Anti-CCP antibodies in children with Juvenile Idiopathic Arthritis (JIA) – diagnostic and clinical significance

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Abstract

Anti-cyclic citrullinated peptide (anti-CCP) antibodies are considered to be a marker of rheumatoid arthritis (RA). However, limited and controversial reports concern this problem in children with juvenile idiopathic arthritis (JIA).

Objective: *To access the prevalence and diagnostic value of anti-CCP antibodies in children with JIA. Sera of 96 JIA children were examined for anti-CCP, IgM rheumatoid factor (IgM-RF) and anti-nuclear (ANA) antibody levels. Anti-CCP-positivity was correlated with the disease characteristics.*

Methods: *Sera of 96 JIA children were examined for anti-CCP, IgM rheumatoid factor (IgM-RF) and anti-nuclear (ANA) antibody levels. Anti-CCP-positivity was correlated positively with the disease characteristics. The control group consisted of 22 healthy children.*

Results: *For anti-CCP, sensitivity was 41.6% and specificity 100%. Anti-CCP were present in both IgM-RF positive and negative sera, in all types of JIA onset, including 40.7% children with early stage of JIA (disease duration <6 months). Anti-CCP levels correlated positively with the high disease activity. All healthy children were anti-CCP-negative. The sensitivity of IgM-RF and ANA were significantly lower (14.5% and 8.3%, respectively).*

Conclusion: *Anti-CCP antibodies are present in sera of JIA children even at the early stage of the disease, in all subtypes of JIA, in both IgM-RF-positive and IgM-RF-negative cases. Moreover, anti-CCP antibodies are good markers of disease activity.*

Key words: *juvenile idiopathic arthritis, anti-CCP antibodies, IgM rheumatoid factor, antinuclear antibodies (ANA).*

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Introduction

In rheumatoid arthritis (RA) in adults, as well as in juvenile idiopathic arthritis (JIA), there are only a few serological markers with confirmed serological value; one of them is IgM class rheumatoid factor (IgM-RF) – the main immunological marker for RA [1-4]. However, contrary to RA, in JIA especially at the onset of the disease IgM-RF is found only in low numbers [3, 5]. Unfortunately, there is no correlation between IgM-RF and severity of the clinical symptoms. IgM-RF could be also present in other disease states and in healthy people. The antinuclear antibodies (ANA) are markers only for early stages of oligoarticular disease with uveitis, and found in low percentage also are not pathognomonic for JIA [1, 5]. The diagnosis of JIA depends mainly on clinical manifestations and it is very difficult to establish the diagnosis of JIA, especially at the early stage of the disease, since the clinical symptoms are often not characteristic [1-6].

Therefore, studies are still conducted to find a new serological marker with significantly high sensitivity and specificity for JIA. Contrary to RA, there are only a few studies concerning the diagnostic efficacy of anti-CCP antibodies in JIA [7-15].

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Table 1. Clinical characteristics group of children with JIA

Patient characteristics	n	(%)
Sex:		
girls	59	61.5
boys	37	38.5
Age:		
<7 years	11	11.5
7-14 years	53	55.2
>14 years	32	33.3
JIA duration time:		
<6 months	27	28.0
6-12 months	30	31.3
>12 months	39	40.7
JIA subtype:		
oligoarthritis	36	37.5
polyarthritis	49	51.0
systemic	11	11.5
JIA activity:		
high	25	26.0
moderate	19	19.8
low	52	54.2

The goal of the study was to access the prevalence of anti-CCP antibodies in children with JIA. Moreover, we aimed to investigate the clinical significance and diagnostic value of the anti-CCP antibodies comparing to standard serological markers assessed in JIA.

Material and Methods

Patients

Ninety six children (59 girls, 37 boys) with JIA, fulfilling the 1997 International League Against Rheumatism (ILAR) classification criteria, aged 3-18 years (mean 12,8±4 years), in different time points of the disease course, were included into the study (Table 1).

