256

IS BETATHROMBOGLOBULIN (BTG) OF PROGNOSTIC VALUE IN PATIENTS WITH TRANSIENT CEREBRAL ISCHEMIA (TIA) ?

A. Strano (1), G. Davì (2), I. Catalano (2), G. Francavilla (2), A. Notarbartolo (2). Medical Pathology, II University of Rome (1) and Dept. of Internal Medicine, Palermo (2), ITALY.

TIA and stroke have for a long time been recognized as being associated with various abnormalities in platelet function. Plasma levels of BTG, a marker of platelet release reaction, have been found raised in TIA patients. However plasma measurements can be influenced by venipuncture or handling of the samples. Therefore we measured urinary BTG levels and compared plasma and urinary levels of this protein in 18 patients with TIA and 18 controls of equivalent age (41-68 years) and sex.

Plasma BTG levels were not different between TIA subjects (24.1 ± 8.6 ng/ml) and controls (20.2 ± 6.4 ng/ml). Urinary BTG levels were significantly different between TIA patients (0.72 ± 0.24 ng/ml) and controls (0.28 ± 0.09 ng/ml) and platelets from TIA patients were more sensitive to ADP than those from controls.

After 6 months of ticlopidine administration urinary BTG levels in TIA patients fell to within normal ranges, plasma BTG values remained in the normal range and the the AC50 for ADP was significantly increased.

We conclude that only urinary BTG levels may have diagnostic usefulness to evaluate a slight but continous platelet activation of circulating platelets, for istance, by ulcerated plaques in cranial arteries. The interpretation of elevated plasma BTG levels is hampered by the influence of the blood sampling technique which may give rise to false high levels due to platelet release during or after blood sampling. Urinary BTG levels provide a means for following the effects of therapy.

In 4 patients,in whom ischemic attacks occured during the course of treatment, urinary and plasma BTG were not higher than in subjects in whom no further events occured. Therefore our data suggest that measurements of urinary and plasma BTG values have no prognostic value in TIA patients.

257

COAGULATION AND FIBRINOLYSIS IN SICKLE CELL DISEASE.

M.D. Dautzenberg, F. Monge, A.M. Fischer, R. Girot, P. Cornu. Laboratoire d'Hématologie, Centre Hospitalo-Universitaire Necker-Enfants Malades, Paris, France.

Sickled erythocytes appear to be primarily responsible for occlusion of microvasculature in patients with homozygous sickle cell disease (SCD), but it is unknown whether the activation of the coagulation pathway is also contributory to these vaso-occlusive crisis and other complications as leg ulcers, aseptic necrosis of bone, strokes. Thus, we studied coagulation and fibrinolysis parameters in 12 patients (ages 2 to 26 years with SCD, in steady-state, far from thrombotic events which occurred in 3 of them) to determine if it would be possible to detect a high-risk group for thrombosis. We were surprised to observe that all the vitamin K dependent factors levels (II, VII+X, IX, protein C) were found next to the lowest values of the normal range. But in 3 out of 12 patients, protein C was significantly lower and 2 of them have had thrombotic events (stroke, leg ulcers). Factor V level was in the normal range except for 3 patients with low levels. As other authors, we observed normal fibrinogen, plasminogen and α 2 antiplasmin values and always very high factor VIII levels. Antithrombin III activity was normal or even high contrasting with the lower levels of the other factors synthesized in the liver. However all these abnormalities seem to balance since the thrombin generation test performed in the patients plasmas are in the normal range. As a marker of high-risk group for thrombosis, fibrin-D-Dimer levels (using a latex bead agglutination assay) were measured and found to be positive in 4 patients, 3 of them having suffered from thrombosis associated in two cases with a protein C deficiency. Thus, if the hemostatic modifications observed are involved in the mechanism of thrombosis, fibrin-D-Dimer and protein C seem to be the most significant parameters in this study.

258

ALTERATIONS OF THE HAEMOSTATIC SYSTEM IN SEVEN PATIENTS WITH HOMOCYSTINURIA.

W. Stenzinger (1), H. Ostermann (1), K. Ullrich (2) and J. van de Loo (1). Dept. of Internal Medicine (1) and Dept. of Pediatrics (2), Univ. of Muenster,FRG.

Homocystinuria is an inborn error of methionine metabolism accompanied by an increased risk of thromboembolism. Seven patients (5 female and 2 male) aged 16 to 25 years were investigated. Some showed highly pathological homocystine serum levels during the last year despite treatment. One patient's history revealed a thrombotic event. All patients were studied for changes in coagulation and the platelet and fibrinolytic systems (the latter before and after venous occlusion). Among the data obtained the following were pathological:

	Range	Mean ± SD	Control ± SD
Protein C Antigen (%)	56-66	58 ± 3	91 ± 27
Fibrinopeptide A (ng/ml)	1.4-10.5	5.8 ± 3.4	1.4 ± 0.76
Tissue-type plasminogen activator (mIU/ml)	0-1040	460 ± 340	50 ± 80

After venous occlusion normal values were obtained. These findings show a weak activation of both coagulation and the fibrinolytic system suggesting a prethrombotic state with consumption of protein C. It remains unclear whether the activation of the fibrinolytic system reflects endothelial cell damage associated with homocystinuria or a reaction to the activation of the coagulation system.

259

DETERMINATION OF SOLUBLE FIBRIN IN PLASMA WITH A CHROMOGENIC KIT METHOD UTILIZING THE HIGH AFFINITY PLASMIN SUBSTRATE S-2390.

E.Ersdal Badju, M. Andersson and S. Rosén, KabiVitrum Diagnostica, Mölndal, Sweden.

A sensitive and quantitative assay of soluble fibrin is of clinically diagnostic relevance in an early thrombotic state where there is a risk for development of DIC. Recently Wiman and Rånby (Thromb. Haemostas 55, 189-193 (1986)) published a spectrophotometric assay which met these criterions. The single-stage assay procedure is based upon activation of Glu-Plasminogen to Plasmin by t-PA in the presence of soluble fibrin and hydrolysis of the chromogenic plasmin substrate S-2390, H-D-Val-Phe-Lys-pNA, which has a high affinity for plasmin. The rate of plasmin generation is correlated to the amount of soluble fibrin monomers present in the sample.

A complete kit containing optimized, stable reagents has now been developed which allows a quantitative determination of soluble fibrin in the range 30-200 nmol/l within 30 min. at room temperature (20-25°C). The assay procedure is straightforward involving addition of 200 µl diluted plasma sample to 200 µl Glu-Plasminogen and 100 ul of a t-PA/S-2390-reagent.

The results show a high resolution of the standard curve as illustrated by a ΔA_{405} amounting to about one absorbance unit between a 200 nmol/l sample of soluble fibrin and the reagent blank, some variation, ± 0.1 absorbance unit, being caused mainly by differences in temperature. In combination with an intra-assay variation coefficient = 6.3% and 5.0% at 150 and 50 nmol/l, respectively, this will allow safe and reliable differentiation of pathological levels of soluble fibrin from levels found in healthy subjects (below 10 nmol/l). A similar precision is also obtained when the assay is performed in microplates.

In the original procedure fresh frozen human plasma was utilized as a dilution medium for soluble fibrin. Comparisons with carefully collected bovine plasma proved this source to be a convenient substitute. Furthermore, lyophilization of the bovine plasma did not produce any significant degradation of fibrinogen which otherwise might interfere in the assay. This simple kit procedure should make it a suitable tool in early determinations of soluble fibrin in a number of pathological states which may result in severe haemostatic disturbances.