Detection of Protamine and Heparin After Termination of Cardiopulmonary Bypass by Thrombelastometry (ROTEM®): Results of a Pilot Study

Markus Mittermayr, MD*
Corinna Velik-Salchner, MD*
Berndt Stalzer, MD*
Josef Margreiter, MD*
Anton Klingler, PhD†
Werner Streif, MD‡
Dietmar Fries, MD*
Petra Innerhofer, MD*

BACKGROUND: Our goal of this study was to determine whether protamine’s effects on coagulation can be detected and differentiated from those of heparin when using thrombelastometry (ROTEM®).

METHODS: To reverse the effects of heparin after cardiopulmonary bypass (CPB), 22 consecutive patients undergoing aortocoronary bypass graft surgery were included. According to clinical routine, all patients received a first dose of protamine calculated from the total amount of heparin given; additional protamine (70 U/kg) was administered to patients with activated clotting time (ACT) above baseline and clinical signs of diffuse bleeding. Simultaneously, routine ACT measurements, ROTEM assays (heparin-sensitive INTEM, and heparinase-containing HEPTEM test) and standard coagulation tests were performed, and the activity of coagulation factors as well as antifactor Xa activity measured.

RESULTS: Administration of additional protamine (n = 16) resulted in a statistically significant increase in coagulation times on the intrinsically activated test (INTEM-CT), namely from (mean ± sd) 219.8 (±19.1) s to 241.1 (±21.7) s (P < 0.001), and on the heparinase-containing test (HEPTEM-CT), namely from 210.2 (±19.9) s to 226.8 (±21.8) s (P < 0.001). These changes were not observed in patients receiving a single protamine dose (n = 6). The INTEM-CT:HEPTEM-CT ratio correctly identified 56 of the 58 samples as not containing residual heparin and correctly detected residual heparin in 3 of the only 6 samples showing elevated antifactor Xa values after CPB.

CONCLUSION: Our preliminary data show that at termination of CPB administration of additional protamine results in a brief prolongation of coagulation times on the INTEM and HEPTEM test and that ROTEM might be useful in excluding residual heparin in cases showing prolonged ACT.

Coagulopathy after cardiopulmonary bypass (CPB) frequently results in significant postoperative bleeding and is caused by platelet dysfunction, hemodilution, coagulation factor depletion, activation of the fibrinolytic system, residual heparin but also excess protamine.1 Activated clotting time (ACT) is commonly measured during and on termination of CPB to guide heparin or protamine dosing. However, Murray et al.2 showed that ACT values are not a sensitive indicator of low heparin concentrations probably present after reversal with protamine. Furthermore, ACT values are not only a function of heparin concentration but are also influenced by hemodilution, low platelet numbers, low fibrinogen, and even excess protamine. At our institution, a fixed dose of protamine is routinely administered on termination of CPB and is calculated from the total heparin dose. Adequate reversal of heparin is confirmed 15 min later when ACT values have returned to near baseline. Prolonged ACT values after protamine administration are often interpreted as suggesting the presence of residual heparin. Thus, repeated doses of protamine are frequently administered although residual heparin, as well as an excess of protamine or other factors, can prolong ACT.3

Thrombelastographic techniques (TEG®) enable measurement of the entire coagulation and clot formation process by assessing initiation and propagation of coagulation as well as final clot strength (Table 1). After intrinsic or extrinsic activation, thrombelastometry (ROTEM®) measures initiation of coagulation as coagulation time (CT). ROTEM-CT is seen to be comparable to the r-time of conventional TEG, and results depend on the concentration of coagulation factors and
the presence of inhibitors like heparin. In a previous *in vitro* study we showed that the ROTEM-derived CT can be used to distinguish the effects of heparin from those of protamine by comparing the CT measured with the intrinsically activated and heparin-sensitive INTEM test with that determined with the intrinsically activated heparinase-containing HEPTEM test (INTEM-CT:HEPTEM-CT ratio).4 In that study in the presence of heparin (0.1–1 U/mL), INTEM-CT increased significantly and dose-dependently above normal values with increasing concentrations of heparin, whereas HEPTEM-CT remained in the normal range. In contrast, the presence of protamine (0.1–1 U/mL) prolonged CT similarly and dose-dependently on both the INTEM and HEPTEM assays.