Methods

The activity of the rheumatoid process was accessed by modified Wilkoszewski's criteria as described previously by Smolewska et al. [17]. Namely, three stages of JIA activity were distinguished, based on clinical and laboratory criteria: low activity (joint movement limited, without pain or swelling, no extra-articular symptoms, ESR <20 mm/h, CRP <10 mg/l); moderate activity (moderate intensity of arthritis, and/or slight temperature, ESR 20-60 mm/1st h, CRP 10–30 mg/l); and high activity (morning stiffness, pain and/or swelling of joints, and/or hepatosplenomegaly, fever, rash, and raised values for laboratory tests ESR >60 mm/1st h, CRP >30 mg/l) (Table 1).

As a control group, 22 sex-, and age-matched children with functional cardio-vascular system dysfunction were also examined. Serum samples were obtained simultaneously with routine laboratory investigations.

The study was approved by the local Ethics Committee. In every case the written informed consent was obtained from the parents.

Measurements of serological markers

Serum samples were analyzed by anti-CCP – Euroimmun Polska Sp. z o.o. (52-219 Wrocław, ul. gen. Grota-Roweckiego 34a) and IgM-RF – Biomedica Poland Sp. z o.o. (05-500 Piaseczno, ul. Raszyńska 13) ELISA kits. ANA were accessed by standard indirect immunofluorescent technique (Euroimmun Polska Sp. z o.o.). According to manufacturers' recommendations, sera contained anti-CCP the levels ≥5 RU/mL, IgM-RF the levels ≥24 RU/mL and ANA at the titers ≥1:320 were considered positive, respectively. All serum samples were tested twice.

The sensitivity and specificity for anti-CCP antibodies were calculated. Correlations between anti-CCP antibodies levels, other serological markers (IgM-RF, ANA) and the disease characteristics were also investigated.

Statistical analyses

The Statistica 6.0 version and MedCalc 6.14 were used for statistical analysis. Comparison of titer distributions was made with the Mann-Whitney U-test. The chi-square test was used for comparisons of sensitivity and specificity. Correlations between variables were evaluated by the Spearman's rank test. Differences were considered significant at p values <0.05.

Results

Prevalence of anti-CCP antibodies in JIA children

Forty out of 96 (41.7%) examined JIA children was anti-CCP positive. Sensitivity was approximately 41.5% at 100% specificity. In contrast, the whole control group were anti-CCP negative. Serum concentration levels of anti-CCP antibodies were found statistically higher in children with JIA comparing to control group (p=0.0049; z=2.808).

The highest prevalence of anti-CCP antibodies was in systemic disease (54.5%, 6/11). In polyarticular type of JIA onset the anti-CCP-positivity was lower (42.8%, 21/49), including 50% (7/14) of anti-CCP-positive cases with simultaneously presence of IgM-RF and the lowest in oligoarthritis (36%, 13/36) (Table 2A). However differences were not statistically significant (p>0,05).

Table 2. The occurrence of the serological markers in children with JIA in relation to JIA subtype (A), the disease duration time (B) and activity of rheumatoid process (C)

Table 2A.

		JIA subtype					
		systemic (n=11)		polyarthritis (n=49)		oligoarthritis (n=36)	
		n	(%)	n	(%)	n	(%)
RF-IgM (+)	≥5 RU/ml	6	54.5	21	42.8	13	36.0
	≥24 RU/ml	1	9.0	11	22.5	2	5.5

Table 2B.

		Activity of rheumatoid process					
		high (n=25)		moderate (n=20)		low (n=51)	
		n	(%)	n	(%)	n	(%)
RF-IgM (+)	≥5 RU/ml	17	68.0	11	55	13	25.5
	≥24 RU/ml	4	16.0	3	15	7	13.7

Table 2C.

