Estimation of anticardiolipin antibodies, anti-$_2$glycoprotein I antibodies and lupus anticoagulant in a prospective longitudinal study of children with juvenile idiopathic arthritis

T. Avčin, A. Ambrožič[1], B. Božič[1], M. Accetto, T. Kveder[1], B. Rozman[1]

Department of Pediatrics and [1]Department of Rheumatology, University Medical Center, Ljubljana, Slovenia.

Abstract

Objective

Anticardiolipin antibodies (aCL) have been frequently detected in juvenile idiopathic arthritis (JIA), but have not been associated with disease activity or clinical features of the antiphospholipid syndrome (APS). Our aim was to determine aCL and anti-$_2$glycoprotein I (anti-$_2$GPI) antibody levels and lupus anticoagulant (LA) in serial samples from children with JIA and to investigate the clinical significance of these antibodies.

Methods

The values of aCL, anti-$_2$GPI and LA were prospectively followed in 28 children with JIA from disease onset. aCL and anti-$_2$GPI were assayed by an ELISA method. Two monoclonal $_2$GPI-dependent aCL (HCAL and EY2C9) were used as calibrators. LA was determined by a modified dilute Russell viper venom time test.

Results

Thirteen (46.4%) children with JIA were already positive for aCL at their first referral to our center. During the follow-up, the frequency of aCL decreased from 46.4% to 28.6%; however, it remained significantly higher compared with healthy children. In contrast, for anti-$_2$GPI the difference in the frequency between the children with JIA and healthy children was not statistically significant. Serial determination of aPL levels in JIA patients revealed frequent fluctuations. Positive aCL persisted over time in 6 (21.4%) children with JIA, 6 (21.4%) children were initially positive for aCL, but became later negative, and 3 (10.7%) children were initially negative for aCL and became later positive. Persistently positive anti-$_2$GPI were observed during the follow-up only in one patient, while none of the patients was persistently positive for LA. No association between aCL, anti-$_2$GPI or LA and disease activity could be established. No patient with positive aCL, anti-$_2$GPI or LA showed any clinical feature of APS.

Conclusion

The discrepancy between the presence of aCL and anti-$_2$GPI might indicate that the production of aCL in JIA is associated with an infectious trigger. Furthermore, the low frequency of anti-$_2$GPI and LA could explain the limited prothrombotic potential of aPL observed in JIA. However, we found a distinct group of JIA patients with persistently positive aCL, the clinical implications of which are at the present time unknown.

Key words

Antiphospholipid antibodies, anticardiolipin antibodies, anti-$_2$glycoprotein I antibodies, lupus anticoagulant, juvenile idiopathic arthritis.

Tadej Avcin, MD; Ales Ambrozic, MD; Borut Bozic, PhD; Meta Accetto, MD; Tanja Kveder, PhD; Blaz Rozman, MD, PhD.

This study was supported in part by a grant from the Slovenian Ministry of Science and Technology (Grant no. J3 7924).

Please address correspondence to:
Tadej Avcin, MD, Division of Allergology, Rheumatology and Clinical Immunology, Department of Pediatrics, University Medical Center, Vrazov trg 1, SI-1000 Ljubljana, Slovenia.
E-mail: tadej.avcin@siol.net

Received on May 22, 2001; accepted in revised form on November 29, 2001.

Introduction

"Antiphospholipid antibodies" (aPL) is an umbrella term used to describe a heterogeneous group of autoantibodies directed against negatively charged phospholipids or phospholipid-binding plasma proteins (1). A large number of clinical studies have shown that aPL may be associated with various clinical manifestations (2, 3). At the 8th International Symposium on Antiphospholipid Antibodies in Sapporo there was a consensus that the term "antiphospholipid syndrome" (APS) should designate the association of medium to high titer anticardiolipin antibodies (aCL) or the presence of lupus anticoagulant (LA) with vascular thrombosis or recurrent fetal loss (4). Besides these 'classical' clinical features, thrombocytopenia, hemolytic anemia and various neurological manifestations have been reported in aPL positive children as well (5).

The presence of aPL in children has been most extensively investigated in pediatric systemic lupus erythematosus (SLE) where numerous clinical manifestations of aPL have been demonstrated (6-10). In addition, aPL have been frequently detected also in the sera from children with other autoimmune diseases and in a variety of non-autoimmune disorders (reviewed in 5 and 11).

The most frequent rheumatic disease encountered in children is juvenile idiopathic arthritis (JIA), which is a heterogeneous group of idiopathic arthritides with an onset in children younger than 16 years (12). A number of cross-sectional clinical studies have shown that aCL may occur in 7-53% of children with JIA (6, 13-17). In the great majority of studies in JIA, no associations between aCL and disease activity have been observed and no clinical manifestations of APS were detected (6, 8, 13-17). There were only two reports of aPL associated thrombosis in children with JIA (18, 19). Therefore, it seems that despite the presumed significant prevalence of aCL in JIA, thrombotic complications are very rare, suggesting that aCL in JIA could have a different pathogenetic potential and, possibly, a different antigenic specificity than in SLE.