Since *in vivo* coagulation factor deficiencies of the intrinsic system may be present (e.g., coagulation factors VIII, IX, XI, XII) and various other factors (e.g., hemodilution, aprotinin) might also influence CT, it is not clear whether these *in vitro* results also apply to clinical routine after CPB.

The goal of this study was to determine whether protamine’s effects on coagulation can be detected *ex vivo* using the ROTEM assay and whether these effects can be differentiated from those of heparin. To answer this question, the ROTEM assays were performed supplementary to the routine ACT measurements during the first 30 min after termination of CPB. Furthermore, antifactor Xa activity and activity of coagulation factors were measured and standard coagulation tests conducted simultaneously.

**METHODS**

The protocol was approved by the local University Ethics Committee. Informed written consent was obtained from 22 consecutive patients (age 19–80 yr) undergoing aortocoronary bypass graft surgery and thus requiring CPB. Exclusion criteria were preoperative congenital or acquired coagulopathy or acute coronary syndrome. All patients received general anesthesia using midazolam, fentanyl, and rocuronium for induction; anesthesia was maintained with sevoflurane in an oxygen/air mixture supplemented with a continuous infusion of remifentanil, which was switched to piritramide at the end of surgery to provide sufficient postoperative analgesia. In addition to standard monitoring in all patients, arterial blood pressure, central venous pressure, and pulmonary artery pressure were measured continuously. For intravascular volume replacement at induction of anesthesia, all patients received 500 mL modified gelatin solution (4% Gelofusin®, B. Braun, Maria Enzersdorf, Austria) followed by Ringer’s lactate solution. After CPB, adequate volume supply was maintained with Ringer’s lactate solution. In all patients a cell saver collection device (Cell Saver, Hemonetics Corporation, Braintree, MA) was used; the salvaged blood was processed and the resulting red cell concentrate retransfused after the last study-related measurements were made. All patients received 5000 IU unfractionated heparin subcutaneously 12 h before surgery and a second-generation cephalosporin after induction of anesthesia. Aprotinin (10000 kallikrein inhibiting units [KIU]/min, up to a maximum total of 3 million KIU) was continuously administered from the beginning of operation to termination of CPB.

During CPB a membrane oxygenator (Capiox RX-25, Terumo Corporation, Tokyo, Japan) was used. Systemic hypothermia was maintained at 32°–34°C; myocardial protection was achieved with intermittent antegrade and retrograde potassium crystalloid cardioplegia (St. Thomas 2, Fresenius Kabi, Graz, Austria). Depending on patient size the CPB was primed with 1000 mL of Ringer’s lactate solution, 500 mL gelatin, 250 mL mannitol, 4500 IU heparin, and 1 million IU aprotinin when body surface area was <2.08 m², or with 1500 mL electrolyte solution, 500 mL gelatin, 250 mL mannitol, 6000 IU heparin, and 1

**Table 1. Abbreviations for Standard ROTEM® Variables and Assays and Their Interpretation**

| Initiation of coagulation (until 2 mm amplitude) | Coagulation time (CT) | INTEM (137–240 s) | EXTEM (42–74 s) | Initial thrombin formation depending on activity of coagulation factors/inhibitors |
| Propagation of coagulation (tangent through 2 mm amplitude point) | α angle | INTEM (71–82°) | EXTEM (63–81°) | Kinetics of clot formation during further thrombin generation depends on activity of coagulation factors interacting with platelets and fibrinogen |
| Strength of the formed clot | 1. Maximum clot firmness (MCF) | INTEM (52–72 mm) | EXTEM (49–71 mm) | Maximal registered clot strength |
| 2. At defined time points | A10, A20, A30 | FIBTEM (9–25 mm) | Detection of heparin |
| 3. After platelet blockage | Fibrinogen/fibrin part of clot | HEPTEM® (phospholipids, ellagic acid) | HEPTTEM® (tissue factor, aprotinin) | Confirms hyperfibrinolysis |

**ROTEM assays**

| INTEM® (phospholipids, ellagic acid) | FIBTEM® (tissue factor, cytochalasin D) | HEPTEM® (phospholipids, ellagic acid, heparinase) | APTEN® (tissue factor, aprotinin) |

