

## ORIGINAL ARTICLE

# Fibrinogen substitution improves whole blood clot firmness after dilution with hydroxyethyl starch in bleeding patients undergoing radical cystectomy: a randomized, placebo-controlled clinical trial

C. FENGER-ERIKSEN,\* † T. M. JENSEN,\* B. S. KRISTENSEN,\* K. M. JENSEN, ‡ E. TØNNESEN,\* J. INGERSLEV † and B. SØRENSEN † §

\*Department of Anaesthesiology, Center for Haemophilia and Thrombosis, Aarhus; †Department of Clinical Biochemistry, Center for Haemophilia and Thrombosis, Aarhus; ‡Department of Urology, Aarhus University Hospital, Aarhus, Denmark; and §Centre for Haemostasis and Thrombosis, St Thomas' Hospital, London, UK

**To cite this article:** Fenger-Eriksen C, Jensen TM, Kristensen BS, Jensen KM, Tønnesen E, Ingerslev J, Sørensen B. Fibrinogen substitution improves whole blood clot firmness after dilution with hydroxyethyl starch in bleeding patients undergoing radical cystectomy: a randomized, placebo-controlled clinical trial. *J Thromb Haemost* 2009; 7: 795–802.

**Summary.** *Background:* Infusion of artificial colloids such as hydroxyethyl starch (HES) induces coagulopathy to a greater extent than simple dilution. Several studies have suggested that the coagulopathy could be corrected by substitution with a fibrinogen concentrate. *Objectives:* The aims of the present prospective, randomized, placebo-controlled trial were to investigate the hemostatic effect of a fibrinogen concentrate after coagulopathy induced by hydroxyethyl starch in patients experiencing sudden excessive bleeding during elective cystectomy. *Methods:* Twenty patients were included. Blood loss was substituted 1:1 with HES 130/0.4. At a dilution level of 30%, patients were randomly selected for intra-operative administration of a fibrinogen concentrate or placebo. The primary endpoint was maximum clot firmness (MCF), as assessed by thromboelastometry. Secondary endpoints were blood loss and transfusion requirements, other thromboelastometry parameters, thrombin generation and platelet function. *Results:* Whole-blood MCF was significantly reduced after 30% dilution *in vivo* with HES. The placebo resulted in a further decline of the MCF, whereas randomized administration of fibrinogen significantly increased the MCF. Furthermore, only 2 out of 10 patients randomly chosen to receive fibrinogen substitution required postoperative red blood cell transfusions, compared with 8 out of 10 in the placebo group ( $P = 0.023$ ). Platelet function and thrombin generation were reduced after 30% hemodilution *in*

*vivo*, and fibrinogen administration caused no significant changes. *Conclusions:* During cystectomy, fluid resuscitation with HES 130/0.4 during sudden excessive bleeding induces coagulopathy that shows reduced whole-blood maximum clot firmness. Randomized administration of fibrinogen concentrate significantly improved maximum clot firmness and reduced the requirement for postoperative transfusion.

**Keywords:** blood coagulation disorders inherited, fibrinogen, hemodilution, hemorrhage, HES 130/0.4.

## Introduction

In severe bleeding, the infusion of artificial colloid plasma expanders such as hydroxyethyl starch (HES) is often used. A series of laboratory studies, pioneering animal studies and numerous anecdotal casuistic reports have suggested that supplementation with colloid plasma expanders induces coagulopathy to a greater extent than simple dilution [1–4]. The coagulopathy is caused by an acquired dysfunctional fibrinogen defect that results in abnormal fibrin polymerization [5]. In a porcine model, dilutional coagulopathy was recently associated with seriously reduced hemostatic capacity and increased bleeding [6]. Initial work in our laboratory [1] and in the research unit of Fries [7] suggested that the coagulopathy may be corrected by substitution with a fibrinogen concentrate. Several subsequent studies have validated the proposed hemostatic effect of fibrinogen in dilutional coagulopathy [8,9]. The fibrinogen molecule has been described as the earliest coagulation factor to reach a critically low threshold level in bleeding patients [10]. Furthermore, fibrinogen has been suggested as an important coagulation factor needed to ensure sufficient and stable hemostasis during serious bleeding [5,11,12].

So far, no prospective, randomized, placebo-controlled study has been performed in humans in order to verify the

Correspondence: Benny Sørensen, Centre for Haemostasis & Thrombosis, Guy's & St Thomas' NHS Foundation Trust, 1st Floor North Wing, St Thomas' Hospital, Westminster Bridge Road, London SE1 7EH, UK.

Tel.: +44 20 7188 2788; fax: +44 20 7401 3125.

E-mail: benny.sorensen@kcl.ac.uk

Received 17 February 2009, accepted 17 February 2009

presence of coagulopathy after dilution with HES and to investigate the hemostatic effect of infusion of a fibrinogen concentrate. However, it seems reasonable to assume that seriously bleeding patients suffering from critically low levels of fibrinogen or acquired functional fibrinogen deficiency may benefit from substitution therapy [13]. Hence, the objectives of the present randomized, controlled, clinical study were to investigate the likelihood of coagulopathy after *in vivo* dilution with HES and to evaluate the hemostatic effect of infusion of a fibrinogen concentrate. We hypothesized that hemodilution with HES induces coagulopathy characterized by a marked reduction in maximum clot firmness. Furthermore, we hypothesized that fibrinogen improves clot firmness and reduces transfusion requirements.