In the last decade it has became apparent that aPL associated with SLE require the presence of cofactor plasma proteins such as $_2$ glycoprotein I ($_2$GPI) for their binding, while aPL associated with infections bind to phospholipids directly (20, 21). Recent evidence suggests that the presence of antibodies against $_2$GPI (anti-$_2$GPI) in patients with SLE confers a high risk of thrombosis and other clinical manifestations of APS (22-24). In contrast, aPL found in the sera of patients with infections are generally not associated with the clinical features of APS (20, 21), similar to patients with JIA. Consequently, one might assume that aPL in JIA could be related to a potential infectious trigger and therefore, do not require the presence of $_2$GPI for their binding.

To address the problem of the true prevalence of aPL in JIA and to ascertain the risk for thrombosis associated with these antibodies, we prospectively followed the values for aCL, anti-$_2$GPI and LA in 28 children with JIA. To our knowledge, there have as yet been no longitudinal studies on aPL in JIA from the very beginning of the disease and no study has addressed the presence of anti-$_2$GPI. Moreover, in our study the serum values of aCL and anti-$_2$GPI in JIA patients were expressed in concentrations of monoclonal antibodies, which could facilitate the comparison of data derived from different centers.

Materials and methods

Study design

The design was a prospective longitudinal study of consecutive unselected children with idiopathic arthritis managed at a tertiary care center. The presence of aCL, anti-$_2$GPI and LA was assessed at three time points: (1) at study entry; (2) 3-6 months after the first determination (mean 3.8 months); and (3) 6-12 months after the first determination (mean 7.9 months). The study was approved by the Ethics Committee of the Slovenian Ministry of Health. Informed consent was obtained from guardians and patients when appropriate.

Patients

The study population consisted of 28 children with idiopathic arthritis, 11 boys and 17 girls, ranging in age from 3.4 to 16.0 years (mean 11.2 years). At the time of the first determination of aPL the duration of arthritis ranged from 6 weeks to 13 months (mean 4.8 months). Classification of the JIA was made 6 months after the onset of the disease, on the basis of the criteria proposed by the Classification Taskforce of the Pediatric Standing Committee of the International League of Associations for Rheumatology (ILAR) (25). Three children were classified as having systemic arthritis, 5 rheumatoid factor (RF) negative polyarthritis, 1 RF positive polyarthritis, 11 oligoarthritis, 1 extended oligoarthritis, 6 enthesitis-related arthritis and 1 psoriatic arthritis. All patients were studied between April 1998 and May 2000. There was no referral bias in the study population, as patients had been referred to our tertiary center by either primary care physicians or subspecialists unaware of the study. There were no exclusion criteria, and informed consent was obtained for all eligible patients.

At the beginning of the study, some patients were receiving non-steroidal anti-inflammatory drugs (NSAIDs), but no one was on therapy with steroids or immunosuppressive drugs. At the times of the second and third determinations of aPL, 21 patients were being treated with NSAIDs alone; associated treatments for the remaining patients were oral methylprednisolone (3 patients), methotrexate (2 patients) and sulfasalazine (2 patients).

The disease activity was determined according to the universally accepted Pavia criteria (26), which include: 1) the physician's global assessment of disease activity; 2) the parent's/patient's global 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 (ESR).

The control group comprised 61 apparently healthy children, for whom data was collected during routine visits to their community based health centers. The clinical parameters of the control group and the statistical test procedures used to determine the cut-off values have been reported elsewhere (27). In addition, 18 children with long-lasting JIA (4 boys and 14 girls; mean age 12.5 years; mean disease duration 5.7 yrs, range 3.7 – 10.2) and 11 children with juvenile-onset SLE (11 girls; mean age 15.4 yrs; mean disease duration 4.6 yrs, range 1.5 – 7.9) served as the pediatric rheumatic disease control groups.

Detection of aCL antibodies

aCL antibodies were detected by a slightly modified aCL ELISA, described previously (28, 29). Microtiter plates (Costar Medium Binding EIA/RIA plates, Costar, Cambridge, MA, USA) were coated with 40 l/well of cardiolipin (Sigma, St. Louis, MO, USA) in ethanol and allowed to evaporate at 4°C overnight. After incubation with 120 l/well of 10% fetal bovine serum (Sigma) in phosphate buffered saline, pH 7.4 (FBS-PBS) for 1 h at room temperature (22-26°C) the plates were washed once with 300 l/well of PBS. Then 100 l/well of standards and serum samples diluted 1:100 in 10% FBS-PBS were applied in duplicate and the plates were incubated for 2.5 h at room temperature. The plates were washed 4 times with PBS, and 100 l/well of alkaline phosphatase-conjugated goat anti-human IgG, IgM or IgA (ACSC, Westbury, NY, USA) diluted in 10% FBS-PBS was added. After 1 h of incubation at room temperature, the plates were washed 4 times with PBS and 100 l/well of p-nitrophenyl phosphate (Sigma) dissolved at 1 mg/ml in 1M diethanolamine buffer (pH 9.8) was added. Optical density at 405 nm (OD) was measured first after 10 min and then every 3 min by a Rainbow Spectra Thermo microtiter plate reader (Tecan, Grödig/Salzburg, Austria) versus a reagent blank until optimal fitting to predicted OD of standards was obtained.

