Transplantation Publish Ahead of Print

DOI: 10.1097/TP.0000000000003763

Coagulation Factors Accumulate During Normothermic Liver Machine Perfusion Regardless of Donor Type and Severity of Ischemic Injury

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NG, MJ, DM, and IJ participated in research design.

NG, MJ, SL, DN, LL, and IJ participated in the performance of the research.

MJ, RL, and KP participated in laboratory analyses.

NG, MJ, DM, and IJ participated in data analyses.

NG, MJ, DM, and IJ participated in writing of the paper.

DN, SL, LL, RJP, PJF, and JP critically revised the manuscript and gave important intellectual contribution.

Disclosure: PJF is a co-founder, chief medical officer and consult to OrganOx Limited and also holds shares in the company. PJF was not involved in the selection, recruitment, or transplantation of patients in this study.

Funding: The COPE liver trial was funded by the European Commission Seventh Framework Programme (grant agreement 305934). For this clinical sub-study, there was no funding.

The Lab of Abdominal Transplantation of KU Leuven has received IGL-1 preservation solution by the Institute George Lopez (Lissieau, France) free of charge to be used in animal experiments. No additional funding was obtained for the experimental work of this study. JP, DM, and IJ are recipients of a CAF chair for abdominal transplant surgery. JP is recipient of an IGL chair for abdominal transplant surgery. DM is a senior researcher of The Research Foundation (FWO) – Flanders. The department of Abdominal Transplant Surgery has received unrestricted grants from Astellas and Roche.

Abbreviations:

ALT, alanine transferase

AST, aspartate transferase

DBD, donation after brain death

DCD, donation after circulatory death

F, coagulation factors

H&E, hematoxylin and eosin

LSEC, liver sinusoidal endothelial cells

NMP, normothermic machine perfusion

Abstract:

Background. Coagulation factors may inform on liver function during normothermic machine perfusion (NMP). We investigated whether graft ischemic injury impairs the accumulation of (anti)coagulation factors during NMP of porcine and human livers.

Methods. Dynamics of FV, FVII, FVIII, FIX, FX during NMP and their correlation with graft injury was investigated in porcine livers with minimal (no warm ischemia, n=5) or severe injury (60 minutes warm ischemia, n=5). Next, FV, FVIII, FIX, fibrinogen, and antithrombin were measured in 35 matched human liver NMPs from the COPE trial. Correlation of these factors with outcomes was explored. Livers were categorized in 4 groups depending on donor type and post-transplant peak aspartate aminotransferase (AST) as surrogate of minimal (peak<500 IU/L) or moderate injury (peak>1000 IU/L).

Results. Factor concentrations increased significantly during NMP regardless of severity of injury. In porcine livers, factor concentrations were 2-6 fold lower in severely injured grafts (all $p<0.05$). All factors negatively correlated with AST (coefficient range -0.50 to -0.93; all $p<0.05$) and lactate (range -0.51 to -0.67; all $p<0.05$). In human livers, no difference in factor accumulation rates and no correlation with other markers was observed. One graft with primary nonfunction had low rate of factor accumulation.

Conclusion. (Anti)coagulation factors accumulate during NMP regardless of donor type and severity of injury. In pigs, severe ischemic injury resulted in significantly lower factor concentrations. In human livers with life-sustaining function, they do not correlate with hepatic injury. Whether low concentrations predict nonfunction in high-risk livers with severe injury requires further investigation.

Introduction

Normothermic Machine Perfusion (NMP) is increasingly used as a platform for organ preservation, viability assessment, and reconditioning.[1] NMP keeps liver grafts at near-to-physiological conditions by recirculating a warm, oxygenated, and nutrient-enriched perfusate through the liver vasculature. Compared to cold storage, NMP preservation has been associated with lower levels of graft injury (measured by transaminase release) and lower organ discard rates.[2] Pioneering centers have shown that the use of NMP as an organ viability assessment and selection tool can enable the transplantation of livers that were initially discarded.[3–6] Human livers have been transplanted successfully[7] after NMP in excess of 24h. Recent advances in MP-technology have enabled the successful ex-situ preservation of livers by NMP for 7 days, with maintenance of metabolic and synthetic function[8]. Such extended preservation times may be important for organ reconditioning and therapeutic intervention.

Despite these major advances, our understanding of liver function during NMP is incomplete, though increasingly important. Initial work investigating markers measurable during NMP that may predict organ quality have focused on transaminase release, lactate clearance, glucose changes, pH regulation, and bile production to assess hepatocellular viability; and bile composition to evaluate the cholangiocytes.[3–5,9,10] Endothelial functioning during perfusion is not understood and markers of liver sinusoidal endothelial cells (LSEC) are currently lacking.[9] (Anti)coagulation factors could relay information on both hepatocyte and endothelial compartments. Indeed, hepatocytes produce factor (F) V, FVII, FIX and FX, and antithrombin,[11,12] whereas LSEC produce FVIII.[13,14] Porcine NMP studies have shown synthesis of FV during perfusion;[15,16] whereas increasing perfusate concentrations of FII, FV, FVII, FX, fibrinogen, Von Willebrand factor, antithrombin III, and plasminogen were measured during NMP of discarded human livers.[17,18] Because human data were derived only from nontransplanted livers, it is currently unclear what the dynamics of (anti)coagulation factors

during NMP are, how they are affected by variables such as donor type or cold and warm ischemia, and whether they correlate with graft injury or whether they can be used to predict post-transplant liver function. Furthermore, the accumulation of LSEC-specific FVIII during NMP has never been investigated. As advocated by others,[19] these are important questions to address.

