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Heparin Monitoring During Cardiopulmonary Bypass Surgery Using the One-Step Point-of-Care Whole Blood Anti-Factor-Xa Clotting Assay Heptest-POC-Hi

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Abstract: The activated clotting time (ACT) generally used for monitoring heparinization during cardiopulmonary bypass (CPB) surgery does not specifically measure heparin anticoagulant activities. This may result in heparin over- or under-dose and subsequent severe adverse events. A new point-of-care whole blood clotting assay (Heptest POC-Hi [HPOCH]) for quantifying heparin anticoagulant activity specifically was compared with ACT and anti-factor Xa (anti-Xa) heparin plasma levels (Coatest heparin) in 125 patients undergoing CPB surgery. The analytical reliability of the HPOCH and the influence of preanalytical variables on assay results were also examined. The ACT and HPOCH clotting times determined throughout the entire observation period correlated closely (n = 683; r = 0.80; p < .0001). Similarly, there was a significant linear correlation between HPOCH and Coatest anti-Xa levels (n = 352; r = 0.87; p < .0001). Pre- and post-CBP values of HPOCH, ACT, and anti-Xa plasma levels correlated closely with each other (correlation coefficients between r = 0.90 and r = 0.99; p < .0001). During CPB, there was no significant relationship between ACT and whole blood or plasma heparin levels determined by HPOCH (n = 157; r = 0.19) and the chromogenic anti-Xa assay (n = 157; r = 0.04), respectively. In contrast, HPOCH and anti-Xa plasma levels correlated strongly during CPB (n = 157; r = 0.57; p < .0001). However, bias analysis showed that the HPOCH and Coatest heparin could not be used interchangeably. The HPOCH was well reproducible and not influenced by aprotinin, hemodilution, or other factors affecting ACT. The HPOCH seems to be a promising new tool for specific on-site measurement of heparin activities in whole blood during CPB.
Keywords: cardiopulmonary bypass, anticoagulation, activated clotting time, Heptest POC-Hi, chromogenic substrate heparin assay. *JECT. 2007;39:81–86*

Activated clotting time (ACT) is commonly used to monitor anticoagulation with unfractionated heparin (UFH) during cardiopulmonary bypass (CPB) surgery. However, this assay has several drawbacks resulting from limited UFH specificity. Clotting factor deficiencies, hemodilution, hypothermia, thrombocytopenia and platelet dysfunction, and the use of aprotinin have been shown to influence assay results, thus mimicking adequate heparinization that in turn may deteriorate clinical outcome significantly (1–3). Several clinical studies have therefore aimed at directly measuring circulating UFH anticoagulant activity levels to optimize anticoagulation during CPB (4–7).

Conventional anti-factor Xa chromogenic substrate and clotting assays have been developed for monitoring UFH in patients receiving heparin prophylaxis or treatment of arterial or venous thromboembolism (8,9). These methods are unsuitable for on-site UFH monitoring in CPB patients because they require sophisticated equipment, centrifugation, frequent calibration procedures, and qualified laboratory technologists. A bedside clotting test (Hepcon/HMS; Medtronic HemoTec, Parker, CO) based on heparin-protamine titration to quantify heparin concentration in whole blood during CPB has also been developed (4). However, this cumbersome assay coupled with some inherent issues has not gained popularity in clinical routine.

In this report, we present data on the performance attributes and clinical reliability of the Heptest POC-Hi (HPOCH), a new easy-to-use single-stage on-site assay for the quantification high levels of anti-FXa and anti-FIIa activities in citrated whole blood. The HPOCH operates on the same biochemical principle as that of the original Heptest heparin assay designed for the laboratory monitoring of UFH and low-molecular-weight heparin preparations in plasma (9,10).

