Anti-Cyclic Citrullinated Peptide (Anti-CCP) Antibodies in Children with Juvenile Idiopathic Arthritis

MARION VAN ROSSUM, RENÉE VAN SOESBERGEN, SANDRA DE KORT, REBECCA TEN CATE, AEILKO H. ZWINDERMAN, BEN DE JONG, BEN DIJKMANS, and WALTHER J. VAN VENROOIJ

ABSTRACT. ***Objective.*** To determine if anti-cyclic citrullinated peptide antibodies (anti-CCP) can be detected in sera of patients with juvenile idiopathic arthritis (JIA) and if they can be used to identify patients with a more destructive course of disease.

Methods. One hundred serum samples of 71 patients with JIA taken at different time points in their disease course were analyzed by a commercially available anti-CCP ELISA. Followup serum samples from 28 patients were also tested. Correlations between anti-CCP and disease characteristics, medication, and radiological damage (presence of joint space narrowing and/or erosions) were also determined.

Results. The serum samples came from patients of all 8 different subtypes of JIA (mean age: 9.6 years, median: 10.5; disease duration mean: 39 months, median: 24) including 11 polyarticular rheumatoid factor positive (IgM-RF) patients. Anti-CCP was positive in 73% of the IgM-RF positive JIA patients and in 3% of the other JIA patients ($p < 0.0001$). Disease duration, medication, and anti-nuclear antibody positivity did not differ significantly between anti-CCP positive and negative patients. Testing of followup samples showed almost identical anti-CCP results. All IgM-RF positive JIA patients had radiological damage ($p < 0.001$). Of the anti-CCP positive patients, 80% had radiological damage resulting in a significant difference between anti-CCP positive and negative patients ($p = 0.009$) with an odds ratio (OR) of 12.7, but corrected for IgM-RF, the OR was no longer significant ($p = 0.88$).

Conclusion. Anti-CCP antibodies can be detected in the sera of patients with JIA but almost exclusively in the subset of patients with polyarticular IgM-RF. (J Rheumatol 2003;30:825–8)

Key Indexing Terms:
JUVENILE IDIOPATHIC ARTHRITIS — ANTI-CCP ANTIBODIES
ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES — RHEUMATOID FACTOR

From the Department of Pediatrics, Leiden University Medical Center, Leiden; the Department of Rheumatology, Slotervaart Hospital, the Department of Rheumatology, Jan van Breemen Instituut, the Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, and the Department of Rheumatology, VU University Medical Center, Amsterdam; the Department of Biochemistry 161, NCMLS, University of Nijmegen, Nijmegen, The Netherlands.

The work of W.J. van Venrooij is supported by Het Nationaal Reumafonds of The Netherlands (the Dutch League against Rheumatism), The Netherlands Foundation for Research (NWO grant 940-35-037), and The Netherlands Technology Foundation (grant 349-5077).

M.A.J. van Rossum, MD, Pediatric-Rheumatologist, Department of Pediatrics, Leiden University Medical Center; R.M. van Soesbergen, MD, PhD, Rheumatologist, Department of Rheumatology, Slotervaart Hospital; S.W.K. de Kort, MD, Trainee Pediatrics; R. ten Cate, MD, PhD, Pediatric-Rheumatologist, Department of Pediatrics, Leiden University Medical Center; A.H. Zwinderman, MSc, PhD, Professor of Epidemiology and Biostatistics, Department of Clinical Epidemiology and Biostatistics, Academic Medical Center; B.A.C. Dijkmans, MD, PhD, Professor of Rheumatology, Department of Rheumatology, VU University Medical Center; B.A.W. de Jong, Biochemist; W.J. van Venrooij, PhD, Professor of Biochemistry, Department of Biochemistry 161, NCMLS, University of Nijmegen.

Address reprint requests to Dr. A.J. van Rossum, Leiden University Medical Center, J-6-S, Department of Pediatrics PO Box 9600, 2300 RC Leiden, The Netherlands. E-mail: m.van_rossum@lumc.nl

Submitted May 28, 2002; revision accepted June 28, 2002.

