Clotting, Activated Partial Thromboplastin and Coagulation Times in Monitoring Heparin Therapy

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ABSTRACT

The automated-activated coagulation time, manual-activated coagulation time and the activated partial thromboplastin time were compared to the whole blood clotting time in the measurement of hypocoagulation of heparinized blood. The normal ranges and degree of reproducibility were determined for each clotting assay. Each method was examined for its sensitivity to various concentrations of heparin. In addition, blood samples from patients treated with heparin were assayed by all four methods and their results were compared. The results indicated that the manual-activated clotting time correlated best with the whole blood clotting time, was sensitive to low concentrations of heparin, formed a discernible clot within a convenient time period in blood containing high concentrations of heparin, was reproducible and was easily performed.

Introduction

Heparin is an acid mucopolysaccharide composed of uronic acid and glucosamine subunits. Because of its powerful anticoagulant effects, it has been a useful agent in the treatment and prevention of thromboembolic disorders.

Its action is to catalyze the neutralizing effects of antithrombin III on thrombin, plasmin and factors Xa, IXa, and XIa. In high concentrations, heparin also retards platelet aggregation.

Since heparin's activity inhibits multiple clotting factors, several coagulation assays are prolonged. As a result, many different measurements have been used to monitor patients treated with heparin.

The whole blood clotting time (WBCT) has been the standard assay for monitoring heparin dosage for many years. Unfortunately, the WBCT lacks precision. Moreover, it requires 30 to 45 minutes for completion at therapeutic levels of heparin.

The activated partial thromboplastin time (APTT) is more reproducible and has a more rapid end point than the whole blood clotting time. The APTT is performed from blood anticoagulated with sodium citrate. As a result, it can be performed at a convenient time in a central laboratory. However, the various com-
mercial partial thromboplastin reagents differ in their sensitivity to heparin.\textsuperscript{4,14} In addition, the APTT can be infinite at high concentrations of heparin.

Several investigators have advocated the activated coagulation time (ACT) for use in monitoring patients treated with heparin.\textsuperscript{2,3,11,12} The ACT appears to be a sensitive indicator of the degree of hypocoagulation of blood from patients treated with heparin. This assay gives more reproducible results and offers a shorter clotting end point than the WBCT. In addition, the ACT has been automated.\textsuperscript{13,17} The Hemochron* is a portable incubator and clot sensing device. The detection of a clot occurs by the adhesion of a fibrin mass onto a magnet causing it to rotate with the tube of blood. The displacement of the magnet triggers an electric timer and the clotting interval is digitally displayed on the front of the instrument.

For over ten years, the WBCT has been the standard assay for monitoring patients treated with heparin in our institution. Since the APTT, Hemochron-ACT (H-ACT), and manual-ACT (M-ACT) offer some theoretical advantages over the WBCT, these three assays have been compared with the WBCT in a variety of studies. The purpose of this report is to describe our experience with these assays in the monitoring of heparin levels.

Materials and Methods

**Specimen**

Using plastic syringes and 20 or 21 gauge needles, whole blood was obtained by atraumatic venipuncture. The first two to three ml of blood were discarded since they could be contaminated by “thromboplastin-like” substances. The specimens were obtained either in the laboratory or on the hospital wards. The WBCT, H-ACT, and M-ACT were performed immediately. The APTT was performed within one hour following the withdrawal of blood.

**Whole Blood Clotting Time (WBCT)**

The WBCT is performed according to a standardized method.\textsuperscript{8} One milliliter of whole blood is added to each of three 13 x 100 mm glass test tubes at 37°C. After five minutes and every 30 seconds thereafter, tube 1 is tilted to check for complete clot formation. After the blood in the first tube has clotted, the process is repeated for tube 2 and then tube 3. The WBCT represents the elapsed time from the appearance of blood in the syringe until clotting occurs in the third tube.

**Activated Partial Thromboplastin Time (APTT)**

Whole blood (4.5 ml) was added to a Monoject tube containing 0.5 ml of 3.8 percent sodium citrate† and centrifuged for 15 minutes at 1200 x g. Duplicate APTT’s were performed on supernatant plasmas using an automated photoelectric clot sensing device,§ 0.02M CaCl\textsubscript{2} and improved ACTIN.\textsuperscript{7}

**Hemochron-Activated Clotting Time (H-ACT)**

H-ACT’s were performed in duplicate according to manufacturer’s instructions using a Hemochron Model 800 dual well coagulation timer and Hemochron evacuated CGA test tubes containing diatomaceous earth, glass particles, a magnet and plastic paddle wheel for clot detection.\textsuperscript{11}

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* International Technidyne Corp., Edison, NJ 08817.

