Reliability of the Heparin Management Test for Monitoring High Levels of Unfractionated Heparin

In Vitro *Findings in Volunteers* versus In Vivo *Findings during Cardiopulmonary Bypass*

Fritz Mertzlufft, M.D., Ph.D.,* Andreas Koster, M.D.,† Roland Hansen, M.D.,‡ Anne Risch, M.D.,§
Herrmann Kuppe, M.D., Ph.D.,‖ Bernhard Kübel, M.D.,# George J. Crystal, Ph.D.**

Background: The authors assessed the heparin management test *in vitro* in volunteers and *in vivo* during cardiopulmonary bypass.

Methods: In *vitro*, the heparin management test was analyzed for heparin levels between 0 and 6 IU/ml using variations in hematocrit, platelets, procoagulants, and storage time. The *in vivo* studies consisted of two groups: In group I (cardiopulmonary bypass ≤ 90 min, n = 40), anticoagulation was performed according to the activated clotting time (with or without aprotinin); in group II (cardiopulmonary bypass ≥ 180 min, with aprotinin) included use (n = 10) and nonuse of coumadin (n = 10) and anticoagulation according to the automated heparin dose–response assay. Tests were performed in duplicate (whole blood, two heparin management test analyzers) and compared with anti-Xa activity (plasma).

Results: In *vitro*, the results of the heparin management test (n = 1,070) correlated well with heparin concentration (r^2 = 0.98). Dilution and storage time did not affect the heparin management test; a hematocrit of 60% and reduced procoagulants (10%) prolonged clotting time. *In vivo*, the correlation (heparin management test *vs.* anti-Xa) was strong in group I (r^2 = 0.97 [with aprotinin] and 0.96 [without aprotinin]; n = 960) and group II without coumadin (r^2 = 0.89, n = 516). In group II with coumadin, the overall correlation was r^2 = 0.87 and 0.79 (n = 484), although the range varied widely (0.57–0.94, between-analyzer differences 0–47%).

Conclusions: The results of the heparin management test were influenced by hematocrit, plasma coagulation factors, and the heparin level, but not by use of aprotinin. The heparin management test provided reliable values *in vitro* in group I, and in group II without coumadin but was less reliable in group II with coumadin. (Key words: Activated clotting time; aprotinin; coumadin; hemodilution; heparin dose–response management; protamine; uncomplicated and prolonged perfusion; within- and between-analyzer reproducibility.)

* Professor and Vice Chair, Department of Anesthesiology and Intensive Care Medicine, University of Homburg-Saar, Germany.

† Consultant, Department of Anesthesiology, German Heart Center, Berlin, Germany.

‡ Consultant, Department of Laboratory Medicine and Pathobiochemistry, Campus Virchow-Klinikum, Charité, Berlin, Germany.

§ Consultant, Department of Anesthesiology and Intensive Care Medicine, University of Homburg-Saar, Germany.

‖ Professor and Chair, Department of Anesthesiology, German Heart Center, Berlin, Germany.

Consultant, Department of Anesthesiology and Intensive Care Medicine, University of Homburg-Saar, Germany.

** Director of Research, Department of Anesthesiology, Illinois Masonic Medical Center, and Associate Professor, Departments of Anesthesiology and of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, Illinois.

THE necessity for anticoagulation during cardiopulmonary bypass (CPB), and the wide variation in heparin sensitivity and rate of heparin metabolism among patients, warrants a reliable method to monitor either anticoagulation or blood heparin concentration at the point of patient care.[1,2]

Measurement of the activated clotting time (ACT) is used widely intraoperatively. Although the ACT provides insights into the overall status of hemostasis, it is nonspecific and correlates poorly with actual heparin levels,[2–11] particularly during prolonged periods of CPB, in which derangements occur throughout the coagulation system. These include[8,9] dilution and depletion of procoagulants because of contact activation on artificial surfaces of the heart–lung machine, activation of the intrinsic pathway that initiates fibrinolysis, and activation of platelets on the artificial surfaces, leading to a

consumption of platelets and an impairment in function of residual platelets.

Although it has not been assessed adequately, maintenance of high heparin levels during CPB has been reported to be associated with a reduced contact activation on the artificial surfaces, decreased consumption of procoagulants, and a reduced inflammatory response.[8,9,12-17] Automated protamine titration and heparin dose-response assays, such as the HepconHMS (Medtronic, Parker, CO), have been used an alternative to the ACT to assess blood heparin levels,[9,13,14,18] but studies have suggested limited reliability of the assay, as evidenced by its correlation with the chromogenic measured anti-Xa activity in plasma (i.e., the reference method).[9,15,17,19-21]

The heparin management test (HMT) has been introduced as an option for point-of-care measurements of heparin effect in citrated or native whole blood.[3] The HMT is based on the automated detection of coagulation applying an opticomechanical principle. Although previous reports[3] and studies[7,10,11] have suggested that the HMT is reliable, the data are limited in scope and do not address the effect of important variables commonly occurring during CPB, with potential influence on the measurements. The current study used in vitro and in vivo approaches to address these issues.

Materials and Methods

Heparin concentrations were measured using the HMT (TAS Analyzer; Cardiovascular Diagnostics, Raleigh, NC; distributed as Rapidpoint Coag by Bayer Diagnostics, Mishawaka, IN). In this technique, a sample of approximately 30 μl citrated whole blood or plasma is spread along a large reaction chamber of the HMT test card. The surface of this chamber is coated with celite and small iron particles. The movement of the iron particles up and down in a magnetic field allows the transmission of light to a photodetector. The formation of a clot impairs this particle movement, which reduces light transmission. Coagulation is defined as the point at which light transmission is reduced to a predetermined percentage of the initial value. The measured time for clot formation is related to a heparin level by use of a previously generated standard curve.

