Clinical and laboratory characteristics of children positive for antiphospholipid antibodies

Paola Giordano[1], Riccardina Tesse[1], Giuseppe Lassandro[1], Deborah Fracchiolla[1], Prudenza Ranieri[2], Antonella Lotito[1], Domenico De Mattia[1], Giovanni Carlo Del Vecchio[1]

[1]Department of Biomedicine of the Developmental Age, University of Bari, Bari; [2]Coagulation Laboratory, Policlinico of Bari, Bari, Italy

Background. It is difficult to estimate the actual prevalence of antiphospholipid syndrome (APS) in the paediatric population since there are no standardised criteria. We aimed to assess clinical and laboratory characteristics of a cohort of children positive for antiphospholipid antibodies (aPL) to contribute to the understanding of the heterogeneous aPL-related features in childhood.

Materials and methods. Forty-four patients with prolonged activated partial thromboplastin time were enrolled and assigned to group I ("transiently positive") or group II ("persistently positive"), based on the detection of elevated aPL plasma levels [lupus anticoagulant (LA), anticardiolipin (aCL), and anti-β2-glycoprotein I (anti-β2GPI) antibodies] on, respectively, one or more occasions, at least 12 weeks apart, by standard procedures. The clinical history and symptoms of all patients were recorded.

Results. Thirty-three (75%) patients were assigned to group I, while the other 11 (25%) formed group II. Major associated diseases in group I were urticarial vasculitis (21%), acute infections (18%) and thalassaemia (12%). Five subjects (15%) were asymptomatic. Four out of the 11 subjects (36%) in group II had thrombotic events; they were all persistently aPL-positive and two of them had concomitant systemic lupus erythematosus. The rate of detection of LA-positivity was not significantly different between the two groups (76% vs 91%, $p>0.05$), whereas the percentage of patients positive for overall aCL was higher in group II than in group I (54% vs 42%, respectively; $p<0.05$). Specifically, aCL IgG and anti-β2GPI IgM subtypes were significantly more represented in group II than in group I (100% vs 62% and 75% vs 33%, respectively; $p<0.05$).

Discussion. Our study shows that aPL-positive children have different features that should be taken into account in the classification of criteria for paediatric APS.

Keywords: activated partial thromboplastin time, antiphospholipid antibodies, children, thrombosis.

Introduction

Antiphospholipid antibodies (aPL) are a heterogeneous class of auto-antibodies directed against plasma proteins with affinity for anionic phospholipids[1]. Among the clinically relevant aPL, the most frequently encountered are lupus anticoagulant (LA), anticardiolipin (aCL) and anti-β2-glycoprotein I (anti-β2GPI) antibodies[1]. The association of thrombotic events or recurrent miscarriages with the presence of circulating aPL on two separate occasions, at 12 weeks apart, defines the antiphosholipid syndrome (APS)[2]. APS may be secondary to other underlying conditions, notably systemic lupus erythematosus (SLE), or may occur as an isolated clinical entity, in which case it is known as primary APS[1,2].

Not all patients with aPL antibodies develop APS, as such antibodies have been found in about 5% of the healthy population[3,4]. There is a high incidence of transient aPL antibodies in children after viral and bacterial infections; these antibodies are thought to be clinically irrelevant[5-8]. Although

© SIMTI Servizi Srl

aPL have been mainly associated with thrombotic features, especially recurrent venous thrombosis, some additional clinical manifestations, including bleeding abnormalities, have been described[1,9,10]. Pathways potentially leading to thrombosis include the direct activation of coagulation, inhibition of anticoagulation and interference with endothelial cells, immunocompetent cells and platelets[11,14]. Because many individuals with high aPL titres remain asymptomatic, a "two-hit" hypothesis has been proposed to explain the mechanisms by which they might cause diseases. According to this hypothesis, the presence of aPL antibodies induces endothelial dysfunction ("first hit") and another condition, such as pregnancy, infection, smoking, hypertension, atherosclerosis, obesity or vascular injury ("second hit") triggers thrombosis[15,16].

