Specific Identification of Fibrin Polymers, Fibrinogen Degradation Products, and Crosslinked Fibrin Degradation Products in Plasma and Serum With a New Sensitive Technique

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A new method is described for identifying low concentrations of circulating derivatives of fibrinogen and fibrin, even when present in heterogeneous mixtures. This technique is applicable to plasma and serum and uses electrophoresis in 2% agarose in the presence of sodium dodecyl sulfate (SDS) followed by immunological identification of separated derivatives, using radiolabeled antigens and autoradiography. Unique electrophoretic patterns distinguish plasmic derivatives of crosslinked fibrin from those of fibrinogen and also identify crosslinked fibrin polymers produced by the combined action of thrombin and factor XIII on fibrinogen. The assay is sensitive to a concentration of 0.1 μg/mL of fibrinogen in serum or plasma. Fibrin polymers, plasmic degradation products of fibrinogen, and plasmic degradation products of crosslinked fibrin were detected in the plasma or serum of a patient with disseminated intravascular coagulation. Plasmic derivatives of both fibrinogen and crosslinked fibrin appeared in serum in the course of fibrinolytic therapy for pulmonary embolism, whereas during acute myocardial infarction a marked increase in the proportion of fibrin polymers in plasma was found in comparison with normal controls. Thus, the procedure can distinguish between the simultaneous processes of fibrin polymer formation, fibrinogenolysis, and fibrinolysis, and is sufficiently sensitive to detect relevant quantities of derivatives in pathologic conditions.

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method to identify particular fibrin and fibrinogen derivatives that is based upon the electrophoretic separation of serum or plasma proteins in 2% agarose gels in the presence of sodium dodecyl sulfate (SDS), followed by sensitive immunologic identification of the separated derivatives, using radiolabeled antifibrinogen antisera and autoradiography. This technique permits the identification of fibrinogen, fibrin polymers, fibrinogen degradation products, and cross-linked fibrin degradation products by their characteristic migration positions. We present technical details of the procedure, as well as an assessment of its sensitivity and specificity and its application to plasma and serum samples from selected clinical cases.

MATERIALS AND METHODS

Human fibrinogen (Grade L) and streptokinase (KabiKininase) were purchased from AB Kabi (Stockholm). Purified fibronectin was a gift from Hoechst-Roussel Pharmaceuticals, Inc. (Somerville, NJ). Human thrombin was kindly supplied by Dr James Fenton (Albany, NY) and human plasmin was kindly supplied by Dr David Aaronson (Bureau of Biologics Standards, Bethesda, MD). Human factor XIII (fraction 3) was prepared by the method of Lowery et al. and assayed by the method of Lorand et al. Agarose was purchased from FMC Corp. (Rockland, Me). Soybean trypsin inhibitor, epsilon-aminocaproic acid (EACA), hirudin and bovine serum albumin were purchased from Sigma Chemical Co. (St Louis). SDS was purchased from Bio-Rad Laboratories (Richmond, Calif). Bromophenol blue and filter paper (Whatman No. 1) were purchased from Fisher Scientific Co (Fair Lawn, NJ). Beef lung heparin (Lipo-Hepin) was obtained from Riker Laboratories (Northridge, Calif). Sephadex G-25, Sepharose CL-4B, and DEAE-Sephacel were purchased from Pharmacia Fine Chemicals (Piscataway, NJ), and monospecific goat anti-human fibrinogen antisera was purchased from Cappell Laboratories (Cochraville, Pa). Aprotinin (Trasylol) was from Mobay Chemical Co. (New York). Von Willebrand protein, human crosslinked fibrin, and plasma digests of crosslinked fibrin and fibrinogen were prepared as previously described.

