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**Validation of rotational thromboelastometry during
cardiopulmonary bypass. A prospective, observational in-vivo study**

THESE

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Validation rotational thromboelastometry during cardiopulmonary bypass

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Résumé

Le ROTEM est un test de coagulation réalisable au près du malade qui permet d'objectiver la coagulopathie, de distinguer la contribution des différents éléments du système de coagulation et de cibler les produits procoagulants comme le plasma frais congelé (PFC), les plaquettes, le fibrinogène et les facteurs de coagulation purifiés ou les antifibrinolytiques.

3 des tests disponibles pour le ROTEM sont: EXTEM, INTEM, HEPTEM. Le premier test est stable sous hautes doses d'héparine alors que le deuxième est très sensible à sa présence. Dans le dernier test on rajoute de l'héparinase pour mettre en évidence l'éventuel effet résiduel de l'héparine en le comparant à l'INTEM.

Idéalement, le ROTEM devrait être effectué avant la fin du bypass cardiopulmonaire (CEC), donc sous anticoagulation maximale pas héparine, afin de pouvoir administrer des produits pro-coagulants dans les délais les plus brefs et ainsi limiter au maximum les pertes sanguines. En effet la commande et la préparation de certains produits procoagulants peut prendre plus d'une heure.

Le but de cette étude est de valider l'utilisation du ROTEM en présence de hautes concentrations d'héparine. Il s'agit d'une étude observationnelle prospective sur 20 patients opérés électivement de pontages aorto-coronariens sous CEC.

Méthode : l'analyse ROTEM a été réalisée avant l'administration d'héparine (T0), 10 minutes après l'administration d'héparine (T1), à la fin de la CEC (T2) et 10 minutes après la neutralisation de l'anticoagulation avec la protamine (T3). L'état d'héparinisation a été évalué par l'activité anti-Xa à T1,T2,T3.

Résultats : Comparé à T0, la phase de polymérisation de la cascade de coagulation et l'interaction fibrine-plaquettes sont significativement détériorées par rapport à T1 pour les canaux EXTEM et HEPTEM. A T2 l'analyse EXTEM et INTEM sont comparables à celles de EXTEM et HEPTEM à T3.

Conclusion: les hautes doses d'héparine utilisées induisent une coagulopathie qui reste stable durant toute la durée de la CEC et qui persiste même après la neutralisation de l'anticoagulation. Les mesures EXTEM et HEPTEM sont donc valides en présence de hautes concentrations d'héparine et peuvent être réalisés pendant la CEC avant l'administration de protamine.

ORIGINAL ARTICLE

Validation of rotational thromboelastometry during cardiopulmonary bypass

A prospective, observational in-vivo study

Fabrizio Gronchi, Anouk Perret, Enrico Ferrari, Carine M. Marcucci, Jérome Flèche, Monique Crosset, Patrick Schoettker and Carlo Marcucci

CONTEXT Rotational thromboelastometry (ROTEM) is a whole blood point-of-test used to assess the patient's coagulation status. Three of the available ROTEM tests are EXTEM, INTEM and HEPTEM. In the latter, heparinase added to the INTEM reagent inactivates heparin to reveal residual heparin effect. Performing ROTEM analysis during cardiopulmonary bypass (CPB) might allow the anaesthesiologist to anticipate the need for blood products.

OBJECTIVE The goal of this study was to validate ROTEM analysis in the presence of very high heparin concentrations during CPB.

DESIGN Prospective, observational trial.

SETTING Single University Hospital.

PARTICIPANTS Twenty patients undergoing coronary artery bypass grafting.

MAIN OUTCOME MEASURE ROTEM analysis was performed before heparin administration (T0), 10 min after heparin (T1), at the end of CPB (T2) and 10 min after protamine (T3). The following tests were performed: EXTEM, INTEM, and HEPTEM. Heparin concentrations were measured at T1 and at the end of bypass (T2).

RESULTS At T1, EXTEM differed from baseline for coagulation time: +26.7 s (18.4 to 34.9, $P < 0.0001$), α: −3° (1.0 to 5.4, $P = 0.006$) and A10: −4.4 mm (2.3 to 6.5, $P = 0.0004$). INTEM at T0 was different from HEPTEM at T1 for coagulation time: + 47 s (34.3 to 59.6, $P > 0.0001$), A10: −2.3 mm (0.5 to 4.0, $P = 0.01$) and α −2° (1.0 to 3.0; $P = 0.0007$). At T2, all parameters in EXTEM and HEPTEM related to fibrin-platelet interaction deteriorated significantly compared to T1. At T3, EXTEM and INTEM were comparable to EXTEM and HEPTEM at T2.

CONCLUSION HEPTEM and EXTEM measurements are valid in the presence of very high heparin concentrations and can be performed before protamine administration in patients undergoing cardiac surgery with CPB.

