Correlations Between Activated Clotting Time Values and Heparin Concentration Measurements in Young Infants Undergoing Cardiopulmonary Bypass

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**BACKGROUND:** Monitoring heparin concentration along with the activated clotting time (ACT) may provide a more accurate guide for the administration of heparin to infants during cardiopulmonary bypass (CPB). However, standard laboratory assays of heparin concentration (antifactor Xa heparin concentration) require plasma instead of whole blood, and results are not immediately available to clinicians. Alternatively, measurements of whole blood heparin concentration may be performed at the bedside using an automated protamine titration device, the Hepcon instrument (Hepcon Hemostasis Management System Plus; Medtronics, Minneapolis, MN). The purpose of this investigation was to compare ACT measurements from 3 commercially available instruments and bedside measurements of whole blood heparin concentration using the Hepcon instrument with laboratory measurements of antifactor Xa plasma heparin concentration in infants younger than 6 months of age undergoing CPB.

**METHODS:** Forty-four pediatric patients younger than 6 months of age scheduled for elective cardiac surgery requiring CPB were enrolled in this prospective study. Blood samples were drawn 3 minutes after the initial heparin bolus and immediately before the termination of CPB to obtain measurements of heparin anticoagulation. Kaolin-activated ACTs were performed with the Hemochron (International Technidyne Corporation, Edison, NJ), Hepcon, and i-STAT (i-STAT Corporation, East Windsor, NJ) instruments. Whole blood heparin concentration was measured using the Hepcon instrument. Plasma heparin concentration was measured using an antifactor Xa chromogenic substrate assay.

**RESULTS:** Immediately after the initial heparin bolus, none of the ACT values correlated with plasma heparin concentration. When measured immediately before the termination of CPB, only the i-STAT ACT showed a moderate correlation. Conversely, bedside measurements of whole blood heparin concentration showed satisfactory agreement with laboratory measurements of plasma heparin concentration at both time points (concordance correlation coefficients 0.30 and 0.67, respectively). There is a bias in that antifactor Xa-measured plasma heparin concentration tends to be higher than Hepcon-measured whole blood heparin concentration.

**CONCLUSIONS:** In infants younger than 6 months old undergoing CPB, caution is warranted when using ACT values as the sole indication of adequate heparin anticoagulation. In general, ACT prolongation correlates poorly with plasma heparin concentration. Only i-STAT ACT values showed a moderate correlation when measured immediately before the termination of CPB. Alternatively, bedside measurements of whole blood heparin concentration measured by the Hepcon instrument agreed well with antifactor Xa laboratory measurements. Our data support the clinical utility of bedside measurements of heparin concentration to provide timely, convenient, and accurate measurements of heparin concentration in these infants. (Anesth Analg 2010;111:173–9)

Effective anticoagulation during cardiopulmonary bypass (CPB) is necessary to prevent excessive activation of the hemostatic system. Unfractionated heparin is most frequently used to achieve this goal. It produces the anticoagulation necessary for CPB primarily by accelerating the inactivation of thrombin by antithrombin (AT). Heparin anticoagulation during CPB may be monitored either by measuring heparin effect or by measuring heparin concentration. Heparin effect is typically measured by the use of the activated clotting time (ACT). However, the ACT is not a direct measurement of the specific end point of thrombin suppression. Rather, it is a measurement of whole blood coagulation initiated by contact activation. Contact activation and thus ACT values are notoriously prolonged in neonates and infants. Additionally, the ACT is affected by other factors during CPB that are not associated with heparin anticoagulation including hemodilution and hypothermia, both of which are common in the pediatric setting. However, maintenance of a defined heparin concentration during CPB has been shown to better estimate thrombin suppression.
The standard laboratory technique of assessing heparin concentration uses an antifactor Xa assay. This assay is difficult to perform, requires plasma instead of whole blood, and results are not immediately available to clinicians. Alternatively, measurements of whole blood heparin concentration can be performed at the bedside using an automated protamine titration device, the Hepcon instrument (Hepcon Hemostasis Management System Plus [Hepcon HMS]; Medtronic, Inc., Minneapolis, MN). Studies in adult cardiac surgical patients assessing the relationship between laboratory-measured antifactor Xa plasma heparin concentration and Hepcon-measured whole blood heparin concentration have found excellent correlation. The only study that examined this relationship in pediatric patients did not find similar results.

