D I C

Pathogenesis, Diagnosis and Therapy of Disseminated Intravascular Fibrin Formation

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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-α), 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 preanhepatic 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 α_1-proteinase inhibitor according to Neumann [7]. Neopterin was estimated by RIA (Henning, Berlin, Germany) and TNF-α 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/µl 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 µg/ml), ADP (2 µmol/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].

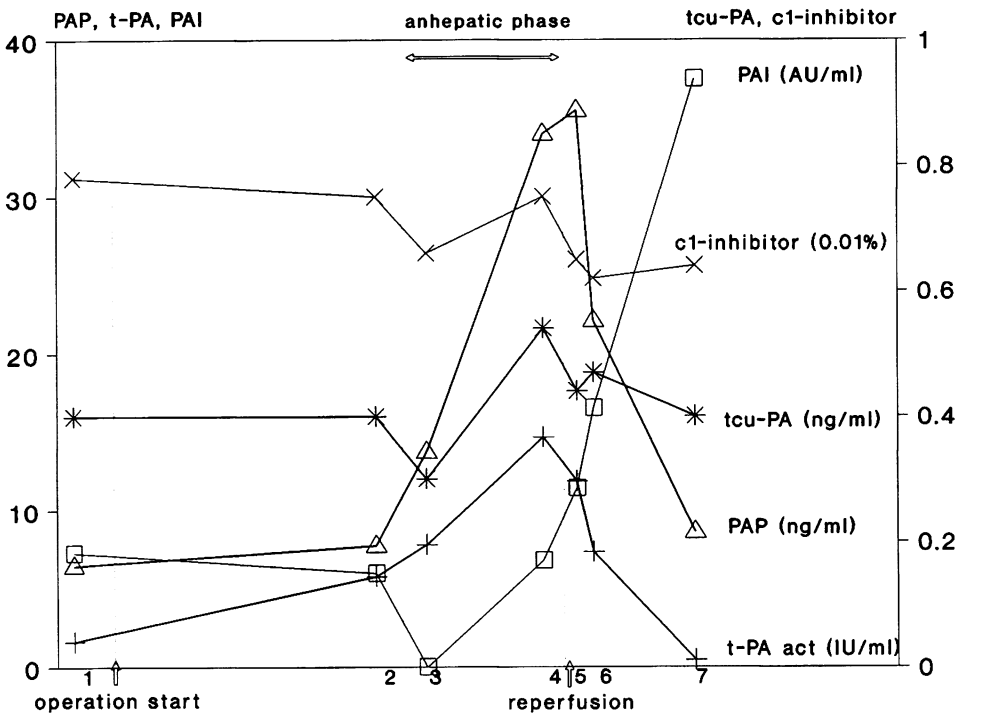


Fig. 1. Parameters of fibrinolysis (t-PA, PAI, C1-inhibitor activities, two-chain u-PA (active form of u-PA, tcu-PA) and PAP complexes) during OLT.