**Variables and Assays and Their Interpretation**

- **Initiation of coagulation:**
  - Coagulation time (CT)
- **Propagation of coagulation:**
  - α angle
- **Strength of the formed clot:**
  - 1. Maximum clot firmness (MCF)
  - 2. A10, A20, A30
  - 3. Fibrinogen/fibrin part of clot
- **ROTEM assays:**
  - INTEM®
  - FIBTEM®
  - HEPTEM®
  - APTEN®
millon IU aprotinin when body surface area was more than 2.08 m². Before starting CPB, all patients were anticoagulated with 300 IU/kg mucosal heparin (Heparin Immuno, EBEWE Pharma, Unterach, Austria) in order to achieve an ACT of more than 400 s. If ACT values were below 400 s, additional heparin (70 IU/kg) was administered. During CPB, further doses of heparin (70 IU/kg, 2000 IU, respectively) were administered to maintain ACT values or when red blood cell concentrates were administered. Leukocyte-depleted red blood cell concentrates were transfused if hematocrit was <20% during CPB. ACT was measured every half hour during CPB. According to clinical routine, after successfully discontinuing CPB, anticoagulation was reversed with a calculated dose of protamine hydrochloride (Protain Valeant, Valeant Pharmaceuticals, Eschborn, Germany), (1 IU [0.01 mg] of protamine per unit of total heparin administered, including the heparin used for the extracorporeal circuit) and ACT was measured 15 min thereafter. In Austria, the licensed product is labeled in IU. According to the manufacturer's instructions, 1 IU protamine hydrochloride antagonizes 1 IU heparin (1 IU protamine hydrochloride is comparable to 0.01 mg protamine sulfate). Additional protamine (70 IU/kg) (0.7 mg/kg) was administered if ACT was above baseline (>10%) and clinical signs of diffuse bleeding occurred.

ACT was measured twice at each time point using the celite-activated HEMOCHRON® Jr. Low Range Activated Clotting Time (ACT-LR) and the kaolin-containing Activated Clotting Time Plus (ACT+) (HEMOCHRON Jr, ITC, Edison, NY). The system uses cartridges in which the blood sample flows into capillaries. The instrument recognizes the clot end-point when the flow decreases below a predetermined rate. Both tests report a celite equivalent ACT value in seconds.

Thrombelastometry (ROTEM, Pentapharm GmbH, Munich, Germany), which is based on the TEG after Hartert, was performed at bedside in citrated whole blood using the intrinsically activated tests (INTEM test: 20 μL CaCl₂ 0.2 M, 20 μL thromboplastin-phospholipid and ellagic acid, 300 μL blood; HEPTEM test: additional 10 μL heparinase) and the extrinsically activated test (EXTEM test: 20 μL CaCl₂ 0.2 M, 20 μL tissue factor, 300 μL blood). In addition, the fibrinogen component of the clot was measured using the platelet-inactivating test (FIBTEM test: 20 μL CaCl₂ 0.2 M plus cytochalasin D, 20 μL tissue factor, 300 μL blood). The variables examined by ROTEM analysis (Table 1) are CT, clot formation time, angle and clot firmness after a defined time (20 min in the present study [A20]). The ROTEM device was checked for proper function using a control serum (ROTROL) in accordance with the manufacturer’s recommendation. All reagents were obtained from Pentapharm GmbH.

In addition to the standard coagulation tests, prothrombin time, partial thromboplastin time (aPTT), antithrombin, concentrations of fibrinogen and blood cell count, we also measured activities of the coagulation factors II, V, VII, VIII, IX, X, XI, XII, XIII (FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII), von Willebrand factor antigen (vWF:Ag) and von Willebrand ristocetin activity (vWF:RistCo) and antifactor Xa activity for unfractionated heparin.

For analysis of all these variables, arterial blood samples were obtained at baseline (before induction of anesthesia) (A), 5 min before termination of CPB (B), 15 min after administering the first dose of protamine (C), 5 min (D), and 15 min (E) after administering additional protamine and at equivalent time points in patients receiving only a first dose of protamine. These timepoints were selected according to clinical routine and the fact that protamine has been reported to have short-lasting effects.

### Statistical Analysis
Data are given as means (±sd). To investigate time dependencies, a repeated measures analysis of variance was applied. In the case of significance a paired t-test was used to compare percentual changes against baseline (100%) and against time point C. A P value <0.05 was considered statistically significant. The sample size of 16 was planned to provide 90% power for detection of a difference in the mean CT of 200 s versus 270 s (sd: ±75) (obtained from our former in vitro study) with a two-sided significance level of 5%.

