

Short Communication

Anti- Carbamylated Protein Antibodies in Juvenile Idiopathic Arthritis: A New Biomarker

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Abstract

Juvenile idiopathic arthritis (JIA) is the most common chronic disease in children. Specific diagnostic biomarkers have been demonstrated in varying amounts in different onset-types. The present study demonstrates the presence of anti-carbamylated (α -CarP) in 20% of the JIA population with a higher percentage in oligoarticular-onset (31%). There were no statistical correlations with disease activity, disease duration, and other previously detected biomarkers. This indicates the uniqueness of this antibody. Further studies are needed to determine its prognostic and diagnostic capabilities.

Keywords

- Juvenile idiopathic arthritis
- Biomarkers
- Anti-Carbamylated antibodies

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of arthritides occurring in children less than 16 years of age and persists for at least six weeks with no known etiology [1]. It is the most common chronic rheumatic disease of childhood. Many different therapies have been developed [2]. The different onset-types have demonstrated varying types of biomarkers [3]. If a more specific biomarker were found, it would help possibly in earlier diagnosis and in developing further treatment regimens. Several studies have indicated the importance of IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide (α -CCP) antibodies and their isotypes in certain onset types of JIA [3-8]. It is known that citrullinating enzymes and citrullinated proteins may have a role in the inflammatory process in the joints [4]. The role of IgG anti-CCP antibodies in rheumatoid arthritis (RA) and JIA is better understood. Recent studies focusing on the identification of targets for the citrulline modification and determining isotype usage of the anti-CCP antibody response in these diseases has been shown [3-9]. Recently, chemical reactions other than citrullination have been shown to produce autoantibodies that may play a part in the inflammatory process in joints. Anti-carbamylated protein (α -CarP) antibodies are known to be predictors of joint damage in RA [10] and precede disease onset in RA and experimental arthritis [11,12]. Moreover, carbamylated proteins appear to be arthritogenic in mice [13].

Considering the high prognostic and predictive value that α -CarP antibodies have demonstrated in patients with RA, the aim of this study was to evaluate the presence or absence of anti-CarP antibodies in a large cohort of JIA patients, RF positive polyarticular-onset, RF negative polyarticular-onset, oligoarticular-onset, and systemic-onset JIA patients.

MATERIALS AND METHODS

Patient population

Sera were collected from a total of 261 patients. A total of 140 patients with JIA including 47 with polyarticular-onset, RF positive (45 females, 2 males; 45 Caucasians, 2 African-Americans), 31 polyarticular-onset, RF negative (28 females, 3 males; All Caucasian), 42 oligoarticular-onset (All female and Caucasian), and 20 systemic-onset (15 females, 5 males; 19 Caucasian, 1 African-American). All JIA patients in this study fulfilled ILAR criteria for the diagnosis of JIA [14]. 74 samples from systemic lupus erythematosus (SLE) patients were evaluated (72 females, 2 males; 61 Caucasians, 11 African-Americans). All SLE patients fulfilled the American College of Rheumatology criteria for the diagnosis of SLE [15]. Any JIA or SLE patients not meeting these criteria [14,15] were excluded from the study. HLAB27 positive patients were also excluded. 37 samples from normal children were evaluated as healthy controls (30 females, 7 males; 27 Caucasians, 10 African-Americans). All samples were collected from the Saint Louis University Pediatric Rheumatology out-patient clinics at the Saint Louis University Medical Center and Cardinal Glennon Children's Hospital following informed consent.

Anti-CarP antibody assays

Anti-CarP antibodies were detected by ELISA as described by Shi et al. [10]. In brief, Nunc Maxisorp plates (Thermo Scientific, Waltham, MA, USA) were coated overnight at +4°C with non-modified fetal calf serum (FCS) (10 μ g/ml in carbonate bicarbonate buffer). After washing, with phosphate-buffered saline (PBS), the plates were blocked with 1% bovine serum albumin (BSA) (Sigma Aldrich, St. Louis, MO, USA) for 6 hr at +4°C. Subsequently, the wells were incubated with serum from

Table 1: Anti- CarP antibodies in JIA patients and disease/healthy controls.

	Anti-CarP antibody positive	Anti-CarP antibody negative	Positivity [%]	p value to healthy controls
RF [+] Polyarthritits	10	37	21%	p<0.05
RF[-] Polyarthritits	4	27	13%	p<0.05
Oligoarthritits	13	29	31%	p<0.05
Systemic	1	19	5%	-----
SLE	3	71	4%	-----
Normal/Healthy	0	37	0%	

patients, diluted 1/50 in PBS/0.05% tween/BSA 1% overnight at +4°C. After four washes with PBS, the plates were incubated for 2 hr at room temperature with goat polyclonal antihuman IgG alkaline phosphatase conjugated antibodies (Sigma-Aldrich, St. Louis, MO, USA) diluted at 1:1000 in PBS/0.05% tween/BSA 1%. After four washes with PBS, a solution of paranitrophenyl phosphate tablets in ethanolamine was used for the enzyme reaction and the plates were read at a 405 nm wavelength after 30 minutes at room temperature. All assays were performed in duplicate and the absorbance of normal/healthy wells was compared.

