Clinical significance of anticardiolipin antibodies in juvenile idiopathic arthritis

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Abstract

Objective

Anticardiolipin antibodies (aCL) have been demonstrated in a large spectrum of autoimmune diseases. However, its occurrence in childhood, in particular in juvenile idiopathic arthritis (JIA), is not well established. The present study addressed the frequency and clinical significance of aCL in a group of JIA patients.

Methods

aCL (IgG and IgM isotypes), antinuclear antibodies (ANA), and rheumatoid factor (RF) were determined in 86 children with JIA (33 systemic, 31 polyarticular and 22 oligoarticular onset type). Thirty-two juvenile systemic erythematosus lupus patients (JSLE) and 52 healthy children formed the control groups. The disease activity and functional status of the JIA patients were scored to study their possible associations with the presence of aCL.

Results

Serum aCL levels above the normal range were detected in 28/86 JIA patients (32.5%), 12/32 JSLE patients (37.5%), and 3/52 healthy children (6%). Positive aCL levels were slightly or moderately elevated (usually below 30 GPL and 20 MPL). The presence of aCL was not associated with the presence of ANA or RF. Associations between aCL and clinical parameters, such as disease onset, duration, activity or severity could not be established. No JIA patient had vascular thrombosis, thrombocytopenia or "livedo reticularis".

Conclusion

aCL occurred in low titers in JIA children, in a similar frequency to that observed in JSLE. No association with JIA clinical parameters or the clinical features classically linked to the antiphospholipid antibody syndrome were observed.

Key words

Juvenile idiopathic arthritis, anticardiolipin antibodies, antiphospholipid antibodies, autoantibodies.

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Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous condition characterized by chronic synovitis of unknown etiology. There are three main onset types: systemic, polyarticular and oligoarticular, which have been recently further reclassified into seven entities (1). A prominent immunologic feature in JIA is the presence of a variety of autoantibodies, such as rheumatoid factor (RF) and antinuclear antibodies (ANA). Autoantibodies are a cardinal element in most of the systemic autoimmune diseases. Lately, there has been great interest in a category of autoantibodies directed toward phospholipid molecules. Antiphospholipid antibodies, specially when present in high serum levels, would confer a higher risk of recurring arterial and venous thrombosis, recurrent fetal loss, and thrombocytopenia (2-5). Among the best studied and clinically most widely accepted antiphospholipid antibodies are the anticardiolipin antibodies (aCL).

Although the incidence and prevalence of the primary antiphospholipid antibody syndrome in childhood have still not been determined (6), there is some information about the occurrence of antiphospholipid antibodies in childhood. Some cases of peripheral vascular thrombosis, central nervous system thrombosis, thrombocytopenia, hemolytic anemia, and Raynaud's phenomenon have been described in association with antiphospholipid antibodies in children (7-13). aCL have been demonstrated in JIA (11, 14-17) and JSLE (18, 19). The frequency of aCL in JIA has been found to vary from 7.3 to 53% (14-17), but the reason for this variability and the exact clinical significance of these antibodies in JIA have not been entirely clarified.

In the present study we evaluated a group of JIA patients with respect to the frequency of antiphospholipid antibodies and its possible association with the thromboembolic manifestations and clinical features of JIA.

Materials and methods

Patients and controls

Eighty-six patients with JIA (38 boys and 48 girls) aged 1.6 to 16.9 years (median 10.7 years) were serially selected from the pediatric rheumatology outpatient clinics in 2 collaborating medical school university hospitals in São Paulo and Rio de Janeiro according to the proposed ILAR criteria (1). Thirty-three patients were classified as having the systemic, 31 the polyarticular, and 22 the oligoarticular onset type of JIA. Blood for laboratory analysis and autoantibody determinations was obtained at the time of the clinical evaluation. The interview was made directly with the children or with a close relative in the case of very young children. Within three months of their enrollment in the study, all patients underwent an ophthalmologic examination, including slit lamp biomicroscopy to detect uveal tract involvement.

Disease activity was defined by Moore's qualitative approach (20): (+) indicated the presence of one or more edematous or painful joints in the polyarticular and oligoarticular onset types (in the systemic onset type, fever and/or extra-articular manifestations, such as hepatosplenomegaly, pleurisy or pericarditis, were also considered as indicative of disease activity); (-) indicated the absence of any of the above conditions. Disease severity was analyzed by functional status, according to the American College of Rheumatology (ACR) criteria (21), where: class I = complete ability to perform the usual activities of daily living (self-care, vocational and avocational activities); class II = ability to perform the usual self-care and vocational activities, but with some limitation in avocational activities; class III = ability to perform the usual self-care activities, but with limitations in both vocational and avocational activities; and class IV = limitation in performing the usual self-care, vocational, and avocational activities.