We had multiple goals for this investigation: to compare ACT measurements using 3 different commercially available instruments, and bedside measurements of whole blood heparin concentration using the Hepcon HMS, with laboratory measurements of antifactor Xa plasma heparin concentration in infants younger than 6 months of age undergoing CPB. Additionally, we sought to evaluate the role of preoperative AT activity on the determination of the heparin dose-response (HDR) assay as calculated by the Hepcon instrument.

METHODS

After obtaining IRB approval and written informed parental consent, 44 pediatric patients younger than 6 months old scheduled for elective cardiac surgery requiring CPB were enrolled in this prospective, observational study. Exclusion criteria included premature infants (gestational age younger than 37 weeks), infants treated with preoperative anticoagulants, infants with a known coagulation defect, the intraoperative administration of an antifibrinolytic drug, and emergency procedures.

All blood samples were drawn from an indwelling arterial line placed after the induction of anesthesia. Five dead-space volumes of blood (5 mL) were aspirated before obtaining a sample to ensure that no heparin from the flush solution was present in the collection sample. Baseline AT activity and a baseline HDR assay were measured in all infants before surgical incision. AT activity was determined using chromogenic assay (Stachrom ATIII; Diagnostica Stago, Parsippany, NJ). The HDR assay, performed by the Hepcon instrument (Hepcon HMS instrument via a protamine titration assay. Plasma heparin concentration was measured in the laboratory using an antifactor Xa chromogenic substrate assay (Stachrom Heparin, Diagnostic Stago, Parsippany, NJ). The antifactor Xa chromogenic substrate assay has been described as the most sensitive and specific method to measure heparin concentration.

All statistical analyses were performed using SAS statistical software (SAS Inc., Cary, NC) and statistical significance was assessed at the α = 0.05 level. Paired, 2-tailed Student t tests were used to compare the means between corresponding Hepcon-measured whole blood heparin concentrations and laboratory-measured plasma heparin concentrations at all time points. Because of non-normal distribution, a nonparametric test of equality (Mann-Whitney) was used to compare median ACT values across time points. Pearson’s product-moment correlation coefficients were calculated to assess linear association between ACT values and plasma heparin concentrations immediately after the initial heparin bolus and immediately before the termination of CPB. Agreement between Hepcon-measured whole blood heparin concentration and antifactor Xa-measured plasma heparin concentration was assessed using concordance correlation coefficients and a Bland and Altman analysis of agreement. Simple linear regression was used to assess AT as a predictor of the HDR assay. A post hoc power analysis, using PASS software (NCSS, Kaysville, UT), was calculated based on the ability to reject the null hypothesis that the correlation between ACT values and plasma heparin concentration is zero. Using a 2-sided hypothesis test with α = 0.05, a sample size of 44 achieves 80% power to detect a difference between the null hypothesis (r = 0) and the alternative hypothesis (r = 0.4).

RESULTS

Forty-four infants younger than 6 months old were enrolled in this study. For 1 infant undergoing tetralogy of Fallot repair, the surgeon decided intraoperatively to perform a Blalock-Taussig shunt without systemic heparinization or CPB. This patient was excluded from all data analyses. Patient demographics, cardiac surgical procedures, and CPB data for the remaining 43 infants are shown in Table 1.

Mean ACT measurements, as measured by all 3 ACT instruments, increased in value throughout the duration of CPB, whereas, conversely, mean heparin concentrations...
Correlation Between ACT Measurements and Antifactor Xa Heparin Concentration

Results of simultaneously measured kaolin-activated ACT values obtained by the 3 different instruments, Hemochron, Hepcon, and i-STAT, were analyzed for correlation with antifactor Xa-measured plasma heparin concentration. Results obtained from the 2 different sampling times were analyzed separately because of major differences in scale and distribution patterns. Three minutes after the administration of heparin, no significant correlations were found among any of the 3 ACT measurements and plasma heparin concentration (Hemochron: $r = 0.19$, $P = 0.22$; Hepcon: $r = 0.13$, $P = 0.42$; i-STAT: $r = 0.2$, $P = 0.21$).

Immediately before the termination of CPB, the Hemochron ACT showed no correlation with plasma heparin concentration ($r = 0.28$, $P = 0.08$); the Hepcon ACT showed a weak positive correlation with plasma heparin concentration ($r = 0.3$, $P = 0.05$); and the i-STAT ACT showed a moderate positive correlation with plasma heparin concentration ($r = 0.53$, $P < 0.001$). Many of the ACT values obtained by the 3 instruments at this time point reached the maximum value of 1000 seconds over a wide range of corresponding antifactor Xa values. Sixty-seven percent of the Hemochron values, 45% of Hepcon values, and 42% of i-STAT values were 1000 seconds or more.