## Patients and methods

### Study design and study subjects

The study was a single-center, prospective, double-blind, placebo-controlled, randomized clinical trial conducted in accordance with the Note for Guidance on Good Clinical Practice (GCP) (CPMP/ICH/135/95) and the Declaration of Helsinki. The study was monitored by the GCP-Unit at Aarhus University Hospital, Denmark. The study was approved by the Danish Medicines Agency (EudraCT number 2007-00026-44), the Regional Biomedical Human Ethics Committee (reference code # 2007-0037), as well as the Danish Data Protection Agency (reference code # 2007-41-05849). The manuscript has been prepared in accordance with the consort statement [14]. Patients older than 17 years of age who were admitted for radical cystectomy at the Department of Urology of Aarhus University Hospital (Skejby, Denmark) and who were suffering from localized bladder cancer ( $T_{1-2}N_0M_0$ ) were considered candidates for inclusion in the study. During the period from June 2007 to March 2008, all patients admitted for elective cystectomy were screened for enrollment by the consulting anesthesiologist responsible for anesthetic procedures during the operation. If the patient met the inclusion and exclusion criteria and provided informed consent, he or she was recruited for the study. The exclusion criteria were as follows: (i) presence of coagulation disorders defined as abnormal values of platelet count, PT, APTT, fibrinogen, antithrombin, or D-dimer; (ii) treatment with oral vitamin K antagonists; (iii) intake of non-steroid anti-inflammatory drugs within 2 days prior to surgery; (iv) renal or hepatic dysfunction; (v) ischemic heart disease; (vi) pregnancy; and (vii) known hypersensitivity to hydroxyethyl starch (HES). Blood samples were obtained at the onset of surgery (baseline), after planned target level of hemodilution and administration of study medication.

### Study outcomes

The primary study endpoint was whole blood maximum clot firmness as determined by thromboelastometry. Pre-specified secondary endpoints were as follows: other thrombelastometric

variables, platelet function, thrombin generation, bleeding and a requirement for peri- and postoperative blood product transfusion.

### Anaesthetic protocol

A lumbar epidural catheter was installed at lower thoracic levels (Th9–Th10 or Th10–Th11) immediately prior to induction of anesthesia, followed by infusion of bupivacain ( $5 \text{ mg mL}^{-1}$ , Bupivacain; SAD, Copenhagen, Denmark) administered as a bolus dose and subsequently by continuous infusion. A radial artery catheter was inserted for continuous monitoring of arterial pressure and for collection of blood samples. General anesthesia was induced with propofol ( $1.5\text{--}2.5 \text{ mg kg}^{-1}$ ) and fentanyl ( $3\text{--}6 \text{ }\mu\text{g kg}^{-1}$ ). Tracheal intubation was facilitated with cisatracurium ( $0.1\text{--}0.15 \text{ mg kg}^{-1}$ ) and anesthesia was maintained with sevoflurane and remifentanyl. A central venous catheter was placed in vena jugularis interna immediately after induction of anesthesia. Minute ventilation and fresh gas flow were adjusted to maintain an end-tidal  $\text{CO}_2$  at  $4.5\text{--}5.5 \text{ kPa}$ . During the operation, hypothermia was prevented by using a circulating warm air blanket. In addition, all infused fluids were preheated. Standard monitoring included pulse oximeter three-lead electrocardiogram, end-tidal  $\text{CO}_2$  and sevoflurane concentrations, invasive blood pressure, central venous pressure and central temperature. Institution of inotropic therapy was based on clinical and individual judgment.

### Fluid regimen, procedure of hemodilution and intervention

The fluid regimen was strictly controlled. From initiation of the surgical procedure, Ringer-Acetat (Fresenius Kabi AB, Uppsala, Sweden) was infused at  $4 \text{ mL kg}^{-1} \text{ h}^{-1}$  in order to cover fluid deficit from the pre-operative period as well as basal fluid requirements. Blood loss was continuously recorded by measurement of drain production as well as the weight of napkins. Furthermore, the level of hemoglobin and hematocrit was measured using a Radiometer ABL 625 analyser (Radiometer Medical A/S, Copenhagen, Denmark). The amount of blood loss was substituted with HES 130/0.4 (Voluven; Fresenius Kabi AB), which is the most commonly used colloid plasma expander in northern Europe. According to Danish national guidelines, the threshold for red blood cell transfusion was a level of hemoglobin at  $4.5 \text{ mmol L}^{-1}$  ( $7.25 \text{ g L}^{-1}$ ). Fresh frozen plasma and platelet pools were administered in accordance with departmental guidelines, based on international transfusion norms. At the 30% dilution level with HES 130/0.4, defined as a 30% reduction in hematocrit level from baseline, patients were randomized using the closed envelope principle to receive either fibrinogen concentrate ( $45 \text{ mg kg}^{-1}$ ) or an equivalent volume of placebo (isotonic saline,  $2.25 \text{ mL kg}^{-1}$ ). Both treatments were administered intravenously. The study medicine was prepared and administered by a second person; thus, the study staff was blinded to infusion of the drug vehicle and to patients participating in the study. Allocation

concealment was sustained until all recruitment, data collection, transfusion requirement data and laboratory analyses were complete.

#### *Fibrinogen concentrate*

A virally inactivated, pasteurized fibrinogen concentrate derived from plasma from the same batch number (85466011A) was evaluated (Haemocomplettan® 20 mg mL<sup>-1</sup> for intravenous administration; CSL Behring, Marburg, Germany). Dosage at 45 mg kg<sup>-1</sup> was chosen based on previously published pharmacokinetic observations, the expected level of fibrinogen at the time of intervention and the summary of product characteristics (SPC) for Haemocomplettan.