Juvenile idiopathic arthritis (JIA, previously juvenile chronic arthritis) is a clinically heterogeneous group of arthritides occurring in children younger than 16 years with an onset characterized primarily by arthritis persisting for at least 6 weeks and without a known cause[1,2]. The diagnosis of JIA is made clinically after exclusion of infections or other inflammatory diseases. The serological support is limited to the determination of antinuclear antibodies (ANA) and rheumatoid factor (IgM-RF). ANA are present in 75-85% of children with oligoarticular JIA and in 40-50% of children with polyarticular JIA. ANA are unusual in patients with systemic JIA. A relation between the presence of ANA in the serum of JIA patients and the occurrence of uveitis has been described but their presence is not related to the disease course nor to the severity of the joint involvement[3]. IgM-RF is present in 5-10% of JIA patients and the clinical pattern of these patients is very similar to rheumatoid arthritis (RA) in adults[4-6].

RA is the most common chronic inflammatory disease of joints in adults (1-3% of the population) and this diagnosis also depends primarily on clinical manifestations. IgM-RF can be detected in about 75% of RA patients but its specifici-

ty is limited[7]. Recently, Schellekens and coworkers described a serological test, the anti-cyclic citrullinated peptide (anti-CCP) ELISA, that is very specific (96-98%) for RA with a sensitivity of more than 60%[8]. Anti-CCP auto-antibodies have been shown to be present in 60-75% of established RA patients[9-11]. The probability of diagnosing RA correctly at an early stage of disease can be significantly increased by testing both IgM-RF and anti-CCP[10,12]. Other studies have shown that anti-CCP positive RA patients developed more severe radiological damage than anti-CCP negative RA patients after 3-6 years of followup[11,13].

The aim of the present study was to determine if anti-CCP can be detected in sera of JIA patients to support the diagnosis and if anti-CCP can be used to identify JIA patients with a more severe destructive course of the disease.

MATERIALS AND METHODS

Sample collection. Serum samples of 71 JIA patients who consecutively visited the departments of (pediatric) rheumatology in Leiden and Amsterdam were analyzed. Informed consent was obtained according to the medical ethical regulations. Two or more sera taken at different time points in the disease course (before change of medication) were available from 28 of the 71 patients to determine if the anti-CCP ELISA converted from positive to negative or *vice versa* during followup. In total 100 serum samples were tested. All patients were classified according to the ILAR criteria[2]. 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. The radiological data collected were roughly from the same time point as serum collection.

Measurements. IgM-RF was measured by ELISA as described previously[14]. ANA was assayed by a standard indirect immunofluorescence technique on ethanol fixed HEp-2 cells (Biomedical Diagnostics, Brugge, Belgium). ANA serum titers at ≥ 1/40 were considered positive. IgM-RF and ANA were determined at disease onset.

Anti-CCP antibodies were tested by a commercially available ELISA kit purchased from Euro-Diagnostica b.v. (Arnhem, The Netherlands) containing the cyclic citrullinated peptide cfc1-cyc as described by Schellekens, *et al*[10]. For the determination of anti-CCP in the sera the cut-off value chosen was 60 units rather than the 50 units recommended by the kit. In our cohort sera, the cut-off of 60 units seemed to guarantee the highest specificity without significant loss of sensitivity as is illustrated in Figure 1. All sera were analyzed at least in duplicate, and the results were averaged. A serum control was included on all plates to monitor plate-to-plate variation. Variation never exceeded 5%, and values were therefore not corrected.

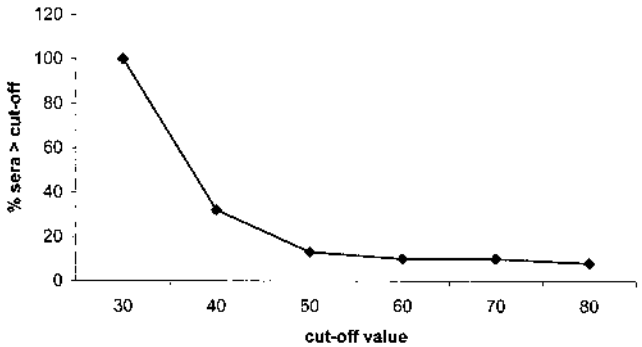

Figure 1. Relationship between the cut-off value of anti-CCP ELISA and the percentage of JIA patients with values above the cut-off. X-axis: values of anti-CCP ELISA (units) (Euro-Diagnostica b.v., Arnhem, The Netherlands). Y-axis: percentage of JIA patients with values above cut-off.

Statistical analysis. Statistical analyses were performed using SPSS software. The Fisher's exact test and Student's t test were used for testing the significance of differences in variables between anti-CCP positive and anti-CCP negative patients. The associations between anti-CCP measurements, disease activity, and radiological data were evaluated with random effects logistic regression to account for the repeated anti-CCP measurements in some of the patients. A p value of ≤ 0.05 was considered statistically significant.

