Point-of-Care Testing for Prothrombin Time, but Not Activated Partial Thromboplastin Time, Correlates With Laboratory Methods in Patients Receiving Aprotinin or epsilon-Aminocaproic Acid While Undergoing Cardiac Surgery

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Abstract

Point-of-care testing (POCT) of coagulation parameters can help optimize transfusion practice in cardiac surgery. Antifibrinolytic agents may interfere with the laboratory and/or POCT coagulation assays. This randomized controlled study compared coagulation parameters obtained from a whole blood POCT coagulation device with a typical laboratory instrument in cardiac surgery patients receiving aprotinin, epsilon-aminocaproic acid, or normal saline before undergoing cardiopulmonary bypass. Aliquots of arterial blood samples from 42 patients were collected perioperatively, and their prothrombin times (PTs) and activated partial thromboplastin times (aPTTs) were measured by POCT and laboratory instrumentation. Linear regression and error analyses were used for the method comparison. For PT, the POCT device compared favorably with the laboratory method. For aPTT, the POCT device did not compare well with the laboratory method. Treatment with antifibrinolytic agents does not interfere with determination of PT.

Bleeding diathesis after cardiac surgery is multifactorial and often has adverse consequences in terms of morbidity and mortality. Point-of-care testing (POCT) can provide a rapid turnaround time for prothrombin time (PT) and activated partial thromboplastin time (aPTT). These rapidly available data not only help to differentiate surgical bleeding from underlying coagulopathy but also have a crucial role in the appropriate use of blood components. The correlation of POCT and laboratory PT and aPTT has been quite variable depending on the assay used, the clinical context, or both. Previous studies have shown that POCT PT results compared well with those obtained from laboratory instrumentation for patients undergoing cardiac operations without antifibrinolytic therapy. Aprotinin, a bovine protein that is a broad-spectrum serine protease inhibitor, and epsilon-aminocaproic acid (EACA), a lysine analogue that inhibits plasmin activity and fibrinolysis, are being used for the vast majority of cardiac surgery patients to reduce blood loss. This raises the concern that antifibrinolytic agents such as aprotinin and EACA may interfere with the determination of PT, aPTT, or both using laboratory or POCT methods. Indeed, Despotis et al demonstrated a dose-dependent prolongation of whole blood aPTT when aprotinin was added in vitro to specimens obtained before and after cardiopulmonary bypass (CPB). Therefore, before POCT devices can be used for patients receiving aprotinin or EACA during cardiac surgery, they must have a one-to-one, first-order, monotonic, linear transformation with typical laboratory instruments, and the ex vivo effect of these antifibrinolytics on any laboratory or POCT coagulation assays should be studied. The present study compared CoaguChek Plus (Roche, Indianapolis, IN), a whole blood POCT coagulation assay, with the ACL 6000 coagulation...
Materials and Methods

Patients
Following institutional review board approval and informed consent, 42 patients who underwent cardiac surgery (primary and redo coronary artery bypass grafting and valve replacements) requiring CPB were randomized in a double-blinded manner to receive aprotinin, EACA, or normal saline perioperatively.

Anesthesia and CPB Conditions
All patients were anesthetized with a narcotic-based anesthetic regimen supplemented with inhalational anesthetic agents, muscle relaxants, and benzodiazepines. CPB was performed using a membrane oxygenator (Gish, Irvine, CA) with nonpulsatile flow using a centrifugal pump (Medtronic, Minneapolis, MN). The CPB circuit was primed with 2 L of lactated Ringer’s solution, 100 mL of 25% albumin, 44.6 mEq of sodium bicarbonate, and 50 g of mannitol. Anticoagulation before CPB was achieved with intravenous bovine heparin, and activated clotting time was monitored using the Medtronic Hepcon system. A minimal activated clotting time of 480 seconds was required before onset of CPB. Antegrade and retrograde hypothermic blood cardioplegia was administrated intermittently during moderate systemic hypothermia (28°C-30°C). Perfusion flow rates were maintained at 2.0 L/min/mm² during hypothermia and at 2.5 L/min/mm² during normothermia.

Dose and Timing of Aprotinin and EACA
The 3 groups of patients received equal volumes of aprotinin, EACA, or saline for (1) a loading dose (200 mL) following heparinization, (2) the CPB pump prime (250 mL), and (3) continuous infusion (50 mL/h). The infusions were all discontinued on arrival in the intensive care unit following surgery. The aprotinin group received a loading dose of 2 × 10⁶ kallikrein inhibiting units (KIU), pump prime of 2 × 10⁶ KIU, and an infusion of 5 × 10⁵ KIU/h. The EACA group received a bolus of 100 mg/kg loading dose, a CPB pump prime of 5 g, and an infusion of 30 mg/kg per hour.