Detection of anti- $_2$GPI antibodies

$_2$GPI was purified from pooled human sera by affinity column chromatography (30). A modified anti- $_2$GPI ELISA was developed on the basis of a method described previously (27, 31). Microtiter plates (Costar High Binding EIA/RIA plates) were coated with 50 l/well of $_2$GPI dissolved at 10 g/ml in PBS for 2 h at room temperature. After one washing with 200 l of PBS containing 0.05% Tween 20 (PBS-Tween), 50 l/well of standards and serum samples diluted 1:100 in PBS-Tween were applied in duplicate and incubated for 30 min at room temperature. After 4 washes, 50 l/well of alkaline phosphatase-conjugated goat anti-human IgG, IgM or IgA (ACSC) diluted in PBS was added. After 30 min of incubation at room temperature and 4 washes, 100 l/well of substrate was added. OD was measured as described for aCL ELISA.

Monoclonal antibodies

Two monoclonal $_2$GPI-dependent aCL were used as calibrators: (i) the chimeric IgG monoclonal antibody HCAL, consisting of human and 1 constant regions and variable regions from the mouse monoclonal $_2$GPI-dependent aCL WBCAL-1 (32); and (ii) the IgM monoclonal antibody EY2C9, derived from a patient with APS (33). Monoclonal antibodies were aliquoted in amounts sufficient for one assay and frozen at -20°C. Monoclonal antibodies in appropriate concentrations were used to create reference curves. Calibrations according to original KAPS standards (34, 35) and calibrations according to monoclonal antibodies were performed simultaneously in the same plates as the tested sera, to allow more accurate comparisons.

The lupus anticoagulant test

Venous blood was drawn into a plastic tube containing 3.8% trisodium citrate solution for a final ratio of one part anticoagulant to nine parts blood. Plasma was immediately separated from the cellular elements by double centrifugation at 2000 x g for 20 min at 4° C. Plasma was subsequently aliquotted and frozen at -30°C.

LA activity was determined by a modified dilute Russell viper venom time test (dRVVT). 200 l of the pre-warmed dRVVT reagent (LA-Screen,

Gradipore, Sydney, Australia) was added to 200 l of test plasma to initiate clotting. Test samples were performed in duplicates with an MCL 2 coagulation analyzer (Instrumentation Laboratory, Barcelona, Spain). If the ratio of the clotting time of a test plasma to that of a normal plasma exceeded 1.2, its LA activity was confirmed by a test in which the assay was repeated exactly as above except that the dRVVT-confirm reagent (LA-Confirm, Gradipore) contained excess phospholipids to neutralize the LA effect. LA activity was considered as positive if the clotting time ratio of dRVVT/ dRVVT-confirm was > 1.2. Tests were also performed in mixtures with pooled normal plasma to exclude factor II, V and X deficiencies. In our previous series, positive LA were identified in 4 (6.6%) out of 61 apparently healthy children (unpublished data).

Antinuclear antibodies (ANA)
ANA were determined using a standard indirect immunofluorescence technique on Hep-2 cells (Immuno concepts, Sacramento, CA, USA). ANA serum titers > 1:40 were considered positive.

Classification of patients according to the presence or absence of aPL
Patients were classified as being persistently positive for aPL if one or more tests were positive at all 3 determinations; as transiently positive if one or more tests were positive at one or two determinations only; and as negative if all tests were negative at all 3 determinations.

Statistical analysis
Student's t-test was used to compare the mean values and Fischer's exact test was used where appropriate. The statistical significance of the intergroup frequency rate was determined by chi-square analysis. Differences were considered statistically significant when $p < 0.05$.

Results
Initial aPL determinations
aCL
Thirteen (46.4%) of the 28 children with idiopathic arthritis were positive for aCL at the first determination. They had positive values for either IgG (42.8%; 12/28) or IgM (7.1%; 2/28) aCL (1 was positive for both isotypes), whereas IgA aCL were initially negative in all patients. Of the 12 patients who were positive for IgG aCL, 11 were low or medium positive (below 42 ng/ml HCAL or 48 GPL), and only one 15-year-old girl with oligoarthritis was highly positive (65 ng/ml HCAL or 100 GPL). Acute infection was confirmed in 3 patients with positive IgG aCL: one patient had beta-hemolytic streptococcus in the throat culture; one had *Klebsiella pneumoniae* in the stool culture; and one had rotaviruses in stool samples. No significant correlations were found between IgG aCL titers and the clinical subtypes of arthritis or disease activity. Both patients who were positive for IgM aCL had levels of IgM aCL below 64 ng/ml EY2C9 (10 MPL). In one of them beta-hemolytic streptococcus was isolated from the throat culture. Among 13 aCL positive patients there were only two who were simultaneously positive for both aCL and anti- $_2$GPI.