Agreement between Hepcon Whole Blood Heparin Concentration and Antifactor Xa Plasma Heparin Concentration

A comparison of the 2 measures of heparin concentration revealed that the mean concentration 3 minutes after the administration of heparin measured by the antifactor Xa assay was statistically significantly larger than the corresponding mean concentration measured by the Hepcon instrument ($P \leq 0.01$; Table 2). When measured immediately before the termination of CPB, the mean antifactor Xa heparin concentration was statistically significantly larger than the mean Hepcon heparin concentration ($P \leq 0.01$; Table 2).

To determine agreement between Hepcon-measured whole blood heparin concentrations and antifactor Xa-measured plasma heparin concentrations, concordance correlation coefficients ($p_c$) were calculated 3 minutes after the initial heparin bolus and immediately before the termination of CPB ($p_c = 0.30$ and 0.67, respectively; Fig. 2). Next, agreement was assessed descriptively between these 2 techniques using the method of Bland and Altman17(Fig. 3). In this analysis, we found a measurement bias in which antifactor Xa values tended to be higher than the Hepcon values by a mean (95% confidence interval) of 0.67 U/mL (−1.52, 2.87) 3 minutes after heparin administration and 0.49 U/mL (−1.64, 2.61) immediately before the termination of CPB.

Table 1. Patient Demographics, Cardiac Surgical Procedures, and Cardiopulmonary Bypass Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Age (d)</td>
<td>121 ± 37</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.9 ± 1.0</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>Gender (male %, female %)</td>
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</tr>
<tr>
<td>AT (%)</td>
<td>80.5 ± 17.8</td>
</tr>
<tr>
<td>Operative procedure (n)</td>
<td></td>
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<tr>
<td>VSD closure</td>
<td>18</td>
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<tr>
<td>AVSD repair</td>
<td>8</td>
</tr>
<tr>
<td>TOF repair</td>
<td>15</td>
</tr>
<tr>
<td>TAPVR repair</td>
<td>2</td>
</tr>
<tr>
<td>HDR assay (U/mL)</td>
<td>4.8 ± 1.7</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>83 ± 33</td>
</tr>
<tr>
<td>AXC time (min)</td>
<td>47 ± 21</td>
</tr>
<tr>
<td>Lowest temperature (°C)</td>
<td>31 ± 1</td>
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</table>

BSA = body surface area; AT = antithrombin; VSD = ventricular septal defect; AVSD = atrioventricular septal defect; TOF = tetralogy of Fallot; TAPVR = total anomalous pulmonary venous return; HDR = heparin dose response; CPB = cardiopulmonary bypass; AXC = aortic cross-clamp time.

Values are expressed as mean ± SD.

Figure 1. Changes in activated clotting time (ACT) values and heparin concentrations during cardiopulmonary bypass. *$P \leq 0.01$ vs time point 1; †$P \leq 0.001$ vs time point 1. Time point 1 = 3 minutes after heparin administration before cardiopulmonary bypass; time point 2 = immediately before the termination of cardiopulmonary bypass; HM = Hemochron; HP = Hepcon; Xa Hep conc = antifactor Xa-measured heparin concentration; HP Hep conc = Hepcon-measured heparin concentration.
**AT Activity as a Predictor of the HDR Assay**

Simple linear regression found AT activity to be a statistically significant predictor of the HDR assay ($\beta = -0.3, P < 0.001$; Fig. 4).

**DISCUSSION**

In this study, we compared ACT values from 3 commercially available instruments and bedside measurements of whole blood heparin concentration with laboratory antifactor Xa measurements of plasma heparin concentration in infants younger than 6 months of age. We found that 3 minutes after the initial administration of heparin, none of the ACT values measured by the 3 instruments showed a significant correlation with plasma heparin concentration. During CPB, both whole blood and plasma measurements of heparin concentration showed a significant decrease. Conversely, the ACT measurements of all 3 devices used increased in value. Immediately before the termination of CPB, only i-STAT ACT values showed a moderate correlation to plasma heparin concentration. However, at both time points, whole blood heparin concentrations measured at the bedside using the Hepcon HMS instrument showed strong agreement with laboratory-measured antifactor Xa plasma heparin concentrations. Additionally, we found that preoperative AT activity had a significant negative association with the HDR assay.