MATERIALS AND METHODS

Selection of Patients and Study Design

After approval by the Institutional Review Board and

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obtaining individual patient consent, we embarked on a study of 125 consecutive patients undergoing elective CPB surgery (83 men and 42 women between 30 and 84 years of age). Patients were included consecutively when they gave informed consent and met the inclusion criteria. Patients were excluded when body mass index was >37 or <18 kg/m^2. Patients <18 or >85 years old or cases of severe liver dysfunction were also excluded. Other exclusion criteria included a history of hemorrhagic events, known platelet dysfunction, platelet count <100/nL, Prothrombin time (PT) international normalized ratio >1.50, and fibrinogen levels <1.50 g/L. Platelet dysfunction was assumed when diagnosed previously in the absence of antiplatelet agents or other drugs impairing platelet function. Thirty-eight of the 125 patients were already under medium- or high-dose UFH before surgery. Heparin was administered intravenously through peripheral lines or subcutaneously. Patients taking aspirin, other antiplatelet agents, or non-steroidal anti-inflammatory drugs within 5 days before surgery were also excluded.

Eighty-three patients underwent coronary artery bypass graft (CABG) surgery, 23 aortic valve replacement, 16 mitral valve replacement, and 3 aortic and mitral valve replacements. Extracorporeal circulation (ECC) was performed under moderate hypothermia (34°C). All subjects received 2 million Kallikrein inactivator units (KIU) aprotinin during the initial phase of surgery and 0.5 million KIU every consecutive hour until the end of operation. UFH was administered as a loading dose of 300 IU/kg of body weight and supplemented to maintain an ACT of >450 seconds during CPB. The membrane oxygenator and circuit were primed with lactated Ringer solution and gelatin. If additional volume was needed during ECC, packed red blood cells and Ringer solution were added to maintain hematocrit levels of 30% ± 5%. After ECC, the UFH was neutralized with protamine sulphate under monitoring with ACT.

ACT and HPOCH were determined simultaneously in duplicate before UFH bolus, 15–30 minutes after UFH bolus, twice to six times during ECC (on pump) at 30-minute intervals, and 10–15 minutes after protamine. Further measurements of ACT and HPOCH were carried out in 26 patients between 30 and 60 minutes after protamine. We also measured anti-FXa heparin activity in 65 patients.

Assays

The HPOCH from Heptest Laboratories (St. Louis, MO) is a point-of-care single-stage assay for the quantification high levels of anti-FXa and anti-FIIa activities in citrated whole blood and a modification of the original two-stage Heptest heparin assay (9,10). In contrast to the original Heptest using two separately lyophilized compounds (factor Xa and Recalmix) that have to be rehydrated with water before use, the HPOCH assay consists of a single vial containing bovine FXa, calcium chloride, phospholipids, antithrombin, prothrombin, factor V, and fibrinogen to compensate for any clotting factor deficiency as a consequence of hemodilution occurring during CPB. Four different batches of HPOCH reagents were used in this study.

Two milliliters of whole blood drawn from one arterial line was added into a 2-mL screw cap bottle containing dry sodium citrate to obtain a final citrate concentration of 0.13 mol/L. Five hundred microliters of citrated whole blood was added to one vial of HPOCH reagent, and the clot timer Heptestor (Behnk Elektronik, Norderstedt, Germany) was started simultaneously. The blood and HPOCH reagent in the assay vial were rapidly mixed and inserted into a measuring channel of the device to detect clot formation. The clotting time was directly proportional to the heparin concentration in the blood samples. Clotting times could be converted to units of UFH per milliliter of blood using a HPOCH batch-specific conversion table supplied by the manufacturer, using a current United State Pharmacopeial (USP) Reference UFH Standard. The linear heparin calibration curves extended from 0 to 10.0 units/mL. Clotting times of non-heparinized citrated blood samples and those containing 10.0 units/mL of UFH were 18 ± 2 and 120 ± 10 seconds, respectively.

The kaolin-ACT HR-ACT was determined in duplicate using an ACT II Automated Coagulation Timer and reagents from Medtronic (Duesseldorf, Germany). Anti-Xa heparin activity was assessed on a STA-R analyzer from Roche (Mannheim, Germany), using a chromogenic substrate assay (Coatest heparin) from Haemochrom (Essen, Germany). Plasma samples were diluted with human blood group AB plasma to meet the measuring range of the assay. Calibration was performed using the Fifth International Standard for Unfractionated Heparin (97/578).