anti- $_2$GPI
Only 3 (10.7%) of the 28 children with idiopathic arthritis were positive for anti- $_2$GPI at the first determination. One girl with systemic arthritis had positive IgG anti- $_2$GPI (11 ng/ml HCAL), one boy with oligoarthritis had positive IgM anti- $_2$GPI (15 ng/ml EY2C9) and one girl with RF negative polyarthritis had positive IgA anti- $_2$GPI. Among 3 anti- $_2$GPI positive patients, 2 were simultaneously positive for both anti- $_2$GPI and aCL.

LA
Four (14.3%) of the 28 children with idiopathic arthritis were initially positive for LA (one high, one medium and two low positive). Two of them were girls with RF negative polyarthritis and the other two were boys with enthesitis-related arthritis. Both of the girls with RF negative polyarthritis were positive for ANA. Two of these 4 patients were at the same time low positive for IgG aCL and none of them had elevated anti- $_2$GPI.

Follow-up aPL determinations
aCL
Persistently positive aCL were observed during the follow-up in 6 patients with JIA (21.4%). All 6 patients were positive for IgG aCL. The serial values of aCL in these patients are illustrated in Figure 1. Four of them were persistently medium or high positive, while 2 of them exhibited low or medium levels of IgG aCL. In spite of the persistent aCL positivity, none of these patients had any evidence of aPL-related clinical manifestations. Moreover, no association between the persistent presence of IgG aCL and the JIA onset type or disease activity was observed. Two patients with persistently positive IgG aCL were positive at one determination also for IgM anti- $_2$GPI; they both had oligoarthritis.
Nine of 28 patients with JIA (32.1%) were classified as transiently positive for aCL. All of them exhibited low positive values for either IgG or IgM aCL. Six of these patients (21.4%) were initially positive and later negative for aCL (5 for IgG and 1 for IgM), and 3 (10.7%) were initially negative and later positive for IgG aCL. Figure 2 illustrates the serial values of IgG aCL in the 5 initially positive patients. None of the transiently aCL positive patients had any evidence of aPL-related clinical manifestations and no association between the presence of aCL and disease activity was observed. An acute infection was confirmed at the time of the first determination in 3 out of 6 patients with initially positive and later negative aCL: 2 patients had beta-hemolytic streptococcus in the throat culture and one had *Klebsiella pneumoniae* in stool culture.
The overall frequencies of aCL in the JIA patients at the first, second and third determinations were 46.4% (13/28), 35.7% (10/28) and 28.6% (8/28), respectively. The differences in frequency rates between various determinations were not statistically significant. Additionally, for all three determinations the overall frequency of aCL was found to be higher in JIA patients than in the apparently healthy children (I. determination: $p < 0.0003$; II. deter-

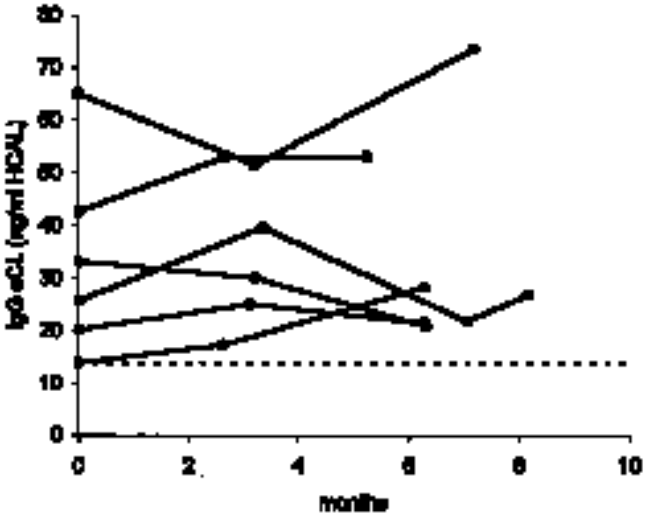

Fig. 1. Serial values of anticardiolipin antibodies (aCL) in 6 patients with juvenile idiopathic arthritis who were persistently positive for IgG aCL. One patient had systemic arthritis, one had RF positive polyartritis, three had oligoarthitis and one had enthesitis-related arthritis. aCL values are expressed as concentrations of HCAL monoclonal antibodies. The cut-off value is indicated as a horizontal dashed line.

