CONCISE REPORT

Diagnostic accuracy of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis

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Objective: To correlate serum anti-cyclic citrullinated peptide antibodies (anti-CCP) levels with juvenile idiopathic arthritis (JIA) subtypes and with an erosive disease course.

Methods: The study group comprised 122 children with JIA; 16 were evaluated during both active disease and remission. Nineteen children with systemic lupus erythematosus (SLE), 27 with rheumatoid arthritis (RA), and 15 healthy children were also included in the study. Twelve children with JIA were rheumatoid factor (RF) positive, and 34 patients had persistent erosive joint disease. Anti-CCP antibody levels were determined by ELISA; values above 5 relative units were regarded as positive.

Results: Three girls with seropositive polyarticular JIA and erosive joint disease had positive anti-CCP values. Children evaluated during active disease and remission, patients with SLE, and healthy children all had negative anti-CCP antibody levels. However, 19/27 (70%) adult patients with RA had positive anti-CCP antibody values.

Conclusions: In contrast with RA, anti-CCP positivity is only rarely found in patients with JIA. In patients with RF positivity and/or in patients with erosive joint disease, anti-CCP can be detected.

Juvenile idiopathic arthritis (JIA) is an autoimmune disease of unknown cause, characterised by chronic, inflammatory changes of the joints, as for rheumatoid arthritis (RA) in adults.[1 2] Limited numbers of serological laboratory tests, such as antinuclear antibodies and rheumatoid factor (RF), are helpful for the classification and diagnosis and for evaluating the clinical status.[1 2] Therefore, continuing efforts are made to find more sensitive and specific markers for diagnosis.

Anti-cyclic citrullinated peptide antibodies (anti-CCP) are autoantibodies with high specificity in adults with RA.[3] Many studies have recently shown that it is as important as RF in the diagnosis of RA. It can be used not only as an early index of the disease but also as a predictor of the erosive form of the disease. Prediction of which patient will develop the erosive form is of great importance in timing the introduction of intensive immunosuppressive treatment in order to prevent late sequelae.[3–8] Only a few studies have shown the diagnostic efficacy of anti-CCP in JIA.[9–11]

This study aimed at relating serum anti-CCP levels to JIA subtypes and to an erosive disease course.

SUBJECTS AND METHODS

Study groups

One hundred and twenty two children (72 female, 50 male) with JIA, all fulfilling the 1997 International League Against Rheumatism (ILAR) classification criteria, were included in the study.[2] Children were randomly entered into the study in the order of admission to the outpatient department. Sixteen of them were evaluated both during active disease and the remission period. The presence of active disease was determined according to the proposed criteria for active disease.[12] Nineteen children with systemic lupus erythematosus (SLE), 27 adults with RA, and 15 healthy children were also included in the study (table 1).

Among the 122 children with JIA, 48 had polyarticular JIA (36 patients had RF negative and 12 patients RF positive polyarthritis), 36 had oligoarticular JIA (33 patients had persistent oligoarticular JIA and 3 had extended oligoarticular JIA), 28 had systemic JIA, 7 had enthesitis related arthritis (ERA), and 3 children had juvenile psoriatic arthritis.

Sixteen children (7 with RF negative polyarticular JIA, 5 with systemic disease, and 4 with oligoarticular JIA) were evaluated both during active disease and the remission period. The first serum sample was obtained during active disease and the second 45 days later, if they were in remission.

Synovial fluid was obtained from 14 children with JIA (10 oligoarticular JIA, 3 RF negative polyarticular JIA, and 1 systemic JIA). Twelve patients with JIA were RF positive.

Most of the children had an *x* ray examination of the affected joint taken during the past 12 months. For those without *x* ray findings, a new radiograph was taken at the time of blood sampling. Radiological joint damage was determined by the presence of joint space narrowing or erosions, or both. Thirty four patients had erosive joint disease. Three patients had oligoarticular JIA, 10 systemic JIA, 1 ERA, and 20 had polyarticular JIA. RF positivity was evident in eight patients with erosive polyarticular JIA. The mean disease duration of patients with erosive joint disease was significantly higher than in those without erosive joint disease (5.8 (3.0) *v* 3.8 (3.1) years; $p = 0.014$).

