Scripta Scientifica Medica, vol. 30 (1998), pp. 115-122

CLASS-SPECIFIC RHEUMATOID FACTORS IN JUVENILE CHRONIC ARTHRITIS. CLINICAL ASSOCIATIONS

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The study aimed at determining the prevalence of the class-specific rheumatoid factors (RFs) in juvenile chronic arthritis (JCA) and evaluating the diagnostic test qualities. A total of JCA 79 patients (48 with pauciarticular form, 17 of whom with extending pauciarthritis), 31 with polyarticular for, as well as 125 control children (58 healthy and 67 disease controls) were tested. Most JCA patients (95%) had negative Waaler-Rose test. ELISA for the detection of IgM-, IgA- and IgG- isotypes of RFs was used. The diagnostic characteristics of the tests were evaluated by clinico-epidemiological methods (assessment of sensitivity, specificity, positive predictive value, and likelihood ratio). The prevalence of IgG-, IgA- and IgM-RF in JCA versus all controls at optimal cut off titres was 16 %, 22 % and 32%, respectively. The test for IgG-RF was not diagnostically significant. IgA-RF had the highest prevalence in the polyarticular form (40 %). The diagnostic qualities of the test were significant, mostly concerning the differentiation of the polyarticular course of JCA and the presence of erosive arthritis. IgM-RF was most typical of the polyarticular form (55 %) followed by the extending pauciarthritis (41 %). The diagnostic test qualities were significant and most prominent to distinguish the polyarticular course and erosive arthritis. IgM- and IgA-RFs had significant diagnostic value in JCA, mainly for the polyarticular and extending pauciarticular form and also in regard to more severe disease course. IgG-RF had no diagnostic significance for JCA.

Key-words: Juvenile chronic arthritis, rheumatoid factors, ELISA, diagnostic tests, epidemiology

Juvenile chronic arthritis (JCA) is a heterogeneous disease that encompasses different forms defined by the type of onset. There is evidence supported by immunogenetic studies that the various subgroups may represent distinct disease entities (1,2). Numerous immunological abnormalities have been detected in JCA, but the most characteristic serological findings are ANA and IgM-rheumatoid factor thought to be useful in patients' classification and management. Antinuclear antibodies are most commonly found in children with early onset pauciarthritis and late onset seropositive polyarthritis (1-3). In

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contrast, the IgM-rheumatoid factor measured by conventional agglutination techniques, is a hallmark only of a polyarthritis with late onset resembling adult rheumatoid arthritits. This group of patients with "seropositive" disease represent less than 20 % of all JCA children. Using more sensitive techniques, it has already been established that rheumatoid arthritis (RA) patients' sera contain not only the "classical" 19S IgM-RF, but also other isotypes of rheumatoid factor (RF). Lots of studies have emphasized the presence of IgG-, IgA-, IgM- and even IgE-RF in patients with "seronegative" RA (4-6).

The aim of this study is to determine the prevalence and to attempt to evaluate the diagnostic and prognostic qualities of the ELISA-tests for rheumatoid factor isotypes in polyarticular and pauciarticular forms at onset of JCA and compare them.

MATERIAL AND METHODS

Patients

We studied serum samples from 204 children. Seventy-nine of them fulfilled the EULAR-criteria for JCA and were classified by subgroup onset types as followed: 48 with pauciarticular disease (17 of whom with extending pauciarticular disease) and 31 with polyarticular form. The patients' age ranged from 1,5 to 18 years. They had active disease for at least one year before the sampling and most of them (82 %) were having active disease at the time of study. All patients were taking NASAIDs and/or slow-acting antirheumatic drugs. Agglutination test for 19S IgM-RF by Waaler-Rose was positive in 4 cases (5 %). Fifty patients had non-erosive and 29 had erosive arthritis. One hundred and twenty five control children were tested simultaneously - 58 healthy controls ranging in age from 2 to 14 years and 67 patients (22 with SLE and 45 with other inflammatory and autoimmune diseases).

Methods

Clinical evaluation of the immunologic findings was made in regard to the following clinical criteria: form of the disease, disease activity and radiological progression.

