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**DDE COMPLEX DETERMINATION AS A SPECIFIC MARKER FOR FIBRINOLYSIS.** **J. Soria (1), C. Soria (2), Mc. Mirshahi (2), S; Mirshahi (2), M. Mirshahi (1), E Pujade (1), C Boucheix (3), M. Samama (1), A. Bernadou (1), J.P. Caen (2). Laboratories of Professors Adolphe, Bernadou, Samama, Hôtel Dieu Paris, France (1) and haematology department, Hôpital Lariboisière, Paris, France (2)., INSERM U. 268, Villejuif, France (3)**

Monoclonal antibodies (McAb) reacting with fibrin degradation products (FbDP), but not with fibrinogen have been produced in order to determine specifically FbDP directly in plasma. Most of the McAb available however do also react with fragment D. Our anti D neo McAb reacts about 10 times less with fragment D than with FbDP but does not react with fibrinogen, fragment X or Y.

In clinical investigation, even in pathological conditions in which there is a great release of tissue-type plasminogen activator (tpA), we have shown that fragment D is not generated in patients plasma. Therefore, the reactivity of our McAb with fragment D did not alter the specificity of FbDP assay.

On the contrary, using polyacrylamide gel electrophoresis in the presence of SDS followed by immunorevelation, we have evidenced that fragment D is generated in patients undergoing thrombolytic therapy even with rtpA. Therefore, using conventional Elisa procedure (capture of FbDP on polystyrene-immobilized anti D neo antibody and detection by peroxidase-labelled anti fragment D immunoglobulins), the presence of fragment D in patients plasma leads to an overevaluation of FbDP. To avoid this overestimation we have modified the Elisa procedure. The structure of FbDP was taken into acount in order to render the technique specific of FbDP. In FbDP fragment D coming from one fibrin monomer is always associated with fragment E from another fibrin monomer, as DDE complex for example. Therefore after capture of fragment D by the polystyrene-bound anti D neo McAb, FbDP were specifically revealed by peroxidase-labelled anti E antibody (polyclonal or monoclonal anti E may be used). For this reason, this test was named DDE determination and DDE determination can be used in any circumstances to evaluate fibrin degradation.

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FIBRIN DEGRADATION PRODUCTS (FbDP) IN HEALTHY VOLUNTEERS AFTER INFUSION OF RECOMBINANT TISSUE-TYPE PLASMINOGEN ACTIVATOR (rt-PA) E. Seifried (1,2), D.C. Rijken (1), B. Hoegee (1), P. Tanswell (3), C. Kluft (1) and W. Nieuwenhuizen (1). Gaubius Institute TNO, Leiden, Netherlands (1), Abteilung Innere Medizin III, Universität Ulm, FRG (2) and Dr. Karl Thomae GmbH, Biberach, FRG (3).

During thrombolytic therapy of myocardial infarction (MI) with urokinase or streptokinase (SK), levels of fibrin(ogen) degradation products in serum are often dramatically elevated as a result of a combination of systemic fibrinogenolysis and local thrombolysis. Others have measured increased levels of D-dimer in serum of MI patients after SK therapy and postulated that thrombolysis could be monitored during SK therapy by measuring D-dimer levels. In the present study rt-PA was infused into healthy volunteers to analyse if elevated FbDP levels in MI patients really reflect coronary thrombolysis or could be due to a systemic effect. Over a period of 60 min., three groups (n = 6 each) were given i.v. 0.25 mg rt-PA/kg (group I), 0.50 mg rt-PA/kg (group II) and a placebo infusion (group III), respectively. Two blood samples were taken from an anticubital vein in the arm contralateral to the site of infusion (one on citrate/aprotinin, the other on citrate alone) at different time points. Using a new enzyme immunoassay (EIA), based on monoclonals and development by us, we measured FbDP in plasma (not serum). Before infusion all volunteers had FbDP levels $<<$ 0.5 µg/ml. Upon infusion FbDP levels in groups I and II increased to average values of 1.0 $\pm$ 0.4 µg/ml and 0.8 $\pm$ 0.2 µg/ml, respectively, for the samples taken in citrate/aprotinin. The values in citrate alone did not differ significantly, and were 1.1 $\pm$ 0.5 µg/ml and 0.8 $\pm$ 0.3 µg/ml for groups I and II, respectively. FbDP levels in group III remained $<<$ 0.5 µg/ml. The results show that FbDP levels increase upon rt-PA infusion, even in healthy volunteers. This suggests lysis of systemic fibrin. We conclude that lysis of systemic fibrin limits the value of FbDP levels as a measure for coronary thrombolysis.



