



## The prevalence of anti-cyclic citrullinated peptide antibodies in juvenile idiopathic arthritis

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### Abstract

**Objectives:** To assess the presence of anti-cyclic citrullinated peptide antibodies in a cohort of patients with juvenile idiopathic arthritis.

**Methods:** Anti-cyclic citrullinated peptide antibodies was tested for with an enzyme linked immunoabsorbent assay (ELISA) in serum samples of patients from the Hospital de Clínicas de Porto Alegre, all less than 18 years old and with previous diagnosis for at least 6 months. IgMRF (rheumatoid factor) and antinuclear antibodies in Hep-2 cells were also assayed.

**Results:** Serum samples were analyzed from 45 patients. The presence of high levels of anti-cyclic citrullinated peptide antibodies was found in the serum of just one child (2%), who presented sero-positive polyarthritis.

**Conclusions:** Anti-cyclic citrullinated peptide antibodies can be detected in children with juvenile idiopathic arthritis, but much less frequently than in adults with rheumatoid arthritis. It still remains to be determined whether anti-cyclic citrullinated peptide antibodies can identify a subset of juvenile idiopathic arthritis patients with the potential to progress to adult rheumatoid arthritis.

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Juvenile idiopathic arthritis (JIA) is defined as a disease with onset before 16 years of age, primarily characterized by the presence of persistent arthritis in one or more joints, for at least six weeks, once other causes have been ruled out.<sup>1,2</sup> To date, JIA diagnosis is based on clinical history and physical examination, with supplementary methods being

used primarily to rule out other diagnostic possibilities and comorbidity. There are no laboratory tests considered specific for confirming diagnosis. Biochemical laboratory tests and serological markers are useful aids to differential diagnosis, for classifying JIA subsets, assessing the extent of inflammation and determining prognosis and response to treatment.<sup>1,2</sup>

A positive serological diagnosis of JIA is restricted to patients with later onset polyarthritis who exhibit IgM-Rheumatoid Factor RF (+), which is detected by nephelometry in 7 to 10% of cases. Antinuclear factors (ANA) were observed in 2 to 70% of the JIA populations studied, with the variability probably due to the different substrates used for measurement and the distinct subtypes of JIA. An elevated prevalence of ANA (between 65 and 85%) has been observed with the JIA oligoarthritis subtype associated with uveitis<sup>1-3</sup>

A new autoantibody, anti-CCP (anti-cyclic citrullinated peptide) has been studied recently. Citrullin is an amino acid that is a post-translation modification of arginine residues.

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It is present in high concentrations in the fillagrin peptide chain. Regions that are rich in citrullin appear to become the target of antifillagrin antibodies, identified in rheumatoid arthritis, which gave rise to the development of a synthetic peptide rich in citrullin to produce a more stable and standardized enzyme immunoassay.<sup>4,5</sup> Schellekens *et al.*<sup>6</sup> observed that anti-CCP is highly specific (96-98%) for rheumatoid arthritis (RA) in adults, with a sensitivity of 60%. Some studies have shown the presence of anti-CCP in 60-75% of patients with confirmed with RA.<sup>7-9</sup>

The frequency of autoantibodies in adults has been reasonably well investigated, but their role in JIA has been little studied. Recent studies of the prevalence of anti-CCP in patients with JIA have observed that it can be detected in patients with polyarthritic onset and positive RF.<sup>10-13</sup>

The objective of this study was to investigate the prevalence of anti-CCP antibodies in serum from patients with JIA, assessing its utility as a serological marker.

### Patients and methods

Forty-five consecutive JIA patients were recruited from those being treated by the Rheumatology Department at our hospital from 01/06/2003 to 31/12/2003. The JIA diagnoses were performed based on criteria published by the International League of Associations for Rheumatology (ILAR)<sup>14</sup> for diagnosing the disease.

