Heparin Detection by the Activated Coagulation Time: A Comparison of the Sensitivity of Coagulation Tests and Heparin Assays

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**Objective:** Laboratory and point-of-care coagulation tests are frequently obtained to determine the presence of heparin after surgical procedures. The objective of this study was (1) to compare the sensitivity of the activated coagulation time (ACT), activated partial thromboplastin time (aPTT), protamine titration (Hepcon; HMS Medtronic, Hemotec, Englewood, CO), and thromboelastography (TEG) with heparin anticoagulation and (2) to determine how frequently residual heparin is present in the 24-hour period after heparin neutralization in cardiopulmonary bypass (CPB) patients.

**Design:** A prospective study.

**Setting:** A tertiary care university teaching center that performs more than 15,000 surgical procedures per year.

**Participants:** Vascular surgical (n = 17) and CPB (n = 29).

**Interventions:** In vascular surgical patients, coagulation tests (ACT, protamine titration [Hepcon], and TEG) were obtained before and 90 minutes after heparin (50 to 60 U/kg IV) and compared with heparin concentration determined by factor Xa inhibition assay. In cardiac surgical patients, ACT and heparin concentrations were measured after anesthesia induction, during CPB, after protamine neutralization, and 3 as well as 6 hours after CPB. In addition to heparin concentrations and ACT measures, platelet counts, fibrinogen levels, and bleeding times were determined before and 3 and 24 hours after CPB.

**Measurements and Main Results:** Ninety minutes after heparin, significant heparin concentrations were present in all vascular surgical patients, but ACT was elevated in only 4 of 17 patients. Protamine titration (Hepcon) correlated with the factor Xa inhibitory assay for heparin (r² = 0.76). All 17 patients had an abnormal TEG (mean “R” time = 81 ± 39 minutes) and a marked elevation of aPTT (135 ± 35 sec [normal 22 to 33 seconds]) 90 minutes after heparin. In CPB patients, ACT did not correlate with heparin assays. After protamine neutralization of heparin in CPB patients, ACT returned to baseline despite the presence of heparin in 3 of 29 patients (0.22, 0.18, and 0.33 U/mL).

**Conclusions:** ACT was less sensitive to residual heparin anticoagulation than aPTT, TEG, and whole blood heparin assay. The whole blood heparin assay (Hepcon) provided sensitive and specific data about the presence of residual heparin. Despite the limitation of ACT in detecting heparin, the investigators found that residual heparin was not common in the period after uncomplicated CPB.

**KEY WORDS:** heparin, activated coagulation time, cardiopulmonary bypass, coagulation tests

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**The Activated Coagulation Time (ACT) is the most common intraoperative hemostatic test to monitor heparin anticoagulation.** ACT testing is used to confirm profound anticoagulation during cardiopulmonary bypass (CPB), to assess protamine neutralization of heparin, and to ensure adequate anticoagulation during vascular surgical procedures and hemodialysis. A variety of studies have correlated ACT with higher heparin doses and concentrations required during CPB, but few studies are available defining the sensitivity of ACT to the smaller heparin doses used in vascular surgery and hemodialysis and in patients anticoagulated for medical disorders. If differences in heparin anticoagulation sensitivity exist among coagulation tests, therapeutic decisions based on test abnormalities could change. The purpose of this study was (1) to measure and compare how sensitive common laboratory and point-of-care coagulation tests are to low heparin concentrations and (2) to determine how frequently measurable heparin concentrations are found during and after CPB.

**METHODS**

After consent was obtained for the protocol from the institution’s Committee for Human Studies, 46 American Society of Anesthesiologists (ASA) physical status 3 patients were studied who required either vascular surgery (n = 17) or CPB (n = 29). The sensitivity of ACT, aPTT, TEG, and Hepcon was compared with heparin anticoagulation as measured by a sensitive heparin assay (factor Xa inhibition). Patients who had received preoperative anticoagulants or who had a clinical history of bleeding or an abnormal prothrombin time (PT), activated partial thromboplastin time (aPTT), or platelet count were excluded from the study.

**Vascular Surgery**

Seventeen ASA physical status III patients who required vascular surgery had a baseline thromboelastograph (TEG), ACT, and a heparin assay (factor Xa inhibitory assay) measured after anesthesia induction but before the start of elective surgery. Fifteen of the patients required revascularization of the lower extremity. Two patients required vascular grafts of the distal aorta and femoral arteries. Before proximal artery cross-clamping, heparin was administered as an intravenous bolus (50 to 70 U/kg). Ninety minutes later, a repeat ACT, TEG, factor Xa heparin inhibitory assay, protamine titration, and PT and aPTT were performed on the same blood sample.