At the time of the study, most patients were receiving non-steroidal anti-inflammatory drugs (NSAIDs), oral glucocorticosteroids or immunosuppressive drugs. Many of them were using more than one drug. Twenty-three patients were under treatment with salicylates and 40 children were receiving other NSAIDs. Prednisone was used by 17 patients, and methotrexate (20 patients), chloroquine (8 patients), and sulfasalazine (1 patient) were being taken by the remaining patients. Two systemic JIA

patients were on a regimen of monthly methylprednisolone pulse therapy. 22 patients were on no medication.

Thirty-two patients (2 boys and 30 girls) aged 7 to 18 years (median 13.2 years), meeting the ACR criteria for the classification of SLE (22), served as the disease control group. The normal control group comprised 52 healthy children (27 boys and 25 girls), ranging in age from 2.1 to 15.7 years (median 7.3 years), recruited from various public schools.

Autoantibodies

Anticardiolipin antibodies were determined by ELISA according to Gharavi *et al*. (23) with minor modifications. Briefly, microtiter plates (Corning Sciences Laboratory, New York, NY) were coated with 30 μl/well of 50 μg/ml cardiolipin (Sigma Chemical Co., St. Louis, Missouri) in ethanol. After overnight incubation at 4° C to evaporate the organic solvent, the plates were washed 3 times (5 min each time) with 100 μl/well phosphate buffered saline pH 7.2 (PBS), blocked with 10% adult bovine serum in PBS (ABS-PBS) for 1 hr at room temperature (75 μl/well), and washed once with PBS (100 μl/well). 50 μl aliquots of test samples at a 1:50 dilution in ABS-PBS were added to duplicate wells and incubated for 1 hr at room temperature. The plates were then washed 3 times with PBS (100 μl/well) and incubated with peroxidase-conjugated rabbit anti-human IgG or IgM (Sigma) in ABS-PBS (50 μl/well) for 90 min at room temperature. The working dilutions were 1:20,000 and 1:10,000 for anti-IgG and anti-IgM, respectively. The plates were washed 3 times with PBS as before and incubated for 30 min in the dark with 50 μl/well chromogenic solution [10 μg of o-phenylenediamine (Sigma) in 25ml 0.1 M citrate buffer pH 5 and 10 μl perhidrol (Merck, Rio de Janeiro, Brazil)]. The reaction was stopped by adding 20 μl 4N sulfuric acid (Labsynth, São Paulo, Brazil) to each well.

Absorbance was read at 492 nm in a spectrophotometer (Multiskan MCC/340, Labsystems Oy, Helsinki). Eight successive double dilutions (starting at 1:50) of two standard aCL sera (200 GPL and 150 MPL, respectively) were run on each plate to build a standard curve from

which the GPL and MPL units for each test serum could be determined using the linear regression equation proposed by Harris *et al.* (24). Test samples were considered positive for aCL when their values exceeded those found in 95% of the 52 healthy control children.

ANA were determined using the standard indirect immunofluorescence technique on HEp-2 cells (Hemagen, Whaltman, MA). The IgM RF was determined by the latex agglutination test adapted for plate-reading (Biolab, São Paulo, Brazil). ANA serum titers > 1:40 and RF > 1:20 were considered positive.

Ethics

The informed consent of the parents or responsible tutors was obtained for the patients and controls according to the standard rules of the Ethics Committee of the Universidade Federal de São Paulo.

Statistics

Comparisons of continuous parameters between the two groups were calculated using the 2-tailed Mann-Whitney U test. The Chi-square test corrected for continuity, or Fisher's test where appropriate, were used when comparing the qualitative variables. Statistical significance was set at 0.05 for all tests performed (p < 0.05).

Results

95% of the 52 healthy control children had aCL serum levels below 9 GPL and 4 MPL. Thus, sera presenting values equal to or above 9 GPL or 4 MPL were

Table I. Frequency of IgG anticardiolipin antibodies in JIA, JSLE, and normal children.

