Heparin monitoring during cardiac surgery. Part 1: validation of whole-blood heparin concentration and activated clotting time

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There is limited published data on the agreement between techniques for monitoring heparin levels. The aim of this study was to validate the Hepcon/HMS, with particular focus on the agreement with laboratory anti-Xa assay. The performances of two ACT instruments – Hemochron and HemoTec – were also evaluated, including an assessment for interchangeability. Blood samples from 42 adult cardiopulmonary bypass (CPB) patients were analysed for activated clotting time (ACT), whole-blood heparin concentration (Hepcon/HMS) and anti-factor Xa (anti-Xa) plasma heparin concentration. Agreement between measures was determined using the method of Bland and Altman. Simple analysis of agreement between the Hepcon and anti-Xa heparin revealed the Hepcon has a mean bias of $-0.46$ U/mL, with the limits of agreement $\pm 1.12$ U/mL. The comparison between ACT instruments indicated a mean difference of $-96$ seconds for the HemoTec, with limits of $\pm 265$ seconds. The Hepcon/HMS instrument displayed satisfactory agreement with anti-Xa plasma heparin concentration, as the expected variation would not be expected to cause problems in the clinical setting. Agreement between the two measurements of ACT may be satisfactory, provided each is assigned a different target value. *Perfusion* (2003) 18, 269–276.

Introduction

Heparin is typically administered in fixed doses prior to initiation of cardiopulmonary bypass (CPB), with the heparin effect monitored by the activated clotting time (ACT) to target values that sustain flow without visible clots. Despite this, significant haemostatic activation occurs.1–5 Improved heparin dosing and monitoring techniques are likely to reduce this activation of coagulation, as intraoperative heparin levels are inversely correlated with generation of fibrin.6–8 Of particular concern is the fact that the ACT has previously been shown to correlate poorly with plasma heparin level during CPB,9–12 due to a wide range in heparin sensitivity10,13 and artificial prolongation by factors such as haemodilution and hypothermia.9,11 Alternatively, whole-blood heparin levels may be determined during CPB using an automated protamine titration device (Hepcon). The few clinical studies evaluating the Hepcon/HMS in comparison with laboratory anti-factor Xa (anti-Xa) heparin determinations have shown varying results.11,14–19 Analysis has also typically relied on the correlation coefficient to evaluate the comparison, which can be misleading as it does not indicate agreement between methods.20,21 This study was designed to assess three instruments for heparin monitoring during CPB currently available on the market: the Hemochron ACT, the HemoTec ACT and the Hepcon/HMS system, and to compare results obtained from each instrument to laboratory anti-Xa heparin concentrations. In particular, the level of agreement between heparin concentration from the Hepcon and the anti-Xa assay was studied in order to discuss the potential for reliable clinical usefulness of this instrument. Agreement between the two different methods of ACT was also measured to assess interchangeability of these results.

Methods

Forty-two adult patients undergoing first-time elective coronary artery bypass grafting (CABG) surgery were enrolled. The study was approved by the ethics
committee of both The Prince Charles Hospital and Queensland University of Technology, with informed consent given by all patients. Exclusion criteria were age less than 50 years, emergency procedures, previous cardiac surgery and previous history of stroke. Three patients received intraoperative aprotinin. This was an observational study, with the surgical team blinded to results.

Systemic anticoagulation was achieved by an initial bolus of bovine lung heparin at a dosage of 200–300 U/kg, with additional 10,000 U in the pump prime. Moderate hypothermia was used for all cases (32°C).

Blood samples obtained before initiation of CPB were drawn from a central venous catheter. Bypass samples were drawn from the CPB arterial port. All samples were obtained after withdrawal of at least three dead space volumes. Specimens were taken at the following times: baseline before surgical incision; 5 min after heparin bolus; 20, 40 and 60 min into CPB; and immediately prior to disconnection of CPB, before protamine administration. During CPB, whole-blood heparin concentration was determined in theatre by the Hepcon/HMS instrument (Medtronic Perfusion Systems, Minneapolis, MN, USA). Additionally, the celite-activated ACT (Hemochron ACT; International Technidyne, Edison, NJ, USA) and the kaolin-activated ACT (HemoTec ACT II; Medtronic Perfusion Systems, Minneapolis, MN, USA) were performed. For the three patients who received intraoperative aprotinin, kaolin-activated tubes were used for the Hemochron ACT according to manufacturer’s instructions. Remaining blood was transferred to a citrate, theophylline, adenosine and dipyridamid (CTAD) tube (Diatube H CTAD; Becton Dickinson, North Ridge, NSW, Australia) and transported to the haemostasis laboratory. At baseline ACT determinations, the Hepcon/HMS instrument was used to determine the individual patient’s heparin dose–response (HDR) curve. The performances of all three instruments were assessed at regular intervals throughout this study using appropriate quality control reagents.