**Point-of-care tests** (ACT, whole blood heparin concentration, and TEG) were conducted in the operating room using whole blood. All tests were conducted in duplicate. ACT was conducted using a dual-chamber (Hemocheck; International Technidyne, Edison, NJ) ACT machine using high-range ACT cartridges. Hepcon (HMS Medtronic, Englewood, CO) was performed using a cartridge containing known protamine concentrations (0, 0.3, 0.6, and 0.9 µg/mL protamine). TEG was measured using a Biclot 916 TEG (Logos Scientific, Chicago, IL).

Laboratory tests included a sensitive heparin assay performed on plasma. The heparin assay uses spectrophotometry to assess a chromogenic substrate released during the inhibition or neutralization of factor Xa by heparin. Commercially obtained factor Xa is added to the patient’s plasma, and the resulting inhibition of factor Xa is proportional to the heparin or heparin-like substances present in the sample. This...
assay measures anticoagulant effects of all types of heparin. aPTT was also performed in the laboratory on plasma samples obtained at the same time as the other coagulation tests (automated aPTT; General Diagnostic Corp, ACL-100 Instrumentation Laboratory).

Crystallloid solutions (lactated Ringer’s or normal saline) were administered during the study period to replace third space fluid and blood losses. None of the patients received blood or blood products during the 90-minute period. The estimated blood loss during the study period was less than 300 mL in all patients. No anticoagulants were administered after the initial heparin dose until blood was obtained for laboratory and point-of-care coagulation tests.

Cardiopulmonary Bypass

All 29 CPB patients underwent elective myocardial revascularization. None of the patients had prior cardiac operations or received oral or intravenous drugs that altered coagulation (eg, thrombolytic therapy, salicylates, corticosteroids). The only exception were patients who had received a heparin dose for cardiac catheterization but who had been neither maintained on nor received heparin in the 24 hours before operation. Patients with a preoperative history of bleeding or abnormalities of PT, aPTT, or platelet count also were excluded.

An ACT and heparin assay (factor Xa inhibition) were obtained at the following times during the perioperative period:
1. after anesthesia induction before sternotomy;
2. before the initiation of CPB (5 minutes after the heparin, 300 U/kg (IV));
3. 10 minutes after initiation of CPB;
4. 60 minutes after initiation of CPB;
5. 20 minutes after protamine administration;
6. 3 hours after CPB;
7. 6 hours after CPB; and
8. 24 hours after CPB.

In addition, fibrinogen levels, platelet counts, bleeding times, and hematocrit and hemoglobin levels were obtained after anesthesia induction and 20 minutes and 3, 6, and 24 hours after protamine neutralization of heparin.

Before CPB, 300 U/kg of heparin were administered. An additional 10,000 units of heparin were included in the bypass circuit. Heparin dosing during CPB was based on ACT results repeated every 30 minutes. An additional heparin bolus dose (50 to 70 U/kg) was administered if an ACT value was less than 450 seconds. At the conclusion of CPB, the total heparin dose including the heparin added to the bypass circuit was antagonized using 1.0 mg of protamine for each 100 U of heparin.

Student’s paired t test was used to compare mean ACT, aPTT, TEG, and Hepcon before and after heparin. Linear regression analysis was used to determine correlation coefficients between heparin assay and ACT, aPTT, TEG, and whole blood heparin assay (Hepcon).

RESULTS

In the 17 vascular surgery patients who had received heparin (50 to 70 U/kg) before arteriotomy, measurable heparin levels were found by heparin assay (factor Xa inhibition) 90 minutes later. The laboratory heparin assay (factor Xa inhibition) ranged between 0.18 to 0.85 U/mL. All 17 patients had an increased aPTT and a prolonged “R” time on TEG measurement (Table 1). Whole blood heparin measures (Hepcon) detected the presence of heparin in 15 of 17 patients. The two patients who had no heparin using the whole blood heparin measure (Hepcon) had the lowest aPTT values of the 17 patients (47 and 85 seconds, respectively [normal 22 to 35 seconds]) and the shortest “R” time (19 minutes and 24 minutes, respectively [baseline 10 to 11 minutes]). The factor X inhibitory assays in both of these patients indicated that heparin levels were 0.18 U and 0.26 U/mL. In contrast to the sensitivity of TEG, aPTT, and whole blood heparin to heparin anticoagulation, only 4 of 17 patients had an abnormal ACT (ie, more than 15 seconds greater than the averaged duplicate ACT before heparin) despite heparin concentrations as great as 0.7 U heparin/mL. In all 13 patients with normal ACT values, abnormalities existed in all other coagulation tests (Table 1). A correlation coefficient was determined for heparin concentrations less than 1 U/mL versus ACT ($r^2 = 0.32$) and for heparin concentrations between 1 to 10 U/mL versus ACT ($r^2 = 0.07$; Figs 1 and 2). The best correlation was observed between heparin assay and Hepcon ($r^2 = 0.76$; Fig 3). Although TEG and aPTT were sensitive to heparin, correlation coefficients between heparin concentrations and aPTT ($r^2 = 0.32$; Fig 4) and TEG $r$ value ($r^2 = 0.21$) indicate a less specific change than with whole blood heparin measures (Hepcon).

