The diagnostic value of anti-cyclic citrullinated peptide (CCP) antibodies in children with Juvenile Idiopathic Arthritis

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Abbreviations:
Ab: antibody;
anti-CCP: cyclic citrullinated peptide antibodies;
DMARD: disease modifiying antirheumatic drugs;
ERA: enthesitis related arthritis;
JIA: juvenile idiopathic arthritis;
NSAID: nonsteroidal antiinflammatory drugs;
PAD: peptidylarginine deiminase;
RA: rheumatoid arthritis;
RF: rheumatoid factor.

ABSTRACT

Background. *Antibodies against cyclic citrullinated peptide (anti-CCP) are considered to be specific for rheumatoid arthritis (RA).*

Objective. *To assess the clinical significance of anti-CCP antibodies in a cohort of patients with juvenile idiopathic arthritis (JIA) and if they can be used to identify patients with an unfavourable course of disease.*

Methods. *68 serum samples were investigated. 45 patients were diagnosed with JIA (15 male and 30 female) aged 1.9-17.3 years (median 12.9 mean 11,0). 5 patients had polyarticular (RF negative), 2 polyarticular (RF positive), 25 oligoarticular JIA, 6 enthesitis related arthritis, 2 psoriatric arthritis, 3 patients had systemic disease and 2 unclassified arthritis. 23 samples were taken from patients with non-inflammatory cardiac diseases undergoing interventional cardiac therapy. Enzyme-linked immunosorbent assay (ELISA; Euroimmun, Lübeck, Germany) was used for the detection and quantification of anti-CCP antibodies in patients with JIA.*

Results. *Overall, anti-CCP antibodies were found in 2.9% (2/68) of all samples and in 4.4 % (2/45) patients with JIA.*

Conclusion *Anti-CCP antibodies are associated with RF positive polyarticular course of JIA. Anti-CCP antibodies are not relevant for other subgroups of JIA. Therefore anti-CCP Abs in patients with JIA should not be investigated routinely.*

Introduction

Juvenile idiopathic arthritis (JIA) is a systemic autoimmune disease of unknown origin, which is characterised by chronic inflammation of the joints similar to rheumatoid arthritis (RA). JIA patients are divided into 7 subgroups, which include systemic arthritis, oligoarthritis (persistent or extended), polyarthritis (rheumatoid factor positive (RF+) and negative (RF-)), psoriatic arthritis, enthesitis-related arthritis (ERA) and undifferentiated arthritis (1). The diagnosis of JIA depends primarily on clinical manifestations of the disease, with only limited serological support (2). Established tests are the presence of the RF and antinuclear antibodies (ANA). The presence of ANA is not related to the disease course nor to the severity of the joint involvement (2).

Autoantibodies directed to citrulline-containing proteins, highlighting anti-citrullinated peptide (anti-CCP), are known to be specifically associated with RA (3-5). The essential part of the antigenic determinant recognized by the anti-CCP antibodies (Abs) is the unusual amino acid citrulline. Citrulline can be generated by post-translational modification of arginine residues, which is catalyzed by peptidylarginine deiminase (PAD) enzymes (4). Anti-CCP Abs can be detected in up to 80% of patients with RA, with 98% specificity and identify patients who develop RA for very early therapeutical intervention (6-8).

Until now anti-CCP Abs have been studied in children only a few times (9-13). Reviewing the literature, Low *et al.* found anti-CCP Abs in 77% of JIA patients (13). It is noteworthy that these results are unique. On the contrary, the majority of the studies revealed that anti-CCP Abs do not have additional diagnostic value in JIA (9-11, 14).

Patients and methods

Patients

A cohort of 45 patients with JIA (15 male and 30 female) recruited from the division of paediatric rheumatology (University of Saarland, Germany) participated in the study. Their mean age was 10,9 years (range 1.9-17.3, median 12.9) duration of the disease was 2.1 years (range 1,77 to 2.77 years). The presenting subgroups of JIA were 5 patients with polyarticular (RF-), 2 with polyarticular (RF+), 25 with oligoarticular, 6 with ERA, 2 with psoriatric arthritis, 3 with systemic disease and 2 with undifferentiated arthritis. As controll anti CCP-Abs were tested in 23 sera of non age- matched patients with non-inflammatory cardiac diseases undergoing interventional cardiac therapy.

At the time of the study, 40 of the 45 patients with JIA were treated with non-steroidal antiinflammatory drugs

(NSAID), 24 patients with disease modifying anti-rheumatic drugs (DMARD), 9 were receiving oral glucocorticosteroids, and 3 patients TNF-α-inhibitors. Data concerning clinical signs of disease (clinical arthritis defined as swelling and/or pain with limitation of motion, fever, rash, visceral involvement), medication use, laboratory variables (IgM-RF, ANA), and radiological joint damage (defined as the presence of joint space narrowing and/or erosions), were collected from the patient files. Seven patients had active disease at the time of anti CCP-determination (15, 16). According to the preliminary criteria clinical remission of JIA, 38 patients are in clinical remission on medication (16).

Methods

Blood (2-3 ml) was collected during routine venepuncture performed for periodic assessment of laboratory tests. Samples were centrifuged, and sera were divided into aliquots and stored at -70°C until assayed. Samples were tested without knowing the clinical details of the patients. Anti-CCP Abs were evaluated by an enzyme linked immunosorbent assay (ELISA; Euroimmun lot 21122m, Germany), which is a second generation anti-CCP test. All sera were analyzed at least in duplicate, and the results were averaged. A cut-off value of 2,5 relative units was established.