Laboratory methods

Anti-CCP antibodies were evaluated by an enzyme linked immunosorbent assay (ELISA; Euroimmun, Germany), which is a second generation anti-CCP test. A cut off value of 5 relative units was established, as recommended by the manufacturer's protocol. IgM RF was studied by a turbidimetric method (Dade Behring, Germany). Values above 40 IU/ml were considered positive.

Abbreviations: AKA, antikeratin antibodies; anti-CCP, cyclic citrullinated peptide antibodies; APF, antiperinuclear factor; ERA, enthesitis related arthritis; JIA, juvenile idiopathic arthritis; PAD, peptidyl arginine deiminase; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus

Table 1 Demographic characteristics of the study groups

Study group	No	Female/ male	Mean age at investigation (years)	Mean disease duration (years)
Juvenile idiopathic arthritis	122	72/50	8.8 (4.4) [1.2–19.3]	4.3 (3.2)
Juvenile systemic lupus erythematosus	19	17/2	13.6 (3.3) [5.3–18]	4.5 (3.6)
Rheumatoid arthritis	27	23/4	45.7 (14.8)	
Healthy children	15	7/8	8.6 (4.2) [3–15.5]	
Total	183	119/64		

Results are shown as mean (SD) [range].

Statistical analysis

All statistical analyses were carried out with the SPSS version 10.0. A p value of <0.05 was considered significant. Patient groups were compared using Student's *t* test, χ^2 test, and Fisher's exact test.

RESULTS

Anti-CCP positivity was present in 3/122 (2%) children with RF positive polyarticular JIA and erosive joint disease. All of them had small joint and finger joint involvement. An orthopaedic operation was performed on one of them because of deformities of the finger joints. Patients with erosive disease were more likely be anti-CCP positive than those with non-erosive disease ($p = 0.02$).

Another three patients with polyarticular joint disease (two with RF positive polyarticular JIA and one with ERA) showed borderline anti-CCP levels (>3 relative units). Erosive joint damage was also present in these children.

The anti-CCP levels of patients who were RF positive were significantly higher than those of patients who were RF negative ($p<0.001$). Anti-CCP values of patients with JIA subtypes other than RF positive polyarticular JIA were not found to be significantly different. The patients studied during both the active disease and the remission periods showed negative anti-CCP values, which were also not statistically significant. Patients with juvenile SLE and healthy children had negative anti-CCP levels.

Synovial fluids of patients with JIA showed negative anti-CCP values. We could not obtain joint fluids from children who had positive or borderline anti-CCP levels.

Nineteen of 27 (70%) adults with RA had positive anti-CCP values.

DISCUSSION

Anti-CCP, which has a high specificity in patients with RA, was investigated at our centre in children with JIA using different control groups. In adult RA, anti-CCP, one of the antibodies directed against citrullinated proteins, provides better diagnostic accuracy than antiperinuclear factor (APF) and antikeratin antibodies (AKA).[3 5]

Citrulline, a non-standard amino acid, is a translational modification of the amino acid arginine by the peptidyl arginine deiminase (PAD). The only manner in which citrulline can become part of a protein is by post-translational modification by PAD enzymes. Citrullinated proteins (for example, fibrin) deposited in the rheumatoid synovial membranes are the major target of antibodies directed against citrullinated proteins. Then, autoimmunisation against deiminated fibrin, which is a key factor in pathogenesis of RA, begins.[13] Reparon-Schuijt *et al* demonstrated that healthy control subjects and patients with RA have a pool of precursor B cells in the circulation that can produce anti-CCP upon activation.[14] Also, it is reported that synovial fluid and bone marrow B cells from patients with RA contain a population of B cells that spontaneously produce anti-CCP. As a result, adults develop erosive joint damage. Positive anti-CCP values in patients with RA are an indicator of forthcoming joint damage.