On arrival at the haemostasis laboratory, blood specimens were centrifuged at 3000g for 10 min. The plasma was then stored at −80°C for later determination of heparin concentration. Plasma heparin concentration was determined using an anti-Xa chromogenic assay (IL Test Heparin, Instrumentation Laboratory, Milano, Italy), performed on the ACL Futura analyser (Instrumentation Laboratory, Milano, Italy). The assay was calibrated using the porcine mucosal standard heparin 97/578 from the National Institute for Biological Standards and Control, UK. To accommodate for the higher heparin concentrations found during bypass, samples were diluted 1:5 with the working buffer. Inter-run precision of test samples demonstrated a mean CV of 9.76% ± 4.6.

Statistics

Agreement between methods was assessed using the technique described by Bland and Altman. First, a plot of the difference between the methods against their mean was constructed: (test method − standard method) versus (test method + standard method)/2. The mean difference between measures was then plotted, with lines representing ±2 SD of the difference giving the 95% confidence range in which the values would be expected to fall. If this range is clinically acceptable, the two methods are in agreement and can be used interchangeably.

For all plots in this series, a relationship appeared to exist between the average and difference, such that the differences were proportional to the mean. Further analysis was performed to account for this, which may provide more accurate limits of agreement. The relationship was first removed by log transformation of the data. Limits of agreement were calculated from the log-transformed data, then back transformed to give the new limits of agreement, expressed as a function of the standard method.

Results

Group data across time points

Values are expressed as mean ± SD. After administration of heparin bolus, mean anti-Xa plasma heparin concentration was 4.0 ± 0.70 U/mL. This level fell to 2.7 ± 0.38 U/mL by 20 min into bypass, then slowly dropped to 2.3 ± 0.46 U/mL at termination. The Hepcon values fell in a similar manner. Conversely, ACT levels did not detect this drop in heparin levels: Hemochron ACT was 638 ± 112.7 seconds after heparin bolus, then continued at this level before dropping at the end of bypass. The Hemotec ACT started at 504 ± 109.4 seconds before first rising and then falling. These results are shown in Figure 1. The marked decline in plasma heparin from the bolus sample to the sample taken after 20 min on bypass was further investigated in a subgroup of four patients. For these patients, an additional blood sample was drawn from the CPB sampling port 3 min after initiation of bypass. Anti-Xa heparin levels significantly declined from 4.4 ± 0.21 U/mL to 2.9 ± 0.13 U/mL (p = 0.005).
Comparison of test methods with anti-Xa

Step 1. Data from each of the three test methods for heparinized samples are visualized by plotting against anti-Xa plasma heparin (Figure 2). The relationship is expressed as the correlation coefficient (r). The results for the two measures of ACT were Hemochron (r = 0.31) and HemoTec (r = 0.26), indicating that both machines have a similar poor relationship with levels of plasma heparin. Analysis of agreement between ACT and anti-Xa is not possible because the results are on a different scale. Results for the Hepcon were more strongly related with plasma heparin (r = 0.74).

Step 2. A plot of the difference between Hepcon measurements and anti-Xa is shown in Figure 3. The mean difference for the Hepcon is −0.46 U/mL, with the limits of agreement ± 1.12 U/mL. However, it appears that there may be a relationship between mean and difference, such that the differences tend to increase as the mean increases. The data were log-transformed; then new limits were generated. The limits of agreement for the logged data are −0.274 and 0.104. The antilog of these limits are 0.53 and 1.27, indicating that, for 95% of cases, the Hepcon will give values that are between 0.53 and 1.27 times the value for anti-Xa. Figure 4 shows these limits of agreement on the scatter plot of original data.

Comparison of two ACT techniques

Results from simultaneous measurements of Hemochron and HemoTec ACT during CPB demonstrate that the two instruments are only moderately corre-
lated \( r = 0.40 \). Mean results for each instrument were Hemochron 604 ± 113 seconds, HemoTec 507 ± 127 seconds, indicating a mean difference of −96 seconds for the HemoTec. The limits of agreement around this mean were ±265 seconds (Figure 5). Again there was a relationship between difference and mean, indicating the use of log transformation. The limits of agreement for the logged data were −0.284 and 0.124, with the antilog indicating that, for 95% of cases, the HemoTec ACT will give values that are between 0.52 and 1.33 times the Hemochron ACT. These limits are shown in the scatter plot of original data (Figure 6).