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

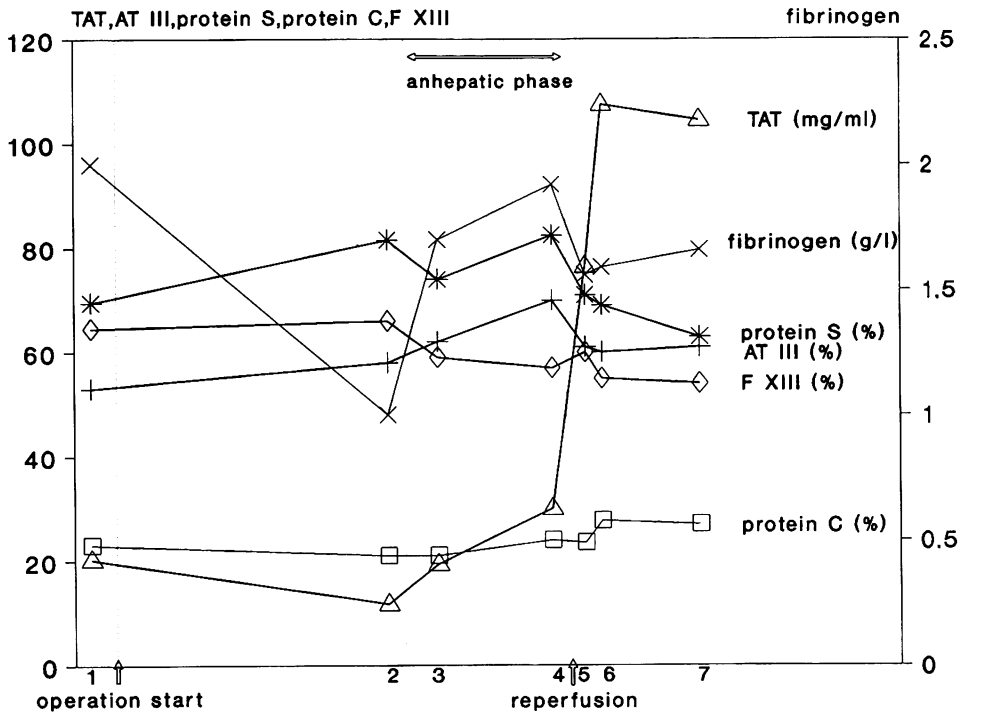
The evaluation of parameters of fibrinolysis (Fig. 1) showed a decrease in t-PA, u-PA and PAP complexes after reperfusion preceded by peak levels at the end of the anhepatic phase. The C1-inhibitor levels significantly decreased with reperfusion whereas the PAI levels demonstrated a highly significant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

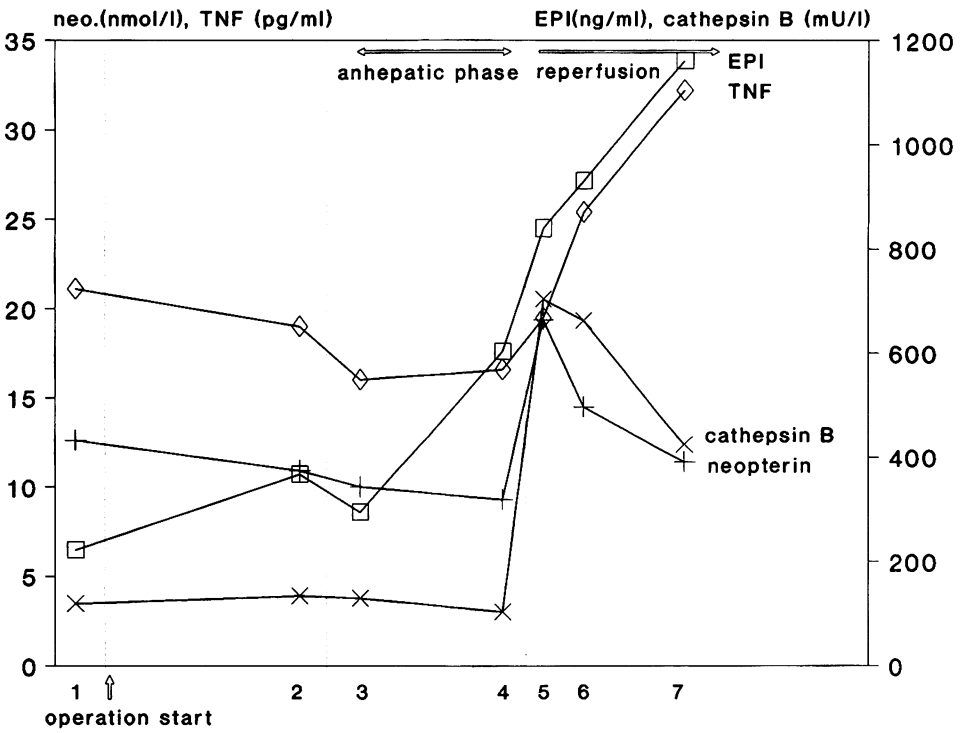
Looking at parameters of coagulation (Fig. 2) a highly significant increase in TAT complexes was accompanied by decreases in the levels of fibrinogen, AT III and protein S, whereas protein C activity levels were rising towards the end of the operation. The F XIII levels demonstrated a short insignificant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