### RESULTS
Twenty-two patients were recruited for the study. Of these, 16 patients received an additional dose of protamine, and their demographic data and details of surgery are shown in Table 2. During the

### Table 2. Demographic Data and Intraoperative Course of patients After Administration of Additional Protamine After Termination of Cardiopulmonary Bypass

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>65 (±10)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>83.1 (±15.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.9 (±8.8)</td>
</tr>
<tr>
<td>Preoperative clopidogrel/ acetylsalicylic acid/both (n)</td>
<td>2/5/2</td>
</tr>
<tr>
<td>Patients with 2/3/4/5 distal anastomoses (n)</td>
<td>2/5/0/0</td>
</tr>
<tr>
<td>Operation duration (min)</td>
<td>277 (±88)</td>
</tr>
<tr>
<td>Bypass duration (min)</td>
<td>118 (±42)</td>
</tr>
<tr>
<td>Cross clamping duration (min)</td>
<td>65 (±25)</td>
</tr>
<tr>
<td>Total heparin administered (IU)</td>
<td>33173 (±4836)</td>
</tr>
<tr>
<td>Total protamine administered (IU/mg)</td>
<td>38925 (±5317)/389.25 (53.17)</td>
</tr>
<tr>
<td>Additional protamine administered (IU/mg)</td>
<td>5800 (±875)/58 (8.75)</td>
</tr>
</tbody>
</table>

Values are means (±sd).
30-min study period after initial protamine administration, no coagulation factors or blood components were administered. Analysis of variance analysis showed significant time effects for all parameters except concentrations of d-Dimers and vWF:Ag (these data are not shown).

**Antifactor Xa Activity**

Of the 22 patients, measurement of antifactor Xa activity after initial protamine administration showed only 3 patients (total from 6 samples) to have detectable residual heparin in the range of 0.11 IU/mL to 0.17 IU/mL. All three patients exhibiting residual heparin received additional protamine and showed ACT values 10% above baseline.

**ACT Measurements**

As compared to baseline, the 16 patients receiving an additional dose of protamine ACT LR and ACT+ showed significant changes until the end of the observation period (Table 3). Administration of additional protamine after time point C did not significantly change ACT values, which remained above baseline during the observation period. Of the 16 patients receiving extra protamine, 14 showed elevated ACT 15 min after initial protamine (time point C) (11 patients > 10% above baseline, 3 patients > 20% above baseline) in addition to clinical signs of bleeding, whereas 2 patients received the additional dose of protamine because of clinical signs of bleeding and because the surgeon so requested. In order to perform an intend-to-treat analysis, data on these two patients were not excluded from analysis.

**ROTEM Assays**

ROTEM data are given in Figure 1 and Table 4. As compared to baseline, CT measurements increased significantly in INTEM and HEPTEM tests. Additional administration of protamine (n = 16) resulted in a significant increase in INTEM-CT (P < 0.001) and HEPTEM-CT (P < 0.001) as compared to values seen after initial protamine administration, with 9 of the 16 patients surpassing the upper normal range of 240 s (Table 1, Fig. 1). Fifteen minutes after additional protamine administration INTEM-CT and HEPTEM-CT had returned to the same values measured after initial protamine administration. The INTEM-CT:HEPTEM-CT ratio remained at about one. By contrast, in patients not receiving additional protamine (n = 6) HEPTEM-CT values remained unchanged after initial protamine administration (time point D) and INTEM-CT values shortened significantly (Fig. 1b).

All other ROTEM parameters and EXTEM-CT (Table 4) remained unchanged after initial protamine administration with the exception that the INTEM test showed an increase in α angle at the last measurement.

In order to test the ability of ROTEM to correctly detect heparin, we compared anti-Xa activity in the 22 patients by measuring the INTEM-CT:HEPTEM-CT ratio after protamine administration (66 samples). Sixty of the 66 samples showed no anti-factor Xa activity, while 6 samples showed anti-factor Xa activities >0.1 U/mL. Of the 60 samples showing no anti-factor Xa activity, 2 were not evaluated by ROTEM, leaving 58 samples without heparin for comparison. From our in vitro data, the presence of heparin can be expected when INTEM-CT reaches pathological values (>240 s) with INTEM-CT at least 10% higher than HEPTEM-CT. When using this threshold in the present study, ROTEM correctly detected heparin in 3 of 6 samples exhibiting antifactor Xa activities >0.1 IU/mL. In addition, 56 of the 58 samples without antifactor Xa activity were correctly identified as not containing heparin.