Statistical analysis

Chi- square test was used to compare the patients with normal/healthy subjects. P values<0.05 were considered statistically significant.

RESULTS

We observed that 20% [28/140] of the JIA patients were positive for α -CarP antibodies versus 0% [0/37] healthy controls and 4% [3/74] SLE patients. The highest percent positivewas found in the oligoarticular-onset patients at 31% [13/42] and 21% [10/47] of the RF positive, polyarticular patients as shown in Table 1. The overall presence of α -CarP antibodies was significantly higher in the RF positive, polyarticular-onset, RF negative, polyarticular-onset,, and oligoarticular-onset groups compared to healthy controls [p<0.05]. There were no statistical correlations with the presence of anti-nuclear antibodies or RF and α -CCP antibody isotypes. There was evidence of correlation with disease activity and duration of disease but did not reach statistical significance.

DISCUSSION

The role of biomarkers has long been an interest of our laboratory, starting with hidden RFs, and continuing with the evaluation of RF isotypes and α -CCP Ab isotypes [3-8]. Our studies have shown that elevated IgA and IgM RFs were more prevalent in polyarticular-onset patients. Therefore, all JIA subtypes should be evaluated for the presence of RF isotypes [3]. We also showed the potential value of measuring IgA, IgG, and IgM α -CCP antibody isotypes in the assessment of patients with all JIA onset types [4]. Later, we showed that multiple citrullinated epitopes are present in the sera of patients with various subtypes of JIA [6-8]. These studies demonstrated the frequent occurrence of α -citrullinated type II collagen and α -citrullinated fibrinogen antibodies in JIA [6,7]. We lastly showed isotypes of α -enolase could also be found in the serum of children with JIA [8]. These studies indicated that

citrullinated autoantibody diversity may indicate a more severe disease course in JIA patients [6-8].

Carbamylation is a chemical reaction mediated by cyanate that modifies lysine residues [16]. Normally the level of cyanate is in equilibrium with urea, but specific conditions like inflammation can change this equilibrium through a myeloperoxidase-dependent mechanism [16]. This leads to the local increase of cyanate levels, thus increasing the degree of carbamylation [17]. Although, there is similarity in structure between citrulline and homocitrulline, cohort studies have demonstrated that α -CCP and α -CarP antibodies are independent antibody subsets that do not cross-react with each other [10,18,19]. Unlike α -CCP antibodies, the presence of α -CarP antibodies has not been associated with the HLA-shared epitope and /or smoking [20]. An interesting finding was the presence of α -CarP antibodies in α -CCP-negative antibody patients with RA and their association with increased diseased activity [21,22] and severe joint damage [22,23]. Moreover, α -CarP antibodies have been detected in patients having arthralgia and their presence has been independently associated with the risk of developing RA [14,24]. α -CarP antibodies are also present in RA serum many years before the clinical appearance of the disease [11,16,25] and have also been identified in healthy first- degree relatives of patients with RA [25].

Shi et al. [10], identified carbamylation as a second post-translational modification frequently targeted by α -CarP antibodies in RA [10]. The presence of α -CarP antibodies in early RA was associated with increased disease severity, manifested by future joint destruction and was detectable in some children with JIA [26]. Thus, α -CarP antibodies alone or in combination with other clinically available related autoantibodies could be useful in the further evaluation of JIA.

Our study shows the presence of another possible biomarker identified in JIA. α -CarP antibodies have been demonstrated in adult patients with RA and predictors of joint damage in RA [10-16]. We have demonstrated α -CarP antibodies in 20 % of JIA patient sera including 31% of oligoarticular-onset, 21% of RF positive, polyarticular-onset, and 13% of RF negative, polyarticular-onset compared to healthy controls. This study shows a higher percentage for α -CarP positivity in oligoarticular-onset than our previous studies in this onset type for RF and anti-CCP isotypes [5,6]. The reason for this is not clear, but correlation with iritis will be further evaluated. However, no correlation was noted with ANA positivity. Overall, this study shows a higher percentage of α -CarP antibodies in JIA than previously shown by Hissink Muller et al. [26], especially in the oligoarticular-onset

group. These studies show the originality of the α -CarP antibodies with no clear correlation with other biomarkers, but confirm the presence of α -CarP antibodies in JIA. This study should promote further investigation in their possible diagnostic value.

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