† HB 1881-040276, Sherwood Medical Industries, St. Louis, MO 63103.
‡ Sherwood Coagulyzer, Sherwood Medical Industries, St. Louis, MO 63103.
§ Dade Diagnostic, Inc., Miami, FL 33152.
\textsuperscript{1} International Technidyne Corp., Edison, NJ 08817.
MANUAL ACTIVATED CLOTTING TIME (M-ACT)

M-ACT's were performed in duplicate according to Hattersley¹¹ as modified by the instructions provided with the Sybron ACT-Stat Model 125925 series 259¹² using Vacutainer tubes containing 12 mg of siliceous earth.** The ACT-Stat is a portable battery operated incubator which maintains the sample at 37.0° ± 0.5°C. In addition, it contains a manually operated timer for the determination of the clotting time performed by the technologist.

DETERMINATION OF NORMAL COAGULATION TIMES

The normal range of the WBCT, the APTT, H-ACT, and M-ACT was determined by performing these assays on at least 41 healthy laboratory personnel of both sexes between the ages of 20 and 50 years.

EVALUATION OF PRECISION

The precisions of the APTT, H-ACT and M-ACT were determined from the results of duplicate assays of normal individuals using the formula:⁶

Coefficient of variation (C. V.) =

\[ \sqrt{\frac{\Sigma d^2}{2n}} \times \frac{1}{\bar{y}} \times 100 \]

where \( d \) = difference between duplicates, \( n \) = number of pairs and \( \bar{y} \) = mean clotting time.

EVALUATION OF THE SENSITIVITY OF CLOTTING ASSAY TO LEVELS OF HEPARIN

Heparin†† was added to 10 normal blood samples to obtain final concentrations of 0.2 u per ml, 0.4 u per ml and 0.6 u per ml. The WBCT and the H-ACT were performed on each sample. In a second study, identical concentrations of heparin were added to blood samples from ten different healthy individuals. The WBCT, APTT and the M-ACT were performed on each of the second samples.

EVALUATION OF CLOTTING ASSAYS IN HEPARINIZED PATIENTS

The WBCT and the H-ACT were performed on 109 identical samples from patients treated with heparin. In a second study, the WBCT and the M-ACT were performed on 79 identical samples from heparinized patients. In 75 of these samples, the APTT was also performed.

STATISTICAL ANALYSIS

Ratios of the experimental clotting time at various heparin concentrations to control clotting time were calculated. The ratios were compared using an analysis of variance, randomized complete-block design.¹⁸

A linear regression analysis was performed to compare the WBCT with the H-ACT and the WBCT with the M-ACT in samples from the two sets of heparinized patients.¹⁸

RESULTS

DETERMINATION OF NORMAL VALUES

The mean values, standard deviation, range and coefficient of variation for the normal population for each assay are summarized in table I.

EVALUATION OF PRECISION

The precision of the duplicate samples is described by the coefficient of variation (C. V.). The C. V. for the H-ACT is 4.1 percent, M-ACT is 4.1 percent and APTT is 1.5 percent.

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¹ Thermolyne Corp., Dubuque, IA 52001.
² No. 3865, lot #8H032, Becton Dickinson and Co., Rutherford, NJ 07070.
† Sodium injection, USP, 1000 units (u) per ml beef lung, Upjohn Co., Kalamazoo, MI.
TABLE I
Normal Values for the WBCT, H-ACT, M-ACT and APTT

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample Number</th>
<th>Sample Mean</th>
<th>Standard Deviation</th>
<th>Normal Range + 2 SD</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood clotting time</td>
<td>41</td>
<td>12.1 min.</td>
<td>1.2</td>
<td>9.7 - 14.6 min.</td>
<td>10.0 %</td>
</tr>
<tr>
<td>H-activated* clotting time</td>
<td>41</td>
<td>129.0 sec.</td>
<td>9.5</td>
<td>110.0 - 147.9 sec.</td>
<td>7.4 %</td>
</tr>
<tr>
<td>M-activated† clotting time</td>
<td>50</td>
<td>90.9 sec.</td>
<td>6.9</td>
<td>77.1 - 104.7 sec.</td>
<td>7.6 %</td>
</tr>
<tr>
<td>Activated partial thromboplastin time</td>
<td>44</td>
<td>22.5 sec.</td>
<td>2.5</td>
<td>17.7 - 27.3 sec.</td>
<td>11.1 %</td>
</tr>
</tbody>
</table>

*H = Hemochron
†M = Manual

EVALUATION OF THE SENSITIVITY OF COAGULATION ASSAYS TO HEPARIN

Ratios of experimental to control clotting times at each concentration of heparin were calculated for the WBCT, H-ACT and M-ACT (table II). It was impossible to determine the ratios using the APTT. At 0.4 units per ml of heparin, the APTT was greater than the 90 second upper time limit of our automated clot sensing device in seven of ten samples. At 0.6 units per ml, a discernible clot did not form in any sample.

The analysis of variance disclosed a significant (p < 0.005) variation in the ratios of clotting times among the concentrations of heparin for the WBCT, H-ACT and M-ACT. Since the heparin treatments were equally spaced, orthogonal polynomial comparisons could be made. This analysis indicated that the data fit a linear (p < 0.005) better than a second-degree response.