**Laboratory Data**

Results of standard coagulation tests and the activity of coagulation factors are shown in Tables 3 and 5. As compared to baseline, all variables except vWF:RiCo changed significantly in all patients.

With the exception of a statistically significant increase in the activity of coagulation factors FVIII, FIX and FXI, 5 min after administration of additional protamine no significant protamine-related changes were observed for prothrombin time, aPTT, platelet or fibrinogen or the activity of coagulation factors FII, FV, FVII, FX, FXII, FXIII or vWF:RiCo.

**DISCUSSION**

The main findings of our study are that heparin anticoagulation is adequately reversed after an initial dose of protamine and that administration of additional protamine showed no measurable benefit. We also show that the ROTEM technique can detect excess protamine as a simultaneously occurring increase in CT in the INTEM and HEPTEM test. Thus, a short-lived prolongation of CT occurring immediately after additional protamine administration should not be misinterpreted as a coagulation factor deficiency with regard to the intrinsic pathway.

Comparison of antifactor Xa measurements with ROTEM results revealed that the ROTEM assay might be a valuable tool for excluding residual heparin after CPB in cases showing elevated ACT values and increased bleeding. If INTEM-CT is not increased to pathological values and the INTEM-CT:HEPTEM-CT ratio remains 1, it is very unlikely that residual heparin is the main problem underlying persistent bleeding or elevated ACT values. Confirming these findings, Nielsen showed in rabbits receiving heparin from 10 IU/kg up to a cumulative dose of 30 IU/kg that nonactivated classical TEG tests were more sensitive to changes in heparin than were aPTT or ACT. We previously showed that ROTEM analysis can detect in vitro heparin concentrations of 0.1–1.0 IU/mL with high sensitivity. Murray et al. showed...
that ACT is not very sensitive to low heparin concentrations. Moreover, elevated ACT after protamine administration can be caused by heparin, protamine or various other factors influencing coagulation including aprotinin. If, in the present study, the ROTEM assay had been used to decide whether to administer additional protamine because of elevated ACT and suspected residual heparin, only one patient would have received an additional dose of protamine. This patient was correctly identified as having residual heparin (anti-Xa = 0.17 IU/mL), whereas the 2 patients showing anti-Xa activity at the detection level (0.11 IU/mL and 0.13 IU/mL) and the 13 patients showing no residual heparin would not have received additional protamine.

Protamine binds ionically to the numerous negative charges of heparin and inactivates it, but also causes anticoagulant effects. One of the methods of calculating the required protamine dose for neutralizing heparin is the use of a fixed-dose ratio of protamine to ing the required protamine dose for neutralizing hep-
anticoagulant effects. One of the methods of calculat-
charges of heparin and inactivates it, but also causes 
additional protamine.

Table 3. Coagulation Measurements in Patients with Additional Protamine

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT (s)</td>
<td>115 (±11)</td>
<td>364 (±37)*</td>
<td>122 (±10)*</td>
<td>123 (±15)*</td>
<td>124 (±16)*</td>
</tr>
<tr>
<td>ACT-LR (s)</td>
<td>153 (±11)</td>
<td>NA</td>
<td>167 (±15)*</td>
<td>163 (±12.4)*</td>
<td>158 (±14)</td>
</tr>
<tr>
<td>PT (%)</td>
<td>98 (±6)</td>
<td>NA</td>
<td>65 (±11)*</td>
<td>64 (±11)*</td>
<td>65 (±13)*</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>33 (±3)</td>
<td>NA</td>
<td>52 (±6)*</td>
<td>51 (±6)*</td>
<td>51 (±9)*</td>
</tr>
<tr>
<td>Fib mg/dL</td>
<td>403 (±88)</td>
<td>210 (±53)*</td>
<td>225 (±63.5)*</td>
<td>229 (±65)*</td>
<td>219 (±62)*</td>
</tr>
<tr>
<td>Platelets G/L</td>
<td>219 (±44)</td>
<td>166 (±37)*</td>
<td>132 (±25)*</td>
<td>138 (±29)*</td>
<td>142 (±27.4)*</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>37 (±3)</td>
<td>25 (±2)*</td>
<td>27 (±1.5)*</td>
<td>27 (±3)*</td>
<td>27 (±2)*</td>
</tr>
<tr>
<td>AT (%)</td>
<td>77 (±12)</td>
<td>48 (±8)*</td>
<td>52 (±9)*</td>
<td>54 (±8)*</td>
<td>53 (±8)*</td>
</tr>
<tr>
<td>vWF:RiCo (%)</td>
<td>113 (±39)</td>
<td>100 (±40)</td>
<td>129 (±33)</td>
<td>131 (±44)</td>
<td>133 (±34)</td>
</tr>
</tbody>
</table>