Blood Collection

Blood samples (10 mL) were routinely collected into citrate (0.1 mL 1.4 mol/L trisodium citrate) and EACA (0.2 mol/L final concentration) and centrifuged at 2,300 g for 15 minutes at 4 °C to prepare plasma. To prepare serum, blood was collected in glass tubes containing EACA (0.2 mol/L final concentration) and thrombin (20 U/mL final concentration), and allowed to clot at 37 °C for two hours. Patient samples were stored at −20 °C or −70 °C prior to study. For some experiments, blood samples (10 mL) were collected into disodiumethylaminoacetate (EDTA) at a final concentration of 0.3% or sodium heparin (20 U/mL final concentration) with EACA (2 mol/L final concentration) or aprotinin at a final concentration of 1,000 U/mL.

Coagulation Studies

Prothrombin time, activated thromboplastin time, and thrombin time were performed according to standard methods using a fibrometer (BBL, Division of Becton Dickinson, Cockeysville, Md). Simplastin used for the prothrombin time and platelet factor 3 reagent for the activated partial thromboplastin time were from General Diagnostics (Morris Plains, NJ). Fibrinogen was determined as thrombin clottable protein according to the method of Ratnoff and Menzies. Serum fibrinogen-fibrin degradation products (FDP) were measured using the Thrombo-Welco test (Burbroughs Wellcome Co, Research Triangle Park, NC).

Preparation of Fibrin Polymers

Fibrinogen was dissolved to a final concentration of 10 mg/mL calcium chloride added to a final concentration of 0.025 mol/L and factor XIII to a concentration of 12 units per milliliter. Thrombin was then added at a final concentration of 0.0025 units per milliliter, the solution was incubated at 37 °C for intervals of ten to 45 minutes, and the thrombin activity was inhibited by addition of hirudin to a final concentration of two units per milliliter.

Preparation of Radioiodinated Crosslinked Fibrin Digest

A stage 2 plasmic digest of crosslinked fibrin was prepared as previously described at an enzyme-substrate ratio of 7 x 10⁵ CTA units plasmin per milligram fibrin and a digestion time of three hours. The digest was labeled to an activity of 1 x 10⁶ Ci/mol by the lactoperoxidase method using Enzymobeads (Bio-Rad Laboratories, Richmond, Calif) and separated from free iodine by chromatography on Sephadex G-25.

Preparation of Antifibrinogen Labeled With Iodine 125 IgG

The IgG fraction of the goat anti-human fibrinogen antisera was purified by ammonium sulfate precipitation and DEAE Sephadex chromatography as previously described. IgG-containing fractions were pooled, and the protein was precipitated at 50% saturated ammonium sulfate, pH 7.9. The precipitate was dissolved to a final concentration of 6 mg/mL in 0.15 mol/L sodium chloride, 0.05 mol/L Tris hydrochloric acid buffer, pH 7.6, and dialyzed against this buffer. Affinity purification of the IgG was performed by passage over a column of Sepharose CL-4B, to which fibrinogen had been coupled following cyano group bromide activation. Prior to the IgG application, the column was washed with borate buffer, pH 8.5 (0.1 mol/L Boric A, 0.025 mol/L sodium borate, 1 mol/L sodium chloride, 0.1% Tween), to remove unbound material and the bound antifibrinogen IgG was eluted with 0.2 mol/L HCl, pH 2.2 (pH adjusted with 2 mol/L glycine), and radioiodinated to a specific activity of 1.1 x 10⁷ Ci/mol using the lactoperoxidase method with Enzymobeads, following which, bound and free iodine were separated by chromatography on Sephadex G-25.