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Introduction

Cardiac surgery using cardiopulmonary bypass (CPB) is frequently complicated by haemorrhage requiring transfusion of blood products.[1] The Society of Thoracic Surgeons (STS) Adult Cardiac Surgery database shows that up to 50% of patients undergoing cardiac surgery receive blood transfusions[2] and this rate is increased in complex procedures.[3] Transfusion of blood products has been shown to negatively influence short-term and long-term outcomes.[4,5]

Classically, the activated clotting time (ACT) is used during CPB to monitor heparin anticoagulation. The ACT test is strongly dependent on, and correlates almost linearly with, high heparin levels. At the end of bypass, the usefulness of the ACT is limited due to this strong heparin dependency, as any underlying coagulopathy will be masked and can only be revealed by more sophisticated viscoelastic coagulation tests. The use of viscoelastic, whole blood point-of-care (POC) coagulation tests, in combination with transfusion protocols, reduces perioperative blood loss and transfusion requirements in high-risk procedures.[6] In cardiac surgery, these tests are usually performed after heparin reversal with

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protamine, but several authors have advocated the use of these tests during CPB, before heparin reversal, arguing that earlier detection of coagulopathies saves precious time and may result in the more effective use of haemostatic blood products. No data on blood loss were presented by these authors.[7,8] A recent study, however, showed a reduction in blood loss and improved survival when bleeding was treated using an algorithm based on rotational thromboelastometry (ROTEM; TEM International, Münich, Germany). Weber et al.[9] found a shorter 'bleed-to-treat' time to be one of the contributing factors leading to the improved outcome.

POC-based treatment algorithms often define precise thresholds for the initiation of procoagulant therapy,[10] and measurement errors may lead to unwarranted transfusion of fresh frozen plasma (FFP), platelets or procoagulant drugs. Validation of these tests in the presence of high heparin levels is, therefore, necessary for them to be used during CPB.

Tuman et al.[11] validated the use of a heparinase-modified thromboelastography (TEG) during CPB. In this study, TEG was activated by kaolin and was, therefore, highly sensitive to the effects of heparin. Heparinase is an enzyme isolated from Flavobacterium heparinum that cleaves the heparin polymer into inactive dimers and oligomers, allowing for the assessment of the underlying coagulation status of the patient.

In ROTEM, coagulation can be initiated using either the contact activator ellagic acid in the INTEM test or recombinant tissue factor (rTF) in the EXTEM test. As with kaolin, the former is highly sensitive to heparin and a heparinase-modified INTEM, called HEPTEM, is available. Conversely, according to the manufacturer, rTF remains stable in the presence of heparin concentrations up to $5\,IU\,ml^{-1}$. The reliability of ROTEM in the presence of very high concentrations of heparin has, to our knowledge, not been tested.

The primary aim of this study was to compare ROTEM results before and after protamine administration, in patients undergoing cardiac surgery with CPB. A significant difference would justify heparin reversal before coagulation testing. Conversely, if the results are comparable, therapeutic decisions based on a ROTEM-based algorithm will be the same whether the test has been performed before or after protamine administration, potentially saving time. The secondary aims were to evaluate the influence of heparin administration and CPB on the ROTEM profile.

Methods
This prospective study was performed in the University Hospital of Lausanne, Switzerland from September 2010 to September 2011. Ethical approval for this study (Ethical Committee nr. 123/10) was provided by the Ethical Committee of the Canton Vaud (Commission Cantonal d'Ethique de la Recherche sur l'Etre Humain, Lausanne, Switzerland) on 2 June 2010 (Chairperson Prof. M. Burnier). Written informed consent was obtained from all participants. Consecutive patients presenting for elective coronary artery bypass grafting were screened for enrolment. Inclusion criteria were patients aged between 18 and 65 years undergoing elective coronary artery bypass grafting, which was anticipated to be uncomplicated. Exclusion criteria were known congenital or acquired coagulopathies, hepatic disease, kidney disease requiring dialysis and preoperative treatment with oral anticoagulants, heparin, low molecular weight heparin or antiplatelet drugs other than aspirin. The latter was not an exclusion criterion owing to the high prevalence of aspirin therapy in this patient group.

All patients were premedicated with 7.5 mg of oral midazolam. Induction of anaesthesia was accomplished with intravenous midazolam 0.05 to $0.1\,mg\,kg^{-1}$, propofol 0.5 to $1\,mg\,kg^{-1}$ and fentanyl $4\,\mu g\,kg^{-1}$. Muscle relaxation was obtained using cisatracurium $0.2\,mg\,kg^{-1}$. Anaesthesia was maintained using sevoflurane before and after CPB, and a propofol infusion during normothermic CPB, titrated to a bispectral index (BIS) value between 40 and 60.