#### *Thrombelastographic whole-blood coagulation analysis*

Blood samples were drawn from the arterial catheter into Venoject® tubes (Terumo Europe, Leuven, Belgium; trisodium citrate, 0.129 M, 3.2 w/v %), at a volume ratio of 1:10. To avoid contact activation, blood sampling tubes were pre-incubated with Corn Trypsin Inhibitor (Haemologic Technologies Inc., Essex Junction, VT, USA) at a final concentration of 100 µg mL<sup>-1</sup> [15].

Dynamic whole-blood coagulation profiles were recorded in parallel using thromboelastometry (ROTEM® Thrombelastometry; Pentapharm, Munich, Germany) as previously described [16]. In brief, blood was rested 30 min prior to analysis, ROTEM® plastic cups pre-warmed to 37 °C were loaded with 300 µL of whole blood and spiked with 20 µL of buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). The coagulation process was activated with tissue factor (Innovin®; Dade Behring, Marburg, Germany) at a final dilution of 1:17 000. In addition, citrated blood was re-calcified with the addition of 0.2 M CaCl<sub>2</sub>. Hence, in all cases the final volume in the ROTEM® cup was 340 µL. All analyses were processed in duplicate for a minimum of 45 min. Standard thromboelastometry parameters such as clotting time (CT, s) and maximum clot firmness (MCF, mm) were recorded. The digitalized raw signal was processed further using DyCoDerivAn software (AvordusoL, Risskov, Denmark) to obtain the dynamic velocity parameter maximum velocity (MaxVel, mm × 100 s<sup>-1</sup>) and time until maximum velocity [t, MaxVel (s)] [16].

#### *Platelet function analysis*

Platelet function was assessed by Multiplate® (Dynabyte GmbH, Munich, Germany) based on whole blood impedance aggregometry [17]. Whole blood (300 µL) was mixed with pre-warmed isotonic saline (300 µL) in the Multiplate® cup, followed by 3 min of incubation time and addition of 20 µL of activator. In the present study we used adenosine diphosphate (ADP) at final concentrations of 0.125 and 0.250 µM as the activator. Change in impedance was transformed into arbitrary aggregation units (AU) and measured over time.

#### *Thrombin generation*

Thrombin generation was measured according to the protocol devised by Hemker [18] using a calibrated, automated thrombogram (Thrombinoscope BV, Maastricht, the Netherlands). Blood samples were centrifuged at 2800 × g for 25 min, at 4 °C to obtain platelet poor plasma. A 96-well microtiter plastic plate (Immulon 2HB clear 96-well; Thermo Electron Corporation, Vantaa, Finland) was prepared with 80 µL platelet poor plasma, followed by 20 µL of activator containing both a mixture of tissue factors (Innovin®; Dade Behring) at a final dilution of 1:7000 and phospholipid-TGT (Rossix, Mölndal, Sweden) at a final concentration of 4 µM. After a brief incubation, 20 µL of thrombin substrate (Fluo-Substrate; Thrombinoscope) was added automatically. All reagents were prewarmed to physiological temperature (37 °C).

Using the 'slow' fluorogenic thrombin substrate and continuous comparison to a simultaneously run calibrator, the continuous development of thrombin was recorded on a Fluoroscan Ascent fluorimeter (Thermo Electron Corporation). The measurements were performed in triplicate, with each well calibrated to a parallel well with a thrombin calibrator (Thrombin calibrator TS 20.0; Thrombinoscope) with known thrombin-like activity. The following parameters were calculated using software provided by Thrombinoscope: lag time (min), endogenous thrombin potential, peak levels of thrombin generation (nm) and time to peak thrombin (min).

#### *Other laboratory variables*

D-dimers were determined by an immunometric technique employing latex particles coated with monoclonal antibodies against fibrin D-dimer. Standard coagulation assays were performed using the STAR Evolution analyser (Diagnostica Stago, Asnières, France) using commercially available reagents: APTT (phospholipids reagent, Platelin LS; Organon, Munich, Germany), PT (calcium-thromboplastin reagent, STA-Neoplastin; Diagnostica Stago) and fibrinogen (Claus's method, thrombin reagent, STA-Fibrinogen; Diagnostica Stago). Levels of hemoglobin and platelet count were obtained using standard laboratory methods. During the cystectomy operation, arterial blood samples were analysed on a Radiometer ABL 625 analyser (Radiometer Medical A/S). Antigen levels of fibrinogen were determined by means of nephelometry (BN ProSpec; Dade Behring) (N antiserum from rabbit against human fibrinogen; Dade Behring).

#### *Statistics*

Statistical analysis was performed using the statistical software Graph Pad InStat (version 3.00; Graph Pad Software, San Diego, CA, USA). Based on our previously published study on HES-induced hemodilution and the effect of fibrinogen, a sample size of 10 patients in each group was required to detect a change in maximum clot strength of 10% that was significant at the two-sided 5% level ( $\alpha = 0.05$ ,  $\beta = 0.2$ ). Groups were

pre-tested for equal standard deviations using the method of Bartlett and were estimated to follow a Gaussian distribution based on the methods of Kolmogorov and Smirnov, as well as based on evaluation of histograms and Q-Q plots. A repeated-measurement analysis of variance was applied to test the influence of dilution and the effect of the fibrinogen administration on thrombelastographic and derived parameters within groups. Between-group variables were evaluated by means of the unpaired *t*-test. Data with no verified Gaussian distribution were evaluated by non-parametric methods (Mann-Whitney or Kruskal-Wallis test). A *P*-value below 0.05 was considered significant. During the trial, no interim analyses were performed.

