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Is there a Role for Anti-phospholipid-binding Protein Antibodies in the Pathogenesis of Thrombosis in Behcet's Disease ?

Dear Sir,

Arterial and venous thrombosis occurs in approximatively one third of patients with Behcet's disease (BD) (1). A superficial thrombophlebitis is observed in a percentage of patients ranging from 8 to 38% (2, 3). Thrombosis also occurs in the deep veins of the limbs or in larger veins such as the superior and inferior vena cava. Budd-Chiari syndrome has also been reported in the course of BD (4). Thrombotic arterial involvement has been observed in 2-10% of patients (5, 6). Pathogenesis of this thrombophilic tendency has not been clearly elucitated as yet. The hypothesis of autoantibody-mediated mechanism is fascinating but not yet confirmed. It is well known that anti-phospholipid antibodies (aPL) are associated with a thrombophilic tendency. The search for serum antibodies directed against cardiolipin, which has for practical reason been the main antigen used to detect aPL, has given conflicting results in BD as these autoantibodies have been detected in 0-46% of sera (7-11). Hull and colleagues (12) found a significant association of retinal vasculitis with anti-cardiolipin antibodies (aCL), while the majority of other reports failed to find any correlation between aCL and any vascular events occurring in BD (7-11). There is now growing evidence that aPL are a heterogeneous population, including antibodies directed against pure phospholipids, antibodies against coagulation-related PL-binding proteins (beta2-GPI, prothrombin, annexin V, protein S), and antibodies which might be directed against complexes of PL and PL-binding proteins (13-14).

As far as we know anti-annexin V, anti-prothrombin and anti-beta2-GPI have never been sought in BD sera before, while more recently an acquired and transient protein S deficiency has been associated with the presence of anti-protein S antibodies (15).

Aim of the study was to investigate the presence of antibodies directed against cardiolipin and some of PL binding proteins (beta2-GPI, prothrombin, protein S, annexin V) in the sera of BD Italian patients.

Twenty-nine outpatients with BD (classified according to ISG criteria) (16) were included in this study: 4 females, 25 males, mean age 36.6, range 22-58). Fourteen out of these 29 patients had experienced at least one episode of arterial or venous thrombosis, all of these patients experienced a venous thrombosis (deep venous thrombosis in all cases but 2 with vena cava thrombosis). A recurrence of venous thrombosis has been observed in six out of the fourteen cases, and a cerebral arterial thrombosis occurred in two of them. The control group consisted of 24 age and sex - matched blood donors.

Anti-CL antibodies were detected by mean of a commercially available ELISA kit (Diamedix corporation, Miami, Florida).

Anti-beta2-GPI, anti-prothrombin, anti-protein S, and anti-annexin V were detected by an ELISA method using γ-irradiated plates (Nunc, Intermed, Roskilde, Denmark) (14). The cut-off value was fixed as mean O.D. + 3 SD of normal human sera. Commercially available aPL binding-proteins polyclonal antibodies were used throughout the experiment as positive controls.

Two patients (one with a previous history of venous thrombosis, the other without) had IgG aCL at a low titre, 17 and 12 GPL respectively. Three patients had anti-annexin V antibodies, only one of them had a previous history of deep venous thrombosis. No sera tested positive for anti-beta2-GPI, anti-prothrombin, anti-protein S antibodies.

In conclusion, a low prevalence of a-CL and anti-annexin V antibodies was found in italian patients with BD. No significant association between these autoantibodies and thrombotic events was found. This finding does not support the hypothesis that anti-PL-binding protein antibodies play a significant role in BD thrombophilic tendency.

R. Priori[1], F. Conti[1], V. Pittoni[1], T. Garofalo[2], M. Sorice[2], G. Valesini[1]
From the [1]Cattedra di Allergologia e Immunologia Clinica III, Clinica Medica I, [2]Dipartimento di Medicina Sperimentale e Patologia, Università "La Sapienza" Roma, Italy

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Correspondence to: Prof. Guido Valesini, Clinica Medica I, Policlinico Umberto I, Viale del Policlinico 00161 Roma, Italy – Tel./FAX Number 064469273; E-mail: gvalesini@mix.it

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Transient Lupus Anticoagulants in Children: Stepwise Disappearance of Diagnostic Features

Dear Sir,

Recently, we have shown in a retrospective cohort study that the majority of lupus anticoagulants (LA) occurring in children are not associated with clinical complications and are transient (1). After a median follow-up of 3.2 years, more than half of children had completely normal coagulation tests. However, 23% of children still had prolonged APTT values, and 15% had prolonged APTTs, positive plasma mixing studies, but negative LA-confirmation assays. These findings suggest that the laboratory abnormalities associated with LA may persist for differing time periods which may cause diagnostic problems.

