

Recombinant factor VIIa in orthotopic liver transplantation: influence on parameters of coagulation and fibrinolysis

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The effect of recombinant factor VIIa (rFVIIa) on blood loss was evaluated in cirrhotic patients undergoing orthotopic liver transplantation. In the present study, we explored the effect of rFVIIa on coagulation and fibrinolysis during orthotopic liver transplantation. Coagulation factors, parameters of thrombin generation and parameters of fibrinolysis were measured in six patients who had received a single dose of 80 µg/kg rFVIIa and in ten controls, during and after orthotopic liver transplantation. Baseline concentrations and course of coagulation factors were similar in patients and controls. Thrombin generation did not rise after the administration of rFVIIa, but showed a sharp increase after reperfusion in patients, as compared with controls. No difference in fibrinolysis was apparent between patients and controls. No evidence of diffuse intravascular coagulation was seen. We conclude that the use of rFVIIa in orthotopic liver transplantation seems to enhance thrombin generation in a localized and time-limited manner, without causing systemic coagulation.

Introduction

Recombinant factor VIIa (rFVIIa; NovoSeven, Novo Nordisk, Copenhagen, Denmark) has been studied as a prohaemostatic drug in a wide range of conditions. We recently conducted a pilot trial of recombinant factor VIIa in six orthotopic liver transplantation (OLT) patients [1] and further studies in this field are underway. In our pilot study, rFVIIa decreased transfusion requirements during OLT. We hypothesized that this effect could be explained from changes in coagulation and fibrinolysis parameters. The aim of this study was to describe the effect of rFVIIa on these parameters during OLT.

The effect of rFVIIa in this specific situation has not been described before. One study was performed in non-bleeding patients with liver cirrhosis and their prolonged prothrombin time could be corrected by administration of rFVIIa [2]. Most available data are from haemophilia patients with inhibitors to factor VIII or IX, in whom rFVIIa is routinely used. Several authors showed that the prothrombin time shortened considerably, with a variable shortening of the activated partial thromboplastin time (APTT) attributed to direct

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activation of factor X by factor VIIa [3–5]. No effect was seen on platelet count, levels of antithrombin, D-dimers or α 2-antiplasmin [4,6]. Thrombin–antithrombin complex (TAT) levels increased to a maximum of twice the baseline value 2 h after administration in non-bleeding patients, but did not increase in bleeding haemophilia patients [6]. Small increases in prothrombin activation fragment F1+2 (F1+2) were reported after administration of rFVIIa [7].

Methods

Patients

This study was reviewed and approved by the ethics committee of our hospital. Six adult patients undergoing OLT for end-stage liver disease enrolled in the pilot trial on rFVIIa and were eligible for the present study. They gave their written informed consent. Controls were ten consecutive OLT patients with end-stage liver disease, who did not receive rFVIIa.

Procedure

A single dose of 80 µg/kg rFVIIa was administered within 10 min before the start of surgery. Transfusions were administered according to predefined criteria [5].

Blood was sampled at baseline; at 30, 90 and 120 min after induction of anaesthesia (phase 1; timepoints 1A, 1B, 1C, 1D); at 30 and 90 min after removal of the liver, and 5 min before reperfusion (phase 2; 2A, 2B, 2C); at 5, 30 and 60 min after reperfusion (phase 3; 3A, 3B, 3C); at the end of surgery; at 4, 8, 18 and 24 h after the end of surgery (phase 4; 4A, 4B, 4C, 4D); and at postoperative days 2, 3, 4 and 5 (phase 5; 5A, 5B, 5C, 5D). Controls were sampled at the same time as patients, except for the first sample, which was taken 30 min after induction of anaesthesia (but before the start of surgery) and less frequent sampling during the first 24 h after surgery. Blood cell counts were performed at all time points.