The following immunological methods were used:

1) IgM-RF ELISA assay (developed in CLB-Amsterdam). In brief, flat-bottomed 96-well microtitre plates (MAXI SORP, NUNC) were coated with 150 l 25 g human gamma globulin/ml in PBS overnight. After washing with 0,1 % Tween 20 in PBS, 100 l serum 1/100 diluted in PBS was added and incubated for 2 hours at 37C. After washing, 100 l conjugated mouse anti-human IgM to HRPO diluted in PBS 0,1 %Tween 20 was incubated for 2 hours in 37C. After washing 100 l substrate (OPD/H_2O_2) was incubated for 10-20 min. The reaction was stopped by 5N H_2SO_4. Absorption was measured by Dynatech reader at 492 nm.

WHO rheumatoid factor standard was used (kindly provided by H. G. M. Geertzen from CLB-Amsterdam). In each plate a control pool of sera from negative healthy children was tested simultaneously. As positive results were considered those exceeding 12,5 IU, weak positive - between 6,25 and 12,5 IU, and negative results - below 6,25.

2) IgG- and IgM-RF ELISA assay (developed in Immunology Lab of The National Centre of Allergology-Sofia). The methods differed from those for IgM-RF at the following points: 1) rabbit IgG was used as antigen; 2) the upper limits for IgG-RF and IgA-RF were defined based on the mean value of the healthy control population plus 2 SD.

The statistical and epidemiological methods included variation analysis, *t*- statistics, Fisher's exact test and test characteristics for diagnostic significance as defined by the clinical epidemiology methods (7,8) (sensitivity, specificity, positive predictive value and likelihood ratio).

RESULTS AND DISCUSSION

The mean values of the different RF isotypes in the control groups and JCA and their upper limits are shown in Table 1.

The prevalence of IgG-, IgA- and IgM-RF in JCA and subgroups are presented in Table 2.

Table 1

Mean values and upper limits of the RF isotypes in JCA

RF isotype	Control group (mean value ± SD)	Upper limits for control sera (x ± 2 SD)	JCA patients (mean value ± SD)
IgG	0,16 ± 0,069	0,30	0,18 ± 0,09
IgA	0,17 ± 0,089	0,35	0,23 ± 0,14
IgM	below 3 IU	6,25 IU	6,45 IU

Table 2

Prevalence of IgG, IgA and IgM-RFs in JCA and subgroups compared to controls

Isotype	healthy controls	control patients		JCA-total		non-extending pauciarthritis		extending pauciarthritis		pauciarticular form total		polyarticular form	
	n=58	n=67	%	n=79	%	n=31	%	n=17	%	n=48	%	n=31	%
IgG-RF	-	8/67	12%	13/79	16%♣	7/31	23%	1/17	6 %	4/48	17 %	5/31	16 %
IgA-RF	-	4/67	6%	17/78	22%*	2/31	6 %	3/17	18 %	5/48	10%♥	12/30	40%*♦
IgM-RF	-	3/67	4%	18/55	33%*	1/21	5%	5/12	42%*#	6/33	8%♠	12/22	55%*#

? p=0.024 compared to control group

**p< 0.001 compared to controls*

#p< 0.001, ? p=0.02 compared to non-extending pauciarticular form

? p=0.0028 and ? p=0.009 compared to polyarticular form

The mean values of IgG-RF in JCA and control groups did not significantly differ from one another. There were no healthy children expressing IgG-RF. Increased antibody levels were found in 12 % of the control patients. Similar prevalence was observed in the JCA group where in 13/79 (16 %) IgG-RF was detected. Diagnostic characteristics of the test were not significant for JCA (Table 3).

No significant clinical associations were established in JCA patients (Fig.1).

Table 3

Diagnostic characteristics of the ELISA-tests for IgG-, IgA- and IgM-RFs

Diagnostic characteristics	JCA/controls			polyarticular form			non- extending pauciarthritis			extending pauciarthritis		
Isotype	IgG-RF	IgA-RF	IgM-RF	IgG-RF	IgA-RF	IgM-RF	IgG-RF	IgA-RF	IgM-RF	IgG-RF	IgA-RF	IgM-RF
Sensitivity	0.17	0.22	0.33	0.16	0.4	0.55	0.23	0.06	0.05	0.06	0.18	0.42
Specificity	0.95	0.97	0.97	0.95	0.97	0.97	0.95	0.97	0.97	0.95	0.97	0.97
Positive predictive value	0.62	0.81	0.83	0.38	0.75	0.79	0.47	0.33	0.53	0.11	0.50	0.64
Likelihood ratio	3.4	8.0	11.0	3.2	13.3	18.3	4.6	2.0	2.0	1.2	6.0	14

