CRITICAL REVIEW OF DIMERTEST LATEX ASSAY

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Introduction:

The ability to differentiate between fibrinogenolysis and fibrinolysis, in vivo, is crucial for clarification of the clinical approach to be taken when elevated levels of fibrinogen-fibrin degradation products (FDP) are observed in patient plasma, particularly when accompanied by bleeding or disseminated intravascular coagulation (DIC). If the unusual occurrence of primary fibrinogenolysis is suspected and the absence of intravascular thrombosis is documented, therapy with fibrinolytic inhibitors (e-aminocaproic acid, tranexamic acid) would be appropriate. However, if secondary fibrinolysis, following initial fibrin formation, is documented, therapy with heparin (with or without antifibrinolytic agents) is indicated.

Most current clinical methods which have been available thus far, while sensitive, have been unable to distinguish crosslinked fibrin derivatives from fibrinogen derivatives. In addition, because all of the commercially available assays cross-react with fibrinogen, their use with plasma has been prevented.

Principle:

About 75% of the body's fibrinogen is present in plasma with a plasma half-life of 3 days. It is a molecule composed of three pairs of disulfide-bound polypeptide chains (Aα,Bβγ) with four major regions: the central domain (E) connected to two terminal domains (D) by alpha helical coils. Each terminal domain has an α-chain polar appendage. Fibrinopeptides A and B, constituting only 2% of the total protein content, are slightly thickened parts of the aminoterminal end of the Aα and Bβ chains.

Fibrin formation from fibrinogen is a central feature of inflammation, tissue repair, and hemostasis and is mediated by thrombin.
DIC is also mediated by activated thrombin. Thrombin first cleaves fibrinopeptides A and B from fibrinogen to form the resultant fibrin monomer \((\alpha_2\beta_2\gamma_2)\). Profound changes follow the loss of electrostatic charges that normally separate fibrinogen molecules. Fibrin monomers polymerize spontaneously to form fibrin polymers. Thrombin also activates factor XIII which then crosslinks these fibrin polymers forming covalent \(\tau-\tau\) crosslinks between opposed terminal domains and an intricate network of crosslinks between alpha chains. The result is a stabilized, protected fibrin clot.

The principle effector of clot removal is the fibrinolytic system (primarily plasmin) which controls the enzymatic degradation of fibrin. The main substrates of plasmin are fibrinogen and fibrin. Both are attacked at the same cleavage points. However, degradation products of fibrin differ from those of fibrinogen by being crosslinked. Initially plasminic cleavage of fibrinogen liberates the polar appendages of the A chains producing fragment X. Next, either alpha helical coiled coil connecting central (E) and terminal domains (D) is cleaved asymmetrically to produce fragments D and Y. Fragment Y is then further degraded to fragment D and fragment E. See diagram A.

**Diagram A. PLASMIN ON FIBRINOGEN**

Plasminolytic degradation of a crosslinked fibrin clot is slower and degradation products are distinctive because of the covalent and noncovalent bonds that hold the domains together even after solubilization of the clot. The unique degradation products are the result of prior crosslinking of fibrin, rather than a unique proteolytic attack by plasmin. As with fibrinogen, initial cleavage occurs at the polar appendage of the A \(\alpha\)-chain followed by cleavage of the alpha helical coiled coil between central and terminal domains. The smallest unique degradation product of crosslinked fibrin is fragment DD which consists of two fragment D moieties joined by crosslinks between their \(\tau\) chains. The smallest complex is a combination of fragment DD with fragment E, held together by noncovalent forces. A variety of large
degradation products is released from less extensively degraded crosslinked fibrin (high molecular weight complexes) and it is likely that these larger complexes circulate in vivo. See Diagram B.

The presence of crosslinked degradation products (XDP) has been demonstrated in conditions associated with intravascular clotting by a combination of immunoprecipitation and gel electrophoresis techniques. The detection of elevated levels of XDP by enzyme immunoassay (EIA) with monoclonal antibodies has also been documented. By this method one study found that 30 healthy volunteers had normal values of XDP while elevated levels were found in 53 patients with ongoing thrombotic processes. Mean values were as follows: normals: 36 ng/ml; pulmonary embolism: 414 ng/ml; deep venous thrombosis: 420 ng/ml; thromboembolic patients: 980 ng/ml; and disseminated intravascular coagulation: 5274 ng/ml. A second study using monoclonal antibody DD-3B6/22 in an EIA also showed clear elevation of XDP in patients as compared to controls. Mean values were as follows: normal: 75 ng/ml; pulmonary embolism: 1251 ng/ml; deep venous thrombosis: 1096 ng/ml; arterial thrombosis: 2817 ng/ml; DIC: 8259 ng/ml. Although both studies show the highest titers of a D-dimer in association with DIC, too much overlap of D-dimer levels exists between these different thrombotic disorders to permit their differentiation with this test. The presence of XDP having been demonstrated in conditions associated with intravascular clotting led to the development of the Dimertest Latex Assay under consideration here.