In Vitro Studies

With written informed consent, citrated whole blood samples (10 ml) were obtained from 10 healthy volun-
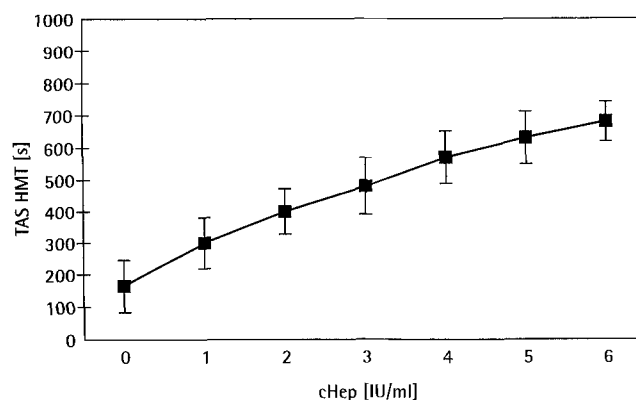
Fig. 1. Linearity of the heparin management test results (standard curve). Data are the mean ± SD (n = 70). Difference within the individual instrument 4% or less; polynomic correlation across the entire range of heparin concentration (cHep) was r^2 = 0.98; slopes were $y = 375 + 49.7x$ (0-3 IU/ml) and $y = 375 + 49.7x$ (4-6 IU/ml).

teers (classified as American Society of Anesthesiologists physical status I; 7 men, 3 women; mean age, 25 yr). The samples from five of the volunteers were used to construct a standard curve for the HMT using unfractionated heparin (UFH) to achieve blood concentrations over the range of 0-6 IU/ml (fig. 1). The relation between the HMT measurement and each heparin concentration was assessed under the following conditions: variation in hematocrit (20, 30, and 60%), obtained by centrifugation and adjustment of the plasma fraction (fig. 2A); dilution of platelets (200, 100, and 30 × 10³/μl), obtained by centrifugation of platelet-rich plasma and addition of platelet-poor plasma (fig. 2B); and dilution of procoagulants (50, 30, and 10% of the initial value), obtained via substitution of platelet-poor plasma with corresponding volumes of a 5% solution of albumin (fig. 2C). In these studies, HMT was assessed two times at each of the seven heparin concentrations to include the reproducibility of measurements within an instrument. Thus, the standard curve was composed of 70 measurements, and the remaining studies were composed of 630 measurements (2 measurements per sample × 5 blood samples × 7 heparin concentrations) at three hematocrit values (n = 210; fig. 2A), three platelet concentrations (n = 210; fig. 2B), or three levels of procoagulants (n = 210; fig. 2C).

The reproducibility of the HMT measurements between instruments and their correlation to five predetermined heparin concentrations (0, 1, 2, 4, and 6 IU/ml) were assessed in the remaining five volunteers using two HMT analyzers in parallel. These studies were composed of 250 measurements (5 measurements per sample × 2
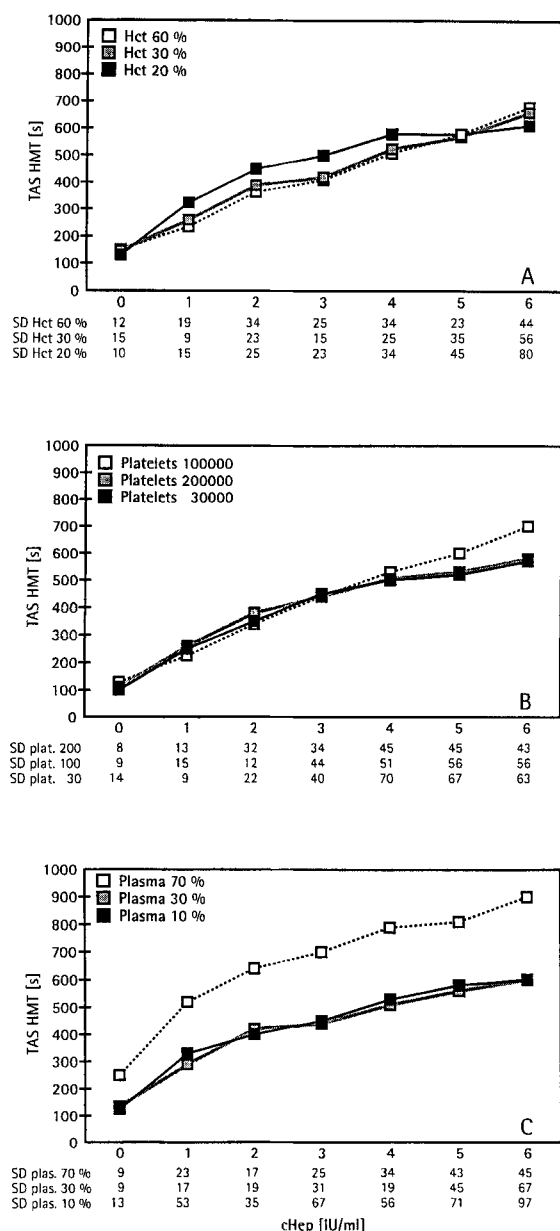
Fig. 2. Relation between the heparin management test measurement and heparin concentration (cHep) at selective variations of hematocrit (n = 210; *A*), platelets (n = 210; *B*), and procoagulants (n = 210; *C*). Data are the mean ± SD. (*A*) Correlation (r^2) between the test and the calibration curve was 0.99 over hematocrit 20 and 30%, and 0.98 at hematocrit 60% (values at hematocrit 60% significantly differed [$P < 0.05$] from hematocrit 20%). (*B*) During dilution of platelets but with constant hematocrit and plasma protein, correlation was 0.99, and values were not significantly different. (*C*) At different plasma protein contents but constant hematocrit and platelets, correlation was 0.98 (values at 10% dilution significantly differed [$P < 0.01$] from values over 50 and 30%). (*A–C*) Within-instrument difference of measurements was 4% or less (variation between the values obtained in the five volunteers averaged 15%).

instruments × 5 blood samples × 5 heparin concentrations; table 1).

