Evaluation of Two New Assays for Determination of Serum Fibrinogen/Fibrin Degradation Products

KENNETH SUMNER, PH.D., ELLEN FELLER, B.S., DENNIS K. GALANAKIS, M.D., and MICHAEL W. MOSESSON, M.D.

Clinical Investigation Department,
General Diagnostics Division,
Warner-Lambert Company,
Morris Plains, NJ 07950
and
The Departments of Medicine and Pathology,
the State University of New York,
Downstate Medical Center,
Brooklyn, NY 11203

ABSTRACT

Two new commercial assays for the detection of degradation products of fibrinogen/fibrin (FDP) were evaluated against two standard procedures. The first, a new hemagglutination inhibition (HAI) assay using glutaraldehyde-treated cells, was compared with the tanned red cell hemagglutination inhibition immunoassay (TRCHII). Analysis of 43 samples from patients with a variety of bleeding disorders and thrombotic conditions showed a high degree of correlation between methods (r = 0.934). The second new assay, a rapid slide test using antibody-coated latex particles, was compared with results obtained by electroimmunoassay. There were no significant differences in the results as assessed by two statistical parameters. It was concluded that both new tests are useful for routine use in clinical laboratories.

Introduction

The ability to detect microgram quantities of fibrinogen/fibrin degradation products (FDP) has proven useful for the diagnosis of disseminated intravascular coagulation and in screening for thrombophlebitis and pulmonary embolism. Recently, the General Diagnostics Division* has developed reagents for two new assays for accurate and convenient measurement of FDP in serum. One assay, designed for use as a rapid screening test, employs latex particles coated with anti-fibrinogen antibodies. The other assay is a hemagglutination inhibition assay (HAI) employing glutaraldehyde-fixed sheep erythrocytes, to which fibrinogen has been covalently coupled.

In this study, the accuracy has been examined of the General Diagnostic as-
TWO NEW ASSAYS FOR FDP DETERMINATION

says on the serum of healthy individuals and on that of hospitalized patients with a wide variety of bleeding and thrombotic disorders. The latex assay was compared against the electroimmunoassay technique of Laurell. The HAI test was compared with the tanned red cell hemagglutination inhibition assay (TRCHII) of Merskey et al.

Methods

COLLECTION OF PATIENT SAMPLES

The blood from 18 healthy individuals and 43 patients was collected in a similar manner. Types of diseases and number of patients in each group were: neoplastic, 13; thrombo-occlusive, 9; hemorrhagic, 6; collagen, 5; hepatic, 4; renal, 2; pregnancy complications, 2; and miscellaneous, 2. Samples were drawn with minimum venous occlusion into citrated Vacutainer® brand tubes. The blood was centrifuged at 1,500 × g for 20 minutes and the platelet-poor supernatant plasma removed with a Pasteur pipet. To remove fibrinogen and minimize fibrinolysis, the plasma was mixed with an equal volume of a clotting solution prepared in lyophilized form. This material, when reconstituted, contained thrombin (50 NIH Units per ml), CaCl₂ (0.013 M) and epsilon-amino caproic acid (0.10 M). After mixing, the plasma was allowed to incubate for 30 minutes at room temperature to allow complete clot formation. The clot was then removed with a wooden applicator stick, and occluded liquid removed by compression against the side of the test tube. Defibrinated plasma prepared in this way was then divided into aliquots for use in each assay.

Negative and positive control materials were assayed on each testing day to assure proper functioning of the reagents. The negative control was lyophilized normal plasma. Lyophilized streptokinase-treated plasma having an FDP concentration of 60 μg per ml served as the positive control.

TESTING DIRECTIONS

Hemagglutination Inhibition Test (HAI). The reagent supplied consisted of a 2 g per 100 ml suspension of glutaraldehyde-treated, fibrinogen-coated sheep erythrocytes in saline solution and antihuman fibrinogen serum (rabbit) in a 0.25 M piperazine dihydrochloride buffer, pH 6.4. The antibody concentration was adjusted to give a sensitivity of 1 μg per ml against a standard serum to which human fibrinogen had been added. Since the glutaraldehyde-treated cells themselves are stable for 18 months, it was not necessary to calibrate them against standard fibrinogen dilutions during the study. A bottle of 0.25 M piperazine dihydrochloride buffer, pH 6.4, was supplied as was a vial of 1.25 g per 100 ml glutaraldehyde in a saline solution.

The assay was performed in U-bottom microtiter trays utilizing 0.05 ml microdilutor loops for the serial transfers.* An aliquot (0.1 ml) of the defibrinated plasma was diluted 1:1 with the piperazine buffer, followed by the addition of 0.05 ml of the glutaraldehyde solution. The remaining steps were similar to the standard TRCHII procedure and involved the transfer of sample to make the serial dilutions, the addition of 0.05 ml of antifibrinogen serum and then the addition of 0.05 ml fibrinogen sensitized cells to each well. After all reagents had been added, the tray was shaken gently, covered with plastic sealing tape and placed over a white background. The tray was left undisturbed for two hours and the results recorded after that time.