All patients under eighteen whose diagnoses had been confirmed for at least six months were included. Patients diagnosed with other autoimmune diseases, including superimposed syndromes, were excluded. Those legally responsible for the patients signed free and informed consent and the project was approved by Ethics Committee at the HCPA.<sup>15</sup>

Venous blood samples (2-3 ml) were collected from all patients by peripheral venopuncture for routine laboratory workup. Serum samples were obtained by centrifuge and frozen at -70 °C until anti-CCP assay, which was performed for all samples at the same time. In addition to anti-CCP, the presence of antinuclear antibodies (indirect immunofluorescence with HEp-2 cells) and Rheumatoid factor IgM (nephelometry, BN2 System, Behring,USA) was also investigated.

Anti-CCP antibodies were determined using second generation ELISA (DIASTAT™, Axis-Shield Diagnostics, Scotland, United Kingdom). The cutoff chosen for a positive result was a concentration above 10 U/ml, as suggested by the manufacturer. The negative controls exhibited concentrations of less than 5 U/ml.

Data from the statistical analysis is presented in the form of descriptive statistics and comparisons between values employ Student's *t* test.

### Results

The ages and clinical characteristics of the 45 patients studied are described in Table 1.

**Table 1** - Demographic data

|                             |                        |
|-----------------------------|------------------------|
| Age (mean)                  | 10 to 11 m (±4 to 1 m) |
| Length of disease (mean)    | 6 to 10 m (±3 to 10 m) |
| Age at disease onset (mean) | 5 to 4 m (±3 to 1 m)   |
| Subtype                     |                        |
| Systemic                    | 7 (16%)                |
| Persistent oligoarthritis   | 9 (20%)                |
| Extended oligoarthritis     | 3 (7%)                 |
| Polyarthritis RF (+)        | 5 (11%)                |
| Polyarthritis RF (-)        | 16 (35%)               |
| Psoriatic arthritis         | 3 (7%)                 |
| Enthesitis arthritis        | 2 (4%)                 |
| Total                       | 45 (100%)              |

Thirty-one of the 45 patients in this sample were female (69%) and 14 were male (31%). Their ages were within the range 3 to 17 years (mean 10 years ,11 months ± 4years, 1 month), and the mean age at onset of arthritis was 5 years, 4 months (±3 years 10 months). Forty-six percent of this sample presented with the polyarticular form of JIA.

Anti-CCP values for each patient are shown in Figure 1, broken down according to JIA subtype. With the exception of a single patient, all levels were below the cutoff for normality established by the laboratory.

Six children in the patient sample had reagent RF IgM (14%): five of these presented polyarticular polyarticular disease and one oligoarticular extended disease.

We found just one case (1/5, 20%) of reagent anti-CCP in the subset of patients with seropositive polyarthritis (reagent FR IgM). None of the other subsets had reagent anti-CCP results. The difference in incidence of anti-CCP between JIA subtypes was not significant.

Additionally, just two patients exhibited reagent ANA, both with non-reagent RF IgM and anti-CCP and suffering from the oligoarticular form of JIA.

### Discussion

Anti-CCP antibodies have been considered an important serological marker for diagnosing RA and also a possible marker of unfavorable prognosis.<sup>6-9</sup>

The present study has demonstrated, in contrast with what has been described with adult patients, that anti-CCP antibodies are rarely detectable in patients with JIA. Anti-CCP was identified in just one case out of a series of 45 patients (≅ 2%) with well-established diagnoses of JIA. Our results are in line with those reported by Avcin *et al.*,<sup>12</sup> who observed anti-CCP positivity in two children out of 109 (1.8%) suffering from JIA.

In contrast, van Rossum *et al.*<sup>10</sup> recently presented the data from their study of 71 JIA patients, 15% of whom exhibited reagent anti-CCP, and 73% of these had reagent RF. This difference in rates of anti-CCP frequency is probably secondary to differences in the populations studied and the assay techniques employed, in addition to the use of different cutoff points. The study undertaken by Rossum *et*



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