We now present two asymptomatic children with LA who were followed-up at short-term intervals. Both patients displayed a similar pattern of sequential normalisation of laboratory features of LA, first the LA-confirmation assay, then the plasma mixing studies, and finally the APTT.

Case reports. Laboratory data of the two patients are summarized in Table 1. Diagnosis of LA was based on three criteria according to ISTH-recommendations (2): 1) prolongation of the APTT or dilute Russell Viper venom time (DRVVT); 2) positive plasma mixing studies; 3) confirmation of phospholipid-dependence; for the reagents used see Table 1. In patient #1 and patient #2, LA were found incidentally on preoperative coagulation screening. They had no history of bleeding or thrombosis and a negative family history. None developed complications at subsequent surgery or during follow-up. Patient #1 had a history or recurrent adenotonsillar infections. His first laboratory investigation showed a moderately prolonged APTT and borderline positive plasma mixing studies. No further tests for LA were done at this time. Adenoidectomy was performed and six months later all coagulation tests had become normal. After 13 months, following a series of tonsillar infections, the patient was reinvestigated for planned tonsillectomy. At this time all diagnostic criteria for a LA were positive. In patient #1 and patient #2, the laboratory features of LA disappeared in a consistent order (Table 1): first, the LA-confirmation assay, then the DRVVT and the plasma mixing study became negative. The APTTs gradually declined but were the last to normalize, after 14 and 10 months, respectively. All other coagulation tests, including individual clotting factor levels, were normal in both patients.

Discussion. The observed pattern of regression of LA most certainly reflects decreasing concentrations of the antiphospholipid antibodies. The lower titer antibodies prolong the APTT to a lesser extent. In the plasma mix, the antibodies are further diluted thus the APTT normalizes. In the confirmation assay, the ratio between the moderately prolonged APTT and the APTT after phospholipid correction does not reach significance. The pattern seen in our patients suggests that the test least

Correspondence to: Christoph Male, M. D., Hamilton Civic Hospitals Research Centre, 711 Concession Street, Hamilton, Ontario L8V 1C3, Canada – Tel.: +1-905-527-2299ext.43782; Fax: +1-905-575-2646; E-mail: cmale@thrombosis.hhscr.org

Pat.	Sex	Age (y)	Observation time (months)	APTT [1]	APTT [1] of 1:1 plasma mix (normal pool)	DRVVT [3]	Confirmation assay [4]
# 1	m	2.7	0	**58**	**43** (36)	-	-
			1.3	adenoidectomy			
			6.2	47	37 (32)	35	negative
			13	**70**	**60** (37)	**57**	**positive**
			14	tonsillectomy			
			15	**60**	**54** (41)	**44**	negative
			19	**58**	47 (43)	41	negative
			27	48	40 (36)	41	negative

Pat.	Sex	Age (y)	Observation time (months)	APTT [2]	APTT [2] of 1:1 plasma mix (normal pool)	DRVVT [3]	Confirmation assay [4]
# 2	m	2	0	**88**	**66** (43)	**45**	**positive**
			1.4	orchidopexy			
			1.7	**63**	**51** (45)	34	negative
			6	**52**	41 (37)	32	negative
			10	49	40 (39)	42	negative

normal values	≤ 48 sec. [1] ≤ 49 sec. [2]	within 5 seconds of normal pool	≤ 42 sec.	negative

Table 1 Time course of laboratory abnormalities associated with LA in two patients

[1] APTT-FSL® [Baxter, Dade]; [2] APTT-LA® [Diagnostica Stago]; [3] DVVtest® [American Diagnostica];
[4] phospholipid confirmation assays: Staclot LA®, Staclot PNP® [Diagnostica Stago] and DVV confirm® [American Diagnostica];

shaded cells: abnormal values