with other rheumatic disorders, including in 60% of patients with mixed connective tissue disease and 25% of those with SLE (unpublished data). Our findings in children with SLE are thus in accordance with results in adults. The rate of anti-RA 33 positivity was significant only in patients with RF positive polyarticular onset JCA, a fact which limits the value of this test. This finding requires confirmation because of the small number of patients in this subgroup.

In conclusion, our results show that APF, AKA, and anti-RA 33 antibodies do not

contribute to the diagnosis or classification of JCA, which still relies mainly on clinical findings and on the presence of RF and ANA. These data are at variance with those found in adult RA serum samples, suggesting that, with the exception of a subset of patients with RF positive polyarticular onset JCA, JCA is a heterogeneous entity different from adult RF positive RA.

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