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FIBRINOLYTIC ACTIVITY AND OTHER COAGULATION PROTEINS IN PATIENTS WITH LUPUS ANTICOAGULANT. M.Borrell, J.Fontcuberta, E.Muñiz, E.Grau, A.Oliver. Servei d'Hematologia, Hospital de Sant Pau, Barcelona (Spain).

Patients with lupus anticoagulant (LA) is a group with recognised risk of thrombosis. In order to study the possible causes of this tendency, we have studied the fibrinolytic activity and some other coagulation parameters considered as thrombotic markers in a group of 12 patients with LA. Half of them had presented thrombotic disease and/or recurrent abortions. 3 patients presented systemic lupus erythematosus and in the other 9 we couldn't detect any connective tissue disorder. The following tests were performed: tissue plasminogen activator (t-PA) activity, t-PA antigen, t-PA inhibitor, fibrin plates with and without kaolin, -antiplasmin, plasminogen, prekallikrein, antithrombin III, protein C, protein S. The results were analysed comparing the values obtained in LA group with respect to normal values, and among the LA group between patients who had suffered from thrombotic disease and those who did not.

The results obtained did not show significant differences between control group and LA patients except in t-PA activity which was found decreased in LA group: 1.86±0.89 versus 3.0±0.94 in control group (mean±standard deviation). Among this group there were no differences between patients who had suffered from thrombotic disease and those who didn't.

From these results we suggest that unpaired t-PA activity may contribute but not be the only responsible factor for the development of thrombosis in these patients.

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DETECTION OF LUPUS ANTICOAGULANTS BY THE "INSIDE-OUT MEMBRANE ADSORPTION TEST": aPTT IS SHORTENED AFTER INCUBATION OF THE PLASMA WITH THE ADSORBENT. A. von Felten. Laboratory of Blood Coagulation, Dept. of Internal Medicine, University Hospital, Zürich, Switzerland

Prolongation of the aPTT in a mixture of a patient's plasma with normal plasma is characteristic for the presence of a coagulation inhibitor. Since inhibitors directed against individual blood coagulation factors differ in their clinical significance from a lupus anticoagulant (LA), a fast and reliable distinction from eachother would be highly desirable. The test presented which is based on the absorption of antiphospholipid antibodies by use of a stable phospholipid adsorbent largely fullfills this demand.

Method 1. Preparation of the LA-adsorbent (modified acc. to Steck et al., Science 168:255, 1970): Fresh human RBC (bl. gr. 0, in 0.5% EDTA) are washed (NaCl, 0.15m / phosphate buffer, 0.005m, pH8.0) and lysed in phosphate buffer alone. The membranes are washed with the same buffer (centrifugations: 40'000g, 10min, 4°C) and incubated 60 min on ice in phosphate buffer, 0.0005m. After centrifugation, the sediment is resuspended in an equal volume of buffer (0.0005m, with merthiolate 0.1%), homogenized and centrifuged in portions of 1 ml; the supernatant is carefully removed. The adsorbent, stored at 4°C in capped tubes, is stable for at least three months.

2. Performance of test: To 600ul of patient's plasma mixed with 600ul normal plasma (citrated), 12ul of heparin (50IU/ml) are added. 600ul of the mixture are given to the LA-adsorbent, vortexed, incubated (room temp., 30min) and centrifuged (40'000g). From the supernatant as well as the not adsorbed portion of the plasma mixture, heparin is eliminated by adding ECTEOLA-cellulose. After centrifugation, aPTT is determined in both supernatant samples.

Results The test is positive, i.e. LA is present, if aPTT is shortened by at least 5 sec after treatment with LA-adsorbent.