HPOCH, ACT, and Coatest heparin assay results were not corrected for hematocrit.

Analytical Reliability

The intra-assay imprecision of the HPOCH was examined by 15-fold determination in citrated whole blood from one healthy individual spiked with 0, 2.5, 5.0, and 7.5 IU/mL UFH. Inter-assay imprecision was checked by analyzing citrated whole blood samples drawn on 15 consecutive days from one healthy subject and also spiked with 0, 2.5, 5.0, and 7.5 IU/mL UFH. The influence of hemodilution on HPOCH results was checked by diluting 10 citrated whole blood samples with priming solution used during CPB 1 + 0.1, 1 + 0.25, 1 + 0.5, and 1 + 1. We examined the influence of aprotinin on HPOCH by spiking 10 citrated whole blood samples with 5 IU/mL UFH and 250, 500, or 750 KIU/mL aprotinin. The sensitivity of the HPOCH was defined as the UFH activity that could be discriminated from the clotting time in samples without heparin with a probability of at least 95%.

Statistical Analysis

Results are reported in means, SD or SE, or as ranges (minimum – maximum). Coefficients of variation (CVs) were calculated to characterize intra-assay imprecision and intra-individual variation. Paired and unpaired data were compared using the paired and unpaired *t* test, respectively. Any two-tailed probability <0.05 was considered significant. The repeated-measures analysis of variance (ANOVA) with post-test and the one-way ANOVA with post-test were used to compare more than two groups of paired or unpaired data, respectively. To compensate for multiple comparisons, $p < .01$ was regarded as statistically significant. Simple regression analyses using several equations were used to find out the closest correlation between results of different assays. Two-tailed $p < .01$ was regarded as statistically significant. Agreement between HPOCH and corresponding values determined by Coatest heparin was established by analyzing the data according to the graphic method described by Bland and Altman (11). We accepted maximum differences between both assays of ±0.7 U/mL heparin as suggested by Hardy et al. (5).

Analyses were conducted using InStat for Windows software, version 3.0, and GraphPad software, version 4.02 (GraphPad Software, San Diego, CA).

RESULTS

The intra-assay and inter-assay imprecision of the HPOCH ranged from 4.3% to 7.5% CV (0 U/mL, 4.3%; 2.5 U/mL, 5.2%; 5.0 U/mL, 4.8%; 7.5 U/mL, 7.5%) and from 4.2% to 7.4% CV (0 U/mL, 4.2%; 2.5 U/mL, 5.4%; 5.0 U/mL, 4.9%; 7.5 U/mL, 7.4%), respectively. HPOCH assay results were neither affected by hemodilution ranging from 10% to 60% (vol/vol) nor by aprotinin at final concentration between 250 and 750 KIU/mL (data not shown). The detection limit of the HPOCH assay was found to be 0.3 U/mL UFH.

Peri-operative ACT and HPOCH values determined in the 125 patients before and after UFH bolus, at intervals during CPB, and after protamine administration are shown in Figure 1. Clotting times determined by both assays changed co-directionally during observation. Baseline ACT (before UFH bolus) were significantly lower than corresponding values determined after protamine (means, 126 vs. 131 seconds; $p = .015$), whereas baseline HPOCH clotting times were significantly greater than corresponding data after protamine (means, 17.2 vs. 16 seconds; $p < .0001$).

A similar graphic pattern to Figure 1 was obtained when HPOCH expressed in units per milliliter was compared with Coatest heparin results (Figure 2). Altogether, HPOCH values (in U/mL) were significantly lower than the corresponding Coatest heparin results ($n = 352$; means, 2.8 vs. 3.1 U/mL; $p < .0001$). However, no significant differences between HPOCH and corresponding Coatest anti-Xa values were observed during CPB ($n = 157$; means, 4.6 vs. 4.6 U/mL; $p = .85$).