Gel Electrophoresis

Plasma or serum samples were prepared for electrophoresis by diluting 1:20 in a solution of 0.01 mol/L phosphate buffer, pH 7 containing 1.7% SDS with bromophenol blue added as a marker and either incubated at 37 °C for 16 hours or at 100 °C for five minutes. Agarose was dissolved to a final concentration of 2% by boiling in 0.05 mol/L phosphate buffer, pH 7, containing 0.1% SDS. Gels (125 mm x 260 mm x 3 mm) were cast at room temperature, using a 20-slot, 10 µL slot-forming template (LKB, Stockholm), and allowed to polymerize for two hours at 25 °C. Electrophoresis was performed toward the anode at 150 V on a flat-bed electrophoresis apparatus (Pharmacia Fine Chemicals, Piscataway, NJ) with cooling to 10 °C. The running buffer, 0.1 mol/L sodium phosphate, pH 7, containing 0.1% SDS, was connected to the gel, using four layers of filter paper as electrodes. Electrophoresis proceeded until the tracking dye had migrated a distance of 8 cm (approximately 60 minutes), and the gels were then fixed in a solution of 10% acetic acid and 25% isopropyl alcohol for one hour at room temperature. Gels were washed twice in 500 mL distilled water for two hours and...
then in two changes of .15 mol/L sodium chloride and 0.1 mol/L phosphate buffer, pH 7.3. The washed gels were overlaid with 4 x 10^3 Ci 125I-labeled antifibrinogen IgG in 75 mL of 0.15 mol/L sodium chloride, 0.1 mol/L sodium phosphate buffer, pH 7.3, containing 10 mg/mL bovine serum albumin for four hours with slow rocking. Gels were then washed with three changes of 500 mL each of 0.5 mol/L sodium chloride, .1 mol/L sodium phosphate buffer, pH 7.3 for two hours with slow rocking. The washed gel was then dried on a slab gel drier (Bio-Rad Model 224) and processed for autoradiography using Kodak X-Omat (XAR-2) film (Eastman Kodak, Rochester, NY) with exposure times up to 72 hours. Gels were stained for protein using Coomassie Blue R-250.25

RESULTS

The specificity of this technique in identifying fibrinogen antigen is illustrated in Fig 1. The Coomassie-stained gel strip of the fibrinogen preparation showed a heavy band corresponding to fibrinogen and a slower migrating band in the position of a dimer, often seen in such preparations. The autoradiogram of the same gel strip was more sensitive, with greater intensity at the monomer and dimer positions, and additional bands were noted that correspond to higher polymers. The Coomassie stain of von Willebrand protein showed a series of bands of high molecular weight (mol wt) typical of this purified protein. However, the more sensitive autoradiogram of an identical sample did not label in this position, but showed bands in positions corresponding to fibrinogen and fibrin polymers, present as trace contaminants in the preparation. Bovine serum albumin showed no reactivity with the antiserum, and no reaction was seen with purified human fibronectin or plasminogen (not shown). The autoradiogram of normal plasma showed a heavy band corresponding in migration to purified fibrinogen and a very light band of slower migration corresponding to the position of a dimer, whereas no bands were seen in serum.

The electrophoretic patterns of fibrinogen, fibrin polymers, and plasmic digests of fibrinogen and cross-linked fibrin were compared in Fig 2. At the concentration used in this experiment, fibrinogen appeared as a single band. Fibrin polymers produced with low concentrations of thrombin in the presence of factor XIII show a characteristic series of bands of slower migration appropriate for dimers, trimers, tetramers, and higher polymers. In contrast, the stage 2 fibrinogen digest principally showed bands of more rapid migration than fibrinogen identified as fragments X, Y, and D.26 Fragment E, with a lower mol wt and faster migration, was not seen, probably due to elution from the gel during processing. The plasmic digest of cross-linked fibrin demonstrated an electrophoretic pattern that was distinctly different from that of polymerizing fibrin or fibrinogen degradation products. Many distinct bands were visible, with a range of migration from M, 190,000 for fragment DD to those of very high mol wt above 10^6. A repeating pattern of three bands was seen for the entire gel strip, with the central band of each triplet characteristically more prominent than the two other bands. The crosslinked fibrin derivatives were identified and labeled according to their known mol wt and comparative migrations in SDS polyacrylamide gels. Distinctions between fibrin
polymers, fibrinogen degradation products, and fibrin degradation products could be based on the presence of a single unique derivative that was absent from the other preparations or on the overall electrophoretic pattern of the derivatives of a sample. A heavy fragment D band strongly suggested fibrinogen degradation products, whereas bands of M, greater than that of fibrinogen would derive from fibrin polymers or plasmic derivatives of crosslinked fibrin. The distinction between polymerizing fibrin and fragments of crosslinked fibrin was most easily made by their characteristic overall electrophoretic patterns. The identification of fragments when present in complex mixtures is considered below (Fig 3A and B).

