D Dimer Assay

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Introduction

A new test for determination of circulating fibrin degradation products has recently been marketed in the U.S. Known generically as the D-dimer assay, it is a direct latex agglutination assay employing monoclonal antibody specific for digestion products of crosslinked fibrin. It is offered as an alternative to or replacement for the widely available Thrombo-Wellcotest assay for fibrinogen and fibrin degradation products. Whereas the present test is used almost exclusively in the setting of disseminated intravascular coagulation (DIC), the D-dimer assay is purported to have diagnostic utility in a wider range of thrombotic disorders eg deep venous thrombosis (DVT) with or without pulmonary embolism (PE), arterial thrombosis, and DIC [1].

Background

DIC

DIC is a clinical entity with diverse presentations characterized by concomitant activation of both the coagulation and fibrinolytic systems intravascularly. It occurs as a complication of a number of serious underlying disorders such as bacterial sepsis, amniotic fluid embolus, and abruptio placentae. It is most commonly manifest as diffuse hemorrhage resulting largely from the anticoagulant properties of fibrin degradation products (FDP) and possibly from consumption of platelets and clotting factors. Thrombotic complications are uncommon. Measurement of circulating FDP has been used as an estimate of the extent of ongoing fibrinolysis and therefore, as an aid in the diagnosis of DIC.

Unfortunately, an elevated level of FDP, or any other single laboratory test, is not diagnostic of DIC. The diagnosis is based largely on clinical impression and is confirmed by a number of laboratory findings, of which, the FDP is just one. This lack of a "gold standard" creates significant difficulty in assessing any new diagnostic tool such as D-dimers. For example, when is a result considered falsely positive or negative and how are diagnostic sensitivity and specificity defined for the test? In most studies, clinical assessment is held as the gold standard. This is complicated however, by the clinical diversity of the syndrome. It occurs relatively commonly in a low grade or chronic form usually without clinical features and is manifest only as laboratory findings consistent with the syndrome [2, 3]. Less commonly, it occurs as a severe or acute form in otherwise acutely ill patients whose underlying disease is the major determinant of mortality [2, 3]. The performance of any laboratory test must be judged within the context of the clinical setting in which it is to be used.

Fibrin Formation and Degradation

Formation of fibrin from circulating-fibrinogen is initiated by the thrombin-catalyzed cleavage of fibrinopeptides A and B [4, 5] (see fig. 1). Fibrin monomers polymerize noncovalently and are then crosslinked via the alpha and gamma chains by FXIIIa [4, 5]. Complete plasmin-mediated digestion of fibrinogen in vitro yields fragments D and E in a 2:1 ratio with intermediate fragments X and Y as seen in fig. 2 [4, 6]. Factor XIIIa-mediated fibrin crosslinks are not subject to hydrolysis by plasmin and therefore, D fragments from adjacent fibrin monomers remain covalently bound after digestion resulting in DD and E as final end products [4, 6] (see fig. 3). The retention of these crosslinks creates antigenic determinants distinct from noncrosslinked species and is the basis of the new assay for crosslinked fibrin degradation products (XDP) including D-dimer.
The situation in vivo is less simple. First, very little DD or E exist as such. The largest fraction of each exist as a noncovalent complex (DD)E. In addition, as with degradation of fibrinogen, intermediate species are present. Thus, clinical specimens will contain mixtures of fibrinogen, fragments X, Y, D, and E, partially and completely crosslinked fibrin, fragments DD, (DD)E, (YD), and X-oligomers (see fig. 4) [4, 6].

Present Technology

The Thrombo-Wellcotest is also a direct latex agglutination assay, but employs polyclonal, polyspecific antisera raised against fragments D and E as its source of antibody [7]. These crossreact strongly with other FDPs and, more importantly, with intact fibrinogen [8-10], necessitating the use of serum as the sample source. The analytic sensitivity of this assay for pure DD is 5,000ng/ml [10].

