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Morbidity of lupus anticoagulants in children: a single institution experience

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ABSTRACT

Introduction: The lupus anticoagulant (LA) and the anticardiolipin antibodies (ACA) are the antiphospholipid antibodies more relevant clinically. Their clinical manifestations are diverse with most patients being asymptomatic while others present venous or arterial thrombosis, and more rarely, bleeding. Our objectives were to evaluate clinical presentation of LA in children and to correlate it to LA behavior. *Patients and methods*: A retrospective cohort of patients (under 18 years old) who had a positive determination of LA followed by at least another determination of LA at a variable period was evaluated. Personal and family history, including infectious diseases temporally related to the event, were recorded. The screening of other coagulation disorders was performed according to symptoms, family history or laboratory results. *Results*: Thirty-six patients were evaluated, median age was 10.8 years old, and 52.8% were female. Asymptomatic patients were 19.4% (7/36) of study population. Bleeding and thrombosis were found in 52.8% and 27.8%, respectively. Median LA determinations per patient were 3. von Willebrand disease was diagnosed in 66.7% of patients consulting for bleeding. A concomitant hemostatic defect was found in 8/10 patients with thrombosis ($p = 0.003$). LA behavior was not uniform and not correlated to symptoms. *Conclusions*: Most LA found in children is incidental and asymptomatic. In children with bleeding, LA might be a fortuitous finding associated with VWD. The persistence of LA does not imply a higher risk of thrombosis.

Introduction

Antiphospholipid antibodies (APA) comprise a heterogeneous group of antibodies directed against phospholipids and negatively charged phospholip-

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id–protein complexes [1]. The lupus anticoagulant (LA) and the anticardiolipin antibodies (ACA) are the most relevant clinically. Clinical manifestations in children and adults include asymptomatic subjects, venous or arterial thrombosis and, more rarely, bleeding.

In asymptomatic children, LA diagnosis is incidental, frequently after investigation of prolonged activated partial thromboplastin time (APTT) prior to surgery or viral infections. Usually, these antibodies are primary, transient and clinically irrelevant [2,3]. It has been demonstrated that LA represents a higher risk of thrombosis than ACA [4], and in children, it has been related with stroke or cerebral venous thrombosis [5,6]. Viral infections might be responsible for thrombotic events in transient LA as well [7]. The hemorrhagic lupus anticoagulant syndrome is rare and due to acquired thrombocytopenia or immune hypo-prothrombinemia [8–10]. The association with congenital bleeding disorders has not been described.

Our purpose was to evaluate clinical presentation and morbidity of LA in children. We investigated whether clinical manifestations were related or not to the LA behavior (i.e., transient or persistently positive) and the concomitancy of other coagulation disorders.

Patients, materials and methods

We retrospectively evaluated a cohort of consecutive children in whom LA was investigated regardless their clinical manifestations or reasons for referral. Patients were referred to the Thrombosis and Hemostasis Department, Institute for Hematological Research, National Academy of Medicine, between 1986 and 2000, by their primary physicians or surgeons. Reasons for referral were thrombosis, bleeding, abnormal coagulation test and presurgical screening.

Patients were included in the study, regardless of clinical manifestations (or reasons for referral), if they were $\leq$18 years old and had a positive LA determination followed by at least another determination at a variable time (minimum: 4 weeks).

Patients were excluded if they had cancer, an autoimmune underlying disease, a thromboembolic condition, such as mechanical heart valve prostheses, or a central venous line or implantable catheter.

Regardless of the reason for referral, all patients were interviewed by a hematologist specialized in coagulation disorders. Symptoms, personal and family history of thrombosis and bleeding, concomitant disorders and infectious diseases temporally related to the LA determination were recorded. Site and magnitude of bleeding and thrombosis location (venous or arterial) were registered.

The screening of other coagulation disorders was performed according to symptoms, family history, laboratory results or medical criteria (i.e., thrombophilic screening was performed in children with thrombosis, congenital bleeding disorders were searched in patients with thrombosis if anamnesis suggested a personal or family history of bleeding, etc.)

Laboratory determinations

Blood was collected in plastic tubes containing 1/10 volume of 0.11 M sodium citrate and centrifuged for 15 min at 1500 $\times$ *g*, supernatant was then recentrifuged and plasma was separated from cells within 20 min of collection. In all cases, the platelet-poor plasma was dispensed in aliquots that were either tested immediately or frozen at -80 °C for future analysis. The anticoagulant used for blood samples for fibrinolytic studies was acid citrate.

