

# D I C

## Pathogenesis, Diagnosis and Therapy of Disseminated Intravascular Fibrin Formation

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## Contents

Preface	v
Acknowledgements	vi
<b>Definition of disseminated intravascular coagulation</b>	
Attempts to define disseminated intravascular coagulation <i>G. Müller-Berghaus, M. Blombäck and J. W. ten Cate</i>	3
<b>Pathogenesis</b>	
Bacteria, cells and disseminated intravascular coagulation in man <i>P. Brandtzaeg and P. Kierulf</i>	11
Role of tissue factor in the coagulant and inflammatory response to LD <sub>100</sub> <i>E. coli</i> sepsis and in the early diagnosis of DIC in the baboon <i>F.B. Taylor Jr</i>	19
Leukocytes, adhesive proteins and disseminated intravascular coagulation: treatment of DIC with plasma/leukapheresis? <i>B. Østerud</i>	32
Tissue factor gene regulation <i>D. von der Ahe, B. Jost and G. Müller-Berghaus</i>	41
Tissue factor expression in endotoxin-activated monocytes is suppressed by agents which increase intracellular cyclic AMP concentration <i>D. de Prost and V. Ollivier</i>	47
Involvement of the vascular endothelium and activation of the coagulation system in human malaria <i>C.J. Hemmer, A. Bierhaus, P. Kern, R. Egbring, R. Seitz, P.P. Nawroth and M. Dietrich</i>	51
Effect of peripheral blood lymphocytes on procoagulant activity of human endothelial cells pretreated with lipopolysaccharide <i>K. Binder, E. Schmid and T.H. Müller</i>	55
Freshly formed thrombi induce procoagulant activity in human vascular endothelial cells <i>E. Schmid, K. Binder and T.H. Müller</i>	63
Hemostasis in orthotopic liver transplantation — a model for studying DIC development? <i>H. Riess, G. Himmelreich, M. Jochum, W. Machleidt, K. Hundt, P. Neuhaus and D. Huhn</i>	71

**Clinical aspects of DIC**

Markers of hemostatic activation in experimental endotoxemia:  
implications for therapy

- H. ten Cate, M. Levi, B.J. Biemond, S.J.H. van Deventer,  
T. van der Poll, H.R. Büller and J.W. ten Cate* 81
- Quantitative evaluation of hypercoagulability by fibrin monomer ELISA  
*C.-E. Dempfle, M. Dollman, S. Pfitzner, H. Lill, W. Mühlhofer,  
A. Dessauer and D.L. Heene* 89
- Determination of plasma fibrin monomers by a newly developed ELISA  
method in patients with disseminated intravascular coagulation  
*K. Okajima, M. Uchiba, K. Murakami, H. Okabe and K. Takatsuki* 99
- Soluble fibrin – a predictor for development and outcome of multiple  
organ failure  
*S. Bredbacka and M. Blombäck* 111
- Monitoring the anticoagulant treatment of DIC and recurrent thrombosis  
in patients with malignancies using the measurement of “soluble fibrin”  
(FM-test, Chromogenix), F1+2 and TAT complexes  
*J. Stibbe* 113
- Degradation of fibrinogen and its derivatives in the course of  
disseminated intravascular coagulation  
*W. Nieuwenhuizen* 117
- Evidence that heparin cofactor II participates in the control of  
disseminated intravascular coagulation  
*T.R. Andersson, P.M. Sandset, K. Nustad and U. Abildgaard* 125
- Cytokine release in leukocytopenic patients with septic shock is  
associated with coagulation activation and consumption of inhibitors  
*H. Ostermann, R. Mesters and J. Kienast* 129
- Increased activated protein C – its inhibitor complex levels in patients  
with disseminated intravascular coagulation  
*H. Wada, K. Minamikawa, S. Shirakawa, T. Hayashi, J. Nishioka  
and K. Suzuki* 131
- Activation of hemostasis and the release of cytokines following the  
injection of bacterial lysates: fibrinolysis precedes fibrinemia  
*J.U. Wieding, H.-J. Veltman, K.-F. Kölmel and M. Köhler* 139
- Alterations of coagulation and fibrinolysis during the preclinical phase  
after polytrauma  
*E. Seifried, L. Lampl, C. Seidl, M. Helm, B. Maier and K.-H. Bock* 147
- Alterations of coagulation inhibitors in consumption coagulopathy  
*B. Kemkes-Matthes and H.-G. Lasch* 159
- Hypercoagulation predicts outcome after isolated brain trauma  
*S. Bredbacka and G. Edner* 163