In this study we investigated the dynamics of hepatocyte (FV, FVII, FIX, FX, fibrinogen, antithrombin) and LSEC (FVIII) specific (anti)coagulation factors during NMP of porcine and human livers and their association with increasing degrees of ischemia and post-transplant outcome.

Materials and Methods

We hypothesized that ischemic injury impairs the accumulation of (anti)coagulation factors during liver NMP and, therefore, that they may provide additional information on the functional state of the graft during perfusion. Because porcine models of liver donation allow for better standardization of hepatic injury than the study of discarded human livers,[20] we first tested this hypothesis in porcine experiments. In these experiments, livers were procured either immediately after organ flush (minimal injury) or after exposure to 60-minutes warm ischemia (severe injury). To explore the relationship of (anti)coagulation factors with outcomes in a clinical setting, a subgroup of livers included in the perfusion arm of the COPE randomized controlled liver trial[2] were included in this study. This trial included human livers of low to intermediate risk donated after brain death (DBD) or after circulatory death (DCD).

Animal experiments

Livers procured from male prepubescent pigs (TOPIGS 20, Nijmegen, The Netherlands; approximately 30 kg) were flushed with 4000 mL of cold IGL-1 preservation solution (Institute George Lopez, Lissieu, France) via the aorta and portal vein, either immediately (minimally injured grafts, n=5) or after exposure to 60-minutes warm ischemia (severely injured grafts,

n=5) (details in Supplemental Digital Content http://links.lww.com/TP/C197). Group allocation was not randomized. Warm ischemia was induced by clamping the thoracic aorta before organ flush. Livers were prepared for NMP while kept on melting ice and subsequently perfused with a heparinized, plasma-free, autologous red blood cell based perfusate (hematocrit 30%) on a circuit replicating NMP as described by Nasralla et al.[2] (details in Supplemental Digital Content http://links.lww.com/TP/C197). The accumulation of coagulation factors was first explored during 24h NMP of five minimally injured livers (immediate organ flush followed by 2h cold storage). Perfusate samples were collected at 15 and 30 minutes, and 1, 2, 3, 6, 12, 18, and 24h to determine the concentration of FV, FVII, FVIII, FIX, and FX. Next, five severely injured livers (exposed to 60-minutes warm ischemia) underwent NMP for 6h, to mimic pretransplant NMP liver quality assessment.[3–6] The perfusate concentration of coagulation factors was measured at 15 and 30 minutes, and 1, 2, 3, and 6h. A wedge liver biopsy was taken after 6h NMP. Porcine livers were not transplanted, though earlier experiments showed that porcine livers exposed to 60-minutes warm ischemia invariably develop primary nonfunction, even when transplanted after 20h NMP.[20,21] The KU Leuven Animal Care Committee approved the study (P027/2016) and the experiments were conducted in line with European guidelines.[22]

Human livers

Human livers allocated to NMP as part of the COPE trial comparing NMP with cold storage[2] were randomized, procured, and perfused with the OrganOx Metra device (OrganOx Ltd, Oxford, UK). Briefly, the device provided continuous flow of a heparinized, plasma-free perfusate based on packed red blood cells (mean hematocrit 25.9% ±5.3) through the liver vasculature (details in Supplemental Digital Content http://links.lww.com/TP/C197). Human livers included in this sub-study were selected and matched for donor type (DBD or DCD), donor age, donor gender, and Eurotransplant-donor risk index[23] to minimize selection bias. Post-transplant peak AST was used to grade the severity of ischaemia-reperfusion injury as

either minimal (peak AST <500 IU/L) or moderate (peak AST >1000 IU/L). Perfusate samples from 10 minimally injured DBDs, 10 minimally injured DCDs, 10 moderately injured DBDs, and 5 moderately injured DCDs were analysed (only 5 DCD livers with peak AST >1000 IU/L were available). Biobanked perfusate samples taken during the randomized trial were used. Samples were taken at 15 minutes of NMP (t1), immediately before the liver was removed from the device (t3) and at an intermediate time point defined by logistics (t2). Perfusate (anti)coagulation factor concentrations were measured at these time-points, and the rate of accumulation in the perfusate was determined as the difference in concentration between t3 and t2, divided by the time difference between the sample points. T1 samples were considered too early to reflect any potential production of factors during NMP and were not used to calculate accumulation rates.

All patients gave written informed consent for samples and data to be used for study purposes; the clinical sub-study was approved by the Research Ethics Committee London-Dulwich, United Kingdom (14/LO/0182).

