Measurement of biomarkers in juvenile idiopathic arthritis patients and their significant association with disease severity: a comparative study

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Abstract

Objective

To evaluate in juvenile idiopathic arthritis (JIA) patients a biomarker panel of anti-cyclic citrullinated peptide (anti-CCP) antibodies, cartilage oligomeric matrix protein (COMP), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), IgM rheumatoid factor (RF), IgG RF, and IgA RF and compare to the presence of joint erosions (JE), joint space narrowing (JSN), and synovitis in order to evaluate aggressive disease.

Methods

Sixty-eight JIA patients (19 RF positive polyarthritis, 23 RF negative polyarthritis, 17 persistent oligoarthritis, and 9 systemic-onset) were evaluated using the biomarker panel and compared to 18 healthy controls. All RF isotypes, anti-CCP antibodies, and COMP were measured by enzyme-linked immunosorbent assays (ELISA). Statistically significant differences and associations were assessed for each biomarker in relation to JE, JSN, and synovitis. Multiple regression analysis was used to find the variables associated with joint damage and synovitis.

Results

Patients with JE and JSN had significantly elevated levels of IgA RF, IgM RF, and anti-CCP antibodies. COMP levels were higher in early disease, but also later in disease in patients with no JE or JSN. ESR, CRP, and IgA RF were significantly elevated in patients with active synovitis. Regression analysis showed IgM RF and disease duration to be associated with JE and JSN. Anti-CCP antibodies and COMP were also associated with JSN. CRP and IgA RF were associated with synovitis.

Conclusion

Our findings demonstrate the importance of measuring IgM RF and IgA RF by ELISA and anti-CCP antibodies by ELISA, in addition to COMP in the assessment of JIA patients to determine severity of disease.

Key words

Juvenile idiopathic arthritis, rheumatoid factor, cartilage oligomeric matrix protein, anti-CCP antibodies, joint erosions, joint space narrowing, synovitis.

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Introduction

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease of childhood, affecting 250,000 children in the United States, and is characterized by chronic inflammation of one or more joints in children and adolescents (1). Because of the heterogeneous pattern of the disease, patients with JIA are divided into seven onset types, dependent upon the number of joints involved and clinical signs and symptoms during the first six months of illness (2-3). These subtypes include IgM rheumatoid factor (RF)-positive polyarthritis, IgM RF-negative polyarthritis, oligoarthritis, systemic-onset arthritis, psoriatic arthritis, enthesitis-related arthritis, and undifferentiated arthritis. The pathology is complicated as it has an unclear etiology, lacks reliable biomarkers, and follows an unpredictable course (1, 4). The disease outcome is complex and relates to several variables such as disease activity, functional status, and joint damage. Furthermore, synovitis commonly results in joint destruction, subluxation, and inflamed periarticular connective tissue.

Several studies have reported that measurement of IgM RF in RA and JIA patients using enzyme linked immunosorbent assays (ELISA) are more sensitive than other methods, including latex agglutination and nephelometry (5-7). In addition, RF measured by such methods does not differentiate between RF isotypes, while ELISAs are able to detect individual RF isotypes. The clinical relevance of the RF isotypes as markers for joint damage remains debatable, with contradicting results being reported (8-9). IgM RF positivity in JIA patients has proven to be a significant marker for radiographic damage and physical disability, especially when considered with other biomarkers (10-12). Van Rossum *et al.* (13) found increased correlation with the presence of IgM RF and radiographic progression in JIA. Recent studies have elucidated a role for anti-cyclic citrullinated peptide (anti-CCP) antibodies in JIA concerning diagnosis and disease outcome, mainly in patients with IgM RF-positive polyarthritis and joint erosions (JE) (14-16). Moreover, cartilage oligomaric matrix protein (COMP) has been identified as a marker of cartilage turnover with putative roles in disease chronicity. In RA, it has been shown that COMP levels are elevated early in the disease course, but as the disease progresses COMP levels decrease (17). Recently, a Japanese study revealed COMP to be a potential marker of impaired growth in systemic JIA (18).