There are no systematic studies on APS in childhood because of the relatively low prevalence and heterogeneity of this syndrome in paediatric patients, although the Ped-APS Register, a collaborative project of the European Forum on Antiphospholipid Antibodies and the Lupus Working Group of the Paediatric Rheumatology European Society currently contains standardised data from 133 children in 14 countries with aPL-related thrombosis[17,18]. One of the aims of this project is to define the prevalence and prognosis of unusual, different foms of APS.

The purpose of this study was to assess clinical and laboratory characteristics of a cohort of aPL-positive children to contribute to the understanding of the heterogeneous aPL-related features in childhood.

Materials and methods

Study population

Between January 2000 and December 2010 we enrolled in a prospective study all examined patients aged 6 months to 18 years with prolonged activated partial thromboplastin time (aPTT), not corrected by the addition of normal plasma, whose parents/guardians gave consent to the children's participation in the study. These subjects were referred to us for pre-operative coagulation counselling or because of abnormal clotting tests, thrombosis or bleeding.

Patients with LA and/or aCL and/or anti-β2GPI antibodies positive on only one occasion were assigned to group I ("transiently positive"), while patients with elevated plasma levels of aPL on two or more occasions, at least 12 weeks apart, were assigned to group II ("persistently positive"). The clinical and laboratory follow-up lasted at least 60 months.

Informed consent was obtained from parents or guardian of all recruited subjects and the local Ethic Committee approved the study.

Clinical and laboratory assessments

Data on past history of thrombosis or bleeding symptoms, concomitant presence of other chronic disorders and previous drug therapies were recorded for all patients.

Peripheral blood was collected into tubes containing sodium citrate (0.129 M) anticoagulant on at least two occasions 12 weeks apart. Platelet-poor plasma (PPP) was obtained by centrifuging these blood samples twice at 2500 g for 15 minutes. Aliquots of PPP were filtered through 0.22-µm cellulose acetate syringe filters and stored at –80 °C until use.

LA were detected in accordance with the recommendations of the Subcommittee on lupus anticoaugulant/antiphospholipid antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis[1], using an automated coagulation analyzer (Instrumentation Laboratory, ACL 7000). The following screening strategies were employed: aPTT time (aPTT-LA, Diagnostica Stago, Asnières, France), kaolin clotting time (Instrumentation Laboratory) and diluted Russell's viper venom time (LAC screen, Instrumentation Laboratory). LA-positive samples were identified by mixing studies and a confirmation test, by means of hexagonal (II) phase phospholipids (STAClot®LA - Diagnostica STAGO, France), as previously described[19].

An enzyme-linked immunosorbent assay (ELISA, Diamedix Diagnostics, Inc-Florida) was used to assess aCL and anti-β2GPI. Both IgG and IgM antibody isotypes were measured and expressed in IgG or IgM Phospholipid Units (GPL or MPL respectively, high titer >40 GPL or MPL, or greater than the 99th percentile)[1].

Statistical analysis

Data are expressed as median (range) and percentages and were analysed using the Stat View

programme (Abacus Concepts, Berkley, CA, USA). Non-parametric tests (Mann-Whitney and Kruskall-Wallis) and the chi-square test were performed. A value of $p>0.05$ was considered not statistically significant (NS).

Results

Forty-four children (male/female ratio 1.1:1, median age 8 years, range 6 months-18 years) who had a prolonged aPTT that was not corrected by the mixing test were enrolled into this study.

Of these 44 children, 33 (75%) were assigned to group I, and the rest of the children (25%) formed group II (Figure 1). There were no age or sex differences between the two groups (p=NS).

Associated diseases in group I were urticarial vasculitis (21%), acute infectious diseases (18%), thalassaemia (12%), glomerulonephritis (6%), lymphadenitis (3%), essential hypertension (3%), Crohn's disease (3%), oesophagitis (3%), neurofibromatosis/epilepsy (3%), arthralgia (3%), SLE (3%), Glanzmann's disease (3%) and thyroiditis (3%). Five subjects (15%) were asymptomatic.

