CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

MEASUREMENT OF HEPARIN CONCENTRATION IN WHOLE BLOOD WITH THE HEPCON/HMS DEVICE DOES NOT AGREE WITH LABORATORY DETERMINATION OF PLASMA HEPARIN CONCENTRATION USING A CHROMOGENIC SUBSTRATE FOR ACTIVATED FACTOR X

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Measurement of circulating heparin concentration has been suggested to optimize anticoagulation during cardiopulmonary bypass. The Hepcon/HMS device (Medtronic HemoTec, Inc., Parker, Colo.) uses heparin/protamine titration to quantitatively determine heparin concentration. Extensive validation of this instrument is still lacking. *Methods:* Agreement between heparin concentrations measured by the Hepcon/HMS system and by laboratory determination was evaluated in 16 patients undergoing cardiac operations. For laboratory determinations, plasma heparin concentration was derived from the measure of anti-Xa activity by means of a chromogenic substrate technique. The Hepcon/HMS instrument and cartridges measured whole blood heparin concentration. Samples were analyzed 5 minutes after administration of heparin, 15 and 30 minutes after the start of cardiopulmonary bypass, 5 minutes after aortic unclamping, at the end of cardiopulmonary bypass, and after administration of protamine. Data were plotted and interpreted according to the method of Bland and Altman: First, a difference less than 1.4 U/ml (i.e., ± 0.7 U/ml) was chosen as acceptable, because it would not cause major difficulties in clinical interpretation; second, the difference between the two measurement techniques was plotted against the mean of the two measures. *Results:* The mean difference (bias) between heparin concentrations derived by the Hepcon/HMS device and those obtained by laboratory determination was as expected for measures performed on whole blood versus plasma (1.45 U/ml). Nevertheless, heparin concentrations derived by the Hepcon/HMS device may be as much as 2.76 U/ml above or 6.17 U/ml below the concentrations measured in the laboratory, differences well outside the predetermined limits of agreement and clearly unacceptable for clinical purposes. *Conclusion:* We conclude that heparin concentrations determined with the Hepcon/HMS instrument do not agree with laboratory determination of heparin concentration. Monitoring of heparin concentrations during bypass with the Hepcon/HMS device cannot be recommended. (J Thorac Cardiovasc Surg 1996;112:154-61)

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Heparin usually is administered according to a fixed dose regimen and anticoagulation is monitored with the activated coagulation time (ACT) during cardiopulmonary bypass (CPB). This method has several drawbacks: the individual response to heparin is notoriously variable and the ACT during CPB can be prolonged by depletion of coagulation factors, hypothermia, hemodilution, and platelet dysfunction.¹ It is thought that inadequate monitoring of heparin effect can lead to subclinical coagulation, deplete clotting factors, and precipitate a post-CPB bleeding diathesis. Although the ACT

correlates well with heparin concentration before CPB, during CPB the ACT cannot be relied on to maintain an appropriate heparin concentration, as determined by the ACT measured before CPB.² Thus measurement of the circulating heparin concentration has been suggested to optimize anticoagulation during CPB.²

Plasma heparin concentration is determined in the hematology laboratory by means of an anti-factor Xa chromogenic substrate assay.³ Purified antithrombin is added to the test plasma to bind all the heparin present in the sample. Factor Xa (in excess) is neutralized by the heparin-antithrombin complex, and the remaining factor Xa hydrolyses a chromogenic substrate, liberating a chromophoric group that is read photometrically at a given wavelength. This method of determining heparin in plasma is not available to clinicians in real time because it requires specialized equipment such as a photometer, centrifugation of each blood specimen, calibration of each new lot number of the heparin kit against a heparin standard of known concentration, and performance of the actual assay by a qualified laboratory technologist.

The Hepcon/HMS hemostasis management system (Medtronic HemoTec, Inc., Parker, Colo.) is a microprocessor-based, multichannel clot-timing instrument that uses the principle of heparin/protamine titration to quantitatively determine heparin concentration in whole blood (Hepcon/HMS operator's manual, Medtronic HemoTec, Inc., 1989). Contrary to the anti-factor Xa chromogenic substrate assay, the Hepcon HMS may be used at the patient's bedside. The device was designed (1) to evaluate, *in vitro*, the patient's response to heparin, (2) to calculate the dose of heparin required on the basis of the desired ACT, estimated blood volume, and extracorporeal circuit parameters, (3) to measure the heparin concentration and calculate any additional heparin required to maintain the desired heparin concentration, and (4) to calculate the dose of protamine required to reverse heparin. Single-use multichannel test cartridges containing thromboplastin as the activator are inserted in the Hepcon/HMS device, which will then deliver a predetermined volume of blood in each sampling channel that contains a known concentration of protamine. The channel that neutralizes the heparin in the sample most closely will be the first to clot, as determined by an automated detection process. Eleven test cartridges are available to determine heparin concentration, ranging between 0 and

8.2 U/ml in increments of 0.4 (cartridge range between 0 and 1.2 U/ml) or 0.7 U/ml (all other cartridges; Heparin Assay Cartridges package insert, Medtronic HemoTec, Inc., 1992).