Analyses

The concentration of coagulation factors in porcine perfusate samples was measured with coagulation assays performed on an ACL-TOP700 coagulometer (Werfen, Bedford, MA, USA), whereas a chromogenic assay (Coatest SP Factor VIII assay; Werfen, Zaventem, Belgium) or an ELISA was used for human perfusate samples. All assays were calibrated with pool of pig or human plasma. Other currently accepted markers of hepatocellular injury (AST; alanine aminotransferase, ALT) and liver function (bile volume, perfusate lactate) were measured (see Supplemental Digital Content http://links.lww.com/TP/C197). Additionally, for human livers, post-transplant AST, ALT, and INR values were determined by the central laboratories of the recipient hospital as part of the randomized controlled trial. The teams performing these analyses were blinded for group allocation.

Porcine liver tissue samples were stored in 6% formalin, paraffin-embedded, and stained with hematoxylin-eosin (H&E). The pathologist assessing H&E sections for features of hepatocellular injury (necrosis) and LSEC damage (necrosis leading to intrahepatic hemorrhage) was blinded for the experimental group.

Statistical analyses

For porcine data, within-group analyses were performed with repeated measure ANOVA. Greenhouse-Geisser correction was applied when appropriate. The concentrations of coagulation factors and other biomarkers were compared between groups with Student's t-tests. Perfusate concentrations of coagulation factors were correlated with other biomarkers with Kendall-tau (τ) correlation coefficient.

For human data, levels of (anti)coagulation factors were compared over time using the t-test for paired samples. The rates of coagulation factor accumulation were compared between groups with the Mann-Whitney U test or Kruskal-Wallis test. Bivariate correlation according to Spearman's Rho were used.

Data are expressed either as mean (± standard deviation) or median (range). All statistics were performed with the InStat3 software (Graph Pad Software, Inc) and SPSS v20 (SPSS, Inc).

Results

Does ischemic injury influence (anti)coagulation factor accumulation during NMP?

Minimally injured porcine livers underwent NMP for 24h with stable flows, normal macroscopic appearance, rapid perfusate lactate clearance, and preserved bile production (Figure S1). All coagulation factors increased over time (Figure S2). While a linear increase in the concentration of FVII ($p=0.08$), FVIII ($p=0.41$), FIX ($p=0.03$), and FX ($p=0.0001$) was observed, FV showed a biphasic kinetic with a significant increase to a peak at 2h NMP ($p=0.001$) and a subsequent decline in concentration at 24h ($p=0.02$).

We next investigated how coagulation factor concentrations during 6h NMP were affected by severe ischemia. Baseline and operative characteristics were comparable between minimally and severely injured grafts, except for a higher liver weight in severely injured grafts (Table S1). Therefore, results are expressed per 100g of liver weight. Perfusate AST concentrations were significantly higher in severely injured grafts at every time-point and reached a peak of 660 (±420) IU/L/100g at 3h NMP (Figure 1). Perfusate lactate concentration was significantly higher in severely versus minimally injured grafts until 2h NMP but was similar thereafter (Figure 1). No difference in hourly bile production was observed. Sinusoidal congestion, necrosis of sinusoidal walls, and hemorrhage provoking necrosis of hepatic muralia was noted more frequently in severely injured livers (Figure S3). Thrombosis of the micro-circulation was not observed. Similar to minimally injured livers, FV ($p=0.001$), FVII ($p=0.008$), FVIII ($p=0.01$), and FIX ($p=0.03$) accumulated significantly over the course of NMP, while FX did not ($p=0.37$). Compared to minimally injured livers, coagulation factor concentrations were significantly lower at every time-point, with an overall 2 to 6-fold reduction (Figure 1).

We also investigated (anti)coagulation factor accumulation during human NMP. Table 1 shows baseline characteristics of human livers included in this study. One liver developed primary nonfunction. Five cases of early allograft dysfunction occurred. Two moderately injured DCD grafts failed after providing initial life-sustaining function: one on postoperative day 16 because of a hepatic artery thrombosis, the other on day 9 after a nonthrombotic infarction. Patient survival 1-year after transplantation reached 86% overall and was similar between groups. Fibrinogen concentrations increased during perfusion of all livers ($p\leq0.01$ in all cases; Figure S4). The rates of fibrinogen increase varied largely between livers, ranging from 0.07 to 0.51 g/L/h (Figure 2; Table S2). Similarly, FV and FIX concentrations increased in the perfusate of all livers ($p\leq0.01$ for all comparisons; Figure S5 and S6), with rates varying largely (from 1.4 to 17.6 IU/dL/h for FV and 2.3 to 29.3 IU/dL/h for FIX; Figure 2; Table S2). Antithrombin

levels also increased in the perfusate of all livers ($p \leq 0.01$ for all comparisons; Figure S7), with rates ranging from 0.8 to 7.8 IU/dL/h (Figure 2; Table S2). FVIII concentration increased over time ($p \leq 0.02$ for all comparisons; Figure S8), with rates varying largely between livers, from 1.3 to 19.5 U/dL/h (Figure 2; Table S2). There was no significant difference in the rates of (anti)coagulation factor accumulation between minimally and moderately injured livers, nor between DBD and DCD or between the four subgroups (Figure 2; Table S2 and S3). Despite the presence of procoagulant factors, no thrombosis of the micro-circulation on histology was observed.