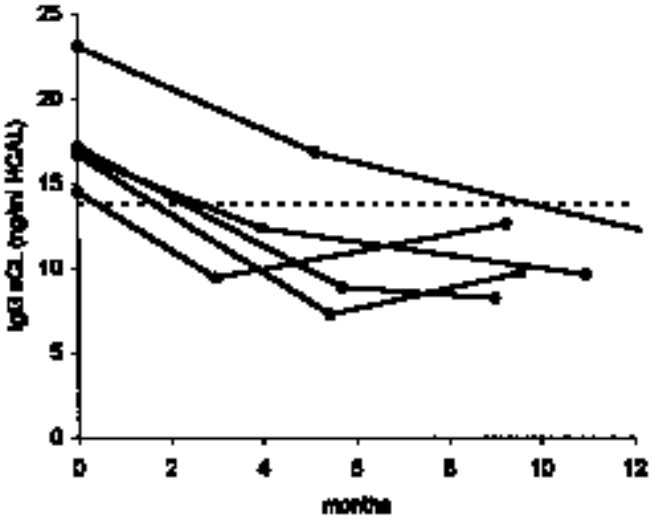

Fig. 2. Serial values of anticardiolipin antibodies (aCL) in 5 patients with juvenile idiopathic arthritis who were transiently positive for IgG aCL. One patient had RF negative polyarthritis, two had oligoarthritis, one had extended oligoarthritis, and one had enthesitis-related arthritis. aCL values are expressed as concentrations of HCAL monoclonal antibodies. The cut-off value is indicated as a horizontal dashed line.

mination: $p < 0.004$; III. determination: $p < 0.05$).

In the disease control group, 27.8% (5/18) of the children with long-lasting JIA were positive for aCL (3 for IgG, 2 for both IgG and IgM). In contrast, aCL were found in 54.5% (6/11) of the juvenile SLE patients.

anti- $_2$GPI

Persistently positive anti- $_2$GPI were observed during follow-up only in one girl (3.6%) who had RF negative polyarthritis and displayed persistent IgA anti- $_2$GPI positivity in the absence of any distinctive clinical feature of APS. She was simultaneously low positive at the second determination for IgG aCL.

Anti- $_2$GPI were detected only transiently in 3 children with JIA (10.7%). Two of these patients were initially positive and later negative for anti- $_2$GPI (1 for IgG and 1 for IgM), and one was initially negative and later positive for IgM anti- $_2$GPI. The quantitative expression of anti- $_2$GPI was low, being generally below 11 ng/ml HCAL and 15 ng/ml EY2C9. None of these patients had evidence of aPL-related clinical manifestations.

The overall frequencies of anti- $_2$GPI in JIA patients at the first, second and third determinations were 10.7% (3/28), 7.1% (2/28) and 7.1% (2/28), respectively. The differences in frequency rates between the various determinations were not statistically significant. For all 3 determinations the overall frequency of anti- $_2$GPI was compared between JIA patients and apparently healthy children and no significant differences were observed. Further, in JIA patients the overall frequency of anti- $_2$GPI for all 3 determinations was found to be significantly lower than the overall frequency of aCL (I. determination: $p < 0.004$; II. determination: $p < 0.006$; III. determination: $p < 0.02$).

In the disease control group, sera from 2 of 18 children with long-lasting JIA (11.1%) showed elevated levels of anti- $_2$GPI (one for IgG and one for IgM). One of these JIA patients was simultaneously positive for both aCL and anti- $_2$GPI. Among the juvenile SLE patients, 45.5% (5/11) were positive for anti- $_2$GPI, a significantly higher number than in JIA patients ($p < 0.04$). All juvenile SLE patients who had elevated anti- $_2$GPI displayed also positivity for aCL.

LA

None of the patients with JIA were persistently positive for LA. Four of the 28 patients with JIA (14.3%) were classified as transiently positive for LA. All 4 were initially positive and later negative for LA. However, in one boy with enthesitis-related arthritis, LA levels fluctuated and returned to low positive values at the time of the third determination. At the first determination this boy was also low positive for IgG aCL. None of these patients had any evidence of aPL-related clinical manifestations. For all 3 determinations the frequency of LA was compared between JIA patients and apparently healthy children and no significant differences

were observed.

Discussion

In recent years intensive work has been carried out to elucidate the pathogenesis of the prothrombotic state associated with aPL. There is accumulating evidence that pathogenic aPL are in fact antibodies directed against various plasma proteins, such as $_2$GPI and prothrombin, which may bind *in vivo* to damaged phospholipid membranes and induce endothelial proinflammatory and procoagulant phenotype (36). The development of new assays to measure antibodies against free cofactors could enable a better approach to identify patients at risk for thrombosis and other clinical features of APS (37).

In the present study, the values of aCL, anti- $_2$GPI and LA were prospectively followed in 28 children with JIA from the beginning of their disease. The first aPL determination in the children with JIA showed that aCL were frequently already positive (46.4%) at the first referral to our center. During the follow-up, the frequency of aCL decreased from 46.4% to 28.6%; however, it remained significantly higher as compared with healthy children. In contrast to the high frequency of positive aCL, only 3 children with JIA (10.7%) were initially positive for anti- $_2$GPI. Furthermore, during the follow-up the frequency of positive anti- $_2$GPI in JIA patients slightly decreased from 10.7% to 7.1%, a value not dissimilar to the frequency of positive anti- $_2$GPI observed in healthy children (6.6%) (27).