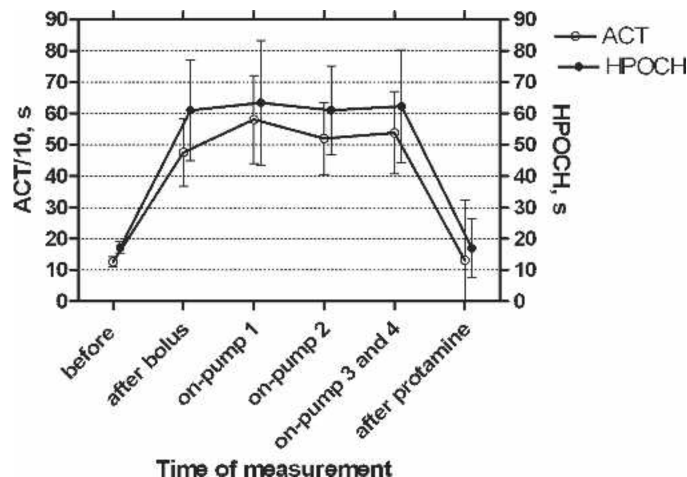

Figure 1. ACT and HPOCH (s) determined in 125 patients before and after unfractionated heparin bolus, during cardiopulmonary bypass (on-pump times 1–4), and after protamine. Left *y*-axis, ACT in seconds divided by 10 (mean ± SD); right *y*-axis, HPOCH in seconds (mean ± SD).

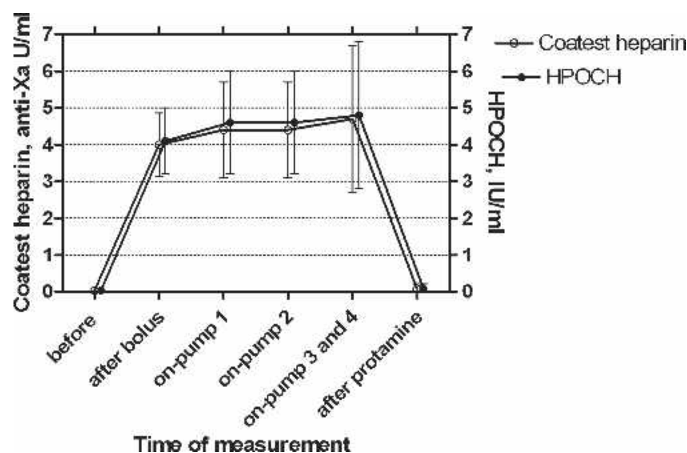

Figure 2. HPOCH (U/mL) and Coatest heparin determined in 65 patients before and after unfractionated heparin bolus, during cardiopulmonary bypass (on-pump times 1–4), and after protamine. Left *y*-axis, Coatest heparin in anti-Xa units per milliliter (mean ± SD); right *y*-axis, HPOCH in units per milliliter (mean ± SD).

The ACT and HPOCH clotting times determined throughout the entire observation period correlated closely (Figure 3). A greater correlation coefficient was observed with one non-linear simple regression model than with linear simple regression ($n = 683$; $r = 0.95$ vs. $r = 0.80$; both $p < .0001$). There was also a strong relationship between corresponding UFH activities determined by HPOCH and Coatest heparin (Figure 4). A simple linear regression model resulted in the highest correlation coefficient ($n = 352$; $r = 0.87$; $p < .0001$).

Table 1 shows the linear correlations between ACT, HPOCH clotting times, HPOCH expressed in units per