Sepsis and septic shock: hemostasis-fibrinolysis equilibrium as a prognostic factor and an indicator during therapy <i>N. Maurin and D. Frank</i>	165
Influence of blood sampling from venipunctures and catheter systems on serial determinations of prothrombin activation fragment 1+2 and thrombin-antithrombin III complex <i>G. Hafner, H. Schinzel, W. Ehrental, C. Wagner, U. Konheiser, R. Zotz, J. Lotz, R. Blank, L.S. Weilemann and W. Prellwitz</i>	171
Hypercoagulable state and DIC in neonatal asphyxia <i>S. Suzuki</i>	175
<b>New therapeutic approaches</b>	
New developments in the field of benzamidine-derived thrombin inhibitors <i>J. Stürzebecher, D. Prasa, E. Bretschneider, W. Bode, M. Bauer, H. Brandstetter, P. Wikström and H. Vieweg</i>	183
Investigation of in vitro antithrombotic potency of a r-hirudin (HBW 023) and a synthetic analogue <i>J. Römisch, W. Stüber and E.-P. Pâques</i>	191
Heparin treatment in thrombin-induced disseminated intravascular coagulation (DIC) in the baboon <i>H. J. Du Toit, A. R. Coetzee and D. O. Chalton</i>	197
Clinical evaluation of low-molecular-weight heparin (FR-860) on disseminated intravascular coagulation (DIC). A multicenter cooperative double-blind trial in comparison with heparin <i>N. Sakuragawa, H. Hasegawa, M. Maki, M. Nakagawa and M. Nakashima</i>	203
Protection of DIC-induced mortality in <i>Klebsiella pneumoniae</i> -infected and LPS-treated rats by antithrombin III <i>G. Dickneite and E.-P. Pâques</i>	215
Results of a double-blind, placebo-controlled trial of antithrombin III concentrates in septic shock with DIC <i>F. Fourrier, J.-J. Huart, I. Runge, C. Caron, J. Goudemand and C. Chopin</i>	221
Antithrombin concentrate dose to patients with acquired antithrombin deficiency <i>J. Albert and M. Blombäck</i>	227
Effect of two-domain tissue factor pathway inhibitor (2D-TFPI) and inactivated factor VIIa on endotoxin-induced DIC in rabbits <i>C. Bregengaard, O. Nordfang, P. Wildgoose and V. Dinness</i>	229
Antithrombotic effects of recombinant soluble human thrombomodulin analogs in animal models of tissue-factor and thrombin induced DIC <i>W. Witt, M. Fredrich, R. Wydro and J. Morser</i>	233

The xanthine derivatives HWA 138 and A 80 attenuate activation of coagulation in porcine endotoxin shock <i>M. Spannagl, G. Frank, M. Goedde and H. Hoffmann</i>	239
Effect of nafamostat mesilate (NM), a synthetic protease inhibitor, on the extrinsic pathway of coagulation <i>M. Uchiba, K. Okajima, H. Abe, H. Okabe and K. Takatsuki</i>	243
The use of Octaplas in patients undergoing open heart surgery <i>B.G. Solheim, J.L. Svennevig, B. Mohr, M. Dragsund, H. Noddeland, S. Töllefsrud, F. Brosstad, H. Rollag and T.E. Mollnes</i>	253
<b>Index of authors</b>	263
<b>Keyword index</b>	265

## Hemostasis in orthotopic liver transplantation — a model for studying DIC development?

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### Introduction

In orthotopic liver transplantation (OLT) hyperfibrinolysis is most prominent immediately before reperfusion — characterized by increased formation of plasmin-antiplasmin (PAP) complexes — and enhanced prothrombin activation thereafter — characterized by rising thrombin-antithrombin III (TAT) complexes — have been described and accepted to occur regularly [1–4]. Paradoxically, the increased prothrombin activation after revascularisation of the graft may be paralleled in the clinical situation by oozing in the operation field, a situation often observed during DIC.

