Hemostatic system activation and reperfusion injury in liver machine preservation and transplantation of extended criteria donor livers

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CHAPTER 3

Activation of fibrinolysis, but not coagulation, during end-ischemic ex situ normothermic machine perfusion of human donor livers

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ABSTRACT

Background: *Ex situ* normothermic machine perfusion (NMP) can be performed after traditional static cold preservation to assess graft function and viability prior to transplantation. It is unknown whether this results in activation of coagulation and fibrinolysis, as may occur upon graft reperfusion *in vivo*.

Methods: Twelve donor livers declined for transplantation underwent 6 hours of end-ischemic NMP using a heparinized plasma-based perfusion fluid. Concentration of prothrombin fragment F1+2 (marker of coagulation activation), D-dimer, PAP complex, tPA and PAI-1 (markers for fibrinolysis) and alanine aminotransferase (ALT; marker of ischemia/reperfusion [I/R] injury) were measured in perfusion fluid at regular intervals. Liver biopsies were examined for the presence of fibrin, using light microscopy after MSB staining.

Results: No significant increase in prothrombin F1+2 was noted during NMP. D-dimer and PAP complex levels increased soon after start of NMP and D-dimer concentrations correlated significantly with levels of tPA. In livers displaying good function during NMP, perfusate levels of ALT and D-dimers were low (≤3,500 ng/mL), whereas significantly higher D-dimer levels (>3,500 ng/mL) were in found in livers with poor graft function. Activation of fibrinolysis correlated significantly with the degree of I/R injury, as reflected by ALT levels.

Conclusion: End-ischemic *ex situ* NMP results in activation of fibrinolysis, but not of coagulation. Markers of fibrinolysis activation correlate significantly with markers of I/R injury. High concentrations of D-dimer early after start of NMP can be considered a marker of severe I/R injury and a predictor of poor liver graft function.

INTRODUCTION

Machine perfusion is receiving increasing attention as a potentially better alternative to traditional static cold preservation of donor livers for transplantation[1-4]. Compared to static cold storage (SCS), machine perfusion may provide better graft preservation and less reperfusion injury, resulting in better outcomes after transplantation[5-8]. When performed at normal core body temperature, machine perfusion mimics physiological circumstances thus limiting cold ischemia-induced injury and allowing for functional assessment of a donor liver[6,9,10]. *Ex situ* normothermic machine perfusion (NMP) can be performed either for the entire preservation period between procurement and implantation, or after a donor liver has undergone a period of traditional SCS for example, during transportation to the transplant centre (end-ischemic machine perfusion)[9,11,12]. The first type of *ex situ* NMP is referred to as normothermic 'preservation machine perfusion[13] and the first clinical series of this novel type of organ preservation was recently reported[14]. The latter type of NMP, referred to as 'post-SCS' normothermic machine perfusion, is performed at the transplant centre and provides the possibility of testing the function and viability of the organ prior to transplantation[13]. Successful transplantation of donor livers initially declined for transplantation but underwent end-ischemic NMP and viability testing has been recently described [15-17].

When *ex situ* NMP is performed after a period of cold ischemic preservation, a donor liver is subjected to re-warming and re-oxygenation which may lead to reperfusion injury[18]; similar to what is generally observed *in vivo* during transplantation. The only difference however is that the perfusion fluid during NMP usually does not contain leucocytes and platelets. This therefore eliminates the detrimental effects of these blood components in the ischemia / reperfusion (I/R) injury cascade. Perfusion solutions based on a combination of red blood cells and fresh frozen plasma or a colloid solution are hence generally used by most research groups.