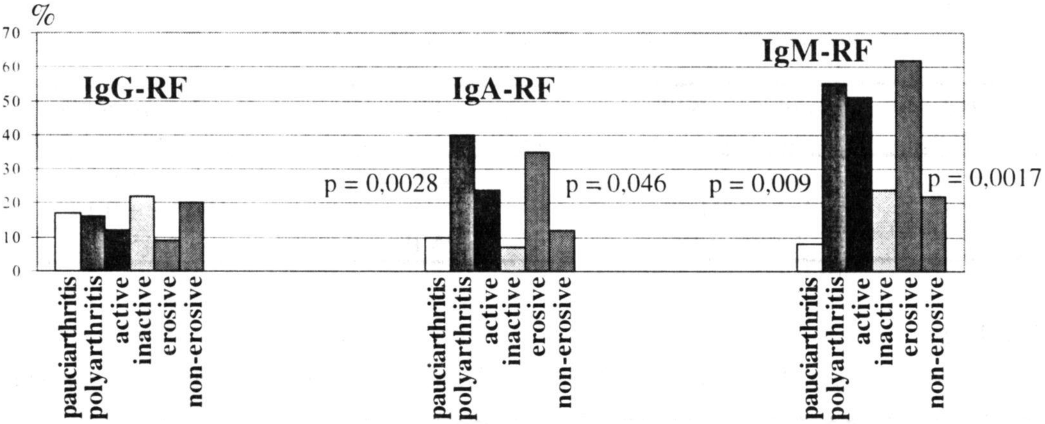

Fig. 1. Clinical associations of class-specific RFs

The mean value of IgA-RF in JCA was found to be higher as compared to healthy children ($p < 0,001$) and disease controls ($p = 0,007$). IgA-RF was not observed in healthy children and was present in 6 % of those from the disease control group only. Positive IgA-RF was established in 17/79 (22 %) of JCA patients. This prevalence was significantly higher when compared to the controls. The highest prevalence of IgA-RF was registered in the polyarticular group 12/30 (40 %) followed by the extending pauciarticular 3/17 (33 %) and non-extending pauciarticular form 2/31 (6 %). Significant difference was also found between the pauciarticular and polyarticular form; non-exetending and extending pauciartritis. Diagnostic characteristics of the test are presented on Table. 3. IgA-RF showed an association with polyarticular form and erosive disease (Fig.1).

IgM-RF was not detected in the healthy children (< 6,25 IU). In the disease control group there were low positive test results (6,25-12,5 IU) in 3 patients (4 %) but a borderline line result of 6,25 UI - in one patient only. The mean value of the IgM-RF in JCA was increased as compared to the healthy children (p < 0,001) and disease controls (p = 0,014). The majority of positive patients expressed weak positive levels of IgM-RF (6,25-12,5 IU) and only 6/18 (33 %) patients all having polyarthritis showed positive levels exceeding 12,5 IU. The prevalence of IgM-RF in JCA was 18/55 (32 %), i. e., significantly higher than that in the control group.

Regarding the distribution of the positive results in JCA we found that the majority of positive patients had polyarthritis 12/55 (22 %), 5/55 (9 %) had extending pauciarticular, and 1/55 (2 %) - non-extending pauciarthritis. All proportions were significantly distinguished from each other - polyarthritis *vs* non-extending pauciarthritis (p < 0,001), polyarthritis *vs* extending pauciarthritis (p = 0,014), and extending pauciarthritis *vs* non-extending one (p = 0,029). On analyzing the prevalence of IgM-RF in the disease subgroups, we established that the antibody was present in 12/22 (55 %) of the patients with polyarthritis, in 5/12 (42 %) of those with extending pauciarticular disease and in 1/21 (5 %) of the patients with non-extending pauciarthritis. There was a significant difference of the prevalence of IgM-RF in the polyarticular form as compared to the control group, non-extending pauciartritis and extending one. Extending pauciarthritis prevalence was higher when compared to the controls and non-extending pauciarticular disease. Diagnostic characteristics of the test for IgM-RF we demonstrated on Table 3. Clinical evaluation of the findings revealed an association of IgM-RF with polyarticular dosease and erosive arthritis (Fig. 1). Eleven of 20 positive patients (55 %) expressed more than one RF isotype.