After an initial bolus of $300\,IU\,kg^{-1}$, heparin sodium derived from porcine intestinal mucosa (Braun Medical AG, Sempach, Switzerland) was titrated to achieve ACT values more than 480 s (Hemochron; ITC, Edison, New Jersey, USA). A total of $30\,mg\,kg^{-1}$ of tranexamic acid was given before heparin and a second dose of $30\,mg\,kg^{-1}$ was given after separation of CPB.

Heparin neutralisation after CPB weaning was achieved with protamine chloride (MEDA Pharmaceuticals, Wangen-Brüttisellen, Switzerland). The protamine dose was calculated as 80% of the total dose of heparin.

Blood sampling
Blood samples for anti-Xa activity measurement and ROTEM analysis were obtained through a central venous catheter and were collected in trisodium citrate anticoagulated 3 ml tubes (S-Monovette; Sarstedt, Nümbrecht, Germany). The first 10 ml of blood drawn were discarded to prevent preanalytical sources of error due to the dead volume of the central venous catheter. Blood samples were collected after sternotomy and before heparin administration (T0, baseline), 10 min after heparin administration before cannulation (T1), at the end of CPB (T2) and 10 min after protamine administration (T3).

Anti-Xa activity measurement
Serum heparin concentrations were determined by measuring anti-Xa activity using spectrophotometry (Sysmax CA-7000; Siemens, Norderstedt, Germany). After centrifugation, a five-fold dilution using standard plasma

was necessary for the heparin concentration to drop within the Sysmax C-7000 detection limits.

Rotational thromboelastometry analysis

In ROTEM, the reagents used have the same names as their specific test. For the sake of clarity, we will use upper case letters to denominate tests and lower case italic to denominate reagents (e.g. EXTEM test and *extem* reagent).

All ROTEM analyses were performed by three regular staff members of the anaesthetic department who were trained and experienced in handling of the ROTEM analyser (FG, CMM, and MC). Test results were read and interpreted by GF and MC who were unblinded for test results at different time points.

Three tests were performed using a ROTEM analyser dedicated to this study:

(1) **EXTEM**: Initiation of coagulation through the extrinsic pathway. Twenty microlitre of *extem* reagent (rTF, phospholipids and heparin inhibitor in buffer) and 20 μl of *startem* reagent (CaCl$_2$ 0.2 mol l^{-1} in HEPES buffer) are added to 300 μl of whole blood.
(2) **INTEM**: Initiation of coagulation through the intrinsic pathway. Twenty microlitre of *intem* reagent (partial thromboplastin phospholipid and ellagic acid in buffer) and 20 μl of *startem* reagent are added to 300 μl of whole blood.
(3) **HEPTEM**: Twenty microlitre of *intem* reagent and 20 μl of *heptem* reagent (heparinase in CaCl$_2$ solution) are added to 300 μl of whole blood

Comparisons

EXTEM

EXTEM at T0 was compared with T1 to detect the influence of heparin administration. EXTEM at T1 was compared with T2 to evaluate the changes in ROTEM parameters due to CPB. EXTEM at T2 was compared with T3 to evaluate whether heparin-induced changes, if present, were reversible with protamine.

INTEM

INTEM at T0 was compared with HEPTEM at T0 to exclude any direct effect of the *heptem* reagent on the INTEM test. INTEM at T1 and T2 could not be measured due to the strong effect of heparin on the test (flat line INTEM tracing during CPB). INTEM at T0 was, therefore, compared to HEPTEM at T1 to detect any changes induced by the high heparin concentration. HEPTEM at T1 was compared to HEPTEM at T2 to evaluate the changes in ROTEM parameters due to CPB. Finally, HEPTEM at T2 was compared to INTEM at T3.

The following parameters were compared for all ROTEM tests: coagulation time expressed in seconds,

angle (α) expressed in degrees, amplitude at 10 min (A10) and maximal clot firmness (MCF) both expressed in millimetres and maximum lysis at 60 min expressed as a percentage.

Reference ranges for EXTEM in our institution are coagulation time 38 to 79 s; angle 63 to 83°; A10 43 to 65 mm; MCF 50 to 72 mm; and maximum lysis less than 15%. Reference ranges for INTEM are coagulation time 100 to 240 s; angle 70 to 83°; A10 44 to 66 mm; MCF 50 to 72 mm; and maximum lysis less than 15%.

Statistical analysis

Distribution analysis was performed with the Kolmogorov–Smirnov test. To assess the mean differences between time points, a one-way analysis of variance (ANOVA) for repeated measurements was performed followed by post-hoc paired Student's t-test or Wilcoxon rank-sum test, when appropriate. P values were adjusted using the Bonferroni correction for multiple comparisons. $P < 0.05$ was considered statistically significant. Data were analysed using JMP 8.0 software (SAS Institute Inc., Cary, North Carolina, USA) and are presented as median (interquartile range, IQR) or mean (SD), unless otherwise specified.