The liver is principally responsible for the synthesis of anti- and pro-coagulant proteins along with components of the fibrinolytic system which play an essential role in the regulation of coagulation and fibrinolysis. One of the features of reperfusion of a donor liver during

transplantation is the activation of these two systems. During ex situ NMP, donor livers resume normal metabolic functions, including the production of bile, proteins, urea and the clearance of lactate[15,19]. Perioperative haemostatic disorders may occur during liver transplantation which could result in an increased risk of the development of bleeding problems in the recipient[19,20]. Although the changes in blood coagulation and fibrinolysis after graft reperfusion during liver transplantation have been described in great detail[21,22], little is known about activation of coagulation and fibrinolysis during end-ischemic *ex situ* NMP. To prevent fibrin formation during NMP as a result of activation of the coagulation cascade, most groups have been adding heparin to the perfusion fluid, however it remains unknown whether coagulation activation still occurs or whether end-ischemic NMP results in the activation of fibrinolysis. The aim of this study is to therefore determine whether activation of coagulation and/or fibrinolysis occurs during end-ischemic NMP of human donor livers and whether this could be used as a marker for graft I/R injury and/or function.

MATERIALS AND METHODS

Donor livers

Twelve human donor livers that were declined for transplantation by all three transplant centres in the Netherlands, as well as other centres within the Euro transplant region, were included in this study. Ten livers were obtained from controlled donation after circulatory death (DCD) donors and two livers from donation after brain death (DBD) donors. The retrieval and preparation procedure of these livers has been previously described[11,23]. Livers were retrieved using a standard surgical technique of *in situ* cooling and flush-out with ice cold preservation fluid (University of Wisconsin [UW] or histidine–tryptophan–ketoglutarate [HTK] solution). The surgical procedure was not started until after a five minute 'no touch' period following declaration of cardiac arrest and circulatory death in case of a DCD donor. In case of DBD liver procurement, the administration of 25.000 units of heparin was given intravenously before cross clamping. The same dose of heparin was added to the preservation solution in case of DCD liver procurement. Livers were subsequently packed

and stored on ice and transported to our center. In all 12 cases, permission for the use of these livers for research purposes was requested from and granted by the relatives. The study protocol was approved by the medical ethical committee of the UMCG and the '*Nederlandse Transplantatie Stichting'*, the national organisation responsible for the coordination and regulation of organ donation in the Netherlands.

End-ischemic *ex situ* normothermic machine perfusion

Upon arrival at our centre, livers were prepared and perfused at 37°C using a pressure controlled liver perfusion device (Organ Assist, Groningen, The Netherlands) which perfused through both the hepatic artery and portal vein, as described previously[11,23]. Livers were perfused for 6 hours with a perfusion solution based on red blood cells and heparinized human plasma fortified with nutrients, calcium, trace elements and antibiotics. Total volume of perfusion fluid was 2120 mL to which 20,000 IE of heparin were added. Samples of the perfusion fluid were collected before the liver was connected to the perfusion machine (baseline) and at 30 minute intervals during the 6 hours of NMP. All samples were centrifuged (2700 rpm for 5 min at 4°C) to remove erythrocytes and plasma was collected, snap-frozen, and stored at -80 °C until analysis.

Following NMP and subsequent evaluation of the liver function, the livers were divided into two groups depending on their functionality. The livers with a high cumulative bile production (≥30 g during 6 hours of NMP) were considered to be "good functioning" livers whereas those with a low bile output (<30 g during 6 hours of NMP) were considered to be "poor functioning" livers[11,23]. Levels of alanine aminotransferase (ALT) and lactate in the perfusate (established markers of hepatocellular I/R injury) were measured at regular intervals using a standard biochemical method.

Assessment of coagulation and fibrinolysis activation

Activation of coagulation leads to the conversion of the zymogen prothrombin to the serine protease thrombin, which releases prothrombin fragment 1+2 as an activation peptide. To

determine whether activation of coagulation occurred during NMP, plasma levels of prothrombin fragment 1 and 2 (F1+2) were determined using the Enzygnost F1+2 ELISA kit (Siemens Healthcare Diagnostics, The Hague, The Netherlands).