We investigated parameters of coagulation, e.g., fibrinogen, factor XIII (F XIII), antithrombin III (AT III), free protein S antigen (protein S) and TAT, parameters of fibrinolysis, e.g., tissue-type plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), plasminogen activator inhibitor (PAI), C1-inhibitor and PAP, mediators of leucocyte activation, e.g., tumor necrosis factor alpha (TNF- $\alpha$ ), neopterin, cathepsin B, neutrophile elastase in complex with protease inhibitor (EPI), platelet count and platelet aggregability as well as the endothelium-derived soluble thrombomodulin (sTM)) in the course of 20 consecutive liver transplantations in order to understand more clearly the pathophysiological changes occurring during OLT, especially with reperfusion of the graft liver.

The impaired hemostasis due to preexisting liver failure is aggravated during OLT in these patients, resulting in a DIC-like constellation that may be considered to represent a human model for studying DIC.

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## Materials and Methods

OLT was carried out by established surgical techniques using a veno-venous bypass [5]. Packed red blood cells (RBC) and fresh frozen plasma (FFP) but neither platelets nor concentrates of hemostatic factors were substituted to compensate for intraoperative blood loss. Patients received a median of 8.6 units (range: 2–26) of RBC and 7.8 units FFP (range: 2–28). Most of these transfusions were given during the pre-anhepatic and anhepatic phases.

Blood samples were taken from the arterial line, after induction of anaesthesia and before operation start <1>, 5 min before <2> and 10 min after <3> the beginning of the anhepatic stage. Further samples were collected 5 min before reperfusion <4>, as well as 5 min <5>, 15 min <6> and 60 min afterwards.

The following parameters were measured: protein C activity, protein S Ag (all: Boehringer, Mannheim, Germany); AT III activity, C1-inhibitor activity, TAT complexes, F XIII activity (all: Behringwerke AG, Marburg, Germany), fibrinogen according to Clauss (Hoffmann-LaRoche, Basel, Switzerland), t-PA and PAI activities (both Chromogenix, Stockholm, Sweden), PAP complexes (Technoclone, Vienna, Austria), sTM Ag, (Stago, Paris, France).

Cathepsin B was measured by its enzymatic activity against the aminopeptidase substrate Z-Phe-Arg-NMec [6]. Elastase was estimated in complex with  $\alpha_1$ -proteinase inhibitor according to Neumann [7]. Neopterin was estimated by RIA (Henning, Berlin, Germany) and TNF- $\alpha$  by IRMA (Medgenix Diagnostics, Fleurus, Belgium). Urokinase-type plasminogen activator (u-PA) antigen was measured by ELISA and u-PA activity were measured by BIA as previously described [8].

Platelet rich plasma (PRP) was obtained by centrifugation for 6 min at 600 U/min. To obtain platelet poor plasma (PPP) another centrifugation for 20 min at 3,000 U/min was added. Platelet counts in PRP were adjusted to 200,000 platelets/ $\mu$ l or PRP with lower platelet counts was directly used in thrombocytopenic patients. In an individual series this platelet concentration was reobtained by diluting with PPP or by a further "concentrating" low speed centrifugation in order to eliminate different aggregating capacities caused by varying platelet counts. Platelet aggregation according to Born [9] registered by an automated platelet aggregation tracer system (APACT, Labor GmbH, Ahrensburg, Germany) was induced by collagen (final concentration 1.0  $\mu$ g/ml), ADP (2  $\mu$ mol/l) or ristocetin (1.2 mg/ml). Before each determination platelet aggregation was calibrated with PRP and PPP. Platelet aggregation was determined by measuring the maximal amplitude.

