Prevalence of anticardiolipin antibodies in juvenile chronic arthritis

R Caporali, A Ravelli, F De Gennaro, G Neirotti, C Montecucco, A Martini

Abstract

The prevalence of anticardiolipin antibodies was evaluated in 70 children with juvenile chronic arthritis (JCA), in 25 adult patients with rheumatoid arthritis, in 42 healthy children and in 40 adult controls. Thirty seven (53%) patients with JCA were positive for IgG or IgM anticardiolipin antibodies, or both, and 30 (43%) for IgG anticardiolipin antibodies. In contrast, only seven (28%) adult patients with rheumatoid arthritis presented anticardiolipin antibodies, which were of IgG class in four (16%) cases. IgG anticardiolipin antibodies were negative in all control subjects while IgM anticardiolipin antibodies were detected in two (5%) children and in four (10%) adult controls. No correlations were found in patients with JCA between the presence or titres of anticardiolipin antibodies and various clinical or laboratory variables. No patient with anticardiolipin antibodies showed any feature of the anticardiolipin syndrome.

Juvenile chronic arthritis (JCA) is an heterogeneous condition, which is currently divided into different subtypes according to symptoms at onset. Among numerous immunological abnormalities seen in JCA only antinuclear antibodies and IgM rheumatoid factor are currently thought to be useful in patient classification and management. Some autoantibodies with defined specificity, such as antihistone and anticolonagen antibodies, have also been detected in patients with JCA, but their significance is still poorly understood.

Anticardiolipin antibodies are antiphospholipid antibodies, which can be found in patients with systemic lupus erythematosus and other autoimmune syndromes. Their presence has been correlated with various clinical manifestations, including thromboembolic phenomena, autoimmune cytopenia, and neurological disease.

In this study we tested 70 patients with JCA for the presence of anticardiolipin antibodies.

Patients and methods

PATIENTS

Seventy children (26 boys, 44 girls), ranging in age from 1 year and 5 months to 17 years and 11 months (mean age 8·3 years), whose parents gave permission for drawing extra blood at the time of routine venepuncture, were studied. All fulfilled the criteria for diagnosis of JCA and had been categorised by onset type according to the criteria of the juvenile rheumatoid arthritis criteria subcommittee of the American Rheumatism Association (ARA). The mean disease duration was 3·5 years. Thirty patients presented a systemic form, eight a polyarticular, and 32 a pauciarticular form. To reduce the heterogeneity of our group patients who were positive for HLA-B27 or rheumatoid factor were excluded from the study. Patients were divided according to disease activity into those with active disease and those in remission, as previously described. At the time of the study 53 patients had active disease and 17 were in remission. Thirteen patients were receiving no treatment, 42 were taking non-steroidal anti-inflammatory drugs (NSAIDs), four were receiving oral slow acting antirheumatic drugs and NSAIDs, and 11 were receiving low dose steroids in combination with NSAIDs on alternate days.

Forty two normal healthy children within the same age range, whose parents gave permission for drawing extra blood at the time of routine examinations, served as controls.

In addition, 25 adult patients (20 female, five male) with rheumatoid arthritis classified according to the ARA revised criteria, ranging in age from 28 to 74 years (mean age 45·3 years), and 40 adult healthy volunteers matched for age and sex were evaluated for the presence of anticardiolipin antibodies.