#### Role of the funding source

The study was supported by an unrestricted research grant from CSL Behring, the University of Aarhus Research Foundation and the A. P. Møller and Hustru Chastine McKinney Møllers Foundation. None of the investigators received any personal honoraria for performing the study. Furthermore,

the sponsors had no influence on the design of the study or on the analysis and interpretation of the results.

#### Results

Over a 9-month period, a total of 38 patients were screened for eligibility; 21 proceeded to randomization and 20 fulfilled the protocol requirements and were included in the final patient sample. A total of 18 patients were ineligible for the study because they failed to meet inclusion criteria, they refused to participate, their operation could not be conducted for technical reasons or their operation deviated from standard protocol in that transfusion was performed before the target level of dilution was achieved (Fig. 1). Hence, the final study group consisted of two females and 18 males with an average age of 63 years (range, 52–77) and an average weight of 77 kg (range, 68–92). All participating patients had levels of hemoglobin, platelet count, activated partial thrombin time, prothrombin time, fibrinogen, antithrombin and D-dimer levels within the normal range and they did not differ significantly between the groups as the groups were comparable with regard

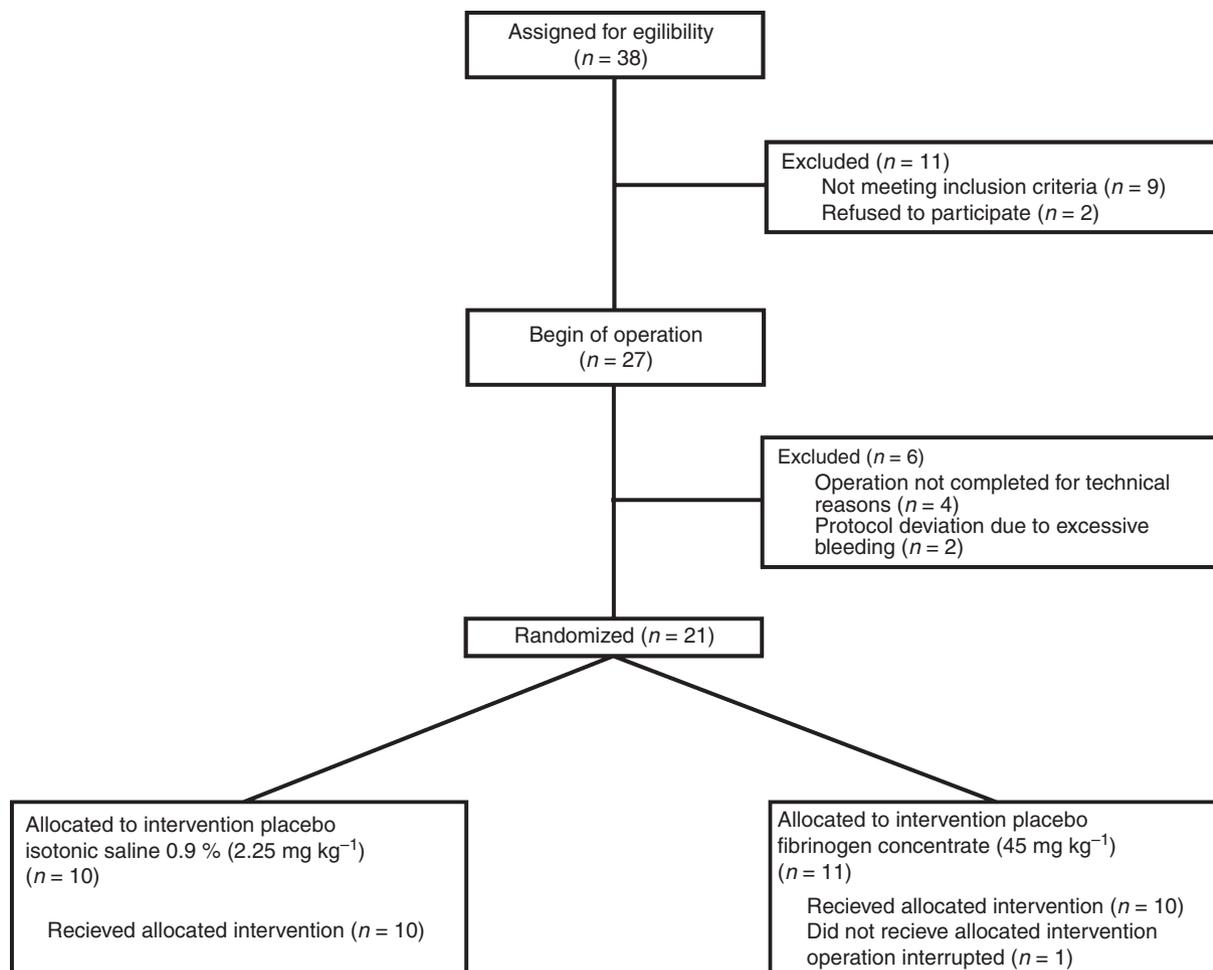


Fig. 1. Flow diagram showing steps of the patient recruitment and randomization process.

