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ORIGINAL ARTICLE

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Anticyclic citrullinated peptide antibodies in juvenile idiopathic arthritis

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Abstract Anticyclic citrullinated peptide (anti-CCP) antibodies have been detected in patients with juvenile idiopathic arthritis (JRA), particularly in those with polyarticular JIA. We analyzed the presence of anti-CCP antibodies of the IgG class in sera of patients with defined juvenile idiopathic arthritis (JIA) of various subgroups. One hundred and fifty-nine serum samples were investigated. Forty-five patients were diagnosed with JIA (15 male and 30 female) aged 1.9–17.3 years (median 12.9, mean 11.0). Thirty-eight samples were taken from patients suffering from other autoimmunopathies and 34 patients with other underlying diseases were taken at different time points in their disease course. Under 42 samples were taken from patients with noninflammatory diseases. Enzyme-linked immunosorbent assay (ELISA) was used for the detection of anti-CCP antibodies. Anti-CCP antibodies were found in 6.9% of all samples and in 4.4% patients with JIA. Disease duration and medication did not differ significantly between anti-CCP positive and negative patients. A review of the literature and our own results shows that anti-CCP antibodies can be detected in the sera of only some patients with JIA. Routine determination of anti-CCP cannot be recommended.

Key words Anticyclic citrullinated peptide (anti-CCP) · Juvenile idiopathic arthritis

Introduction

A number of autoantibodies, including autoantibodies directed to citrulline-containing proteins highlighting anticyclic citrullinated peptide (anti-CCP), are known to be specifically associated with rheumatoid arthritis (RA).[1–3] The essential part of the antigenic determinant recognized by the anti-CCP Abs is the unusual amino acid citrulline.[2,4] Citrulline can be generated by post-translational modification of arginine residues (guanido group 3 ureido group) (Fig. 1). This modification is catalyzed by peptidylarginine deiminase (PAD) enzymes.[5] Anti-CCP antibodies (Abs) can be detected in up to 80% of patients with RA, with 98% specificity and identify patients who develop RA for very early therapeutic intervention.[6–8]

Juvenile idiopathic arthritis (JIA) is a systemic autoimmune disease of unknown origin, which is characterized by chronic inflammation of the joints similar to RA. It is one of the most common chronic illnesses of childhood. Patients with JIA are divided into seven subgroups, which include oligoarthritis, rheumatoid factor (RF) positive polyarthritis, RF-negative polyarthritis, extended oligoarthritis, systemic arthritis, psoriatic arthritis, and enthesitis-related arthritis (ERA).[9] The diagnosis of JIA depends primarily on clinical manifestations of the disease.[10] Rheumatoid factor (RF) and antinuclear antibodies (ANA) are established tests. Antinuclear antibodies are considered to be a marker for early onset oligoarticular disease with uveitis.[10,11] However, their presence is not related to the disease course nor to the severity of the joint involvement.[10] Anti-CCP Abs have been studied in children a few times.[11–15] Reviewing the literature, Low et al. found anti-CCP Abs in 77% (51/66) patients with JIA, including 15/18 (83%) RF-negative polyarthritis, 12/16 (75%) RF-positive polyarthritis, 16/19 (84%) oligoarthritis, 8/13 (62%) systemic arthritis.[15] Noteworthy is that these results are unique. In contrast, Avčin et al. found positive anti-CCP values in sera of 1.8% (2/109) patients with JIA. Both of them were relatively low. One of 25 (4%) patients with polyarticular onset, 1/64 (1.6%) with oligoarticular onset, and none of the 20 patients with systemic onset type were anti-CCP positive. The patient with polyarticular onset was RF-negative.[12] In another study, Hromadnikova et al. scrutinized the presence of anti-CCP Abs in sera of 140 patients with JIA aged 2–47 years. Overall, anti-CCP Abs were found in 5% (7/140) patients includ-