As shown in Figure 1, thrombotic events were recorded in four of the 11 (36%) patients in group II. Of the four patients who had thrombotic events, two had primary APS: one patient had cerebral venous thrombosis and episodes of otitis (1 month after the first identification of of aPL antibodies), while the other one had thrombophlebitis of the left leg associated with a large haemangioma (36 months after antibody identification). The other two patients who had thrombotic events had secondary APS, in both cases associated with SLE: one had deep vein thrombosis of the left leg (after 60 months) and the other had cerebral artery thrombosis (after 60 months).

These four patients were investigated for inherited prothrombotic disorders (mutations of methylenetetrahydrofolate reductase, factor V Leiden, prothrombin factor, protein C, protein S and antitrombin mutations) and resulted negative. None of them had a family history of any of these disorders. The treatment was anticoagulation with low weight molecular heparin (LWMH) for 1 month and subsequent anti-aggregation therapy for 3 months in the patient with ischaemic stroke, LWMH for 3 months in the patient with cerebral venous thrombosis, LMWH for 2 months in the patient with deep venous thrombosis of the leg and LWMH for 1 month followed by oral anticoagulation with warfarin for 3 months in the remaining patient. Seven subjects (64%) who were persistently aPL-positive did not have thrombotic events. Three of them had thrombocytopenia, two were affected by juvenile idiopathic arthritis, one had mononucleosis infection and the others had episodes of otitis.

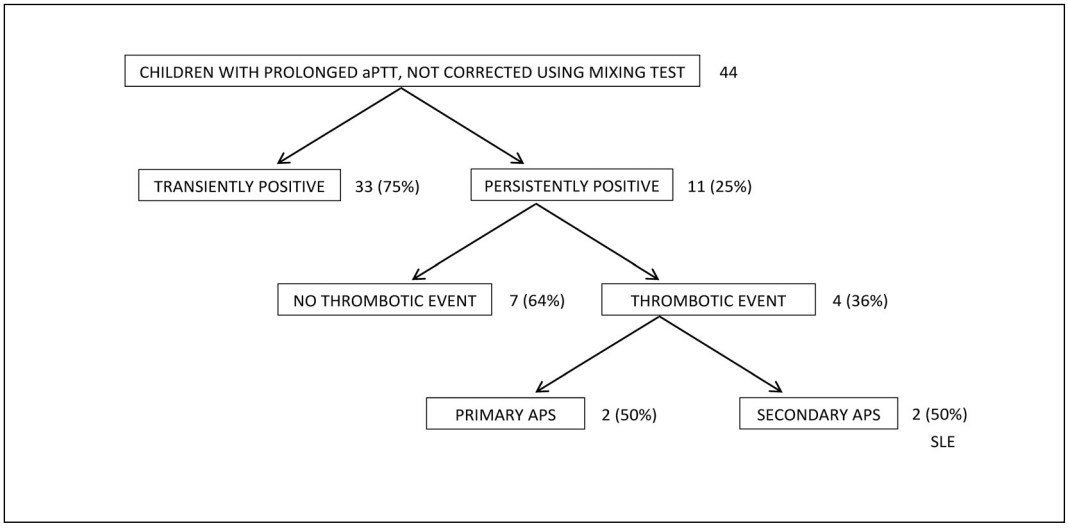

Figure 1 - Flowchart of the characteristics of the study population.
Thirty-three of 44 patients were transiently positive, and 11 persistently positive for antiphospholipid antibodies (aPL), based on the definition reported in the text. Four of the 11 aPL persistently positive subjects had thrombotic including two who had concomitant systemic lupus erythematosus (SLE).