**Table 1** Hemoglobin levels, total blood loss, fluid administered (at time of intervention) and transfusion requirements during the operation and within 48 h after

	Placebo (n = 10)	Fibrinogen (n = 10)	P-value
Hemoglobin levels ( $\mu\text{M}$ )			
Baseline	8.8 $\pm$ 0.6	9.0 $\pm$ 0.5	0.29
Third postoperative day	6.11 $\pm$ 0.5	6.40 $\pm$ 0.64	0.27
Blood loss at time of intervention (mL)			
Total	2933 $\pm$ 1320	2682 $\pm$ 962	0.40
Fluid administered at time of intervention (mL)			
HES 130/0.4	1600 $\pm$ 175	1625 $\pm$ 339	0.73
Ringers lactate	1195 $\pm$ 285	1405 $\pm$ 620	0.97
Transfusion, red blood cells			
During operation	2.5 (0–6)	2 (0–5)	0.91
Postoperative period (48 h)	1.5 (0–2)	0 (0–2)	<0.05*
Total	4.0 (0–6)	3.5 (0–5)	0.34

HES, hydroxyethyl starch.

Data are presented as mean  $\pm$  SD. Transfusion data are presented as median (range). \*Significantly different from placebo ( $P < 0.05$ ).

to age and body mass index. All patients underwent radical cystectomy with a mean duration of surgery of 312 min (SD, 83 min); none of the patients underwent re-operation. The total amount of fluid administered at the time of the study intervention was equal in the two groups (Table 1). During the operation, blood loss and fluid substitution resulted in a significantly decreased baseline hematocrit value from 0.43 (SD, 0.028) to 0.29 (SD, 0.022) at the time of intervention ( $P < 0.05$ ), corresponding to a dilution of approximately 33%. Comparison of hematocrit values between the groups revealed no significant differences at baseline [0.44 (SD, 0.03) vs. 0.43 (SD, 0.03)], after [0.30 (SD, 0.02) vs. 0.29 (SD, 0.02)] intervention, at the end of the operation [0.34 (SD, 0.06) vs. 0.32 (SD, 0.03)] or 6 h after the end of the operation [0.33 (SD, 0.06) vs. 0.32 (SD, 0.02)]. Furthermore, no significantly different levels of haemoglobin between the groups were observed after the 48-h observation period (Table 1). No adverse events or side effects were observed in any of the study participants.

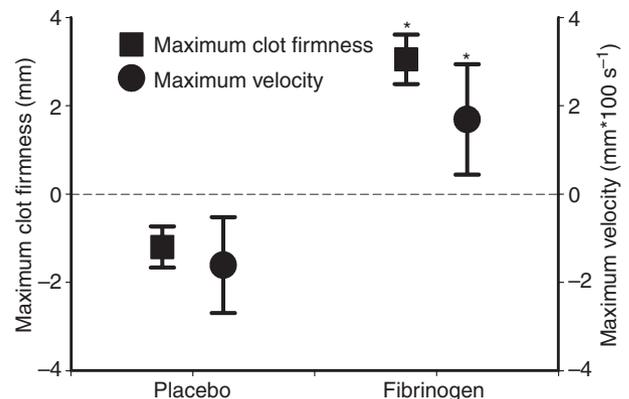
#### Thromboelastometric whole-blood coagulation analysis (Table 2 and Fig. 2)

ROTEM results and derived parameters after *in vivo* 33% hemodilution with HES are depicted in Table 2. At a 33% dilution level, the primary endpoint – maximum clot firmness (MCF) decreased significantly from 59.2 mm (SD, 5.8) to 50.6 mm (SD, 4.7), corresponding to an average reduction of 14.5%, whereas the clot initiation and clot propagation remained unchanged. Patients treated with the fibrinogen concentrate experienced significantly better maximum velocity of clot formation and significantly better maximum clot firmness than the placebo group. *In vivo* intervention with Haemocomplettan<sup>®</sup> induced no changes on the clot initiation and clot propagation, as evaluated by clot formation time and time until maximum velocity.

**Table 2** Laboratory parameters of hemostasis before (baseline) and after 30% *in vivo* resuscitation with hydroxyethyl starch (HES) 130/0.4

	Baseline	30% Hemodilution	P-value
Clot initiation			
Clotting time (s)	404 $\pm$ 94	398 $\pm$ 91	0.98
Clot propagation			
Clot formation time (s)	190 $\pm$ 78	173 $\pm$ 38	0.28
Maximum velocity (mm $\times$ 100 s <sup>-1</sup> )	12 $\pm$ 4.0	11.2 $\pm$ 1.9	0.32
Time until maximum velocity (s)	541 $\pm$ 150	496 $\pm$ 121	0.19
Clot strength			
Maximum clot firmness (mm)	59.2 $\pm$ 5.8	50.6 $\pm$ 4.7	<0.05*
Thrombin generation			
Lag time (min)	6 $\pm$ 1.0	5.2 $\pm$ 0.79	<0.05*
Endogenous thrombin potential (nM $\times$ min)	1471 $\pm$ 309	1532 $\pm$ 247	0.33
Peak (nM)	162 $\pm$ 29	167 $\pm$ 21	0.32
Time to peak (min)	11.0 $\pm$ 1.74	9.6 $\pm$ 1.25	<0.05*
Platelet function			
AUC (ADP 5 $\mu\text{M}$ )	444 $\pm$ 144	199 $\pm$ 125	<0.05*
AUC (ADP high)	488 $\pm$ 129	247 $\pm$ 141	<0.05*

AUC, area under the aggregation curve; ADP, adenosine 5'-diphosphate.  $n = 20$ . \*Significantly different from baseline ( $P < 0.05$ ).