To date, no study has evaluated in JIA a comprehensive panel of biomarkers, including anti-CCP antibodies, COMP, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), IgA RF, IgG RF, and IgM RF for their association with disease severity. In this cross-sectional study, we determined which biomarkers provided useful indication of joint damage and synovitis in JIA patients.

Materials and methods

Serum samples

Sera were collected from the outpatient clinics of the Saint Louis University Medical Center and Cardinal Glennon Children's Medical Center. A total of 68 JIA patient samples were collected, including 19 IgM RF-positive polyarthritis, 23 IgM RF-negative polyarthritis, 17 persistent oligoarthritis, and 9 systemic-onset with polyarthritis course. All patients in this study fulfilled the International League of Associations for Rheumatology criteria for JIA (2). Eighteen healthy controls with no symptoms of connective tissue disease were also analyzed. The study was approved by the Institutional Review Board of the Saint Louis University Health Sciences Center.

Clinical and radiographic evaluation

The presence of joint swelling and active synovitis was determined upon examination by the pediatric rheumatologists of the Saint Louis University Division of Pediatric Rheumatology. To determine the number of active joints involved, joint counts on all JIA patients were performed by pediatric rheumatologists. The mean ± standard deviation (SD) active joint count for the JIA patient population was 7.4±4.1 joints. JE and joint space narrowing

(JSN) were evaluated by a musculoskeletal radiologist at Cardinal Glennon Children's Medical Center and reviewed by pediatric rheumatologists using radiographs (AP and lateral) of hands, wrists, knees, ankles, and feet. The radiological data was obtained at approximately the same time as collection of sera from the JIA cohort.

At the time of sample collection, 9 of the IgM RF-positive polyarthritis patients were on methotrexate and a biologic, 10 of the IgM RF-negative polyarthritis were on methotrexate and two were on a biologic, 5 systemic-onset arthritis patients were on methotrexate with 3 on a biologic. None of the oligoarthritis patients were on an immunosuppressive therapy at the time of sample retrieval. Also, none of the patients had received intra-articular steroids in 6 months from the time of sample collection.

Laboratory evaluation

Serum and plasma samples were collected from patients at inclusion of the study. Heparin treated plasma and sera were frozen in 50µl aliquots and stored at -80ºC. ESR was determined according to modified Westergren and CRP by electroimmunoassay. Initial determination of IgM RF positivity was performed by nephelometry or latex agglutination, which is how patients were classified in their disease subtypes. The QUANTA Lite RF ELISA (Inova Diagnostics, Inc., San Diego, CA) for the detection of IgA RF, IgG RF, and IgM RF in patient plasma and sera and the QUANTA Lite CCP IgG ELISA (Inova Diagnostics, Inc.), a second generation anti-CCP antibody test, for the detection of IgG anti-CCP antibodies in patient plasma and sera, were used following manufacturer's instructions. The cut-off values for RF and anti-CCP antibodies were 6 U/L and 20 U/L, respectively. The COMP ELISA (AnaMar Medical, Lund, Sweden) for detection of COMP in patient plasma and sera was used according to manufacturer's instructions. The cut-off value for COMP was 12 U/L. All controls were negative or in the normal range for all measured laboratory markers. All sera were tested in duplicate to ensure consistency and the results were averaged. A control serum was included on all plates to monitor plate-to-plate variation. Variation between duplicates and control serum were never significant and therefore values were not corrected.

Table I. Demographic and laboratory features of JIA patients (n=68) and healthy controls (n=18).