Only one study has compared the Hepcon/HMS automated protamine titration method with a measurement of heparin concentration obtained by a standard laboratory assay.² Whole blood heparin concentration was found to correlate extremely well with plasma heparin concentration. However, the correlation coefficient determines if two methods are related, not if they agree.⁴ Agreement between two methods means that they can be used interchangeably, which, in the present case, would be a major advantage in the clinical setting. Also, although the rationale for determining and maintaining the optimal heparin concentration during CPB is attractive, introduction of the Hepcon/HMS instrument into routine clinical practice requires extensive validation because of the initial cost of the device and, more important, of the single-use cartridges.

The aim of the study was to evaluate the agreement between determinations of circulating heparin concentration with the Hepcon/HMS device and laboratory methods to confirm the (potential) usefulness of the instrument under clinical conditions.

Methods

The study was approved by the Ethics Committee of the Montreal Heart Institute. Sixteen patients scheduled to undergo cardiac operations with extracorporeal circulation were studied. No attempt was made to standardize anesthetic, surgical, or perfusion techniques because, in actual practice, these may vary widely among professionals. Although these practice variations may be expected to result in different heparin concentrations, they should not influence agreement between the two methods of measurement.

Blood specimens were obtained from a central venous catheter, after removal of at least three dead spaces, at the following time intervals: 5 minutes after the intraauricular injection of a 300 U/kg dose of pork mucosal heparin by the surgeon; 15 and 30 minutes into CPB; 5 minutes after aortic unclamping; at the end of CPB; and 10 minutes after the administration of protamine. A completely filled 3 ml Monoject syringe (Sherwood Medical, Inc., St. Louis, Mo.) was attached to a 19-gauge 1½-inch blunt needle and inserted into the syringe holder of the automated Hepcon/HMS sample dispenser. Another 4.5 ml of blood was transferred into a blue top Vacutainer tube (Becton Dickinson & Co., Rutherford, N.J.) containing 0.5 ml sodium citrate, mixed gently, and sent to the hematology laboratory.

A cartridge with the range estimated to be appropriate for the expected heparin concentration was inserted in the Hepcon/HMS device ahead of time, inasmuch as possible.

Fig. 1. Bland-Altman plot in view of determining agreement between laboratory anti-Xa determination of plasma heparin concentration (*plasma [Hep]*) and whole blood determination of heparin concentration (*whole blood [Hep]*) with the Hepcon/HMS instrument. The *shaded area* shows the predetermined limits of agreement (± 0.7 U/ml, see text for details).

This allowed it to stabilize at approximately 37° C, the heat block temperature of the instrument. When heparin concentration measured with the Hepcon/HMS device was at the limit of detection of any cartridge, this concentration was confirmed with the use of a cartridge with a more appropriate range. Only the results obtained from (or confirmed by) central channels are reported.

On arrival at the hematology laboratory, the blood sample was centrifuged at 3500 rpm for 15 minutes. The decanted plasma was centrifuged for another 10 minutes and two aliquots were then frozen and stored at -70° C for later determination of heparin concentration. Plasma heparin concentration was derived from the measure of anti-factor Xa activity by means of a chromogenic substrate technique (Coatest heparin, Chromogenix A.B., Mölndal, Sweden).³ For heparin concentrations above 0.7 U/ml, samples were diluted as required with human normal plasma and the results multiplied by the appropriate dilution factor.

Agreement between the two methods of measurement was established by analyzing the data according to the graphic method described by Bland and Altman.^{4,5} In summary, this statistical approach involves two major steps.

Step 1. A decision must be made as to how large a difference between the two methods is permissible and will still support the conclusion that the two methods are interchangeable. Given heparin concentrations are expected to range between 3 and 6 U/ml during CPB, and

since the Hepcon/HMS device cannot discriminate between heparin concentrations smaller than 0.7 U/ml, a difference less than 1.4 U/ml (or ± 0.7 U/ml) was arbitrarily determined to be acceptable because it would not cause problems in clinical interpretation.