EVALUATION OF CLOTTING ASSAYS IN HEPARINIZED PATIENTS

The results of a second study comparing the WBCT and the M-ACT in 79 identical patient samples are given in figure 2. The correlation coefficient for this relationship was r = 0.78. There was one sample with a WBCT greater than its normal range and a M-ACT within the normal limits. In 12 samples, the M-ACT was longer than the normal range and the WBCT was within the normal limits. In 75 of these samples the APTT was also performed. In figure 3 are displayed the results of 59 samples comparing the APTT with the WBCT in patients treated with heparin. In 16 samples, the APTT results were greater than 90 seconds and are not recorded. The WBCT's in these 16 samples were longer than the normal range. In ten additional samples, the APTT was outside the normal range with the WBCT within the normal limits. In contrast, there was only one sample in which the APTT was within normal limits and the WBCT was abnormally prolonged.

TABLE II
Mean Ratios of Experimental to Control Clotting Times at Various Concentrations of Heparin

<table>
<thead>
<tr>
<th>Assay</th>
<th>Units of Heparin</th>
<th>Probability of Larger F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>WBCT</td>
<td>2.8450.09</td>
<td>2.2750.15</td>
</tr>
<tr>
<td>H-ACT</td>
<td>1.1950.02</td>
<td>1.3480.02</td>
</tr>
<tr>
<td>M-ACT</td>
<td>1.4000.06</td>
<td>1.7880.05</td>
</tr>
</tbody>
</table>

*Hemochron-activated clotting time
†Manual-activated clotting time
The normal range of these measurements is similar to previous reports.\textsuperscript{7,11} However, the coefficient of variation of the normal population for the WBCT was considerably smaller than in other studies.\textsuperscript{1,8}

The reproducibility of results between normal duplicate samples was excellent for the APTT and acceptable for the H-ACT and M-ACT. Similar results were obtained by Allison,\textsuperscript{1} Bull\textsuperscript{3} and Hattersley.\textsuperscript{11,12}

The WBCT, H-ACT and the M-ACT were sensitive to the various concentrations of heparin. A significant variation in the ratios of the experimental to control clotting times was observed among the three concentrations of heparin for each of the assays tested. In addition, the orthogonal comparisons indicated that the ratios of the clotting times and the concentrations of heparin fitted best a linear response.

The APTT was very sensitive to low concentrations of heparin. However, at 0.4 u per ml or 0.6 u per ml of heparin, the APTT usually exceeds the 90-second upper time limit of our automated clot sensing device. It is possible that with newer models of this instrument or with other manufacturers' machines having a longer automatic time interval, end points would be detected at the higher heparin concentrations.

When the APTT, H-ACT and M-ACT were compared to the WBCT on specimens from heparinized patients, the best correlation was observed with the M-ACT. Discordant values were observed in 13 of 79 samples. In twelve samples, the M-ACT was abnormally long and the WBCT was within normal limits, while in one case the M-ACT was within normal limits but the WBCT was prolonged. These data suggest that the M-ACT is more sensitive than the WBCT to concentrations of heparin.

In 32 of 109 samples, the H-ACT was discrepant with the WBCT. In 28 samples the H-ACT was within normal limits while the WBCT was longer than the normal range. In four samples the reverse situation existed. In these studies the H-ACT appeared less sensitive than the WBCT to concentrations of heparin.
MONITORING OF HEPARIN THERAPY

There were discrepant results in 27 of 75 samples from heparinized patients when the APTT was compared to the WBCT. In ten samples, the APTT was longer than the normal range while the WBCT was within normal limits. In 16 samples, the APTT exceeded the upper time limit of the machine. In only one case was the APTT value within normal limits, while the WBCT results were outside the normal range. These data suggest that the APTT is very sensitive to blood levels of heparin.

Although the WBCT has been a good assay for the monitoring of heparinized patients, it requires too much time to go to completion in heparinized patients. Our data suggest that the APTT is too sensitive to therapeutic concentrations of heparin to make it a useful assay in monitoring patients on moderate to large dose heparin (20,000 to 100,000 units per 24 hours), at least with our automated clot sensing device.

In contrast, the H-ACT may be too insensitive to therapeutic concentrations of heparin to endorse its use as a method to monitor hypocoagulation in patients treated with heparin. The major disadvantages to this method, however, were the numerous mechanical problems experienced with the employment of two of these machines.

The best results were repeatedly obtained with the M-ACT. This method detected both low and high concentrations of heparin. There was a linear relationship between the ratio of experimental and control clotting times and the concentration of heparin in the blood. The precision of the technique was within an acceptable range. It did not require a long time for completion even at 0.6 units of heparin per ml of blood. Finally, our technologists have found the M-ACT an easy assay to perform.

References

7. Davey, F. R. and Oates, R. P.: Evaluation of an automated method for the determination of the...