LA determinations were performed according to SSC-ISTH recommendations [11]. Tests performed were as follows: APTT, diluted Russell viper venom test, mixing studies and platelet neutralization procedures of both tests. Anticardiolipin IgG and IgM antibodies were tested (anticardiolipin IgG and IgM EIA, The Binding Site). Prothrombin time and prothrombin levels as well as the platelet count were determined in all patients.

Established methods were applied to carry out the coagulation and fibrinolytic tests whenever appropriate: Antithrombin III (ATIII) activity (Immunochrom ATIII, IMMUNO) and antigen (Laurell technique, rabbit anti-human Antithrombin III, DAKO), Protein S activity (IL Test Protein-S, Instrumentation Laboratory) and free antigen (Laurell technique, rabbit anti-human Protein-S, DAKO), protein C (IL Test ProChrom, Instrumentation Laboratory), modified activated Protein C resistance (APCR; COATEST APC Resistance-V), Factor V Leiden (FV Leiden) mutation.

Fasting total homocysteine levels were measured by high-performance liquid chromatography (HPLC) with fluoresce detection.

Fibrinolysis evaluation included Euglobulin Lysis time (ELT), biological and immunological tests for plasminogen activator inhibitor-1 (PAI-1; COATEST 5PAI, Chromogenix and Asserachrom PAI, Diagnostica Stago, respectively) and tissue-type plasminogen activator (t-PA; COATEST t-PA, Chromogenix and Asserachrom t-PA, Diagnostica Stago, respectively).

Normal reference values considered are as follows (both activity and antigen): ATIII: 80–140%; PS: 60–120%; PC: 60–120%; homocysteine levels <15 μmol/l; Fibrinogen: 200–400 mg/%. PAI–1 activity: 9–28 ng/ml; PAI-1 antigen: 4–43 ng/ml; t-PA activity: 0.2–1.4 UI/ml; t-PA antigen: 1–12 ng/ml. APCR: 1.95–3.24; ELT: 1.5–4 h. ACA IgG <10 GPL and IgM <10 MPL. Values outside the reference values were considered abnormal.

Impaired fibrinolysis was defined as abnormal basal levels of ELT (prolonged), PAI-1 (increased) or t-PA (decreased). No venous occlusion tests were performed in children.

Factor von Willebrand Antigen, Ristocetin cofactor activity and Factor VIII (FVIII) levels were evaluated. Blood sampling, phenotypic analysis and laboratory assays were performed as previously reported [12]. We defined Von Willebrand disease (VWD) as a patient with a personal history of excessive bleeding and a ristocetin cofactor activity (VWF:RCo) lower than 50 U/mL measured on at least two different blood samples [13,14]. The effect of blood group in von Willebrand Factor level and ristocetin cofactor was not taken into account [15]. In most cases, family screening was performed.

LA was defined as *transient* if it became negative in the second test or *persistent* if it remained positive in the second determination. An LA alternatively positive and negative in at least three determinations was described as *fluctuating*.

Statistical analysis

Values are expressed as means or percentages. Results were compared using the chi-square test or Fisher's exact test, as appropriate. Statistical significance was set at p values <0.05. Calculations were performed with the Statistical Package for Social Sciences (SPSS, Chicago IL, USA, version 10.0 for Windows) software and Epi Info, 6.04 version from the Center for Disease Control and Prevention (CDC), Epidemiology Program Office, USA.

Results

One hundred and twenty-four consecutive children were screened for LA during the study period. Thirty-six patients who fulfilled the inclusion criteria were evaluated. The remaining 88 patients were not included due to the following reasons: initial LA determination was negative (52 patients), and in 36 patients, a second LA determination was not performed, but reasons for referral and symptoms were similar between patients included and not included.

Median age was 10.8 years old (range: 3–18), and 52.8% were female. None had an underlying disease; an infectious concomitant event was diagnosed in six patients. Table 1 shows the main reasons leading to consulting.

Bleeding was the reason for consulting in 47.2%, although after anamnesis, bleeding episodes were found in 52.8% (19/36). Nine patients sought medical attention for an incidental prolonged APTT during a preoperative coagulation screening; however, bleeding symptoms were detected during anamnesis in only two patients. There were only two major hemorrhages. A 6-year-old girl presented gastrointestinal bleeding that required no transfusional support. She was diagnosed VWD, as well as her father. A 10-year-old boy consulted for hemathroses, he had a personal history of hematomas. He was diagnosed to have a sporadic mild hemophilia A since he had no family history of hemophilia or bleeding disorders. Bleeding symptoms were mostly minor and muco-cutaneous, i.e., epistaxis, ecchimosis, gum bleeding, hematomas and bleeding after dental extractions.

