A Latex Agglutination Assay for D Dimer: Evaluation and Application to the Diagnosis of Thrombotic Disease


Although latex agglutination assays have been used for some years to diagnose thrombotic disorders, only recently has it been possible to measure specifically the products of fibrin breakdown in the presence of fibrinogen degradation products, by using monoclonal antibodies. We have evaluated a preparation of latex particles coupled to the monoclonal antibody DD-3B6/22, which is specific for cross-linked fibrin degradation products (XDP) and allows accurate discrimination between normal and pathological conditions. Of samples from 515 apparently healthy volunteers, 97.7% failed to agglutinate the latex; the normal reference interval for XDP determined by enzyme immunoassay was <78–320 µg/L. The use of different anticoagulants with or without the addition of a protease inhibitor had no significant effects on the results of the latex assay. The latex preparation provides a useful, rapid diagnostic tool for assaying small numbers of samples or as an emergency test.

Additional Keyphrases: monoclonal antibodies · enzyme immunoassay · fibrin degradation products · reference interval

Fibrin clots are formed as a response to tissue damage, by polymerization of soluble fibrin to a network of fibrin fibers (1). The network is stabilized by the action of factor XIIIa transglutaminase, which covalently links Gln 397 to Lys 405 between two D domain gamma chains of fibrinogen (2, 3). This cross-linked region, which is specific to fibrin derivatives, can be utilized to differentiate between fibrin and fibrinogen breakdown products. The smallest and best characterized of the fibrin breakdown products (XDP) is D dimer, comprising two cross-linked D domains. Gaffney proposed in 1972 that an assay specific for this region would be pathognomonic of fibrin clot breakdown (4) and would be the assay of choice for diagnosing thrombosis and monitoring thrombolytic therapy. Using monoclonal antibody technology, Rylatt et al. developed such an assay in 1983 (5). Their enzyme immunoassay (EIA) has since been used to monitor patients with ovarian carcinoma (6) as well as patients with thrombotic disorders (7).

Fibrin/fibrinogen breakdown products (FDP) have been measured traditionally by agglutination assays—initially by staphylococcal clumping (8), hemagglutination immunoassay (9) and, more recently, by the use of polystyrene latex particles to which an antiserum has been attached (10, 11). Based on the use of polyclonal antibodies, these assays, although rapid and widely used, were not specific for fibrin breakdown products but also measured fibrinogen derivatives. The use of the monoclonal antibody DD-3B6/22 to D dimer introduced the specificity required to differentiate between fibrin and fibrinogen breakdown products.

Initial studies (12, 13) to show the feasibility of this monoclonal antibody method for determination of XDP indicated that it was an improvement over the older FDP tests, being more specific for the presence of clots. This is particularly important during therapy with fibrinolytic agents, which break down both fibrin and fibrinogen.

For routine clinical application a robust, stable, and reproducible reagent is essential. To this end, a new covalently coupled latex has been produced. We report here the evaluation of this latex preparation and of its application to the diagnosis of thrombotic disorders.

Patients and Methods

Patients and Volunteers

Blood was taken from three groups of subjects for the following three studies. The data were analyzed separately.

Effect of sample preparation. To study whether serum or plasma was the more appropriate sample for the latex assay, we used blood from nine patients with coagulation-associated conditions and from 11 ostensibly healthy volunteers. Blood was transferred to tubes containing either no anticoagulant or heparin, EDTA, or citrate, in the presence or absence of aprotinin ("TrasyloL"; Bayer Australia, Botany, NSW 2019); or to FDP tubes containing soybean trypsin inhibitor and thrombin (Dade Diagnostics Inc., Aguada, PR 00602). Samples were centrifuged and the plasma or serum was separated and transported on ice. Aliquots of plasma and serum were stored as detailed below.

Normal reference interval. A series of citrated plasma samples, taken from an unselected cohort of 504 volunteers and stored at -20 °C for as long as four months, were assayed to determine the normal reference interval. Also included in the statistical analysis were the Day 0 results for citrated blood from the control group above.

Thrombotic patients. Plasma samples, taken for routine hematological analysis, were used to measure D dimer concentrations in patients with various thrombotic diseases: seven with deep venous thrombosis, 22 with pulmonary embolism, and 21 with disorders associated with disseminated intravascular coagulation.

Assays

Samples were analyzed for D dimer and larger derivatives of fibrin containing D dimer (XDP) by two-site EIA (3) ("Dimertest EIA") and by a semi-quantitative latex agglutination test ("Dimertest latex"; both from MABCO Ltd., Springwood, Queensland 4127, Australia), in which monoclonal antibody DD-3B6/22 was coated onto polystyrene beads 0.8 µm in diameter. We gently mixed 25 µL of bead suspension with 10 µL of sample for 3 min on a glass slide, then observed the agglutination pattern.

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4 Nonstandard abbreviations: XDP, cross-linked fibrin degradation products; EIA, enzyme immunoassay; FDP, fibrin/fibrinogen degradation products.
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Design of the Studies and Statistical Analyses

Sample preparation. The purpose of this study was to assess the effect of the following factors on the latex assay results:

- The use of serum vs the use of plasma with heparin, EDTA, or citrate as anticoagulant.
- The effect of adding aprotinin to plasma or serum.
- The stability of samples when stored at room temperature for 0 and 24 h; at 4 °C for three, seven, 10, and 14 days; and at −20 °C for one and 17 days.