In the 29 patients who had CPB, the mean duration of CPB was 72 ± 12 minutes. The mean age and weight of patients was 63.8 ± 8 years and 87.2 ± 9 kg. Twenty-seven men and two women participated in the study of hemostasis during CPB. The

Table 1. Comparison of ACT, Hepcon, TEG, and aPTT and Heparin Assays in Vascular Surgery Patients

<table>
<thead>
<tr>
<th></th>
<th>Preheparin</th>
<th>90 Minutes Postheparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin assay (Xa inhibition)</td>
<td>0.03 ± 0.01</td>
<td>0.42 ± 0.24 U/mL*</td>
</tr>
<tr>
<td>ACT</td>
<td>118 ± 14</td>
<td>125 ± 13 s</td>
</tr>
<tr>
<td>Prothrombin time (Hepcon)</td>
<td>0</td>
<td>0.45 ± 0.28 U/mL*</td>
</tr>
<tr>
<td>TEG &quot;R&quot; wave (min)</td>
<td>12 ± 4</td>
<td>61 ± 39 min*</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>28 ± 4</td>
<td>135 ± 36 s*</td>
</tr>
</tbody>
</table>

*p < 0.05 from preheparin.

Fig 1. ACT versus Heparin assay (factor X inhibitory assay) in patients with heparin concentrations less than 1.0 U/mL (correlation coefficient [$r^2 = 0.32$]). Shaded area represents usual heparin concentrations in anticoagulated patients with aPTT between 1.5 to 2.5 x control.
ACT values before CPB were 129 ± 14 seconds (range 102 to 152 seconds). All 29 patients had an internal mammary artery graft in addition to saphenous vein bypass grafts.

In addition to the heparin, 300 U/kg, and an additional 10,000 units of heparin in the prime of the bypass circuit, 15 of the 29 received additional heparin doses in 5,000-U increments to maintain an ACT value greater than 450 seconds during bypass. The mean heparin dose administered was 476 ± 40 U/kg using the heparin dosing protocol. Simultaneous heparin concentrations using the factor X inhibitory assay and ACT were measured during and after CPB (Table 2).

After heparin reversal, post-CPB ACT (113 ± 13 seconds) was less than ACT before CPB (129 ± 14 seconds). Post-CPB platelet counts and fibrinogen levels decreased, and bleeding time increased when compared with pre-CPB (Table 2). Trace heparin levels (less than 0.04 U/mL) were present in most of the patients 20 minutes after protamine, but 3 patients had heparin concentrations of 0.33, 0.20, and 0.18 U/mL, respectively. In these 3 patients, ACT values were 102, 109, and 118 seconds. Three hours after bypass, 2 of these 3 patients had persistent heparin levels that were elevated (0.21 to 0.33 U/mL). The ACT remained in the normal range in both patients. In the other 27 patients, heparin levels remained in the trace range after CPB. No evidence of heparin rebound was observed. None of the patients had any evidence of increased bleeding. None of the patients received platelets, fresh frozen plasma, or cryoprecipitate during the intraoperative or postoperative period. None of the patients received pharmacologic agents to prevent or treat postoperative bleeding.

The mean hemoglobin (13.0 ± 1.2 g/dL) and hematocrit (38.7% ± 3.2%) before initiation of bypass decreased to 8.6 ± 0.7 g/dL and 25.1% ± 2.0%, respectively, at the conclusion of bypass. Patients had a mean hematocrit of 28.4% ± 2.6% 24 hours after surgery. The volume and hemoglobin concentrations of mediastinal drainage after operation were used to estimate postoperative blood loss. Mean blood loss of 460 ± 240 mL was observed in the postoperative period. Three patients received packed cells during hospitalization.

**DISCUSSION**

When compared with other coagulation tests, the ACT was less sensitive to heparin anticoagulation than other coagulation tests. Heparin concentrations as high as 0.7 U/mL were not detected by ACT. If one of the primary goals of coagulation test monitoring in the post-CPB period is to exclude the presence of heparin, then the ACT is the least effective measure. TEG and aPTT were sensitive methods to detect the presence of heparin but were nonspecific. Whole blood heparin measures (Hepcon) provide a sensitive and specific guide to residual heparin anticoagulation as measured by an accepted laboratory standard of heparin measurement, the factor X inhibitory assay for heparin.