METHODS

Serum anticardiolipin antibodies were detected by an immunosorbent assay by the method of Gharavi et al with slight modifications. Briefly, serum samples were diluted 1/50 in 10% fetal calf serum in phosphate buffered saline, pH 7·4 (PBS-FCS), and incubated in triplicate for three hours in polyvinyl microtitre plates previously coated with 25 ml of 50 mg/ml cardiolipin in ethanol (Sigma Chemical, St Louis, Missouri, United States), washed in PBS, and blocked with PBS-FCS for one hour. After three additional washings the plates were incubated with a peroxidase conjugated rabbit antiserum selectively directed to human IgG, IgM, or IgA (Dakopatts, Denmark) at 1/1000 dilution in PBS-FCS. After three washings 100 ml of 2 mg/ml o-phenylenediamine (Sigma) in 0·1 M phosphate buffer pH 6 plus 30% H2O2 was added to each well. The reaction was stopped by adding 2 M H2SO4, and the optical density was read at 492 nm with a Titertek multispecan reader (Flow Laboratories). Upper normal limits for IgG, IgM, or IgA antibodies were set as the mean normal values plus five standard deviations of normal control samples.
IgG and IgM antibody titre was expressed as arbitrary units using reference standard serum samples: serum samples with IgG values ranging from 8 to 15 arb units or IgM from 5 to 15 arb units were defined as low positive, while values above 15 arb units were considered as high positive. No standard reference serum samples were available for titration of IgA antcardiolipin antibodies.

Statistical analysis was by Wilcoxon's test for comparison of differences between means and by linear regression analysis for calculation of correlation coefficients.

Results
The table and figure show the results of IgG and IgM antcardiolipin antibody determinations in patients with JCA, in adults with rheumatoid arthritis, and in healthy controls.

Of the 70 patients with JCA tested, 37 (53%) were positive for IgG or IgM antcardiolipin antibodies, or both, and 30 (43%) for IgG antcardiolipin antibodies. In contrast, only seven out of 25 (28%) adult patients with rheumatoid arthritis presented antcardiolipin antibodies, which were of IgG class in four cases (16%). IgG antcardiolipin antibodies were negative in all control subjects while IgM antcardiolipin antibodies were present in two children (5%) and in four adult controls (10%).

Of the 30 patients with JCA positive for IgG antcardiolipin antibodies, eight (27%) were in the high positive group and 22 (73%) in the low positive group. All adult patients with rheumatoid arthritis were in the low positive group. No significant correlations were found in patients with JCA between antcardiolipin antibody titres and clinical subtypes, disease activity defined on clinical grounds, onset age, disease duration, presence of iridocyclitis or other extra-articular manifestations, erythrocyte sedimentation rate, antinuclear antibody positivity, partial thromboplastin time, outcome, and treatment. All patients with a high titre of IgG antcardiolipin antibodies had a raised erythrocyte sedimentation rate. No patient presented with acute occlusive vascular episodes or cytopenia.

Discussion
Our results show a high prevalence (53%) of antcardiolipin antibodies in patients with JCA. IgG antcardiolipin antibodies were present in 43% of patients with JCA and a quarter of these positive cases presented high antibody titres. The prevalence and the titre of IgG antcardiolipin antibodies in patients with JCA were also markedly higher than those found in a group of adult patients with rheumatoid arthritis. This finding may be regarded as a further distinctive feature between JCA and adult onset rheumatoid arthritis.

Previous studies on antcardiolipin antibodies in childhood have focused primarily on systemic lupus erythematosus and related autoimmune conditions. In a study of patients with JCA, Leek found a low titre of antcardiolipin antibodies in 29% of patients with antinuclear antibodies in association with active arthritis and a poor outcome. Moreover, recently, Shergy et al reported IgG antcardiolipin antibodies in five of 12 patients with JCA; four of these patients had also a positive antinuclear antibody test.

Our study was carried out on a larger series of patients with JCA and did not show any association between antcardiolipin antibodies and antinuclear antibodies or between antcardiolipin antibodies and any other clinical or laboratory feature. Indeed, the prevalence of antcardiolipin antibodies in our antinuclear antibody positive patients was 50% and was similar to that found in patients with a negative antinuclear antibody test. A lack of correlation between a positive antinuclear antibody test and antcardiolipin antibodies has been found also in other autoimmune disorders.

It should be noted that all patients with high titre IgG antcardiolipin antibodies had a raised...
Prevalence of antiphospholipid antibodies in juvenile chronic arthritis