Activation of fibrinolysis was assessed by measuring the concentrations of tissue plasminogen activator (tPA) antigen, plasminogen activator inhibitor-1 (PAI-1) antigen and plasmin-antiplasmin (PAP) complexes, using an IMUBIND® tPA ELISA kit, (Sekisui (USA) via Werfen, Breda, Netherlands), Quantikine Human Serpin E1/PAI-1 ELISA kit (Duoset DY1786 R&D systems, Abingdom, UK) and TECHNOZYM® PAP complex ELISA kit (Technoclone, Vienna, Austria), respectively. All ELISAs were performed according to the manufacturers' instructions. In addition, concentration of D-dimers in the perfusion fluid was measured using an automated latex enhanced immunoassay (D-dimer HS 500, ACL 300 TOP, Instrumentation Laboratory, Breda, The Netherlands). D-dimer is a fibrin degradation product, a small protein fragment that is released after crossed linked fibrin is degraded by fibrinolysis.

Histological evaluation

Biopsies of the liver parenchyma were taken before machine perfusion and immediately stored in formalin. Paraffin-embedded slides of the liver biopsies were stained using Maurits, Scarlet and Blue (MSB) stain, a trichome staining technique particularly used for the selective demonstration of fibrin[24]. A strong bright red stain was representative of fibrin deposition. The slides were subsequently analysed by light microscopy (at a magnification of x40, x100 and x200) to determine whether microthrombi or fibrin depositions were present in the liver microcirculation. All histological analyses were supervised by an experienced hepato-pathologist (ASHG).

Statistical analysis

Statistical analyses were performed using SPSS version 20 for Windows (SPSS Inc., Chicago, Ill, USA). Continuous variables were presented as medians and interquartile range (IQR)] and categorical variables were presented as total numbers and percentages. The Mann-Whitney U test was used for comparison of continuous variables between groups and the Wilcoxon's signed rank test for comparison within a group. Categorical variables were compared using the Fischer's exact test. Correlations between continuous variables were determined by the Pearson correlation coefficient or by linear regression analysis, as appropriate. A p-value of <0.05 was considered statistically significant.

RESULTS

Characteristics of the twelve donor livers that underwent end-ischemic NMP are presented in Table 1. We performed NMP for 6 hours because based on the experience we have gathered whilst conducting machine perfusion (studies performed by colleagues' op den Dries et al and Sutton et al from our research group[11,24]), we concluded that 6 hours were sufficient to draw credible conclusions on graft viability and function. During the 6 hours of NMP, as Table 2 illustrates, six livers produced ≥30 g of bile[11], which contained significantly higher bilirubin (measure of bile quality). These livers also exhibited higher lactate clearance rates and were thus classified as "good functioning" livers. The remaining six livers produced <30 g bile, with significantly less bilirubin, and lower lactate clearance, were grouped as "poor functioning" livers.

Table 1: Donor characteristics

	Total livers (n=12)
Type of donor	
DCD	10 (83%)
DBD	2 (17%)
Age (years)	61 (50-64)
Gender	
Male	8 (67%)
Female	4 (33%)
Height (m)	1.77 (1.67-1.80)
Weight (kg)	88 (76-98)
Reason for rejection	
DCD + age > 50 years	5 (41%)
DCD + high BMI	3 (25%)
DCD + other reason*	2 (17%)
Severe steatosis**	2 (17%)
Donor risk index	2.35 (2.01-2.54)
ALT (IU/L)* **	38 (24-59)

Preservation solution	
UW solution	6 (50%)
HTK solution	6 (50%)
Total donor warm ischemia time (min)∞	43 (33-71)
Cold ischemia time (min)	389 (458-585)
Liver weight (kg)	2.09 (1.60-2.24)

** One DCD donor with history of iv drug abuse and one donor with prolonged low oxygen saturation sO2 (30%) after withdrawal of life support. ** defined as macro-vesicular steatosis with more than 60% of hepatocytes involved. *** last known value before procurement.*