The influence of storage time on the measurements of the HMT (and their relation to four predetermined heparin concentrations) was evaluated by performing three measurements on the same sample with two HMT analyzers in parallel after 10, 30, 60, 90, and 120 min of storage (n = 120; table 2).

In Vivo *Studies*

With written informed consent and approval of the local ethics committees, 60 patients undergoing cardiac surgery with CPB at either of two university hospitals participated. Forty of the patients were scheduled for elective coronary artery bypass grafting of one or two vessels (group I; table 3). The remaining 20 patients were scheduled for complex cardiac surgery (group II), including coronary artery bypass grafting in patients with impaired ventricular function (< 20%), combined coronary artery bypass grafting and valve replacement or repair, pulmonary thrombendarterectomy, thoracoabdominal aortic aneurysm repair, and heart and lung transplantation (tables 3 and 4). In this group, 10 of the patients were on therapy with coumadin (group II_{cou}); the remaining 10 patients were not (group II_{ncou}).

In the 40 patients of group I and the 10 patients of group II_{ncou}, preoperative antiaggregative therapy with salicylic acid was stopped 10 days before surgery and replaced with either a single subcutaneous bolus injection (7,500 IU) of UFH (n = 34 patients) or continuous infusion of UFH (n = 16 patients), according to values of the activated partial thromboplastin time of 40 to 60 s. In the 10 patients of group II_{cou}, preoperative anticoagulation consisted of coumadin, according to INR values of 1.8 to 2.5 (INR = international normalized ratio), which could not be replaced before surgery.

Premedication consisted of the oral administration of either midazolam, 0.05–0.1 mg/kg (group I), or clorazepate, 20 mg (group II). Induction of anesthesia was accomplished according to the standards of the two hospitals. Anesthesia was maintained using a total intravenous technique: sufentanil (0.05 $\mu g \cdot kg^{-1} \cdot h^{-1}$), propofol (100 $\mu g \cdot kg^{-1} \cdot h^{-1}$), and pancuronium bromide (supplementary if needed).

Surgery was performed under moderate (36°C rectal temperature, group I) or deeper hypothermia ($\geq$ 28–30°C, 14 patients in group II) or deep hypothermic cardiac arrest (18°C, 6 patients in group II) (tables 3 and 4).

In 20 of the patients in group I (14 males, 6 females;

Table 1. Reproducibility of the Heparin Management Test (HMT) Measurements Obtained in Two HMT Analyzers and their Correlation to Heparin Concentration (n = 125)

cHep [IU/ml]	0	1	2	4	6
n	25	25	25	25	25
HMT_1 [s]*	178.8	278.4	372.8	442.4	512.8
SD	±8.73	±2.9	±18.9	±15.1	±12.5
SEM	3.90	1.29	8.49	6.76	5.60
n	25	25	25	25	25
HMT_2 [s]†	181.2	280.0	379.6	449.2	517.6
SD	±6.1	±1.8	±11.5	±6.5	±7.3
SEM	2.73	0.84	5.16	2.92	3.28

Data are mean ± SD. Differences within an individual instrument were ≤ 4 % of the mean value; between-analyzer P values were 0.59-> 0.99 (non significant).

* Polynomic correlation HMT_1 versus cHep: r^2 = 0.9888 (y = 185,438 + 99,925x − 7,737x^2); slope HMT_1 versus cHep 0–4 IU/ml: y = 204.72 + 64.789x; slope HMT_2 versus cHep 2–6 IU/ml: 302.667 + 35x.

† Polynomic correlation HMT_2 versus entire cHep: r^2 = 0.9886 (y = 186,867 + 102,699x − 8,102x^2); slope HMT_2 versus cHep 0–4 IU/ml: y = 206.8 + 66.114x; slope HMT_2 versus cHep 2–6 IU/ml: y = 310.8 + 34.5x.

cHep = heparin concentration; HMT_1 and HMT_2 = HMT analyzers 1 and 2; SD = standard deviation; SEM = standard error of the mean.

mean age, 63 yr) and in the 20 patients in group II (13 males, 7 females; mean age, 68 yr), antifibrinolytic therapy was given using aprotinin. The therapy consisted of a 2 × 10⁶ kallikrein inhibiting units (KIU) bolus given to the patient and a bolus of 2 × 10⁶ KIU added to the priming solution, followed by a continuous infusion of 500,000 KIU/h.

In group I, systemic anticoagulation was achieved using UFH (350 IU/kg intravenous). Five minutes later, the ACT was measured (in seconds) using the Hemochron 401 device (International Technidyne Corporation, Edison, NJ). If the ACT exceeded 400 s, the extracorporeal circulation was initiated. Otherwise, an additional bolus

of 5,000 IU of UFH was given. ACT values were obtained at intervals of 30 min; if the ACT value was less than 400 s, additional intravenous bolus injections of UFH (5,000–10,000 IU) were given. At the end of CPB, heparin was reversed with an initial fixed dose of protamine (0.8 mg per milligram of total heparin). In group II, the anticoagulation protocol was based on the automated heparin dose–response assay using the HepconHMS II device (Medtronic). According to departmental standards, an ACT value of 480 s was the predetermined target, and the necessary individual heparin concentration was calculated by means of the heparin titration cartridge of the heparin dose–response assay. Five min-