Sample size analysis

The sample size needed for a paired Student's t-test with Bonferroni correction was calculated using the results of ROTEM measurements performed after protamine administration in historical patients in our institution. The cut-off values for treatment were defined in our local treatment algorithm (equal to the minimal normal values provided by the manufacturer). For the estimation of relevant differences between T2 and T3, we calculated the change in results in our historical patients that would have led to unwarranted therapeutic interventions according to our algorithm. Sample size analysis for a power of 0.8 and α of 0.016 (0.05/3) yielded a sample size of 10 patients for coagulation time, 11 patients for A10, five patients for MCF and 19 patients for alpha.

Results

A total of 22 patients were enrolled. The blood samples of the first two patients were used for calibration and validation of the dilution method for spectrophotometric anti-Xa measurement. Complete data for EXTEM, INTEM and HEPTEM were obtained in 20 patients. Patient characteristics are summarised in Table 1.

The results for activated partial thromboplastin time, ACT and anti-Xa activity are represented in Table 2. The mean (SD) total heparin dose used during CPB was 576 (99) IU kg^{-1}. The mean ACT was 110 (11) s at T0, 552 (122) s at T1, 495 (53) s at T2 and 118 (14) at T3. There were no significant differences for ACT between T1 and T2 ($P = 0.06$). Mean CPB duration was

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Table 1   Patient's characteristics

Patients (n)	20
Age (years)	69 (11)
Male sex	15 (75%)
BSA (m²)	2.1 (0.4)
ASA 2	2 (20%)
ASA 3	18 (80%)
Arterial hypertension	16 (76%)
Diabetes	3 (14%)
Peripheral artery disease	4 (20%)
Renal insufficiency	3 (14%)
History of myocardial infarction	5 (25%)
History of angina	5 (25%)
History of cerebrovascular disease	1 (5%)
Aspirin therapy	20 (100%)
Tobacco use	7 (33%)

ASA, American Society of Anesthesiologists; data are presented as number (proportion) or mean (SD).

77 (22) min and duration of aortic cross-clamping was 64 (22) min. The mean anti-Xa activity at T1 was 7.77 (2.38) IU ml^{-1} and remained stable throughout CPB [7.17 (2.84) IU ml^{-1} at T2, $P = 0.6$]. The mean protamine dose administered for heparin reversal was 393 (111) IU kg^{-1}.

The results for the EXTEM, INTEM and HEPTEM tests are presented in Tables 3 and 4, respectively. ROTEM parameters at T0 for EXTEM and INTEM were within normal ranges for all patients.

At T0, there was no difference between INTEM and HEPTEM for any of the parameters.

At T1, after the addition of heparin, EXTEM significantly differed from baseline for all parameters except for MCF (Fig. 1). Coagulation time was prolonged by 26.7 s (95% confidence interval, CI 18.4 to 34.9, $P < 0.0001$). The α angle was 3° lower (95% CI 1.0 to 5.4, $P = 0.006$). A10 was reduced by 4.4 mm (95% CI 2.3 to 6.5, $P = 0.0004$) and finally maximum lysis was 4% less (95% CI 2.1 to 6.0, $P = 0.0007$).

Compared to the INTEM test at T0, HEPTEM at T1 showed markedly prolonged coagulation time by 47 s (95% CI 34.3 to 59.6, $P > 0.0001$), A10 was reduced by 2.3 mm (95% CI 0.5 to 4.0, $P = 0.01$) and the α angle was slightly but significantly reduced by 2° (95% CI 1.0 to 3.0, $P = 0.0007$). Maximum lysis increased by 4.5% (95% CI 3.4 to 5.7, $P < 0.0001$; Fig. 1)

At T2, all parameters related to fibrin-platelet interaction (ALPHA, A10 and MCF) deteriorated significantly compared to the values before bypass at T1 in both EXTEM and HEPTEM tests.

Finally, at T3 after heparin reversal, EXTEM and HEPTEM were comparable to EXTEM and HEPTEM at T2, except for A10 in the EXTEM test, which improved by 4.2 mm (95% CI 1.3 to 7.0, $P = 0.0004$).

Table 2   Values for activated partial thromboplastin time, activated clotting time and anti-Xa activity at baseline (T0), after administration of heparin 300 IU kg^{-1} (T1), at unclamping of the aorta (T2) and after administration of protamine (T3)

	T0	T1	T2	T3
aPTT (s)	32.1 (3.7)	>150	>150	41.8 (15)
ACT (s)	110.2 (10.6)	552.7 (122.2)	495.8 (53.7)	118.4 (14)
Anti-Xa activity (U ml^{-1})	–	7.8 (2.4)	7.2 (2.8)	0.2 (0.1)

ACT, activated clotting time; aPTT, activated partial thromboplastin time. Data are presented as mean (SD).