Table 2: Classification of livers according to function

	Good functioning (high bile output)	**Poor functioning (low bile output)**	**P-value**
Amount of bile[11] (g in 6 hours)	*>30*	*<30*	
Bilirubin in bile (µmol/L)	*1100 [968-1398]*	*270 [215-525]*	**0.02**
Lactate clearance (mmol/L)	*2 [1-4]*	*6 [3-11]*	**0.03**

No activation of coagulation during end-ischemic NMP

An overview of baseline values and changes in parameters of coagulation and fibrinolysis activation during NMP of all twelve livers is presented in Table 3. At baseline, before the liver was connected to the perfusion device, very small amounts of prothrombin fragment F1+2 (*good* functioning livers - median 278 pmol/L; IQR 120- 556 and *poor* functioning livers - median 127 pmol/L; IQR 127- 648) were detected in the perfusion fluid. During 6 hours of NMP, F1+2 concentrations remained stable and there was no significant difference in the delta increase or decrease of F1+2 during 6 hours of NMP between the groups of livers with good or poor function (P=0.86) (Figure 1A).

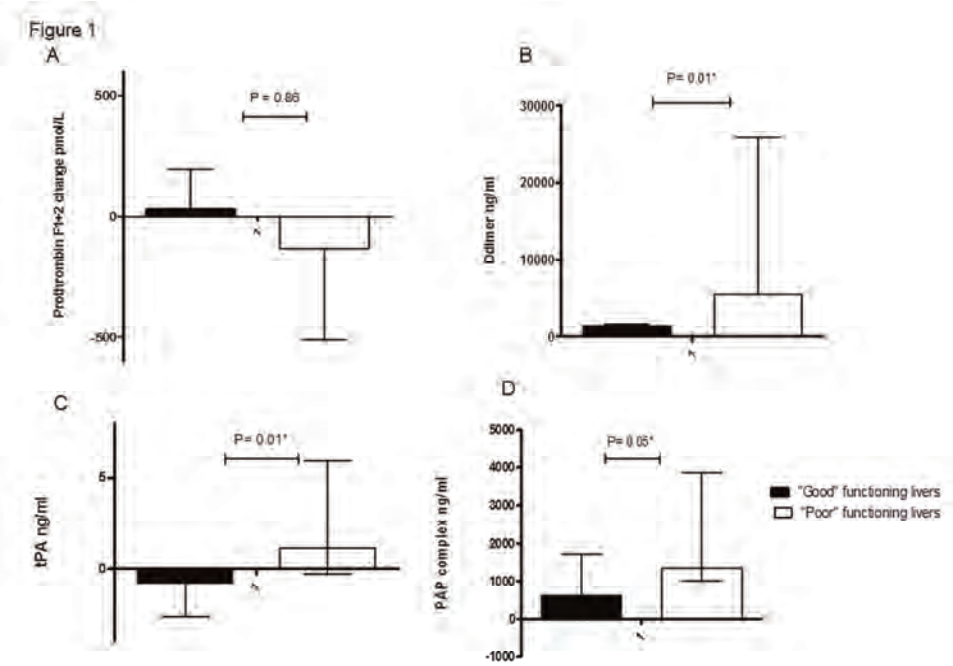

Figure 1. ***Comparison of the changes in concentrations of coagulation and fibrinolysis proteins in livers with good or poor function during 6 hours of NMP.*** *Panel A; Prothrombin fragment F1+2. Panel B: Tissue plasminogen activator. Panel C: D-dimers. Panel D: Plasmin-antiplasmin complexes.*
P values <0.05 represent statistical significance between the two groups

