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ANTIPHOSPHOLIPID ANTIBODIES IN 57 CHILDREN AND ADOLESCENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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CAMPOS LMA et al. - Antiphospholipid antibodies in 57 children and adolescents with systemic lupus erythematosus. **Rev. Hosp. Clín. Fac. Med. S. Paulo 58**(3):157-162, 2003.

OBJECTIVE: To investigate the frequencies and behavior of antiphospholipid antibodies in 57 children and adolescents with systemic lupus erythematosus.

METHODS: Anticardiolipin antibodies were investigated by ELISA and lupus anticoagulant antibodies by the international tests recommended. The antiphospholipid antibodies analyses were performed in frozen samples (mean of 5.3 samples per patient obtained during a mean follow-up period of 3 years and 7 months) and on blood samples collected between January 1997 and November 1998 (mean of 2.5 samples per patient during a 2-year follow-up period).

RESULTS: The frequencies of antiphospholipid antibodies (anticardiolipin and lupus anticoagulant) were similar in the samples collected prospectively and in the frozen samples (retrospective study): 63.2% and 75.4% respectively. Positivity for these antibodies fluctuated during the follow-up period and was not associated with any clinical or laboratory parameters of lupus erythematosus, including autoantibodies and also including disease activity and/or severity scores.

CONCLUSIONS: The frequencies of antiphospholipid antibodies in children and adolescents with lupus erythematosus were similar to those observed in adults. The positivity fluctuated during the follow-up and was not correlated with clinical and/or laboratory disease parameters.

DESCRIPTORS: **Antiphospholipid antibodies. Children. Systemic lupus erythematosus.**

The antiphospholipid (aPL) antibodies constitute a heterogeneous family of autoantibodies that react against antigenic epitopes present in negatively charged phospholipids, in complex phospholipid-plasmatic proteins, or even directly in plasmatic proteins[1].

These antibodies are not always pathogenic. They are present in several situations, such as infections, tumors, use of drugs, and even in 3% to 4% of normal individuals, without being related to thrombotic phenomena[2,3]. The aPL antibodies can also be detected in several autoimmune diseases, for example, systemic lupus erythematosus (SLE), Sjögren's syndrome, systemic sclerosis, eosinophilic fasciitis, vasculitis, Behçet's disease, Lyme disease, sarcoidosis and rheumatoid arthritis[4].

Antiphospholipid syndrome (APS) is an entity characterized by the presence of arterial or venous thrombotic phenomena or by the occurrence of reccurented abortions associated with the presence of antiphospholipid (aPL) antibodies, positive on at least 2 occasions[5]. In medical practice, only the anticardiolipin antibodies (aCL) and lupus anticoagulant (LAC) antibodies are available for detecting the aPL antibodies.

Only a few studies have evaluated the frequency and behavior of the aPL antibodies and the APS in children with SLE. The positivity of the aPL antibodies varies from 19% to 87% (mean 56%) for the aCL antibodies and from 11% to 62% (mean 31%) for LAC antibodies. Antiphospholipid syndrome is present in 9% to 24% of the cases[6]. Clinical manifestations relative to the presence of aPL antibodies are similar in children and adults, with a prevalence of venous thrombosis.

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This study was designed to evaluate the frequency and behavior of aPL antibodies in Brazilian children and adolescents with SLE.

PATIENTS AND METHODS

A total of 57 children and adolescents with SLE (according to the criteria revised in 1982 by the American College of Rheumatology[7]) were evaluated from January 1997 to November 1998. The 57 children and adolescents were selected from a sample of 109 patients with SLE that were diagnosed at this service. The inclusion criteria were the diagnosis of SLE and correct follow-up (appointments, laboratory tests, and treatment) between January 1997 and November 1998. Patients with more than 1 hospitalization due to severe infectious events were excluded.

The patients presented a mean age of 14.7 years (range, 6 to 22 years) and a female to male ratio of 5.3 to 1. The mean age of disease onset was 10.2 years (range, 1 to 16 years).

The determination of the presence of aPL antibodies was performed on frozen samples obtained during a mean follow-up period of 3 years, 7 months (mean of 5.3 samples per patient, range, 1 to 10 samples), collected at patient admission, annually, and/or according to clinical indication during the course of the disease, as well as on blood samples collected prospectively (mean of 2.5 samples (range, 1 to 3) per patient during a 2-year follow-up period from January 1997 to November 1998).