TAT,AT III,protein S,protein C,F XIII
fibrinogen
anhepatic phase
TAT (mg/ml)
fibrinogen (g/l)
protein S (%)
AT III (%)
F XIII (%)
protein C (%)
120
100
80
60
40
20
0
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
operation start
reperfusion


neo.(nmol/l), TNF (pg/ml)
EPI(ng/ml), cathepsin B (mU/l)
anhepatic phase
reperfusion
EPI
TNF
cathepsin B
neopterin
35
30
25
20
15
10
5
0
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
operation start

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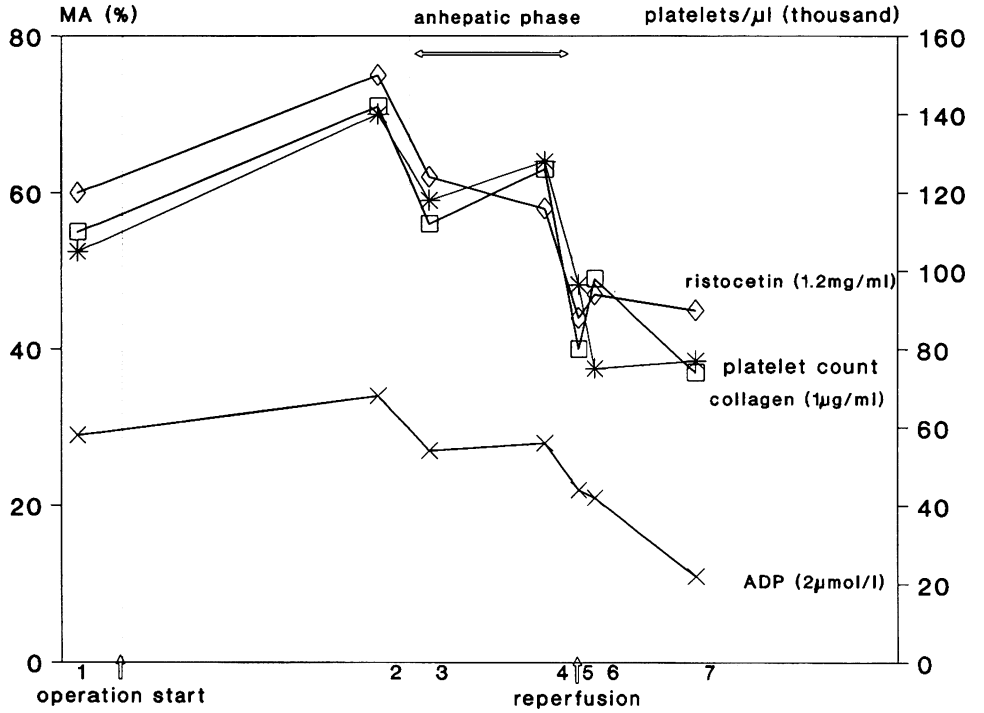
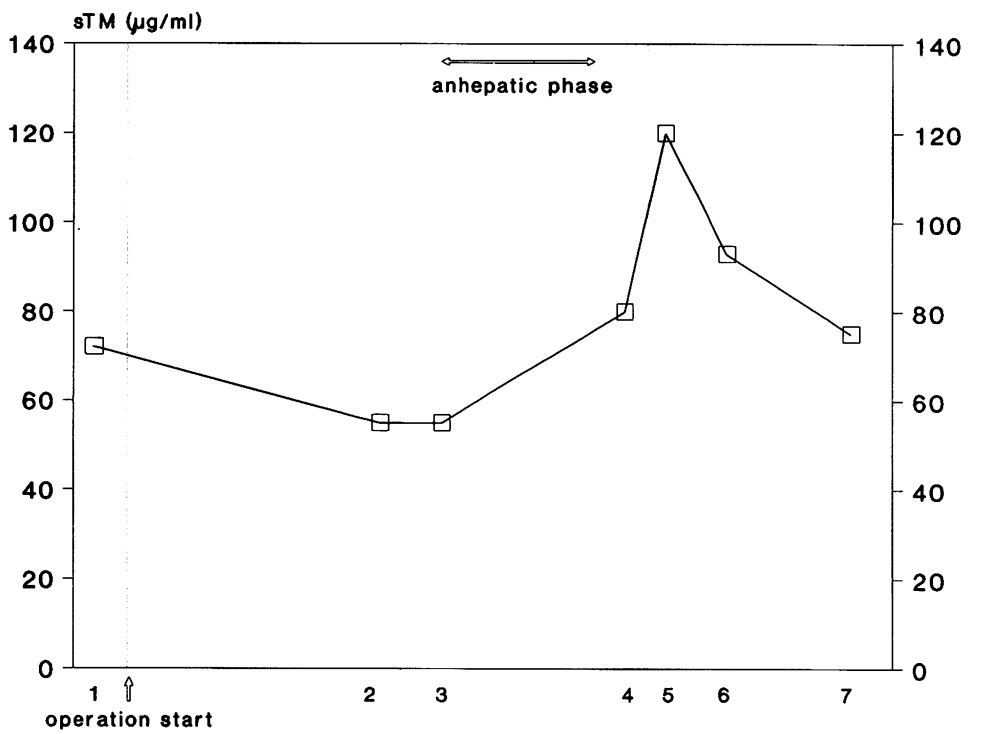
Fig. 2. (Facing page, top). Parameters of coagulation (fibrinogen, F XIII, AT III, TAT complexes, free protein S antigen) during OLT.