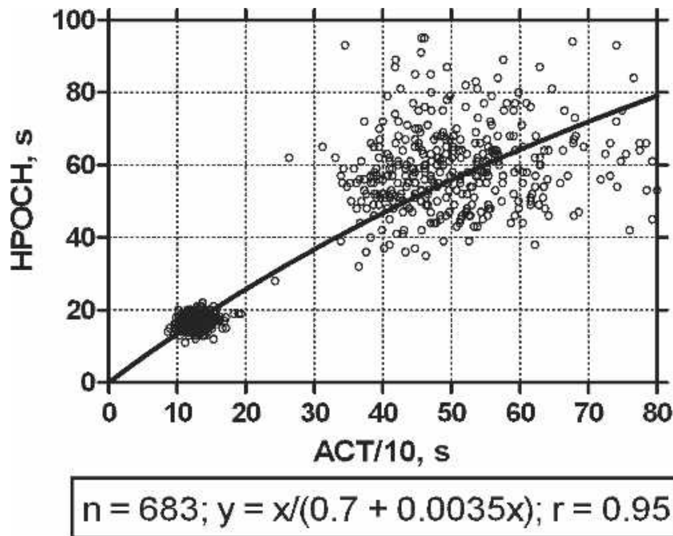

Figure 3. Correlation between ACT and HPOCH determined in whole blood samples, using a simple non-linear regression approach ($n = 683$; $r = 0.95$; $p < .0001$). *x*-axis, ACT in seconds divided by 10; *y*-axis, HPOCH in seconds.

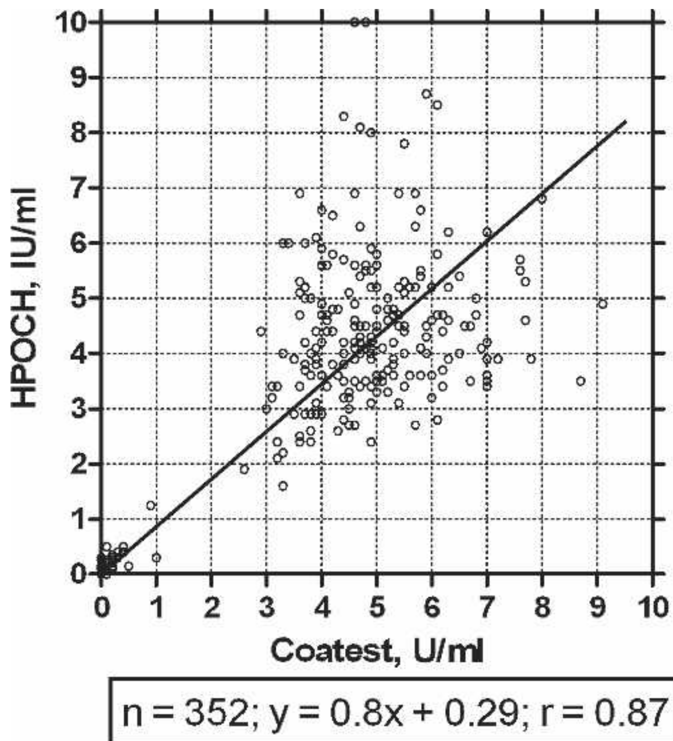

Figure 4. Correlation between Coatest heparin determined in plasma samples and HPOCH determined in whole blood samples, using a simple linear regression approach ($n = 352$; $r = 0.87$; $p < .0001$). *x*-axis, Coatest heparin in anti-Xa units per milliliter; *y*-axis, HPOCH in units per milliliter.

milliliter, and Coatest heparin levels in three different settings. In the top, when all data measured before and after UFH bolus, at intervals during CPB, and after protamine administration were compiled and analyzed as a group, there was a close relationship between all three assays. This result was especially influenced by the data shown in the middle, where only the pre- and post-UFH bolus and the post-protamine data were considered. However, when only the on-pump data were analyzed (bottom), the ACT did not correlate significantly with the HPOCH ($r = 0.19$) and the Coatest anti-Xa activity ($r = 0.04$). In contrast, there was a strong linear relationship between the HPOCH and Coatest anti-Xa results ($n = 157$; $r = 0.57$; $p < .0001$).

Table 1. Simple linear regression (Pearson correlation coefficients, level of significance 0.01).