The laboratory technique used for all samples to detect the ACL antibody was the enzyme-linked immunosorbent assay (ELISA) using the commercial kit Hemagen Anticardiolipina®, with results given in international units (GPL and MPL). The values were considered positive when above 10 GPL or 10 MPL. The following international recommendations[8] were used to detect the LAC antibodies in all the samples: 3 screening tests were performed (activated partial thromboplastin time - aPTT, dilute Russell's viper venom test – DRVVT and kaolin clotting time - KCT), and when indicated, the confirmatory tests (PNP (platelet neutralization procedure) of aPTT and/or PNP of the DRVVT).

The evaluation of clinical and laboratory manifestations and the disease course was based on a retrospective analysis of the records of each patient, from admission to the end of the study in November 1998. The activity of the SLE was checked at the time of each blood sample collection, based on the erythrocyte sedimentation rate (ESR) value and the systemic lupus erythematosus disease activity index (SLEDAI).

The statistical analysis was performed using the chi-square test, Fisher's exact test, *t* test for 2 independent samples, Kruskal Wallis test, and Cochran's Q test, with statistical significance established with a probability of 95%. Spearman's coefficient of correlation was used for the correlation analysis.

RESULTS

Of the 57 children and adolescents with SLE studied, aPL antibodies were positive in 63.2% and 75.4% of the cases, respectively, in the samples collected between January 1997 and November 1998 and in frozen samples (retrospective analysis) (Tables 1 and 2). The number of samples collected per patient varied from 1 to 3 (mean 2.5 samples per patient) in the 2-year study and 1 to 10 (mean 5.3) in the retrospective study.

Both analyses yielded similar results regarding positivity of the aPL antibodies and their isotypes. Positivity for aPL antibodies varied during the study, being increased when only 1 sample was necessary to define this positivity, as compared to the necessity of 2 or more samples (Tables 1 and 2).

To relate the presence of aPL antibodies, demographic data, and clinical and laboratory manifestations, the frequencies of aPL antibodies found in the retrospective study (frozen samples) were used.

There was no significant difference

Table 1 - Frequency of antiphospholipid antibodies (aPL) in 57 children and adolescents with systemic lupus erythematosus in the samples collected between January 1997 and November 1998.

	anticardiolipin (aCL)				
	IgG n (%)	IgM n (%)	IgG and/or IgM n (%)	LAC n/N (%)	aPL n (%)
1st sample (n=57)	6 (10.5)	18 (31.6)	20 (35.1)	7/52 (13.5)	23 (40.3)
2nd sample (n=48)	5 (10.4)	19 (39.6)	19 (39.6)	7/47 (14.9)	24 (50.0)
3rd sample (n=36)	1 (2.8)	13 (36.1)	13 (36.1)	2/21 (9.5)	13 (36.1)
+ in at least one sample (n = 57)	9 (15.8)	29 (50.9)	30 (52.6)	12/55 (21.8)	36 (63.2)
+ in two or more samples (n=48)	3 (6.2)	15 (31.2)	16 (33.3)	3/45 (6.7)	17 (35.4)

+ = positive for antibody; LAC = lupus anticoagulant antibody; n/N = number of positive samples / total number of samples.

Table 2 - Frequency of antiphospholipid antibodies (aPL) in 57 children and adolescents with systemic lupus erythematosus, in at least 1 sample and in 2 or more samples, via retrospective analysis (mean follow up = 3.7 years).

	anticardiolipin (aCL)				
	IgG n (%)	IgM n (%)	IgG and/or IgM n (%)	LAC n/N (%)	aPL n (%)
+ in at least 1 sample (n = 57)	29 (50.9)	33 (57.9)	40 (70.2)	16/55 (29.1)	43 (75.4)
+ in 2 or more samples (n=54)	12 (22.2)	20 (37.0)	26 (48.1)	7/47 (14.9)	31 (57.4)

+ = positive for antibody; LAC = lupus anticoagulant antibody; n/N = number of positive samples / total number of samples.

between the group of patients with positive aPL (n = 43) and the group of patients with negative aPL (n = 14) in terms of age, age at presentation, follow-up, gender or race (Table 3). The number of samples was higher in the group that was positive for aPL (P = 0.031).

There was no association between the presence of the aPL antibodies and the involvement of any organs/systems evaluated (Table 4), except in relation to impairment of the reticuloendothelial system, where a higher frequency of hepatomegaly was observed in the children and adolescents with SLE and positive aPL antibodies (P = 0.021).

