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Anticarbamylated protein (anti-CarP) antibodies are present in sera of juvenile idiopathic arthritis (JIA) patients

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Abstract

Objectives

Anti-citrullinated protein antibodies (ACPA) have a low prevalence in juvenile idiopathic arthritis (JIA) patients. Recently, autoantibodies recognizing carbamylated proteins (anti-CarP) were observed in rheumatoid arthritis (RA) patients and were reported to be associated with a more severe clinical course in ACPA negative RA. We investigated the presence of anti-CarP antibodies in JIA patients and their relation to ACPA and IgM-rheumatoid factor (IgM-RF).

Methods

Cross-sectional samples of 234 JIA patients and 107 age-matched controls were analyzed for the presence of ACPA, IgM-RF and anti-CarP antibodies against carbamylated Fetal Calf Serum (FCS) or carbamylated Fibrinogen (Fib). The samples were obtained from patients in all different categories of JIA. Cut-off for positivity of anti-CarP antibodies was determined as the mean plus two times standard deviation of levels in sera of healthy controls.

Results

Anti-CarP FCS antibodies were present in 8.1% (19/234) of all JIA patients versus 4.7% (5/107) of the controls. Anti-CarP FCS antibodies were predominantly present in IgM-RF positive polyarticular JIA patients (42.1%, p<0.0001 vs other JIA categories). A similar observation was made using anti-CarP Fib antibodies.

Conclusions

Anti-CarP antibodies can be detected in sera of JIA-patients, especially in the polyarticular IgM-RF positive patients, the category most similar to RA.
Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of chronic arthritides that starts before the age of 16. Different categories according to ILAR criteria are discerned [1] and the diagnosis is made clinically after exclusion of other causes of arthritis. Prognosis is currently largely unpredictable, except for rheumatoid factor (RF)-positive individuals. Reaching sustained remission is still scarce [2] although treatment options have improved with biologicals [3], resulting in better outcome [4]. Research to identify markers predicting disease flares is ongoing [5-7] but markers that predict severe disease are currently lacking.

The discovery of anti-citrullinated protein antibodies (ACPA), often detected using assays based on cyclic-citrullinated peptides (CCP), has contributed substantially to the understanding of rheumatoid arthritis (RA) [8]. The presence of ACPA is now part of the 2010 EULAR/ACR criteria for RA. ACPA-positive RA patients have generally more severe disease courses with increased joint destruction [9]. Incidence rates for ACPA-positivity, usually detected by a CCP-assay, in JIA are low (2-5%) [10-13]. The presence of ACPA in JIA is merely confined to the polyarticular IgM-RF positive JIA category that resembles RA.

Recently, anti-carbamylated protein antibodies (anti-CarP) were described as a novel serological marker [14-16]. These antibodies are directed against proteins that have been modified by a post-translational modification named carbamylation. The physiological process of carbamylation increases during inflammation. In carbamylated proteins lysines are converted into homocitrullines. Anti-CarP antibodies were detected in sera of approximately 45% of RA patients and importantly in sera of 16-20% ACPA-negative RA patients, in comparison to less than 3% in healthy controls [15, 17, 18]. Within the ACPA-negative patients the presence of anti-CarP antibodies was associated with more severe radiographic progression [15]. Therefore anti-CarP antibodies could serve as a new prognostic marker in ACPA-negative RA patients [19]. Since the majority of JIA patients are ACPA-negative we analyzed whether anti-CarP antibodies can be detected in sera of JIA patients and whether their occurrence correlates with the presence of ACPA and/or IgM-RF.

Materials and methods

Sample collection

JIA patients from three Dutch sources were included. The first group (n=33) consisted of patients participating in the BeSt for Kids trial, (NTR 1574) a treatment strategy study enrolling JIA patients. The second group (n=48) contained patients in early years of disease...
participating a retrospective study described by Albers et al.[20] The third cohort (n=153) comprised participants of the Arthritis and Biologicals in Children (ABC) Register, an ongoing prospective observational study initiated in 1999, that aims to include all Dutch JIA patients treated with biologicals.[21] Healthy controls (n=107, mean age 11 years, range 2-20 years) were anonymous pediatric donors of allogeneic hematopoietic stem-cell grafts. Written informed consent was obtained from all patients and controls. Patients’ disease characteristics and part of laboratory data (IgM-RF, ANA) were collected from patient files. Blood collection and storage are comparable among different cohorts. Median disease duration of the 234 JIA patients at the time of serum collection was 2.3 years (IQR 0.7-6.8) (Table 1). All International League against Rheumatism JIA categories were included with polyarticular JIA over-represented.[1, 22]