Results in the figures are given as medians and the non-parametric Wilcoxon signed-ranks test was used to evaluate differences of levels between the timepoints; values of  $p \leq 0.5$  were considered to be significant.

## Results

Most of the results obtained have been reported and discussed in detail [2,10–14].

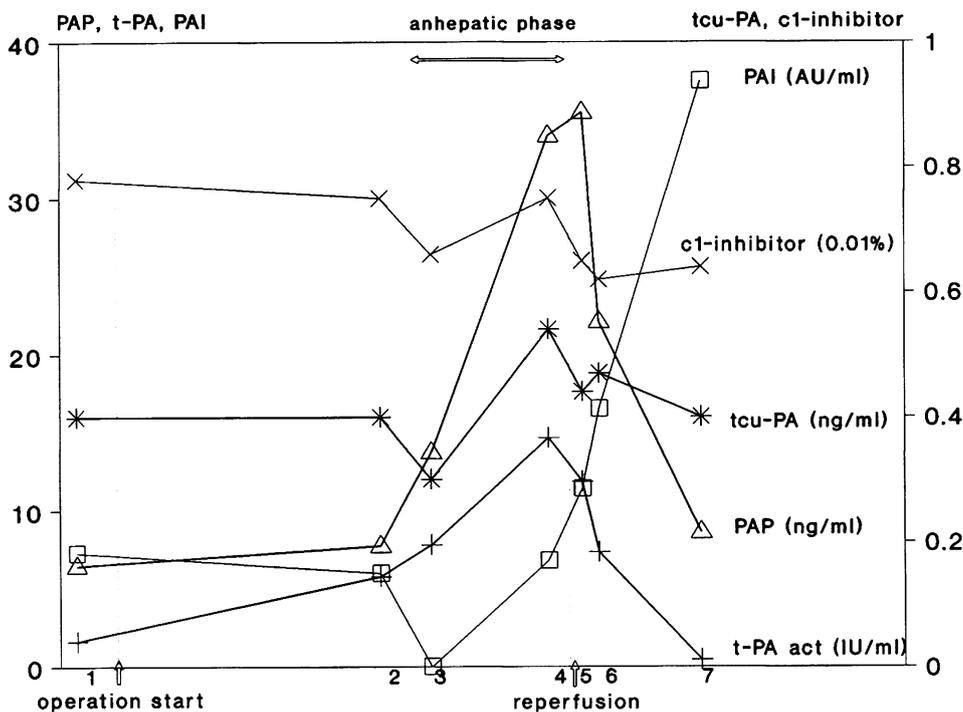


Fig. 1. Parameters of fibrinolysis (t-PA, PAI, C1-inhibitor activities, two-chain u-PA (active form of u-PA, tcu-PA) and PAP complexes) during OLT.

Here a comprehensive review focussing on the changes after revascularisation of the liver graft will be given.