There was insufficient blood from some subjects for analysis of all of these combinations.

Before statistical analysis, we transformed the latex assay results to a numerical scale closely approximating the log2 value of the lowest dilution that gave a positive latex result, as follows:

<table>
<thead>
<tr>
<th>Latex result</th>
<th>Transformed variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg., no dilution of sample</td>
<td>0</td>
</tr>
<tr>
<td>Pos., no dilution</td>
<td>1</td>
</tr>
<tr>
<td>Pos., twofold dilution</td>
<td>2</td>
</tr>
<tr>
<td>Pos., fourfold dilution</td>
<td>3</td>
</tr>
<tr>
<td>Pos., eightfold dilution</td>
<td>4</td>
</tr>
<tr>
<td>Pos., 16-fold dilution</td>
<td>5</td>
</tr>
</tbody>
</table>

Results from patients were analyzed by analysis of variance. Results from normal volunteers, always 0 or 1 on the transformed scale, were analyzed by analysis of deviance with a binomial model (14). Results of EIA determinations, although not analyzed in detail here, are briefly compared with results by latex agglutination, taking into account the dilution by citrate.

Normal reference interval. The upper one-tailed 95% confidence limit for EIA was used to define the reference interval and was estimated directly from the 95th percentile in the distribution of 515 readings from ostensibly normal subjects. For the latex assay, a negative agglutination was normal; we assessed the reliability of this observation by considering the proportion of normals who responded in this way.

Thrombotic patients. Graphical comparisons with normal reference intervals were used to illustrate the distinction between results from patients and those from normal volunteers and to show the close association between the EIA and latex assay systems.

Results

Comparison of sample preparations. There were no statistical differences between concentrations of XDP in plasma prepared with heparin, EDTA, or citrate as anticoagulants. Serum without thrombin gave higher results, probably because of incomplete clotting (Figure 1a).

Storage conditions did not affect patients' test results (Figure 1b), and aprotinin caused no significant effects when added to plasma or serum. Mean transformed latex data with and without aprotinin for patients' samples were 2.477 and 2.491, respectively (standard error of difference = 0.047). The proportion of negative:positive latex agglutination results in normal volunteers was not influenced by either storage conditions or aprotinin.

The distribution of latex results on Day 0 of this study is shown in Figure 2a, and the corresponding figures for EIA in Figure 2b. In both cases, only results for serum showed any overlap between patients' and normal subjects' results.

Normal reference intervals. The distribution of 515 results for normal citrated plasma is shown in Figure 3. Because 97.7% of the normal results were latex negative, a negative result is an acceptable normal reference value, having substantially more than the usual 95% confidence limits. In the EIA, 169 volunteers had circulating concentrations of XDP below the detection limit (78 μg/L); fewer than 5% of the volunteers had XDP concentrations >320 μg/L. The normal reference interval is thus <78–320 μg/L (95% confidence limit).

Thrombotic patients. All patients presenting with thrombotic disorders had measurable concentrations of XDP, 94% of which considerably exceeded the normal reference interval by EIA (Figure 4). Only one of these patients, who had been treated with heparin, had a negative result for the latex agglutination test.

Correlation between methods. A highly significant positive association between XDP measured by latex and by EIA was indicated by the use of Kruskal–Goodman statistics for the normal group and Spearman's rank correlations for the group of patients (Spearman's rank correlation of patients' data = 0.82; P <0.0001). The relationship between the logarithms of the EIA results and the transformed latex results is essentially linear (Figure 5), with the bulk of normal being at one extreme and the patients'
results being distributed over intermediate and higher ranges of both tests. There was a relatively high degree of overlap between neighboring ranges.

Discussion

These results clearly show that the latex agglutination assay for D dimer-containing XDP provides a rapid and accurate test for the presence of thrombotic disease. It requires no special equipment and is ideally suited for use in the clinicians’ office or casualty department as well as the
routine laboratory. A semiquantitative result can be obtained by serial dilution of samples. In contrast to the FDP latex assays, plasma can be used, which allows very rapid processing, there being no need to wait for the sample to clot for serum formation. Most tests for FDP require special tubes containing thrombin and trypsin inhibitor. The latex assay can be performed on routine samples taken for hematological examination and need no special treatment. The discrimination between normal subjects and thrombotic patients is less pronounced with serum unless tubes containing thrombin are used. Samples are stable in storage and can be kept at 4°C for as long as two weeks, allowing batching of samples, where required.

Samples were also suitable for assay after 24 h at room temperature and after frozen storage. There was no advantage conferred by the addition of aprotinin to the plasma or serum samples, and there were no statistical differences between any of the anticoagulants at any of the times, tested with or without aprotinin.

Increased XDP concentrations were seen in 47 of 50 patients with coagulation-associated disorders by EIA; 49 showed positive latex agglutination (Figure 5). The patient with the negative latex test, whose concentration of D dimer by EIA was 612 μg/L, had been treated with heparin. Because purified D dimer cross-reacts less in the latex test than in the two-site EIA (12), the overlapping results seen in these studies may reflect the size distribution of the cross-linked polymers circulating in patients with coagulation disorders. If so, using both of these techniques to measure XDP would provide additional information about patients with thrombotic diseases.

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References