Table 1 Disease characteristics of 234 juvenile idiopathic arthritis (JIA) patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
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<tbody>
<tr>
<td>Gender m/f (%f)</td>
<td>76/158 (67.5%)</td>
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<tr>
<td>Median age (years) (IQR)</td>
<td>12.1 (8.4–16.2)</td>
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<tr>
<td>Median disease duration (IQR)</td>
<td>2.3 (0.7–6.8)</td>
</tr>
<tr>
<td>Median age at JIA onset (IQR)</td>
<td>8.8 (3.4–12.4)</td>
</tr>
<tr>
<td>ANA-positive at disease onset</td>
<td>64 (27.4%)</td>
</tr>
<tr>
<td>Systemic JIA</td>
<td>35 (15.0%)</td>
</tr>
<tr>
<td>Polyarticular JIA RF-negative</td>
<td>90 (38.5%)</td>
</tr>
<tr>
<td>Polyarticular JIA RF-positive</td>
<td>19 (8.1%)</td>
</tr>
<tr>
<td>Oligo-articular JIA extended</td>
<td>41 (17.5%)</td>
</tr>
<tr>
<td>Oligo-articular JIA persistent</td>
<td>18 (7.7%)</td>
</tr>
<tr>
<td>Juvenile psoriatic arthritis</td>
<td>24 (10.3%)</td>
</tr>
<tr>
<td>Enthesitis-related arthritis</td>
<td>5 (2.1%)</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>2 (0.8%)</td>
</tr>
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ANA, anti-nuclear antibodies; RF, rheumatoid factor.

The correlation between anti-CarP antibodies and time in active disease as a measure of severity was investigated in the second cohort. The correlation between anti-CarP antibodies and ACRpedi30 response [23] (at least 30% improvement from baseline in 3 of 6 variables in the core set, with no more than 1 of the remaining variables worsening by >30%. Core set variables are 1) physician global assessment of disease activity; 2) parent/patient assessment of overall well-being; 3) functional ability; 4) number of joints with active arthritis; 5) number of joints with limited range of motion; and 6) erythrocyte sedimentation rate) after start of a biological was determined in the group from the ABC register.
Detection of anti-CarP antibodies, anti-CCP antibodies, ANA and IgM-RF

Anti-CarP antibodies were measured by ELISA using carbamylated Fetal Calfs-Serum (Ca-FCS) and human Fibrinogen (Ca-Fib) as antigens as described previously.[15] ACPA were measured using the CCP2 ELISA (Immunoscan RA Mark 2; Eurodiagnostica). Samples with a value above 25 units/ml were considered positive according to the manufacturer’s instructions. IgM-RF and ANA levels were determined at disease onset as part of routine patient care. For measuring ANA levels most Dutch hospitals use a standard indirect immunofluorescence technique on ethanol fixed HEp-2 cells and IgM-RF levels are usually determined by ELISA.

Statistical Analysis

Statistical analyses were performed with SPSS 17.0. Fisher’s exact test was used for testing the significance of differences between the percentages anti-CarP-positive and -negative patients. Pearson’s chi square and student’s t-test were used for identifying differences in time–in-active-disease [20] and ACRpedi30 response [23] between anti-CarP-positive and negative patients. Binary logistic regression with sensitivity analysis was used to test the interaction between age and the presence of anti-CarP antibodies. A p-value of less than 0,05 was considered statistically significant.

Results

Anti-CarP antibodies are present in JIA patients

All sera were tested for the presence of anti-CarP antibodies using Ca-FCS and Ca-Fib as antigens. In the total JIA cohort 8,1% (19/234) of the patients were positive for antibodies reacting to Ca-FCS versus 4,7% (5/107) of controls (p=0,20). In addition 13,2% (31/234) of patients versus 2,8% (3/107) of controls were positive for antibodies reacting to Ca-Fib (p=0,003). Not all individuals harbored both reactivities and (39/234) 16,7% of patients and 8/107 (7,5%) of controls were positive for at least one anti-CarP antibody (p=0,028) (data not shown) and 11/234(4,7%) vs 0 of the controls (p=0,017) were positive for both anti-CarP reactivities.

Since the cohort of JIA patients consisted of different disease categories these were analyzed separately (Figure 1). Anti-CarP antibodies were predominantly present in polyarticular IgM-RF positive patients (8/19, 421%) as compared to the other JIA categories (p<0.0001). This observation was made for both Ca-FCS and Ca-Fib as detecting antigens (Figure 1).
Together, these data indicate that the presence of anti-CarP-antibodies in JIA is mainly confined to polyarticular IgM-RF positive patient group.