Examples:

plasma samples	aPTT before	aPTT after ads.	Difference (sec)
normal plasma *	39	41	+ 2
plasma deficient in FVIIIC	41	45	+ 4
hemophilia A with FVIIIC-inhibitor	49	54	+ 5
aquired hemophilia A	50	52	+ 2
plasma with lupus anticoagulant	92	59	- 33

* not diluted; other samples are mixed 1:1 with normal plasma

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HIGH INCIDENCE OF CARDIOLIPIN-BINDING ANTIBODIES IN PATIENTS TAKING ORAL ANTICOAGULANTS.
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It is known that patients with thrombosis in autoimmune disease often suffer recurrent thrombotic episodes on withdrawl of oral anticoagulants, frequently have raised ACA and may require long term therapy. In view of the association between lupus inhibitors, phospholipid binding antibodies and thrombotic episodes we investigated a broad group of patients (n=140) taking oral anticoagulants for anti-cardiolipin antibodies (ACA).

The incidence of raised ACA (defined here as more than 5 S.D. above normal) was 10%, in comparison with less than 2% in both healthy volunteers and randomized hospital patients. Analysis of patients with raised ACA indicated an approximately equal distribution among those with artifical valves and patients treated for thrombotic episodes. ANF and DNA binding studies were negative in most cases and a lupus inhibitor (KCT mixing test) was detectable only in 1/15. APTT/PT ratios were not significantly high in comparison with other clinic patients. The ACA were predominantly IgG (12/15) in contrast to those found in females with recurrent abortions which are frequently IgM's. The binding antibodies appeared to be much weaker in the presence of calcium than in phosphate buffered saline.

The patients having high ACA had no excess thrombotic or bleeding complications during their treatment compared with others in this group. This suggests that thrombotic or occlusive events associated with ACA may be adequately treated with oral anti-coagulants.

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LUPUS ANTICOAGULANT (LA) COEXISTENT WITH TRANSIENT PROTHROMBIN (FII) INHIBITOR: FII DEFICIENCY DUE TO CLEARANCE OF THE IMMUNOCOMPLEX. R.Redaelli, F.Baudo, G.Busnach*, T.M.Caimi, L.Perrino*, L.Pezzetti, F.deCataldo. Departments of Haematology and Nephrology*, Ospedale Cà Granda Niguarda, Milano, Italia.

23 y.o. man with acute nephritis and bleeding (epistaxis, ecchymosis) at presentation. Family and personal past history negative for bleeding. Laboratory data consistent with SLE. Coagulation tests: PT Ratio (R) 1.8, aPTT R 2.4, FII:C <1%, FIIR:Ag 5%, other coagulation factors normal. Tissue thromboplastin inhibition test (TTIT) R 2.8, congenital FII deficiency (6%) R 1.6.

1. FII survival time (FII-concentrate infusion - 60 U/kg) t½: 9 hours.
2. FII neutralizing activity (FII:C normal plasma (NP) + buffer 55%; NP + patient plasma (PtP) 50%): absent.
3. Immunocomplex formation

FII (in the supernatant) %	C	RAg	Radioactivity % in the pellet	
NP + buffer	38	36	^{125}I FII + NP	11
NP + PtP	21	23	^{125}I FII + PtP	75
NP + FII (6%)	34	36	^{125}I FII + antiFII Ig*	90

Method: incubation x 16 h at 4°C→addition of $(NH_4)_2SO_4$→30' at 4°C→2.000 g x 30' at 4°C→dialysis of supernatant.

4. FII inhibitor characterization (purified FII coupled to CNBr-activated Sepharose →PtP incubation with FII-Sepharose→specific antiFII immunoglobulins (Ig)* elution at acid pH→identification by double immunodiffusion): precipitin line with anti IgA, anti IgG_2, anti k, anti l.
5. LA characterization (after FII inhibitor disappearance): TTIT on mixtures NP + PtP or N Ig in equal volumes.

Protein A Sepharose	$IgG_{1,2,4}$	R 1.82	IgG_3, IgA, IgM	R 1.21
Ultrogel AcA 22	IgM	R 0.98	IgA, IgG	R 1.66

Diagnosis: SLE, LA (IgG); polyclonal (IgA, IgG_2, k, l) not neutralizing FII inhibitor; hypoprothrombinemia due to clearance of the immunocomplex.

FII inhibitor was transient. Bleeding was rapidly controlled by replacement therapy. LA persits after FII inhibitor disappearance.

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