The final concentration of FDP was calculated by multiplying the last dilution factor of the sample giving positive agglutination by the sensitivity of the system (1 μg per ml). Thus, agglutination in a

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* Cooke Engineering Company, Alexandria, VA.
well having a final dilution factor of 1:20 equalled a final FDP concentration equivalent to 20 × 1 μg per ml or 20 μg per ml.

**Latex Test.** The latex reagents consisted of a 1 g per 100 ml suspension of antifibrinogen coated latex particles and 0.25 M piperazine dihydrochloride buffer, pH 6.4. The latex preparation was sensitive to 0.25 μg fibrinogen per ml. The test was performed as follows: defibrinatated plasma was diluted 1:20 or 1:80 with the piperazine buffer (yielding a final plasma dilution of 1:40 or 1:160). A 0.05 ml sample of each dilution was placed on separate ovals on black, disposable, laminated reaction slides. One drop (0.05 ml) of the latex suspension was then placed next to each of the test dilutions and mixed with an applicator stick. The slide was then rocked gently for two minutes. Positive reactions showed macroscopically visible agglutination within two minutes.

Since the test has a sensitivity of 0.25 μg fibrinogen per ml and the samples were diluted 1:40 and 1:160, the interpretation of results was straightforward. A positive result (agglutination) with both dilutions corresponded to an FDP concentration in the original sample of greater than or equal to 40 μg per ml. A positive result at a 1:40 dilution, but a negative one at 1:160 indicated that the original sample had an FDP concentration of 10 to 40 μg per ml. Failure to observe agglutination at either dilution indicated an FDP level of less than 10 μg per ml.

**Tanned Red Cell Hemagglutination Inhibition Immunoassay (TRCHII).** This procedure was performed as described by Merskey et al.6

**Electroimmunoassay.** This procedure, using rabbit anti-human fibrinogen serum, was performed as described by Laurell.4

**Results**

**HEALTHY INDIVIDUALS**

Specimens from 18 healthy men and women between the ages of 18 and 60 were tested. All specimens had a level of <5 μg per ml FDP using either the HAI or the TRCHII procedures. As assessed by latex agglutination or electroimmunoassay, these same specimens had levels of FDP < 10 μg per ml.

**Patient Specimens**

**COMPARISON OF HAI WITH THE TRCHII PROCEDURE**

The results of 43 specimens assayed with the HAI and TRCHII procedures are shown as a frequency matrix in table I. Values of 10 μg per ml of FDP or more for the HAI and 9 μg per ml or more for the TRCHII were considered to represent abnormally elevated levels. The difference in sensitivity for a particular dilution with each method is related to the sensitivity of the tanned red cells in the TRCHII procedure or the glutaraldehyde treated cells in the HAI procedure. A correlation coefficient (r) of 0.934 for these two assays was calculated from these data.
Since a level of 10 μg per ml of FDP or more is the concentration usually regarded as elevated, it is useful to compare the data with regard to this parameter. In table II, the data are arranged in a way that makes this comparison simple. Note that of the 19 elevated FDP levels determined by TRCHII, the HAI test agreed in 16 of these (84 percent). Of the 24 specimens having FDP levels of < 9 μg per ml by TRCHII, the HAI test found 21 to be < 10 μg per ml of FDP (88 percent). Statistical comparison of paired samples by the chi-square test gave a value of 0.167. This value indicated that there were not significant differences between the results obtained by these two procedures.

Finally, when the data in table II were analyzed using the binomial distribution as a measure of statistical significance, the correlation between the two procedures was not the result of a chance relationship (α = 0.5) at the p < 0.001 level.

TABLE II
Comparison of FDP* Levels in 43 Specimens Assayed by TRCHII+ and General Diagnostics HAI§ Procedures

<table>
<thead>
<tr>
<th>FDP* Determined by HAI§</th>
<th>10 μg/ml</th>
<th>&lt;10 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 9 μg/ml</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>&lt; 9 μg/ml</td>
<td>24</td>
<td>21</td>
</tr>
</tbody>
</table>

*Degradation products of fibrinogen/fibrin. fTanned red cell hemagglutination inhibition. §Hemagglutination inhibition.

