CHAPTER 5

In vitro evaluation of pro- and anticoagulant drugs in children with end-stage liver disease undergoing liver transplantation

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ABSTRACT

Background: Pro- and anticoagulant drugs are commonly used in pediatric liver transplantation to prevent and treat thrombotic and bleeding complications. However, the combination of baseline hemostatic changes in children with liver disease and additional changes induced by transplantation makes this very challenging.

Objectives: This study aimed to analyze the efficacy of clinically available pro- and anticoagulant drugs in plasma from children undergoing liver transplantation.

Methods: *In vitro* effects of pro- and anticoagulant drugs on thrombin generation capacity were tested in plasma samples of 20 children (≤ 16 years) with end-stage liver disease undergoing liver transplantation, and compared to 30 age-matched healthy controls.

Results: Addition of pooled normal plasma had no effect in patients or controls, while 4-factor prothrombin complex concentrate increased thrombin generation in both patients and controls, with enhanced activity in patients. At start of transplantation, dabigatran and unfractionated heparin had a higher anticoagulant potency in patients, whereas 30 days after transplantation low molecular weight heparin was slightly less effective in patients. Effects of rivaroxaban were comparable between patients and controls.

Conclusion: This study revealed important differences in efficacy of commonly used pro- and anticoagulant drugs in children with end-stage liver disease undergoing liver transplantation. Therefore, dose adjustments of these drugs may be required. The results of this study may be helpful in development of urgently needed protocols for strategies to prevent and treat bleeding and thrombotic complications in pediatric liver transplantation.

INTRODUCTION

Outcomes after pediatric liver transplantation (LT) have improved over the years. At present, two of the main challenges remain bleeding and thrombotic complications, which significantly contribute to morbidity and mortality of pediatric LT.[1-3] Prevention and treatment of these complications are essential.

The hemostatic system plays a key role in the pathogenesis of these complications. The liver produces thrombopoietin, pro- and anticoagulant factors along with pro- and antifibrinolytic factors and regulates hemostasis. In patients with end-stage liver disease (ESLD), a proportionate decrease in both pro- and antihemostatic factors occurs, resulting in a 'rebalanced hemostasis'.[4-7] This rebalanced hemostatic state is very delicate and can easily be disturbed and turn into a hypo- or hypercoagulable state, especially during invasive procedures such as transplantation.[8,9] We have recently reported the hemostatic changes observed during pediatric liver transplantation.[4] At baseline, these children were characterized by thrombocytopenia which appeared balanced by high von Willebrand factor and low ADAMTS13 levels, normal thrombin generating capacity, and a preserved fibrinolytic status in most patients. During transplantation, profound hemostatic changes as a result of surgical stress, hemodilution, and the anhepatic phase occur. However, hemostatic balance remains intact with the exception for a temporary intraoperative hyperfibrinolytic state, which converts to a persistent post-operative hypocoagulable state.

Blood transfusion requirement remains substantial in pediatric LT.[1,10] Along with red blood cell transfusion, fresh frozen plasma (FFP) and 4-factor prothrombin complex concentrates (PCC) are frequently administered as prohemostatic agents in clinical practice.[11-13] However, these strategies to prevent or treat bleeding complications are largely based on empiric considerations. In adults with cirrhosis, *in vitro* addition of FFP to plasma of patients showed a lack of prohemostatic effect, whereas an exaggerated response was observed after addition of the 4-factor PCC.[14,15] The effects in children undergoing LT remain unknown.

At present, there is a wide range in post-operative thromboprophylactic strategies employed in pediatric transplant centers, with unfractionated heparin, low molecular weight heparin (LMWH) and acetylsalicylic acid as most commonly used agents.[16,17] Yet, an evidence-based decision for the type of drug, dosing, and timing is lacking. Current anticoagulant therapy protocols are mainly based on experiences in pediatric cardiology or adult LT.[17,18] However, in addition to the hemostatic changes associated with liver disease, children have lower levels of hemostatic proteins compared to adults, as hemostasis is an evolving age-depending process with most maturation during childhood.[19,20]

Due to the complex alterations in hemostasis of patients with ESLD, the efficacy of pro- and anticoagulant drugs are unpredictable.[14,21] We have previously demonstrated that *in vitro* effects of pro- and anticoagulants in adults with ESLD are highly different when compared to

healthy controls. Dabigatran, unfractionated heparin and LMWH were more potent in patients with cirrhosis, whereas rivaroxaban had an impaired anticoagulant effect.[14,22,23] Up to now, it remains unclear how different anticoagulant drugs affect hemostasis in children with ESLD, and which is the optimal antithrombotic therapy strategy.