Figure 1 IgG anticarbamylated protein (anti-CarP) antibodies are present in juvenile idiopathic arthritis (JIA) sera. A cut-off for positivity (horizontal line) was determined using the mean plus two times the SD of the healthy controls. Antibodies against Ca-FCS (A) and Ca-Fib (B) in the sera of JIA patients and healthy controls are depicted in aU/mL. (C) Results of anti-CarP antibodies: positivity above cut-off per JIA category in absolute number, percentage and significance (NS, not significant, *p<0.05, **p<0.01). FCS, fetal calves serum; RF, rheumatoid factor.

<table>
<thead>
<tr>
<th></th>
<th>anti-CarP-FCS</th>
<th>anti-CarP-Fib</th>
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<tbody>
<tr>
<td>All JIA in total</td>
<td>19/234 (8.1%)</td>
<td>31/234 (13.2%)</td>
</tr>
<tr>
<td>Systemic onset JIA</td>
<td>2/35 (5.7%)</td>
<td>2/35 (5.7%)</td>
</tr>
<tr>
<td>Polyarticular RF negative</td>
<td>3/90 (3.3%)</td>
<td>9/90 (10%)</td>
</tr>
<tr>
<td>Polyarticular RF positive</td>
<td>8/19 (42.1%)</td>
<td>11/19 (57.9%)</td>
</tr>
<tr>
<td>Oligo articular extended</td>
<td>3/41 (7.3%)</td>
<td>4/41 (9.8%)</td>
</tr>
<tr>
<td>Oligo articular persistent</td>
<td>2/18 (11.1%)</td>
<td>2/18 (11.1%)</td>
</tr>
<tr>
<td>Juvenile Psoriatic Arthritis</td>
<td>1/24 (4.2%)</td>
<td>3/24 (12.5%)</td>
</tr>
<tr>
<td>Enthesitis related Arthritis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Controls</td>
<td>5/107 (4.7%)</td>
<td>3/107 (2.8%)</td>
</tr>
</tbody>
</table>

Anti-CCP and IgM-RF in JIA and in relation to anti-CarP

Comparing anti-CarP antibodies to anti-CCP antibodies and IgM-RF revealed that 53% (8/15) of anti-CCP-positive children and 42.1% (8/19) of IgM-RF-positive children were also positive for anti-CarP antibodies. Importantly, anti-CarP antibodies were also found
in ACPA and IgM-RF-negative children as 57.9% (11/19) of anti-CarP-positive children were negative for anti-CCP antibodies and 27.3% (3/11) were negative for IgM-RF. In total 9 JIA patients were positive for IgM-RF, anti-CCP and anti-CarP (Ca-FCS and/or Ca-Fib). All triple positive patients were part of the ABC register.

**Correlation with clinical features**

Disease duration at sample collection, ANA status or age (at onset or at sample collection) was not associated with the presence of anti-CarP-antibodies. In addition, in the group previously described by Albers[20] we did not find an association of anti-CarP positivity with disease activity measured by time-in-active-disease at the time of sampling. Within the ABC register cohort no association was found between the presence of anti-CarP antibodies and ACR-Pedi 30 response[23] or reaching inactive disease at 15 months after start of anti-TNF treatment.[24] The cross-sectional nature of this study comprising three cohorts did not allow further detailed clinical association studies.

**Discussion**

Here we report that anti-CarP antibodies, initially identified in samples of adult RA-patients, are also present in (categories of) JIA. The detection of anti-CarP antibodies, especially in the IgM-RF-positive JIA category, reflects the similarity between RA and polyarticular IgM-RF positive JIA. The presence of anti-CarP antibodies in anti-CCP-negative RA is associated with a more severe disease course as expressed by radiological damage.[15] In our analyses information on radiographic damage is currently not available. We did not observe an association between anti-CarP antibodies and time-in–active-disease or clinical response to anti-TNF therapy but this could be due to lack of power. Disease severity differed across the three studies, but in general a severely affected group was collected as represented by the high percentages of polyarticular JIA and the use of biologicals. Although the numbers are too small to draw conclusions we observed that the triple positive sera (IgM-RF, anti-CCP and anti-CarP) were all confined to the ABC group that represent severe cases.

One limitation of this study is the cross-sectional nature of the sera used. Although this contributed to a large sample size detailed analyses of the association between the presence of anti-CarP antibodies and clinical outcome were not possible, as patients were included at different follow-up times and clinical parameters were recorded differently in each study.

In conclusion, anti-CarP antibodies are not only present in RA patients, but are also detectable in patients with JIA. They are present predominantly in the polyarticular RF-positive JIA category in both anti-CCP-positive and negative patients. Studies dedicated to
the diagnostic and prognostic value of anti-CarP-antibodies for (categories of) JIA patients can now be conducted.
References