Measurements taken at baseline (before induction of anesthesia) (A), 5 min before termination of cardiopulmonary bypass (B), 15 min after initial administration of protamine (C), 5 min after administration of additional protamine (D), and 10 min thereafter (E).

Values are means (±so).

Activated clotting time high range (ACT+), low range (ACT-LR), PT, aPTT, fibrinogen (Fib), platelets, hematocrit (HCT) and ristocetin cofactor activity (vWF:RiCo).

* P < 0.05 as compared to time point A.

that ACT is not very sensitive to low heparin concentrations. Moreover, elevated ACT after protamine administration can be caused by heparin, protamine or various other factors influencing coagulation including aprotinin. If, in the present study, the ROTEM assay had been used to decide whether to administer additional protamine because of elevated ACT and suspected residual heparin, only one patient would have received an additional dose of protamine. This patient was correctly identified as having residual heparin (anti-Xa = 0.17 IU/mL), whereas the 2 patients showing anti-Xa activity at the detection level (0.11 IU/mL and 0.13 IU/mL) and the 13 patients showing no residual heparin would not have received additional protamine. Protamine binds ionically to the numerous negative charges of heparin and inactivates it, but also causes anticoagulant effects. One of the methods of calculating the required protamine dose for neutralizing heparin is the use of a fixed-dose ratio of protamine to heparin, without considering that most of the initial administered heparin has already been metabolized. Hereby 0.8 mg\(^7\) up to 1.3 mg\(^8\) of protamine for each 100 IU of total heparin are reported in the literature. At our institution, 1 mg protamine per each 100 IU of total heparin are administered. Despite this rather high dosing of initial protamine, three patients showed residual heparin, measured by elevated anti-factor Xa activity.

Protamine has been shown to inhibit platelet function in several studies. It alters platelet aggregometry,\(^3\) decreases platelet sensitivity for aggregation,\(^9\) inhibits glycoprotein Ib-vWF activity,\(^10\) shows a more pronounced platelet-inhibitory effect under conditions of high shear stress\(^11\) and decreases P-selectin expression.\(^12\) Furthermore, when investigating plasma samples, Nielsen demonstrated that after adding tissue-type plasminogen activator protamine significantly enhances fibrinolysis in a concentration-dependent fashion by inhibiting tissue factor-initiated thrombin generation.\(^13\) Using low and high concentrations of tissue factor for activation, he also found that protamine, especially high doses (50 \(\mu\)g/mL), affects the extrinsic system. In contrast, our study found no protamine-related changes in extrinsically activated ROTEM variables (EXTEM and FIBTEM tests). The discrepancy in these results might be explained by the fact that the ROTEM assays were performed in whole blood, as is customary in clinical practice, and the fact that different concentrations of protamine and tissue factor also influence results.

In the present study, protamine’s effects were mainly reflected by an increase in the intrinsically activated coagulation time (INTEM, HEPTEM). We found a statistically significant increase in the activity of FVIII, FIX, and FXI after additional protamine, which should have resulted in a shortening of intrinsically activated CTs while, contrarily, a significant prolongation was observed. We thus hypothesize that these findings reflect protamine’s effects on coagulation.