		JIA duration time					
		<6 months (n=27)		6-12 months (n=30)		>12 months (n=39)	
		n	(%)	n	(%)	n	(%)
RF-IgM (+)	≥5 RU/ml	11	40.7	7	23.3	22	56.4
	≥24 RU/ml	2	7.4	5	16.7	7	17.9

Prevalence of anti-CCP antibodies depending on disease activity

Serum concentration levels of anti-CCP antibodies were found to be significantly higher in children with higher activity of rheumatoid process (p=0.014; R=0,25). Anti-CCP antibodies were most frequently present in sera of children with high (68%) and moderate (55%) JIA activity (Table 2B).

Moreover, the positive correlations between anti-CCP and CRP levels (p=0.027; R=0.22) and thrombocyte count were observed (p=0.03; R=0.22).

Prevalence of anti-CCP antibodies depending on disease duration

Anti-CCP-positivity was most frequently observed in children with the disease duration time above 1 year (56.4%; 22/39), than below 6 months (40.7%; 11/27) and were rarely found in sera of children with clinical symptoms observed less then 12 months (23.3%; 7/30) (Table 2C).

Diagnostic utility of other serological markers

Sera of 14/96 (14.5%) children with JIA and one child (4.5%) from the control group were IgM-RF-positive. The IgM-RF sensitivity was 14.5% at 95.5% specificity. Serum concentration levels of IgM-RF did not differ statistically comparing to control group. The highest IgM-RF-positivity (22.5%) was observed in JIA children with polyarticular onset (Table 2). No correlation between IgM-RF serum concentration levels and disease activity was observed. IgM-RF prevalence raised slightly with the longer disease duration time – from 7.4% (2/27) to 18.0% (7/39).

The ANA with spotted type of luminescence, at the titers ≥1:320 considered as positive, were present in 8.3% (8/96) children with JIA, mainly with oligoarthritis – 22.2% (8/36). The sensitivity for ANA was 8.3%. No child from control group had ANA at the titers ≥1:320. There was no correlation between ANA-positivity and activity of rheumatoid process or the disease duration time. The presence of ANA was also not associated with anti-CCP-positivity in examined JIA children.

Discussion

According to recent findings in adults with RA anti-CCP antibodies are believed to be pathognomonic for rheumatoid process [4, 5, 18, 19]. Moreover, they are also present in 20-25% of RF-negative RA cases [5, 18, 19]. Furthermore, anti-CCP antibodies may be an indicator of the activity and severity of the rheumatoid process and can be predictors of progressive radiological damage in bones [4, 5, 7]. However, contrary to RA, there are only a few studies evaluating

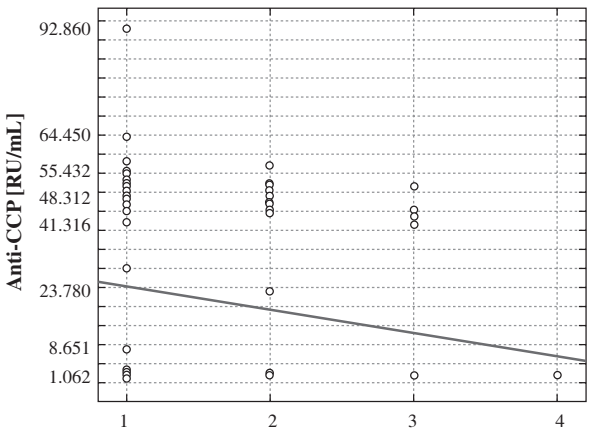

Fig. 1. Anti-CCP serum titers in JIA and control group children (1 – polyarthritis; 2 – oligoarthritis; 3 – systemic disease; 4 – control group)

anti-CCP antibodies in JIA and the opinions about their value in children are controversial [7-16].