Table 2. Influence of Storage Time on Heparin Management Test (HMT) Measurements and their Relationship to Heparin Concentration Obtained in Two HMT Instruments in Parallel

| cHep (IU/ml) | Storage Time (min) | | | | |
| | 10 (n = 6) | 30 (n = 6) | 60 (n = 6) | 90 (n = 6) | 120 (n = 6) |
	HMT_1/HMT_2	HMT_1/HMT_2	HMT_1/HMT_2	HMT_1/HMT_2	HMT_1/HMT_2
1	277.7/279.0	281.7/281.7	276.3/278.3	282.0/282.7	279.0/282.7
SD	±3.8/±1.7	±3.5/±1.5	±3.1/±2.1	±5.3/±3.1	±8.9/±3.2
SEM	2.2/1.0	2.0/8.8	1.8/1.2	3.1/1.8	5.1/7.6
2	369.7/371.7	374.3/378.7	373.7/375.0	368.7/369.7	370.3/372.7
SD	±9.3/±6.7	±6.7/±1.5	±7.2/±8.7	±9.5/±8.5	±9.2/±7.4
SEM	5.4/3.8	3.8/8.8	4.2/5.0	5.5/4.9	5.3/4.3
4	445.7/449.3	439.0/439.7	447.3/447.0	446.3/442.3	437.7/438.0
SD	±7.1/±3.2	±4.6/±5.5	±7.8/±4.4	±13.1/±7.8	±9.0/±6.6
SEM	4.1/1.9	2.6/3.2	4.5/2.5	7.5/4.5	5.2/3.8
6	504.3/510.3	506.7/508.3	508.3/506.3	494.3/497.3	500.3/502.0
SD	±11.8/±10.4	±10.3/±10.6	±13.7/±6.8	±8.4/±2.9	±14.2/±13.1
SEM	9.6/6.0	5.9 /6.1	7.9 /3.9	4.8/1.7	8.2/7.6
Correlation	r^2 = 0.981	r^2 = 0.971	r^2 = 0.978	r^2 = 0.983	r^2 = 0.972

Data are mean ± SD, n = 120. Within-instrument difference ≤ 4 %; between-group P values, 0.58-> 0.99.

cHep = heparin concentration; HMT_1 and HMT_2 = HMT analyzers 1 and 2; SD = standard deviation; SEM = standard error of the mean.

MERTZLUFFT *ET AL.*

Table 3. Reproducibility of HMT (s)-Measurements* and their Correlation to Chromogenic Heparin Anti-Xa Activity [U/ml; Reference Method] during Cardiopulmonary Bypass

	HMT (baseline)	HMT (5 min after heparin)	Anti-Xa (5 min after heparin)	HMT (during CPB)	Anti-Xa (during CPB)	HMT (5 min after protamine)	Anti-Xa (5 min after protamine)	HMT versus Anti-Xa
Group I								
Without aprotinin (16 M, 4 F)	151 ± 3.8 (n = 80)	342 ± 27.4 (n = 80)	4.6 ± 0.8	372 ± 27.7 (n = 240)	3.7 ± 0.83	183 ± 15.5 (n = 80)	0.05 ± 0.0	r^2 = 0.97
With aprotinin (14 M, 6 F)	149 ± 3.7 (n = 80)	389 ± 32.5 (n = 80)	4.7 ± 0.9	346 ± 23 (n = 240)	4.1 ± 0.95	194 ± 13.8 (n = 80)	0.02 ± 0.0	r^2 = 0.96
Group II$_{ncou}$								
Without coumadin (6 M, 4 F)	163 ± 4.2 (n = 40)	493 ± 42.8 (n = 40)	5.9 ± 1.1	543 ± 68 (n = 396)	5.65 ± 1.4	175 ± 16.3 (n = 40)	0.04 ± 0.01	r^2 = 0.89

Data are mean ± SD. Group I = uncomplicated CPB (≤ 90 min) either with or without aprotinin, 40 patients (30 men, 10 women, ASA III, NYHA IV, left ventricular ejection fraction 45–70 %, mean age 63 ± 8.4 yr), target ACT = 400 s (celite-based), n = 960. Group II$_{ncou}$ = complicated surgery with extended CPB (190–270 min, with aprotinin) in patients not receiving coumadin, 10 patients (ASA III–IV, NYHA IV, mean age 62 ± 2.4 yr), target ACT 480 s (heparin dose–response assay), n = 516. Within-analyzer difference ≤ 4 % of the mean value; between-analyzer P values = 0.55 –> 0.99 in group I and 0.54 –> 0.98 in group II$_{ncou}$. Surgeries in group I = 16 one-vessel coronary bypass grafting, 24 two-vessel CABG. Surgeries in group II$_{ncou}$ = three thoracoabdominal aortic aneurysm replacement (two with and one without deep hypothermic cardiac arrest); two three-vessel coronary artery bypass grafting (LVEF < 20 %); two aortic and mitral valve replacement plus two-vessel CABG; two thrombendarterectomy of the pulmonary artery in deep hypothermic cardiac arrest; one aortic arch aneurysm replacement in deep hypothermic cardiac arrest. 24 h blood loss = 185 ± 76 ml (range, 45–630 ml); 12 patients = 1–5 U packed erythrocytes and 2–6 U fresh frozen plasma; 38 patients = no allogeneic product.

* s, performed in duplicate on two analyzers in parallel.

HMT = heparin management test; Anti-Xa = antifactor Xa; CPB = cardiopulmonary bypass; CABG = coronary artery bypass graft.

utes after injection of the initial individual bolus of UFH, both the ACT and the heparin level (as provided by the heparin dose–response assay) were controlled. During the course of the surgery, the heparin concentration was measured at intervals of 30 min and adjusted to the individual target according to the calculations of the heparin dose–response assay. By the end of CPB, the protamine dose was based on the residual heparin concentration. In both groups, infusion of fresh-frozen plasma and erythrocytes was based on clinical demands and departmental standards.