	IgM RF-pos. polyarthritis n=19 Mean ± SD	IgM RF-neg. polyarthritis n=23 Mean ± SD	Oligoarthritis n=17 Mean ± SD	Systemic-onset arthritis n=9 Mean ± SD	Healthy Controls n=18 Mean ± SD
Age (years)	18.8 ± 4.7	12.1 ± 6.8	9.7 ± 4.3	12.2 ± 5.5	5.5 ± 6.1
Sex (female/male)	18/1	20/3	13/4	5/4	12/6
Disease duration (years)	5.6 ± 5.2	5.3 ± 5.9	4.1 ± 4.6	7.5 ± 5.2	N/A
Active joint count (n)	9.7 ± 3.0	9.1 ± 2.4	1.6 ± 0.7	9.0 ± 3.3	N/A
Anti-CCP antibodies (U)	98.7 ± 89.9	14.4 ± 42.7	3.7 ± 20.1	26.8 ± 58.8	5.4 ± 3.2
Postivity n (%)	12 (63.2)	4 (17.4%)	1 (5.8%)	2 (22.2%)	0 (0.0%)
COMP (U/l)	9.2 ± 6.4	12.1 ± 5.8	16.7 ± 7.1	11.7 ± 5.1	7.7 ± 1.6
Positivity n (%)	5 (26.3%)	12 (52.2%)	11 (64.7%)	3 (33.3%)	0 (0.0%)
CRP (mg/dl)	2.7 ± 6.9	4.3 ± 10.9	0.6 ± 0.2	5.1 ± 5.9	N/A
Positivity n (%)	4 (21.1%)	8 (34.8%)	3 (17.6%)	5 (55.6%)	
ESR (mm/hr)	16.3 ± 12.4	24.6 ± 24.5	14.5 ± 10.0	69.4 ± 39.6	N/A
Positivity n (%)	6 (31.6)	11 (47.8%)	5 (29.4%)	8 (88.9%)	
IgA RF (U)	26.0 ± 29.1	4.1 ± 3.5	2.2 ± 1.6	11.0 ± 21.0	0.98 ± 0.94
Positivity n (%)	13 (68.4%)	4 (17.4%)	0 (0.0%)	2 (22.2%)	0 (0.0%)
IgG RF (U)	17.0 ± 23.6	4.2 ± 9.5	0.05 ± 5.1	14.6 ± 18.7	3.3 ± 1.7
Positivity n (%)	10 (52.6%)	9 (39.1%)	4 (23.5%)	6 (66.7%)	0 (0.0%)
IgM RF (U)	82.9 ± 74.5	3.6 ± 4.6	1.7 ± 4.1	37.4 ± 68.5	3.1 ± 1.9
Positivity n (%)	19 (100%)	9 (39.1%)	1 (5.8%)	6 (66.7%)	0 (0.0%)

Continuous variables are expressed as means ± SD. Anti-CCP antibodies: anti-cyclic citrullinated peptide antibodies; COMP: cartilage oligomeric matrix protein; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; N: number of patients; RF: rheumatoid factor; U: units.
Cut-points for biomarker positive values: anti-CCP antibodies (≥20 U), COMP (≥12 U/l), RF isotypes (≥6 U), ESR (≥20mm/hr), CRP (≥0.8mg/dl).

Statistical analysis

Statistical analysis was performed using the Statistical Package Social Science (SPSS) program version 13.0 (Chicago, IL). Mean ± SD were used to describe biomarker concentrations. Comparison of the groups was performed with Student's *t*-test. To compare percentages, χ^2 analysis was used. Correlations were analyzed using the Pearson's correlation coefficient. Multiple logistic regression analysis was utilized to identify which variables were associated with joint destruction and synovitis. Regression analysis results were expressed as odds ratio (OR) with 95% confidence intervals (CI) and *p*-values. $P \leq 0.05$ was considered statistically significant in all analyses.

Results

Demographic and laboratory features of the JIA patients and healthy individuals are illustrated in Table I. The RF isotypes all correlated significantly with each other (IgA RF and IgG RF, r=0.326; IgM RF and IgG RF, r=0.632; IgA RF and IgM RF, r=0.665; $p \leq 0.001$). Anti-CCP antibodies also significantly correlated with IgA RF, IgG RF, and IgM RF (r=0.411, r=0.407, r=0.561, respectively; $p \leq 0.001$). IgA RF and IgM RF correlated with age (r=0.251, $p \leq 0.05$ for IgA RF; r=0.324, $p \leq 0.001$ for IgM RF). COMP had a significant negative correlation with IgM RF (r=-0.308, $p \leq 0.05$), along with age and disease duration (r=-0.484, $p \leq 0.001$ for age; r=-0.301, $p \leq 0.05$ for disease duration). Anti-CCP antibodies also correlated significantly with age (r=0.329, $p \leq 0.001$). CRP and ESR demonstrated significant correlation (r=0.487, $p \leq 0.001$).