The lack of correlation between ACT measurements and antifactor Xa-measured heparin concentration is not surprising given the sensitivity of ACT values to hemodilution, thrombocytopenia, and hypothermia, all of which are common in infants younger than 6 months old undergoing CPB. The i-STAT was the only ACT instrument to show any significant correlation with antifactor Xa-measured heparin concentration. Additionally, it was the only ACT instrument that did not produce significantly increasing values from the beginning to end of CPB. The end point used in the i-STAT determination of ACT relies on the detection of a certain amount of thrombin generation and not on the formation of fibrin strands. This may be an inherently more sensitive method of ACT determination because the formation of fibrin strands occurs when only minimal levels of prothrombin have been converted to thrombin. Nevertheless, all ACT tests, including the i-STAT ACT, are notoriously influenced during CPB by factors other than heparin that frequently lead to an artificial prolongation of their values. Thus, although the ACT test is a convenient and efficient measure of heparin effect, sole reliance on this value to regulate heparin administration may not be advisable.

Agreement between whole blood heparin concentration measured at the bedside using the Hepcon HMS instrument and plasma heparin concentration measured in the laboratory using an antifactor Xa assay has been extensively studied in the adult cardiac population and found to be excellent. However, agreement between these different methods of measuring heparin concentration has not been extensively studied in the pediatric population. Gruenwald et al., who conducted an investigation evaluating correlation only and not agreement, reported a poor correlation between these 2 methods in 51 patients younger than 1 year of age undergoing CPB. The authors postulated that the inaccuracy of the Hepcon instrument in these infants resulted from the extreme hemodilution often encountered during pediatric CPB. Although Gruenwald et al. did not quantify the amount of priming volume used in their protocol, it is likely that it was much larger than the priming volumes used today. Recent modifications in CPB circuits to reduce the priming volume of oxygenators, arterial line filters, and circuit tubing have ameliorated...
some of the impact of hemodilution. The priming volume used in our study was limited to 300 mL for all infants. Given this restriction, our study found strong agreement between bedside measurements of whole blood heparin concentration and laboratory measurements of plasma heparin concentration.

When considering the relationship between Hepcon-measured whole blood heparin concentration and laboratory-measured plasma heparin concentration, it can be argued that correlation is a measure of association and not necessarily a measure of agreement. Clinical methodologies designed to measure the same quantity are often linearly associated and therefore correlate and, yet, may not be interchangeable within a clinically acceptable range. Thus, we performed an analysis of agreement between Hepcon-measured whole blood heparin concentration and laboratory-measured antifactor Xa heparin concentration using the method of

Figure 3. A, Bland and Altman plot of agreement between Hepcon whole blood heparin concentrations and antifactor Xa plasma heparin concentrations when measured immediately after heparin administration before the initiation of cardiopulmonary bypass. Solid line represents the mean bias between the 2 measures (0.67 U/mL); dotted lines represent the limits of agreement between the 2 measures (−1.52, 2.87). B, Bland and Altman plot of agreement between Hepcon whole blood heparin concentrations and antifactor Xa plasma heparin concentrations when measured immediately before the termination of cardiopulmonary bypass. Solid line represents the mean bias between the 2 measures (0.49 U/mL); dotted lines represent the limits of agreement between the 2 measures (−1.64, 2.61).

Figure 4. Scatterplot of baseline antithrombin (AT) activity versus heparin dose-response (HDR) assay. Solid line represents line of best fit; dotted lines represent 95% confidence interval.
Bland and Altman, an analysis frequently used to compare a new methodology against a previously established one. Using this analysis, we showed that, 3 minutes after the administration of the heparin bolus, >95% of the sample values were within the limits of agreement or within 2 standard deviations. Immediately before the termination of CPB, 93% of the values were within this range. This analysis indicates satisfactory agreement between these 2 methodologies and supports the use of the Hepcon HMS instrument to monitor heparin concentration during CPB in infants younger than 6 months. Additionally, it should be remembered that the Hepcon HMS instrument is not intended to replace laboratory determinations of heparin concentration but, rather, to enhance our clinical ability to determine an accurate degree of anticoagulation in a timely fashion, which we otherwise assess only by the ACT.