Regarding the disease activity, no linear correlation was found between the titers of IgG or IgM aCL antibodies and the activity of SLE, when considering the mean titers of aCL antibodies and mean values of ESR and SLEDAI throughout the follow-up period. A weak correlation (r = 0.38), albeit significant (P = 0.009), was observed between the IgM aCL antibody and the ESR levels when considering only an isolated sample (the first) of the 57 patients. Regarding the course of the disease, remission was found more frequently (P = 0.035) in the group negative for aPL antibodies when compared to the group presenting aPL antibodies (46.1% and 16.3%, respectively).

Antiphospholipid syndrome was diagnosed in 8 patients (14%) (Table 5). All patients presented positive IgG aCL antibodies, 7 presented IgM aCL antibodies, and 6 presented LAC antibodies. Arterial events were found in 4 patients (ischemic vascular cerebral accident (IVCA) - 3, amaurosis fugax - 2, transient ischemic attack (TIA) - 1, renal thrombosis - 1), and deep venous thrombosis (DVP) – 1. Two patients presented osteonecrosis, and transverse

Table 3 - Antiphospholipid antibodies (IgG and IgM anticardiolipin antibodies) and demographic data in 57 children and adolescents with systemic lupus erythematosus.

	positive aPL n=43	negative aPL n=14
AGE (mean/median)	15.1 / 14	13.6 / 16
AGE AT PRESENTATION (mean/median)	10.4 / 11	9.6 / 10
FOLLOW-UP (mean/median)	3.9 / 3	3.2 / 3
FEMALE:MALE RATE	36:7	12:2
CAUCASIANS	22 (51.2%)	4 (28.6%)
ADMIXTURE	16 (37.2%)	6 (42.8%)
NUMBER OF SAMPLES (mean/median)	5.6 / 6	4.1 / 4*

aPL = antiphospholipid antibodies; *P = 0.031

Table 4 - Antiphospholipid antibodies (IgG and IgM anticardiolipin antibodies) and organs/systems involvement in 57 children and adolescents with systemic lupus erythematosus

	positive aPL n=43 n (%)	negative aPL n=14 n (%)
HEPATOMEGALY	18 (41.8)	1 (7.1)*
LIVEDO RETICULARIS	11 (25.6)	2 (14.3)
OSTEONECROSIS	2 (4.6)	0 (0)
VALVULITIS	10 (23.2)	2 (14.3)
HEMOLYTIC ANEMIA	17 (39.5)	6 (42.8)
THROMBOCYTOPENIA	12 (27.9)	3 (21.4)
HEADACHES	12 (27.9)	6 (42.8)
CHOREA	2 (4.6)	0 (0)
STROKES	3 (6.9)	4 (28.6)
PSYCHOSIS	3 (6.9)	0 (0)
VASCULAR CEREBRAL ACCIDENT	3 (6.9)	2 (14.3)
TRANSIENT ISCHEMIC ATTACKS	1 (2.3)	1 (7.1)
AMAUROSIS FUGAX	2 (4.6)	0 (0)
TRANSVERSE MYELITIS	1 (2.3)	0 (0)
COAGULATION ABNORMALITIES	9 (20.9)	0 (0)
BFP-STS	1 (2.3)	0 (0)

aPL = antiphospholipid antibodies; BFP-STS = biologic false positive serological test for syphilis; *P = 0.021

Table 5 - Characteristics of antiphospholipid syndrome (APS) in 8 patients with systemic lupus erythematosus (SLE).

CASE	Thrombotic manifestations	Other manifestations related to APS	Interval until the diagnosis of APS, after the diagnosis of SLE
1	IVCA	hemolytic anemia, thrombocytopenia, livedo reticularis	3 yr 3 m
8	DVT (2x)	hemolytic anemia, thrombocytopenia	3 yr 2 m
9	Renal thrombosis	hemolytic anemia, thrombocytopenia, valvulitis	7 m
19	Osteonecrosis	thrombocytopenia, migraine	5 yr 11 m
22	Amaurosis fugax, TIA, IVCA (2x)	hemolytic anemia, thrombocytopenia, behavioral disturbances	6 m
24	Osteonecrosis	hemolytic anemia, thrombocytopenia, migraine	3 yr 7m
26	Amaurosis fugax, IVCA	hemolytic anemia, thrombocytopenia, migraine	2 yr 11m
49	Transverse myelitis	-	3 yr 10m