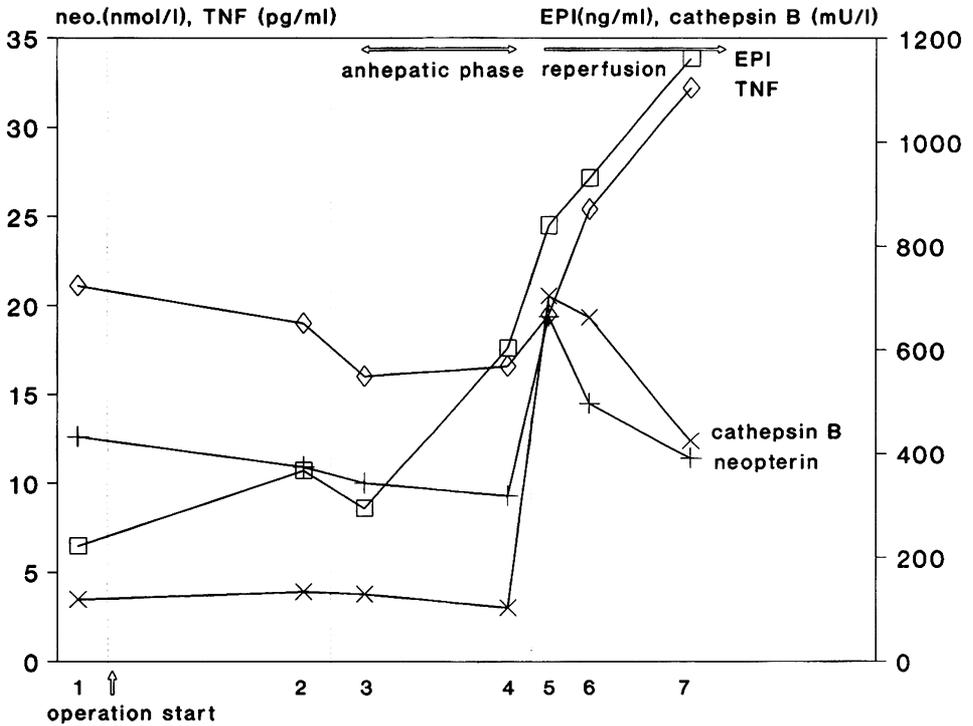
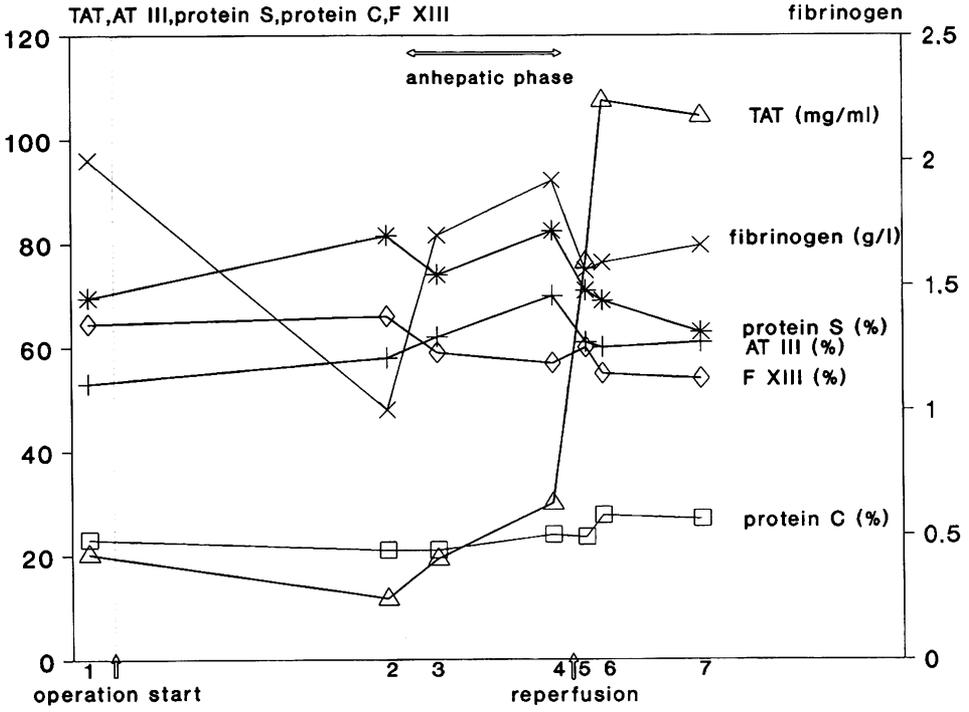
The evaluation of parameters of fibrinolysis (Fig. 1) showed a decrease in t-PA, u-PA and PAP complexes after reperfusion preceded by peak levels at the end of the anhepatic phase. The C1-inhibitor levels significantly decreased with reperfusion whereas the PAI levels demonstrated a highly significant rise.

Looking at parameters of coagulation (Fig. 2) a highly significant increase in TAT complexes was accompanied by decreases in the levels of fibrinogen, AT III and protein S, whereas protein C activity levels were rising towards the end of the operation. The F XIII levels demonstrated a short insignificant increase in the very early reperfusion phase followed by a significant decrease 10 min later.