Do (anti)coagulation factors correlate with severity of injury?

As hepatocyte function depends upon viable hepatocytes, we investigated whether (anti)coagulation factor concentrations correlate with hepatocyte injury markers.

In porcine experiments, all hepatocyte-specific coagulation factors (FV, FVII, FIX and FX) were inversely correlated with AST levels during NMP, with coefficients ranging from -0.50 to -0.93 (all $p<0.05$; Table 2). The strongest correlations with AST were observed for FV (τ=-0.93, p=0.001) and FIX (τ=-0.86, p=0.003), both at 1h NMP. All hepatocyte-specific coagulation factors were inversely correlated with lactate concentration, with coefficients ranging from -0.51 to -0.67 (all $p<0.05$; Table 2). The strongest correlations with lactate were observed for FVII (τ=-0.67, p=0.006) and FX (τ=-0.67, p=0.02), at 1h and 2h NMP, respectively. There was no correlation with bile production. As vascular resistance is considered a surrogate marker of endothelial injury, we investigated whether the LSEC-specific FVIII correlated with portal vein and hepatic artery resistances. A negative correlation with portal vein resistance at 30 minutes of NMP (τ= -0.56, p=0.03), but not with hepatic artery resistance was observed (Table 2).

In human livers, correlations between the rate of increase of fibrinogen, FV, FIX, and antithrombin and the rate of change in perfusate ALT during NMP were tested, none were significant (data not shown). There was also no correlation with the total volume of bile produced during NMP. Correlation with the rate of lactate change was not considered as lactate remained low and stable during NMP in all livers.

There was no correlation between the rate of increase of fibrinogen, FV, or FIX and post-transplant peak AST. There was a weak correlation between the rate of antithrombin accumulation during NMP and post-transplant peak-AST (rho=0.34, p=0.04; Figure S9). There was no correlation between the rate of FVIII increase and post-transplant peak-AST levels (rho=0.11, p=0.51), or hepatic artery or portal vein flow during NMP (data not shown). An exploratory analysis revealed no differences in fibrinogen, FV, FVIII, FIX, or antithrombin between livers that developed early allograft dysfunction and those that did not, nor between livers with peak AST>2000 IU/L and those with lower peak (data not shown).

Did the liver with primary nonfunction have a different pattern of (anti)coagulation factors accumulation?

A steatotic DBD liver from a 62-year-old donor with normal transaminases at time of donation developed primary nonfunction. No significant problems regarding flow were encountered during 10h NMP. An initial lactate of 16 mmol/L fell to 5.1 mmol/L after 1h NMP and remained between 4.5 and 5 mmol/L for the remainder of the perfusion. Only minimal bile was produced. The liver was transplanted into a 68-year-old male with a MELD-score of 17. The recipient developed postreperfusion syndrome needing high doses of inotropes and continued to require cardiorespiratory support in the intensive care. The patient was relisted for super urgent transplantation but died 3 days after transplantation.

Although this liver released considerable transaminase during NMP (peak ALT of 4113 IU/L), there were five livers with even higher peak ALT (the maximal being 16772 IU/L; Figure S10)

that all functioned immediately, did not develop early allograft dysfunction, and were still functioning 1-year after transplantation. In contrast, the rate of accumulation of fibrinogen, FV, FIX, and antithrombin was lower than the median rates of accumulation observed (Figure 3) and were equal to or below the 25th percentile of those measured in all livers. The accumulation of FVIII was below the 5th percentile of all livers. The two grafts that failed beyond the first week had rates of (anti)coagulation factor accumulation during NMP comparable to those of grafts that did not fail (Figure 3).

Discussion

Because (anti)coagulation factors are synthetized by the liver, they may provide unique information on liver function during NMP and may play a role in graft selection.[19] This study describing the dynamics of (anti)coagulation factors during NMP investigated their association with hepatic injury and post-transplant outcomes. It is the first study to measure the LSEC-specific FVIII and investigate its association with other markers.

This observational study suggests that the accumulation of (anti)coagulation factors during NMP is universal, regardless of donor type or degree of ischemic injury sustained by the graft. Indeed, porcine experiments showed that coagulation factors accumulate at measurable levels during NMP, even in grafts that would have led to primary nonfunction if transplanted. All human livers, including the liver with primary nonfunction, produced measurable amounts of (anti)coagulation factors during NMP. Successfully transplanted human livers showed considerable variation in factor accumulation, giving the first insight into upper and lower 'limits of normal' of on-pump (anti)coagulation factors.