Serial dilutions of purified proteins were electrophoresed and processed for autoradiography to define the sensitivity of the method (Fig 4). In this series, the band corresponding to fibrin monomer could be clearly detected at a final concentration of 0.1 μg/mL, corresponding to 0.001 μg of protein applied to the gel. Fibrinogen and fibrin digests were detected to concentrations of 1.0 μg/mL (0.01 μg protein applied), reflecting the multiple derivatives present and smaller proportions of protein in each band. The intensity of bands varied with the duration of developing of the autoradiograms, with greater sensitivity at longer exposure times. To determine the recovery of crosslinked fibrin derivatives in serum, a stage 2 plasmic digest of crosslinked fibrin was radiolabeled, and 20,000 cpm/mL was added to normal citrated plasma containing 10 μg/mL of unlabeled digest. After clotting with thrombin at a concentration of 20 U/mL, the recovery of radiolabel in the serum was 91% of that initially added, with 9% in the clot.

Electrophoretic patterns of artificial mixtures of fibrinogen and fibrin derivatives, mixed in buffer or in plasma, are shown in Fig 3A and B. When added to plasma, the electrophoretic mobilities of bands in the preparations of fibrin polymers, fibrinogen digest, and fibrin digest were not altered. The pattern of fibrin polymers was easily identified, although the band corresponding to fibrinogen or fibrin monomer was increased in intensity. In plasma, the bands corresponding to fragments X, Y, and D in the stage 2 fibrinogen digest could be distinguished, but those corresponding to fragment X and fibrinogen partially overlapped. The bands of crosslinked fibrin fragments YY and DY were of similar migration to that of fibrinogen and were obscured in mixtures with plasma. However, the larger crosslinked fibrin fragments could be detected since they retained their distinctive slower migration even after addition to plasma. In other experiments (not shown), the addition of fibrin polymer, fibrinogen digest, and fibrin digest to serum caused no alteration in their electrophoretic mobilities.

Figure 3B shows the electrophoretic patterns of mixtures of equal amounts of purified fibrinogen and fibrin derivatives and demonstrates some overlapping of bands in these complex mixtures. In the mixture of fibrin and fibrinogen digests, the most prominent derivatives and most rapidly moving fragment, fragment D, can be easily distinguished. However, fibrino-
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Fig 4. Electrophoresis of serial dilutions of fibrin polymers to determine the sensitivity of the autoradiographic technique. Fibrin polymers were prepared as described in Materials and Methods and diluted in phosphate buffer to the concentrations indicated. 10-μL samples of each dilution were applied to the gel, electrophoresed toward the anode (bottom), and processed for autoradiography with a 12-hour exposure.

Fig 5. Effect of storage conditions and anticoagulant on plasma electrophoresis pattern. Normal plasma samples in different anticoagulants (see Methods) were electrophoresed immediately or stored at −20 °C or −70 °C for 60 days prior to dilution and electrophoresis. The proportion of polymers was determined after densitometry.

Plasma from eight normal individuals was collected into citrate, EDTA, or heparin and stored at −20 °C or −70 °C prior to electrophoresis to compare the effects of anticoagulants and storage on the gel pattern. As shown in Fig 5, the proportion of the dimer varied with different anticoagulants and storage conditions. Samples that were processed immediately showed similar amounts of polymer in each of the three anticoagulants. Polymerization increased with storage and was greater at −20 °C than at −70 °C. EDTA was most effective in preventing polymer formation, whereas heparin was least effective, with up to 63% polymer formation at −20 °C for 60 days.