With regard to the behaviour of platelets (Fig. 3) a significant decrease is seen in platelet count as well as in collagen-, ADP- and ristocetin-induced platelet aggregability with reperfusion.

Mediators of leucocyte activation, TNF- $\alpha$ , EPI complexes, cathepsin B, and neopterin increase clearly after reperfusion (Fig. 4).

Looking at endothelium-derived parameters (Fig. 5) we observed a significant increase of sTM.



## Discussion

During OLT a strongly enhanced prothrombin activation paralleled by an increased consumption of inhibitors and fibrinogen can be demonstrated after revascularisation of the graft. The thrombin formation is reflected in the increase in TAT and in the enhanced formation of fibrin monomers as well as d-dimers (not shown here, [3,12]). Furthermore the thrombin formation may play an important role in the impairment of platelet count and function. These data strongly favour that there is a DIC-like situation regularly occurring during reperfusion thereby confirming previous investigations [1–4].

The rise of protein C is probably reflecting an early synthesis by the graft liver. The increase in F XIII levels in the very early reperfusion phase is explained by hepatic release as elevated F XIII levels are seen as well in the perfusate [14].

Furthermore, we tried to evaluate the pathomechanisms leading to increased prothrombin activation. The extrinsic and intrinsic fibrinolytic systems are activated in the anhepatic phase — probably by contact activation in the veno-venous bypass and by reduced activator clearance — reaching maxima at its end. Hyperfibrinolysis as reflected in thromboelastography [2] may play an important role in influencing the anhepatic blood loss and may favour the generation of weak clots in the operation field. After revascularisation of the graft liver plasminogen activators can be cleared out of the systemic circulation. PAI may be released by (thrombin-) stimulated platelets and in addition PAI activity steeply rises due to its acute-phase properties. Thereby decreases in t-PA and u-PA activities are explained. The decrease of C1-inhibitor is caused by consumption during the anhepatic phase.