Although the accumulation of (anti)coagulation factors during NMP might be universal, their concentrations and rate of accumulation drop at the extremes of injury. Indeed, in porcine experiments, the absolute values of factor concentrations in severely injured grafts were 2 to 6-fold lower compared to minimally injured grafts. These are grafts at a high risk of post-

transplant failure as previous porcine studies showed that livers exposed to 60-minute warm ischemia invariably developed primary nonfunction, even when undergoing 20h NMP before transplantation.[20,21] The human liver with primary nonfunction had low rates of factor accumulation when compared to livers providing life-sustaining function, suggesting that the measurement of (anti)coagulation factors may be of value as markers of viability. However, there seems to be a nonlinear relationship between the amount of injury sustained by the graft and the quantity of (anti)coagulation factors accumulated during NMP, as their accumulation rates had limited value in discriminating human livers that sustained moderate injury.

Other groups have proposed that a lactate concentration falling below either 2.5 or 1.7 mmol/L within 2h NMP can discriminate which high-risk grafts will not develop primary nonfunction.[4,6] In this light it is noteworthy that severely injured porcine livers cleared lactate to levels comparable to that of minimally injured grafts within 2h NMP, despite extensive necrosis and pan-lobular haemorrhage on histology and high AST values. Because of the considerable capacity of the liver to metabolize lactate,[9] it may be that the small number of surviving hepatocytes was sufficient to clear the lactate in the small volume of the perfusate during NMP. In line with this, Watson et al. described a case of primary nonfunction after transplantation of a high-risk graft that underwent NMP and rapidly cleared the perfusate lactate below the currently proposed cut-offs,[3] suggesting that livers which fail shortly after transplantation may be misclassified as functioning during NMP based on perfusate lactate clearance. In contrast, coagulation factor concentrations in severely injured grafts were significantly and considerably lower compared to minimally injured porcine grafts, differentiating between these degrees of injury where lactate did not. This was, however, not observed in human livers, where there were similar rates of factor accumulation during perfusion of DBD and DCD livers with minimal and moderate injury and no association with on-pump or post-transplant transaminase release.

Because most human grafts had good clinical outcomes and only one primary nonfunction occurred, we could not investigate whether (anti)coagulation factor accumulation during NMP predicts post-transplant liver function. The human liver with primary nonfunction accumulated little hepatocyte-specific coagulation factors and minimal LSEC-specific FVIII, similar to what observed during NMP of porcine livers with high likelihood of failure after transplantation. Although speculative, this similarity suggests that (anti)coagulation factors could provide useful additional information about 'on-pump' liver function, at least for livers that suffered severe injury and are at high risk of failure. In that context, it would be valuable to investigate the value of (anti)coagulation factors in livers initially declined for transplantation that undergo NMP after (prolonged) ischemia.

If the accumulation of (anti)coagulation factors is universal, even in severely injured grafts with extensive necrosis and pan-lobular hemorrhage, it may be reasoned that their accumulation relates to the release of intracellular deposits after hepatocellular lysis. If (anti)coagulation factors are to serve as markers of hepatocyte viability, i.e. functional reserve and not surrogates of hepatocyte injury,[9] this is an important issue to address. The significant, strong inverse correlation in porcine NMP and the lack of correlation in human NMP of all hepatocyte-specific (anti)coagulation factors with transaminase levels suggests that these factors are not (only) released by necrotic cells. Also, a correlation with lactate clearance during NMP, a marker of hepatocellular function, was seen in porcine livers. Current standard clinical-grade coagulation assays return results in about 2 hours, which is a timeframe compatible with real-life clinical utilization. As associations with transaminase were observed only at the extremes of ischemic injury, future research should focus on the association of (anti)coagulation factors with markers of function and outcomes especially in high-risk grafts.

The LSEC-specific FVIII might serve as a marker of LSEC-viability. In porcine experiments, FVIII negatively correlated with portal vein resistance early during NMP. Animal data suggests that higher portal vein resistance and flow may be predictive of outcome.[21,24,25] However, a correlation between FVIII and portal vein resistance was not observed in human livers.

This observational study has limitations. Because porcine livers were not transplanted, no association of 'on-pump' and post-transplant levels of coagulation factors could be assessed. Human liver grafts were part of a trial that randomised livers only when they were considered transplantable before undergoing NMP. These livers were indeed of good quality with only one case of primary nonfunction out of 222 livers randomised and transplanted.[2] The quality of the grafts included in the study prevented us from analysing human livers with severe injury. Even though the analysis on the four sub-groups of human livers of low to intermediate risk is based on small samples size, it provides relevant information as it suggests that (anti)coagulation factors have limited informative and discriminative value in the "grey zone" of livers that sustained moderate injury. Sampling time points for human livers in the randomized trial were limited; therefore, we looked at accumulation rates over time. The estimation of factor accumulation as the delta between two sampling points may have been an over-simplification of the actual trend of accumulation. However, the perfusate concentration of (anti)coagulation factors increased linearly during NMP of human livers in preliminary studies.[18] A similar linear increase in (anti)coagulation factors concentration was seen during 24h perfusion of minimally injured porcine livers, except for FV. The biphasic kinetics of FV in our porcine experiments remains unexplained and does not seem to be present in human livers. [18] We therefore assume that the calculated rates provide a good approximation of the actual trend of accumulation during perfusion. The correlation between accumulation during NMP and post-transplant production of (anti)coagulation factors could not be explored since blood samples in humans were collected only after 1h of graft reperfusion in the recipient. As the shortest half-life of the

coagulation factors (that of FVIII) is 12h, any factor measured at this time-point would reflect the residual concentration in the recipient before transplantation.

