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Anti-type II collagen antibodies detection and avidity in patients with oligoarticular and polyarticular forms of juvenile idiopathic arthritis

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ABSTRACT

Juvenile idiopathic arthritis (JIA) refers to a heterogeneous group of illnesses that have in common the occurrence of chronic joint inflammation in children younger than 16 years of age. The diagnosis is made only on clinical assessment. The identification of antibody markers could improve the early diagnosis, optimizing the clinical management of patients. Type II collagen is one potential autoantigen that has been implicated in the process of arthritis development. The aims of our study were to investigate the occurrence of anti-type II collagen antibodies and also to determine the avidity of the antibody–antigen binding. Ninety-six patients with oligoarticular or polyarticular JIA, 13 patients with ankylosing spondylitis (AS) and 61 healthy controls (HC) were tested for anti-type II collagen antibodies by ELISA and avidity ELISA. Sensitivity and specificity were determined by the receiver operating characteristic (ROC) curve analysis. Forty-two JIA patients (44%) were positive for antibodies against type II collagen. Its detection was significantly higher in JIA patients than in AS patients ($p = 0.006$) and HCs ($p < 0.0001$). Furthermore, anti-type II collagen antibody detection was significantly more frequent in patients with JIA of ≤6 months duration ($p = 0.0007$). Antibodies displaying high avidity to type II collagen were associated with disease activity ($p = 0.004$). This study demonstrates that antibodies against type II collagen are present in the serum of patients with oligoarticular and polyarticular JIA, being its presence more prevalent in patients with early disease. It also demonstrates that JIA patients with active disease present antibodies with high avidity against type II collagen.

1. Introduction

Juvenile idiopathic arthritis (JIA), the most common chronic inflammatory rheumatic disorder of childhood, is characterized by the onset of chronic arthritis of unknown etiology in children younger than 16 years of age [1]. According to the International League of Associations for Rheumatology criteria, JIA comprises seven subtypes, being oligoarticular and polyarticular the most common [2]. Arthritis that affects four or fewer joints after the first six months of the disease is classified as persistent oligoarticular JIA. Although sometimes thought of as a benign condition, persistent oligoarticular JIA may, in fact, lead to a wide spectrum of outcomes, ranging from complete remission after discontinuation of medication to development of severe damage to affected joints. The extended oligoarticular JIA subtype has a worse prognosis and includes patients who have five or more joints affected after the first 6 months of disease [3]. Arthritis that affects five or more joints during the first 6 months of the disease is called polyarticular JIA, which is classified as rheumatoid factor-negative (RF^-) or rheumatoid factor-positive (RF^+). JIA also encompasses juvenile psoriatic

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arthritis, enthesis-related arthritis and systemic arthritis. All JIA subtypes are of unknown causes. Diagnosis is based on the combined evaluation of medical history, clinical presentation and, to some extent, laboratory abnormalities [4,5].

Type II collagen (CII) is the predominant collagen type in joint cartilage and anti-CII antibodies have been reported to be present in serum, synovial fluid and eluted from cartilage explants of Rheumatoid Arthritis (RA) patients [6,7]. Studies have shown that antibodies from patients with RA react to various species of CII such as chicken, bovine, porcine and humans [6,8,9]. However, few studies have evaluated the presence of anti-CII antibodies in JIA [10–13]. Avidity ELISA has been used for measuring antibodies–antigen interaction in many diseases, including RA, in which different profiles could be detected with or without washing with denaturing agents [6,14–16]. There are no published studies evaluating the avidity of anti-CII antibodies in JIA.

In the present study we investigated whether antibodies present in serum of children with oligoarticular and polyarticular JIA reacts with type II collagen. In addition, we measured the level of avidity between type II collagen and IgG antibodies by using the avidity ELISA assay.

2. Materials and methods

2.1. Patients

For this study, serum samples were obtained from Brazilian and Portuguese donors. A total of 96 patients (72 female and 24 male, mean age 13.6 ± 6.8 years) who fulfilled the diagnostic criteria for oligoarticular and polyarticular JIA, according to the International League of Associations for Rheumatology [2], 13 patients with ankylosing spondylitis (AS) were used as an inflammatory rheumatic disease control (2 female and 11 male, mean age 49.3 ± 7.9 years) and 61 Healthy Controls (HC) (41 female and 20 male, mean age 11.7 ± 4 years), were enrolled in this study. Twenty-seven (27.8%) patients had samples collected in the early phase of the disease (≤6 months duration). Seventy-one (73.2%) patients had active disease at the time of sample collection. The criteria adopted to classify active disease were that defined by Consolaro et al. [17] and include 4 measures: positivity for CRP or ESR into a continuous measure of inflammation; presence of swollen joints; a physician's global assessment of disease activity, and a parent/patient global assessment of well-being.