The applicability of this technique to the study of clinically relevant pathologic conditions is illustrated by samples from a patient with consumption coagulopathy (Fig 6), a patient undergoing fibrinolytic therapy for pulmonary embolism (Fig 7) and patients with acute myocardial infarction (Fig 8). The first patient was a 77-year-old man with acute myelomonocytic leukemia complicated by consumption coagulopathy. In comparison with the purified protein standards, the electrophoretic pattern in this patient’s plasma closely resembled that of fibrin polymers produced in vitro by using purified proteins. In the serum sample, the polymers were removed and a different pattern was obtained. Bands with mobilities slower than fibrinogen had a pattern characteristic of plasminic derivatives of crosslinked fibrin; bands corresponding to plasminic derivatives of fibrinogen were not seen. The overall findings in this patient indicate significant thrombin and factor XIIIa activity that produced fibrin polymers and concomitant plasmin action on crosslinked fibrinogen fragments Y and D migrated more rapidly than fibrin monomer and were easily identifiable. Although fibrinogen fragment X and fibrin monomer partially overlapped, the bands corresponding to fibrin dimers and higher polymers were clearly evident.
fibrin, resulting in circulating crosslinked fibrin degradation products.

The patient illustrated in Fig 7 received urokinase for 12 hours for angiographically documented pulmonary embolism with sequential blood samples before, during, and after successful treatment as documented by repeat angiography. The serum sample preinfusion shows a band corresponding to fragment Y, indicating a basal level of fibrinogenolytic activity. Bands migrating more slowly than fibrinogen with a pattern typical of plasmic derivatives of crosslinked fibrin appear at two hours and are more prominent at 24 hours, consistent with the demonstrated lysis of fibrin in the embolus. Evidence of increased fibrinogenolysis is also evident in the 24-hour sample, with persistence of the fragment Y band and appearance of a band migrating as fragment D. Plasma samples taken at the same time show prominent fibrinogen–fibrin polymers in addition to evidence of fibrinogen products D and Y, although the significance of the presence of polymers is difficult to assess in stored citrated plasma (Fig 5).

Application of the method to plasma samples from two patients with acute myocardial infarction is shown in Fig 8. Both samples were obtained within four hours of the onset of ischemic pain, collected into EDTA, EACA, and Trasylol and electrophoresed within four hours of collection to minimize in vitro crosslinking and degradation. In both cases, an increase in plasma fibrin polymers was seen, accounting for 15% and 20%, respectively, of the total fibrinogen antigen as determined by densitometric analysis, in contrast to the amount of 7.7% ± 2.1% found in eight normal subjects whose samples were processed in the same way. In addition, the presence of bands corresponding to fibrinogen fragments Y and D, most prominent in patient 1, demonstrated an activated fibrinolytic system.

DISCUSSION

We have developed a method to characterize derivatives of fibrinogen and fibrin in clinical samples based on SDS agarose electrophoretic separation and specific immunological identification, a technique similar to that which has been widely applied to the study of von Willebrand protein multimers. The method is specific (Fig 1), and the sensitivity of 0.1 μg of fibrinogen antigen per milliliter is greater than that of the tanned red cell hemagglutination inhibition immunoassay,2 the most sensitive of the clinically available assays for serum fibrinogen–fibrin degradation products.