Step 2. The difference between the two measurement techniques is plotted against the mean of the two measures. The mean difference line (bias) and the lines representing two standard deviations on either side of the mean difference are plotted as well. The two measurement methods are judged to be interchangeable if the limits of agreement (± 2 standard deviations) do not exceed the chosen acceptable difference (step 1, represented as the *shaded area* in Fig. 1). Ideally, the bias should be minimal. However, if there is a consistent bias, the operator of either device can adjust for the bias. The Bland-Altman analysis was repeated at each measurement interval. The results of these repeated analyses are summarized in Fig. 2.

Retrospectively, to allow comparisons with the study by Despotis and associates² and to help explain the bias between plasma and whole blood heparin concentration, we used hematocrit values to convert the latter into plasma-equivalent heparin concentrations using the standard formula: Plasma-equivalent heparin concentration = (Whole blood heparin concentration $\times$ 100)/(100 - Hematocrit).

Hematocrit value was obtained from arterial blood gas analysis with the Stat Profile 9 instrument (Nova Biomed-

Fig. 2. Mean difference (black circles) ± 2 standard deviations (arrows) of the differences between laboratory anti-Xa determination of plasma heparin concentration (plasma [Hep]) and whole blood determination of heparin concentration with the Hepcon/HMS instrument (whole blood [Hep]) at different time intervals during the operation. Ao, Aortic.

ical, Waltham, Mass.) determined at 30-minute intervals during CPB. Results of measurements made 5 minutes after injection of heparin were not converted because we could not determine retrospectively whether the patient was already supported by CPB at that time. The data were analyzed by the method of Bland and Altman⁴ at each measurement interval. The results of these repeated analyses are presented in Fig. 3. Again, to allow comparisons, we calculated correlation coefficients between plasma and whole blood heparin concentration and between plasma and plasma-equivalent heparin concentration.

Results

Eighty-six pairs of measurements were available for final analysis. The correlation between plasma (laboratory) and whole blood (Hepcon/HMS device) heparin concentration was highly significant ($p < 0.0001$), with an $r = 0.769$. Fig. 1 shows the data plotted in accordance with the method of Bland and Altman.⁴ The mean difference (bias) between plasma and whole blood determination of heparin concentration was 1.45 U/ml and the standard deviation of the differences was 1.65 U/ml. It was calculated that the limits of agreement for 95% of all measurements ranged between 4.75 and -1.85 U/ml.

Fig. 2 plots the mean difference ± 2 standard deviations between plasma and whole blood heparin

concentration at each measurement interval. The bias ranged between 0.08 and 2.14 U/ml, and the standard deviations of the differences ranged between 0.71 and 2.23 U/ml. Because the limits of agreement exceeded the chosen acceptable difference of 1.4 U/ml, the two methods were judged not to be interchangeable.

Hematocrit value was available for conversion of 64 whole blood heparin concentrations into plasma-equivalent heparin concentrations. Fig. 3 shows the correlation for the 64 converted data points. The correlation between plasma and plasma-equivalent heparin concentrations was highly statistically significant ($p < 0.0001$), with an $r = 0.791$.

The mean difference ± 2 standard deviations between plasma and plasma-equivalent heparin concentration at each measurement interval is presented in Fig. 4. The bias for the converted data ranged between 0.02 and 1.74 U/ml, and the standard deviation of the differences ranged between 0.79 and 1.73 U/ml. Overall, for the 64 measurement pairs, the calculated bias and the standard deviation of the differences were 0.79 and 1.42 U/ml, respectively. The limits of agreement for 95% of all converted measurements ranged between 3.63 and -2.04 U/ml. Again, because the limits of agreement exceeded the chosen acceptable difference of 1.4 U/ml, the two methods were judged not to be interchangeable.

Fig. 3. Correlation between laboratory anti-Xa determination of plasma heparin concentration (*plasma [Hep]*) and plasma equivalent heparin concentration calculated from hematocrit value and whole blood determination of heparin concentration with the Hepcon/HMS instrument (*plasma equivalent [Hep]*).

Discussion

The attributes of the ideal operating room monitor of anticoagulation include simplicity of operation necessitating short operator attention, quickly available and reproducible results, minimal equipment using whole blood rather than plasma, and stable reagents.⁶ The Hepcon/HMS device possesses several of the characteristics required, but whole blood heparin concentration may be as much as 2.76 U/ml above or 6.17 U/ml below plasma heparin concentration measured in the hematology laboratory (see Fig. 2). Overall, only 35% of measurements (30/86 data points) were within the predetermined limits of agreement (see Fig. 1, *shaded area*). These differences are clearly unacceptable for clinical purposes.