Serum samples collected from AS patients were used as a rheumatic disease control group and healthy blood donors were used as the HC group. The study protocol involving Brazilian patients was approved by the ethics committee of the Federal University of Uberlândia whereas the study protocol involving Portuguese patients was approved by the ethics committees of the Centro Hospitalar Lisboa Norte, Hospital de Santa Maria, Lisbon Academic Medical Centre. The Portuguese samples were obtained from the Biobanco-IMM, Lisbon Academic Medical Centre. After approval of the consent procedure by the ethics committee, written informed consent was obtained from every parent of each child included in this study. The school-aged children provided their verbal and written informed consent to participate in this study, while children under 6 years of age only provided their verbal informed consent. After signed all informed consent were scanned and the originals were filed. The study was conducted in accordance with the Declaration of Helsinki.

2.2. Blood tests for inflammation detection

C-reactive protein (CRP) detection was determined by latex agglutination test (LAT) and a value of ≥0.8 mg/dl was considered elevated. Latex fixation test was utilized for the detection of IgM RF positivity and erythrocyte sedimentation rate (ESR) was determined by modified Westergren technique and considered elevated at ≥15 mm/h.

2.3. ELISA and avidity ELISA

For antibody analysis we used serum samples stored at −80, collected between 2011 and 2014. The best serum dilution, CII concentration, coating/blocking/washing buffers and anti-Human IgG were determined after several tests. The best ELISA condition for CII measurement was chosen based on the difference of reactivity between patients with JIA and HCs. ELISA and avidity ELISA were performed simultaneously. The avidity of CII-specific IgG antibodies was determined as previously described [18], with some modifications. Briefly, 96-well microtiter plates (NUNC MaxiSorp) were coated overnight at 4 °C with bovine CII 1.5 μg/ml (Becton Dickinson Biosciences, San Jose, CA) previously diluted in 0.06 M carbonate buffer (pH 9.6). To verify the presence of cross-reaction between antibodies and the ELISA plate, each serum sample was tested alone as blank control (without CII). The microplates were blocked with PBS supplemented with 3% of Bovine Serum Albumin (BSA) for 1 h at 37 °C. Serum samples diluted at 1:100 in PBS–BSA 1% plus 0.05% Tween 20 were added in duplicate on separate plates. After incubation for 1 h at 37 °C, the plates were washed with PBS Tween 0.05% and then subjected to differential washing as follows: one plate was incubated with 6 M urea solution diluted in PBS for 10 min, while the other plate was incubated with PBS Tween 0.05% for 10 min. Anti-CII antibodies were detected using horseradish peroxidase-conjugated anti-IgG (Sigma Chemical Co., St. Louis, Mo.) diluted 1:1000 and incubated for 1 h at 37 °C. The reaction was revealed with a substrate solution consisting of ortho-phenylenediamine (Sigma Chemical Co.) at 1 mg/ml in 0.01 M citrate–phosphate buffer (pH 5.0) and 0.03% H_2O_2. After incubation for 15 min at room temperature, the reaction was stopped with 2 N H_2SO_4 and read at 492 nm.

For ELISA analysis, the final optical density (OD) was normalized by the ratio of OD readings for each JIA and AS sample divided by the mean of OD value obtained by the HC group. Receiver operating characteristic (ROC) curve was constructed comparing the ELISA results from JIA with HC group. Based on the ROC curve, a cut-off point was determined as the value corresponding to the highest sensitivity without lowering the specificity. Differences in anti-CII antibodies reactivity between JIA patients and control groups are expressed in Reactivity index (RI), which was calculated based on the cut-off point (RI = normalized OD of each sample/cut-off). Samples with RI > 1.49 (cut-off) were considered positive.

The criteria adopted to classify the avidity index (AI) were defined by Marcolino et al. [18]. AIs were calculated as the ratio between the absorbance (Abs) obtained for the plate washed with urea (U^+) and the plate without urea (U^-), and was expressed as a percentage: $AI(\%) = Abs(U^+)/Abs(U^-) \times 100$. Avidity index was arbitrarily defined as: less than 30%, which was considered low avidity, between 30% and 60%, classified as average avidity and higher than 60%, corresponding to high avidity. Data were presented as a mean value ± standard deviation.

2.4. Statistical analysis

Unpaired *t* test with Welch's correction was used to evaluate the differences among JIA vs HC and JIA vs AS groups. Kruskall–Wallis test was used when three or more groups were compared. ELISA data were normalized based on the overall average absorbance obtained in the detection of anti-CII antibodies in the HC group. Cut-off that allowed best sensitivity and specificity was determined using the ROC curve. The area under the curve (AUC) was also

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