In all 60 patients, whole blood samples were obtained before heparinization (baseline), 5 min after the initial bolus of UFH, during CPB (30-min intervals), and 5 min after the administration of protamine. The samples were analyzed immediately using both the HMT (whole blood) and the chromogeneic standard laboratory measurement of the heparin anti-Xa activity (citrated plasma), which

Table 4. Reproducibility of HMT [s] Measurements* and their Correlation to Chromogenic Heparin Anti-Xa Activity† during Complicated Cardiac Surgery with Cardiopulmonary Bypass‡ in Patients Treated with Coumadin (group II$_{cou}$)

HMT Analyzer	HMT (5 min after heparin, n = 40)	Anti-Xa (5 min after heparin)	SEM HMT I/II	HMT (during CPB, n = 364)	Anti-Xa (during CPB)	SEM HMT I/II	HMT (5 min after protamine, n = 40)	Anti-Xa (5 min after protamine)	SEM HMT I/II	HMT versus Anti-Xa
HMT I	547 ± 63.2	3.7 ± 0.51	12 ± 4.1	687 ± 83.2	3.4 ± 0.67	27 ± 6.7	189 ± 23.5	0.02 ± 0.0	5.2 ± 1.7	r^2 = 0.87 ± 5.1
HMT II	467 ± 47.8	3.7 ± 0.51		521 ± 48	3.4 ± 0.67		79 ± 27.1	0.03 ± 0.001		r^2 = 0.79 ± 4.7

Data are mean ± SD; target ACT = 480s (heparin dose–response assay); 10 patients (7 men, 3 women, ASA III–IV, NYHA IV, mean age 73 ± 6.3 yr); HMT I/II = HMT analyzers I and II (n = 484). HMT baseline = 159 ±5.2 s (HMT I, n = 20) and 147 ± 4.9 s (HMT II, n = 20); SEM–HMT I/II = 8.7 ± 1.2; within-analyzer difference ≤ 4 % of the mean value; between-analyzer P values = 0.001–< 0.05. Surgical procedures = three heart transplantation; two bilateral lung transplantation; one closure of infarction ventricular septal defect plus three vessel CABG; one aortic and mitral valve replacement plus tricuspid valve reconstruction; two aortic valve replacement plus three-vessel CABG; one aortic arch aneurysm replacement in deep hypothermic cardiac arrest. 24 h blood loss = 623 ± 1,230 ml (range 415–1435 ml); transfusion requirement = 2.35 ± 1.24 U packed erythrocytes, 2.51 ± 0.74 U fresh frozen plasma; 0.1 ± 0.26 U random donor platelets.

* s, performed in duplicate on two analyzers in parallel.

† U/ml, reference method.

‡ 190–320 min, with aprotinin.

HMT = heparin management test, Anti-Xa= antifactor Xa; SEM = standard error of the mean, CPB = cardiopulmonary bypass; CABG = coronary artery bypass graft.

was obtained using our current anti-Xa analyzer (ACL 3000, Instrumentation Laboratory, Kirchheim, Germany). All measurements were performed in duplicate using two HMT analyzers in parallel.

Statistic Analysis

The reproducibility of the HMT measurements (between and within instruments) and their correlation with heparin concentration were analyzed using multifactorial analysis of variance (StatView 4.02 for Macintosh; Abacus Systems, Berkeley, CA) and post hoc analysis according to the Scheffé test; the correlation was obtained by polynomic (entire range of heparin concentrations) and linear regression analysis (slope of the relation for heparin concentrations over 0–3 and 4–6 IU/ml). The differences between the HMT measurements caused by selective variations in erythrocytes, platelets, and procoagulants were analyzed using the Wilcoxon test and multifactorial analysis of variance. For all studies, $P < 0.05$ was defined as statistically significant.

Results

In Vitro Findings

The correlation between the measured HMT values and heparin concentration over the entire range of heparin concentrations was $r^2 = 0.98$, indicating a strong relation between the two variables (figs. 1 and 2 and tables 1 and 2). The slope of the curve relating HMT to heparin concentration was greater for heparin concentrations over the range 0–3 IU/ml (i.e., linearity) than it was for heparin concentrations over the range 4–6 IU/ml (fig. 1 and table 1). There was no significant difference between the measured values for HMT obtained in the two analyzers; the difference between the values obtained within a given analyzer did not exceed 4% of the mean value (figs. 1 and 2 and tables 1 and 2), and the between-analyzer P values varied between 0.58 and more than 0.99 (tables 1 and 2). The HMT values for hematocrits of 20 and 30% were similar to each other greater, but they were less than those for an hematocrit of 60% at heparin concentrations less than 3 IU/ml (fig. 2A). Selective dilution of platelets did not alter the HMT values (fig. 2B). Selective dilution of plasmatic procoagulants to 50 and 30% of baseline did not alter the HMT values, whereas dilution to 10% of baseline nearly doubled the HMT values across the entire range of heparin concentrations (fig. 2C). The HMT values were not affected by storage time (table 2).

In Vivo Findings

In the 40 patients in group I, CPB did not exceed 90 min. Systemic anticoagulation required heparin doses from 25,000 to 35,000 IU, resulting in heparin concentrations during CPB of 3.2–4.7 IU/ml, assessed by the reference anti-Xa procedure (table 3). The total heparin requirement ranged from 26,200 to 164,000 IU. The corresponding HMT values averaged 146–155 s (baseline) and 326–422 s during CPB, and they correlated well with the anti-Xa values in the absence and presence of aprotinin ($r^2 = 0.96$ and 0.97, respectively; table 3). At the termination of CPB, coagulation time ranged from 168 to 208 s, despite total reversal of heparin (table 3). The difference between HMT values obtained from the two HMT analyzers never exceeded 4% of the mean value; the between-analyzer P values were between 0.55 and more than 0.99.