The presence of synovitis correlated significantly with CRP and ESR (r=0.355, r=0.38, respectively;

Table II. Comparison of laboratory parameters between JIA patients with and without JE.

	Patients with JE (n=19) Mean ± SD	Patients with no JE (n=49) Mean ± SD	*p*-value
Age (years)	18.1 ± 6.7	11.4 ± 5.4	0.001
Disease Duration (years)	9.4 ± 5.8	3.4 ± 3.9	0.001
Anti-CCP antibodies (U)	64.6 ± 73.3	17.9 ± 65.7	0.022
CRP (mg/dl)	5.1 ± 7.9	2.7 ± 7.6	0.364
COMP (U/l)	10.2 ± 4.1	13.2 ± 7.3	0.046
ESR (mm/hr)	32.2 ± 31.1	27.0 ± 28.0	0.539
IgA RF (U)	18.1 ± 20.8	6.8 ± 17.9	0.045
IgG RF (U)	11.7 ± 18.6	6.8 ± 15.2	0.315
IgM RF (U)	62.2 ± 80.7	17.8 ± 35.2	0.031

Continuous variables are expressed as means ± SD. Anti-CCP antibodies: anti-cyclic citrullinated peptide antibodies; COMP: cartilage oligomeric matrix protein; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; JE: joint erosions; RF: rheumatoid factor; U: units.
Cut-points for biomarker positive values: anti-CCP antibodies (≥20 U), COMP (≥12 U/l), RF isotypes (≥6 U), ESR (≥20mm/hr), CRP (≥0.8mg/dl).

Table III. Comparison of laboratory parameters between JIA patients with and without JSN.

	Patients with JSN (n=18) Mean ± SD	Patients with no JSN (n=50) Mean ± SD	*p*-value
Age (years)	19.0 ± 6.1	11.1 ± 5.2	0.001
Disease duration (years)	10.4 ± 5.8	3.1 ± 3.3	0.001
Anti-CCP antibodies (U)	80.8 ± 81.9	12.8 ± 56.6	0.004
CRP (mg/dl)	5.4 ± 8.2	2.7 ± 7.5	0.337
COMP (U/l)	9.5 ± 4.2	13.4 ± 7.1	0.009
ESR (mm/hr)	33.2 ± 31.9	26.7 ± 27.7	0.454
IgA RF (U)	19.4 ± 20.8	6.5 ± 17.7	0.027
IgG RF (U)	15.8 ± 24.4	5.4 ± 11.1	0.097
IgM RF (U)	68.6 ± 81.7	16.4 ± 33.2	0.016

Continuous variables are expressed as means ± SD. Anti-CCP antibodies: anti-cyclic citrullinated peptide antibodies; COMP: cartilage oligomeric matrix protein; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; JSN: joint space narrowing; RF: rheumatoid factor; U: units.
Cut-points for biomarker positive values: anti-CCP antibodies (≥20 U), COMP (≥12 U/l), RF isotypes (≥6 U), ESR (≥20mm/hr), CRP (≥0.8mg/dl).

$p \leq 0.001$). JE demonstrated significant correlations with IgA RF and IgM RF, along with anti-CCP antibodies (r=0.268, $p \leq 0.05$ for IgA RF; r=0.364, $p \leq 0.001$ for IgM RF; r=0.302, $p \leq 0.05$ for anti-CCP antibodies). Age, disease duration, and JSN also correlated with JE (r=0.471 for age; r=0.53 for disease duration; r=0.814 for JSN; $p \leq 0.001$). All three RF isotypes correlated significantly with JSN (r=0.30, $p \leq 0.05$ for IgA RF; r=0.268, $p \leq 0.05$ for IgG RF; r=0.421, $p \leq 0.001$ for IgM RF). COMP demonstrated a negative correlation with JSN (r=-0.271, $p \leq 0.05$), and anti-CCP antibodies also correlated significantly with JSN (r=0.432, $p \leq 0.001$). Disease duration and age showed significant correlation with JSN (r=0.627, $p \leq 0.001$ for disease duration; r=0.546, $p \leq 0.001$ for age).