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, x2 test, and Fisher's exact test.

Results

Serial determinations of anti-CCP in serum were carried. The results were always identical. Positive anti-CCP values were detected in sera of 2.9% (2/68) of all samples and in 4.4 % (2/45) patients with JIA. These 2 patients had RF+ JIA. None of the 43 patients with other subtypes of JIA had positive anti-CCP Abs (Fig. 1). There was no statistically significant association between anti-CCP positivity neither the presence of C reactive protein nor the disease duration and medication. A statistically significant association was found between positive anti-CCP test and the presence of ANA (p = 0,008). The 23 samples from the controll group demonstrated no anti-CCP Abs (Table I).

Table I. The cumulative results of anti-CCP assays in all the patients studied. The cut off was 2,5 RU/ ml.

Anti CCP	JIA	Healthy donors
Positive	2	0
Negative	43	23
%	4	0
∑	45	23

Discussion

Anti-CCP Abs are an important serological marker for the diagnosis of RA (4, 7). In JIA, which is not a homogeneous disease like RA, development of Anti-CCP Abs is not well understood (13). The anti CCP-Abs are traceable in 76% in patients with RA, with a very high specificity ranging between 95% and 100% (13, 14). In our cohort of patients with JIA, anti-CCP Abs were found in only 2/45 (4,4%) patients with JIA.These patients represent the RF+ polyarticular subgroup of JIA. This small fraction of the heterogeneous group of JIA can be considered the paediatric equivalent of RA. So far, eight studies have evaluated anti-CCP levels in JIA (9-11, 13, 14, 17-19). Six of them investigated children with JIA and in two of them, patients with JIA served as the control group for RA. In the study of Lee, all anti-CCP positive patients had a RF+ polyarticular JIA (18) and Bizarro found none of his three JIA patients anti CCP positive (17). Studies regarding Anti CCP Abs in childhood reported 2–5% anti-CCP positive. In one study, only 1 patient with polyarticular RF+ JIA was included, who was negative for anti-CCP Abs. Most of the patients (up to 75 %) presented with RF+ polyarticular JIA. Patients who had polyarticular-onset JIA and were RF+ were much more likely to have anti-CCP Abs (9-11, 19). These results are comparable to our findings. Anti CCP- positivity is related to RF+ polyarticular onset and course of JIA. Anti-CCP Abs are also significantly associated with the presence of HLA–DR4 alleles in JIA patients (14, 19). This aspect orchestrates the idea that citrullination of peptides leads to better insertion in the HLA–DR4 antigen binding site and results in a anti-CCP Ab production (19).

The high prevalence of anti-CCP Abs in polyarticular RF+ JIA could be interpretated as an immunologic character in this subset of patients, who might have the childhood equivalent of RA (17). Low *et al.* published a unique antithetic investigation with detection of anti-CCP Abs in 88% of patients with RF+ polyarticular disease but also in 75% of patients suffering from the

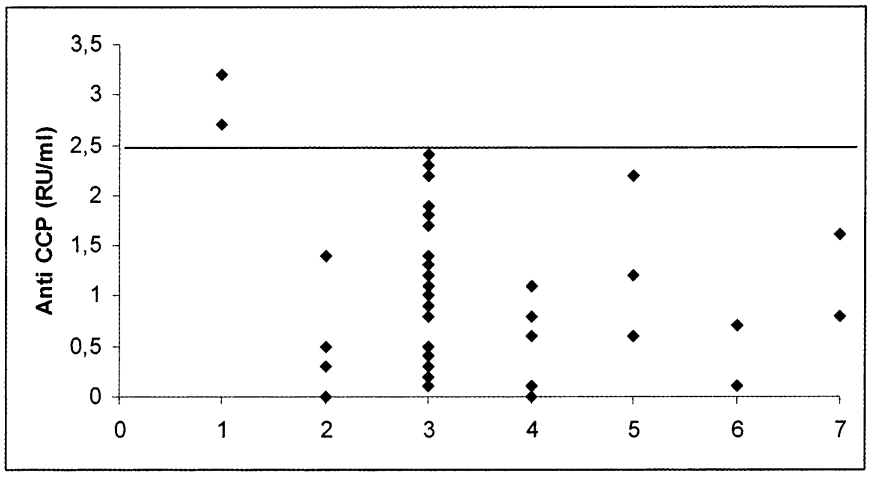

Fig. 1. Anti-CCP values in patients with different subtypes of JIA (polyarticular RF+ (1), polyarticular RF- (2), oligoarticular (3), ERA (4), systemic disease (5), psoriatric arthritis (6) and unclassifiable arthritis (7)). The cut-off value is indicated as a horizontal dashed line.

other subtypes. Therefore the authors postulate citrulline as an antigen heralding for JIA (13, 17). However, the majority of the studies demonstrated anti-CCP positivity in patients with RF+ polyarticular JIA. The RF+ polyarticular type of JIA might be a paediatric pendant of RA (19). Furthermore, the presence of anti-CCP was associated with ANA, which is not consistent with other results (9).

In conclusion, anti-CCP Abs can be detected exclusively in the subset of patients with polyarticular RF+ JIA, indicating that this subtype is more like adult RA than the other forms of JIA. A larger seropositive polyarticular cohort might reveal a significantly higher correlation with anti-CCP Abs in this subgroup. Therefore anti-CCP Abs in patients with non-polyarticular RF+ JIA should not be investigated routinely.

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