Merskey in 1972 showed that the concentration of FDPs is greater in serum than in the initial specimen when either whole blood or plasma was allowed to clot at room temperature [11]. The increase was time and temperature dependent, did not occur in citrated whole blood or plasma incubated under identical conditions, and was not prevented by the addition of plasmin or trypsin inhibitors. The increase could be minimized by the addition of thrombin, but even under optimal conditions, concentrations of FDP in the range of 1000ng/ml were found in the sera of normal individuals. The Thrombo-Wellcotest is designed to give positive results at FDP concentrations of 2000 ng/ml (fibrinogen equivalents) [7]. This artifactual FDP occurring in a specimen from a patient with apparent low grade DIC contributes to the already difficult interpretation of a positive result at low titer.

More recently, Gaffney [9] measured FDP concentrations in sera generated with varying concentrations of thrombin and in the supernatant from a clotted solution of purified fibrinogen. All sera and the supernatant had FDP concentrations greater than 1,000ng/ml. He also found that in the formation of serum, concentrations of XDP fell by approximately 25-75% from the original concentration of approximately 40ng/ml (range 10-100). This latter finding was felt to be due either to 1) physical trapping of a portion of the XDP fraction in the clot, or 2) consumption of thrombin-clottable, partially crosslinked X-oligomers.

Thus, the need to generate serum in order to perform the FDP assay creates two in vitro artifacts contributing to reduced specificity for in vivo fibrinolysis.

D Dimer Assay

Advantages

The D dimer assay employs a monoclonal antibody, DD-3B6/22, specific for all gamma-gamma crosslinked species [12-15], but with highest affinity for DD [10, 14]. It does not crossreact with intact fibrinogen or non-crosslinked degradation products and thereby obviates the need to perform the test on serum samples [10, 14]. This fact alone represents an advantage over the present methodology in that the two artifacts inherent in the process of generating serum (discussed above) are eliminated. It seems reasonable to suggest that this might render a positive result at low levels of XDP more specific for increased fibrinolysis in vivo.

Other advantages are the increased analytic sensitivity and the specificity for crosslinked species. The latter ensures that the results reflect solely fibrinolysis and not fibrinogenolysis. The distinction is important in distinguishing primary fibrinogenolysis from increased fibrin/fibrinogenolytic activity secondary to increased thrombotic activity of any cause. However, the former appears to be a rare phenomenon [4, 6, 16] and will not be discussed here.

Methodology

The test is simple to perform. Antibody-coated latex beads are mixed with the specimen of serum or plasma and incubated. Results are recorded as positive or negative for agglutination. The analytic sensitivity of the system is set so as to give a positive result at concentrations over 200ng/ml of a mixture of crosslinked fibrin degradation products (D dimer equivalents) [1] or at 2,000ng/ml of a solution of pure DD [10]. Semi-quantitative estimates of XDP concentration can be obtained by serial dilution of the specimen.
Clinical Trials

Most of the data currently available on the utility of the XDP assay on clinical material have been obtained using the monoclonal antibody of the latex agglutination assay (DD-3B6/22) in an ELISA capture-tag system developed by Rylatt et al. [12]. The system employs the monospecific DD-3B6/22 as the capture antibody, and horseradish peroxidase-labeled, panspecific DD-4D2/182 as the tagging agent. Unless otherwise stated, the studies cited below employ this method.

In six studies, totaling 208 normal controls, mean normal values of XDP have varied from 13.6-75 ng/ml [13-15, 17-19]. Ranges have not been reported in some studies, but where noted have been 3-144 ng/ml [14,19]. In four studies reporting on DVT, PE, and DIC, there has been no overlap between patient (n=235) and control (n=178) groups [13-15, 17]. Means for DVT have ranged from 255-1096ng/ml [13,14,17,18]. In cases of PE, the mean concentration has varied from 414-1251 ng/ml [13,14,18]. The mean in DIC has ranged much higher, from 5274-8259 ng/ml [13,14] with individual values as high as 390,000 seen in obstetrical cases [17]. Elevated XDP has also been seen in arterial thromboembolism with mean values of 980-1918 [13,18].