Blood Collection
Arterial blood samples were collected during 3 time periods: (1) preoperatively (before induction of anesthesia and heparinization), (2) following heparin reversal (after separation from the CPB circuit), and (3) 2 hours following CPB. Aliquots from the same blood samples obtained via radial or femoral intra-arterial catheters after removal of 6 dead-space volumes were used for coagulation testing by both POCT and laboratory methods.

POCT Coagulation Assays
Whole blood PT and aPTT were determined by using the CoaguChek Plus. The CoaguChek Plus accepted 1 of 2 disposable cartridges, which contained the reagents for either the PT or aPTT test. The PT reagent was rabbit brain thromboplastin. The aPTT reagents were bovine brain sulfatide as the activator and soybean phosphatide as the platelet substitute. Each cartridge contained an application well, a reagent chamber, and a reaction path. After the cartridge was prewarmed to 37°C, a drop of nonanticoagulated whole blood (minimum of 25 µL) was applied to the test cartridge sample application well. Blood was drawn by capillary action into the reagent chamber where it mixed with either PT or aPTT reagents to initiate coagulation. The blood-reagent mixture moved along the reaction path—a capillary tube—until a clot formed. The laser optical system detected the clot by monitoring blood flow, and the end point was reached when the blood stopped moving. The elapsed time from applying the sample to detecting the clot was converted mathematically to a plasma equivalent PT or aPTT.

Laboratory Coagulation Assays
One-stage PT, a modification of the method initially described by Quick et al., was performed on citrate anticoagulated plasma with IL Test PT-Fibrinogen reagents (Instrumentation Laboratory) using the ACL 6000 automatic coagulation analyzer. The PT-Fibrinogen reagent contained an extract of rabbit brain thromboplastin. The aPTT assay, a modification of the method initially described by Proctor and Rapaport, was performed on citrate anticoagulated plasma with IL Test APTT-SP (Instrumentation Laboratory). The APTT-SP contained silica as the activator and synthetic phospholipids as surfaces to form complexes that activated factor X and prothrombin. The sampler probe aspirated and dispensed precisely measured samples, diluents, and controls into cuvettes. The cuvettes were transferrred to the transport belt, which moved the cuvettes into a heater trough, and reagents then were added. At the end of incubation or activation time, the transport belt moved the cuvettes into the stations where precisely controlled light sources and photo sensors detected the onset of fibrin clot formation. The principle of photometric clot detection was used in the ACL 6000. The time elapsed between reagent addition by the system and the beginning of clot formation was measured to determine the clot time.

Statistical Analysis
Quality control was performed in accordance with the manufacturer’s instructions for the POCT and laboratory
assays. Means of duplicate measurements of PT and aPTT from POCT and laboratory assays were obtained. Least squares linear regression analysis was used for the method comparison. Error analyses using the regression equations were performed to evaluate the percentage of error at 1.5 times the mean reference range of PT and at 1.5 times the top of the reference range of aPTT. An error of less than 10% was considered clinically acceptable.

**Results**

**Prothrombin Time**

We analyzed 126 sets of paired data. The values of PT obtained by the laboratory ranged from 10.6 to 20.9 seconds and had a median of 14.8 seconds. The values of PT obtained by POCT ranged from 10.9 to 20.6 seconds and had a median of 15.7 seconds. The reference range of PT used in the laboratory was 10.7 to 13.1 seconds. For PT, the POCT device compared favorably with the laboratory method \((POCT = 0.89[\text{laboratory}] + 2.13; \text{correlation coefficient}, r = 0.89)\), and there was good agreement between POCT and laboratory PT among the 3 treatment groups [Table 1].

Using the regression equation of \(POCT = 0.89[\text{laboratory}] + 2.13\) and at 1.5 times the mean of the reference range, the ratio of prolonged laboratory PT (18/12 = 1.5) would be slightly less if determined by POCT PT (18.2/12.8 = 1.42). This represents an error of 5.3% (0.08/1.5 × 100%), which we find acceptable using an acceptable error limit of less than 10% for clinical decision making [Table 2]. Furthermore, the difference between actual and predicted PTs demonstrates this low error across the range of expected PT values.