Parameter	HPOCH (s)	HPOCH (U/mL)	Coatest Heparin (U/mL)
All data			
ACT (s)	$r = .80$	$r = .81$	$r = .78$
	$p < .0001$	$p < .0001$	$p < .0001$
	$n = 683$	$n = 352$	$n = 352$
HPOCH (s)		$r = .99$	$r = .85$
		$p < .0001$	$p < .0001$
		$n = 352$	$n = 352$
HPOCH (U/mL)			$r = .87$
			$p < .0001$
			$n = 352$
Data before and after UFH bolus and after protamine			
ACT (s)	$r = .90$	$r = .90$	$r = .91$
	$p < .0001$	$p < .0001$	$p < .0001$
	$n = 399$	$n = 195$	$n = 195$
HPOCH (s)		$r = .99$	$r = .95$
		$p < .0001$	$p < .0001$
		$n = 195$	$n = 195$
HPOCH (U/mL)			$r = 0.95$
			$p < .0001$
			$n = 195$
Data during CPB (on-pump)			
ACT (s)	$r = .23$	$r = .19$	$r = .04$
	$p = .002$	NS	NS
	$n = 284$	$n = 157$	$n = 157$
HPOCH (s)		$r = .99$	$r = .45$
		$p < .0001$	$p < .0001$
		$n = 157$	$n = 157$
HPOCH (U/mL)			$r = .57$
			$p < .0001$
			$n = 157$

Anti-Xa chromogenic assay (coatest heparin) in 125 (ACT and HPOCH in s) and 65 patients, respectively. UFH, unfractionated heparin; NS, not significant.

Figure 5 shows the data plotted according to Bland and Altman (11). The mean difference (bias) between both methods was 0.3 U/mL, and the SD of the differences was 1.2 U/mL. The limits of agreement for 95% of all measurements (±1.96 × SD) were 2.65 and –2.05 U/mL, respectively, which exceeded the acceptable range of ±0.7 U/mL considerably. Only 52% of the measurements (167 of 352 data points) were within the predetermined limits of agreement (dotted area).

DISCUSSION

High doses of UFH are necessary to mitigate the severe coagulation and platelet activation occurring at foreign

surfaces of the set-ups used for CPB (12). Anticoagulation has to be monitored precisely to minimize the potential intra- and post-operative bleeding and thromboembolism potentially resulting from either UFH over-dosing or under-dosing. ACT is still the gold standard for UFH monitoring during extracorporeal circulation despite the fact that it is adversely affected by many undesirable interferences such as hemodilution, hypothermia, thrombocytopenia, platelet dysfunction, coagulation factor deficiencies, and aprotinin (1–3). Consequently, this assay neither reflects plasma heparin levels accurately during CPB nor can it be reliably used to predict protamine requirement (1,4,13–15). It has been suggested that the use of an assay that is more specific than ACT toward heparin activity could be a promising measure to improve the safety and efficacy of anticoagulation in CPB surgery (4,16–18). A commercial on-site assay based on heparin-protamine titration for quantifying heparin concentrations in whole blood samples during CPB surgery is also available (Hepcon/HMS). However, the reliability of this test system has been discussed rather controversially (4–7). This test has not been widely used in clinical routine because of several limitations. Highly sophisticated instruments and calibration procedures are needed, and several heparin levels observed during CPC lie outside the measuring range of the assay. Apart from the fact that the Hepcon/HMS only provides a rough heparin concentration value, it also records clinically inactive, low antithrombin-binding UFH present in all commercial UFH preparations. A prospective randomized trial comparing the Hepcon/HMS and ACT for monitoring UFH in CPB patients revealed significantly increased post-operative blood loss in the Hepcon/HMS group (19).

This is the first study using a highly specific, simple on-site whole blood clotting assay for monitoring UFH in a large number of patients undergoing CPB surgery. Our pre-clinical validation studies (data not shown) showed that the HPOCH is well reproducible (intra-assay and inter-assay imprecisions of <7.5%), not affected by hemodilution up to 60% (vol/vol) nor by aprotinin or protamine concentrations commonly used in CPB surgery (750 KIU/mL and 1.25 mg/mL, respectively). Batch-specific linear calibration curves covering the whole range of values expected during operation allowed easy conversion of clotting times into units per milliliter of UFH anticoagulant activities.