Laboratory assays

Blood for coagulation assays in study patients and controls was collected in 0.129 mol/l citrate, immediately separated and plasma frozen at -80°C until assayed. Blood for tissue plasminogen activator (t-PA) activity was collected in Stabilyte (Biopool, Umea, Sweden). Fibrinogen was measured according to the Clauss method, using bovine thrombin (Biopool). Factors II, V, VII, VIII, IX, X and XI were assayed by a one-stage method based on either prothrombin time (Thromboplastin IS; Dade Behring, Marburg, Germany) or APTT (Actin FS; Dade Behring), using the appropriate factor deficient plasma (factor II-deficient plasma from Dade Behring; all others from Organon Teknika, Durham, North Carolina, USA). The Coamatic assay (Chromogenix AB, Molndal, Sweden) was used for antithrombin. TAT, F1+2 and plasmin- α 2antiplasmin complex (PAP) were measured by enzyme-linked immunosorbent assay (Enzygnost TAT micro, Enzygnost F1,2 micro and Enzygnost PAP micro respectively; Dade Behring) as were t-PA antigen (Asserachrom t-PA; Roche, Basel, Switzerland) and plasminogen activator inhibitor type 1 (PAI-1) antigen (Chromolize PAI-1; Biopool). The Chromolize t-PA assay was used for t-PA activity (Biopool). A latex agglutination test was used for D-dimers (Minutex; Biopool). Levels of fibrinogen, factors II, V, VII, VIII, IX, X, XI and antithrombin and platelet counts were adjusted for changes in haematocrit as compared with baseline.

Analysis

Data are presented in median values of patients and controls. As the groups were small and the aim of the study was exploratory, no statistical comparisons were made.

Results

Characteristics of the six patients and ten controls are summarized in Table 1. Transplantation in study patients was performed with preservation of the retrohepatic caval vein (piggy back technique). In controls,

Table 1 Baseline characteristics of study patients and controls

	Study patients (n = 6)		Controls (n = 10)	
Age (years)	43	37–61	39	22–48
Female (n)	2		4	
Child–Pugh	A (n)	0	4	
	B (n)	3	6	
	C (n)	3	ND	
Cholestatic liver disease	2		6	
Urea nitrogen (mmol/l)	3.6	2.4–9.5	5.1	2.8–10.0
Haemoglobin (g/dl)	11.8	8.4–13.2	10.6	7.3–16.6
PT (s)	21.8	15.2–23.6	16.8	13.4–21.0
APTT (s)	38.5	32.8–51.9	40.5	33.9–46.4
Fibrinogen (g/l)	1.7	1.4–3.9	2.3	1.4–5.1
Antithrombin (%)	39	27–69	65	37–135
Platelet count ($10^9/l$)	78	38–134	107	19–194

PT, prothrombin time; APTT, activated partial thromboplastin time. Values in median and range, unless otherwise denoted.

venovenous bypass was used in nine of the 10. Fresh frozen plasma was infused in three of six patients and nine controls, median 1 unit (range 0–7 units) and 3.5 units (range 0–8 units), respectively. Fibrinogen concentrate in one patient and four controls, 0 g (0–2 g) and 0 g (0–4 g). Platelet concentrate was given in two of six patients and two of ten controls, 0 units (0–1 units) and 0 units (0–1 units).