Using the latex agglutination method, Elms et al [15] found negative results in 40 normal controls. Greenberg et al [10] reported no agglutination in 80 normal controls. Both Elms and Hafter [15,17] have demonstrated good correlation between ELISA and latex methods in controls and patients. Elms found found XDP levels of <250ng/ml by ELISA and no agglutination in his control group. XDP levels in 88 patients with DVT, PE, or DIC ranged from 250-5,000ng/ml. Latex agglutination was positive in 87 of these 88. Hafter found levels of XDP >200ng/ml by latex in 18 patients DVT or PE, whose ELBA levels were 259-1,250ng/ml (see figs 5 and 6). In both studies, individual results by the two methods correlated well (see figs. 5-7).

Three studies have compared FDP and XDP in the same patients [10,14,18]. Whitaker and Greenberg reported results for individuals while Hunt gave only group means. Whereas all of Whitakers patients with DVT or PE had elevated XDP, only 10 of 14 PE patients and 2 of 7 DVT patients also had elevated FDP. He does not report cutoff values for for "elevated", but assuming the use of positive at 1:20 as in DIC [2,20,21], the discrepancy can be attributed to the high range of normal or uncertain values in the FDP test. This lends support to the notion that XDP is more sensitive for increased fibrinolysis in vivo. Unfortunately, detection of an elevated XDP level is insufficient evidence for a diagnosis of either DVT or PE (or arterial thromboembolism) and the appropriate imaging studies would still be required. In DIC, where no such definitive study exists, an interpretable value for XDP (i.e. either pos. or neg. and without uncertainty as to its significance) would be more helpful, Whitaker found both FDP and XDP to be elevated in all his patients.

Greenberg compared XDP by latex with FDP in 152 patients "suspected" of having DIC by chart review. Criteria for the diagnosis were not given. Fibrin monomer by protamine sulphate precipitation was also measured. With fibrin monomer positivity as the standard (n=40), XDP had a sensitivity of 92%. Specificity was not reported and cannot be calculated from the data presented. The corresponding sensitivity of FDP was also not presented and cannot be calculated. In evaluating the entire group though, it was noted that 34 of 40 patients (85%) with FDP of <10,000ng/ml (neg. at 1:5 dilution) and 87 of 105 patients (83%) with FDP of <40,000ng/ml (neg. at 1:20 dilution), neither of which would be diagnostic of DIC, had "measurable" XDP in plasma. This again supports the contention that XDP is more sensitive for DIC than FDP.

Though Hunt reported only the mean values of XDP and FDP for his patients, other findings are of note. First, he found statistically significant elevations of XDP in several common conditions among patients without any other evidence of thromboembolic disorders or DIC. These included patients with disseminated malignancy, "severe infection or inflammation", postoperative status, and "symptomatic chronic arterial disease". Nonsignificant increases were seen in hepatic dysfunction and localized malignancy. Elevations in similar groups of patients was also noted by Greenberg (see fig. 8).If one argues that circulating crosslinked fibrin derivatives may represent markers for a prethrombotic state as Gaffney, and Graeff and Hafter have [9,22], then the finding of elevated XDP in populations with a known propensity to thrombotic disorders could be seen as useful information. But, since the data do not show the test capable of selecting those patients who will have thromboses from those who will not, the results must be taken as false positives (for the diagnosis of DIC) that severely compromise the test's specificity for fibrinolysis of clinical concern.