IgA RF, IgG RF, and IgM RF isotypes were positive in 19 (27.9%), 29 (42.6%), and 35 (51.5%) patients, respectively. Fourteen (20.6%) patients were positive for all three RFs, and 43 (63.2%) patients were positive for at least one RF. Elevated COMP levels were found in 31 (45.6%) patients, and high levels of anti-CCP antibodies were detected in 19 (27.9%) patients.

Joint erosions

Nineteen patients (28.4%) had JE. Nine patients with JE were in the IgM RF-positive polyarthritis group, 6 had RF-negative polyarthritis, and 4 patients had systemic-onset arthritis. None of the oligoarthritis patients had JE. Comparisons of the laboratory parameters according to JE are shown in Table II. Levels of anti-CCP antibodies, IgA RF, and IgM RF were significantly higher in patients with erosive disease (p=0.022, p=0.045, and p=0.031, respectively). Interestingly, patients with non-erosive disease demonstrated increased levels of COMP compared to those with JE (p=0.046). Patients with erosive disease were older and had a longer disease duration than those patients with no JE (p=0.001). Age, disease duration, anti-CCP antibodies, COMP, IgA RF, and IgM RF were included in the multiple regression analysis. Levels of IgM RF (OR 1.017, 95% CI 1.004-1.029, p=0.008) and disease duration (OR 1.325, 95% CI 1.134-1.548, p=0.001) were the independent factors correlating with JE. Age, anti-CCP antibodies, COMP, and IgA RF were not independently associated with JE.

Joint space narrowing

Eighteen patients (26.9%) had JSN. Ten of the IgM RF-positive polyarthritis patients had JSN, 4 IgM RF-negative polyarthritis and systemic-onset arthritis patients had JSN. None of the oligoarthritis patients had developed JSN. Comparisons of the laboratory parameters according to JSN are illustrated in Table III. Anti-CCP antibodies, IgM RF, and IgA RF levels were significantly higher in patients with JSN (p=0.004, p=0.016, and p=0.027, respectively). Again, COMP levels were elevated in those patients without JSN compared to those with JSN (p=0.009). JIA patients with JSN had a longer disease duration and were older than patients with no JSN (p=0.001). The differences between CRP, ESR, and IgG RF in the two groups were not statistically significant. Multiple logistic regression analysis showed that IgM RF (OR 1.020, 95% CI 1.001-1.039, p=0.043), anti-CCP antibodies (OR 1.014, 95% CI 1.001-1.027, p=0.037), disease duration (OR

1.636, 95% CI 1.249-2.141, p=0.001), and COMP (OR 0.212, 95% CI 0.060-0.741, p=0.015) were independent factors correlating to the presence of JSN. IgA RF and age were not independently associated with JSN.

Synovitis

Twenty-nine patients (43.3%) had synovitis. Patients with synovitis included 11 of the IgM RF-positive polyarthritis patients, 8 IgM RF-negative polyarthritis patients, 6 patients with oligoarthritis, and 4 of the systemic-onset patients. Table IV shows the comparisons of the laboratory parameters according to synovitis. In JIA patients with synovitis, CRP and ESR levels were significantly higher than those patients without synovitis (p=0.032 and 0.004, respectively). The JIA patients with synovitis also demonstrated elevated levels of IgA RF, but did not reach statistical significance (p=0.064). The differences between the remaining biomarkers, age, and disease duration were not statistically significant. CRP, ESR, and IgA RF were included in the multiple regression analysis, which showed CRP (OR 2.271, 95% CI 2.112-22.012, p=0.001) and IgA RF (OR 1.482, 95% CI 1.181-16.405, p=0.027) were the factors correlating with the presence of synovitis.

Table IV. Comparison of laboratory parameters between JIA patients with and without synovitis.