Coagulation factors

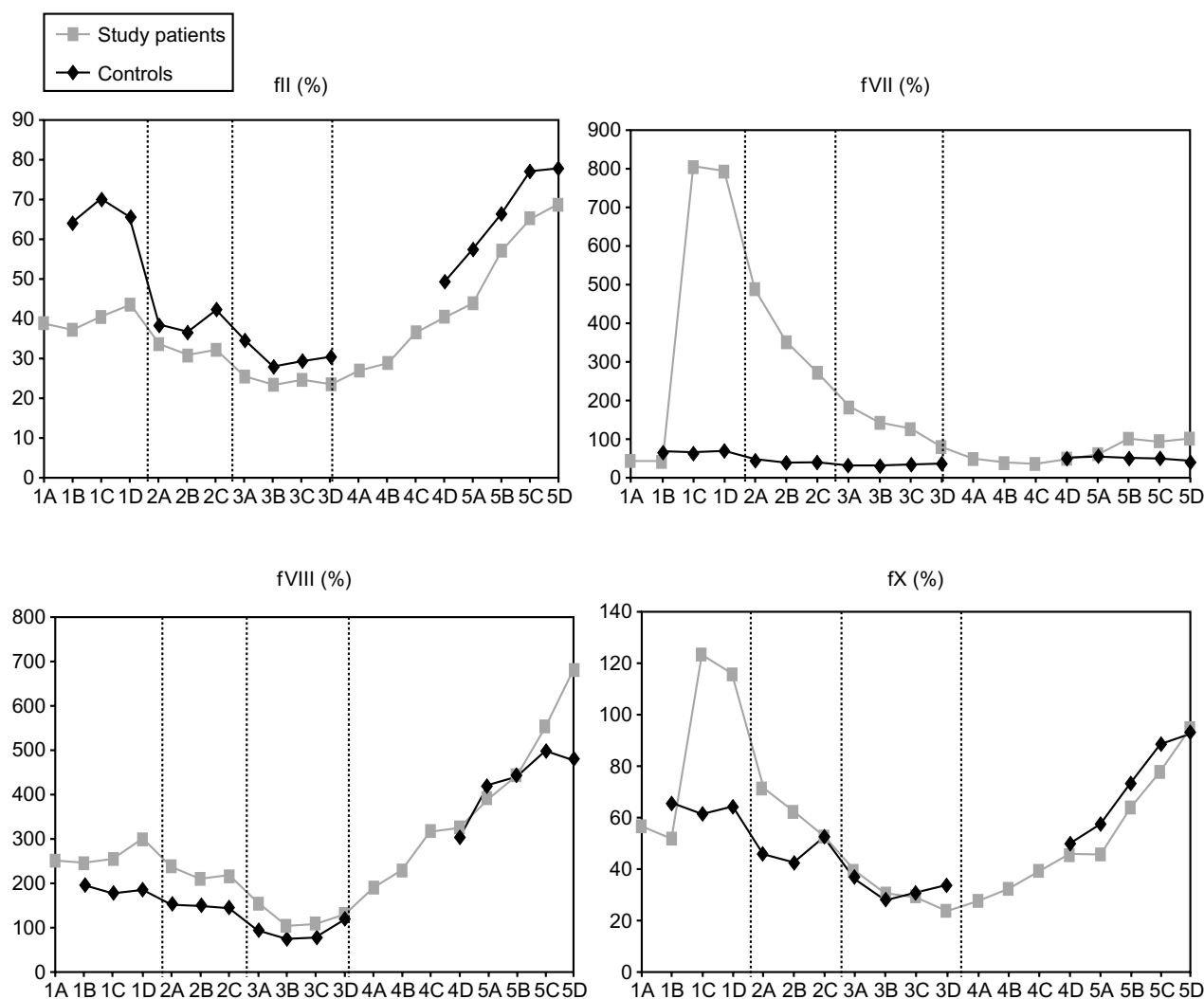
Data from study patients and controls on factors II, VII, VIII and X are shown in Figure 1 (data on other factors not shown). Before the start of transplantation, plasma levels of fibrinogen, factors II, V, VII, IX, X, and XI as well as antithrombin levels were decreased, while factor VIII levels were increased. During surgery, all factors showed a steady decline from the anhepatic phase until after reperfusion, with the exception of factor VII, which was largely influenced by the administration of rFVIIa. Postoperatively, all coagulation factors recovered. Values at the end of the study period exceeded the preoperative values. The same course was seen in study patients and controls. Pre-operative baseline levels of fibrinogen and factors II and VII were somewhat higher in controls than in study patients. Simultaneously with the peak value of factor VII (time points 1C–1D), factors V, VIII, X, IX and XI increased. This increase was not observed for fibrinogen, factor II and antithrombin. Patterns were similar in individual patients (data not shown). In controls, no peak values were seen in phase 1.

Thrombin time in all study patients was slightly longer than that of normal plasma (18.6–24.1 s at time point 1A versus 18.2 s), and did not change after administration of rFVIIa (20.4–23.2 s at time point 1C).

Thrombin generation

In study patients, both TAT and F1+2 showed a gradual increase during phases 1 and 2, with a sharp

Fig. 1



Course of coagulation factor levels in study patients and controls. Values in median; phases of orthotopic liver transplantation divided by dotted lines. f, factor.

increase after reperfusion (time points 2C-3A) (Fig. 2). In contrast to TAT, F1+2 remained above baseline values during the postoperative days. In controls, maximum levels of F1+2 and TAT were lower. Values increased gradually during transplantation, without peak levels after reperfusion.