In the 10 patients in group II_{ncou}, CPB duration was between 190 and 270 min (227.5 ± 27.5 min). Systemic anticoagulation required heparin doses from 27,000 to 55,000 IU, resulting in heparin concentrations during CPB of 4.1–10.2 IU/ml, assessed by the heparin dose-response assay (table 3). The total heparin requirement ranged from 65,000 to 145,000 IU. The corresponding HMT values averaged 143–179 s (baseline) and 451–790 s during CPB, and they correlated well with the anti-Xa reference ($r^2 = 0.89$; table 3). At the termination of CPB, coagulation time ranged from 79 to 203 s, despite total reversal of heparin (table 3). The difference in HMT values obtained from the two HMT analyzers never exceeded 4%; the between-analyzer values varied between 0.54 and more than 0.98.

In the 10 patients in group II_{cou}, CPB duration was between 190 and 320 min (242 ± 34.4 min). The amount of heparin necessary to achieve systemic anticoagulation ranged from 25,000 to 55,000 IU. The total heparin requirement during CPB ranged between 42,000 and 125,000 IU, producing heparin levels during CPB of 3.7–10.5 IU/ml, assessed by heparin dose-response assay (table 4). The corresponding HMT values averaged 144–167 s (baseline) and 385–768 s during CPB. The difference between the duplicate measurements within a HMT analyzer never exceeded 4% of the mean, but the difference between HMT values obtained from the two analyzers ranged from 0 to 47%. The correlation of the two HMT devices with the anti-Xa control within each patient covered a relatively wide range ($r^2 = 0.57$–0.94), although the mean correlation of each of the two analyzers (HMT vs. anti—Xa) in the total of 10 patients was acceptable ($r^2 = 0.79$ and 0.87, respectively) (table 4).

After reversal of heparin, the reproducibility was comparable to that found *in vitro* and in groups I and II$_{ncou}$, despite a wider range in coagulation time of 75–210 s (table 4).

Discussion

The HMT is an extension of the TAS Analyzer line of point-of-care anticoagulation testing, that is, activated partial thromboplastin time[22] and ecarin clotting time.[23] Although the HMT originally was designed for the requirements of the cardiac catheter laboratory, preliminary studies have reported successful use of the HMT during CPB in adults.[3] Recent studies, addressing the suitability during standard CPB,[7,10,11] have reported similar[11] or better[7,10] performance of the HMT *in vitro* and *in vivo* compared with the ACT. The current study extended this previous work by assessing under well-controlled conditions *in vitro* the influence of variables common to CPB, *e.g.*, dilution of erythrocytes and platelets, on the HMT; and by evaluating the reliability of the HMT to assess the heparin activity during different conditions of CPB, such as standard and complex (prolonged) surgeries (CPB ≤ 90 min *vs.* CPB ≤ 180 min), use or nonuse of aprotinin, different anticoagulation protocols during perfusion (ACT *vs.* automated protamine titration and heparin dose–response assay), and preoperative use or nonuse of coumadin.

Our *in vitro* studies indicated that the HMT values were reliable and reproducible (within and between instruments) and were independent of storage time (up to 120 min). The relation between the HMT values and heparin concentration was strong across the entire range of heparin concentrations (*i.e.*, a definite relation between HMT and predetermined heparin levels), but linearity was restricted to heparin concentrations less than 4 IU/ml (fig. 1 and table 1). Although a concentration of 4 IU/ml UFH rarely is achieved during cardiologic intervention, heparin levels of more than 4 IU/ml often occur during CPB and would be easier to calculate if there was linearity over the entire range of heparin.

Reductions of hematocrit to 30 and 20% had no effect on HMT results, but an increase in hematocrit to 60% was associated with a marked prolongation of the coagulation time (fig. 2A). This effect may be relevant in patients with cyanotic heart disease, who often present with significantly increased hematocrit values. The HMT result was independent of decreases in platelet concentration to as low as 30 × 10³/μl (fig. 2B). It was also

independent of decreases in procoagulant concentration to 50 and 30% of baseline, although decreases to 10% of baseline nearly doubled the HMT values (fig. 2C). Clinically, this effect could lead to underheparinization during and overprotaminization following CPB, and to clotting within the bypass circuits following the administration of coagulation factors *via* transfusion of fresh-frozen plasma, even without the administration of protamine.

During standard CPB with a fixed regimen of anticoagulation controlled by monitoring of the ACT (group I), the HMT technique also showed good reproducibility and reliability (table 3). In keeping with previous data,[3,7,10,11] there was a strong correlation between the HMT values and the chromogeneic heparin anti-Xa activity (laboratory reference) during the entire period of extracorporeal circulation. In contrast to findings obtained with the celite-based ACT,[3,6,17,18,22] this correlation was not affected by aprotinin.

The HMT method also revealed good reproducibility and reliability (table 3) even under the conditions of complex cardiac surgery and global alterations of the coagulation system. This included an extended CPB of 180 min or more in patients without preoperative therapy with coumadin, hypothermia (≥ 28–30°C), marked hemodilution (tolerated hematocrit ≈ 20%), and maintenance of the heparin levels according to the automated heparin dose–response assay compared with patients undergoing standard CPB (group II$_{ncou}$ *vs.* group I).

