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COAGULATION, FIBRINLYSIS AND KALLIKREIN ACTIVATION IN SEVERE INFECTION AND SEPSIS: RELATION TO OUTCOME. M. Blombäck (1), F. Hesselvik (2), B. Brodin (2), R. Maller (3), P. Gaffney (4). Dept of Clinical Chemistry and Blood Coagulation, Karolinska Hospital, Stockholm, Sweden (1), Depts of Anaesthesiology (2), and Infectious Diseases, University Hospital, Linköping, Sweden (3), and National Inst Biol Standards and Control, London, UK (4).

Fatal multiple organ failure following severe infection may be related to early activation of protease cascade systems. The study aimed to relate changes in the below mentioned components to shock and outcome. Of 53 patients with severe infection, 30 did not develop shock (group I); 12 survived septic shock (group II); and 11 died from organ failure after septic shock (group III). No patient had overt DIC. During the first 3 days after admission, blood was sampled daily for assay of: platelet count, fibrinogen, prothrombin complex, F XII, F VIII:C, vWF:Ag, F VII, F V, antithrombin, protein C, plasminogen, antiplasmin, plasminogen activator inhibitor (PAi), X-oligomers, D-dimers, prekallikrein, functional kallikrein inhibition (fKI), and fibronectin, by chromogenic substrate and immunochemical techniques. The Proenzyme functional index (PFI) was calculated combining the results of antithrombin, plasminogen, antiplasmin, prekallikrein and fKI (Aasen, Acta Chir Scand 1985; 522: 211).

Low ($p<.001$) initial values for F XII, prothrombin complex, F VII, antithrombin, protein C, prekallikrein, and fibronectin were seen in all groups. The shock groups (I-III) had in addition significant decreases in platelet count, antiplasmin, and plasminogen. Fibrinogen, F VIII:C, vWF:Ag, X-oligomers, and D-dimers were significantly higher than normal in all groups. Shock patients had higher X-oligomers and D-dimers, but lower fibrinogen than non-shock patients. PAi was within the normal range in survivors (I-II), but was elevated ten-fold and increased progressively over 3 days in the non-survivors. vWF:Ag showed a similar progressive increase in non-survivors; these two variables were the best early indicators of non-survival. PFI was significantly lower in shock patients (II-III), but did not discern between survivors and non-survivors during days 1-3. The results indicate a marked activation of coagulation in patients with severe infection, with more fibrin formation and fibrinolysis in the shock groups. High vWF:Ag and PAi in non-survivors may indicate more endothelial damage, and potentially harmful fibrinolysis inhibition.

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PROTEIN C IS ACTIVATED AND GIVES 110,000 MW COMPLEXES AND PROTEIN S IS CLEAVED AND DECREASED IN VIVO IN PATIENTS WITH INTRAVASCULAR COAGULATION (DIC). M.J. Heeb(1), D.F. Mosher(2), and J.H. Griffin(1), Scripps Clinic and Research Fndn, La Jolla, CA (1) and Univ. of Wisconsin, Madison, WI U.S.A. (2).

Immunoblotting studies using denaturing and nondenaturing polyacrylamide gel electrophoresis conditions were performed on 100 plasmas from 88 patients with suspected DIC, in order to determine whether the anticoagulant regulatory proteins C and S (PC and PS) are altered in vivo during DIC. 70 of these plasmas from 65 patients contained 5-35% of PC antigen in the form of activated protein C (APC) complexed with inhibitor(s). 24 normal plasmas showed no detectable APC-inhibitor complexes. The complexes in DIC plasmas had a MW of 110 K on SDS-PAGE, as did complexes formed when APC was incubated with plasma immunodepleted of PC, or when PC in normal plasma was activated with Protac C. On nondenaturing gels, the complex present in 69 of 70 patient plasmas had the same mobility as one of two major bands of complexed APC observed in Protac C-activated normal plasma. One patient plasma contained two forms of PC antigen complex. This patient had suffered a perforated uterus during an abortion. After Protac C activation of the patient plasmas, two APC complexed bands were seen. The 16 patients with >15% complexed PC antigen included 3 with severe infection, 5 with solid tumors, 3 with leukemias, 2 with vascular disease and 3 with other diagnoses. These patients had a higher mortality (69%) than the group as a whole and higher levels of fibrin degradation products. 13 of these 16 plasmas and 56 of the entire group of 100 contained a higher than normal proportion of PS in a cleaved form with an apparent molecular weight lower than intact PS on reduced SDS-PAGE. Mean levels of PS antigen determined by electroimmunoassay for 95 of the plasmas were as follows: entire group, 86% (92%); patients with infection (n=34), 76% (81%); patients with malignancy (n=37), 102% (105%); all others (n=24), 70% (74%) where numbers in parentheses exclude patients with liver disease and 100% = normal pooled plasma. These studies suggest that PS is cleaved and decreased in vivo and that PC is activated in vivo and complexed with a 50K MW inhibitor(s) during DIC.