**Discussion**

Sufficient heparin levels must be maintained during CPB to prevent excessive haemostatic activation. In this study, the anti-Xa determinations of plasma heparin show a marked decline from the initial...
sample, suggesting that the heparin level drops immediately upon connection to bypass. This cannot be simply explained by blood dilution with prime, as this had heparin added to 5.0 U/mL, greater than the bolus dose given to patients. The rapid disappearance of heparin from the circulation may in part be due to distribution to another body compartment, and also possibly to heparin binding to the artificial surfaces. This decline in heparin level was not detected by either ACT techniques; however, Figure 1 shows that the Hepcon results dropped accordingly. The Hepcon may, therefore, be able to provide a valuable assessment of heparin concentration during CPB. However, there are few data on the agreement with plasma heparin in the clinical setting. In a previous study using 86 measurement pairs, a distinct lack of agreement between the Hepcon and anti-Xa plasma heparin concentration was reported, showing the bias and limits of agreement as \(-1.45 \pm 3.30\) U/mL.\(^{19}\) The same year a smaller study reported the bias \pm limits for Hepcon as \(0.79 \pm 2.00\) U/mL below anti-Xa heparin,\(^{18}\) although this may be unreliable due to the small number of measurements. A subsequent report by Despotis et al.\(^{16}\) did not support earlier findings, based on a bias and limits of agreement of \(0.002 \pm 1.06\) U/mL in their series of 310 Hepcon measurements converted to plasma equivalent heparin.

One other study has reported a lack of correlation between the Hepcon and anti-Xa plasma heparin concentration (\(r = -0.04\)) in 51 paediatric patients.\(^{14}\) The authors concluded that this was due to extreme haemodilution affecting Hepcon results, yet this is unlikely to be the explanation, as the end point is which channel clots first rather than actual length of the test. If the test were indeed prolonged by haemodilution, the resolution between channels (separation in clotting time) would actually improve, resulting in increased accuracy. Other studies have reported a strong correlation with plasma heparin, ranging from \(r = 0.77\) to \(r = 0.95.\)^{11,15,19} While it is difficult to explain these differences, an explanation of the variability between studies may be the inherent variability of the anti-Xa assay.\(^{24,25}\) This study supports previous findings of a strong association between the Hepcon and anti-Xa plasma heparin (\(r = 0.74\)). Furthermore, the level of agreement between measures was strong, showing the mean bias for the Hepcon as \(0.46\) U/mL below anti-Xa measurements, with the limits of agreement \(\pm 1.12\) U/mL. Log transformation of data was also used to assess agreement because there appears to be a relationship between the mean and difference. The limits generated in Figure 3 appear too wide for the lower values, although it is difficult to assess the ideal limits for results of higher magnitude from our data due to the small number of determinations above \(4\) U/mL. Closer inspection reveals that the widest band of difference relates to the Hepcon concentration of \(3.4\) U/mL. Because this value is the upper limit for one of the cartridges used during bypass, it was hypothesized that the larger difference between measures may have been caused by using cartridges covering too low a range. A review of the raw data revealed that this was not the case, as cartridges covering a high range of heparin concentration were used when anti-Xa measurements exceeded \(3.4\) U/mL. This may indicate a tendency toward greater variation at higher magnitude, although the limited data above this range in our study cannot confirm this conclusion.

The limits generated through log transformation have shown that the Hepcon may vary from 0.53 to 1.27 times the value for anti-Xa. The average heparin concentration encountered during cardiac surgery is around \(3\) U/mL. At this concentration, the limits of agreement for the Hepcon are \(1.4\) U/mL below and \(0.8\) U/mL above the anti-Xa assay. The question of what are acceptable limits for a method comparison is ideally answered before analysis of the data plot.\(^{20,21}\) Hardy et al.\(^{19}\) used \(\pm 0.7\) U/mL, as the Hepcon cannot discriminate between concentrations below \(0.7\) U/mL. However, this definition may be too strict for the range of heparin concentrations encountered during cardiac surgery. Furthermore, the Hepcon is not designed to replace laboratory anti-Xa determinations, rather to be used as a point of care monitor that is considerably more accurate than the currently used method (ACT).