The combination of the rebalanced hemostasis, transplantation, and the altered hemostatic state in children compared to adults, makes it difficult to translate existing pro- and anticoagulant treatment regimens used in adults to the pediatric liver transplant recipient. With this study we aimed to examine the efficacy of clinically available pro- and anticoagulant drugs in children undergoing LT.

METHODS

Setting and participants

This prospective cohort study included 20 children (≤ 16 years) with ESLD that underwent a primary LT in the University Medical Center Groningen between 09-2017 and 10-2018. Exclusion criteria were acute liver failure, combined organ transplantation or retransplantation. All recipients received thromboprophylaxis according to our standard protocol which consisted of continuous intravenous heparin for one week posttransplantation followed by 3 months of acetylsalicylic acid as outlined previously.[10] An age-matched group of 30 otherwise healthy children admitted for a minor surgical intervention (i.e. inguinal surgery (n = 20), resection of thyroglossal duct cysts (n = 3) or benign soft tissue tumors (n = 7)), served as normal controls. Exclusion criteria for the control group were preterm birth, a medical history of bleeding or thrombosis, and comorbidities, or use of medication affecting hemostasis. Informed consent was obtained from all patients (if ≥ 12 years) and/or parents/guardians. The study protocol was approved by our local Medical Ethical Committee (NL61164.042.17).

Blood samples

At each time point 4.5 ml of blood was drawn by venipuncture or from a central venous catheter into 3.2% sodium citrate and double centrifuged at 18°C for 10 minutes at 2,000 *g* and 10 minutes at 10,000 *g*, respectively, and subsequently stored at -80°C. For the study group, blood samples were obtained at the following three predefined time points:

1. Shortly after induction of anesthesia for LT, further abbreviated as ‘pre-LT’
2. During reperfusion phase of LT (30 minutes after reperfusion, which is a crucial phase in liver transplant procedures that may be complicated by significant hyperfibrinolysis-associated bleeding), abbreviated as ‘during LT’
3. Thirty days posttransplantation or at day of discharge, whichever came first, abbreviated as ‘post-LT’

For the control group, blood samples were taken immediately after induction of anesthesia, prior to the start of any surgical intervention.

In vitro added pro- and anticoagulants

In vitro, pro- and anticoagulant drugs were added at a single, clinically relevant dose to the platelet poor plasma samples, as outlined previously.[22] Procoagulants were added to the plasma samples derived from controls and study patients pre-LT and during LT. Anticoagulants were added to plasma samples derived from controls and patients pre-LT and post-LT.

The tested procoagulants were:

- *Pooled normal plasma (PNP);* final concentration 20%. To simulate FFP transfusion. (A generous gift from Dr. J.C.M. Meijers, Academic Medical Center Amsterdam, the Netherlands)
- *The 4-factor PCC Cofact* (Sanquin, Amsterdam, the Netherlands); final concentration 0.5 U/mL

The tested anticoagulants were:

- *Dabigatran* (Alsachim, Illkirch Graffenstaden, France); final concentration 300 ng/mL
- *Rivaroxaban* (Alsachim, Illkirch Graffenstaden, France); final concentration 25 ng/mL
- *Unfractionated heparin* (Heparine Leo, Leo Pharma, Ballerup, Denmark); final concentration 0.1 U/mL.
- *The low molecular weight heparin Clexane* (Sanofi-Aventis BV, Gouda, the Netherlands); final concentration 0.2 U/mL

Thrombin generation capacity

The hemostatic effect of the above-mentioned pro- and anticoagulants was assessed by using thrombin generations assays. These assays were performed according to the fluorimetric method as previously described by Hemker et al.[24] Thrombin generation capacity was measured with calibrated automated thrombography, in clotting plasma in presence of thrombomodulin using a micro titer plate reading fluorometer (Fluoroskan Ascent, Helsinki, Finland) with reagents and protocols from Thrombinoscope (Maastricht, The Netherlands). Thrombin generation capacity was expressed as the endogenous thrombin potential (ETP; nM IIa*min). We assessed ETP in absence and presence of study drugs, and calculated percentual changes in ETP after addition of the pro- and anticoagulants.

Prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratios (INR), plasma levels of fibrinogen, factor II, VIII and antithrombin were analyzed by using an automated coagulation analyzer (300 TOP) with reagents and protocols from the manufacturer (Werfen, Breda, The Netherlands).