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MOLECULAR STUDIES OF FIBRINOGEN NEW YORK I : ANALYSIS OF THE GENOMIC DISORDER FOR THE DELETION OF AMINO ACID SEQUENCE 9-72 OF THE Bβ-CHAIN. Chung Y. Liu (1), Dominic Chung, Earl Davie, & Leonard Chess (1). Columbia University, New York City, New York (1) and University of Washington, Seattle, Washington, U. S. A.

Fibrinogens New York I and Ia (NY-I and NY-Ia) have been purified from blood plasma samples of a sister and a brother in a white family with thrombotic tendency. Both are heterozygous and contain both thrombin-clottable fibrinogen with two normal Bβ-chains and thrombin-nonclottable fibrinogen with two abnormal Bβ-chains. The abnormal Bβ-chains result from deletions of amino acid residues 9-72, which are encoded exactly by exon II of the gene. To study the genomic disorder for this deletion, genomic DNAs were isolated respectively from leukocytes of NY-Ia, NY-Ib (a nonaffected brother), and four normal individuals outside the NY-I family, and analysed in Southern blotting experiments with a human genomic DNA probe containing exons I-V. Digestion of various DNAs were performed with two different restriction enzymes, and these digestions were analyzed respectively by agrose electrophoresis.

Digestion with Hind III reveals 3 cleavage sites (one site in intron A near exon II) with formation of two fragments of equal size (2 bands : 3.1 kb and 3.1 kb) in normal, NY-Ia and NY-Ib, but an extra fragment (one band = 6.0 kb) in NY-Ia. Digestion with Pvu II reveals 3 cleavage sites (one site in exon II) with formation of two fragments (2 bands : 7.5 kb and 2.9 kb) in normal, NY-Ia and NY-Ib, but an extra fragment (one band = 5.7 kb) in NY-Ia. These results show that one Hind III and one Pvu II cleavage sites which are present in the normal allele are absent in the abnormal allele of NY-Ia. Thus, these studies indicate that genomic disorder is associated with the patient (NY-Ia) with a thrombotic tendency, and further suggest that the genomic defect in the abnormal allele is near the junction of intron A and exon II. A possible mechanism for this genomic disorder is due to that an inverse double crossover have taken place in a region covering this junction, resulting in an abnormal RNA-splicing site in this junction. Thus, exon II is eliminated with intron A during RNA processing and absent in the abnormal mRNA. Accordingly, the Bβ(9-72) amino acid sequence disappears from the abnormal Bβ-chains.

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ABNORMAL FIBRINOGEN (FIBRINOGEN NAPLES) CHARACTERIZED BY DEFECTIVE INTERACTION WITH THROMBIN AND PLASMIN IN TWO YOUNG SIBLINGS WITH ARTERIAL THROMBOSIS. G. Di Minno, A.M. Cerbone, F. Cirillo, M. Colucci, N. Semeraro, G. Di Santo, P.L. Mattioli, M. Mancini and A. Quattrone. Department of Experimental Medicine, Reggio Calabria University at Catanzaro and Department of Internal Medicine and Metabolic Disease, II Medical School, Naples University, and Department of Clinical Pathology, Bari University, Italy.

Prolonged thrombin time (partially corrected by calcium chloride) and normal reptilase time were found in the plasma of two siblings with arterial thrombosis. Their purified fibrinogen showed similar abnormalities as well as impaired fibrinopeptide release in response to thrombin, delayed polymerization of pre-formed fibrin monomers and normal sialic content. Binding of radiolabelled thrombin by patient's fibrin was 30% of normal. Supernatants from patients' fibrin clots contained abnormal amounts of thrombin (not adsorbed by fibrin) and caused abnormal enhancement of platelet aggregation and ATP secretion from platelets exposed to sub-threshold concentrations of ADP or epinephrine. Hirudin suppressed the enhancing effect of the supernatant and substitution of γ-thrombin for α-thrombin led to normalization of platelet response. Studies on fibrinolysis showed that the abnormal fibrinogen from these patients as well as its naturally occurring derivative fibrin are highly resistant to lysis by plasmin. Thus our data support the concept that, in addition to the enhanced activation of platelets by residual free thrombin, thrombosis in these patients is the result of an impaired sensitivity of fibrinogen the lytic effect of plasmin.