Comparison of Electroimmunoassay and the TRCHII Procedure

Since two standard procedures were used in this analysis for comparison with the General Diagnostics products, the results of these two procedures were compared. This comparison is shown in table V. In 35 of the 42 specimens (83 percent) there was agreement between the methods. This agreement is highly signifi-

TABLE III
Frequency Matrix Showing Levels of FDP* Determined by General Diagnostics Latex Assay and by Electroimmunoassay Procedure

<table>
<thead>
<tr>
<th>FDP* Level Determined by General Diagnostics Latex Procedure ug/ml</th>
<th>0-9</th>
<th>10-40</th>
<th>&gt;40</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDP* Level by Electroimmunoassay ug/ml</td>
<td>11</td>
<td>8</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>0-9</td>
<td></td>
<td>13</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>&gt;40</td>
<td></td>
<td>13</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Frequency</td>
<td>13</td>
<td>22</td>
<td>6</td>
<td>41</td>
</tr>
</tbody>
</table>

*Degradation products of fibrinogen/fibrin.
TABLE IV
Comparison of General Diagnostics FDP* Latex Assay with Electroimmunoassay

<table>
<thead>
<tr>
<th>FDP* Determined by Electroimmunoassay</th>
<th>FDP* Determined by Latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 10 µg/ml</td>
<td>≥ 10 µg/ml</td>
</tr>
<tr>
<td>10 µg/ml</td>
<td>&lt; 10 µg/ml</td>
</tr>
<tr>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>&lt; 10 µg/ml</td>
<td>8</td>
</tr>
<tr>
<td>19</td>
<td>11</td>
</tr>
</tbody>
</table>

*Degradation products of fibrinogen/fibrin.

icant (p < 0.001). In two specimens, the level by TRCHII was elevated when that by electroimmunoassay was normal. In five specimens, (12 percent), the level of electroimmunoassay was elevated when that by TRCHII was normal.

Discussion

Two new products for the determination of FDP have been evaluated against two established techniques currently used in our laboratory.* The hemagglutination inhibition assay (HAI) using glutaraldehyde fixed red cells was compared against the standard tanned red cell hemagglutination inhibition immunoassay (TRCHII). The results of this study, in which two serial dilution procedures were compared, were analyzed by three statistical tests. The correlation coefficient (r) was calculated to be 0.934. Two other statistical tests (chi-square and binomial distribution) demonstrated no significant differences between results obtained by the two methods. A recent comparison of a glutaraldehyde fixed sheep cell assay with the tanned cell assay also showed a similar high degree of correlation between the two procedures.¹

Comparison of a commercially available tanned red cell kit,* with the standard TRCHII procedure has also been reported. In that study,² a correlation coefficient (r) of 0.54 was noted.

The red cell reagents tested by us have several advantages over those prepared by the classical procedure. The aldehyde treated cells for the HAI test are stable for at least eighteen months at 4°(tanned cells are stable for only one month). Thus, unlike the TRCHII procedure, a fibrinogen standard is not required to determine cell sensitivity each day. Therefore, the General Diagnostics HAI assay procedure requires a shorter period of time for completion.

In the second assay evaluated, latex particles coated with antibodies to human fibrinogen were compared with the Laurell electroimmunoassay technique.

The rapid results of the latex assay were easy to read owing to the uniformity of the latex suspension and the dark background of the disposable slides. As shown in table IV, the tests showed agreement in 76 percent of the cases. Statistical testing by χ² and binomial distribution showed no significant differences between methods. That 20 percent of the specimens had elevated FDP levels by the latex test, but not by electroimmunoassay is a phenomenon

* All FDP assays are based on the immunological reaction of the degradation products with antibodies to either fibrinogen or to the degradation products themselves. Because all four assays in the experiments of this report used antibody to human fibrinogen, comparison of these results with those obtained using a different antibody preparation should be made with caution.

TABLE V
Comparison of Electroimmunoassay and TRCHII* Procedure for Determination of FDP† in Clotted Plasma

<table>
<thead>
<tr>
<th>FDP† Determined by Electroimmunoassay</th>
<th>FDP† Determined by TRCHII* Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 10 µg/ml</td>
<td>≥ 9 µg/ml</td>
</tr>
<tr>
<td>10 µg/ml</td>
<td>&lt; 9 µg/ml</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>&lt; 10 µg/ml</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>18</td>
</tr>
</tbody>
</table>

*Tanned red cell hemagglutination inhibition immunoassay.
†Degradation products of fibrinogen/fibrin.

* Wellcome FDP Kit* (Wellcome Reagent Division, Burroughs Wellcome Company, Research Triangle Park, NC.)
seen with most latex tests.\textsuperscript{5} Certain clinical conditions such as rheumatoid arthritis, lupus erythematosus, as well as other autoimmune and hepatic disorders cause the appearance in serum of macroglobulins (anti-human IgG). Because the latex particles were coated with rabbit globulin, these macroglobulins probably cross-reacted with the rabbit IgG on the latex to give positive results.\textsuperscript{7} The fact that none of the healthy individuals showed this discrepancy between the methods lends support to the finding that this is only a problem with certain pathological specimens. Published studies comparing another commercial FDP latex test, Thrombo Wellcotest\textsuperscript{®} with the TRCHII procedure yielded similar results.\textsuperscript{3,8} It has been concluded from this evaluation that both new assays should be most useful for routine clinical assessment of FDP.

\textsuperscript{©} Wellcome Reagents Division.

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References