IVCA= ischemic vascular cerebral accident; TIA= transient ischemic attack; DVT = deep venous thrombosis

myelitis was found in the last one. Three patients presented more than 1 event (mean interval of 13 months between them). Hemolytic anemia and thrombocytopenia were found in 6 and 7 patients, respectively. Other manifestations related to APS found in these patients included headaches (3), valvulitis (1), behavioral disturbances (1) and livedo reticularis (1). The interval between SLE and APS diagnosis was 3 years (ranging from 6 months and 5 years, 11 months).

DISCUSSION

In this study, the frequencies of aPL antibodies presented a very well-defined behavior. The number of individuals positive for aPL antibodies on at least 1 occasion was larger than the number of individuals positive on 2 or more occasions. This fact was observed both in the samples collected between January 1997 and November 1998 (63.2% and 35.4%, respectively) and in the frozen samples (retrospective study) (75.4% and 57.4%, respectively) for aCL antibodies and LAC antibodies, which suggest a cyclical behavior of these autoantibodies.

Several works have verified that the positivity of the aPL antibodies is linked to the time of follow-up and the number of times that it is tested[9;10;11]. PÉREZ-VÁZQUEZ et al.[10] demonstrated that when they extended the follow-up of a group of 667 lupus patients from 7.5 months to 3.1 years, the frequency of aPL antibodies increased from 52% to 66%, with a consequent increase in the number of cases in which it was possible to diagnose APS (from 10% to 15%).

Regarding the isotypes, the aCL antibody was detected more frequently than the LAC antibody (52.6% and 21.8%, respectively, in the samples collected in the 2-year study and 70.2% and 29.1% in the retrospective study – frozen samples). This finding is in agreement with data reported in literature, where the positivity for aPL antibodies among lupus children varies from 19% to 87% (mean 56%) for the aCL antibody and from 11% to 62% (mean 31%) for LAC antibodies[6].

The IgM aCL isotype was found more frequently than the IgG aCL isotype in both analyses. This difference was maintained when we selected the patients that were positive on 2 or more occasions. In the literature, the IgG isotype is reported to be the most common in adults and children with lupus[12].The clinical manifestations related to the presence of aPL antibodies in children are similar to those found in adults, in agreement with the literature review performed by Ravelli & Martini in 1997[6].

In this study, the presence of the aPL antibodies was not correlated with a specific clinical or laboratory profile among the children and adolescents studied. Hepatomegaly was the only manifestation that was statistically more frequent in the group with positive aPL antibodies. In the literature, there are reports of patients with APS, hepatomegaly, and an increase in the hepatic enzymes, which is attributed to hepatic microthrombosis without any relationship to infections, drugs, congestion, and degenerative or metabolic causes [13]. In this study, the children that presented hepatomegaly associated with the presence of aPL antibodies (n = 18) did not present any other clinical or laboratory manifestations of hepatic abnormality. All the patients, except 3, presented other signs of involvement of the reticuloendothelial system, such as adenomegaly (n = 14) and/or splenomegaly (n = 6).

Other manifestations usually reported in the literature in association with the aPL antibodies, such as livedo reticularis, osteonecrosis, valvulitis, hemolytic anemia, and thrombocytopenia, were also not found frequently in this study. Even involvement of the central nervous system (with manifestations such as headaches, strokes, transient ischemic attacks, amaurosis fugax, psychosis, chorea, and transverse myelitis), which in the studies by RAVELLI et al. [12] and SHERGY et al. [14] was reported to be associated with the presence of aPL antibodies, was

not observed in the patients of this study. Coagulation abnormalities, characterized by prolongation of the prothrombin time and/or activated partial thromboplastin time, were only found in the patients with positive aPL antibodies (20.9%) and in none of the patients with negative aPL antibodies. The low frequency of these abnormalities indicates the low sensitivity of these parameters as screening methods for the detection of aPL antibodies, as has already been observed by PETRI [2]. The same is true in relation to the biologic false-positive serological test for syphilis (BFP-STS) that was found in just 1 patient with positive aPL antibodies.