Category	Anticardiolipin antibodies		
	Positive		Negative
	n	(%)	n
A. JIA	21	(24)	65
Systemic	10	(30)	23
Polyarticular	7	(22)	24
Oligoarticular	4	(18)	18
B. JSLE	10	(31)	22
C. Normal	2	(4)	50

Systemic x Polyarticular x Oligoarticular
  * $x^2 = 1.14$; p > 0.05
A x B x C * $x^2 = 12.30$; p < 0.01
A x B ** $x^2 = 0.56$; p > 0.05
A x C **' $x^2 = 9.87$; p < 0.01
B x C   Fisher's exact test: p < 0.01
* critical $x^2 = 5.99$; ** critical $x^2 = 3.84$

considered positive for IgG and IgM, respectively. Figures 1 and 2 show that most of the children with JSLE and JIA exhibited undetectable or low titers of IgG and IgM aCL. Twenty-one of the 86 JIA patients (24%) were considered positive for IgG aCL. No association between IgG aCL and the JIA onset type was observed (Table I). Ten of the 32 JSLE patients (31%) and two of the 52 healthy children (4%) were positive for IgG aCL. A statistically significant difference was observed between the frequency of IgG aCL in healthy children and in JIA or JSLE patients, but not between JIA and JSLE patients (Table I). No statistically significant association was observed between the presence of IgG aCL and age, sex, disease duration, disease activity, or disease severity (Table II).

Table II. IgG anticardiolipin antibodies and clinical variables in children with JIA.

	Anticardiolipin antibodies		
	Positive	Negative	x^2 or Mann-Whitney*
Age in years (median)	9.1	10	Z = 0.09
Sex (M/F)	8/13	30/35	$x^2 = 0.16$
Duration (5/ > 5a)	12/9	50/15	$x^2 = 2.18$
Disease activity (present/absent)	13/8	31/34	$x^2 = 0.78$
Functional status			$x^2 = 2.86$ **
I	9	36	
II	7	16	
III	1	7	
IV	4	6	

* For p < 0.05, Z > 1.96 or $x^2 > 3.84$.
** Chi square partition: for p < 0.05, $x^2 > 7.82$
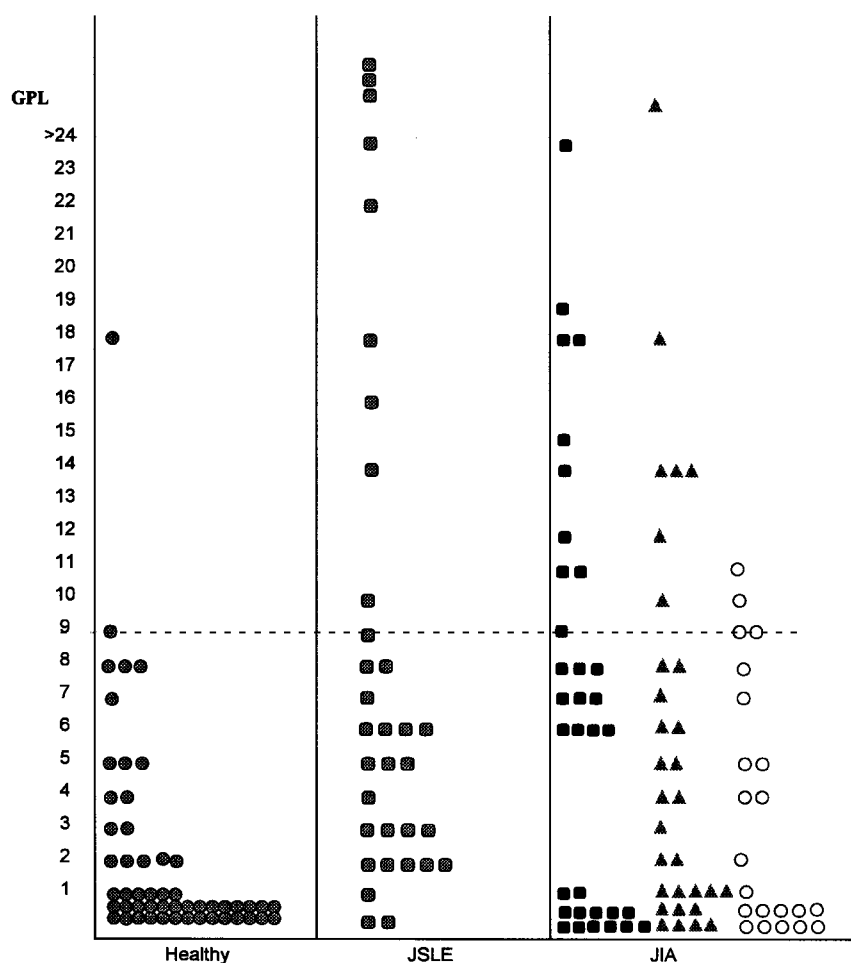
Fig. 1. Distribution of healthy, JSLE, and JIA children according to their GPL values.
● Healthy children; ▨ JSLE; ■ systemic JIA; ▵ polyarticular JIA; ○ oligoarticular JIA.
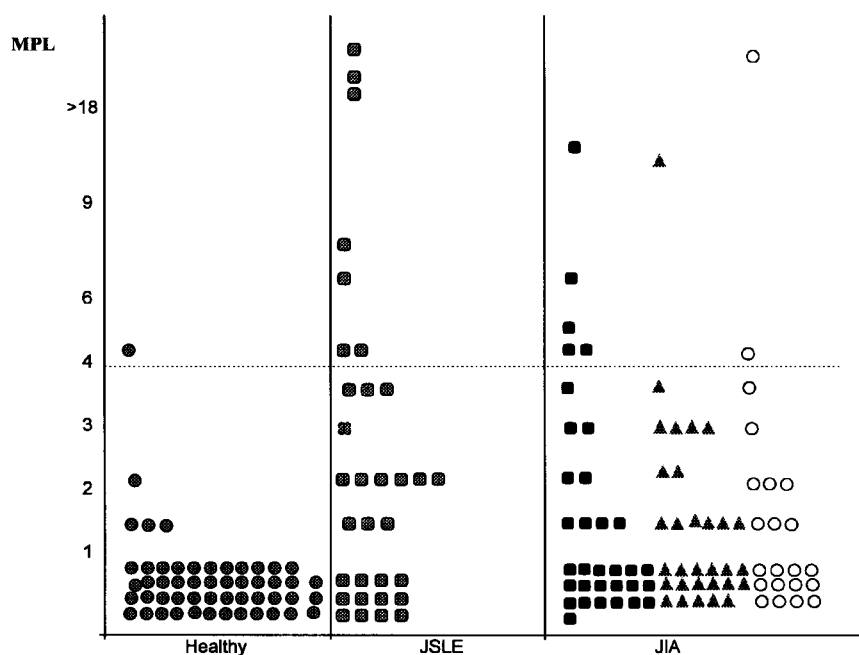
Fig. 2. Distribution of healthy, JSLE, and JIA children according to their MPL values.
● Healthy children; ▨ JSLE; ■ systemic JIA; ✕ polyarticular JIA; ○ oligoarticular JIA.