In JIA, which is not a homogeneous disease like RA, development of antibodies directed against citrullinated proteins is not well understood. The prevalences of APF and AKA in JIA, the first defined group of antibodies directed against citrullinated proteins, are 1–37% and 2–50%, respectively.[11] The methodological difficulties in evaluating the levels of these antibodies preclude their measurement in routine practice.[3–5 11] For that reason we did not determine these measures in the current study.

Five studies have evaluated anti-CCP levels in JIA.[4 6 9–11] Three of them investigated children with JIA, but in the other studies, patients with JIA served as the control group for RA. The first two studies reported 2–5% anti-CCP positivity and found no correlation with erosive disease or disease subtype.[9 10] Avcin *et al* reported on 109 patients with JIA, and only two cases (one with oligoarticular and the other with RF negative polyarticular disease) were found to be anti-CCP positive.[9] Neither had erosive joint disease; thus, their results are not similar to our findings. Another study by Hromadnikova *et al* in 140 children with JIA also investigated AKA.[10] Seven children (5%) had anti-CCP; two children with anti-CCP positivity were diagnosed as RF positive polyarticular JIA. Other patients with anti-CCP had a heterogeneous distribution of subtype diagnoses. Despite the fact that the anti-CCP positivity in those studies is comparable to our findings, relationships to subtype distribution and the presence of erosive disease are not.[9 10]

van Rossum *et al* reported 10 cases (14%) of anti-CCP positivity in 71 children with JIA and found that this finding was significantly related to RF positive erosive disease.[11] However, in this study 11/71 patients had RF positive polyarticular JIA and 8 (73%) had anti-CCP positivity. The percentage of anti-CCP positivity in a cohort of patients with JIA seems to be related to the number of IgM RF positive patients with JIA included in the study. Additionally, 30 children had radiological damage and 8 (27%) of them had anti-CCP positivity. Our findings are consistent with this study. We also found that anti-CCP positivity is associated with erosive disease and RF positive patients.

Two other studies also found anti-CCP positivity at different levels. Bizarro *et al* reported that three patients with unknown subtype had negative anti-CCP values.[4] Lee *et al* studied 21 adults with JIA (mean age 31 years) as controls and found anti-CCP in 6 (29%) of them.[6] They did not discuss the subtype of the disease in those patients; however, they found that the anti-CCP reactivity did not correlate with radiological joint destruction. Possibly, such a high rate of positivity may be related to longer disease duration (mean disease duration 21 years).

The fact that anti-CCP positivity is found with high specificity only in patients with JIA with RF positive polyarthritis may be explained as this specific form of the disease may be the paediatric form of RA.[1]

A relationship between anti-CCP and erosive joint disease has been shown by some researchers.[3 5 6 8] We also demonstrated anti-CCP positivity in children with erosive joint disease; this promotes the use of this test for diagnosis of patients with JIA. Anti-CCP positivity in an RF positive patient indicates that we should consider strong immunosuppressive treatment.

Patients with juvenile SLE and healthy children, as the control group of the study, did not show anti-CCP positivity. In patients with SLE, autoimmune antibody responsiveness is very well known. The absence of anti-CCP positivity in patients with SLE supports the suggestion that these antibodies are of different origin than antinuclear antibodies.

Some studies demonstrated variable anti-CCP values according to clinical activity status.[15] Our study fails to demonstrate such variations in the antibody titres, although serum samples of the active and remission period were evaluated within 45 days. All these children were RF negative, for that reason we can say that anti-CCP is not an activity based antibody.

By the design of the study, positivity of anti-CCP in 70% of the patients with RA may be accepted as an index of performance of the test.

In conclusion, we can state that anti-CCP positivity is not as common in a heterogeneous disease like JIA as in RA. Nevertheless, anti-CCP positivity is found in RF positive patients with erosive joint disease. Anti-CCP determination should not be used as a screening method for patients with JIA, but it can be used to predict erosive joint damage, especially in children with polyarticular disease.

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