**Fig. 2.** Absolute changes in maximum clot firmness in patients receiving fibrinogen or placebo after 30% *in vivo* hemodilution with hydroxyethyl starch (HES) 130/0.4.  $n = 10$  in each group. Data presented as mean ( $\pm$  1 SD). \*Significant different from placebo ( $P < 0.05$ ).

#### Blood loss and transfusion requirements (Table 1)

Blood loss and transfusion requirements during the operation were comparable in the two groups. Postoperatively, in the placebo group, 8 out of 10 patients (80%) required red blood cell transfusion within 48 h after the end of the operation, whereas only two patients out of 10 (20%) in the group treated with the fibrinogen concentrate required additional red blood cell transfusion ( $P < 0.05$ ). Median units of red blood cells transfused in the postoperative period were 1.5 in the placebo group vs. 0 in the fibrinogen group ( $P < 0.05$ ). The total median amount of red blood cell transfused during and 48 h

after the operation was 3.5 (range, 0–5) in the fibrinogen group and 4 (range, 0–6) in the control group (Table 1). During or after the operation, no significant differences in transfusion requirements of fresh frozen plasma were found. No patients received platelet transfusions.

#### Platelet function (Table 2)

Platelet function, as assessed by impedance aggregometry activated with ADP at two different concentrations, decreased significantly after 30% *in vivo* hemodilution with HES 130/0.4 (Table 2). Fibrinogen substitution had no measurable effect on platelet function (data not shown).

#### Thrombin generation (Table 2)

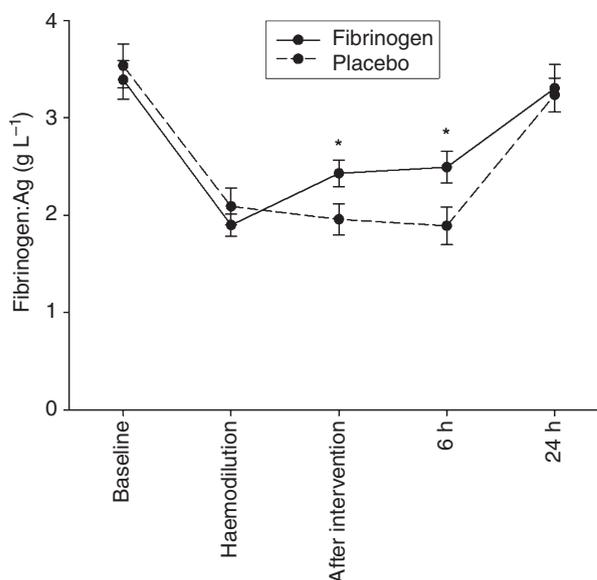
Paradoxically, the lag time and time to peak were both significantly reduced after *in vivo* hemodilution with HES 130/0.4, whereas peak levels of thrombin generation and endogenous thrombin potential remained unaffected (Table 2). Intervention with the fibrinogen concentrate had no immediate effect. At 24 h after study drug administration, thrombin generation was significantly compromised in the placebo group, as evidenced by a significantly reduced endogenous thrombin potential and peak levels, as well as diminished peak values and prolonged time until peak compared with the fibrinogen group (data not shown).

#### Levels of fibrinogen

Quantitative levels of plasma fibrinogen decreased significantly after 30% hemodilution from  $3.23 \text{ g L}^{-1}$  (SD, 0.65) to  $1.74 \text{ g L}^{-1}$  (SD, 0.27). After administration of the study medicine, the functional fibrinogen level was significantly higher in the intervention group  $2.41 \text{ g L}^{-1}$  (SD, 0.14) vs. the placebo group  $1.9 \text{ g L}^{-1}$  (SD, 0.5). Antigen levels of fibrinogen also decreased significantly after 30% hemodilution. After intervention, fibrinogen levels were significantly higher in patients exposed to fibrinogen administration. Noteworthy, the difference was maintained up to 6 h after the intervention. Twenty-four hours after the operation, the levels of fibrinogen were significantly increased in both groups compared with baseline, with no detectable difference between the groups (Fig. 3).

#### Discussion

The present randomized, placebo-controlled clinical trial verified the presence of significant coagulopathy after fluid resuscitation with HES 130/0.4 during severe bleeding in patients undergoing elective cystectomy. The coagulopathy was characterized by a pronounced and significant reduction in the primary end-point maximum clot firmness. Significant improvements in the primary endpoint maximum clot firmness as well as maximum velocity of clot formation were observed in patients randomized to receive intra-operative administration



**Fig. 3.** Absolute changes in maximum velocity of clot formation in patients receiving fibrinogen or placebo after 30% *in vivo* hemodilution with hydroxyethyl starch (HES) 130/0.4.  $n = 10$  in each group. Data are presented as mean ( $\pm 1$  SD). \*Significant different from placebo ( $P < 0.05$ ).

of a fibrinogen concentrate. Furthermore, the need for postoperative transfusions of red blood cells was significantly lower in the group of patients receiving fibrinogen. To our knowledge, the present study is the first randomized, placebo-controlled clinical trial on monotherapy with a fibrinogen concentrate in massively bleeding patients suffering from dilutional coagulopathy caused by infusion of the colloid plasma expander HES 130/0.4 in patients undergoing elective cystectomy.