The percentages of all auto-antibodies and the distribution of aPL subtypes in the two groups in which our patients were divided are shown in Table I. There were no differences in LA tests within the two groups. Positivity for aCL overall was more frequent in group II than in group I (54% vs 42%, p<0.05). Specifically, aCL IgG subtypes were significantly more represented in group II (100% vs 62%, p<0.05). The frequency of anti-β2GPI antibodies did not differ significantly between the two groups, but anti-β2GPI IgM subtypes were more frequent in group II than in group I (75% vs 33%, p<0.05). Among the 11 patients who had thrombotic events, the frequency of these events among subjects who were positive for two or more antibodies (3 out of 6 patients; 50%) and that in patients who had one antibody (1 in 5 patients, 20%) was not statistically significant (p=NS).

Table I - Distribution of antiphospholipid antibodies between group I "transiently positive" and group II "persistently positive" patients.

Biomarkers	Group I n. tests/total (%)	Group II n. tests/total (%)	*p*
LA	25/33 (76)	10/11 (91)	NS
aPTT LA	8/25 (33)	4/10 (40)	NS
KCT	12/25 (48)	6/10 (60)	NS
DRVTT	16/25 (64)	7/10 (70)	NS
aCL	13/31 (42)	6/11 (54)	<0.05
IgM	6/13 (46)	4/6 (67)	NS
IgG	8/13 (62)	6/6 (100)	<0.05
anti-β2GPI	3/19 (16)	4/8 (50)	NS
IgM	1/33 (33)	3/4 (75)	<0.05
IgG	2/3 (67)	3/4 (75)	NS

LA: lupus anticoagulant; aCL: anticardiolipin; anti-β2GPI: anti-β2-glycoprotein I antibodies; aPTT: activated partial thromboplastin time; KCT: kaolin clotting time; dRVV: diluted Russell's viper venom time.

Discussion

In this study we assessed the characteristics of aPL-positive children who had prolonged aPTT not corrected by the addition of normal plasma. We divided these children into two groups depending on whether they were transiently aPL positive (75%) or showed persistent aPL positivity (25%). It is currently thought that aPL alone are not sufficient to trigger the coagulation cascade, and the mechanism that converts reactive aPL into pathogenic ones remains to be clarified. An *in vitro* study shows that incubation of endothelial cells with aPL is able to promote several harmful effects, such as up-regulation of pro-inflammatory and chemokine expression, interference with protein C/S activation and apoptosis[20].

Some data suggest that there are no differences in the characteristics of aPL in subjects who develop a thrombotic event and in those who remain asymptomatic, but that the presence of the antibodies over time might be the trigger starting the autoimmune activity[6]. The transiently positive children in our study were mainly affected by urticaria/vasculitis or infectious diseases, and 15% of them were asymptomatic. It has been hypothesised that non-pathogenic aPL are generated through immunological mechanisms such as nutritional antigens[21]. Ambrozic *et al.* reported that levels of non-thrombogenic anti-β2GPI antibodies are higher in children with atopic dermatitis who have an exaggerated immune response to nutritional antigens[22].

The association between the presence of aPL and infection has been extensively described. Acquired infections can be responsible for transient and asymptomatic positivity for aPL antibodies in infants; thus, the presence of aPL is not considered an epiphenomenon, but can be an important step in the course of immunity against pathogens[5-8]. The risk of manifestations of APS with post-infectious aPL is not, however, completely absent. Paediatric cases of varicella zoster virus infection complicated by purpura fulminans and/or thromboembolism linked to the presence of aPL and protein S deficiency have been described[7,23].

The estimated frequency of aPL in children without any underlying disorder ranges from 3 to 28% for aCL and from 3 to 7% for anti-β2GPI[3].

The higher frequency of aPL in children than in adults has been suggested to be related to the higher rate of recurrent infections during childhood. The reported prevalence rates for aCL in healthy children vary widely[24,26]. These differences may reflect variability in assays used for aPL testing or age-related differences in immune response. The presence of LA with prolonged aPTT that spontaneously corrects to values within the normal range in healthy children has also been documented[27, 28].

