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PROGNOSTIC SIGNIFICANCE OF HAEMOSTATIC VARIABLES IN ACUTE STROKE. Jessie T Douglas (1), G.D.O.Lowe (1), R. Balendra (1), C D. Forbes (1), L.J. Creighton (2), P J. Gaffney (2), D.A. Lane (3) and C. Kluft (4). University Department of Medicine, Royal Infirmary, Glasgow (1), Department of Haematology, Charing Cross Hospital, London (2), National Institute for Biological Standards and Controls, London (3) and TNO Gaubius Institute for Cardiovascular Research, Leiden, Netherlands (4).

Acute stroke is normally the result of thromboembolism. Such thromboemboli form and extend by the interaction of platelets and fibrin and elicit a fibrinolytic response. Hence laboratory indices of platelet activation, thrombin formation and plasmin formation may be related to thrombus size and progression, and hence to clinical outcome, ie disability and death. We studied 100 patients with acute paretic stroke and followed them for 1 year. Plasma levels of betathromboglobulin (BTG), fibrinogen, fibrinopeptide A (FPA), fibrin(ogen) fragment BB15-42, serum fragment E, high molecular weight cross-linked fibrin degradation products (X-L FDP) D-dimer, total and tissue plasminogen activator activity, tissue plasminogen activator inhibition and serum fibrin(ogen) degradation products (FDP) were related to death and functional recovery in the 1 year follow up period. The levels of BTG, fibrinogen, FPA, BB15-42, tissue plasminogen activator inhibition and serum fragment E were significantly higher on the first day following stroke, in patients who subsequently died within 1 year when compared to patients who survived. Lowered levels of fibrin plate lysis area as well as raised tPA activity, X-L FDP and D-dimer levels did not achieve significance in patients who subsequently died. In the patients who survived only increased BB15-42 and X-L FDP levels were predictive of functional dependence when compared to patients who became independent. Increased fibrinogen, FPA and fibrin plate lysis area, fragment E and tPA activity and reduced D-dimer did not achieve significance in patients with greater disability when compared to patients with minimal disability. We conclude that several measures of activation of haemostasis are predictive of death in the year following stroke, but only BB15-42 and X-L FDP predict disability in survivors.

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DETECTION OF FACTOR X ACTIVATION IN HUMANS. K.A. Bauer (1), B.L. Kass (1), M. Bednarek (2), M. Kloczewiak (2), J. Hawiger (2) and R.D. Rosenberg (1,3). Beth Israel Hospital (1) and New England Deaconess Hospital (2), Harvard Medical School, Boston and Department of Biology (3), Massachusetts Institute of Technology, Cambridge, MA, U.S.A.

The activation of factor X by factor VII/VIIa-tissue factor or factor IXa plays a pivotal role in the hemostatic mechanism. This reaction results in the liberation of a peptide from the zymogen for which we have developed a sensitive and specific radioimmunoassay (RIA). The native peptide was purified from activated human factor X by hydroxylapatite chromatography and reverse-phase high pressure liquid chromatography (HPLC). Gel filtration experiments demonstrated that the peptide was not physically associated with the enzyme. A 15 amino acid peptide with the COOH-terminal sequence of the activation fragment was synthesized using the solid-phase method of Merrifield. Antisera were raised in rabbits to the synthetic analogue coupled to bovine serum albumin with glutaraldehyde. The antibody population obtained was used to construct a double antibody RIA and was able to measure as little as 0.02 nM of this component. The antibody reactivity toward the factor X zymogen was negligible (less than 1/36,000 that of the activation peptide on a molar basis). However because other plasma constituents contributed to a nonspecific basal signal in the RIA, we developed an extraction procedure for the native peptide utilizing perchloric acid. Plasma peptide levels in normal individuals were ~0.1 nM, and elevations up to 0.8 nM were observed in patients with evidence of disseminated intravascular coagulation. Individuals chronically anticoagulated with coumarin derivatives had plasma levels of this peptide suppressed to ~0.02 nM. The validity of our measurements of factor X activation in vivo is supported by the fact that the immunoreactive signal migrates on reverse-phase HPLC in a manner identical to that of the native activation peptide and can be quantitatively recovered. This assay should be useful for studying the pathophysiology of thrombotic as well as bleeding disorders in humans.