Fig. 3 demonstrates two common errors in the medical literature. First, the plot of the data shown in the figure helps gauge the degree of agreement between the two methods of measurement of heparin concentration, but use of the correlation coefficient is inappropriate in this case and would lead the unwary reader into thinking that the two methods are interchangeable. The null hypothesis here is that the two methods are not linearly related and, as stated by Bland and Altman: "It would be amazing if two methods designed to measure the same quantity were not related."⁴ Moreover, a large range

of measures lends itself to high correlation coefficients.¹ Inasmuch as investigators usually compare two methods over the whole range of values that may be encountered in clinical practice, a high correlation is almost certain.⁴ Second, comparison of measurement techniques by the use of replicate observations from one individual may falsely improve the estimate of precision owing to the elimination of interpatient variability (Reich DL, Newsletter of the Society of Cardiovascular Anesthesiologists, April 1995, page 4). Thus, although more difficult to grasp as a whole, only the data presented in Figs. 2 and 4, summarizing the results of the Bland-Altman analyses obtained at each measurement interval, should be used to reach conclusions pertaining to the agreement between the two methods of measurement.

Theoretically the two instruments should agree, but a bias between whole blood and plasma heparin concentration is expected, because the same amount of heparin is diluted in a different volume. Predictably, whole blood heparin concentration underestimates plasma heparin concentration, and the mean bias between these two measures (1.45 U/ml) fell within the expected range of difference. Whole blood heparin concentration was retrospectively converted into plasma-equivalent heparin concentration to determine if bias and agreement could be

Fig. 4. Mean difference (black circles) $\pm$ 2 standard deviations (arrows) of the differences between laboratory anti-Xa determination of plasma heparin concentration (*plasma [Hep]*) and plasma equivalent heparin concentration calculated from hematocrit value and whole blood determination of heparin concentration with the Hepcon/HMS instrument (*plasma equivalent [Hep]*). Ao, Aortic.

improved. Conversion corrected the bias in part but did not improve substantially the agreement between the two methods. This conversion was retrospective and may have been imperfect owing to suboptimal determination of hematocrit value, but it should be recalled that such a comparison was not the primary objective of our study.

Both methods have been shown to be accurate. The performance characteristics of the Coatest heparin assay indicate a coefficient of variation between series of 2.6% and within series of 2.3% at the 0.7 U/ml level (therapeutic levels in the medical context), and the assay allows detection of 0.05 U/ml of heparin (Coatest heparin package insert, Chromogenix A.B., 1992). Some variability in the results may be introduced by inaccurate blood sampling, plasma treatment, and dilution of the sample. However, our personnel were highly trained and adhered scrupulously to the manufacturer's instructions. Similarly, a high degree of precision and accuracy was obtained with the Hepcon/HMS heparin assay cartridges in tests run on heparinized, recalcified plasma (Heparin Assay Cartridges package insert, Medtronic HemoTec, Inc., 1992). Yet a majority of cartridges allows detection of heparin concentration by only 0.7 U/ml increments. This represents an important and inherent variability of 15% or more, considering the heparin concentration usually at-

tained during CPB (4 to 5 U/ml). Precision of the whole blood determination of heparin concentration can probably be improved by using the mean of two simultaneous measures, as in the study by Despotis and associates,² but this practice is both uncommon and expensive.

Given its similarity with an ACT (whole blood assay, addition of an activator, detection of clotting as the end point), it can be postulated that the Hepcon/HMS whole blood heparin assay is affected also by those factors affecting the ACT: for example, hemodilution, hypothermia, and platelet dysfunction. In theory, all channels should be affected in like manner and the end result should not be altered. However, contrary to that of the different ACT-measuring instruments available, the reproducibility of the heparin/protamine titration instruments has not been scrutinized closely under the clinical conditions of CPB, in tests run on whole blood.¹ The coefficients of variation of five clotting methods of heparin assay in plasma have been shown to range between 3% and 78%, depending on the method tested, the heparin concentration, and the normality/abnormality of the plasma sample.⁷ If this variability of whole blood heparin/protamine titration methods in patients undergoing CPB is confirmed by future studies, such poor internal reproducibility could be one of the factors explain-

ing the lack of agreement observed between the two methods examined in the present study.