Table 3   Rotational thromboelastometry values for EXTEM at baseline (T0), after administration of heparin 300 IU kg^{-1} (T1), at unclamping of the aorta (T2) and after administration of protamine (T3)

|	EXTEM T0	EXTEM T1	EXTEM T2	EXTEM T3
CT	50 [45 to 53]	72 [70 to 81]*	66 [59 to 72]§	62 [50 to 69]
α	75 [73 to 80]	73 [68 to 77]*	66 [60 to 70]§	69 [64 to 70]
A10	59 [56 to 63]	56 [49 to 60]*	48 [41 to 51]§	52 [46 to 54]†
MCF	66 [64 to 70]	64 [61 to 68]	60 [57 to 63]§	61 [55 to 63]
ML	6 [4 to 9]	1 [0 to 3]*	0 [0 to 1]	3 [2 to 4]†

CT, coagulation time; MCF, maximal clot firmness; ML, maximum lysis. *Different from EXTEM at baseline (T0). $P < 0.05$. §Different from EXTEM at T1. $P < 0.05$. †Different from EXTEM at T2. $P < 0.05$. Data are presented as median [interquartile range, IQR].

Table 4   Rotational thromboelastometry values for INTEM and HEPTEM at baseline (T0), after administration of heparin 300 IU kg^{-1} (T1), at unclamping of the aorta (T2) and after administration of protamine (T3)

	INTEM T0	HEPTEM T0	HEPTEM T1	HEPTEM T2	INTEM T3
CT	157 [137 to 168]	156 [139 to 164]	193 [183 to 214]*	183 [164 to 201]	189 [164 to 200]
α	78 [76 to 79]	78 [76 to 80]	76 [74 to 79]*	72 [67 to 74]#	74 [70 to 75]
A10	59 [55 to 61]	58 [54 to 61]	55 [53 to 61]*	49 [41 to 51]#	51 [44 to 54]
MCF	64 [61 to 68]	62 [60 to 68]	64 [61 to 68]	58 [54 to 61]#	60 [53 to 63]
ML	6 [4 to 8]	6 [4 to 8]	1 [0 to 2]*	1 [0 to 2]	3 [2 to 5]†

During cardiopulmonary bypass (CPB; T1 and T2,) INTEM yields no results. CT, coagulation time; MCF, maximal clot firmness; ML, maximum lysis. *Different from INTEM at baseline (T0). $P < 0.05$. #Different from HEPTEM at T1. $P < 0.05$. †Different from HEPTEM at T2. $P < 0.05$. Data are presented as median [interquartile range, IQR].

Fig. 1
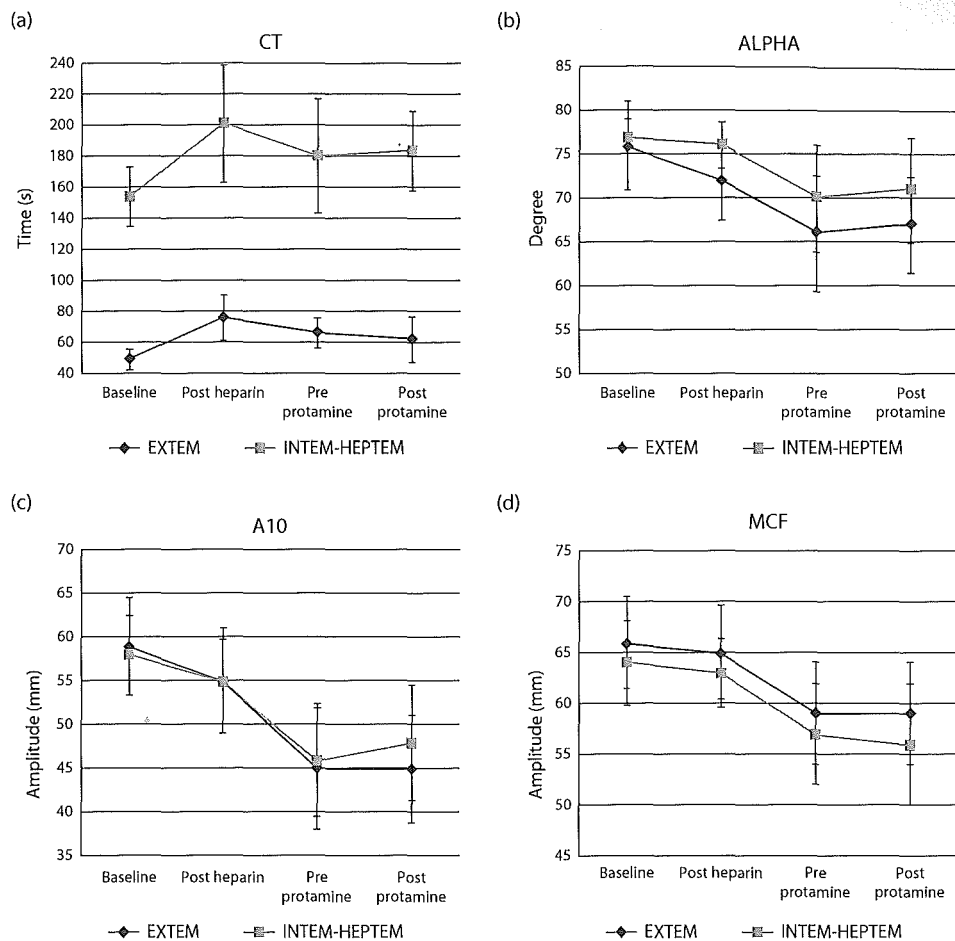
Graphical representation of rotational thromboelastometry (ROTEM) parameters at the various time points. (a) Coagulation time (CT) expressed in seconds. (b) Angle (ALPHA) expressed in degrees. (c) Amplitude at 10 min (A10) expressed in millimetres (mm). (d) Maximal clot firmness (MCF) expressed in millimetres (mm).