	Patients with synovitis (n=29) Mean ± SD	Patients with no synovitis (n=39) Mean ± SD	p-value
Age (years)	13.4 ± 6.1	13.2 ± 6.9	0.878
Disease duration (years)	4.1 ± 3.6	6.1 ± 6.1	0.126
Anti-CCP antibodies (U)	39.9 ± 81.7	25.9 ± 61.4	0.448
CRP (mg/dl)	6.4 ± 11.1	0.95 ± 1.4	0.032
COMP (U/l)	11.6 ± 6.5	12.7 ± 6.8	0.541
ESR (mm/hr)	40.9 ± 33.8	18.9 ± 20.0	0.004
IgA RF (U)	15.3 ± 26.1	6.3 ± 11.3	0.064
IgG RF (U)	11.6 ± 19.5	5.6 ± 13.1	0.156
IgM RF (U)	42.3 ± 63.7	21.9 ± 47.4	0.156

Continuous variables are expressed as means ± SD. Anti-CCP antibodies: anti-cyclic citrullinated peptide antibodies; COMP: cartilage oligomeric matrix protein; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; U: units. Cut-points for biomarker positive values: anti-CCP antibodies (≥20 U), COMP (≥12 U/l), RF isotypes (≥6 U), ESR (≥20mm/hr), CRP (≥0.8mg/dl).

Discussion

Currently, there is a need for biomarkers that can differentiate between patients who are prone to progressive and destructive disease than those with self-limiting or non-erosive disease. While previous studies have evaluated long-term outcome in JIA cohorts, with an emphasis on physical disability as the outcome, the use of radiographic progression as the outcome has received less attention (3). Although the sample size in this study is relatively small, with the limitations of cross-sectional design, it provides a comprehensive assessment of the clinical relevance of a group of laboratory biomarkers for their association with aggressive joint destruction and synovitis in JIA patients.

Surprisingly, 4 of the IgM RF-negative polyarthritis patients with JE, as determined by nephelometry, were positive for IgM RF by ELISA. These results parallel a previous study showing that seronegative JIA patients with deforming joint disease demonstrated higher levels of IgM RF (19). This study found that 45% of children who were seronegative by latex agglutination were positive for IgM RF by ELISA. As expected, all of the IgM RF-positive polyarthritis patients were positive for IgM RF by ELISA. Two of the systemic-onset arthritis patients were positive for IgM RF, while all of the oligoarthritis patients were negative for IgM RF and had no JE or JSN.

A variety of studies in human and experimental arthritis indicate that changes in serum COMP concentrations are related to processes involving cartilage turnover (20-22). Therefore, increased serum levels may occur early in the disease course as a sign of cartilage involvement. Although mean COMP levels were significantly lower in the groups with JE and JSN, the groups with no JE or JSN exhibited elevated levels of COMP.

A significant number of oligoarthritis patients demonstrated elevated levels of COMP (64.7%), and these were the youngest patients in the JIA patient population. IgM RF-positive polyarthritis patients were the oldest and exhibited the least number of patients with elevated COMP (26.3%). Decreased mean levels of COMP in IgM RF-positive polyarthritis patients indicated that joint destruction had already occurred. However, elevated COMP levels in 5 IgM RF-positive polyarthritis may indicate future joint damage. In addition, 7 IgM RF-negative polyarthritis patients who had joint damage were negative for COMP, while 11 with no joint erosions were positive for COMP. Similar results were also noted in the systemic-onset arthritis group. This data, along with the inverse relationship between JSN and COMP, lends further support to the presence of COMP prior to development of joint damage.

In conclusion, our data indicates the utility of measuring IgM RF and IgA RF by ELISA, anti-CCP antibodies by ELISA, and COMP levels in the assessment of JIA patients of all onset types for JE and JSN. These findings suggest that measurement of IgM RF and IgA RF by ELISA are more clinically useful than measurement of RF by nephelometry or latex agglutination. CRP and ESR remain the distinctive biomarkers exhibiting a significant association with active synovitis, along with elevated levels of IgA RF. Further studies are needed to longitudinally evaluate the group of biomarkers in JIA patients to confirm their association with aggressive disease, and possible predictive value. Overall, the presence of IgM RF, IgA RF, anti-CCP antibodies, and elevated COMP levels may indicate a JIA patient with aggressive disease.

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