Some of the children transiently positive for aPL in our study also had thalassaemia. We first reported the association between these two conditions in

a previous work in which we described a high prevalence (34%) of anti-β2GPI independent aCL in thalassaemic patients, which was related to hepatitis C virus infection; none of the patients developed any complications related to the aPL[29]. These results were confirmed 10 years later by Kashef *et al*., who described a high prevalence of aCL in Iranian thalassaemic patients, irrespective of previous history of thrombosis and presence of hepatitis C virus infection[30].

The frequencies of aPL in patients with venous thrombosis, the most common clinical manifestation of APS, have been reported to range from 5.2 to 30% for any aPL, 0.6-16% for LA, and 4-24% for aCL[3].

We found that 4 out of the 44 (9%) enrolled patients with non-corrected prolonged aPTT had thrombotic events; they were persistently aPL positive and thrombosis occurred between 1 month and 3 to 5 years after the first appearance of the auto-antibodies. Male *et al*. found that LA was the strongest predictor of the risk of thrombosis[31], but the presence of multiple aPL subtypes has been shown to have a stronger association with thromboembolic events than single factors. In our patients we found that the overall rate of LA auto-antibodies was higher than that of aCL and anti-β2GPI antibodies, particularly in subjects persistently positive for aPL antibodies. Moreover, half of patients who developed thrombotic events had primary APS while the other half had APS associated with SLE; these patients were mainly positive for a combination of aPL rather than a single aPL.

In conclusion, in contrast to the extensive information on diagnosis and management of APS in adults, there are only few standardised data on paediatric aspects of APS[25,26]. The particular features of this syndrome in children are the absence of prothrombotic risk factors, an increased incidence of infection-induced aPL, unusual clinical manifestations, differences in the cut-off values for determining aPL and different treatment[24].

Recently the Ped-APS International Registry has collected more data on aPL and the clinical manifestations and laboratory characteristics of paediatric APS patients[17,18]. One of the main purposes of this registry has been to establish how often some non-thrombotic manifestations occur in aPL positive children and whether they can be included in the classification criteria for paediatric APS.

Our study corroborates other recent reports in the literature: the clinical and biochemical features of children positive for aPL differ from those of adults and should be taken into account in the classification of criteria for paediatric APS .

The Author declares no conflicts of interest.

References

1) Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006; **4**: 295-306.
2) Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. Lancet 2010; **376**: 1498-509.
3) Biggioggero M, Meroni PL. The geoepidemiology of the antiphospholipid antibody syndrome. Autoimmun Rev 2010; **9**: A299-304.
4) Hedge VAP, Vivas Y, Shah H, et al. Cardiovascular surgical outcomes in patients with the antiphospholipid syndrome - a case series. Heart Lung Circ 2007; **16**: 423-7.
5) Avcin T, Toplak N. Antiphospholipid antibodies in response to infection. Curr Rheumatol Rep 2007; **9**: 212-8.
6) Frauenknecht K, Lackner K, von Landenberg P. Antiphospholipid antibodies in pediatric patients with prolonged activated partial thromboplastin time during infection. Immunobiology 2005; **210**: 799-805.
7) Kurugol Z, Vardar F, Ozkinay F, et al. Lupus anticoagulant and protein S deficiency in otherwise healthy children with acute varicella infection. Acta Paediatr 2000; **89**: 1186-9.
8) Loizou S, Singh S, Wypkema E, Asherson RA. Anticardiolipin, anti-beta2-glycoprotein 1 and antiprothrombin antibodies in black South African patients with infectious diseases. Ann Rheum Dis 2003; **62**: 1106-11.
9) Greaves M. Antiphospholipid syndrome: unusual clinical presentations. Thromb Res 2011; **127**: 47-50.
10) Hunt BJ. Pediatric antiphospholipid antibodies and antiphospholipid syndrome. Semin Thromb Hemost 2008; **34**: 274-81.
11) de Groot PG. Mechanisms of anti-phospholipid antibody formation and action. Thromb Res 2011; **127**: S40-2.
12) Gharavi AE, Wilson W, Pierangeli S. The molecular basis of antiphospholipid syndrome. Lupus 2003; **12**: 579-83.
13) Meroni PL, Riboldi P. Pathogenic mechanisms mediating antiphospholipid syndrome. Curr Opin Rheumatol 2001; **13**: 377-82.
14) Weiler H. Tracing the molecular pathogenesis of antiphospholipid syndrome. J Clin Invest 2008; **118**: 3276-8.