One of the most remarkable findings of the present study was that the majority of aCL-positive JIA children were not positive for anti- $_2$GPI at the same time. In fact, among 13 aCL-positive patients there were only 2 who were simultaneously positive for both aCL and anti- $_2$GPI. Assuming that post-infectious aPL do not require the presence of $_2$GPI for their binding to cardiolipin (20, 21), this finding suggests that the production of aCL in JIA could be associated with an infectious trigger. Therefore, the high frequency of positive aCL at the beginning in our JIA patients may reflect environmental influences (viruses, bacteria), which are possible triggers of the disease process. Such a hypothesis seems particularly applicable to the group of 6 patients (21.4%) who were initially low positive and later negative for aCL. In 3 of these 6 patients an acute infection was confirmed at the time of the first visit, which further suggests a possible causative association between infections and the presence of elevated aCL values at the beginning of the disease.

However, we found a distinct group of 6 JIA patients (21.4%) with persistently positive aCL, where an antigen-driven production could not be excluded. Since pure cardiolipin is poorly immunogenic, we hypothesize that the aCL production in these patients might have been induced by the binding of microorganisms or their products to self-phospholipids, thus forming immunogenic complexes against which aCL were produced. A similar mechanism of aCL induction has already been established in experimental animal models. Gharavi *et al*. demonstrated that aCL and anti- $_2$GPI in mice could be induced by immunization with phospholipid-binding viral and bacterial peptides (38). In our study, 2 patients with persistently positive IgG aCL were also positive at one determination for IgM anti- $_2$GPI. Alternatively, the finding of patients with persistently positive aCL but without anti- $_2$GPI can be interpreted as antibody production against the complexes of phospholipids with other protein cofactors, e.g. prothrombin, protein C, protein S or anexin V (39).

To date the published clinical studies on aCL in children with JIA have been primarily cross-sectional and have not addressed the question of the presence of anti- $_2$GPI. There has been a wide variation in the frequency of aCL observed in JIA patients, ranging from 7% to 53% (6, 8, 13-17). These disparities in the frequency of aCL reported in previous studies may reflect several issues: (i) substantial differences in the assays used for the detection of aCL; (ii) different ways of establishing the cut-off values; (iii) variations in the criteria for disease classification; and (iv) heterogeneity in the study population regarding the disease duration, disease activity and use of immunosuppressive therapy. Only Gattorno *et al*. have reported the results of two sequential aCL tests carried out in 16 patients with juvenile chronic arthritis who were followed for 4-7 months (8). In that study, transiently positive aCL were observed in 5 patients (4 of them were initially positive and later negative) and only one patient had persistently positive aCL (8). Similarly to previous studies, we observed no association between the presence of aCL and the clinical presentation of JIA, disease activity or positivity for ANA.

It is worth noting that during the follow-up none of our patients developed aPL-related clinical manifestations, which is in accordance with previous reports of aCL to be non-thrombogenic in children with JIA (8, 15, 17). Even the 4 JIA patients with persistent medium or highly positive aCL fulfilling the laboratory criteria for APS had no clinical manifestations of APS. Since anti- $_2$GPI and LA are reportedly more specific for thrombosis than aCL (37), the low frequency and values of anti- $_2$GPI and the low frequency of LA found in our patients could explain the limited prothrombotic potential of aPL in JIA. Another reason for the absence of thrombotic complications in our JIA patients might also be the short follow-up time and the lack of additional prothrombotic risk factors such as atherosclerosis and cigarette smoking.

Nevertheless, it should not be overlooked that besides thrombosis, elevated aPL in childhood have also been associated with conditions such as subtle neurological disorders (migraine, memory loss, etc.) (40). Therefore, the potential clinical importance of persistently positive aPL in JIA patients should not be underestimated and deserves to be further investigated.

In conclusion, our study showed a high frequency of aCL, but not anti- $_2$GPI and LA at the beginning of the JIA. The discrepancy between the presence of aCL and anti- $_2$GPI might indicate that the production of aCL in JIA is associated with an infectious trigger. In addition, the low frequency and values of anti- $_2$GPI and the low frequency of LA could explain the limited prothrom-

botic potential of aPL observed in JIA. However, we found a distinct group of JIA patients with persistently positive aCL, the clinical implications of which are at the present time unknown.

Acknowledgements

We wish to thank Professor Takao Koike (Hokkaido University School of Medicine, Sapporo, Japan) for kindly providing the monoclonal antibodies HCAL and EY2C9, Dr. Rolando Cimaz (Istituti Clinici di Perfezionamento, Milan, Italy) for his critical reading of the manuscript and helpful suggestions, and Mrs. Breda Slavec, B.Sc., for her help in carrying out the LA assays.