In conclusion, (anti)coagulation factors accumulate during liver NMP regardless of donor type and severity of ischemic injury. Our observations give a first indication of what could be considered normal values of (anti)coagulation factors in livers that provide life-sustaining function and show that there is no relationship with 'on-pump' or post-transplant transaminases in low to moderate risk grafts. Whether low values of (anti)coagulation factors are predictive of nonfunction for high-risk livers near the extreme of ischemic injury is unclear but the experimental and clinical observations in this study seem to point in that direction.

Acknowledgments

We would like to thank the patients, donors, and donor families as well as all transplant centres that included livers in this trial. We are indebted to Sarah Mertens, Rajeev Kumar, and Bhumika Patel for help with ethics approval, database management, and sample bio banking and shipment.

The COPE Liver Trial was performed by the Consortium for Organ Preservation in Europe (COPE) and was funded by a European Commission Seventh Framework Programme (FP7) Grant (No 305934). We thank the European Commission for their support through the Seventh Framework Programme.

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Figures legend

Figure 1. Severely injured porcine livers accumulated significantly less coagulation factors during 6-hours NMP. The perfusate concentration of AST was significantly higher in severely injured porcine livers at every time-point considered (panel A), but lactate concentration was similar starting from the second hour of perfusion (panel B). The perfusate accumulation of coagulation factor V (panel C), VII (panel D), VIII (panel E), IX (panel F), and X (panel G) was significantly reduced in severely injured porcine livers (AST, aspartate transaminase; F, coagulation factor). *$p<0.05$ (Student's t-test).

Figure 2. The rate of accumulation of (anti)coagulation factors during NMP of minimally and moderately injured livers showed considerable variation but did not differ between groups. The rate of accumulation (determined as the difference in concentration between two time-points, divided by the time difference between them) of fibrinogen (panel A), coagulation factor V (panel B), IX (panel C), antithrombin (panel D), and coagulation factors VIII (panel E) are depicted in the dot-plots. The median rate of increase in (anti)coagulation factors is indicated by the horizontal bar. The differences in rate of increase were not significantly different between the groups according to donor type and severity of ischemic injury. The open arrow indicates the liver with primary non function; the black arrows corresponds to the livers with post-transplant peak AST>1000 IU/L but with life sustaining function. Fg, fibrinogen; AT, antithrombin.

Figure 3. The human liver that developed primary nonfunction accumulated only minimal hepatocyte-specific and LSEC-specific coagulation factors during perfusion. Dot plots representing rates in increase of fibrinogen (panel A), coagulation factor V (panel B), IX (panel C), antithrombin (panel D), and coagulation factor VIII (panel E) during NMP of human livers, split according to donor type (DBD, DCD), ischemic injury (minimal, moderate), and outcome (immediate function, primary non function). Grafts that failed are presented by black dots. (DBD, donation after brain death; DCD, donation after circulatory death).

Table 1. Donor, procedural, recipient characteristics and outcomes after transplantation of human livers included in the sub-study, according to donor type and severity of ischemic injury.

	DBD minimal injury (n=10)	DCD minimal injury (n=10)	DBD moderate injury (n=10)	DCD moderate injury (n=5)	*P* value
Donor age (y)	55 (22-78)	64 (24-75)	52 (21-73)	35 (17-62)	0.13
Donor gender (M/F)	5/5 (50/50)	4/6 (40/60)	4/6 (40/60)	4/1 (80/20)	0.54
Cause of death					0.82
Trauma	2 (20)	0 (0)	1 (10)	1 (20)	
Cerebrovascular accident	5 (50)	4 (40)	6 (60)	2 (40)	
Hypoxia	2 (20)	5 (50)	2 (20)	2 (40)	
Other	1 (10)	1 (10)	1 (10)	0 (0)	
Donor BMI (kg/m²)	26,2 (21.4-30.6)	26,2 (20.0-31.0)	24,9 (21.5-37.1)	23,7 (17.7-31.1)	0.69
Donor Peak AST (IU/L)	63 (56-76)	49 (22-360)	61 (44-109)	104 (27-181)	0.96
Donor Peak ALT (IU/L)	63 (11-452)	33 (19-261)	40 (7-950)	57 (20-390)	0.93
ET-DRI	1,70 (0.99-2.17)	1,94 (1.60-4.31)	1,77 (1.50-3.09)	1,31 (1.06-1.97)	0.05
Total preservation time (min)	668 (258-1300)	813 (440-1335)	654 (382-878)	671 (561-854)	0.43
NMP Time (min)	525 (156-1117)	600 (255-1095)	467 (224-717)	525 (375-660)	0.60