In devising their procedure for method comparison, Bland and Altman argued against the use of correlation analysis to describe agreement between measures, because ‘...it would be amazing if two methods designed to measure the same quantity were not related.’\(^{20}\) While all instruments were related to anti-Xa at the level of significance, both instruments for ACT are only weakly related to plasma heparin concentration. It can be seen that ACT determinations are a poor indicator of heparin concentration. The ACT is, though, an effective measure of heparin effect, and it is not advised to replace the ACT by a device such as the Hepcon; however, the sole reliance on the ACT may not give satisfactory control over anticoagulation and subsequent reversal by protamine. The inherent variability of the ACT and the artificial prolongation during bypass lead to overestimation of heparin levels. There is no indication that influences, such as
hypothermia and haemodilution, prolonging the ACT in vitro, convey sufficient protection against clot formation in vivo. Young et al. recommended a therapeutic value for ACT during cardiac surgery of greater than 400 seconds, based on findings in a small group of monkeys, the appearance of fibrin monomer when values fell below this level. Values between 400 and 480 seconds are now widely used today. However, this describes only a minimum safe level of anticoagulation, rather than an optimal level for patient safety. Evidence of subclinical coagulation is repeated in the literature despite maintenance of ‘safe’ ACT values. This may result in a consumptive coagulopathy and increased postoperative bleeding, and may also possibly play a role in thromboembolic damage to the brain. Because generation of fibrin is inversely related to heparin concentration, maintaining ideal heparin concentration during cardiac surgery may improve patient outcomes. Previous studies have shown that heparin maintenance with the Hepcon leads to patients receiving more heparin and having evidence of reduced formation of fibrin compared with controls. Providing satisfactory agreement between Hepcon and the true concentration of heparin in the patients’ circulation, this instrument may offer valuable information on the state of anticoagulation during CPB. While there is no true ‘gold standard’ for assessing heparin concentration, the anti-Xa plasma heparin assay is usually used. We have shown that the Hepcon may vary from 0.53 to 1.27 times the value for anti-Xa plasma heparin. The slant toward lower values compared with anti-Xa heparin is expected, as the Hepcon assays whole blood rather than plasma. By taking this into account, using mean bias between measurements, the limits would be around ±1 U/mL for most cases, which are the same limits around the bias generated in Figure 3 before log transformation. This is also comparable with previous results by Despotis et al. These limits would not be expected to cause inappropriate heparin or protamine management during cardiac surgery. Importantly, the limits of agreement between measures are very tight at low heparin concentrations, when it is critical not to incorrectly assume adequate anticoagulation. This level of agreement, in conjunction with other beneficial features, such as the rapid turnaround time for results, individualized heparin-dosing protocols and more accurate protamine dosing, makes the Hepcon a useful tool in the monitoring of anticoagulation during CPB.

Comparison of the two measures of ACT during heparinization revealed that they are only moderately correlated (r = 0.40). Values from the Hemo-chron were on average 96 seconds greater than simultaneous HemoTec results, consistent with previous evaluations. The limits of agreement around this bias were ±265 seconds, although there does appear a definite relationship between difference and mean. By accounting for this relationship, it was calculated that the HemoTec may vary from 0.52 to 1.33 times the Hemochron. Further analysis revealed that the difference between measurements did not correlate with mean (r = 0.12; p = 0.14), i.e., the bias does not change with magnitude, although the range of differences does appear to increase. Therefore, the determination of agreement between measurements depends on the possible range of limits at any given magnitude, rather than the bias, because this can be accounted for using a different target ACT value. The limits of agreement that would indicate interchangeability between HemoTec and Hemochron should be similar to the level of agreement that any individual Hemochron instrument displays with another Hemochron, testing duplicate samples. We did not assess agreement between individual Hemochron instruments, though this needs to be performed before any true assessment of agreement between different methods can be accomplished.

In conclusion, this study supports the use of the Hepcon to monitor heparinization during cardiac surgery. This instrument provides a rapid, quantitative assessment of heparin concentration that correlates well with the anti-Xa heparin assay. Values obtained from the Hepcon also agree well with plasma heparin, particularly at heparin concentrations usually encountered during cardiac surgery. The difference between measures would not be expected to cause problems with clinical interpretation, and may possibly lead to improved patient outcomes over ACT-based protocols. Although the ACT correlates poorly with heparin concentration, it remains an essential part of anticoagulant monitoring to ensure adequacy of heparin effect. Provided a different target value is used, the two ACT instruments may be in satisfactory agreement with each other, although further evaluation on the agreement between instruments of the same type is warranted.

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