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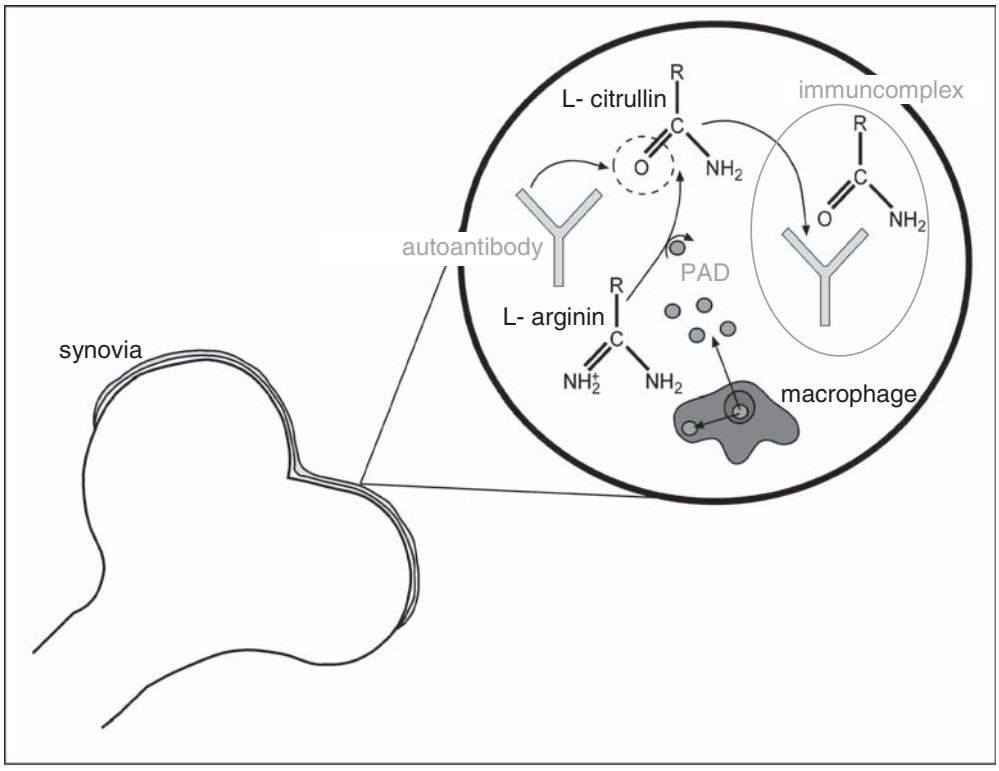

Fig. 1. Citrulline can be generated by posttranslational modification of arginine residues (guanido group 3 ureido group), which is catalyzed by peptidylarginine deiminase (PAD) enzymes

ing 3/52 RF-negative polyarthritis, 2/18 RF-positive polyarthritis, 1/15 ERA, and 1/5 unclassifiable arthritis.[13] Kasapçopur et al. detected anti-CCP Abs in 2% (3/122) of children with JIA. Twelve of them had RF-positive polyarticular JIA. In the sera of 25% (3/12) of the patients with RF-positive polyarticular JIA, anti-CCP Abs were verifiable. Anti-CCP values of patients with JIA subtypes other than RF-positive polyarticular JIA were not found to be significantly different.[14] Van Rossum et al. detected anti-CCP Abs in 15% (10/71) of patients. Eight of these JIA patients were also RF-positive, 1 patient had an oligoarthritis, and 1 an unclassifiable arthritis.[16] However, anti-CCP Abs do not have any usefulness in the diagnosis and prognosis of JIA. In this study we evaluate the relevance of anti-CCP Abs in JIA.

Patients and methods

The cohort consisted of 45 patients with JIA (15 male and 30 female). Their mean age was 11.0 years (range 1.9–17.3); duration of the disease was 2.1 years. Serum samples were obtained from 159 patients. Forty-five patients had JIA: 5 with polyarticular (RF-negative), 2 with polyarticular (RF-positive), 25 with oligoarticular, 6 with ERA, 2 with psoriatric arthritis, 3 with systemic disease, and 2 with unclassified arthritis. Thirty-eight samples were taken from patients suffering from other autoimmunopathies (12 with Crohn's disease, 8 with reactive arthritis, 7 with diabetes mellitus type I, 5 with uveitis, 4 with systemic lupus erythematosus, 2 with myositis). Sera from 34 patients with other underlying diseases were taken at different time points in their disease course (9 with infectious diseases, 5 with endocrinopathies and arthralgias, 3 with cystic fibrosis, galactosemia and hemophilia, 2 with attention deficit order, and 1 with epilepsy, Raynaud's phenomenon, osteochondroma, and fibromyalgia). For control, 23 samples were taken from patients with noninflammatory cardiac diseases undergoing interventional cardiac therapy and 19 samples were taken from healthy newborns.