RESULTS

One hundred sera from 71 JIA patients at different time points in their disease course were analyzed. Patient and disease characteristics at the time of serum collection are shown in Table 1. The 100 sera belonged to 71 JIA patients [age 2-20 years, mean 9.6 years (SD 4.5), median 10.5 years] of all 8 different JIA subtypes. One or more followup sera were available from 28 patients randomly distributed over the different JIA subtypes. The interval between the tested serum samples of one patient ranged between 1 and 84 months (mean 26 ± 20 months, median 23).

The disease duration had a mean of 39 ± 47 months (median 24 months, and range 3-245). Seventy-seven percent of the patients had clinical arthritis at the time of serum collection. Radiological data were available for 66 of the 71 patients.

Anti-CCP test results. Ten JIA patients (15%) tested anti-CCP positive and 8 of these anti-CCP positive JIA patients were also IgM-RF positive. A positive anti-CCP test occurred significantly more often in polyarticular IgM-RF positive patients compared to the other JIA subtypes ($p < 0.0001$). The occurrence of anti-CCP among the other subtypes of JIA ($p = 0.17$) was rare as is shown in Table 2. Of the 11 IgM-RF positive patients, 8 (73%) had a positive anti-CCP test with values ranging between 60 and 1915 units. One JIA patient with persistent oligoarthritis had a clearly positive anti-CCP test (674 units), while one JIA patient with other arthritis (extended oligoarthritis with a second degree relative with psoriasis) gave different results at the 2 occasions that serum was collected. This patient first tested anti-CCP positive (77 units) but the serum collected 2 years later was anti-CCP negative. This was the only patient who had different anti-CCP results when tested on more than one occasion.

Clinical correlation. Disease duration did not differ significantly between anti-CCP positive and anti-CCP negative patients ($p = 0.34$).

All anti-CCP positive patients had clinical arthritis at the time of serum collection, as was the case in only 39 of 61 (64%) anti-CCP negative patients ($p = 0.025$). There was no significant difference in the use of disease modifying antirheumatic drugs between anti-CCP positive and negative patients in this study.

Radiological damage was observed on the radiographs of 30/66 (46%) of the evaluable patients: 8/30 (27%) of the patients with radiological damage were anti-CCP positive,

Table 1. Patient and disease characteristics of 71 JIA patients at the time point of serum collection.

JIA Subtype	Patients, n	Tested Sera*, n	Disease Duration mos**, Mean (range)	DMARD Use at Time Point of Serum Collection***, n	Clinical Arthritis Present at Time Point of Serum Collection†, n	Radiological Damage Present‡ (No Data Available), n
Systemic arthritis	10	18	60 (3–141)	10	13	10 (1)
Persistent oligoarthritis	11	13	33 (3–134)	1	8	5 (4)
Extended oligoarthritis	6	11	53 (3–223)	4	8	0 (1)
IgM-RF negative polyarthritis	24	35	26 (3–71)	20	25	14 (1)
IgM-RF positive polyarthritis	11	12	26 (6–245)	5	12	11 (1)
Psoriatic arthritis	3	3	45 (36–65)	2	2	1
Enthesitis related arthritis	2	2	6 (3–12)	1	1	1
Other arthritis	4	6	67 (15–122)	5	4	1

* 28 patients had more than one serum sample tested at different time points in their disease course; ** The disease duration is calculated at the time point of serum collection; *** DMARD included sulfasalazine, hydroxychloroquine, methotrexate, etanercept; † Clinical arthritis defined as swelling and/or pain with limitation of motion; ‡Radiological damage defined as swelling and/or joint space narrowing (at the time point of serum collection). Other arthritis is defined as not fulfilling one category within the JIA definition[2].

Table 2. Results of anti-CCP ELISA using cfc1-cfc peptide in sera of 71 JIA patients.