We found that the difference between Hepcon-measured heparin concentration and laboratory-measured heparin concentration became greater as mean heparin values increased. This divergence is observed in our results in 2 ways. First, we found a greater degree of agreement between these 2 methodologies at lower heparin concentrations. Concordance was stronger when heparin concentration was measured immediately before the termination of CPB, a time when mean heparin concentration is lower, than when it was measured immediately after the initial heparin bolus, a time when mean heparin concentration is larger. Second, as observed in the Bland and Altman plot performed before the termination of CPB (Fig. 3B), there is a greater discrepancy between the 2 methods as heparin concentration increases above approximately 3 U/mL. Similarly, in an earlier study of adult cardiac patients, Raymond et al. found greater variation between the 2 methods as the antifactor Xa heparin concentration exceeded 3.4 U/mL. Perhaps the most important finding was that agreement is tighter at lower heparin concentrations, a time when it is most critical to be able to detect inadequate anticoagulation.

Although the chromogenic antifactor Xa assay is considered to be the “gold standard” for measuring heparin concentration, different commercially available products do not yield equivalent results. It has been suggested that these differences may be attributable to the differing sensitivities of each product to endogenous AT levels. Because the principal action of unfractionated heparin is to accelerate the catalytic action of AT, a decrease in endogenous AT for any reason would result in a decrease in heparin effectiveness. Thus, most antifactor Xa assays are performed with the addition of exogenous AT to standardize for differing endogenous AT levels seen in different clinical settings or in different patient populations. This effect becomes important particularly in the pediatric population in which infants younger than 3 months have significantly low endogenous AT levels. In this setting, the addition of exogenous AT to the antifactor Xa assay could be considered an artificial measurement, not accurately reflecting the true in vivo anticoagulant effect of heparin. Therefore, it is not surprising that we found an increased measurement bias in heparin concentration when determined by the antifactor Xa assay as compared with the Hepcon HMS instrument. Further studies are needed to determine whether the use of exogenous AT in the antifactor Xa assay introduces a source of error to this gold standard when performed in neonates and young infants.

As previously described, heparin’s primary anticoagulant action is a result of its ability to facilitate the inhibition of circulating thrombin. This action is dependent on the binding of heparin to AT, causing a conformational change in its molecular structure, thus transforming it from a poor to a rapid inhibitor of thrombin. Indeed, heparin effect has been shown to be dependent on coupling with AT. Based on this physiology, any device proclaiming to accurately project an HDR assay, i.e., the determination of the amount of heparin required to reach a desired ACT value, should be influenced by AT activity. We found that the heparin amount determined by the HDR assay did indeed have a strong negative association with AT activity. Those infants with the least AT activity required the largest amounts of heparin to reach the desired ACT. This finding helps confirm the validity of the HDR assay.

It is clear that the number of neonates and young infants undergoing early reparative cardiac surgery requiring CPB continues to increase and that the management of these infants continues to be refined at the biochemical and nanomolecular levels. A limitation of this investigation is that we did not measure differences in thrombin generation with the different methodologies. Although our data are significant in the laboratory arena, further work is needed to determine its usefulness in improving clinical outcomes. However, our intent is that these current data will provide practical and more specific insights into the optimal effectiveness of heparin anticoagulation.

In summary, of the 3 commercially available ACT instruments used in this study, none showed a significant correlation to plasma heparin concentration when measured immediately after the initial heparin bolus. When measured immediately before the termination of CPB, only the i-STAT ACT showed a moderate correlation. Additionally, we found that bedside measurements of whole blood heparin concentration using the Hepcon HMS instrument showed satisfactory agreement with laboratory measurements of plasma heparin concentration using an antifactor Xa chromogenic assay. The level of agreement found in the Bland and Altman analysis was strong enough to support the use of the Hepcon HMS instrument to monitor heparin concentration during pediatric CPB in infants younger than 6 months old. Indeed, the level of agreement seems greatest when heparin concentrations are low, a time when the clinical assessment of adequate anticoagulation is most important. Finally, our study showed that the HDR assay performed by the Hepcon HMS instrument does have a strong inverse relationship to baseline AT activity.

**AUTHOR CONTRIBUTIONS**

NAG helped design the study, conduct the study, analyze the data, and write the manuscript. This author has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files. HGM helped conduct the study and analyze the data. This author has seen the original study data and approved the final manuscript. JDF helped conduct the study. This author has seen the original study data and approved the final manuscript. TMF helped analyze the data.
and write the manuscript. This author has seen the original study data, reviewed the analysis of the data, and approved the final manuscript. AK helped analyze the data and write the manuscript. This author has seen the original study data, reviewed the analysis of the data, and approved the final manuscript. BEM helped design the study, conduct the study, analyze the data, and write the manuscript. This author has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

REFERENCES