Determination of Fibrinogen in Plasma

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ABSTRACT

This paper compares two specific, rapid, functional methods for the measurement of fibrinogen. Both methods demonstrate a high degree of reproducibility (thrombin clotting time CV = 3.7 percent, clot density rate CV = 7.6 percent). Measurement of plasmas from 20 patients by both techniques showed excellent correlation between the two methods with a correlation coefficient of 0.93 by least squares method. The initial dilution step in the thrombin clotting time method bypasses interference by small amounts of heparin or other inhibitors in jaundiced samples which may present a problem in the measurement of the rate of density development in the clot density method.

Introduction

Accurate determination of fibrinogen by chemical techniques requires considerable time and careful technical manipulation.6,7,8,12,15,23 Fibrinogen can be separated from other plasma proteins by nonspecific protein precipitants such as ammonium sulfate and sodium sulfite,7,8,10,12 by precipitation with heat,3,12,19 by precipitation with antibody1,12 or by specific coagulation with thrombin and calcium.2,5,7,9,10,12,15,17,22,23,24

Recent reviews have indicated that fibrinogen determination by heat precipitation at 56°C is rendered inaccurate by the presence of cryoglobulins or myeloma proteins.3 The method is insensitive and lacks precision below 100 mg per dl.19

The time honored clot weight method requires two to five ml of plasma for accurate determination9 and trapping of other plasma proteins in the clot has recently been demonstrated using radioactive plasma proteins.4,16 This trapping may account for 17 to 25 percent of the clot weight.16 Chemical precipitation techniques are not specific and do not detect small changes in fibrinogen level.6,8 Immunological techniques, such as immunodiffusion,1 require excessive time. Measuring the density of the plasma clot may be affected by heparin, hemolysis and chyle.5,16,17,24 It has been reported, however, that this method is not influenced by bilirubin.24

Coagulation of fibrinogen by thrombin is the most specific method that has been accepted by many as the reference method. However, the clot must be washed and digested with alkali then reacted with biuret reagent23 or Folin ciocalteu phenol.15 While these methods
are accurate and, with care, reproducible, they require at least two ml of plasma and two hours of technician time. The thrombin clotting time method is now widely used because it is rapid, simple, sensitive and reproducible. Thus far, its only disadvantage has been low values produced by high concentrations (greater than 40 μg per ml) of high molecular weight fibrin split products. New automated clotting instruments now allow the rapid determination of fibrinogen on small samples (0.1 to 0.2 ml) of plasma after the addition of thrombin, by measurement of either the clotting time or the development of clot density.

The present paper compares the thrombin time method with a thrombokinetic method for measuring the rate of clot density development.

Methods

Whole blood was collected in 3.8 percent citrate from normal controls and from patients with a variety of illnesses. The blood was centrifuged at 3,000 g for 10 minutes within one hour of collection. The plasma was separated and frozen or was tested within two hours. For the thrombin time method, plasma was diluted 1:10 in Owren’s buffer. Exactly 0.2 ml of diluted plasma was clotted by the addition of 0.1 ml thrombin (100 U per ml), using an automatic pipet and the clotting time was determined in an electronic timer. All determinations were made in duplicate. Fibrinogen was chemically determined using the biuret method of Ware et al. Clot density thrombokinetic determinations were made using the Coagulation Profiler with scale set at X2. Precisely 0.2 ml of undiluted plasma was placed in each of two 7X 70 mm test tubes in the test well of the Coagulation Profiler, and 0.2 ml of thrombin was added by automatic pipet. The clotting time was recorded automatically on the digital timer and a curve of the rate of clot density development was produced by a recording photometer. The height of maximum amplitude was measured and recorded for both known standard plasma and for patient samples to determine the fibrinogen concentration.

Results

Normal plasma was serially diluted in Owren’s buffer and the fibrinogen concentration was determined by thrombin clotting time and by thrombokinetics (table I). Fibrinogen concentration was determined by comparison with a standard, the concentration of which had been determined by biuret measurement. The fibrinogen concentration shows a linear relationship with dilution in both methods. The thrombin time method is ten times more sensitive than the thrombokinetic method.