Activation of fibrinolysis during end-ischemic NMP

The concentration of D-dimer and PAP complexes in the perfusion fluid of both good and poor functioning livers increased more than 20-fold soon after the start of NMP (Table 3). Concentrations of tPA remained relatively stable during NMP. However, when comparing the change in tPA concentration during NMP in the group of livers with good or poor function, a significant difference was noted. While tPA in the perfusion fluid decreased during the 6 hours of NMP in the good functioning livers, an increase was noted in livers with poor function (Figure 1B). Similarly, the increase in D-dimer and PAP complexes during NMP was significantly higher in the group with poor functioning livers, compared to good functioning livers (Figure 1C and D). PAI-1 concentrations were low for both groups during the first

hours of NMP, but a sharp increase was noted during the second part of the 6 hours of NMP (Table 3). Although the increase in PAI-1 was more pronounced in livers that displayed good function during NMP, compared to poor functioning livers, this difference did not reach statistical significance (data not shown).

Table 3: Concentrations of coagulation and fibrinolytic proteins in the perfusion fluid during 6 hours of normothermic machine perfusion

	Baseline	Hours of NMP					
		1	2	3	4	5	6
Prothrombin F1+2 (pmol/L)	278 [120-556]	251 [213-663]	257 [243-776]	332 [227-714]	339 [203-619]	324 [168-503]	347 [169-449]
	348 [127-648]	367 [334-753]	395 [349-877]	349 [250-884]	303 [206-830]	260 [170-747]	188 [135-481]
D-dimer (ng/ml)	114 [31-178]	2004 [1583-3343]	2228 [2228-3359]	1958 [1667-3126]	1904 [1546-2542]	1835 [1448-2292]	1772 [1377-2234]
	103 [69.3-154]	4125 [3273-10432]	6090 [5030-21968]	5723 [5376-27147]	5509 [5215-27368]	5604 [5248-28231]	5618 [5031-26062]
tPA (ng/ml)	13.5 [12.7-14.4]	12 [11.3-13.4]	12 [11.5-13.4]	11.9 [11.4-12.8]	11.8 [11.6-12.5]	11.9 [11.6-12.5]	12.1[11.4-12.4]
	12.2 [12.0-12.83]	12.8 [11.9-14.1]	13.5 [12.2-15.2]	14.0 [12.4-16.3]	14.8 [12.2-16.4]	15.6 [12.2-17.7]	13.9 [12.1-18.0]
PAP (ng/ml)	588 [546-728]	1198 [1072-1586]	1316 [889-2314]	1521 [1098-2230]	1269 [1269-2037]	1480[1093-2660]	1439 [1439-2062]
	611 [541-646]	1480 [1104-2050]	1868 [1651-3852]	2103 [1703-4956]	2161 [1668-4134]	2243 [1556-3694]	1950 [1603-4457]
PAI-1 (ng/ml)	0.61 [0.36-1.16]	0.83 [0.42-3.12]	1.67 [1.24-4.96]	5.12 [3.82-10.3]	9.36 [7.01-14.5]	13.35 [10.02-19.15]	17.5 [13.60-25.29]
	0.58 [0.47-0.70]	0.46 [0.41-1.38]	0.75 [0.55-4.59]	1.2 8[1.0-10.13]	1.50 [1.33-16.13]	2.56 [2.13-19.43]	8.64 [3.28-30.72]

Figure 2.