At the time of the study, 41 patients of the 83 with JIA or other autoimmunopathies were treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and 27 with disease-modifying antirheumatic drugs (DMARDs), 16 were receiving oral glucocorticosteroids, and 3 were on tumor necrosis factor α (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 and laboratory variables (IgM-RF, ANA) were collected from the patient files.

Blood (2–3 ml) was collected during routine venipuncture performed for periodic assessment of laboratory tests. The samples from the newborn were taken from the blood collected for screening 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 antibodies were evaluated by an enzyme-linked immunosorbent assay (ELISA; Euroimmun lot 21122m, Germany), which is a second-generation anti-CCP test with a suggested cutoff from 5 units per ml. All sera were analyzed at least in duplicate,

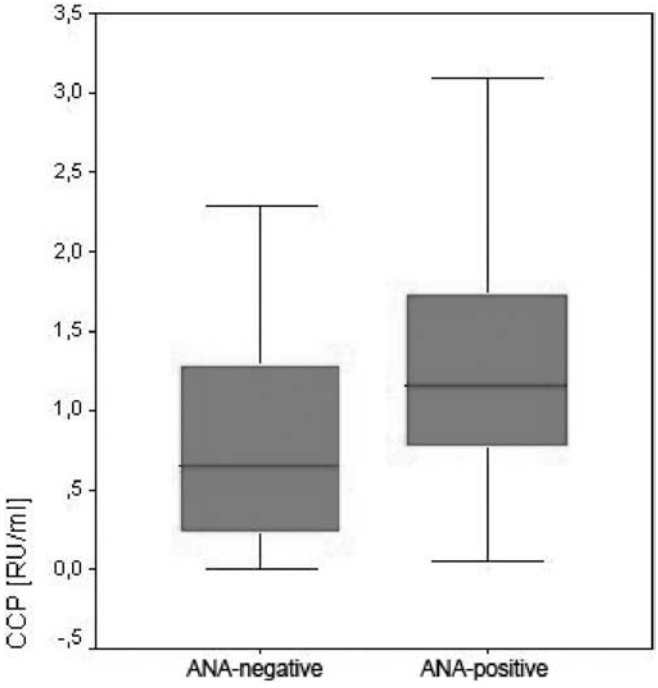

Fig. 2. Relationship between serum anticyclic citrullinated peptide (*CCP*) antibodies and presence of antinuclear antibodies (*ANA*)

and the results were averaged. By defining optimal cutoff values, the optimal performance of tests in different patient populations can be compared. This is useful because the sensitivities and specificities of tests, and therefore the optimal cutoff values, may vary between different populations. For optimal use of the anti-CCP ELISA in specific patient populations, it is therefore advised to determine and use the optimal cutoff value. The cutoff value of 2.5 relative units (RU) was established.

Results

Positive anti-CCP values were detected in sera of 6.9% (11/159) of all samples and in 4.4% (2/45) patients with JIA. The values were 2725 and 3275 U/ml. These two patients had RF positive JIA. A statistically significant association was found between positive anti-CCP test and the presence of ANA ($P = 0.008$) (Fig. 2). Positive anti-CCP results were also found in the group with other autoimmunopathies. One out of 4 (25%) patients with SLE (3092 U/ml) and 3/7 (42%) of diabetes mellitus type I (3639 U/ml, 2758 U/ml, 3889 U/ml) presented anti-CCP values. In the group of 34 patients with other underlying diseases, 5 patients had a detectable anti-CCP value, 2 with cystic fibrosis (5%) (3454 U/ml and 3889 U/ml) and 1 with galactosemia, osteochondroma, and fibromyalgia (2.9%). The patient with fibromyalgia had an anti-CCP titer of 5376 U/ml. The 42 samples from the healthy donors demonstrated no anti-CCP Abs (Table 1).