Our method offers several advantages over other techniques that have been described for the study of
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Fig 8. Electrophoresis of plasma from two patients with acute myocardial infarction. The samples from patient 1 and patient 2 were obtained three and four hours after the onset of chest pain, respectively. Blood was drawn into EDTA (0.3% final concentration), EACA (2 mol/L final concentration) and trasylol 1,000 μg/ml, centrifuged to prepare plasma, and electrophoresed within 30 minutes of collection. Autoradiogram exposure time was 12 hours.

plasma and serum fibrin and fibrinogen derivatives. Global immunological methods measure all unclottable fibrin–fibrinogen derivatives and are rapid, quantitative, and sensitive, but they do not distinguish between the derivatives of fibrinogen or fibrin, and they are not applicable to plasma because of the high concentration of crossreacting fibrinogen. Antisera specific for epitopes of crosslinked fibrin derivatives that are lacking in fibrinogen or its plasmic fragments may be used in radioimmunoassays, but factor XIII-crosslinked fibrin polymers have many structural similarities and may be expected to cross-react with the antisera, thereby complicating the analysis of samples containing both types of derivatives. The electrophoretic method that we have used resolves derivatives over a wide mol wt range, and the autoradiographic step allows for the detection of lower concentrations of fibrinogen antigen than do those electrophoretic methods that utilize protein staining techniques. Furthermore, the method described here uses direct analysis of diluted plasma or serum by electrophoresis and is consequently simpler than those techniques that require immunological or physicochemical extraction of fibrinogen from plasma or serum prior to electrophoretic analysis. Techniques are available that are specific for particular enzymatic reactions with fibrinogen, such as radioimmunoassay of fibrinopeptide A (FDP) to reflect thrombin activity or measurement of Bβ 1–42 as a marker of plasmin action on fibrinogen. However, each assay measures only a single specific derivative, so that assay of FPA gives no indication of factor XIIIa or plasmin activity, and Bβ 1–42 measurement does not reflect thrombin or factor XIIIa activity or identify plasmin degradation of crosslinked fibrin. The method described here gives a more comprehensive evaluation of fibrinogen and fibrin derivatives, identifying polymers produced by the combined action of thrombin plus factor XIII in addition to distinguishing between degradation products of fibrinogen and crosslinked fibrin in serum.

Applicability of the technique to both plasma and serum offers distinct advantages. With plasma, it allows the identification of high mol wt factor XIIIa crosslinked polymers that would be removed by clotting and would be absent from serum. Factor XIIIa has been shown to crosslink fibrinogen directly, although the reaction proceeds faster with fibrin due to self-alignment of γ chains, enabling the enzyme to interact with the paired chains as a single moiety rather than two separate moieties. Although the gel system cannot directly distinguish polymers of fibrinogen from those of fibrin, the presence of either in plasma reflects the action of both thrombin, which converts fibrinogen to fibrin and activates factor XIII, or of active factor XIII, which directly crosslinks the monomers. The high concentration of fibrinogen in plasma obscures the presence of degradation products of fibrinogen or crosslinked fibrin that have similar molecular size and electrophoretic mobility, such as fragments X, DY, or YY (Figs 2 and 3). Even so, other fragments of fibrinogen (Y and D) or crosslinked fibrin (larger than DXD) are likely to be present concurrently and permit proper conclusions regarding fibrinogenolytic or fibrinolytic processes in the patient. Furthermore, the analysis of serum samples that lack fibrinogen and polymerizing fibrin species permits the sensitive identification of all nonclottable derivatives, even those with molecular size similar to that of fibrinogen. The high recovery (>90%) of crosslinked fibrin derivatives in serum after clotting in vitro permits detection of small amounts of fibrin derivatives in plasma containing a large excess of fibrinogen.