The questions arise why the intrinsically activated CT, but not aPTT, was prolonged after administration of additional protamine and whether a solely prolonged INTEM CT is of clinical importance and related to bleeding. It must be noted that ROTEM assays use activators of a different type and concentration than do classical coagulation tests.\(^14\) The INTEM test contains phospholipids and ellagic acid. In contrast, the aPTT test used for this study contains silicon dioxide particles. In addition, aPTT is measured in plasma and strongly reflects the concentration of coagulation factors. Interestingly, the activity of the aPTT-sensitive coagulation factors FVIII, FIX and FXI increased after administering additional protamine in our patients. Thus an increase in measured aPTT cannot be expected. By contrast, ROTEM CT is derived from whole blood, reflects functional consequences of initial thrombin generation in the presence of platelets and is thus not directly comparable with aPTT. Regarding bleeding tendency, it needs to be noted that heparin or a deficiency in coagulation factors FVIII or FIX also only prolongs intrinsically activated tests and
these deficiencies are associated with a considerable bleeding tendency.

Nevertheless, the question whether a protamine excess has a clinically important effect on the coagulation system is the subject of continuing controversy. Despotis et al. demonstrated that a reduction in the protamine:heparin ratio reduced postoperative bleeding and the need for fresh frozen plasma and platelet administration. Despotis et al. demonstrated that a reduction in the protamine:heparin ratio reduced postoperative bleeding and the need for fresh frozen plasma and platelet administration. DeLaria et al. also demonstrated that a reduction in protamine significantly reduces postoperative bleeding and blood product administration. In contrast, Svenarud et al. reported observing no differences in blood loss or in the incidence of allogeneic transfusion when protamine dose was reduced after CPB. Moreover, assuming a heparin rebound phenomenon, continuous postoperative protamine infusion has been shown to diminish postoperative bleeding. The half-life of circulating protamine in cardiac patients is reported to be about 5 min. This finding coincides with the results of our study showing detectable protamine effects when using the ROTEM assay at 5 min after additional administration; 15 min later the effects had already disappeared. Although the effects of protamine administration were

Figure 1. Coagulation time (CT, left side) and INTEM-CT:HEPTEM-CT ratio (right side) measured in citrated whole blood using the INTEM test (normal range, 100–240 s) and the HEPTEM test in patients with (a) or without (b) additional administration of protamine after point C. Values are means (±sd). *P < 0.05 as compared to time point A, #P < 0.05 as compared to time point C. Measurements were taken at baseline (before induction of anesthesia) (A), 5 min before termination of cardiopulmonary bypass (B), 15 min after initial administration of protamine (C), 5 min after additional administration of protamine (D) and 10 min thereafter (E). In patients needing no additional protamine blood samples were drawn at equivalent time points (D, 20 min after initial protamine; E, 30 min after initial protamine).
of short duration, in light of the possible side effects (hypotension, pulmonary edema, pulmonary hypertension) and the fact that retrospective data show a possible association between protamine administration and mortality.\textsuperscript{17} We feel that unnecessary administration should be avoided. For this reason the ROTEM assay may be helpful in deciding for or against additional protamine administration, namely by excluding residual heparin as a possible cause of prolonged ACT.

Limitations of the present study need to be discussed. First, the study was designed and powered to answer the question whether protamine acting 	extit{in vivo} can be detected by ROTEM assays. In this study ROTEM correctly excluded residual heparin in 56 of 58 cases and identified heparin’s presence in 3 of the 6 heparin-containing samples. However, these findings are partly related to the unexpectedly low prevalence of residual heparin. To exactly determine sensitivity and specificity, further data obtained in a larger patient population are needed. Another limitation of the present study is the observation period of only 30 min after protamine administration. In order to eliminate the influence of patient-specific differences in intravascular volume replacement, administration of coagulation factors, fresh frozen plasma and platelets on ROTEM values, we decided to avoid later measurement times. In addition, the present pilot study was not intended to evaluate differences in blood loss or transfusion requirements associated with additional protamine.

In conclusion, we demonstrated that heparin anticoagulation is adequately reversed after the initial dose of protamine and that additional protamine showed no measurable benefit. Administration of clinically relevant doses of additional protamine after CPB is detectable as a brief prolongation of intrinsically activated CT (INTEM and HEPTEM test) as measured with the ROTEM technique. Therefore, the prolongation of CT observed immediately after protamine administration is not indicative of the need for coagulation factors. We further show that ROTEM might be useful in excluding residual heparin after CPB in cases showing prolonged ACT. Combining these methods might reduce the frequency of unnecessary additional protamine administration.

ACKNOWLEDGMENTS

We thank Tiroler Landeskrankenanstalten GmbH (TILAK) for laboratory testing and Pentapharm for providing the reagents.
REFERENCES