Statistical analysis

Data are presented as mean (standard deviation), median (interquartile range) or number (percentage) as appropriate. To test for differences between study and control group, two sample independent t-tests or Mann-Whitney U test and Pearson chi-square or Fisher exact test were used for continuous and categorical variables, respectively. To examine the pro-

or anticoagulant potency of study drugs, percentual changes in thrombin generation were determined after addition of the study drugs. Percentual changes between groups were compared with One-way ANOVA with Tukey's post-test or Kruskal-Wallis with Dunn's post-test. Associations between percentual changes and hemostatic proteins were analyzed by using Spearman's correlation. All reported *P*-values are two-sided and considered statistically significant if < 0.05. Statistical analyses were performed using GraphPad Prism, version 7.02 (San Diego, USA) and IBM Statistics SPSS, version 23 (IBM Inc. Chicago, IL).

RESULTS

Baseline characteristics

A total of 20 children undergoing primary LT were included in the study group and 30 age-matched children in the control group. As Table 1 shows, main indications for LT were biliary atresia (45%) and congenital cholestasis (30%). A total of 13 living and 7 post-mortal donors donated 16 partial and 4 full-size grafts (Supplementary Table 1). Gender (female 55 vs. 37%; *P* = 0.20*)*, age (2.3 vs. 3.3 years; *P* = 0.62) and weight (13.9 vs. 15.5kg; *P* = 0.31) were comparable for study and control group.

Table 1 | Demographic characteristics and coagulation parameters in study and control group.

	Study group (n=20)		**Control group (n=30)**		***P*-value**
Basic characteristics					
Gender, female	11	(55%)	11	(37%)	0.20
Age, yr	2.3	(0.6-6.0)	3.3	(1.2-5.7)	0.62
Weight, kg	14	(8-21)	16	(11-22)	0.31
Length, cm	90	(68-114)	108	(83-124)	0.09
Indication for transplantation					
Biliary atresia	9	(45%)			
Congenital cholestasis	6	(30%)			
Metabolic	4	(20%)			
Malignancy	1	(5%)			
Coagulation parameters					
Prothrombin time, sec	19.1	(13.2-24.2)	12.1	(13.2-24.2)	**<0.001**
APTT, sec	39.5	(32.1-53.7)	31.8	(30.3-36.0)	**0.005**
INR	1.8	(1.2-2.2)	1.1	(1.1-1.2)	**<0.001**
ETP, nM IIa*min	629	(417-765)	556	(409-655)	0.36
Fibrinogen, g/L	1.6	(1.1-2.2)	2.2	(1.8-2.6)	**0.001**
Factor II, %	46	(25-59)	85	(79-92)	**<0.001**
Factor VIII, %	157	(118-225)	80	(76-119)	**<0.001**
Antithrombin, %	48	(18-86)	113	(104-122)	**<0.001**

Data presented as median (interquartile range; IQR) or number (%) where appropriate. *P*-value using two sample Mann-Whitney U test and Pearson chi-square tests. Abbreviations: INR, International normalized ratios; APTT, Activated partial thromboplastin time; ETP, Endogenous thrombin potential.

Coagulation in study group and control group

At baseline, ETP values were comparable between study and control group (629 vs. 556 nM IIa*min; *P* = 0.36). A significant decrease in ETP occurred in liver transplant patients during LT (to 160 nM IIa*min; *P* = 0.002), which recovered after LT (to 700 nM IIa*min; *P* = 0.81). Although baseline ETP values were comparable between study and control group, other hemostatic parameters were significantly different (Table 1).

In vitro effect of pro-coagulants

As shown in Figure 1A, addition of PNP to control samples did not change ETP values (556 vs. 567 nM IIa*min; *P* = 0.66). In pre-LT samples, a slight, but not significant ETP increase of 18% was observed after addition of PNP (*P* = 0.21). Importantly, initial ETP values in pre-LT samples were normal, and increased to 788 with PNP, which is supra-physiological when compared to ETP in controls (556; *P* = 0.002). In samples derived from patients during LT, low ETP values were measured (160 nM IIa*min) which increased with 35% (*P* = 0.14) after PNP addition. Notably, ETP values during LT did not reach physiologic levels comparable with controls, despite PNP administration (*P* = 0.008).