Discussion

In this study, we validated the EXTEM and HEPTEM assays during CPB. The administration of heparin immediately induced significant changes in rTF and ellagic acid activated ROTEM parameters. Heparinase efficiently inactivated heparin in the HEPTEM test during bypass.

The comparison of INTEM and HEPTEM at T0 showed that the *heptem* reagent does not influence any of the parameters of the INTEM test.

Comparison of the ROTEM parameters over time showed that the administration of heparin 300 UI kg^{-1} induced a significant change in all EXTEM and HEPTEM parameters except for the MCF, that there is a progressive decline of the parameters during CPB, and finally, that all parameters remain stable after the administration of protamine.

The changes we observed immediately following the administration of heparin (Fig. 2) are in contrast with the results reported by Gertler *et al.*[12] who studied the influence on ROTEM of incremental doses of heparin added *in vitro* to whole blood. They found no difference between INTEM at baseline and HEPTEM at heparin concentrations up to 8 IU ml^{-1}.

Our finding can either be the consequence of a direct influence of heparin on the ROTEM test reagents (in which case, the tests would not be valid during CPB) or they may reflect real changes in haemostasis. On one hand, concerning a possible interaction with the ROTEM tests, the manufacturer guarantees stability of the *extem* reagent up to 5 UI ml^{-1} of heparin, whereas we measured concentrations in excess of 7 UI ml^{-1}. The *extem* reagent contains a non-specified heparin inhibitor, the concentration of which might not be sufficient to

Fig. 2
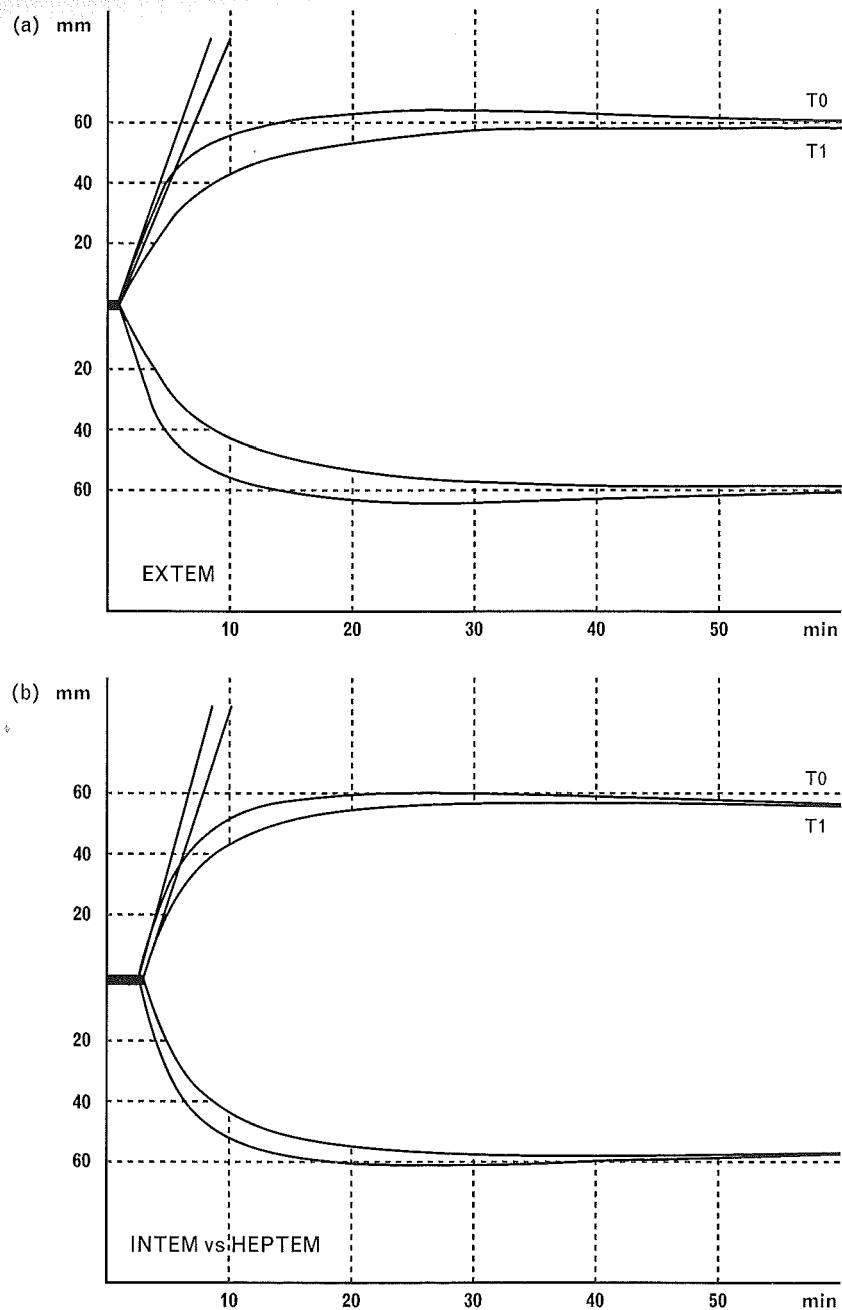
(a) EXTEM traces at baseline (T0, outer trace) and after 300 IU kg^{-1} of heparin (T1, inner trace). (b) INTEM traces at baseline (T0, outer trace) and HEPTEM after 300 IU kg^{-1} of heparin (T1, inner trace).

