Detection of fibrinogen-fibrin degradation products by counterelectrophoresis

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SYNOPSIS Counterelectrophoresis using a discontinuous buffer system permits detection of fibrinogen-fibrin degradation products (FDP) under a variety of clinical circumstances. The method is sensitive, reliable, and is easily performed using conventional equipment in any clinical laboratory assuming the responsibility for assaying fibrinogen-fibrin degradation products.

This report describes the use of counterelectrophoresis as a sensitive, reliable, and easy method of detecting fibrinogen-fibrin degradation products (FDP). Because the effects of these materials may be deleterious their recognition is important and, therefore, multiple techniques have been devised to delineate their presence. Certain of these procedures, such as the tanned red cell haemagglutination inhibition assay (Merskey, Lalezari, and Johnson, 1969) and the staphylococcal clumping test (Hawiger, Niewiarowski, Gurewich, and Thomas, 1970), have a high degree of sensitivity but the manner in which they are carried out, their complexity, and the reagents they require often limit their availability to highly specialized laboratories. In contrast, counterelectrophoresis, performed under optimal conditions as described, has neither of these drawbacks and maintains the desirable feature of permitting detection of FDP generated by a number of mechanisms in a variety of clinical conditions.

Methods

Patients

Tests for FDP were performed on three classes of individuals. The first included 15 actively bleeding patients. Eight of these had septicaemia and developed auxiliary evidence of disseminated intravascular coagulation. The remainder were patients with cirrhosis whose clinical symptom complex, hospital course, and associated laboratory data supported the diagnosis of primary fibrinolysis. The second group of 39 patients had a multiplicity of disorders or states each of which, inherently and potentially (Deykin, 1970), could lead to the development of FDP. In this category were seven healthy, asymptomatic females taking oral contraceptives; 10 immediately postoperative patients (surgical procedures represented were lobectomy, cholecystectomy, prostatectomy, gastrectomy, dilatation and curettage, meniscus repair, and cystoscopy); two patients with metastatic gastric adenocarcinoma; eight patients with disorders of haematoipoiesis (included were multiple myeloma, chronic lymphocytic leukaemia, sickleemia, erythremic myelosis, and polycythaemia vera); 12 patients with miscellaneous diseases (von Willebrand's disease, Felty's syndrome, acute proliferative glomerulonephritis, chronic pyelonephritis, vitamin K deficiency, rheumatoid arthritis, diabetes mellitus, viraemia, mitral valve prosthesis, and coronary artery disease). Finally, studies were also performed on 11 completely healthy normal volunteers from the hospital's professional and technical staffs. Thus, there were 65 participants in the project.

Tests for FDP

Using a discontinuous buffer system (Alter, Holland, and Purcell, 1971), counterelectrophoresis was carried out on ordinary 1 x 3 inch microscope slides precoated with 1.5% agarose (Calbiochem, Los Angeles, Calif) in distilled water and dried at 37°C. To prepare supporting templates, four slides were placed in a 15 cm diameter Petri dish which contained a solidified 3 mm agarose gel layer formed previously to provide a perfectly level surface (Brody, Mobarak, and Haidar, 1967). Seventy ml of a 1%
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agarose solution, made in a buffer containing 0.01M Tris, 0.1M NaCl, and 0.001M EDTA at pH 9.6, was then poured into the Petri dish giving a slide layer 1 mm thick. The gels should not be used for 12 hours, remain stable in the refrigerator for five days, and should be discarded after this period. On each slide, two parallel rows of six wells each (three pairs) were cut in the long axis to permit two separate assays per slide. The arrangement was such that each well was 5 mm in diameter, each of the paired antigen-antibody cups was separated from one another by 3 mm, and each pair of wells placed 5 mm distant from the following pair.

Before use in counterelectrophoresis, serum always was allowed to clot in glass at room temperature for 90 minutes; 100 units (0.1 ml) of bovine thrombin (Thrombin, Topical, Parke, Davis and Co, Detroit, Mich) was then added to each ml serum removed from the clot and incubation was carried out at 37°C for an additional 30 minutes. Undiluted antifibrinogen antibody (Hyland Laboratories, Costa Mesa, California) was placed in anodal cups and test serum, in full strength and diluted 1:2 and 1:4, filled the cathodal wells.

