Heparin Monitoring During Cardiopulmonary Bypass*

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ABSTRACT

Three procedures have been compared for monitoring heparin in patients undergoing cardiopulmonary bypass: (1) activated clotting time (ACT) (2) protamine titration, and (3) fluorometric substrate assay. The ACT monitors the degree of anticoagulation. It is easy to perform and is relatively inexpensive; however, it does not correlate well with heparin levels and may not accurately predict the protamine dose for neutralization of heparin at the completion of bypass. A protamine titration assay or an assay using a thrombin-sensitive fluorometric substrate measures the heparin level and calculates the protamine requirement at the completion of surgery; however, these assays do not indicate the degree of anticoagulation. The fluorometric assay is the less expensive of the two assays measuring heparin, but it requires an experienced technologist to perform the test.

Introduction

Large doses of heparin are administered to patients during cardiopulmonary bypass (CPB) to prevent blood from clotting in the patient and in the pump oxygenator. At the completion of CPB, the heparin is neutralized with protamine sulfate to prevent excessive blood loss from the operative site. Since the decision to adjust the heparin dose or to go ahead with its neutralization is made in the operating room, the laboratory tests for monitoring heparin levels are limited. The routine coagulation tests, such as activated partial thromboplastin time and thrombin time, are of limited value as the levels of heparin achieved during the CPB exceed the sensitivity of these tests. Other tests such as whole blood clotting time, which can be done at the bedside, lack precision and accuracy.

Several investigators have recommended the use of the ACT for determining the heparin dose and for calculating the amount of protamine sulfate necessary for heparin neutralization at

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the completion of CPB.\textsuperscript{1,9} They found ACT most satisfactory because of its rapidity, accuracy, and reliability. The assay can be performed at the bedside and has a better precision than the whole-blood clotting time test. The ACT, however, has been criticized for lack of correlation with heparin levels.\textsuperscript{4,7}

A few medical centers have used a semiautomated system\textsuperscript{*} to determine the heparin level by protamine titration.\textsuperscript{4,5,12} Fluorometric and chromogenic substrates are now available which claim to give accurate and precise plasma heparin levels.\textsuperscript{3,15} Plasma methods have not gained popularity for patients requiring CPB because centrifugation of the blood increases the turnaround time. In our laboratory a modified fluorometric procedure using whole blood instead of plasma has been successfully used to determine heparin levels. In this study, the ACT, the protamine titration system and the fluorometric procedure have been compared to determine the usefulness, accuracy, precision, turnaround time, and cost per test for these procedures.

Materials and Methods

Patients

Fourteen patients undergoing myocardial revascularization were studied. A standard initial dose of four mg per kg body weight of beef lung heparin\textsuperscript{†} was injected intravenously. Fifteen to 30 minutes after the heparin injection, CPB was instituted. The pump was primed with 2000 ml of Plasmalyte\textsuperscript{§} containing 50 mg of heparin. A baseline ACT was obtained before heparinization. Samples of blood were drawn for measuring the ACT and the heparin level by the protamine titration and fluorometric method at the following intervals: (1) ten minutes after heparinization (prepump); (2) ten minutes after initiation of bypass (post-pump); and (3) at 30 minute-intervals while on bypass. Additional heparin was given if the ACT level was below 480 seconds. At the termination of the bypass, heparin was neutralized with protamine sulfate. In eight patients, the dose of protamine was equal to the amount of heparin given (mg for mg), excluding the amount of heparin in the pump prime. In six patients, the protamine dose was calculated by the Hepcon A-10\textsuperscript{*} system.\textsuperscript{*} The ACT assay was performed as recommended by the manufacturer.\textsuperscript{1,5} Mean values of the ACT 10 minutes following heparinization and 10 minutes after initiation of CPB were compared with the baseline ACT using the student "t" test for paired variables; p values <0.05 were considered significant.

Fluorometric Assay

A thrombin-sensitive synthetic substrate which releases a fluorophore, 5 aminoisophthalic acid dimethyl ester (AIE), is used in this assay. The patient sample is mixed with pooled plasma to provide adequate antithrombin. Thrombin is added to the mixture. Thrombin is partially neutralized by antithrombin in presence of heparin. Residual thrombin splits the substrate and the released AIE is measured fluorometrically.