A fundamental advantage of the electrophoretic method used here is that it separates fibrinogen and fibrin fragments of widely differing molecular weights, from approximately 100,000 for fragment D of fibrinogen to over one million for fibrin–fibrinogen polymers.
or plasmic derivatives of crosslinked fibrin. In samples with only one type of derivative, the unique pattern of the entire series identifies the process as having involved plasmin alone, thrombin plus factor XIII, or thrombin plus factor XIII plus plasmin (Fig 2). Although overlapping bands necessarily occur in mixtures of these groups of derivatives, the contributions of each can still be distinguished, whether in a plasma or buffer milieu (Fig 4). Bands resulting from fibrinogen degradation are easily distinguished from those of either polymerizing fibrin or degradation products of crosslinked fibrin, allowing identification of fibrin and fibrinogen products in the same sample. This has been an especially difficult laboratory and clinical distinction in patients with the various consumption coagulopathies, and our approach now allows for clear distinction between intravascular coagulation–fibrin lysis and primary hyperfibrinogenolytic syndromes and for determining whether thrombus dissolution has occurred during thrombolytic treatment (Fig 7). More difficult to distinguish from each other when present together are fibrin polymers and plasmic derivatives of crosslinked fibrin. For example, fibrin polymers that result from the action of thrombin and factor XIII on fibrinogen produce a series of ever-closer bands of slower mobility than fibrinogen, whereas plasmin action on crosslinked fibrin produces a unique triplet pattern of large mol wt products.26 A mixture of the two necessarily results in some overlap of bands, but with a pattern that nevertheless should permit a conclusion that both processes are occurring concurrently. In cases of ambiguous results, the use of serum as well as plasma helps to demonstrate fibrin degradation products even in low concentration (Fig 6), since fibrin–fibrinogen polymers can be selectively removed by clotting.

The technique will not distinguish plasmic derivatives of fibrinogen from those of noncrosslinked fibrin, since they have virtually the same electrophoretic mobilities,28 but the formation of noncrosslinked fibrin without the formation of crosslinked fibrin is an unlikely phenomenon except in patients with factor XIII deficiency. The system will not identify covalently bound complexes that are released from crosslinked fibrin by plasmin or noncovalent complexes of fibrinogen, fibrin monomer, or plasmic derivatives of fibrin,35,37 since these complexes would be disrupted by SDS and migrate as their component fragments in the electrophoretic system. Such complexes would be useful to identify, but the identification of their covalently bound fragments should assist in characterizing the pathophysiologic processes of fibrin formation and dissolution.

The applicability of this method to the study of patient samples is illustrated by the cases shown in Figs 6, 7, and 8. In consumption coagulopathy complicating acute leukemia (Fig 6), the predominant finding plasma was that of circulating fibrin polymers, indicating the combined action of thrombin and factor XIII in the formation of crosslinked fibrin, and the presence in serum of nonclottable derivatives of crosslinked fibrin, indicating plasmic action on the accumulated thrombus. A distinct alteration in plasma fibrinogen antigen was seen in acute myocardial infarction (Fig 8), with the presence of a marked increase in polymeric forms indicative of in vivo thrombin and factor XIII activity, findings consistent with previous reports of an increase in high mol wt fibrinogen antigen in acute myocardial infarction, shown by means of chromatographic analysis.38,39 The electrophoretic method allows greater specificity, identifying at least four polymeric forms and separating fibrinogen fragments Y and D in addition. In the patient with pulmonary embolism, crosslinked fibrin derivatives were not present in the serum prior to treatment but appeared after treatment with urokinase, concomitant with the less-pronounced increase in fibrinogen degradation products. This demonstrates the ability to distinguish between simultaneous processes of fibrinogenolysis, caused by the systemic lytic state producing fibrinogen degradation products, and lysis of crosslinked fibrin in thrombus, the desired clinical result. It further shows that the method is sufficiently sensitive to detect clinically relevant serum concentrations of crosslinked fibrin derivatives and could prove useful in following the course of lytic therapy in patients in whom repeated angiography is not feasible. These cases demonstrate the applicability of this noninvasive technique in important clinical situations and emphasize the advantages of specific identification of multiple derivatives of fibrinogen when fibrin formation and dissolution are occurring concurrently.

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Specific identification of fibrin polymers, fibrinogen degradation products, and crosslinked fibrin degradation products in plasma and serum with a new sensitive technique

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