No hypo-prothrombinemia or thrombocytopenia was detected in the study population. In 66.7% of patients consulting for bleeding, VWD was diagnosed vs. 33.3% of patients with bleeding but without low ristocetin cofactor ($p=0.002$). Specific features regarding VWD in these patients were reported elsewhere [12]. Persistent or fluctuating LA and VWD was diagnosed in 9/17 (53%) patients consulting for bleeding.

Thrombosis was observed in 27.8% of patients (10/36). Thrombotic episodes were deep venous thrombosis in four patients and cerebral venous thrombosis in a 6-year-old boy. Patients with venous thrombosis received standard long-term oral

Table 1 Reasons for consulting

Consult	*n*	%
Screening (prior to surgery or prolonged APTT or family history)	9	25.0
Bleeding		
Easy bruising/postsurgery or dental extractions	15	40.8
Major	2	6.4
Thrombosis		
Venous	5	13.9
Arterial	5	13.9
Total	36	100

anticoagulation therapy [16] without recurrence even after stopping anticoagulant treatment. Five patients had ischemic stroke. A 14-year-old girl who had a history of stroke 12 years ago had LA and high PAI-1 levels. Clinical data about the acute event was not available but a concomitant infection had been excluded. Patients with arterial thrombosis remained symptom-free during follow-up (median 24 months).

A concomitant hemostatic defect was found in 80% (8/10) of patients with thrombosis ($p = 0.003$). Prothrombotic associated defects were hyperhomocysteinemia (3 patients), anticardiolipin antibodies (5 patients), and abnormal activated protein C resistance (1 patient). Fibrinolytic disorders (i.e., prolonged ELT or high PAI-1 levels) were found in 3 patients.

Median number of LA determinations per patient was 3 (range: 2–8). Median interval between LA determinations was 26 months (range: 1 month to 4 years).

LA behavior

LA behavior was not uniform: 44.5% persisted in all determinations (range: 2 months to 4 years); 47.2% (17/36) became negative (2 months to 2 years, only one patient was restudied 10 years after) and 8.3% were alternately positive and negative in several determinations during a 1-year period.

LA was more persistent and fluctuating among children with thrombosis than among the asymptomatic or those with bleeding. Only one of the patients with thrombosis had a persistent LA.

There was no relationship between sex or age and LA behavior.

Discussion

Most LA diagnosed in asymptomatic children is incidental but LA has also been associated with bleeding due to hypo-prothrombinemia or more commonly with thrombosis, both related or not to viral infections. Hypo-prothrombinemia associated with LA might be the cause of mild and severe bleeding [8–10]. Schmugge et al. [17] recommend considering this syndrome in children with newly appearing bleeding symptoms or unclear prolonged prothrombin time or APTT. In our population, no hypo-prothrombinemia or thrombocytopenia was found; bleeding symptoms were mostly minor and associated to VWD. VWD is the most prevalent inherited bleeding disorder, characterized by variable clinical manifestations, although usually mild [18,19]. The high prevalence of this congenital disorder increases the chances for coincidence with other disorders. This entity should always be considered in a child with muco-cutaneous bleeding and prolonged APTT, regardless of coexistence with LA. As the quantification of FVIII activity in the presence of LA can be inaccurate, a chromogenic assay should be performed in order to make certain the measurement of Factor VIII levels in the presence of strong lupus anticoagulants [11,20,21].

An increased prevalence of VWD due to referral bias cannot be excluded in our population because our prevalence is higher than that reported in general population. It has been reported that APTT values are longer in children than in adults, although the cause remains unclear [22]. Neither LA nor a clotting factor deficiency was found to explain these findings. A recent retrospective study on LA in children [3] observed that APTT values shortened with time; a similar tendency was seen among our patients (data not shown). Clinical implications of these observations are not clear.

LA-related thrombosis is often associated with concomitant diseases, including viral infections and other coagulation disorders [6,23,24]. In agreement with other reports [25], we observed thrombotic complications mostly in children with LA and a concomitant thrombophilic defect. That observation is in contrast with what has been reported in pediatric patients with systemic lupus erythematosus in whom a significant relationship between the presence of a lupus anticoagulant and thrombotic events has been found [26].

Recent recommendations [27] emphasize that the detection of one thrombophilic disorder does not exclude the existence of another; similarly, the coexistence of LA and VWD should be considered in patients with LA and bleeding.

Conclusions

In spite of the limitations of a small retrospective study, our observations may have important clinical implications. In our study, the persistence of LA does not in itself represent a higher risk of thrombosis in the pediatric population.

In children with muco-cutaneous bleeding, no hypo-prothrombinemia or thrombocytopenia were observed. Instead, VWD was the reason for bleeding and LA diagnosis was a fortuitous finding.

Prospective multicenter studies are required to understand clinical outcomes and laboratory correlation among pediatric patients with LA.

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