JIA type	n	Anti-CCP Positive, n	Value ELISA, U Range	Anti-CCP Negative, n	Value ELISA, u Range
Systemic arthritis	10	—		10	30–52
Persistent oligoarthritis	11	1	674	10	33–47
Extended oligoarthritis	6	—		6	35–47
IgM-RF negative polyarthritis	24	—		24	32–51
IgM-RF positive polyarthritis	11	8	60–1915	3	36–47
Psoriatic arthritis	3	—		3	32–37
Enthesitis related arthritis	2	—		2	37–50
Other arthritis	4	1*	77	3	34–38

Anti-CCP positive: values ≥ 60 units; Anti-CCP negative: values ≤ 59 units. *One patient was tested with different results: at disease duration of 8 yrs: anti-CCP positive (77 units); 24 mos later the results were: anti-CCP negative (35 units)

and 11/30 (37%) were IgM-RF positive. All 11 IgM-RF positive JIA patients had radiological damage ($p < 0.001$) compared to 8 out of 10 anti-CCP positive patients ($p = 0.009$). The 2 positive anti-CCP patients without IgM-RF had no radiological abnormalities. Radiological damage occurred significantly more in the anti-CCP positive patients than in the anti-CCP negative patients ($p = 0.009$) with an odds ratio (OR) of 12.7 (95% confidence interval 1.5-108), but when corrected for IgM-RF status the OR was no longer significant ($p = 0.88$).

DISCUSSION

The anti-CCP ELISA is a new diagnostic test with extremely high specificity for RA[8]. We investigated whether anti-CCP antibodies could also support the diagnosis of JIA. We did not test sera from healthy children or those with infections or other autoimmune diseases since such analyses have been performed extensively in adults[8,10,15]. The high prevalence of anti-CCP in polyarticular IgM-RF positive JIA patients shows that anti-CCP selects for a specific subgroup of JIA patients but is not supportive for the diagnosis of JIA in general.

The anti-CCP test originates from the detection of other autoantibodies commonly seen in RA: antiperinuclear factor (APF) and anti-keratin antibodies (AKA). Schellekens, *et al* have shown that APF and AKA specifically bind to substrates containing the modified amino acid citrulline[9]. The methodological difficulties in the assessment of APF and AKA are summarized by Van Boekel, *et al*[8] and the anti-CCP assay might be looked at as a simple, more specific, and functional replacement of the immunofluorescence tests used for the detection of APF and AKA.

Published studies have reported substantial differences of occurrence of APF and AKA in patient populations with JIA; results varied from 1% to 37% for APF[16,17] and 2% to 50% for AKA[18,19]. These discrepancies were attributed to either methodological differences in the detection of the autoantibodies or differences in the JIA population studied. Several authors have noted that APF or AKA were most frequently detected in the subgroup of IgM-RF positive JIA patients[17-21]. These observations are in agreement with our results of predominance of occurrence of anti-CCP in this subgroup.

In the literature, reports of anti-CCP ELISA in JIA are very

scarce. Bizzaro, *et al*[15] describe negative anti-CCP results in 3 tested JIA patients (subtype unknown). Recently, Avcin, *et al*[22] described anti-CCP positivity in 2 out of 108 tested JIA patients (oligoarticular and polyarticular IgM-RF negative patients). One polyarticular IgM-RF positive JIA patient tested negative. Although their cut-off value, using the same commercial anti-CCP test we used, was 70 units, our results were very similar. In our study only one patient with a value of 60 units (Table 2) was considered anti-CCP positive, while all others had values above 70 units. Therefore our results are in agreement with the results published by Avcin and coworkers[22] and indicate that anti-CCP antibodies are present in only a subset of JIA patients.

Because of the very high specificity of this autoantibody system for adult RA (more than 97%)[8], it seems likely that this subset includes JIA patients who are developing a pattern of involvement like that of adult RA. The observation that the titer values in the anti-CCP positive JIA patients generally were lower than those observed in adults with RA is in line with this assumption of a developing disease. Longer followup of this group of JIA patients will provide a definitive answer as to whether anti-CCP antibodies can predict the development of an adult RA-like disease pattern in JIA.

It is known that IgM-RF positive JIA often has a disease course similar to RA in adults[6]. Our study confirms this similarity.

Although a role for anti-CCP in RA has been suggested, the significance of anti-CCP in the disease pathogenesis remains unclear[23]. All IgM-RF positive JIA patients had clinical arthritis and radiological damage, and almost all were anti-CCP positive. These results confirm previous studies in adult early RA that both IgM-RF and anti-CCP antibodies can predict the development of a more severe destructive disease course[11-13] and that their simultaneous presence may be an indication for earlier immunosuppressive treatment[24].