To determine the relative reproducibility of the methods, different plasma samples were measured six times by each method (table II). The mean and standard

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Fibrinogen by Thrombin Time mg per dl</th>
<th>Fibrinogen by Thrombokinetics mg per dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>---</td>
<td>230</td>
</tr>
<tr>
<td>1/2</td>
<td>---</td>
<td>120</td>
</tr>
<tr>
<td>1/4</td>
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<td>60</td>
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<td>1/8</td>
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</tr>
<tr>
<td>1/16</td>
<td>12</td>
<td>---</td>
</tr>
<tr>
<td>1/32</td>
<td>5</td>
<td>---</td>
</tr>
</tbody>
</table>

* Owren’s buffer—(pH 7.35) 11.75 g of sodium diethylbarbiturate and 14.67 g of sodium chloride dissolved in 1,570 ml distilled water and 430 ml 0.1 N HCl.
† Thrombin topical—Parke, Davis and Co., Detroit, MI.
‡ Fibrometer BBL—Baltimore Biological Laboratories, Baltimore, MD.
deviation for each method was determined. The coefficient of variation is smallest for the thrombin clotting time and largest for the biuret measurement with the thrombokinetic method falling between.

Twenty plasma samples from patients were obtained for fibrinogen determination by each micro method. The correlation between the thrombin time measurement and the thrombokinetic measurement was determined by least squares method and was 0.93 (figure 1). The values of fibrinogen in these samples varied from 150 mg per dl to 960 mg per dl. The two methods show excellent agreement in 18 samples. Two plasmas were icteric and while normal fibrinogen levels were obtained by the thrombin time method, no clotting curve was obtained by the thrombokinetic method. It must be presumed that an inhibitor was present in

neat plasma which could be diluted out in the thrombin time method. Unfortunately, there was insufficient plasma to determine the nature of the inhibitor. Studies in the presence of heparin showed no interference with the thrombin time method (table III).

**TABLE II**

Comparison of Precision of Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean ± SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin Time</td>
<td>296 ± 11</td>
<td>3.7%</td>
</tr>
<tr>
<td>Bio Data Profiler</td>
<td>250 ± 20</td>
<td>7.6%</td>
</tr>
<tr>
<td>Biuret</td>
<td>225 ± 30</td>
<td>13.0%</td>
</tr>
</tbody>
</table>

**TABLE III**

Effect of Heparin on Fibrinogen Assay*

<table>
<thead>
<tr>
<th>Before Heparin</th>
<th>100 mg IV</th>
<th>After Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin time</td>
<td>11.2 s</td>
<td>10.7 s</td>
</tr>
<tr>
<td>Biuret fibrinogen</td>
<td>296 mg/dl</td>
<td>298 mg/dl</td>
</tr>
<tr>
<td>Partial Thromboplastin time</td>
<td>30 s</td>
<td>&gt;300 s</td>
</tr>
</tbody>
</table>

*A normal volunteer received 100 mg heparin IV. Blood samples were obtained in 3.8 percent sodium citrate just prior to and eight minutes after the injection for the measurements listed.

**Discussion**

In 1957, Clauss developed a micro method for manually determining the fibrinogen of plasma using the platinum wire. Morse and others subsequently automated the technique utilizing the principle of substrate limited end point. The fibrinogen is diluted to the point where, in the presence of excess thrombin, the clotting time is inversely proportional to the fibrinogen concentration. The method has been compared with chemical determination and with clot density methods and shows good correlation with these methods.

The thrombin time appears more sensitive and more precise than the other methods. Low concentrations of heparin and other inhibitory products are diluted during the initial steps and do not interfere. The method is simple enough to be performed by technically trained personnel at any time. Reagents are readily available and easily stored. Abnormal fibrinogen values should be confirmed using a chemical, immunologic technique or clot weight method to detect the rare
One theoretical advantage of the thrombokinetic or clot density method is the potential for detecting abnormal polymerization in the increasing clot density by the recording photometer. The thrombokinetic method provides this information in seconds$^{13,20}$ while those instruments measuring the creasing clot density by the recording polymerization in the tracing of the increasing clot density take 12 to 20 minutes.$^{5,6,17,22,24}$ Clot density measurement, whether end point determination or thrombokinetic (initial rate of polymerization), appears to be less sensitive and less precise than the thrombin clotting time of diluted plasma. In addition, other factors present in undiluted plasma may interfere.

Summary

In summary, this paper has compared two specific functional assays of fibrinogen and demonstrates good reproducibility and excellent correlation between them. The initial dilution step in the thrombin clotting method bypasses interference by small amounts of heparin or other substances which may present a problem in the measurement of the rate of density development in the thrombokinetic method.

References