Recipient age (y)	57 (42-69)	47 (36-70)	57 (22-70)	60 (51-67)	0.68
Recipient gender (M/F)	8/2 (80/20)	7/3 (70/30)	8/2 (80/20)	3/2 (60/40)	
MELD	12 (8-26)	12 (7-23)	13 (6-21)	12 (8-26)	0.94
Transplantation indication					0.26
Postalcoholic cirrhosis	2 (20)	5 (50)	0 (0)	2 (40)	
Hepatocellular carcinoma	1 (10)	1 (10)	4 (40)	0 (0)	
Biliary diseases	4 (40)	3 (30)	3 (30)	2 (40)	
Retransplant	0 (0)	0 (0)	2 (20)	0 (0)	
Other	3 (30)	1 (10)	1 (10)	1 (20)	
Peak post-transplant AST (IU/L)	162 (68-196)	176 (75-305)	1905 (1344-5101)	1437 (1191-2767)	<0.001
Peak post-transplant INR	1,7 (1.4-2.1)	1,6 (1.2-3.0)	1,6 (1.3-5.1)	1,7 (1.3-2.96)	0.63
Primary non function	0 (0)	0 (0)	1 (10)	0 (0)	0.46
Early allograft dysfunction	1 (10)*	0 (0)	1 (10)*	1 (20)*	0.20
Patient survival 1 year	90%**	100%	80%**	60%	0.19

Median (range) for continuous variables, number (%) for categorical variables; * 1 unknown, ** 1 died with functioning graft

BMI, body mass index; ET-DRI, Eurotransplant donor risk index; MELD, model of end stage liver disease; NMP, normothermic machine perfusion.

Table 2. Overview of the correlations between coagulation factors and other available markers of hepatocellular injury and function at different time-points during six-hour normothermic machine perfusion of porcine livers.

Coagulation factor	Time-point	AST	Bile volume	Lactate
Factor V	15min	τ: -0.79, p=0.006	-	τ: -0.41, p=0.11
	30min	τ: -0.87, p=0.0005	-	τ: -0.42, p=0.09
	1h	τ: -0.93, p=0.001	τ: 0.23, p=0.37	τ: -0.51, p=0.04
	2h	τ: -0.71, p=0.02	τ: 0.38, p=0.17	τ: -0.39, p=0.14
	3h	τ: -0.83, p=0.002	τ: 0.22, p=0.41	τ: -0.05, p=0.85
	6h	τ: -0.62, p=0.05	τ: 0.28, p=0.28	τ: 0.09, p=0.71
Factor VII	15min	τ: -0.64, p=0.03	-	τ: -0.63, p=0.01
	30min	τ: -0.47, p=0.06	-	τ: -0.57, p=0.03
	1h	τ: -0.57, p=0.05	τ: 0.14, p=0.59	τ: -0.67, p=0.006
	2h	τ: -0.52, p=0.10	τ: 0.49, p=0.07	τ: -0.44, p=0.10
	3h	τ: -0.50, p=0.06	τ: 0.36, p=0.17	τ: -0.19, p=0.46
	6h	τ: -0.52, p=0.10	τ: -0.28, p=0.28	τ: 0.01, p>0.90
Factor IX	15min	τ: -0.57, p=0.05	-	τ: -0.41, p=0.10
	30min	τ: -0.64, p=0.009	-	τ: -0.29, p=0.25
	1h	τ: -0.86, p=0.003	τ: 0.41, p=0.10	τ: -0.42, p=0.09

	2h	τ: -0.52, p=0.10	τ: 0.49, p=0.07	τ: -0.33, p=0.21
	3h	τ: -0.72, p=0.007	τ: 0.41, p=0.12	τ: 0.01, p>0.90
	6h	τ: -0.52, p=0.10	τ: 0.28, p=0.28	τ: 0.09, p=0.71
Factor X	15min	τ: -0.05, p=0.87	-	τ: 0.01, p>0.90
	30min	τ: -0.11, p=0.69	-	τ: 0.04, p=0.90
	1h	τ: -0.48, p=0.11	τ: 0.23, p=0.41	τ: -0.44, p=0.10
	2h	τ: -0.51, p=0.12	τ: 0.29, p=0.32	τ: -0.67, p=0.02
	3h	τ: -0.67, p=0.02	τ: 0.39, p=0.15	τ: -0.31, p=0.25
	6h	τ: -0.72, p=0.03	τ: 0.40, p=0.13	τ: 0.18, p=0.51

AST, aspartate transferase.