References

1. ROUBEY RAS: Autoantibodies to phospholipid-binding plasma proteins:A new view of lupus anticoagulants and other "antiphospholipid" autoantibodies. *Blood* 1994; 84: 2854-67.
2. LOVE PE, SANTORO SA: Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann Intern Med* 1990; 112: 682-98.
3. CERVERA R, PIETTE JC, FONT J, *et al.*: Antiphospholipid syndrome: Clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum* (in press).
4. WILSON WA, GHARAVI AE, KOIKE T, *et al.*: International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. *Arthritis Rheum* 1999; 42: 1309-11.
5. AVCIN T, CIMAZ R, MERONI PL: Recent advances in antiphospholipid antibodies and antiphospholipid syndromes in pediatric populations. *Lupus* (in press).
6. SHERGY WJ, KREDICH DW, PISETSKY DS: The relationship of anticardiolipin antibodies to disease manifestations in pediatric systemic lupus erythematosus. *J Rheumatol* 1988; 15: 1389-94.
7. RAVELLI A, CAPORALI R, DI FUCCIA G, ZONTA L, MONTECUCCO C, MARTINI A: Anticardiolipin antibodies in pediatric systemic lupus erythematosus. *Arch Pediatr Adolesc Med* 1994; 148: 398-402.
8. GATTORNO M, BUONCOMPAGNI A, MOLINARI AC, *et al.*: Antiphospholipid antibodies in paediatric systemic lupus erythematosus, juvenile chronic arthritis and overlap syndromes:SLE patients with both lupus anticoagulant and high-titre anticardiolipin antibodies are at risk for clinical manifestations related to the antiphospholipid syndrome. *Br J Rheumatol* 1995; 34: 873-81.
9. SEAMAN DE, LONDINO V, KWOH CK, MEDSGER TA, MANZI S: Antiphospholipid antibodies in pediatric systemic lupus erythematosus. *Pediatrics* 1995; 96: 1040-5.
10. BERUBE C, MITCHELL L, SILVERMAN E, *et al.*: The relationship of antiphospholipid antibodies to thromboembolic events in pediatric patients with systemic lupus erythematosus: a cross-sectional study. *Pediatr Res* 1998; 44: 351-6.
11. RAVELLI A, MARTINI A: Antiphospholipid antibody syndrome in pediatric patients. *Rheum Dis Clin North Am* 1997; 23: 657-76.
12. WOO P, WEDDERBURN LR: Juvenile chronic arthritis. *Lancet* 1998; 351: 969-73.
13. SOUTHWOOD TR, ROBERTS-THOMSON PJ, AHERN MJ, *et al*.: Autoantibodies in patients with juvenile chronic arthritis and their immediate family relatives. *Ann Rheum Dis* 1990; 49: 968-72.
14. SIAMOPOULOU-MAVRIDOU A, MAVRIDIS AK, TERZOGLOU C, FILOPOULOU S, ATHANASIADOU S, TZIOUFAS AG: Autoantibodies in Greek juvenile chronic arthritis patients. *Clin Exp Rheumatol* 1991; 9: 647-52.
15. CAPORALI R, RAVELLI A, DE GENNARO F, NEIROTTI G, MONTECUCCO C, MARTINI A: Prevalence of anticardiolipin antibodies in juvenile arthritis. *Ann Rheum Dis* 1991; 50: 599-601.
16. GEDALIA A, MOLINA IF, GARCIA CO, DOGGETT S, ESPINOZA LR, GHARAVI AE: Anticardiolipin antibodies in childhood rheumatic disorders. *Lupus* 1998; 7: 551-3.
17. SERRA CRB, RODRIGUES SH, SILVA NP, SZTAJNBOK FR, ANDRADE LEC: Clinical significance of anticardiolipin antibodies in juvenile idiopathic arthritis. *Clin Exp Rheumatol* 1999; 17: 375-80.
18. CAPORALI R, RAVELLI A, RAMENGHI B, MONTECUCCO C, MARTINI A: Antiphospholipid antibody associated thrombosis in juvenile chronic arthritis. *Arch Dis Child* 1992; 67: 1384-5.
19. ANDREWS A, HICKLING P: Thrombosis associated with antiphospholipid antibody in juvenile chronic arthritis. *Lupus* 1997; 6: 556-7.
20. HUNT JE, MCNEIL HP, MORGAN GJ, CRAMERI RM, KRILIS SA: A phospholipid- $_2$-glycoprotein I complex is an antigen for anticardiolipin antibodies occurring in autoimmune disease but not with infection. *Lupus* 1992; 1: 75-81.
21. MCNALLY T, PURDY G, MACKIE IJ, MACHIN SJ, ISENBERG DA: The use of an anti- $_2$-glycoprotein-I assay for discrimination between anticardiolipin antibodies associated with infection and increased risk of thrombosis. *Br J Haematol* 1995; 91: 471-3.