Fibrinolysis

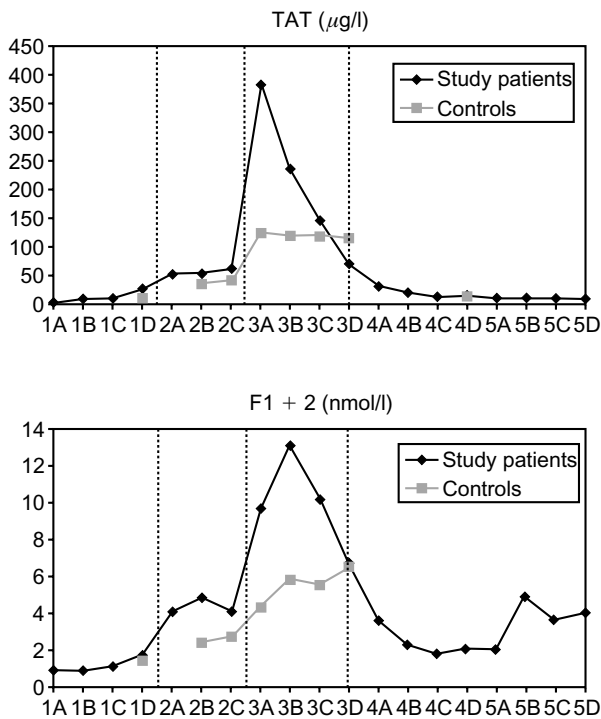
In study patients, no increase in parameters of fibrinolysis was seen in phase 1 (Fig. 3). PAP and D-dimers increased during phase 2. While PAP levels fell after reperfusion, levels of D-dimers increased further to a maximum in the middle of phase 3. During the postoperative days, both PAP and D-dimers increased again slightly. In controls (Fig. 3), D-dimers increased similarly during surgery but to lower maximum values. PAP levels were not measured in controls. During phases 1

and 2, tPA antigen levels were rising, and were comparable in study patients and controls (Fig. 4). At 5 min after reperfusion (3A, data available only in controls), the maximum value of t-PA antigen was measured. From 3B on, values levelled off in study patients, while controls showed a new increase until the first preoperative day. In controls, the course of t-PA activity was comparable with t-PA antigen: a steady increase from the beginning of phase 2 until immediately after reperfusion (time point 3A). PAI-1 antigen levels in study patients and controls were similar: both groups showed stable, low levels with a sharp increase at the end of the reperfusion phase.

Platelets

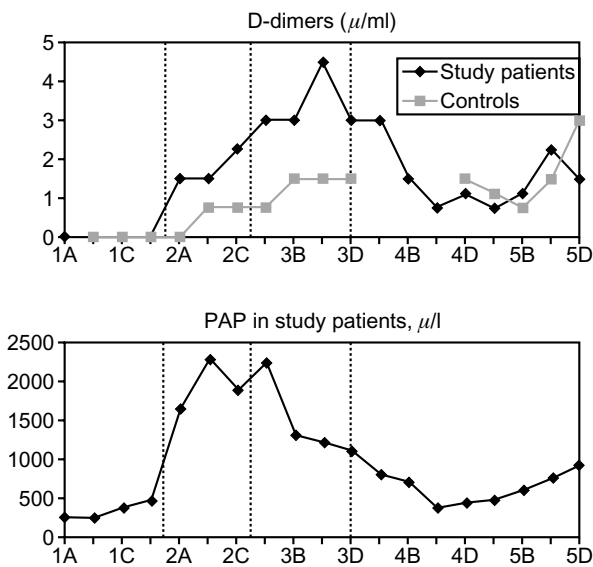
Peroperatively, platelet counts in study patients were

Fig. 2



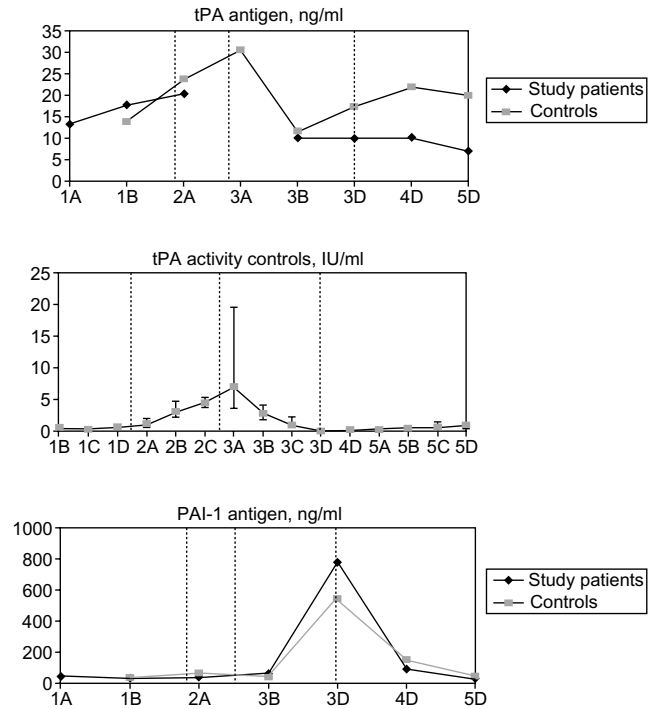
Parameters of thrombin generation in study patients and controls. Values in median; phases of orthotopic liver transplantation divided by dotted lines. TAT, thrombin-antithrombin complex.