The present clinical observations validate several previously published laboratory and experimental investigations suggesting that resuscitation with HES induces coagulopathy characterized by a significantly reduced maximum clot firmness that can be partially corrected by fibrinogen supplementation [1,7,19]. As a secondary endpoint, we report a significant reduction in postoperative transfusion requirements in the group of patients randomized to receive treatment with the fibrinogen concentrate. Transfusion data are the subject of considerable bias, although clinicians state they follow transfusion guidelines. The levels of hematocrit were comparable in each group at all the time points, suggesting that patients receiving placebo experienced more bleeding being corrected by blood transfusion. In the pioneering porcine model developed by Fries *et al.* [5], it was possible to show a significant reduction in intra-operative bleeding after intervention by the fibrinogen concentrate. The dosage used in the pig study was more than four times the fibrinogen dosage used in the present clinical study. Hence, we speculate that treatment with a higher dose of fibrinogen concentrate or earlier administration for example, at the beginning of operation, may also reduce intra-operative blood loss and total transfusion requirements during cystectomy in humans.

The maximum clot firmness was improved after treatment with the fibrinogen concentrate; however, it was not returned to pre-operative levels. Assuming that compromised fibrin polymerization is the primary cause of HES-induced coagulopathy, fibrinogen substitution at higher dosages may be expected to reverse the condition completely. Another possible explanation for the incomplete reversal observed here is that the infused fibrinogen was consumed.

The reduced maximum clot firmness reported here after *in vivo* hemodilution with HES 130/0.4 may reflect not only functional fibrinogen deficiency but also a lack of platelets and impaired platelet aggregation. The average platelet count was  $181 \text{ (SD, 49)} \times 10^9 \text{ L}^{-1}$  at the time of hemodilution. According to recent studies by Larsen *et al.* [20] using the same method and activation with minimal amounts of tissue factor, platelet counts above  $100 \times 10^9 \text{ L}^{-1}$  cause only minor and insignificant changes to the MCF. Evaluation of platelet function using impedance aggregometry showed a significant reduction after hemodilution. Whether this reflects a specific inhibition of the platelets or represents simple dilution was not elucidated in this investigation. However, previous studies have shown that low-molecular starches do not impair platelet function [8,21]. Finally, substitution with the fibrinogen concentrate did not alter platelet aggregation. Lag time and time to peak in thrombin generation were significantly impaired after hemodilution but unaltered after fibrinogen administration.

As Nielsen *et al.* [22] have reported recently, dilution with HES 130/0.4 seems to reduce thrombin-substrate interaction rather than thrombin generation. Fluid regimens in this study involved HES 130/0.4 as a replacement for blood loss, in addition to basic crystalloid infusion. This may have led to an underestimation of the effect of HES on the thrombelastometric variables. However, based in the pharmacokinetic properties of crystalloids [23], we assume the effects of this potential confounder to be limited. Some researchers are more sceptical regarding the use of thromboelastometry/thrombelastography, probably because studies have shown a limited positive predicted value [24]. However, the main advantage of thromboelastometry is detection of coagulopathies in bleeding patients and guidance of rational hemostatic intervention. Blood lost during the operation may have been diluted by urine, lymphatic fluid and intestinal transudation thereby increasing the volume registered. However, we assume this confounder is equally distributed between the groups. Further a potential dilution would underestimate the effect on fibrinogen on blood loss. The cystectomy model of bleeding and *in vivo* hemodilution has been chosen as a result of its high external validity. First, included patients were healthy, except for their bladder cancer. Second, the bleeding experienced during the operation was short, intense and uniform among the patients. With the reservation that the power calculation was not pointed at blood loss and transfusion requirements, we conclude that it is likely that hemostatic intervention with a fibrinogen concentrate can improve hemostasis and reduce blood loss after dilutional coagulopathy induced by HES 130/0.4 during massive bleeding. In contrast, other hemostatic

drugs have shown only modest effects on blood loss [25]. Large-scale clinical investigations are required to clarify the optimal dosage as well as timing of the intervention, which might be controlled by the use of thrombelastometry as a monitoring and guiding tool.

In conclusion, we have demonstrated that fluid resuscitation with HES 130/0.4 in massive bleeding during elective cystectomy can induce coagulopathy characterized by reduced maximum clot firmness. In the present randomized, placebo-controlled clinical study, hemostatic intervention with a fibrinogen concentrate significantly improved maximum clot firmness and maximum velocity of clot formation, and it reduced the requirements for postoperative transfusion with red blood cells.

### Acknowledgements

The authors are grateful for the excellent laboratory assistance of K. Christiansen and L. Norengaard. We also thank M. S. Petersen, Department of Clinical Immunology, for retrieval of transfusion data, and we appreciate the support of the entire staff at the Department of Urological Anaesthesiology.

### Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

### References

- 1 Fenger-Eriksen C, Anker-Moller E, Heslop J, Ingerslev J, Sorensen B. Thrombelastographic whole blood clot formation after ex vivo addition of plasma substitutes: improvements of the induced coagulopathy with fibrinogen concentrate. *Br J Anaesth* 2005; **94**: 324–9.
- 2 Felfernig M, Franz A, Braunlich P, Fohringer C, Kozek-Langenecker SA. The effects of hydroxyethyl starch solutions on thromboelastography in preoperative male patients. *Acta Anaesthesiol Scand* 2003; **47**: 70–3.
- 3 Brummel-Ziedins K, Whelihan MF, Ziedins EG, Mann KG. The resuscitative fluid you choose may potentiate bleeding. *J Trauma* 2006; **61**: 1350–8.
- 4 de JE, Levi M. Effects of different plasma substitutes on blood coagulation: a comparative review. *Crit Care Med* 2001; **29**: 1261–7.
- 5 Fries D, Krismer A, Klingler A, Streif W, Klima G, Wenzel V, Haas T, Innerhofer P. Effect of fibrinogen on reversal of dilutional coagulopathy: a porcine model. *Br J Anaesth* 2005; **92**: 172–7.
- 6 Haas T, Fries D, Holz C, Innerhofer P, Streif W, Klingler A, Hanke A, Velik-Salchner C. Less impairment of hemostasis and reduced blood loss in pigs after resuscitation from hemorrhagic shock using the small-volume concept with hypertonic saline/hydroxyethyl starch as compared to administration of 4% gelatin or 6% hydroxyethyl starch solution. *Anesth Analg* 2008; **106**: 1078–86.
- 7 Fries D, Haas T, Klingler A, Streif W, Klima G, Martini J, Wagner-Berger H, Innerhofer P. Efficacy of fibrinogen and prothrombin complex concentrate used to reverse dilutional coagulopathy – a porcine model. *Br J Anaesth* 2006; **97**: 460–7.
- 8 Kozek-Langenecker SA. Effects of hydroxyethyl starch solutions on hemostasis. *Anesthesiology* 2005; **103**: 654–60.
- 9 Konrad C, Markl T, Schuepfer G, Gerber H, Tschopp M. The effects of in vitro hemodilution with gelatin, hydroxyethyl starch, and lactated

- Ringer's solution on markers of coagulation: an analysis using SONOCLOT. *Anesth Analg* 1999; **88**: 483–8.
- 10 Hiippala ST, Myllyla GJ, Vahtera EM. Hemostatic factors and replacement of major blood loss with plasma-poor red cell concentrates. *Anesth Analg* 1995; **81**: 360–5.
  - 11 Nielsen VG, Levy JH. Fibrinogen and bleeding: old molecule – new ideas. *Anesth Analg* 2007; **105**: 902–3.
  - 12 Nielsen VG. Colloids decrease clot propagation and strength: role of factor XIII-fibrin polymer and thrombin-fibrinogen interactions. *Acta Anaesthesiol Scand* 2005; **49**: 1163–71.
  - 13 Danes AF, Cuenca LG, Bueno SR, Mendarte BL, Ronsano JB. Efficacy and tolerability of human fibrinogen concentrate administration to patients with acquired fibrinogen deficiency and active or in high-risk severe bleeding. *Vox Sang* 2008; **94**: 221–6.
  - 14 Hopewell S, Clarke M, Moher D, Wager E, Middleton P, Altman DG, Schulz KF; CONSORT Group. CONSORT for reporting randomised trials in journal and conference abstracts. *Lancet* 2008; **26**: 281–3.
  - 15 Mann KG, Whelihan MF, Butenas S, Orfeo T. Citrate anticoagulation and the dynamics of thrombin generation. *J Thromb Haemost* 2007; **5**: 2055–61.
  - 16 Sorensen B, Johansen P, Christiansen K, Wöelke M, Ingerslev J. Whole blood coagulation thrombelastographic profiles employing minimal tissue factor activation. *J Thromb Haemost* 2003; **1**: 551–8.
  - 17 Gerotziafas GT, Depasse F, Busson J, Leflem L, Elalamy I, Samama MM. Towards a standardization of thrombin generation assessment: the influence of tissue factor, platelets and phospholipids concentration on the normal values of Thrombogram–Thrombinoscope assay. *Thromb J* 2005; **3**: 16.
  - 18 Hemker HC, Wienders S, Kessels H, Beguin S. Continuous registration of thrombin generation in plasma, its use for the determination of the thrombin potential. *Thromb Haemost* 1993; **70**: 617–24.
  - 19 Fries D, Innerhofer P, Reif C, Streif W, Klingler A, Schobersberger W, Velik-Salchner C, Friesenecker B. The effect of fibrinogen substitution on reversal of dilutional coagulopathy: an in vitro model. *Anesth Analg* 2006; **102**: 347–51.
  - 20 Larsen OH, Ingerslev J, Sorensen B. Whole blood laboratory model of thrombocytopenia for use in evaluation of hemostatic interventions. *Ann Hematol* 2007; **86**: 217–21.
  - 21 Franz A, Braunlich P, Gamsjager T, Felfernig M, Gustorff B, Kozek-Langenecker SA. The effects of hydroxyethyl starches of varying molecular weights on platelet function. *Anesth Analg* 2001; **92**: 1402–7.
  - 22 Nielsen VG. Hydroxyethyl starch does not decrease prothrombin to thrombin conversion. *Acta Anaesthesiol Scand* 2008; **52**: 163–4.
  - 23 Hiippala S, Teppo AM. Perioperative volume effect of HES 120/0.7 compared with dextran 70 and Ringer acetate. *Ann Chir Gynaecol* 1996; **85**: 333–9.
  - 24 Watson HG, Greaves M. Can we predict bleeding? *Semin Thromb Hemost* 2008; **34**: 97–103.
  - 25 Brandsborg S, Sorensen B, Poulsen LH, Ingerslev J. Recombinant activated factor VIIa in uncontrolled bleeding: a haemostasis laboratory study in non-haemophilia patients. *Blood Coagul Fibrinolysis* 2006; **17**: 241–9.