The procedure is modified from the method described by Choo et al.\textsuperscript{3} The blood is collected in a plastic syringe and a 1-in-10 and 1-in-20 dilution of whole blood is made in normal saline. Five \(\mu\)l of the diluted blood is added to 0.2 ml of pooled normal plasma; 0.05 NIH units of thrombin is added, and the mixture is incubated exactly 60 seconds. Residual

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\textsuperscript{*} Hepcon A-10\textsuperscript{*}, Hematec Inc., Englewood, CO.
\textsuperscript{†} Upjohn Company, Kalamazoo, MI.
\textsuperscript{§} Travenol Laboratory, Inc., Deerfield, IL.

\textsuperscript{1} Hemochron Model 400, International Technidyne Corp., Edison, NJ.
thrombin activity is measured kinetically by its reaction with fluorometric substrate in a fluorometer.*

Heparin standards are prepared by diluting stock heparin to give concentrations ranging from 0 to 0.8u per ml. Corresponding values of percent thrombin remaining are obtained for each heparin standard from the fluorometer and are plotted on a graph. The test result is read off the standard curve, and the final result is multiplied by the dilution of whole blood used.

**Results**

The average pump time for the 14 patients was 75 minutes.

**Activated Clotting Time:** In figure 1 is shown the mean ± 1 S.D. of ACT: (1) before heparinization (baseline); (2) 10 minutes after the administration of heparin (prepump); and (3) 10 minutes after the initiation of bypass (post-pump). The baseline ACT in the 14 patients was 154 ± 25 seconds. After heparinization the ACT levels rose in all 14 patients. Ten minutes post-heparin the ACT was 589 ± 131 seconds. Ten minutes post-pump the ACT level was 668 ± 169 seconds. The difference in prepump and post-pump ACT values was statistically significant (p < 0.05). The average increase of ACT corresponding to a unit increase of plasma heparin was 91 seconds in prepump samples and 123 seconds in post-pump samples. The difference was statistically significant (p < 0.05). The ACT also correlated poorly with heparin levels (figure 2).

**Comparison of Fluorometric and Titrimetric Methods**

**Standards:** Six standards ranging from 1.0 to 10.0 units per ml were prepared by diluting heparin with fresh whole blood. The standards were run by both methods. The mean bias (assayed value – true value) by the fluorometric assay was −3.5 percent and the titrimetric assay was +18.0 percent. The precision of duplicate determination (1 S.D.) was 5.0 percent by the fluorometric assay and 17.5 percent by the titrimetric assay.

**Patient Samples:** Thirty-nine blood samples obtained during surgery were analyzed by both procedures. The mean

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* Protopath®, American Dade, Miami, FL

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**Figure 1.** The activated clotting time in 14 patients undergoing CPB at (1) Baseline, (2) 10 minutes post heparin, (3) 10 minutes after initiation of bypass (post-pump). The solid line indicates the mean, and the dotted lines indicate 1 S.D. from the mean.

**Figure 2.** Correlation between the activated clotting time and heparin level.
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Heparin Level (u/ml) - Fluorometric Assay

Figure 3. Comparison of the protamine titration assay and fluorometric assay for heparin.

± 1 S.D. of heparin levels by the fluorometric procedure was 3.4 ± 0.96 units per ml. By the titrimetric method, it was 4.1 ± 0.74 units per ml. Although the correlation between the two procedures (figure 3) was satisfactory \( r = 0.7 \), the protamine titration procedure gave significantly higher heparin levels \( p < 0.01 \).