Fig. 3



Parameters of fibrinolysis in study patients and controls. Values in median; phases of orthotopic liver transplantation divided by dotted lines. PAP, plasmin-α2antiplasmin.

Fig. 4



Parameters of activation of the fibrinolytic system in study patients and controls. Values in median, and interquartile ranges for tissue plasminogen activator (t-PA) activity levels; phases of OLT divided by dotted lines. PAI-1, plasminogen activator inhibitor type 1.

stable. Values decreased from 80 to about $50 \times 10^9/l$ postoperatively (data not shown).

Discussion

The course of coagulation factors during phases 2 and 3 did not differ between study patients and controls. They are consistent with data from the literature [8–10]. Most coagulation factors showed a peak activity parallel to the sharp increase in factor VII activity. The most pronounced increase was seen in factor X. In *in-vitro* studies, high concentrations of factor VIIa – similar to those reached in plasma after therapeutic administration of rFVIIa – directly activated factor X on the surface of activated platelets [5,11]. Subsequent thrombin generation was seen in the *in-vitro* experiments. In our study, the peak levels of factor X activity at time points 1C and 1D were not associated with increased thrombin generation as measured by levels of TAT and F1+2 at that stage of transplantation. This makes a testing artefact more likely, caused by high levels of factor VIIa influencing the prothrombin time/APTT-based assays.

Plasma levels of TAT and F1+2 did increase strongly after reperfusion in study patients, in contrast with

controls. This could be explained by reperfusion of a donor liver with substantial endothelial damage [12] in the presence of rFVIIa levels that, though declined in comparison with phase 1, were still supraphysiological. The TAT levels we found in controls were similar to those reported previously [13,14]. Literature data on F1+2 during OLT is not available, but the postoperative levels in our study patients are consistent with findings from others after general surgery [15]. There was no evidence that rFVIIa caused systemic activation of coagulation as the course of coagulation factor levels was similar in study patients and controls, whereas platelet counts showed no decline during surgery.

We could not demonstrate a clear effect of rFVIIa on parameters of fibrinolysis. Both study patients and controls showed increasing levels of t-PA in the anhepatic phase and PAI-1 after reperfusion. The only difference between study patients and controls were higher levels of D-dimers in study patients after reperfusion. Since the peak of D-dimers followed the peaks of TAT and F1+2, it can be speculated that more fibrin was formed and consequently the levels of D-dimers rose in study patients.

Controls had a lower Child–Pugh score (i.e. had less severe liver disease) than patients. This was of no consequence for the present study as their actual preoperative and nadir values of coagulation factors were not clearly different from study patients. It must be stressed, however, that these control results are unfit to report on effects of rVIIa on blood loss, as their less severe liver disease could significantly decrease their transfusion requirements [16].

The generally held view is that rFVIIa initiates coagulation, limited to sites of injury. This occurs after complexing with tissue factor or in interaction with activated platelets [11]. The model of localized action of rFVIIa might, however, not be attributable to OLT. Instead of one localized site of haemorrhage, an extensive wound bed is expressing tissue factor. Vascular anastomoses are made that are prone to thrombosis; thrombosis of the hepatic artery and portal vein are recognized, serious complications of OLT [17]. In addition, extensive endothelial injury, which is present within the hepatic sinusoids due to ischemia/reperfusion injury [13], will lead to expression of tissue factor. All these factors can theoretically cause extensive coagulation activation and thrombosis in the donor liver and hepatic vessels. Patients could be protected from the development of thrombosis by fibrinolysis after reperfusion, counteracting the large increase in thrombin generation at that point. The fact that D-dimer levels were higher in the study patients than in controls indicates that this mechanism is active after the administration of rFVIIa.

In conclusion, the very high levels of exogenous FVIIa during the pre-anhepatic phase did not lead to increased thrombin generation. After reperfusion, a sharp rise of thrombin generation markers was seen in patients receiving rFVIIa. This was not associated with diffuse systemic coagulation. No effect on fibrinolysis was seen. The use of rFVIIa in OLT seems to enhance thrombin generation in a localized and time-limited matter, without causing systemic coagulation.