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


MA (%)
anhepatic phase
platelets/µl (thousand)
80
60
40
20
0
160
140
120
100
80
60
40
20
0
ristocetin (1.2mg/ml)
platelet count
collagen (1µg/ml)
ADP (2µmol/l)
1
2
3
4
5
6
7
operation start
reperfusion


sTM (µg/ml)
140
120
100
80
60
40
20
0
anhepatic phase
1
2
3
4
5
6
7
operation start

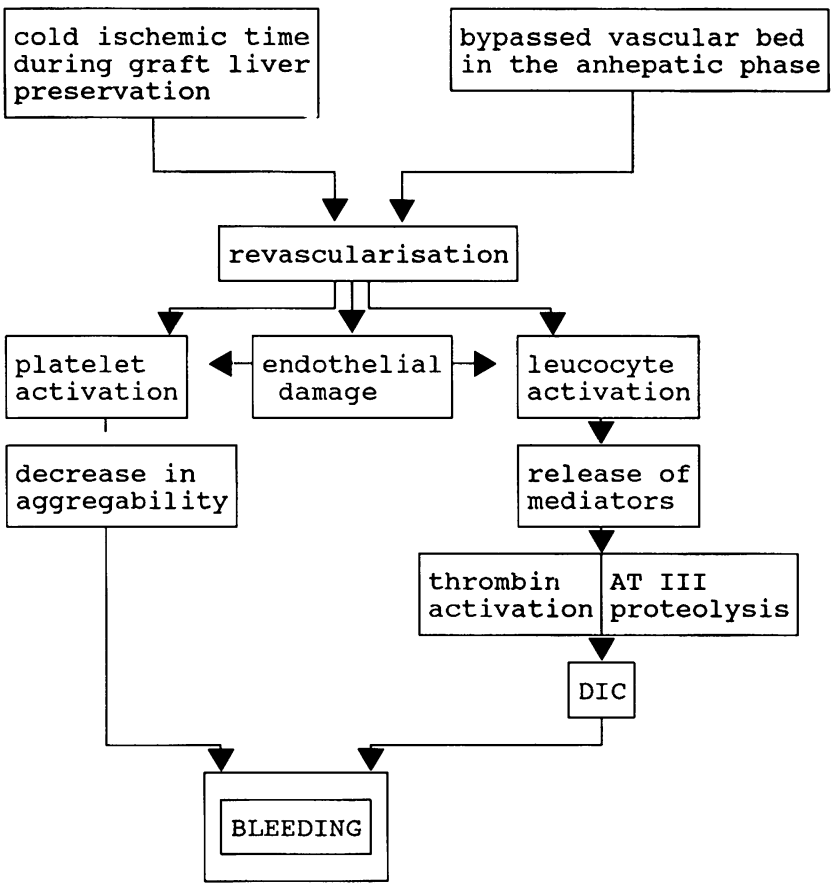


Fig. 6. Suggested pathomechanism leading to oozing and increased blood loss during the reperfusion phase in orthotopic liver transplantation.

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

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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