Also of note from the Hunt study is the observation that mean XDP levels were elevated to a greater degree than mean FDP in postoperative patients and in patients with disseminated malignancy or severe infection. After arbitrarily assigning a value of one to the XDP/FDP ratio in normals, the ratio in postoperative patients was 5.79,
5.18 in patients with infection, and 13.96 in those with disseminated malignancy. Since only means were reported, we do not know if these high ratios resulted from a few very high levels or from consistently higher levels of XDP. If it is the latter, one would have to conclude that the XDP assay is actually too sensitive to be of clinical utility as the patients listed above with the high ratios comprise a very significant proportion of all hospitalized patients. It is also possible that a few patients with very high levels raised the mean and that these patients are identified as being at risk for thromboembolic phenomena. But since this has not been shown, the significance of an elevated XDP in these conditions remains uncertain.

XDP has also been found elevated in 2 additional populations: men immediately status-post myocardial infarction (MI), and sickle cell patients in vaso-occlusive crisis [19,23]. The diagnosis of MI in Rogers’ group was based on traditional criteria and the significance of the finding was not discussed. In sickle cell patients on the other hand, a positive XDP (by latex agglutination) was relatively specific for vaso-occlusive crisis or other complications and the level seemed to correlate well with the patient’s clinical status. Thus, the XDP assay may have a role in the management of complications of sickle cell disease in patients whose clinical status is difficult to interpret.

Cost
The following prices are based on the price structures of the distributors presently available to the SFVAMC.

A) Thrombo-Wellcotest = $80 / 20 tests incl. special tubes.

B) Dimertest Latex = $65 / 30 tests or $98 / 60 tests

Summary
Regarding the D dimer assay:

1. It appears to be of no diagnostic utility in DVT, PE, MI, or arterial thromboembolism.

2. With fibrin monomer positivity as a standard, it has a sensitivity for DIC of 92%.

3. In patients suspected of having DIC clinically (though criteria were not given), but in whom the FDP is negative, it is approximately 85% sensitive.

4. It has a large number of false positives and a positive result is not specific for DIC.

5. It may have a role in the management of complications of sickle cell disease and in distinguishing DIC from primary fibrinogenolysis.

Discussion
A major question to be answered by this review is whether the D dimer assay should be adopted as a replacement for the FDP assay, used in conjunction with it, or ignored altogether for the time being. In general, there are two reasons to adopt a new laboratory test: 1) It should bring a capability the lab presently lacks; or 2) It should fill a pre-existing role at a lesser price. The first has two parts: a) It can identify a group of patients with the diagnosis of interest that present methods cannot; or b) It can confirm a diagnosis with greater confidence.

Can the D dimer assay specifically diagnose DIC better than FDP? Clearly it cannot. Does it identify a group of patients heretofore missed? It may. Unfortunately, it also identifies a population of patients who have no clinical indications of DIC and no data exist to indicate whether it does this to a greater or lesser degree than FDP. Data to indicate that this can be corrected by adjustment of the cutoff point also do not exist (manufacturers representative, personal communication). And finally, there is no indication that the additional patients identified by the new test are in need of treatment or that they will benefit from treatment (the ultimate goal of making any diagnosis). Since most of the patients detected by XDP who are FDP negative have only "measurable...D dimer...material" [10], they have probably only low grade DIC. Most of these patients have no clinical manifestations of the syndrome [2,3] and it resolves spontaneously [20,21]. Thus, it seems possible that D dimers will identify a population that neither needs nor will get treatment, yet clinicians will feel compelled to follow the course of the patients "DIC" with further XDP tests, platelet counts, coagulation panels etc. Despite the per test savings to the lab, the XDP test could result in
greater total costs. I would therefore conclude that until data are generated to show that XDP positive, FDP negative patients do in fact represent a population with treatable entities, that it not be adopted as a replacement for the current FDP assay, but that it be available for the following settings:

1. Cases of suspected primary fibrinogenolysis (in conjunction with FDP test).

2. Management of complications of sickle cell disease in cases where a clinical interpretation alone is difficult.

3. Suspected cases of significant DIC where there is a willingness to treat (ie FFP, platelets, or heparin) and the FDP is <1:20.
References