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References

- Hendriks HGD, Meijer K, De Wolf JThM, Klompmaaker IJ, Porte RJ, De Kam PJ, *et al.* Reduced transfusion requirements by recombinant factor VIIa in orthotopic liver transplantation, a pilot study. *Transplantation* 2001; **71**:402–405.
- Bernstein DE, Jeffers L, Erhardtsen E, Reddy R, Glazer S, Squiban P, *et al.* Recombinant factor VIIa corrects prothrombin time in cirrhotic patients: a preliminary study. *Gastroenterology* 1997; **113**:1930–1937.
- Lindley CM, Sawyer WT, Macik G, Lusher J, Harrison JF, Baird-Cox K, *et al.* Pharmacokinetics and pharmacodynamics of recombinant factor VIIa. *Clin Pharmacol Ther* 1994; **55**:638–648.
- Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA. Prospective, randomised trial of two doses of rFVIIa (NovoSeven) in haemophilia patients with inhibitors undergoing surgery. *Thromb Haemost* 1998; **80**:773–778.
- Telgt DS, Macik BG, McCord DM, Monroe DM, Roberts HR. Mechanism by which recombinant factor VIIa shortens the aPTT: activation of factor X in the absence of tissue factor. *Thromb Res* 1989; **56**:603–609.
- Macik BG, Lindley CM, Lusher J, Sawyer WT, Bloom AL, Harrison JF, *et al.* Safety and initial clinical efficacy of three dose levels of recombinant activated factor VII (rFVIIa): results of a phase I study. *Blood Coagul Fibrinolysis* 1993; **4**:521–527.
- Ingerslev J, Holm M, Christiansen K, Knudsen L, Négrier C. Levels of prothrombin activation peptide F1+2 in patients with a bleeding tendency. *Blood Coagul Fibrinolysis* 1998; **9**:S129–S134.
- Kratzer MAA, Dieterich J, Denecke H, Knedel M. Hemostatic variables and blood loss during orthotopic human liver transplantation. *Transplan Proc* 1991; **23**:1906–1911.
- Lewis JH, Bontempo FA, Awas SA, Kang YG, Kiss JE, Ragni MV, *et al.* Liver transplantation: intraoperative changes in coagulation factors in 100 first transplants. *Hepatology* 1989; **9**:710–714.
- Owen JA, Rettke SR, Bowie EJW, Cole TL, Jensen CC, Wiesner RH, *et al.* Hemostatic evaluation of patients undergoing liver transplantation. *Mayo Clin Proc* 1987; **62**:761–772.
- Monroe DM, Hoffman M, Oliver JA, Roberts HR. Platelet activity of high-dose factor VIIa is independent of tissue factor. *Br J Haematol* 1997; **99**:542–547.
- Clavien PA. Sinusoidal endothelial cell injury during hepatic preservation and reperfusion. *Hepatology* 1998; **28**:281–285.
- Bakker CM, Metselaar HJ, Gomes MJ, Porte RJ, Groenland TN, Schalm SW, *et al.* Intravascular coagulation in liver transplantation: is it present or not? A comparison between orthotopic and heterotopic liver transplantation. *Thromb Haemost* 1993; **69**:25–28.
- Porte RJ, Bontempo FA, Knot EAR, Lewis JH, Kang YG, Starzl TE. Systemic effects of tissue plasminogen activator-associated fibrinolysis and its relation to thrombin generation in orthotopic liver transplantation. *Transplantation* 1989; **47**:978–984.
- Richards EM, Alexander GJM, Calne RY, Baglin TP. Thrombocytopenia

following liver transplantation is associated with platelet consumption and thrombin generation. *Br J Haematol* 1997; **98**:315–321.

- 16 Hendriks HGD, Van der Meer J, Klompmaker IJ, Choudhury N, Hagens JAM, Porte RJ, *et al.* Blood loss in orthotopic liver transplantation: a retrospective analysis of transfusion requirements and the effects of autotransfusion of cell saver blood in one hundred and sixty-four consecutive patients. *Blood Coagul Fibrinolysis* 2000; **11**: S87–S93.
- 17 Sánchez-Bueno F, Hernández Q, Ramírez P, Robles R, Acosta F, Rodríguez JM, *et al.* Vascular complications in a series of 300 orthotopic liver transplants. *Transplant Proc* 1999; **31**:2409–2410.