In the studies by RAVELLI et al. [12] and SHERGY et al. [14] in children with SLE, it can be seen that the positivity of the aPL antibodies fluctuated during the follow-up. RAVELLI et al. [12] demonstrated a relationship between the titers of the aCL antibody and disease activity when the ESR, the reduction in the C3 fraction of the complement, and Systemic Lupus Activity Measure (SLAM) were analyzed, but this correlation was not maintained when SLEDAI and anti-DNA antibody were used as parameters of disease activity.

In this study, it was observed that fewer patients with positive aPL antibodies tended to experience disease remission, when compared to patients with negative aPL antibodies (P = 0.035). It is possible that patients with a remittent disease course produce fewer autoantibodies and consequently present a lower frequency of aPL antibodies.

This study, and its similarity to others in the literature, presents some interesting aspects.

The behavior of the aPL antibodies was cyclical in terms of positivity, with a higher frequency in the longer periods of follow-up and/or larger number of samples collected. There was no clear relationship of the occurrence of aPL antibodies with disease activity, severity, or clinical and laboratory manifestations. It is possible that their occurrence has a relationship with the etiopathic mechanism of the SLE itself (production of multiple autoantibodies) or with the appearance of neoantigens due to the process of chronic vasculitis or also due to the chronic use of corticosteroids, causing abnormalities in lipid metabolism. This second hypothesis (exposure to neoantigens) could explain the late emergence of the manifestations related to the aPL antibodies during the follow-up of patients with SLE.

There was also no correlation between the presence of aPL antibodies and specific clinical manifestations, which does not support a pathogenic role for these antibodies. It is possible that there are pathogenic aPL antibodies and nonpathogenic aPL antibodies, which would explain the high frequency of patients with SLE that have aPL antibodies and the low relative frequency of APS in these patients. In fact, various authors have suggested that the pathogenic aPL antibodies could be those dependent on the β2-GP1[3;15]. While studying the aCL and anti-β2-GP1 antibodies in patients with SLE, AMENGUAL et al.[15] observed that the former were present in 70.4% of the cases with APS and in 24.5% of the patients without APS, while the anti-β2-GP1 were present in 53.5% and 4.1%, respectively. Thus, the anti-β2-GP1 antibody was more specific, while being less sensitive. Patients that are positive for aCL antibodies and negative for anti-β2-GP1 antibodies could correspond to the cases in which the aCL antibodies are independent from the β2-GP1 and are therefore nonpathogenic, or also pathogenic, through the connection with other plasmatic proteins. Further studies with the intention of identifying the pathogenic role of the aPL antibodies and their connection with specific plasmatic proteins will be important to establish the real role of aPL antibodies in patients with SLE.

RESUMO

CAMPOS LMA e col. - Anticorpos antifosfolípides em 57 crianças e adolescentes com lúpus eritematoso sistêmico. **Rev. Hosp. Clín. Fac. Med. S. Paulo 58**(3):157-162, 2003.

OBJETIVO: Investigar a freqüência e o comportamento dos anticorpos antifosfolípides em 57 crianças e adolescentes com lúpus eritematoso sistêmico.

MÉTODOS: A técnica laboratorial para a pesquisa do anticorpo anticardiolipina foi ELISA e para a pesquisa do anticorpo anticoagulante lúpico foram seguidas as recomendações internacionais. A pesquisa dos anticorpos antifosfolípides foi realizada em soros

estocados (média de 5,3 amostras por paciente durante o período de seguimento de em média 3 anos e 7 meses) e em soros coletados no período de janeiro de 1997 à novembro de 1998 (média de 2,5 amostras por paciente em um período de dois anos).

RESULTADOS: A freqüência dos anticorpos antifosfolípides (anticorpo anticardiolipina e anticoagulante lúpico) foi semelhante nos soros coletados prospectivamente e nos soros estocados (estudo retrospectivo): 63,2% e 75,4% respectivamente. A positividade destes anticorpos flutuou durante o seguimento e não esteve associado a nenhum parâmetro clínico e laboratorial do lúpus eritematoso sistêmico, incluindo auto-anticorpos e também atividade e/ou gravidade da doença.

CONCLUSÕES: A freqüência dos anticorpos antifosfolípides em crianças e adolescentes com lúpus eritematoso sistêmico foi semelhante à observada em adultos. A positividade dos anticorpos flutuou durante o seguimento dos pacientes e não foi evidenciada associação com parâmetros clínicos e/ou laboratoriais da doença.

DESCRITORES: **Anticorpos antifosfolípides. Crianças. Lúpus eritematoso sistêmico.**

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