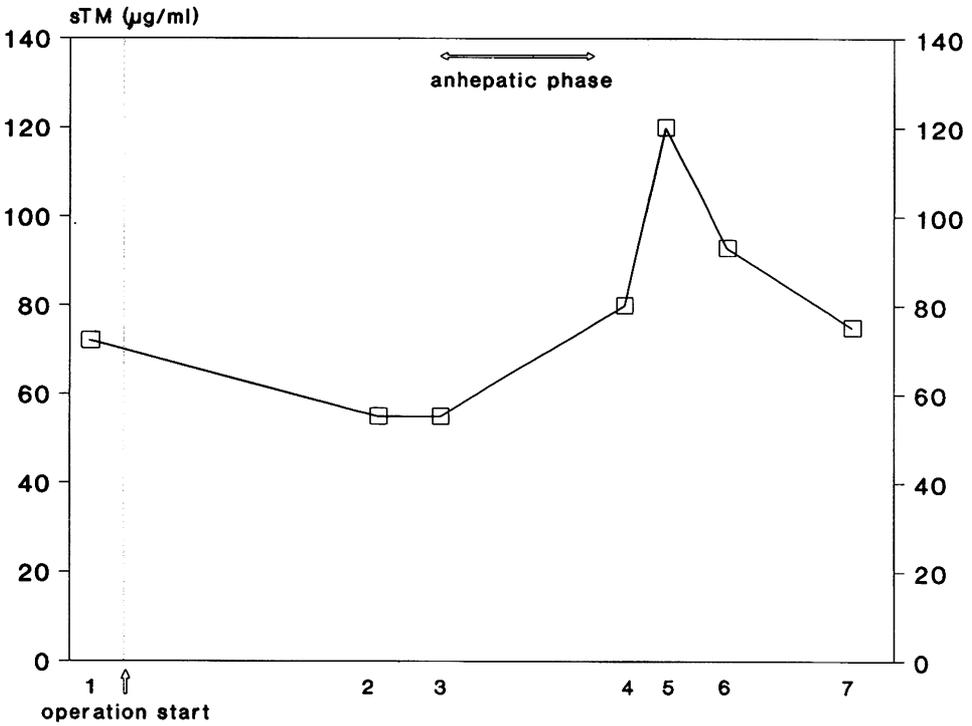
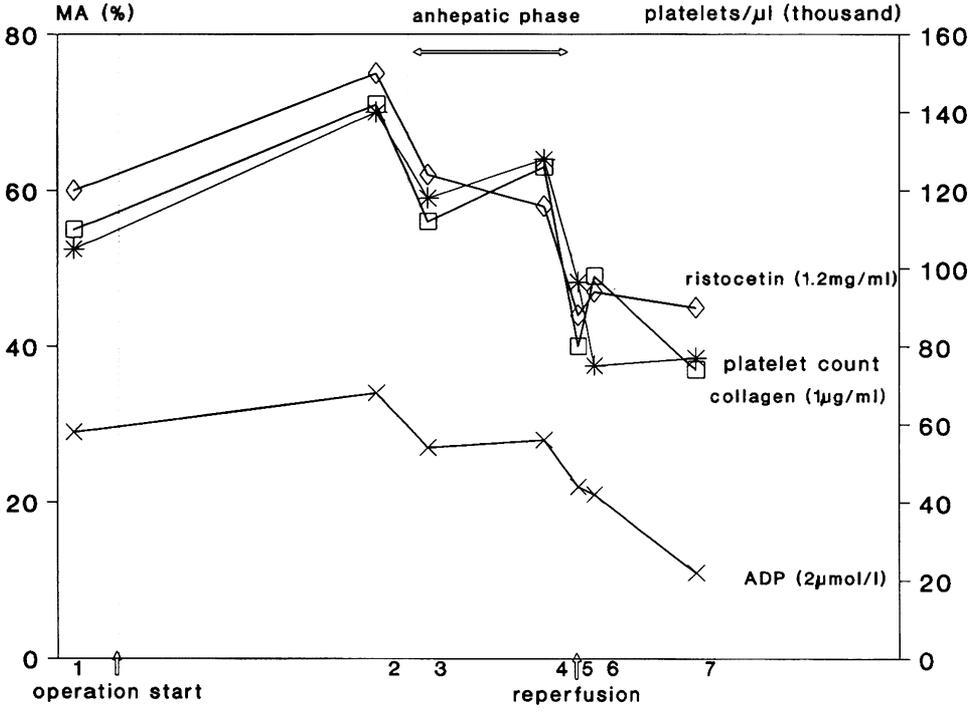
However, no correlation could be demonstrated between parameters of increased fibrinolysis and parameters of increased thrombin formation, making the possibility unlikely, that the observed “DIC” is secondary to hyperfibrinolysis.

In parallel with increased prothrombin activation in the reperfusion phase a significant decrease in platelet count and platelet aggregability and an increase in leucocytic mediators could be observed suggesting platelet and leucocyte activation, as had been described during DIC [6]. In addition, increasing levels of sTM in the reperfusion phase suggest endothelial damage. Endothelial damage is able to induce both, platelet and leucocyte activation and vice versa. Mediators released upon leucocyte activation like elastase may induce thrombin activation, degrade AT III and may thus induce a DIC-like state [15] as well as modulate platelet function [16]. TNF- $\alpha$  released from leucocytes on the other hand alters the endothelium [17]. Furthermore mediators released from leucocytes in addition to those measured exhibit a variety of degrading properties. The oozing observed exclusively in the reperfusion phase may thereby result from the proteolysis of non-resistant clots formed during the

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*Fig. 2.* (Facing page, top). Parameters of coagulation (fibrinogen, F XIII, AT III, TAT complexes, free protein S antigen) during OLT.

*Fig. 3.* (Facing page, bottom). Mediators of leucocyte activation (TNF- $\alpha$ , neoptein, cathepsin B, EPI complexes) during OLT.