The prevalence of anti-CCP antibodies in our study was higher than that obtained in children sera by other authors. Avčin et al. [8] and Kasapcopur et al. [7] reported that anti-CCP antibodies were rare in patients with JIA (1.8% out of 109; 2% out of 122). As in the work of Avčin et al. [8] the JIA children were from multicenter study, with no inclusion criteria other than their clinical diagnosis, that could be one reason of such low prevalence of these autoantibodies. In addition, the time at which the sera were obtained, as it was speculated their correlation with disease activity, would influence the anti-CCP antibody concentration. Slightly higher prevalence of anti-CCP antibodies, 5% from the group of 140 children with JIA, was described by Hromadnikova et al. [10]. Similarly Ferrucci et al. [15] reported anti-CCP-positivity in 5.6% out of 230 patients. However, van Rossum et al. [11] indicated presence of anti-CCP antibodies in sera of 15% out of 71 children with JIA, what was almost three time more frequently comparing to previous studies. In another research, Lee et al. [12] gained anti-CCP positive sera in 28.6% cases. The divergence of the results in various researches could be due to different groups (criteria of inclusion) of children with JIA included into the studies and different cut-off values approved as the normal value. Such high prevalence of anti-CCP antibodies in our study (41.6%) might support the previous work of Low et al. [13] and confirm authors' opinion that the obtained results could be also depend on different ELISA kit used in the researches. In children with JIA authors observed from 19.7% to 77% anti-CCP positive sera according to the usage of various epitopes.

It should be stressed that, like in other studies [7-15], we did not find increased levels of anti-CCP antibodies in the group of healthy children. As IgM-RF or ANA, were present in sera of patients with other diseases and even in healthy people, anti-CCP antibodies are very specific marker also for JIA comparing to classical markers of rheumatoid process.

We found anti-CCP-positive cases in all subtypes of JIA. Since previous observations indicated that anti-CCP antibodies were found in patients with RA, it was not surprising to find them in children with IgM-RF-positive polyarthritis that resembles disease course similar to RA in adults. However, the presence of anti-CCP antibodies did not correlate directly with presence of IgM-RF as it was proved in adults [5, 12]. Controversially, in our cohort of JIA patients, anti-CCP antibodies were most frequently found in sera of children with systemic disease. These data are at variance with those published previously by van Rossum et al. [11] and Ferruci et al. [15]. However, in our cohort anti-CCP antibodies were present in polyarthritis and oligoarthritis just in slightly lower percentages than in systemic disease.

Comparing the occurrence of anti-CCP antibodies with IgM-RF in children with JIA, classical rheumatoid factor was present above 3 or almost 4 times less frequently than anti-CCP antibodies. It should be underlined, that majority of IgM-RF-positive sera were also anti-CCP-positive. On the other hand, half of RF-positive children had simultaneously present anti-CCP antibodies in their sera (7/14). Observations Ferruci et al. [15] are in agreement with our results of probable anti-CCP positivity in all subgroups of JIA, however, contrary to our findings authors underlined the predominance of anti-CCP occurrence in RF-positive cases.

It was confirmed in our study, like in previous researches, that anti-CCP-positivity correlated with activity of rheumatoid process. It could be reassumed that similarly in our cohort of JIA children, those with systemic disease had high rheumatoid process activity and that was the reason of such high prevalence of anti-CCP antibodies in that subgroup.

In our study no tendency for anti-CCP to increase or decrease during disease course was shown. The approximate percentages of anti-CCP positivity in sera of children with short and lasting over one year disease course were observed. Interestingly, in the subgroup of JIA children with clinical history between 6 months up to one year the presence of anti-CCP antibodies was the lowest. It could be speculated that large part of children with oligoarthritis and the low rheumatoid process activity (also resulting from applied treatment) in that group could be the main reason of that phenomenon. Nevertheless, because of further researches are needed to confirm that fact.

Although a role for anti-CCP in RA has been suggested, the significance of anti-CCP in the disease pathogenesis remains unclear [4, 5, 18]. Further follow up studies would 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 destructive disease course. Longer observation will also provide a definitive answer as to whether anti-CCP antibodies concentration values could change over the time.

To sum up, it was indicated that anti-CCP antibodies are present in sera of JIA children even at the early stage of the disease, in all subtypes of JIA, in both IgM-RF-positive and IgM-RF-negative cases. Moreover, anti-CCP antibodies are good markers of disease activity. The anti-CCP antibodies seem to be more useful in JIA than other investigated serological markers and their inclusion into classification criteria for JIA should be considered.

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