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LUPUS ANTICOAGULANT AND REPEATED ABORTIONS: A CASE- CONTROL STUDY. T. Barbui, S. Cortelazzo, M. Galli, F. Parazzini (1), E. Radici (2) E. Rossi (3). Divisione di Ematologia and Divisione di Ostetricia e Ginecologia (2), Ospedali Riuniti, Bergamo; Centro Trasfusionale, Istituti Clinici di Perfezionamento (3), Milano; Istituto di Ricerche Farmacologiche "Mario Negri" (1), Milano, Italy.

In the last few years a role of Lupus Anticoagulant (LAC) in the ætiology of repeated spontaneous abortions and intrauterine deaths has been repeatedly suggested. To quantify this association few data are available, since the published reports are generally based on uncontrolled and small clinical series. We have analyzed data from a case-control study conducted in Bergamo and Milan, two contiguous provinces in Lombardia, Italy. Cases were 63 women, mean age 30 years, range 23-40, with 2 or more "sine causa" spontaneous abortions (repeated abortions) admitted between March 1985 and December 1986 to the Ospedali Riuniti of Bergamo and Istituti Clinici di Perfezionamento of Milan. Controls were 63 women, mean age 32 years, range 20-49, with 1 or more live births and without spontaneous abortions, admitted to the same Institutions for neither gynaecological nor cardiovascular acute conditions. Informations were collected on sociodemographic factors, gynaecological and obstetrical data and related medical history. LAC was diagnosed according to the Working Party reccomandations (1983) and Systemic Lupus Erythematosus (SLE) according to the revised criteria of the American Rheumatism Association (1982). 11 out of 63 cases (17%) (95% confidence interval ranging from 9.5% to 34% based on the Poisson's approximation) were LAC positive, whereas in none of 63 controls this inhibitor was detected ($\chi^2_1$ adjusted for age = 10.1, p= 0.02). Similarly SLE was diagnosed in 4 cases (all having a Lupus Anticoagulant) and in none control ($\chi^2_1$ adjusted for age= 4.17, p=0.02). These findings confirm that LAC is associated with a positive history of repeated abortions, being present in about 10% of the cases. Conclusive estimate of relative risk is prevented by the small control group size (i.e. lack of positivity for LAC in controls), but very elevated risk (many tenfold increase) is suggested.

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THE "LUPUS ANTICOAGULANT" INDUCES FUNCTIONAL CHANGES IN ENDOTHELIAL CELLS AND PLATELETS. Anna E. Schorer and Kathleen V. Watson, Minneapolis VA Medical Center and the University of Minnesota, Minneapolis, MN U.S.A.

The presence of the "lupus anticoagulant" (LA) predicts a clinical syndrome of excessive arterial, venous and microvascular thrombosis. LA is an antibody which reacts with negatively charged phospholipid (PL) species in vitro. Since PL is involved in many aspects of the regulation of thrombosis, we postulated that LA might modify one or more of the membrane-(PL-)dependent reactions of platelets and endothelial cells (EC). Blood samples from 20 patients with a history of thrombosis were tested for the presence of LA (kaolin PTT) and titres determined. LA-positive (LA+) sera and plasma were compared to LA-negative (LA-) samples from normal donors (n=6) or patients who had lupus but no clinical thrombosis (n=4). These specimens were tested in a panel of assays. The thrombin-stimulated release of prostacyclin (PGI2) from cultured human EC was markedly reduced (52%$\pm$12.5 s.e.) by preincubation of the EC with LA+ sera (30 minutes). Purified LA+ IgG from one patient reproduced this effect. Thrombin induction of EC synthesis of the procoagulant, tissue factor--which is dissociable from prostaglandin metabolism--was also inhibited by LA+ sera. Normal platelets incubated in LA+ plasma became refractory to thrombin (1 unit/ml) but retained their responsiveness to epinephrine and ADP. The reduced responsiveness to thrombin was not due to altered (specific or total) binding of thrombin. The cleavage of Factor X by Factor VII requires PL as a co-factor for the EC procoagulant, tissue factor (TF). Unlike the inhibitory effect of LA on thrombin activation of EC and platelets, this distinct membrane-(PL-) dependent function was variably **enhanced** by LA+ sera. Brief (20 min) exposure of EC to LA+ sera increased TF co-catalysis of Factor VII cleavage of Factor X (measured by chromogenic Xa substrate, S-2222) by up to 10 fold ($p<0.05$, unpaired t test). This effect was not the result of EC disruption or changes in whole-cell TF content. These data suggest multiple, complex and heterogenous effects of LA, including impaired production of PGI2, impaired EC modulation, and heightened ability of endogenous EC tissue factor to initiate coagulation. These (and perhaps other) membrane-dependent effects may contribute to the tendency of LA+ patients to develop clots.