Eight JIA patients (4 boys and 4 girls) ranging in age from 1.6 to 13.5 years (median 6.4 years) were positive for IgM aCL. Only one child exhibited concomitantly abnormal levels of IgM and IgG aCL. Seven JSLE patients (all girls) and only one healthy child (also female) had IgM aCL. Table III shows that the frequency of IgM aCL in JIA did not differ significantly according to the disease onset type. On the other hand, the frequency of IgM aCL was significantly higher in JSLE patients than in healthy children ($p < 0.01$), but no statistically significant difference was observed between healthy children and JIA patients nor between JIA patients and JSLE children (Table III). In the JIA group no statistically significant association was observed between IgM aCL and sex (Fisher's exact test: $p > 0.05$), age [$Z(U) = 3,03; p > 0,05$], disease duration (Fisher's test: $p > 0.05$) or disease activity (Fisher's test: $p > 0.05$) (data not shown). Concerning functional status, three IgM aCL-positive JIA children were class I, whereas four of them were class II and III (two in each class); only one child had severe functional status impairment (class IV). The small size of the sample precluded statistical analysis for this variable.

ANA were detected in 36 of the 86 JIA patients (42%). The concomitant occurrence of ANA and IgG aCL was observed in 11 JIA patients (3 with the systemic,

Table III. Frequency of IgM anticardiolipin antibodies in groups JIA, JSLE, and normal children.

Category	Anticardiolipin antibodies		
	Positive		negative
	n	(%)	n
A. JIA	8	(9)	78
Systemic	5	(15)	28
Polyarticular	1	(3)	30
Oligoarticular	2	(9)	20
B. JSLE	7	(22)	25
C. Normals	1	(2)	51

Systemic x Polyarticular x Oligoarticular
* $x^2 = 2.70; p > 0.05$
A x B x C * $x^2 = 9.25; p < 0.01$
A x B   Fisher's exact test: $p > 0.05$
A x C   Fisher's exact test: $p > 0.05$
B x C   Fisher's exact test: $p < 0.01$
* Critical $x^2 = 5.99$.