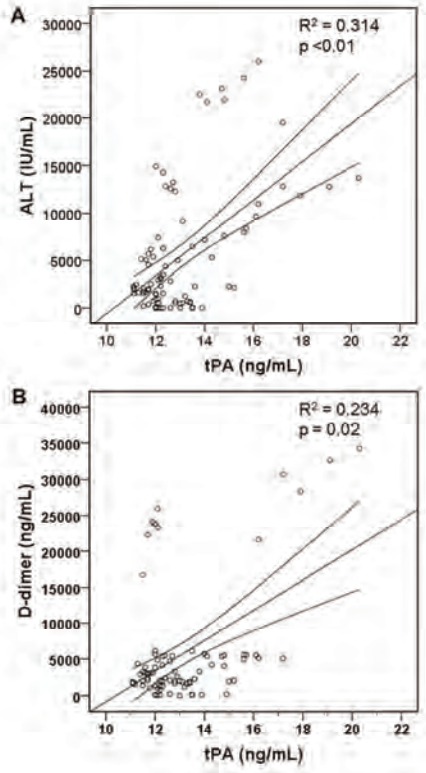

Figure 2. ***Scatter plots illustrating the correlations between markers of fibrinolysis (tPA and D-dimer) and I/R injury (ALT) in perfusion fluid during 6 hours of NMP.*** *Panel A: Correlation (incl 95% confidence interval) between ALT and tPA concentration. Panel B: Correlation (incl 95% confidence interval) between D-dimer and tPA concentration. Abbreviations: tPA, tissue plasminogen activator; ALT, alanine aminotransferase.*

Correlation between activation of fibrinolysis and I/R injury

We next examined whether activation of fibrinolysis during NMP correlated with the degree of I/R injury. ALT levels in the perfusion fluid were used as an established marker of hepatocellular I/R injury. Significant correlations were observed between tPA and ALT concentrations measured at all time-points, and between tPA and D-dimer (Figure 2A and B). In accordance with this, a significant correlation was observed between ALT and D-dimer concentrations (Figure 3A). Moreover, livers that displayed good function during NMP were

found to have lower levels of ALT and D-dimer in the perfusion fluid, compared to poorly functioning livers. In fact, very high D-dimer levels were only found in the group of poorly functioning livers (Figure 3A). Altogether, these data suggest that *ex situ* end-ischemic NMP is associated with activation of fibrinolysis, the degree of which is linked to the severity of I/R injury.

As could be expected from the lack of change in prothrombin F1+2 concentrations, there was no correlation between this marker of coagulation activation and ALT levels in the perfusion fluid (Figure 3B).

Figure 3.

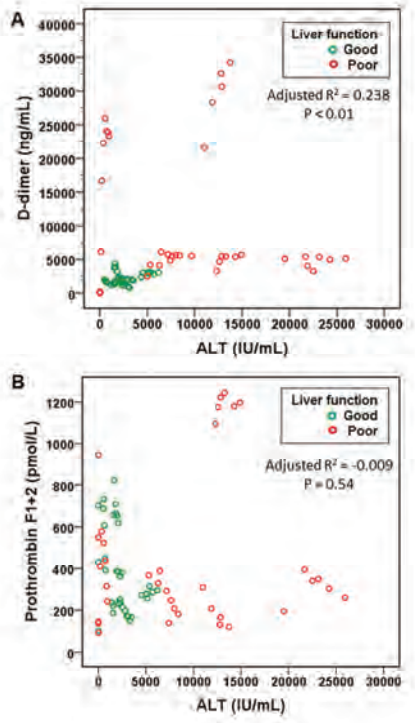

Figure 3. ***Scatter plots illustrating the correlations between D-dimer (marker of fibrinolysis) and prothrombin fragment F1+2 (marker of coagulation) with I/R injury (ALT) in perfusion fluid, separated for livers with good or poor function during 6 hours of NMP.*** *Panel A: Correlation D-dimer and between ALT per subgroup. Panel B: Correlation prothrombin fragment F1+2 and ALT per subgroup. Abbreviations: tPA, tissue plasminogen activator; ALT, alanine aminotransferase.*

Histological detection of fibrin in donor livers

Since we found no evidence of coagulation activation during end-ischemic NMP, yet a significant release of D-dimers into the perfusate soon after the start of NMP, it is likely that the D-dimers released were derived from lysis of (micro)clots or fibrin depositions already present in the liver grafts prior to *ex situ* NMP. To investigate the presence of (micro)thrombi or fibrin depositions in the liver microcirculation, the liver biopsies obtained before NMP underwent MSB trichome staining. A total of 292 portal venous structures and 213 central venous structures were examined in biopsy slides of the twelve liver grafts. In 4 of these 292 (1.35%) portal tracts and in none of the 213 central venous tracts, MSB staining revealed signs of intravascular fibrin. In the four portal venous tracts with positive fibrin staining, only small amounts of free floating fibrin were detected, without evidence of vessel-occluding microthrombi (Figure 4A and B). There was no significant difference in the presence of fibrin in portal venous tracts of good or poor functioning livers.