From a fundamental point of view, two other differences, related to the use of whole blood rather than plasma, must be sought to explain the lack of agreement between the two methods. First, the effects of heparin on platelets may increase the variability of whole blood clotting assays. Many of these effects demonstrated *in vitro* occur abruptly and should be applicable to patients undergoing CPB. It is clear that heparin binds avidly to platelets and induces a platelet release reaction.¹ Platelet inhibition (by prostacyclin) or platelet activation (by adenosine diphosphate) prolongs the ACT by 61% and 52%, respectively, in healthy donor blood containing heparin 2 U/ml. In blood taken from the CPB circuit of 10 patients undergoing cardiac operations and containing, presumably, a higher heparin concentration, the ACT was prolonged indefinitely by addition of the platelet-inhibitor carbacyclin, a prostacyclin analog.⁸ Moreover, impairment of collagen-induced platelet aggregation is proportional to the dose of heparin administered during CPB in human beings.⁹ Thus the effect of heparin on platelet function appears to vary with heparin concentration. It remains uncertain whether the effects of heparin on platelet function are neutralized as rapidly as those of circulating heparin. Consequently, it can be hypothesized that the lack of agreement between the plasma chromogenic assay and the Hepcon/HMS device may be explained by the confounding effect of heparin concentration on platelet function, an important component of the latter (clotting) assay. Also, inhibition of platelet activity by heparin/protamine complexes themselves^{10,11} could alter the results of the clotting assay.

Second, both techniques determine the concentration of active heparin molecules, but the methods used are different. Heparin activity depends on its dramatic acceleration of the effect of antithrombin III, a naturally occurring, slow-acting inhibitor of coagulation present in human blood. The concentration of antithrombin III present in the samples tested by the two methods varies considerably. For the plasma determination of heparin concentration, exogenous antithrombin III is added in excess to bind all the heparin molecules responsible for anticoagulation. Consequently, the activated factor X method measures the optimal effect of all the active heparin molecules present in the sample. On the other hand, during CPB, blood fed into the Hepcon/HMS analyzer contains antithrombin III concentra-

tions that vary between 47% and 75% of normal values.¹²⁻¹⁴ Theoretically, only the effect of those heparin molecules bound to antithrombin III and active *in vivo* are measured by the Hepcon/HMS device. Thus such differences in antithrombin III concentrations may certainly contribute to the lack of agreement observed between the two methods.

The costs involved by the application of new technologies with respect to the expected benefits of their introduction must be examined. The cost considerations for the purchase of instruments and disposables for automated tests are certainly among the factors that will affect the choice of a monitor of coagulation.⁶ The issue of cost is a relative one, but it cannot be argued that the cost per patient of monitoring heparin concentration is much more than that associated with the ACT. Despite appealing arguments in favor of monitoring heparin concentration, a majority of articles published up to 1993 did not demonstrate conclusively that such monitoring is responsible for decreased postoperative bleeding and use of allogeneic blood products.¹ More recently, maintenance of a "therapeutic" heparin concentration and determination of an appropriate protamine dose have been shown to reduce blood product use in patients requiring CPB.¹⁵ Alternatively, the favorable outcome observed could result simply from the use of a larger dose of heparin and a reduced protamine dosage (inasmuch as the Hepcon/HMS device tends to underestimate plasma heparin concentration) and, largely, be independent of the device used to monitor heparin concentration.

During the same period, several investigators demonstrated decreased allogeneic transfusions using different, often less costly, methods—the administration of heparin and protamine according to *in vitro* predictive tests integrating drugs, tests, and patient response (RxDx System, International Technidyne Corporation, Edison, N.J.)¹⁶; the management of patients with a transfusion algorithm based on on-site coagulation data (platelet count, prothrombin time, and activated partial thromboplastin time)¹⁷; or the institution of a thromboelastographically guided coagulation monitoring program in patients undergoing CPB.¹⁸ Thus good results (i.e., the reduction of patient exposure to allogeneic blood products¹⁹) can be achieved by a number of different approaches involving more or less sophisticated technology, the costs of which can vary considerably.

In conclusion, this study was unable to confirm the agreement between the Hepcon/HMS device

and the laboratory determination of circulating heparin concentration in patients undergoing CPB for cardiac surgical procedures. Use of the Hepcon/HMS instrument to monitor heparin concentration during CPB cannot be recommended. The physician must remember that monitoring of heparin concentration is only a surrogate for monitoring of anticoagulation. Clinicians still need a monitor of (anti-) coagulation that is as simple as the ACT, that uses whole blood rather than plasma, and that is capable of producing as rapid but more informative results.

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