4 with the polyarticular, and 4 with the oligoarticular onset type). No statistically significant association was demonstrated between these two groups of autoantibodies. Only one patient presented both IgM aCL and ANA. Four JIA patients presented RF, one of whom also had IgM aCL.

In general, aCL occurred in low levels in both JIA and in JSLE patients in this series (Figs. 1 and 2). The three JIA patients exhibiting the highest levels of these antibodies (> 20 GPL or MPL) were more than 10 years of age: one patient with systemic onset disease had 24 GPL, one with a polyarticular onset had 26 GPL, and one child with oligoarticular onset disease had 25 MPL. The systemic onset patient presented active disease and was positive for ANA. High aCL levels were demonstrated in four SLE patients; one of them (a 7-year-old girl) had the highest levels detected in this study (65 GPL and 29 MPL).

None of the JIA patients with IgM or IgG aCL had clinical evidence of thromboembolic events, livedo reticularis, heart valve manifestations, or thrombocytopenia. Only three children presented signs of anterior uveitis detected by ophthalmologic examination and none of them were positive for aCL.

Discussion

Antiphospholipid antibodies have been widely studied due to their association with vascular thrombosis and fetal loss. High levels of circulating aCL have been demonstrated in a variety of infectious and autoimmune diseases. Since low levels of these antibodies can also be found in healthy people, it is important to establish the normal range and to determine the quantitative risk associated with different levels of aCL (25). The fact that the majority of normal people have very low or undetectable aCL levels (26-30) suggests that this parameter does not follow a Gaussian distribution in healthy subjects. Therefore, some authors have claimed that the best way to define the normal range for aCL is using the percentile principle (25, 29, 30).

Many studies have assumed that the normal range for aCL established for the adult population can be applied to the infant and juvenile populations (15, 17,

18) while others have tried to establish the normal pediatric range derived from healthy children (9, 19). In both cases, however, the mean and standard deviation have been used to define the normal range. In the present study we decided not to treat aCL as a Gaussian variable and instead considered the normal range as that running within 95% of the healthy control children. Using this method, abnormal aCL levels were defined as being equal to or higher than 9 GPL and 4 MPL for IgG and IgM aCL, respectively. According to this definition, a considerable portion of patients with JSLE and JIA presented abnormal levels of these antibodies. The quantitative expression of aCL, however, was not high, being generally situated between 9 and 20 GPL/MPL.

The frequency of aCL in our JIA patients (32.5%) was in accordance with the estimates reported in the literature (7.3% to 53%) (14-17). As per Malleson et al. (17), we found no significant difference in aCL frequency among the three JIA onset types. The frequency in our JSLE patients (37.5%) resembled the most conservative estimates reported in the literature, which vary from 38% to 87% of the cases (18, 19, 31). It is relevant to point out that the highest aCL levels were predominantly detected in JSLE patients. The predominance of low aCL levels in aCL-positive JIA might be related to the fact that no clinical counterpart of the antiphospholipid syndrome was detected in these patients.

In our study, we observed no association between the presence or levels of aCL (both isotypes) and sex, disease duration, disease activity defined on clinical grounds, the presence of iridocyclitis, and the severity of JIA. Similar observations were reported by Caporali et al. (15), who reported a 53% frequency of aCL in JIA with no particular clinical associations. Also in accordance with this latter study, we could not demonstrate a significant association between aCL and ANA.

A low frequency of vascular thrombosis associated with antiphospholipid antibodies has been reported in children with rheumatic autoimmune diseases (9, 12, 13, 15, 18, 19, 32). The only case described in JIA was a child with a posi-

tive lupus anticoagulant test who developed popliteal vein thrombosis after prolonged immobilization in plaster of the affected limb (11), which is in itself a known thrombogenic factor. In our JIA patients, we did not find any clinical manifestations of vascular thrombosis or any other clinical feature associated with the antiphospholipid antibody syndrome. As mentioned above, the low levels of aCL observed in the aCL-positive JIA patients may explain these results. In addition, the lack of other risk factors for thromboembolism commonly found in adults (such as contraceptive medication, cigarette smoking, and arteriosclerosis) might also be related to the absence of thrombogenic expression of antiphospholipid antibodies in these young patients.

In conclusion, our study demonstrated a considerable frequency of aCL in JIA, similar to the figures observed in our juvenile SLE patients. Although relatively common, these antibodies occurred in low levels, did not distinguish a particular disease onset type, and were not associated with the clinical features of JIA such as disease activity or severity, nor with the clinical manifestations of antiphospholipid antibody.

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