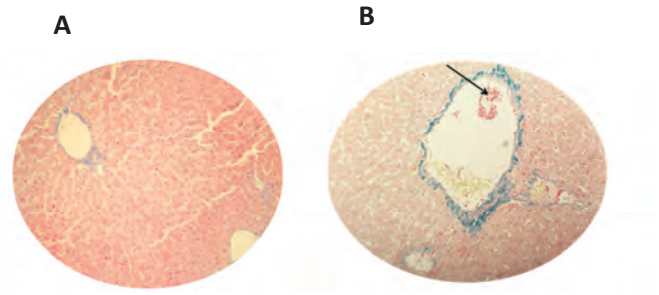

Figure 4. Light microscopy of baseline liver biopsies taken after Maurits, Scarlet and Blue (MSB) staining. *Panel A: MSB stained tissue- slice image from a biopsy showing no fibrin deposition. Panel B: MSB stained tissue- slice image from a biopsy exhibiting non-occluding fibrin deposition (arrow). Bright red stain = fibrin; yellow staining = erythrocytes; blue stain = collagen in vascular wall*

DISCUSSION

Ex vivo normothermic machine perfusion has been proposed as an alternative preservation method to traditional SCS as well as a novel method to resuscitate extended criteria donors livers and to assess their function and viability prior to transplantation[11,15,23,25]. When used as a selection tool to identify donor livers that are transplantable after initially being declined for transplantation based on high donor risk factors, NMP could potentially contribute to an increase of the number of donor livers for transplantation[23,26].

If *ex situ* NMP is performed after a period of traditional cold ischemic preservation, the donor liver is subject to re-warming and re-oxygenation which potentially leads to I/R injury. Proteolysis and activation of fibrinolysis have been identified as key events in clinical reperfusion of cold stored donor livers[19,22]. The main finding of this study is that end-ischemic *ex vivo* NMP of donor livers results in activation of fibrinolysis, but not of coagulation. Markers of fibrinolysis activation correlate significantly with markers of hepatocellular I/R injury during NMP. High concentration of D-dimers in the perfusion fluid soon after the start of NMP can be considered a marker of severe I/R injury and a predictor of poor liver graft function.

D-dimer is a fibrin degradation product, a small protein fragment that is released during fibrinolysis after crossed linked fibrin is degraded. It is named so because it contains two cross linked D fragments of the fibrin protein[27]. D-dimers are a specific marker for degradation of cross linked fibrin. The sharp increase in D-dimers in the perfusion fluid during end-ischemic NMP in the absence of an increase in prothombin fragment F1+2 suggests that this D-dimer increase is a result of degradation of pre-existing rather than newly formed fibrin in the donor livers. Prothrombin fragment F1+2 is a reliable indicator of coagulation activation. Activation of coagulation leads to the conversion of the zymogen prothrombin to the serine protease thrombin, which releases prothrombin fragment F1+2 as an activation peptide. During NMP, prothrombin fragment F1+2 remained stable and levels did not change significantly compared to baseline values. Although the increase in D-dimers