Classical 19S IgM-RF is an indicator of erosive arthritis and serological marker for the late-onset polyarticular JCA (less than 20 % of the disease population). The presence of this antibody in younger patients and such with other forms is unusual (2,9). Over the last 15 years a lot of studies have demonstrated that "seronegative" patients with RA and JCA express different isotypes of RF in their sera (6,10-14). Moreover, Moore et al. established "hidden" IgM-RF in the immune complexes of sera from JCA children correlating with the disease activity (1,4). In our study we found IgG-, IgA- and IgM-RF in the sera of "seronegative" patients with JCA. Our results showed that the prevalence of IgG-RF was not significantly higher than that of the controls. Approximately the same prevalence was observed in the SLE patients (14 %). Epidemiological patterns of diagnostic value of this test were insignificant. No significant clinical associations were found. These results correspond to other studies (11,12,15,16) which have reported a prevalence of IgG-RF ranging from 4 to 8 % with no clinical significance. In our study low IgA-RF levels were demonstrated in the JCA patients with a significant prevalence of 22 % as compared to the controls. The highest prevalence of 40 % was observed in the polyarticular group where the highest antibody levels were detected. The extending pauciarthritis showed a lower prevalence than the polyarticular form but a significantly higher one than the non-extending pauciarthritis. According to the epidemiological characteristics, IgA-RF had diagnostic value mainly for the polyarticular JCA. We also found that IgA-RF correlated with erosive arthritis, which indicates a more severe disease. IgA-RF was reported to be a highly specific marker for disease activity and severity in RA (5,17,18) with recently confirmed diagnostic significance (19). Walker et al. (20) demonstrated IgA-RF in 58 % of the JCA patients with polyarthritis, strongly correlating with the disease activity, whereas the antibody was absent in the pauciarticular and systemic form. In contrast, Saulsbury (9) observed the highest prevalence of IgA-RF in the pauciarticular form.

We found IgM-RF in 1/3 of the patients with JCA which was significantly higher as compared to the controls. The WHO-standard enabled us to quantify the test results precisely. No positive results were yielded in the group of healthy controls and the majority of them (95 %) had levels below the maximal dilution of the standard (<3 IU). Only 4 % of the disease controls had low IgM-RF levels (6,25-12,5 IU) and two of them were SLE patients. The remainder had insignificant levels mostly below 3 IU. An impressive difference was observed between the prevalence of IgM-RF in the subgroups. JCA patients with polyarticular disease expressed significantly higher IgM-RF levels than the controls. Polyarticular and extending pauciarthritis showed a higher prevalence than non-extending pauci-arthritis. These findings suggest that the term "seronegativity" in JCA needs to be discussed. The results also indicate that RF-positivity is not obligatory linked to the late-onset polyarticular form. Our data also suggest that the extending pauciarticular form is an entity which is clearly distinguished from that with non-extending oligoarticular onset and course of the disease. Forty-two per cent of these patients had IgM-RF and 1/5 of them - IgA-RF. All of them had more than two relapses of the disease, duration over 3 years and the majority had active disease at the time of the investigation. It might be more appropriate to refer the extending pauciarthritis to the polyarticular subgroup considering the course of the disease rather than the type of onset. The diagnostic characteristics of the IgM-RF test are significant. It could be used in the diagnosing the JCA and distinguishing the polyarticular disease, in particular, since after the test the likelihood for diagnosing the polyarticular JCA increases 18-fold and for the extending pauciartritis - 14-fold. We also prove that IgM-RF is a marker for disease severity, since a significant correlation with erosive disease has been found. However, according to our findings, IgM-RF is not dependent on disease activity.

In conclusion, we consider that JCA patients express IgG-, IgA- and IgM-RFs as measured by ELISA. IgA- and IgM-RFs. ELISA-tests have a diagnostic significance and could be an alternative for the agglutination assays in seronegative patients with polyarticular disease. IgA-RF and IgM-RF are serological markers for disease severity and radiological progression of JCA. Extended clinical study will enable us to assess the real clinical importance of these data.

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