22. CABIEDES J, CABRAL AR, ALARCÓN-SEGOVIA D: Clinical manifestations of the antiphospholipid syndrome in patients with systemic lupus erythematosus associate more strongly with $_2$-glycoprotein I than with antiphospholipid antibodies. *J Rheumatol* 1995; 22: 1899-906.
23. TSUTSUMI A,MATSUURA E, ICHIKAWA K, *et al*.:Antibodies to $_2$-glycoprotein I and clinical manifestations in patients with systemic lupus erythematosus. *Arthritis Rheum* 1996; 39: 1466-74.
24. İNANÇ M, DONOHOE S, RAVIRAJAN CT, *et al*.: Anti- $_2$-glycoprotein I, anti-prothrombin and anticardiolipin antibodies in a longitudinal study of patients with systemic lupus erythematosus and the antiphospholipid syndrome. *Br J Rheumatol* 1998; 37: 1089-94.
25. PETTY RE, SOUTHWOOD TR, BAUM J, *et al.*: Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J Rheumatol* 1998; 25: 1991-4.
26. GIANNINI EH, RUPERTO N, RAVELLI A, LOVELL DJ, FELSON DT, MARTINI A: Preliminary definition of improvement in juvenile arthritis. *Arthritis Rheum* 1997; 40: 1202-9.
27. AVÇIN T, AMBROZIC A, KUHAR M, KVEDER T, ROZMAN B: Anticardiolipin and anti- $_2$ glycoprotein I antibodies in sera of 61 apparently healthy children at regular preventive visits. *Rheumatology* 2001; 40: 565-73.
28. LOIZOU S, MCCREA JD, RUDGE AC, REYNOLDS R, BOYLE CC, HARRIS EN: Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA):Standardization and quantitation of results. *Clin Exp Immunol* 1985; 62: 738-45.
29. STEGNAR M,BOZIC B, PETERNEL P, KVEDER T, VENE N, ROZMAN B: Prevalence of antiphospholipid antibodies in deep vein thrombosis and their relationship to blood coagulation and fibrinolysis. *Thromb Res* 1991; 63: 433-43.
30. MATSUURA E, IGARASHI Y, FUJIMOTO M, *et al.*: Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. *J Immunol* 1992; 148: 3885-91.
31. CUCNIK S, AMBROZIC A, BOZIC B, SKITEK M, KVEDER T: Anti- $_2$-glycoprotein I ELISA: Methodology, determination of cut-off values in 434 healthy Caucasians and evaluation of monoclonal antibodies as possible international standards. *Clin Chem Lab Med* 2000; 38: 777-83.
32. ICHIKAWA K, TSUTSUMI A, ATSUMI T, *et al.*: A chimeric antibody with the human 1 constant region as a putative standard for assays to detect IgG $_2$-glycoprotein I-dependent anticardiolipin and anti- $_2$-glycoprotein I antibodies. *Arthritis Rheum* 1999; 42:2461-70.
33. ICHIKAWA K, KHAMASHTA MA, KOIKE T, MATSUURA E, HUGHES GRV: $_2$-glycoprotein I reactivity of monoclonal anticardiolipin antibodies from patients with the antiphospholipid syndrome. *Arthritis Rheum* 1994; 37: 1453-61.
34. HARRIS EN, GHARAVI AE, PATEL SP, HUGHES GRV: Evaluation of the anti-cardiolipin antibody test: Report of an international workshop held 4 April 1986. *Clin Exp Immunol* 1987; 68: 215-22.
35. HARRIS EN: The second international anticardiolipin standardization workshop/The Kingston Anti-Phospholipid Antibody Study (KAPS) group. *Am J Clin Pathol* 1990; 94: 476-84.
36. MERONI PL, RIBOLDI P: Pathogenic mechanisms mediating antiphospholipid syndrome. *Curr Opin Rheumatol* 2001; 13: 377-82.
37. CARRERAS LO, FORASTIERO RR, MARTINUZZO ME: Which are the best biological markers of the antiphospholipid syndrome ? *J Autoimmunity* 2000; 15: 163-72.
38. GHARAVI EE, CUCURULL E, TANG H, CELLI

CM, WILSON WA, GHARAVI AE: Induction of antiphospholipid antibodies by immunization with viral peptides. *Lupus* 1999; 8: 449-55.
39. OOSTING JD, DERKSEN RHWM, BOBBINCK IWG, HACGENG T, BOUMA BN, DE GROOT P: Antiphospholipid antibodies directed against combination of phospholipids with prothrombin, protein C or protein S. An explanation for their pathogenic mechanism. Blood 1993; 81: 2618-25.
40. ANGELINI L, ZIBORDI F, ZORZI G, *et al.*: Neurological disorders, other than stroke, associated with antiphospholipid antibodies in childhood. *Neuropediatrics* 1996; 27:149-