Table 1. Results of anticyclic citrullinated peptide assays

Anti-CCP	JIA	Other autoimmunopathies	Other diseases	Healthy donors
Positive	2	4	5	0
Negative	43	34	29	42
%	4	11	14	0
Σ	45	38	34	42

Cutoff was 2.5 RU/ml

Discussion

Anti-CCP antibodies are now considered as an important serological marker for the diagnosis of RA.[2,7] In JIA, which is not a homogeneous disease like RA, development of anti-CCP Abs is not well understood.[15] We examined if anti-CCP Abs could facilitate the diagnosis of JIA. In this study we demonstrated that anti-CCP can be detected also in patients with JIA, but they are generally present at low levels and less common than in adults with RA. The anti-CCP Abs are traceable in 76% in patients with RA, with a very high specificity ranging between 95% and 100%.[15,16] In our cohort of patients with JIA, anti-CCP was found in only 2/45 (4.4%) patients.[17] These two patients suffer from RF-positive polyarticular JIA. This small fraction of the heterogeneous group of JIA can be considered the pediatric equivalent of RA.

So far, eight studies have evaluated anti-CCP levels in JIA.[12–19] Five of them investigated children with JIA, but in the other studies, patients with JIA served as the control group for RA. In the study of Lee all anti-CCP-positive patients had an RF-positive polyarticular JIA,[19] and Bizarro et al. found none of their three JIA patients to be anti-CCP positive.[18] The first three studies about anti-CCP Abs in childhood reported 2%–5% anti-CCP positivity.[12–14] Hromadnikova et al. found seven children (5%) with anti-CCP. Two of them were diagnosed as having RF polyarticular JIA.[13] Avčin et al. found two patients (1.8%) with positive but low anti-CCP values. One patient had polyarthritis and one, oligoarthritis, and both of them were RF negative. In the cohort there was only one girl with RF-positive polyarticular disease, who was negative for anti-CCP.[12] Kasapçopur et al. discovered anti-CCP positivity 2% of children with JIA with RF-positive polyarticular subset.[14] The anti-CCP positivity in these studies is comparable to our findings.[17] Anti-CCP- positivity is supposed to be related to RF-positive disease. Van Rossum et al. reported 14% of anti-CCP positivity in 71 children with JIA. This was significantly related to RF-positive polyarticular disease.[16] It is known that RF-positive JIA often has a disease course similar to RA.[18] Low et al. published a unique antithetic investigation with detection of anti-CCP Abs in 77% of the JIA patients.[15] These authors found anti-CCP Abs in 88% RF-positive polyarthritis patients but also in 75% (30/40) of the other subtypes. Therefore Low et al. postulate citrulline as an antigen heralding JIA more than other diseases.[15,18] However, the majority of the studies demonstrated anti-CCP positivity in patients with JIA with RF-positive pol-

yarthritis. This fact may be allegeable. The RF-positive polyarticular type of JIA might be a pediatric pendant of RA. The presence of anti-CCP Abs is also possible in the sera of SLE patients. One of our four patients with SLE demonstrated positive anti-CCP Abs. In SLE, autoimmune antibody reaction is known. In other studies none of the patients with juvenile-onset SLE was positive for anti-CCP,[18,19] whereas positive anti-CCP values were found in adult patients with SLE up to one third of the patients.

Based on our own results anti-CCP Abs are not specific enough to develop diagnostic concepts in SLE.[20] Furthermore, the presence of anti-CCP was associated with the presence of ANA. In contrast, other studies demonstrated no statistically significant association between the presence of anti-CCP and ANA.[12,21] In conclusion, anti-CCP Abs can be detected in the sera of patients with RF-positive JIA, heralding that these subtypes should be more like adult RA than the other forms of JIA.

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