completely inhibit heparin at these concentrations. For the HEPTEM test, after the degradation of heparin by heparinase we expected the results to be identical to the INTEM before heparin administration. Comparing HEPTEM at T1 with baseline INTEM, however, we found most parameters to be altered significantly. This is in line with the findings of the study by Tuman *et al.*[11] conducted on TEG. One possible explanation could be

that the dimer and oligomers resulting from the cleavage of heparin may still have some minor anticoagulant effect.

On the other hand, apart from the inhibition of factors IIa and Xa through enhancement of antithrombin, heparin has numerous endothelium-related effects on various elements of haemostasis. We believe that the changes measured at T1 are actual alterations in haemostasis

rather than heparin-induced artefacts. First, in-vivo heparin rapidly displaces tissue factor pathway inhibitor (TFPI) from the endothelium.[13] The effect of heparin on TFPI is dose-dependent and peaks as soon as 3 to 10 min after intravenous heparin administration.[14] As its name suggests, TFPI inhibits the TF-VIIa complex, but it also inhibits factor Xa. Heparin increases the affinity of TFPI for Xa and accelerates the initial interaction between both molecules.[15] Second, intravenous heparin inhibits platelet macro-aggregation.[16] This effect is related to endothelial release of lipoprotein lipase.[17] The resulting increase in plasmatic lipophospholipids and non-esterified fatty acids may inhibit synthesis of thromboxane A2, a potent platelet activator.[18] Due to the endothelial contribution to heparin's inhibitory effect on platelet aggregation, this phenomenon is not observed during in-vitro studies, and yet is always present after intravenous heparin administration.[19] Heparinase neutralises heparin but has no influence on the anticoagulant proteins originating from the initial heparin-endothelial interaction. The absence of the endothelial effect of heparin in in-vitro study by Gertler *et al.* explains the difference in observations and illustrates the importance of in-vivo validation of tests on haemostasis.

Comparison of the parameters at T2 and T3 showed that there is no influence of heparin reversal. ROTEM parameters at the end of bypass (T2) were identical to the parameters after protamine administration (T3). This confirms that the endothelial related mechanisms, rather than a direct effect of heparin on the tests, were responsible for the initial changes, as any direct effect on the test should dissipate after protamine administration. Moreover, the fact that heparin reversal shows no influence on ROTEM parameters implies that ROTEM measurements can reliably be performed by the end of bypass. The opportunity to evaluate the patient's haemostatic capacity before protamine administration can possibly eliminate any unnecessary delay before the initiation of procoagulant therapy.

Although the differences we measured between time points were statistically significant, none of them were clinically relevant. With only a few exceptions, all values, for all participants, remained within normal limits, demonstrating that ROTEM is a highly sensitive tool, detecting subclinical changes in haemostatic mechanisms.

In this study, all patients were given tranexamic acid before baseline measurements. The influence of heparin on the measurement of clot lysis is, therefore, biased. The administration of antifibrinolytic drugs in cardiac surgery, is a class I(A) recommendation,[20] and part of routine patient management in our centre. Therefore, we decided not to omit this drug in our study. Moreover, the omission would not reflect clinical reality and the

results of this study would not have been applicable to our daily practice.