Figure 1

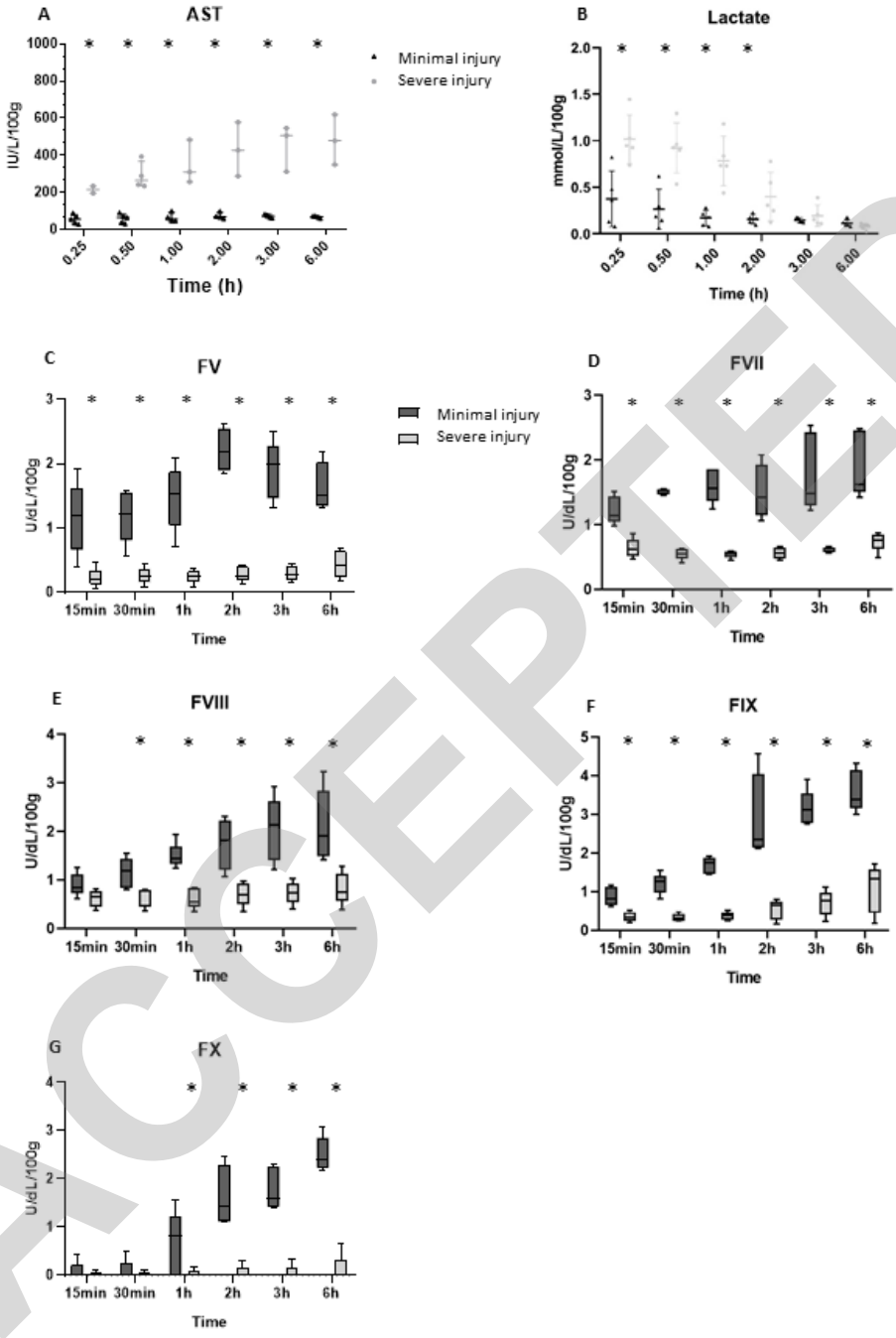

Figure 2

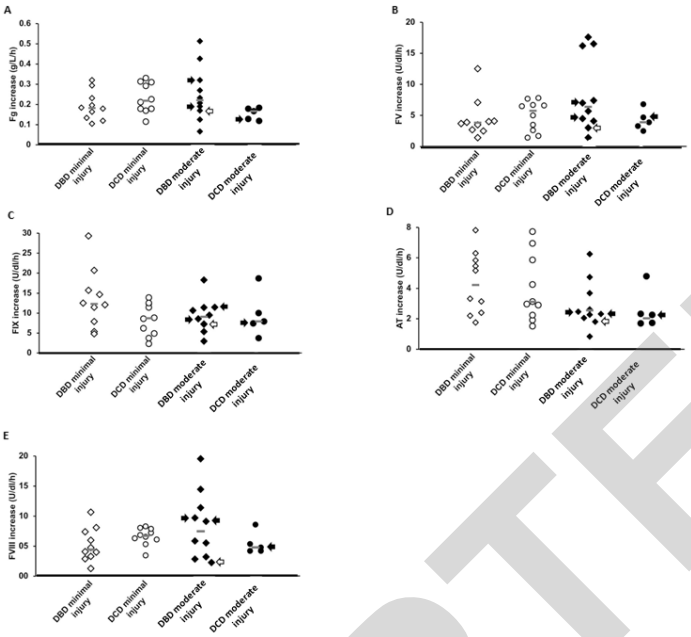

Figure 3