We have shown that anti-CCP antibodies can be found incidentally in the serum of children with several subtypes of JIA, but that they are commonly present in polyarticular IgM-RF positive JIA patients. Further followup studies will more firmly establish whether the presence of anti-CCP antibodies in JIA patients predicts the development of a disease course like adult RA and selects JIA patients with a more severe destructive disease course.

ACKNOWLEDGMENT

We thank Rob van der Stadt (clinical chemist) for his contribution and support.

REFERENCES

1. Woo P, Wedderburn LR: Juvenile chronic arthritis. Lancet 1998;351:969-73.
2. Petty RE, Southwood TR, Baum J. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. J Rheumatol 1998;25:1991-4.
3. Cassidy JT, Petty RE. Textbook of pediatric rheumatology. 4th ed. Philadelphia: W.B. Saunders Company; 2001:254-5, 260-2.
4. Andersson Gäre B, Fasth A. The natural history of juvenile chronic arthritis: A population based cohort study. I. Onset and disease process. J Rheumatol 1995;22:295-307.
5. Prieur AM: Juvenile (chronic) arthritis is not juvenile rheumatoid arthritis. Rev Rhum 1996;63:1-4.
6. Cassidy JT, Petty RE. Textbook of pediatric rheumatology. 4th edition Philadelphia: W.B. Saunders Company; 2001:233-5.
7. Smolen JS. Rheumatoid arthritis. In: van Venrooij WJ, Maini RN, editors. Manual of biological markers. Amsterdam: Kluwer Academic Publishers 1996: C1.1;1-18.
8. Van Boekel MAM, Vossenaar ER, van den Hoogen FHJ, van Venrooij WJ. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. Arthritis Res 2002;4:87-93.
9. Schellekens GA, De Jong BAW, Van den Hoogen FHJ, Van de Putte LBA, Van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis specific antigens. J Clin Invest 1998;101:273-81.
10. Schellekens GA, Visser H, De Jong BAW, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-63.
11. Kroot EJA, De Jong BAW, Van Leeuwen MA, et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831-5.
12. Visser H, Le Cessie S, Vos K, Breedveld FC, Hazes JMW. How to diagnose rheumatoid arthritis early. Arthritis Rheum 2002; 46:357-65.
13. Van Jaarsveld CHM, Ter Borg EJ, Jacobs JWG, et al. The prognostic value of the antiperinuclear factor, anti-citrullinated peptide antibodies and rheumatoid factor in early rheumatoid arthritis. Clin Exp Rheumatol 1999;17:689-97.
14. Otten HG, Daha MR, de Rooij HH, Breedveld FC. Quantitative detection of class-specific rheumatoid factors using mouse monoclonal antibodies and the biotin/streptavidin enhancement system. Br J Rheumatol 1989;28:310-6.
15. Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli R. Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem 2001;47:1089-93.
16. Gabay C, Prieur AM, Meyer O. Occurrence of antiperinuclear, antikeratin, and anti-RA 33 antibodies in juvenile chronic arthritis. Ann Rheum Dis 1993;52:785-9.
17. Nesher G, Moore TL, Grisanti MW, El-Najdawi E, Osborn TG. Antiperinuclear factor in juvenile rheumatoid arthritis. Ann Rheum Dis 1992;51:350-2.
18. Abreu I, Melo Gomes JA. Antikeratin antibodies in chronic arthritis of childhood [letter]. Clin Exp Rheumatol 1998;16:516-7.
19. Hromadnikova I, Vavrincova P, Stechova K, Hridelova D. Anti-keratin antibodies in patients with juvenile idiopathic arthritis. Clin Exp Rheumatol 2001;19:470-3.
20. Essen R von, Ylijoki H, Savolainen A, Haapasaari J, Oksala U. Antiperinuclear factor in chronic juvenile arthritis (letter). Ann Rheum Dis 1992;51:1264-8.
21. Pelkonen P, Kurki P. Antibodies to stratum corneum in juvenile chronic arthritis. Clin Exp Rheumatol 1993; Suppl 11:S-73.
22. Avcin T, Cimaz R, Falcini F, et al. Prevalence and clinical significance of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis. Ann Rheum Dis 2002; 61:608-11.
23. Reparon-Schuijt, van Esch WJE, van Kooten C, et al. Secretion of anti-citrulline-containing peptide antibody by B lymphocytes in rheumatoid arthritis. Arthritis Rheum 2001;44:41-7.
24. O'Dell JR. Treating rheumatoid arthritis early: a window of opportunity? Arthritis Rheum 2002;46:283-5.