Despite the limitations of ACT in detecting residual heparin concentrations, no bleeding complications were found after uncomplicated vascular surgery and CPB. This finding might suggest that ACT is an effective qualitative guide to heparin
anticoagulation in uncomplicated surgical procedures. In contrast, limited correlation and lack of sensitivity of ACT to heparin anticoagulation could be interpreted as an indication to implement more sensitive and specific coagulation tests to monitor heparin anticoagulation during the perioperative period. When specific coagulation tests are used to guide therapy in bleeding CPB patients, blood loss is reduced and fewer blood products administered compared with diagnostic methods that rely primarily on ACT monitoring. The role of the ACT as a monitor of profound heparin anticoagulation has also been questioned. Metz et al found that maintaining a minimum ACT during CPB was not necessary if a 300-U/kg intravenous heparin dose was administered before CPB.

During the last 30 years, reagents and testing methodology for the aPTT have continuously improved the sensitivity of this test to both reduced coagulation factor levels and heparin. At present, aPTT measures that are extremely sensitive to heparin and coagulation factor decreases cannot be used to differentiate between these causes of postsurgical bleeding. When initially introduced by Hattersley 30 years ago, the ACT had similar sensitivity to reduced coagulation factor levels compared to PTT measurements. Current ACT measurements remain similar in heparin sensitivity to original ACT determinations.

The progress in developing more sensitive aPTT tests may explain why marked differences were found in sensitivity between ACT and aPTT when compared with earlier studies. If these differences in heparin sensitivity among coagulation tests are not considered when interpreting test results, then coagulation factor deficiency might be diagnosed rather than residual heparin.

Residual heparin or heparin rebound was uncommon with the protamine neutralization protocol that was used for all CPB patients. This finding suggests that a heparin neutralization protocol for uncomplicated CPB of 1 mg protamine/100 U heparin is effective in neutralizing heparin and only infrequently associated with residual heparin.

Heparin concentrations as measured both by laboratory (factor Xa inhibitory assay) and point-of-care (whole blood heparin measures) are consistent with pharmacokinetics of a bolus dose of heparin. Whole blood heparin assays provided a rapid and specific guide to heparin determinations that were similar to a laboratory assay based on factor Xa inhibition to quantify heparin. As expected, when heparin concentrations were between 0.1 and 0.25 U/mL, aPTT results were similar to those found in patients being treated for thrombotic disorders. The ACT results in this study are consistent with those reported by Martin et al 30 and 60 minutes after the administration of 5,000 U of heparin.

In summary, the ACT is the least sensitive hemostatic test to detect residual heparin anticoagulation. Whole blood heparin provided more sensitive and specific information about residual heparin. Diagnostic algorithms to assess causes of bleeding in CPB patients should include tests more sensitive and specific than the ACT to exclude residual heparin.

REFERENCES


| Table 2. Heparin Assay (Factor Xa, ACT, Fibrinogen, Bleeding Time, and Platelet Count) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | ACT (s)         | Heparin (U/mL)  | Fibrinogen (mg/dL) | Bleeding Time (s) | Platelet Count  |
| Baseline                        | 129 ± 14        | 0.02 ± 0.04     | 379 ± 76           | 511 ± 756        | 229 ± 64        |
| Heparin + 5 min                 | 616 ± 140       | 5.5 ± 1.5       | 618 ± 129          | 4.5 ± 1.4        | 212 ± 36        |
| Bypass + 10 min                 | 629 ± 129       | 6.4 ± 1.3       | 211 ± 44           | 612 ± 222        | 174 ± 55        |
| Bypass + 90 min                 | 614 ± 124       | 0.04 ± 0.08     | 178 ± 36           | 358 ± 144        | 212 ± 183       |
| Protamine + 20 min              | 112 ± 13        | 0.07 ± 0.08     | 110 ± 12           | 0.02 ± 0.06      | 164 ± 24        |
| Protamine + 3 h                 | 108 ± 16        | 0.04 ± 0.06     | 110 ± 24           | 164 ± 24         | 153 ± 45        |
| Protamine + 6 h                 | 108 ± 16        | 0.07 ± 0.08     | 110 ± 12           | 0.02 ± 0.06      | 164 ± 24        |
| Protamine + 24 h                | 108 ± 16        | 0.04 ± 0.06     | 110 ± 24           | 164 ± 24         | 153 ± 45        |


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