In conclusion, we found that HEPTEM measurements are valid in the presence of very high heparin concentrations and that the *extem* reagent is stable in the presence of heparin concentrations exceeding $5\,UI\,ml^{-1}$. Both HEPTEM and EXTEM can, therefore, be used to evaluate haemostasis during CPB.

References

1   Ascione R, Williams S, Lloyd CT, et al. Reduced postoperative blood loss and transfusion requirement after beating-heart coronary operations: a prospective randomized study. *J Thorac Cardiovasc Surg* 2001; **121**:689–696.
2   Mehta RH, Sheng S, O'Brien SM, et al. Reoperation for bleeding in patients undergoing coronary artery bypass surgery: incidence, risk factors, time trends, and outcomes. *Circ Cardiovasc Qual Outcomes* 2009; **2**:583–590.
3   Genovese EA, Dew MA, Teuteberg JJ, et al. Incidence and patterns of adverse event onset during the first 60 days after ventricular assist device implantation. *Ann Thorac Surg* 2009; **88**:1162–1170.
4   Koch CG, Li L, Duncan AI, et al. Transfusion in coronary artery bypass grafting is associated with reduced long-term survival. *Ann Thorac Surg* 2006; **81**:1650–1657.
5   Murphy GJ, Reeves BC, Rogers CA, et al. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. *Circulation* 2007; **116**:2544–2552.
6   Rahe-Meyer N, Solomon C, Winterhalter M, et al. Thromboelastometry-guided administration of fibrinogen concentrate for the treatment of excessive intraoperative bleeding in thoracoabdominal aortic aneurysm surgery. *J Thorac Cardiovasc Surg* 2009; **138**:694–702.
7   Royston D, von Kier S. Reduced haemostatic factor transfusion using heparinase-modified thrombelastography during cardiopulmonary bypass. *Br J Anaesth* 2001; **86**:575–578.
8   Ho AM, Lee A, Chan SK, Ng SK. The timing for the use of heparinase and tromboelastography to prevent excessive bleeding coronary artery bypass graft surgery. *J Cardiothorac Vasc Anesth* 2003; **17**:413–414; author reply 414–415.
9   Weber CF, Gorlinger K, Meininger D, et al. Point-of-care testing: a prospective, randomized clinical trial of efficacy in coagulopathic cardiac surgery patients. *Anesthesiology* 2012; **117**:531–547.
10 Schochl H, Maegele M, Solomon C, et al. Early and individualized goal-directed therapy for trauma-induced coagulopathy. *Scand J Trauma Resusc Emerg Med* 2012; **20**:15.
11 Tuman KJ, McCarthy RJ, Djuric M, et al. Evaluation of coagulation during cardiopulmonary bypass with a heparinase-modified thrombelastographic assay. *J Cardiothorac Vasc Anesth* 1994; **8**:144–149.
12 Gertler R, Wiesner G, Tassani-Prell P, et al. Are the point-of-care diagnostics MULTIPLATE and ROTEM valid in the setting of high concentrations of heparin and its reversal with protamine? *J Cardiothorac Vasc Anesth* 2011; **25**:981–986.
13 Sandset PM, Abildgaard U, Larsen ML. Heparin induces release of extrinsic coagulation pathway inhibitor (EPI). *Thromb Res* 1988; **50**:803–813.
14 Rapaport SI, Rao LV. The tissue factor pathway: how it has become a 'prima ballerina'. *Thromb Haemost* 1995; **74**:7–17.
15 Hansen JB, Sandset PM, Huseby KR, et al. Depletion of intravascular pools of tissue factor pathway inhibitor (TFPI) during repeated or continuous intravenous infusion of heparin in man. *Thromb Haemost* 1996; **76**:703–709.

16   Belcher PR, Muriithi EW, Milne EM, *et al.* Heparin, platelet aggregation, neutrophils, and cardiopulmonary bypass. *Thromb Res* 2000; **98**:249–256.

17   Muriithi EW, Belcher PR, Day SP, *et al.* Lipolysis generates platelet dysfunction after in vivo heparin administration. *Clin Sci (Lond)* 2002; **103**:433–440.

18   Bengtsson G, Olivecrona T. Lipoprotein lipase. Mechanism of product inhibition. *Eur J Biochem* 1980; **106**:557–562.

19   Muriithi EW, Belcher PR, Day SP, *et al.* Heparin-induced platelet dysfunction and cardiopulmonary bypass. *Ann Thorac Surg* 2000; **69**:1827–1832.

20   Ferraris VA, Brown JR, Despotis GJ, *et al.* 2011 update to the Society of Thoracic Surgeons and the Society of Cardiovascular Anesthesiologists blood conservation clinical practice guidelines. *Ann Thorac Surg* 2011; **91**:944–982.