**Activated Partial Thromboplastin Time**

We analyzed 91 sets of paired data. The values of aPTT obtained by the laboratory ranged from 20.1 to 75.3 seconds and had a median of 32.2 seconds. The values of aPTT obtained by POCT ranged from 18.0 to 49.6 seconds and had a median of 30.5 seconds. The reference range of aPTT used in the laboratory was 24.0 to 35.6 seconds. For aPTT, the POCT device did not compare well with the laboratory method \((r < 0.6; \text{the composite data showed } POCT = 0.31[\text{laboratory}] + 19.9; \text{correlation coefficient}, r = 0.54)\). There were strong proportional and constant biases for the POCT aPTT. The second-order and third-order polynomial regressions fit the data better than did the linear regression.
Furthermore, the values for POCT drop off as they exceed 35 seconds, indicating that the coagulation cascade has been activated for their samples in the device. The device loses usefulness because it fails to respond above 35 seconds, which is about the upper limit of the normal range. Thus, the strong proportional bias results in loss of sensitivity of POCT aPTT in detecting factor deficiency or a change in factor levels. For example, at 1.5 times the top of the reference range, the ratio of prolonged laboratory aPTT (53.4/35.6 = 1.5) would be significantly less if determined by POCT aPTT (36.5/30.9 = 1.18). This represents an error of 21.3% (0.32/1.5 × 100%), which we find unacceptable using an allowable error limit of less than 10% for clinical decision making; furthermore, the difference between actual and predicted error is excessively large with prolonged aPTT values.

In addition, depending on the type of drug treatment and the range of aPTTs, the POCT device did not compare well with the laboratory method. We found POCT aPTT results did not compare well with laboratory aPTT results. In an initial evaluation by Ansell et al, the whole-blood aPTT result correlated well with the laboratory aPTT result in nonsurgical patients receiving heparin, warfarin, or both, with correlation coefficients ranging from 0.79 to 0.83, depending on the comparison reagent and instrumentation used. The correlation coefficient was in the same range as that obtained for laboratory aPTTs with different reagents ($r = 0.79$). The ranges of the aPTT values were from 25 seconds to 140 seconds, with most values clustered around 22 to 80 seconds. However, at higher aPTT values, the whole-blood values varied greatly from the laboratory values, and their statistical analysis was insufficient, being correlation coefficient dependent. In contrast, other studies using either linear regression or bias analysis demonstrated discrepant results between POCT and laboratory aPTT methods. The variability was attributed to probable differences in reagents and clot timers. In 1 study, variability between POCT and laboratory aPTT was found to be most likely related to differences in normal reference ranges and responsiveness to coagulation factor concentrations between the 2 assay systems. In addition to the differences in reagents and clot timers, we propose that the effect of contact activation

Discussion

In the present study, we found that POCT PT results compared favorably with those obtained from the laboratory for patients receiving aprotinin, EACA, or saline placebo while undergoing CPB. Errors at 1.5- to 2-fold the mean normal of POCT PT are less than 10%, and they are acceptable for clinical decision making. This is in accord with previous studies that demonstrated POCT PT results were as accurate and reliable as laboratory PT results for patients not receiving antifibrinolytic therapy. In addition, our study demonstrated that treatment with aprotinin or EACA does not interfere with determination of PT over a wide range of values commonly seen in cardiac surgery patients. Our study differs from previous ones in that we studied POCT PTs on specimens from patients receiving aprotinin or EACA, where previous studies did not. The accuracy and rapid turnaround time of POCT PT may have a significant role in clinical decision making to ensure that patients with surgical bleeding do not receive transfusions with plasma products unnecessarily and that patients with multiple factor deficiencies receive transfusions without delay.

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In addition to the differences in reagents and clot timers, we propose that the effect of contact activation
may contribute to the variability of aPTT between POCT and laboratory methods. In the POCT aPTT assay, the whole blood-reagent mixture moves along a capillary tube until a clot forms, the laser optical system detects the clot by monitoring blood flow, and the end point is reached when the blood-reagent mixture stops moving. The increase in surface area for contact activation along the reaction path may accelerate clot formation in the determination of aPTT compared with a plasma-reagent mixture incubating in a cuvette in the laboratory assay system. In the setting of cardiac surgery in which coagulation factors are activated, the increase in surface area of contact activation in vitro may result in shortened aPTT by the POCT assay compared with the laboratory assay. This is most noticeable when the aPTT is substantially prolonged (>1.5 times the upper of normal reference range), as well as in the range in which a transfusion decision is made. Further refinement of the POCT aPTT method is needed to improve the comparability with the laboratory method.

We found that the results of POCT PT compared well with those obtained from the laboratory for patients receiving aprotinin or EACA while undergoing CPB. There is no detectable interference from these antifibrinolytic agents on the determination of POCT and laboratory PT. The POCT PT may provide useful information for rapid clinical decision making regarding not transfusing plasma products if the PT is within the acceptable range. On the other hand, the POCT aPTT is not sensitive to the change in coagulation factors in this surgical setting. These POCT devices were designed using specimens from patients receiving treatment with coumadin or heparin, not aprotinin or EACA, and, thus, their use in a special surgical setting may be counterproductive in that they may provide misleading information for physicians making transfusion decisions.

References