levels after the start of NMP is most likely explained by the breakdown of pre-existing fibrin in the livers, histological evidence of fibrin in the hepatic microvasculature was found only sporadically. After microscopic evaluation of 292 portal and 213 central venous branches in parenchymal biopsies, traces of fibrin were found in only 4 of the 292 (1.4%) portal venous branches. Moreover, no signs of vessel-occluding microthrombi were seen. This observation is in accordance with a recent clinical study on histological analyses of bile duct biopsies of 128 human donor livers prior to transplantation in which the incidence of microthrombi in the peribiliary vascular plexus after static cold storage was 2.7%[8,28]. A calculation of the estimated amount of fibrin that must have been present in livers, based on molecular weight of D-dimers and fibrin is approximately 14 mg per liver (equal to the size of a pinhead). Based on an average liver weight of 2000 g, this would amount to an approximate value of 0.0007% of the total liver weight. This information adds an interesting new perspective to the discussion about whether thrombolytic therapy of (DCD) liver grafts prior to transplantation is potentially beneficial. Thrombolytic therapy of DCD livers grafts with plasminogen activators such as recombinant tPA or streptokinase has been proposed as a method to reduce the incidence of ischemic cholangiopathy after DCD liver transplantation[29,30]. On the other hand, several studies have indicated that hypoxia due to circulatory arrest (as occurs in a dying person) is associated with a pronounced stimulation of the fibrinolytic system because of the release of endogenous plasminogen activators[31,32]. In accordance with this, it was recently reported that evidence of clinically relevant endogenous hyper-fibrinolysis can be found in DCD donors (Maastricht type II) which therefore argued against the need for additional thrombolytic treatment of DCD donors or their livers[33]. Our study demonstrated that end-ischemic NMP also results in activation of endogenous fibrinolysis (with no particular difference noted in regard to whether or not the donor liver is derived from a brain or circulatory death donor). This may be a clinically relevant aspect of NMP that contributes to the improved preservation of a donor liver during this type of machine perfusion. Activation of fibrinolysis was most pronounced in livers of poor quality as illustrated by a high release of

ALT and low bile production during NMP. In fact, high levels of D-dimers in the perfusion fluid correlated with liver function and ALT levels, reflecting I/R injury.

The observation that end-ischemic NMP of suboptimal quality donor livers is associated with activation of fibrinolysis is very much in line with data from clinical studies that have identified proteolysis and fibrinolysis as key components of graft reperfusion and I/R injury[20,22,34]. Hyper-fibrinolysis has been proposed as an explanation for the higher risk of blood loss in recipients after reperfusion of extended criteria donor livers[35,36]. However, clinical studies determining whether end-ischemic NMP of donor livers of suboptimal quality prior to transplantation leads to a reduction hyper-fibrinolysis in recipients after transplantation are still lacking.

In contrast to the activation of fibrinolysis, we found no evidence of activation of coagulation during NMP. When the perfusion fluid used for NMP is based on human plasma (as was the case in this study) or whole blood, the addition of an anticoagulant to the perfusion fluid is necessary. In fact, even when plasma is replaced by a (synthetic) colloid solution, which does not contain coagulation proteins, investigators generally still add heparin to the perfusion fluid[12,15,37]. Given the restoration of metabolic function of the liver during *ex situ* NMP, coagulation activation and subsequent fibrin formation may occur. However, the half-life of most proteins involved in the coagulation cascades is relatively long and large amounts of de novo production during a few hours of NMP are not to be expected. Nevertheless, we would certainly advise to include heparin or another potent anticoagulant drug to the perfusion fluid during liver machine perfusion. Additionally an important detail to take into consideration in regard to maintaining a favourable haemostatic balance of these livers during NMP is the donor type. As mentioned previously, depending on the donor type (DBD or DCD), a hypercoagulable or hyperfibrinolytic state may be seen. This difference however, was not observed in this study as data from DBD livers were comparable to the DCD livers. This may have been attributed to the small sample size in which a conclusive comparison between the DBD and DCD livers could not be made.

In conclusion, end-ischemic *ex situ* NMP results in activation of fibrinolysis, but not of coagulation. Markers of fibrinolysis activation correlate significantly with markers of I/R injury and high concentrations of D-dimer early after start of NMP can be considered a marker of severe I/R injury and a predictor of poor liver graft function.

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