15) Giannakopoulos B, Passam F, Rahgozar S, Krillis SA. Current concepts on the pathogenesis of the antiphospholipid syndrome. Blood 2007; **109**: 422-30.
16) Levine JS, Ware Brancch D, Rauch J. The antiphospholipid syndrome. N Engl J Med 2002; **346**: 752-63.
17) Avcin T, Cimaz R, Rozman B, Ped-APS Registry Collaborative Group. The Ped-APS Registry: the antiphospholipid syndrome in childhood. Lupus 2009; **18**: 894-9.
18) Avcin T, Cimaz R, Silverman ED, et al. Pediatric antiphospholipid syndrome: clinical and immunologic features of 121 patients in an international registry. Pediatrics 2008; **122**: e1100-7.
19) Tripodi A. Testing for lupus anticoagulants: all that a clinician should know. Lupus 2009; **18**: 291-8.
20) Meroni PL, Borghi MO, Raschi E, et al. Inflammatory response and the endothelium. Thromb Res 2004; **114**: 329-34.
21) Avcin T, Ambrozic A, Kuher H, et al. Anticardiolipin and anti-β2GPL antibodies in sera of 61 apparently healthy children at regular preventive visits. Rheumatology 2001; **40**: 565-73.
22) Ambrozic A, Avicin T, Ichikawa K, et al. Anti- ß2GP1 antibodies in children with atopic dermatitis. Int Immunol 2002; **14**: 823-30.
23) Josephson C, Nuss R, Jacobson L, et al. The varicella-autoantibody syndrome. Pediatr Res 2001; **50**: 345-52.
24) Avcin T, Silvermann ED. Antiphospholipid antibodies in pediatric systemic lupus erythematosus and the antiphospholipid syndrome. Lupus 2007; **16**: 627-33.
25) Cimaz R, Descloux E. Pediatric antiphospholipid syndrome. Rheum Dis Clin North Am 2006; **32**: 553-73.
26) Ravelli A, Martini A. Antiphospholipid syndrome in pediatrics. Rheum Dis Clin North Am 2007; **33**: 499-523.
27) Male C, Lechner K, Eichinger S, et al. Clinical significance of lupus anticoagulant in children. J Pediatr 1999; **134**: 199-205.
28) Siemens HJG, Gutsche S, Bruckner S, et al. Antiphospholipid antibodies in children without and in adults with and without thrombophilia. Thromb Res 2000; **98**: 241-7.
29) Giordano P, Galli M, Del Vecchio GC, et al. Lupus anticoagulant, anticardiolipin antibodies and hepatitis C virus infection in thalassaemia. Br J Haematol 1998; **102**: 903-6.
30) Kashef S, Karimi M, Amirghofran Z, et al. Antiphospholipid antibodies and hepatitis C virus infection in Iranian thalassemia major patients. Int J Lab Hem 2008; **30**: 11-6.
31) Male C, Foulon D, Hoogendoorn H, et al. Predictive value of persistent versus transient antiphospholipid antibody subtype for the risk of thrombotic events in pediatric patients with systemic lupus erythematosus. Blood 2005; **106**: 4152-8.

Arrived: 3 July 2011 - Revision accepted: 9 August 2011
Correspondence: Paola Giordano
Department of Biomedicine of the Developmental Age
Pediatric Unit "F. Vecchio", University of Bari
Piazza Giulio Cesare 11
70124 Bari, Italy
e-mail: giordano@bioetaev.uniba.it