Methodology:

The Dimertest Latex Assay (American Diagnostica) uses an immunological reaction to determine the presence of fibrin degradation products (XDP) in serum or plasma. Monoclonal antibody DD-3B6/22 was raised against a highly purified preparation of D-dimer by conventional hybridoma technology. The antibody was attached to latex beads. One drop of latex beads is placed on a slide and .01 ml of plasma or serum is added. The slide is rocked for three minutes and then checked for agglutination against a black background. Positive agglutination is obtained with a specimen containing greater than 200 ng of D-dimer per ml of specimen. Serial doubling dilutions can be used to obtain estimates of higher levels of crosslinked derivatives. The mean range of plasma and serum XDP in normal subjects is less than 200 ng/ml.

Test Performance:

In a recent study using monoclonal antibody coated latex particles for rapid detection of XDP, plasma samples from 40 normal subjects all having D-dimer levels below 250 ng/ml as measured by enzyme immunoassay were all negative by latex assay. In contrast, positive latex agglutination titers were obtained in 87 of 88 patients with demonstrated deep venous thrombosis, pulmonary embolism, or disseminated intravascular coagulation. Precision studies of 10 plasma and 10 serum specimens taken from 10 individuals (2 normal; 2 DIC; 4 PE; 2 DVT) with a range of XDP levels from less than 200 to 4000-8000 ng/ml indicated that 8 replicates of each specimen gave identical results. No false
positive reactions were found with Dimertest Latex Assay in 27 serum and plasma specimens taken from normal individuals also determined to be normal with a reference latex test for serum. Sixty-four plasma and 59 serum specimens taken from patients judged to be suffering from thrombotic disorders by both clinical evaluation and a reference latex test gave no false negatives when tested with Dimertest Latex. The test has the advantage of use with plasma which is more convenient and economical than special serum specimens.

Limitations:

As with EIA, data studying the latex assay indicate that the antibody DD-3B6/22 has a lower affinity for purified high molecular weight derivatives and a D-dimer-E than for D-dimer. In D-dimer-E, the antigenic site seems partially obscured by the noncovalent association of D-dimer with fragment E and this attenuating effect also seems to apply to larger complexes. Because crosslinked high molecular weight fibrin complexes are frequently the major circulating fibrin derivatives in DIC, the use of the D-dimer standard will underestimate the quantity of XDP in a sample.

Using enzyme immunoassay, many assays on serum were lower than those obtained using the corresponding plasma. There are two possible explanations for this difference. One is that plasma may contain nonclottable fibrin fragments which are occluded in the clot or perhaps bound to fibrin when blood is allowed to clot in vitro. A second mechanism may be that the procedure is able to detect clottable, soluble fibrin complexes which circulate in plasma. Similarly, although serum can be validly assayed by latex agglutination, its use can give false negative results in some patients. The differences are often not detectable by Dimertest Latex Assay unless there is a greater than two-fold difference between serum and plasma levels. In parallel plasma and serum specimens from 75 patients suffering from DIC, PE, or DVT, 78.6% had equivalent Dimertest Latex plasma and serum titres, 17.3% of specimens differed by one doubling dilution, and 4% differed by 2 doubling dilutions.

With some samples, the presence of rheumatoid factor may cause false positive results by reacting with heterologous antibodies and agglutinating latex particles. Therefore a test for rheumatoid factor should be run if rheumatoid arthritis is suspected. If the rheumatoid factor test is negative the XDP level can be read as correct. If both tests are positive pretreatment of the sample with dithiothreitol to reduce the IgM rheumatoid factor can be attempted. Finally since the level of sensitivity appears to be just above the normal range it is possible that slightly elevated levels may be missed although false negatives are infrequent provided plasma rather than serum is assayed.

Conclusion:

Conformational and structural changes on conversion of fibrinogen to fibrin and its crosslinking by Factor XIIIa lead to the development of new antigenic determinants that permit differentiation between their
plasminolytic cleavage products. A monoclonal antibody (DD-3B6/22) that is specific for crosslinked fibrin derivatives containing the D-dimer configuration has been used in developing the Dimertest Latex assay. Neither fibrinogen or its degradation products cross-react with this antibody. The Latex assay provides a rapid and simple means to detect elevated levels of crosslinked fibrin degradation products in patients with thrombin-induced fibrin formation. Thus, this assay permits the laboratory differentiation between primary fibrinogenolysis and disseminated intravascular coagulation. With this information available, a rational basis for decision making with regard to therapy (antifibrinolytic inhibitors v. heparin) is provided. Furthermore, the test has the advantage of being a plasma assay. Lastly, however, it should be noted that although the test is very sensitive for D-dimers, preliminary data indicate that quantitation of the D-dimer levels cannot yet be used to differentiate DIC from other thrombotic disorders; i.e., pulmonary embolism, deep venous thrombosis, or arterial thrombosis. In light of this lack of specificity, the definitive diagnosis of DIC must depend upon the use of additional laboratory tests: the platelet count and fibrinogen levels.


10. Victor Marder, Charles Francis, Russell Doolittle: Fibrinogen structure and physiology. In Robert Colman, MD; Jack Hirsch, MD; Victor Marder, MD; Edwin Salzman, MD (Eds.): Hemostasis and Thrombosis--Basic Principles and Clinical Practice. Philadelphia: J.B. Lippincott Company.