The impaired interinstrument reproducibility of the HMT values in the presence of coumadin (group II$_{cou}$ *vs.* group II$_{ncou}$ and group I) is another noteworthy finding (tables 3 and 4). Figure 3 demonstrates the poor reproducibility in the duplicate HMT values and anti-Xa measurements plotted separately for 3 of the 10 patients in group II$_{cou}$. This finding may be explained by the alterations in the coagulation system caused by coumadin, which were aggravated by extended perfusion times (up to 320 min), the hypothermia (18–30°C), low hematocrits (≈ 20%), and maintenance of heparin levels according to the automated heparin dose-response assay. The baseline values for the HMT were comparable to group I and group II$_{ncou}$, and the reproducibility of the HMT measurements improved after total reversal of heparin by the end of CPB (anti-Xa = 0). After total reversal of heparin, the difference between the two instruments decreased to 4%, although the HMT values after heparin reversal showed significant variation (between 75 and 210 s; fig. 3, table 4). For example, in patient 2 (fig. 3), the overestimation of blood heparin level could prompt
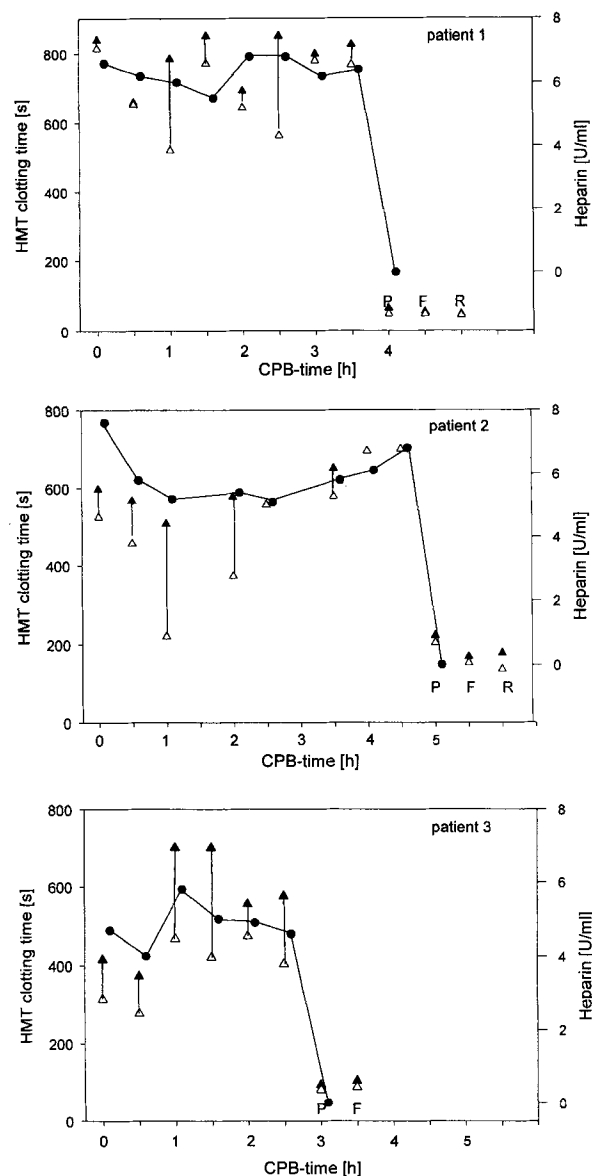
Fig. 3. Relation of duplicate heparin management test (HMT) values (two analyzers [HMT$_1$ and HMT$_2$] in parallel) and measurements of anti-Xa activity (●) shown for 3 of the 10 patients in group II$_{cou}$ (use of coumadin) during complex cardiopulmonary bypass (> 180 min; table 4). Data are plotted separately for each of the three patients: Patient 1 underwent heart transplant; patient 2, combined valve replacement (aortic, mitral) plus tricuspid reconstruction; and patient 3, closure of a ventricular septal defect (following acute infarction) plus three coronary artery grafts. P = protamine; F = fresh frozen plasma; R = random donor platelets; ▲ = HMT analyzer$_1$; △ = HMT analyzer$_2$. Correlation (r^2) of HMT to anti—Xa activity within the 3 patients was 0.91 (patient 1), 0.84 (patient 2), and 0.94 (patient 3) using HMT$_1$, and 0.93, 0.67, and 0.84 with HMT$_2$; mean correlation (r^2) of each of the two instruments in the three patients was 0.89 (HMT$_1$) and 0.80 (HMT$_2$). Between-analyzer difference covered more than 18% (patient 1), more than 40% (patient 2), and more than 23% (patient 3).

the use of excessive protamine to restore clotting (despite total reversal of heparin). Because protamine has direct anticoagulant influence on platelets and proteins, including fibrinogen,[1,16,18] this could further aggravate post-CPB disorders of the hemostatic system.[9,19] The findings in group II$_{cou}$ are consistent with the results obtained in vitro, demonstrating that a depletion of the procoagulants to less than 10% causes a marked prolongation even of the baseline HMT value.

The current study suggests the need for improvement in the reliability of the HMT. This may be achieved by the use of a more potent stimulator of coagulation in manufacturing of the HMT card (i.e., kaolin in place of celite) or coating of the card with lyophilized plasma.

The hardware may require additional features for routine clinical use. Although the HMT has satisfactory electronic quality control and several notable assets, including simplicity, compact size, and silence of operation, the absence of a digital display of clotting time is a disadvantage. As advocated for the ACT,[12] the capability for duplicate HMT measurements, i.e., two channels, is most desirable.

In conclusion, our findings suggest that the HMT (originally developed for monitoring in the catheterization laboratory) is well-suited for use in standard and even complex surgeries with prolonged CPB of 180 min or more, including deep hypothermia and use of aprotinin. The current results, however, also suggest that the HMT test card is not suitable as the sole criterion for therapeutic decisions during complex surgery with extended CPB in patients preoperatively treated with coumadin. In these patients, further studies must provide evidence that anticoagulation according to a specific range for the HMT ensures safety compared with the anticoagulation protocol based on the kaolin-based ACT (400–480 s).