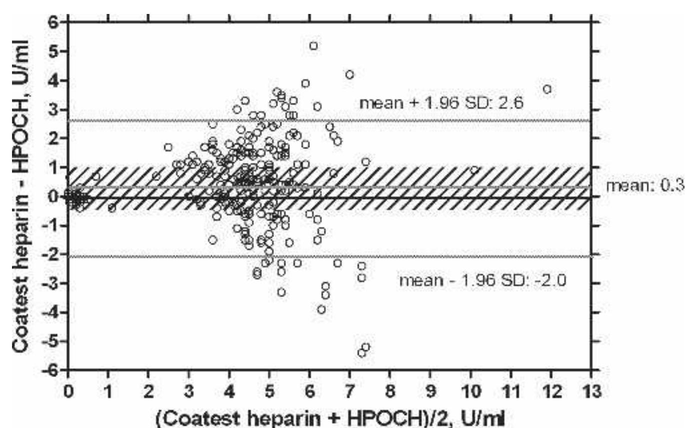

Figure 5. Bland and Altman plot for determining the agreement between Coatest heparin and HPOCH based on 252 pairs of data. Dotted area shows acceptable limits of agreement (±0.7 U/mL).

In agreement with previous findings of others (4,13–15), ACT and plasma anti-Xa heparin levels correlated closely with each other when all values or only those determined pre- and post-CPB were included in simple regression analyses (Table 1; Figures 1–4). Similarly, a close correlation between HPOCH and plasma anti-Xa activity was observed (Table 1). However, in contrast to these two findings, there lacked a relationship between ACT and heparin level measurements during CPB (Table 1). Subsequent bias analysis by the Bland and Altman method (11) showed that HPOCH and Coatest heparin assays cannot be used interchangeably (Figure 5). Our study using HPOCH is in agreement with a previous study in 16 patients using the Hepcon/HMS and Coatest heparin for measurements of whole blood and plasma heparin concentrations, respectively (5), but contradicts another trial in 62 patients showing close agreement between Hepcon/HMS and Coatest heparin (6). It can be speculated that this may be caused by the fact that heparin-protamine titration assays like the Hepcon/HMS detect both clinically active high and clinically non-active low antithrombin affinity heparins and that the percentages of high and low affinity heparins may vary from batch to batch of UFH preparations. Therefore, when comparing the Hepcon/HMS with anti-Xa assays detecting clinically active heparin only, agreement between methods will depend significantly on the percentage of clinically active, high antithrombin affinity heparin. Like the Coatest heparin, the HPOCH only measures clinically active heparin activities.

The mean bias between our whole blood HPOCH and plasma anti-Xa heparin activities was low (0.3 U/mL). However, no significant difference between assay results was observed when only corresponding values obtained during CPB were compared. Unlike previous studies (4,5), we did not convert whole blood heparin measurements to plasma equivalent values by correcting for the influence of hematocrit. There were slight but significantly lower UFH levels determined by the HPOCH compared with Coatest anti-Xa assay. This difference is mainly because of different detection limits. Different calibration procedures might also have contributed to bias between assay results. The HPOCH was calibrated against the USP standard and the Coatest heparin against the Fifth International Standard for Unfractionated Heparin (97/578). A collaborative

study for establishing the 97/587 showed that the USP standard gave 4.5% lower UFH potencies than 97/587 (20). The Coatest heparin was markedly more sensitive than the HPOCH (0.04 vs. 0.3 U/mL) and had a narrow measuring range, requiring pre-dilution of all plasma samples obtained after UFH bolus and during CPB. Although we used blood group AB plasma for pre-dilution, influences on assay results caused by matrix alterations cannot be excluded completely.

On the whole, the HPOCH clotting times in samples after protamine were significantly shorter than the baseline, pre-heparin values, whereas ACT behaved complementary.

In summary, we showed that the HPOCH is a simple, reliable, and easy-to-use point-of-care assay for measuring whole blood heparin activities in CPB surgery. Unlike the current methods for monitoring heparin in CPB surgery, it is more specific for the anticoagulant. The clinical significance of heparin monitoring by the HPOCH has to be determined in prospective clinical studies comparing this new assay with established measures using the ACT.

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