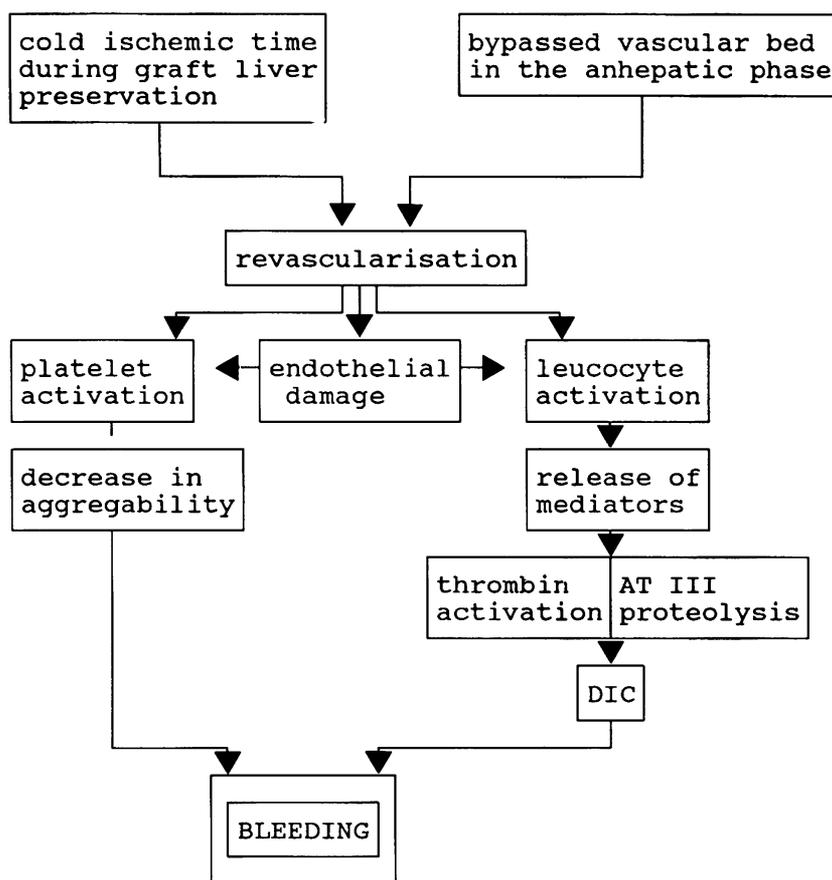


Fig. 6. Suggested pathomechanism leading to oozing and increased blood loss during the reperfusion phase in orthotopic liver transplantation.

anhepatic phase when systemic signs of hyperfibrinolysis are present. On the other hand, locally restricted reactive (“secondary”) hyperfibrinolysis cannot be ruled out by our investigations. This view is supported by the protective effect of aprotinin, an antifibrinolytic protease inhibitor, on oozing in OLT [18].

Comparative studies in patients undergoing OLTs and heterotopic liver transplantation [19] stress the importance of the diseased host liver preventing the deterioration in hemostasis observed during the anhepatic and reperfusion phases.

Facing the question raised in the headline of this article we are convinced that

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Fig. 4. (Facing page, top). Platelet count and parameters of platelet aggregability (ADP-, collagen- and ristocetin-induced platelet aggregability) measured by maximal amplitude (MA, see text) during OLT.

Fig. 5. (Facing page, bottom). Soluble thrombomodulin (sTM) as endothelial-derived parameter during OLT.

hemostasis in OLT is a valuable model in humans to study DIC development because the changes described are regularly observed, though differently pronounced, in every patient. There is a well accepted underlying disease, e.g., terminal liver failure with impaired hemostasis. OLT represents an “exogenous” trigger resulting in increased fibrin formation, bleeding and the risk of multi-organ failure. Therefore the main criteria for the diagnosis of DIC are fulfilled. Our investigations provide evidence for a hypothetical pathophysiological pattern (Fig. 6) leading to increased bleeding tendency in the reperfusion phase in OLT and are offering the opportunity to study different therapeutic options to ameliorate the clinical picture.

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