References

1. Stoelting RK: Anticoagulants, Pharmacology and Physiology in Anesthetic Practice, 1st edition. Edited by Stoelting RK. Philadelphia, JB Lippincott, 1987, pp 444–53

2. Mochizuki T, Olson PJ, Szlam F, Ramsay JG, Levy JH: Protamine reversal of heparin affects platelet aggregation and activated clotting time after cardiopulmonary bypass. Anesth Analg 1998; 87:781–5

3. Risch A, Mertzlufft F: Impact of aprotinin on ACT and HMT during CABG. Anesth Analg 1998; 86(suppl 1):S95

4. Despotis GJ, Alsoufiev AL, Spitznagel E, Goodnough LT, Lappas DG: Response of kaolin ACT to heparin: evaluation with an automated assay and higher heparin doses. Ann Thorac Surg 1996; 61:795-9

5. Wang JS, Lin CY, Hung WT, Karp RB: Monitoring of heparin-induced anticoagulation with kaolin-activated clotting time in cardiac surgical patients treated with aprotinin. ANESTHESIOLOGY 1992; 77:1080-4

6. Culliford AT, Gitel SN, Starr N, Thomas ST, Baumann FG, Wessler S, Spencer FC: Lack of correlation between activated clotting time and plasma heparin during cardiopulmonary bypass. Ann Surg 1981; 193: 105-11

7. Gibbs NM, Weightman WM, Thackray NM, Michalopoulos N: Evaluation of the TAS coagulation analyzer for monitoring heparin effect in cardiac surgical patients. J Cardiothorac Vasc Anesth 1998; 12:536-41

8. Moore RA: Intraoperative evaluation of hemostasis, Blood: Hemostasis, Transfusion, and Alternatives in the Perioperative Period. Edited by Lake CL, Moore RA. New York, Raven Press, 1995, pp 179-201

9. Gravlee GP: Anticoagulation for cardiopulmonary bypass, Cardiopulmonary Bypass. Edited by Gravlee GP, Davis RF, Utely JR. Baltimore, Williams & Wilkins, 1993, pp 340-80

10. Fitch JCK, Geary KLB, Mirto GP, Byrne DW, Hines RL: Heparin management test versus activated coagulation time during cardiovascular surgery: Correlation with anti—Xa activity. J Cardiothorac Vasc Anesth 1999; 13:53-7

11. Flom-Halvorsen HI, Øvrum E, Abdelnoor M, Bjørnsen S, Brosstad F: Assessment of heparin anticoagulation: Comparison of two commercially available methods. Ann Thorac Surg 1999; 67:1012-7

12. Despotis GJ, Joist JH, Hogue CW Jr, Alsoufiev A, Joiner-Maier D, Santoro SA, Spitznagel E, Weitz JL, Goodnough LT: More effective suppression of hemostatic system activation in patients undergoing cardiac surgery by heparin dosing based on heparin blood concentrations rather than ACT. Thromb Haemost 1996; 76:902-8

13. Yamanashi H, Watanabe S, Hayashi K, Tomioka H, Minami M, Nozaki Y, Aoki T, Kawai Y, Kishino K, Ohta S: A clinical evaluation of the Hepcon/HMS: A new device of monitoring hemostasis management during cardiopulmonary bypass. Kyobu Geka 1997; 50:459-62

14. Solomon HM, Mullins RE, Lyden P, Thompson P, Hudoff S: The diagnostic accuracy of bedside and laboratory coagulation: Procedures used to monitor the anticoagulation status of patients treated with heparin. Am J Clin Pathol 1998; 109:371-8

15. Shore-Lesserson L, Reich DL, DePerio M: Heparin and protamine titration do not improve haemostasis in cardiac surgical patients. Can J Anaesth 1998; 45:10-8

16. Royston D, Bidstrup BP, Taylor KM, Sapsford RN: Effect of aprotinin on need for blood transfusion after repeat open-heart surgery. Lancet 1987; ii:1289-91

17. Okita Y, Takamoto S, Ando M, Morota T, Yamaki F, Matsukawa R, Kawashima Y: Coagulation and fibrinolysis system in aortic surgery under deep hypothermic circulatory arrest with aprotinin: The importance of adequate heparinization. Circulation 1997; 96(Suppl II): 376-81

18. Salmenperä MT, Levy JH, Harker LA: Hemostasis and cardiopulmonary bypass, Cardiopulmonary Bypass. Edited by Mora CT. New York, Springer, 1995, pp 88-113

19. Wolzt M, Weltermann A, Nieszpaur—Los M, Schneider B, Fassolt A, Lechner K, Eichler HG, Kyrle PA: Studies on the neutralizing effects of protamine on unfractionated and low-molecular-weight heparin (Fragmin) at the site of activation of the coagulation system. Thromb Haemost 1995; 73:439-43

20. Hardy JF, B'élisle S, Robitaille D, Perrault J, Roy M, Gagnon L: 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. J Thorac Cardiovasc Surg 1996; 112:154-61

21. Baugh RF, Deemar KA, Zimmermann JJ: Heparinase in the activated clotting assay: Monitoring heparin-independent alterations in coagulation function. Anesth Analg 1992; 74:201-5

22. Mertzlufft F, Risch A, Seyfert UT: Der "TAS-Analyzer:" Ein neues patientennahes Verfahren zur Bestimmung von Gerinnungsparametern (Thrombolytic Assessment System). Anaesthesist 1997; 46:233-5

23. Koster A, Kuppe H, Hetzer R, Sodian R, Crystal GJ, Mertzlufft F: Emergent cardiopulmonary bypass in five patients with heparin-induced thrombocytopenia type II employing recombinant hirudin. ANESTHESIOLOGY 1998; 89:777-80