Neutralization of Heparin by Protamine

The standard protocol called for administration of protamine equal to the amount of heparin given to the patient. The mean of protamine dose by the standard protocol was 375 mg per patient compared to the mean calculated dose by the titration method of 200 mg; the latter represents an average of 53 percent of the mean dose by the standard protocol. Eight patients were administered protamine according to the standard protocol, while six patients were given protamine as calculated by the titration protocol. The 10 minutes post-protamine ACT (mean ± 1 S.D.) for both groups (14 patients) was 142 ± 10 seconds. This level showed no statistical difference from the baseline ACT \( p > 0.1 \). No heparin was detected in the 10 minutes and the one-hour post-protamine samples. The amount of postoperative blood loss in eight patients who received the standard protamine dose was not significantly different from the amount of blood loss in six patients receiving the calculated dose.

Turnaround Time

The average turnaround time for the ACT varied from two minutes to over 16 minutes, depending on the level of anticoagulation. The average turnaround time of the protamine titration method was two minutes, and that of the fluorometric procedure was five minutes.

Cost Per Test

The initial cost of instruments was $5,000 for the Hemochron (ACT), $10,000 for the Hepcon A-10 system (protamine titration), and $5,000 for the Protopath Fluorometric system. The cost per test was $0.50 for ACT, $8.00 for protamine titration, and $2.50 for the fluorometric procedure. An average of five tests were performed for each patient undergoing CPB.

Discussion

Several studies have established the sensitivity of the ACT to plasma heparin for monitoring heparinization in patients undergoing CPB. The present authors confirm earlier reports that with standard heparin dose the ACT varies from patient to patient and that there is poor correlation between heparin level and the ACT. Although heparinization prolonged the ACT in all 14 patients in our study, the increase of the ACT per unit of heparin was quite variable from patient to patient, as well as in the same patient at different stages of surgery. The average increase of the ACT per unit of heparin after the bypass was significantly higher \( p < 0.05 \) than the average increase in the ACT before the bypass. This increase in the sensitivity of the
ACT to heparin after the bypass has been attributed to dilution of the clotting factors. Hypothermia has also been reported to increase significantly the ACT, independent of heparin level, and prewarming of the blood sample to 37°C has been recommended prior to performance of the test.

Although the ACT does not accurately predict the heparin level, it does represent the degree of anticoagulation for an individual patient. In a patient with anti-thrombin III deficiency, the heparin levels are not meaningful, and a lack of expected rise of the ACT despite heparin injection may warn the surgeon of thrombotic diathesis in the patient.

Several studies have emphasized the need for accurate neutralization of heparin after the bypass. Guffin et al used a protamine dose based on half-life of heparin. Compared with the standard protocol, their average protamine dose was 46 percent. They observed a decrease in postoperative blood loss in patients using the lesser dose of protamine. In six patients in our study, the protamine dose was administered based on heparin level. The average dose was 52% of the dose used in the standard protocol. However, the postoperative chest drainage did not show any statistical difference, whether the standard or the calculated dose of protamine was used. Also, unlike others, the present authors did not detect heparin rebound in the 14 patients studied using either regimen of protamine neutralization.

In this study the suitability of the three tests has been compared: the ACT, the fluorometric assay, and the protamine titration assay for patients undergoing CPB. The ACT reflects the degree of anticoagulation rather than the heparin level. The assay is easy to perform, requires 2.0 ml of blood, and the results are available in five to 10 minutes. The fluorometric assay gives a more accurate heparin level (mean bias – 3.5 percent) compared with the titrimetric assay (mean bias + 18.0 percent). The reason for the slightly higher heparin values by the titrimetric assay is not entirely clear and may represent a difference of methodologies. Both methods have good precision. The fluorometric assay requires smaller sample volume (0.1 ml) compared with the protamine titration method (6.0 ml). Both methods are quick (two minutes turnaround for the protamine titration and five minutes for the fluorometric assay) and easy to perform. The fluorometric assay requires the attention of an experienced medical technologist while the protamine titration assay can be performed by the same person performing the ACT. Taking only the cost of disposable material into account, the ACT appears to be the least expensive.

From this study it is concluded that a protocol for monitoring heparin requires a combination of the ACT with one of the heparin assay methods. Each surgical team may set up a protocol to suit its need and circumstances, taking into consideration the accuracy, precision, turnaround time, cost per test, and availability of a trained medical technologist in the operating room laboratory.

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References