Counterelectrophoresis was performed with a Shandon Vokam 2541 power supply (Shandon Instrument Company, Sewickley, Pa), and its accompanying electrophoresis cell which contained 0.05M barbital buffer, pH 8.6, in its compartments. The discontinuous buffer system with agarose dissolved in Tris-EDTA and barbital buffer in the electrophoretic vessel produces precipitin lines which are well defined over the widest range of antigen-antibody concentrations. Sensitivity to high and low titre antigens, especially important in this form of assay, is also increased by making the agarose wells 5 mm in diameter and by maintaining the interwell distance at 3 mm. A constant current of 10 ma per slide was applied for 80 minutes after which the slides were removed and immediately viewed against an obliquely placed fluorescent light. Using these laboratory specifications this method can detect plasma fibrinogen up to and including a concentration of 3 µg/ml.

For comparative purposes, auxiliary tests for FDP were also done in the form of serial thrombin and thrombin clotting times (Brody, Mobarak, Lau, and Beizer, 1966; Bloom and Campbell, 1965), immunodiffusion, and the Fi test (Hyland Laboratories) as determinations with low and intermediate sensitivities (Marder, Matchett, and Sherry, 1971).

Results

The results of all the tests for FDP are summarized in the Table. Counterelectrophoresis detected degradation products in all 15 actively bleeding patients and always beyond undiluted serum (Fig. 1A). In contrast, a consistent diagnostic paradigm was not obtained with the other assays. Fibrinogen-fibrin degradation products were observed by counterelectrophoresis in 53% of patients in group 2, whereas the other techniques were entirely unable to delineate their presence. Split products were seen only transiently in the postoperative patients and disappeared within 48 hours. None of the patients in this category had any form of inappropriate bleeding, and a precipitin line formed only with full-strength serum (Fig. 1B). However, FDP were seen in a 1:4 serum dilution from the patient with acute proliferative glomerulonephritis. Had counterelectrophoresis not been performed their presence would have been overlooked. Finally, degradation products were not observed in any of the normal sera subjected to counterelectrophoresis.

Comment

Fibrinogen-fibrin degradation products have heterogeneous biochemical features and may be produced

<table>
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<th>Group</th>
<th>No.</th>
<th>Test¹</th>
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<th>STT</th>
<th>TCT</th>
<th>Fi</th>
<th>DD</th>
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<tr>
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<td>+ (1)</td>
<td>+ (2)</td>
<td>+ (4)</td>
<td>+ (2)</td>
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</table>

Table Assays for fibrinogen-fibrin degradation products

¹STT = serial thrombin time; TCT = thrombin clotting time; DD = immunodiffusion
Fig. 1A  Precipitin bands formed after counterelectrophoresis using serum from a patient in the first study group with septicaemia. Note immunoprecipitation in the 1:2 and 1:4 dilutions.

Fig. 1B  Characteristic band following counterelectrophoresis with serum from a patient after cholecystectomy.

by several mechanisms with diverse degrees of potency functioning for variable time periods. These modifiers make multiple testing necessary and also account for the discrepant results which appear among the several techniques employed. Thus, the staphylococcal clumping test reacts poorly with late stage (D,E) digests of fibrinogen and fibrin, the Fi test detects early split product fragments moderately well but is insensitive to later digests, immuno-

diffusion will discern all forms of degradation products, but only in high concentration (50 µg/ml), and the thrombin clotting times are relatively insensitive to both early and late stage breakdown products (300-1200 µg/ml). The data presented in the Table are consistent with the acknowledged limitations of these assays.

In contrast, counterelectrophoresis appears capable of detecting split products in numerous clinical situations in which the generation of anti-thrombic, inhibitory fragments must be produced, separately or in combination, by disseminated activation of the haemostatic mechanism, systemic fibrinolysis, or fibrin deposition with ultimate release of FDP. In this regard it compares quite favourably with the tanned red cell haemagglutination inhibition assay (Marder et al, 1971) but is less cumbersome to perform and does not require preparation and storage of special reagents. While there is no intention to indicate that counterelectrophoresis should supplant present techniques now employed in the study of patients with this form of clotting abnormality, its ready availability, sensitivity, and facility of performance using conventional equipment, should make it a valuable and reliable addition to any laboratory with the responsibility for detecting fibrinogen-fibrin degradation products.

References


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