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DETECTION OF ACTIVATED PLATELETS WITH MONOCLONAL ANTIBODIES
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Blood tests reflecting in-vivo activation of platelets are potentially useful in evaluating patients with thrombotic diseases. Recently monoclonal antibodies have been described that react preferentially with activated platelets. We prepared an IgG2b antibody, designated RUU-AP 2.28, that reacted with a 53.000 MW protein that is located in a special subclass of platelet granules in unstimulated platelets and that is exposed on the surface of activated platelets. Increased numbers of platelets that expressed the 2.28 antigen on their surface were observed in patients undergoing cardiopulmonary bypass and in patients with acute deep venous thrombosis. The percentage of RUU-AP 2.28 positive platelets in the circulation was 3,9 ± 2.7 (SD)% in the controls (n = 20), 24.6 ± 13.5% in patients after cardiopulmonary surgery (n = 10) and 8.5% in patients with acute deep venous thrombosis (n = 2).

In order to detect also earlier stages of platelet activation, such as secretion-independent phenomena, we produced new monoclonal antibodies by fusing spleen cells from Balb/c mice, immunized with thrombin stimulated, paraformaldehyde fixed platelets, with Ag 8653 myeloma cells. As a screening assay we used an ELISA with freshly fixed platelets or fixed thrombin-activated platelets. We detected six monoclonal antibodies (RUU-AP 1-6) specific for thrombin-activated platelets. The results of the ELISA were confirmed by flow cytofluorometry.

None of the antibodies inhibited platelet aggregation induced by ADP, collagen or ristocetin. Ascites of IgG1 antibody RUU-AP 3 reacted with normal thrombin-activated platelets but did not react with thrombin-activated platelets from a patient with Glanzmann's disease. In addition antibody RUU-AP 3 reacted with normal platelets stimulated with 1 μM of ADP. These data suggest that antibody RUU-AP 3 detects a secretion-independent conformational change in the platelet membrane glycoprotein IIb-IIIa complex.

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DETECTION OF ACTIVATED PLATELETS IN WHOLE BLOOD BY FLOW CYTOMETRY. S.J. Shattil (1), J.A. Hoxie (1), M. Cunningham (1), C.S. Abrahms (1), J. O'Brien (2) and A. Z. Budzynski (2). University of Pennsylvania (1) and Temple University (2) Schools of Medicine, Philadelphia, PA., U.S.A.

Platelets may become activated in a number of clinical disorders and participate in thrombus formation. We have developed a direct test for activated platelets in whole blood that utilizes dual-color flow cytometry and requires no washing steps. Platelets were distinguished from erythrocytes and white blood cells in the flow cytometer by labeling the platelets with biotin-AP1, an antibody specific for membrane glycoprotein Ib, and analyzing the cells for phycoerythrin-streptavidin fluorescence. Membrane surface changes resulting from platelet activation were detected with three different FITC-labeled monoclonal antibodies: 1) PAC1, an antibody specific for the fibrinogen receptor on activated platelets; 2) 9F9, which binds to the D-domain of fibrinogen and detects platelet-bound fibrinogen; and 3) S12, which binds to an alpha-granule membrane protein that associates with the platelet surface during secretion. Unstimulated platelets demonstrated no PAC1, 9F9, or S12-specific fluorescence, indicating that they did not bind these antibodies. Upon stimulation with agonists, however, the platelets demonstrated a dose-dependent increase in FITC-fluorescence. The binding of 9F9 to activated platelets required fibrinogen. Low concentrations of ADP and epinephrine, which induce fibrinogen receptors but little secretion, stimulated near-maximal PAC1 or 9F9 binding but little S12 binding. On the other hand, a concentration of phorbol myristate acetate that evokes full platelet aggregation and secretion induced maximal binding of all three antibodies. When blood samples containing activated and non-activated platelets were mixed, as few as 0.8% activated platelets could be detected by this technique. There was a direct correlation between ADP-induced FITC-PAC1 binding and binding determined in a conventional ^{125}I-PAC1 binding assay ($r = 0.99$; $p < 0.001$). These studies demonstrate that activated platelets can be reliably detected in whole blood using activation-dependent monoclonal antibodies and flow cytometry. This method may be useful to assess the degree of platelet activation and the efficacy platelet inhibitor therapy in thrombotic disorders.