Four-factor PCC (Cofact) had a pronounced procoagulant effect in both study and control group (Figure 1B). In controls, addition of 4-factor PCC resulted in ETP values of 788 (*P* < 0.001), a 60% ETP increase. In pre-LT samples a comparable effect was seen, with a percentual increase of 69% (*P* = 0.46). During LT 4-factor PCC had an exaggerated effect on ETP with a 311% increase (*P* < 0.001). Although the initial ETP levels were very low during LT, addition of 4-factor PCC resulted in supraphysiological ETP levels up to 1051 nM IIa*min, which is almost twice as high as ETP levels in controls.

A significant negative correlation was found between the percentual increase in ETP after addition of 4-factor PCC and Factor II levels in pre-LT samples (R = -0.75, *P* < 0.001) and during LT-samples (R = -0.66, *P* = 0.002; Supplementary Figure 1). Furthermore, a positive correlation between percentual increase in ETP after addition of 4-factor PCC and PT was observed pre-LT (R = 0.64; *P* = 0.003) and during LT (R = 0.51; *P* = 0.05).

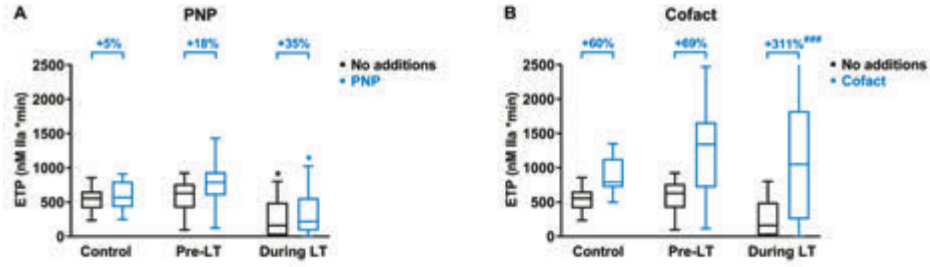

Figure 1 | *In vitro* effects of procoagulant drugs on thrombin generation capacity in study patients and controls.

*Shown are ETP values in samples from healthy controls and in samples from patients prior to and during LT in absence (black) and presence (blue) of in vitro added procoagulant agents. The values on top represent median percentual increases in ETP after addition of the procoagulant drug. In 4 patients ETP values were zero. After addition of procoagulant drugs, two of them resulted in an increase in ETP, whereas the other two did not, and therefore no percentual difference was calculated for these two. *P < 0.05 when comparing ETP values of patients to that of healthy controls; #P < 0.05 and ###P < 0.001 comparing the percentual increase in ETP between patients and controls. P-values using Kruskal-Wallis with Dunn's post-test. Abbreviations: ETP, endogenous thrombin potential; LT, liver transplantation; PNP, pooled normal plasma.*

In vitro added anti-coagulants

Addition of a direct thrombin inhibitor (dabigatran) to samples derived from controls caused a significant decrease in ETP, from 556 to 393 nM IIa*min ($P = 0.007$), a 32% difference (Figure 2A). Pre-LT addition of the same concentration of dabigatran led to a more pronounced ETP decrease from 629 to 17 nM IIa*min; a 96% reduction ($P < 0.001$). In post-LT samples, the effect of dabigatran was comparable with the effect in controls, with a 34% ETP reduction ($P > 0.99$) from 700 to 392 nM IIa*min.

Addition of a direct anti-Xa inhibitor (rivaroxaban) to controls led to a 33% decrease in ETP. Addition of rivaroxaban to pre-LT samples resulted in a percentual ETP decrease of 28% ($P = 0.20$) to 492 nM IIa*min, and in post-LT samples of 34% ETP decrease ($P > 0.99$), to 465 nM IIa*min. Effects of rivaroxaban were thus comparable between controls and patients (Figure 2B).

Addition of unfractionated heparin reduced the ETP with 87%, to a median ETP of 75 nM IIa*min ($P < 0.001$) in controls (Figure 2C). In pre-LT samples, heparin had an even larger ETP reduction of 97% to 14 nM IIa*min ($P = 0.08$). Post-LT heparin decreased ETPs with 82%, which was comparable to its effect in controls ($P = 0.15$).

Addition of LMWH to samples of controls decreased ETPs from 556 to 316 nM IIa*min, a 46% reduction (Figure 2D). In patients LWMH led to an ETP decrease of 43% in pre-LT samples (629 to 365; $P = 0.47$) and 33% in post-LT samples (700 to 464; $P = 0.07$). Although addition of LMWH resulted in a significant ETP decrease for all samples, ETP values in the absence of

anticoagulant differed between time points. After LMWH addition to post-LT samples, ETPs were comparable to physiological ETPs in controls without any additions ($P = 0.32$).

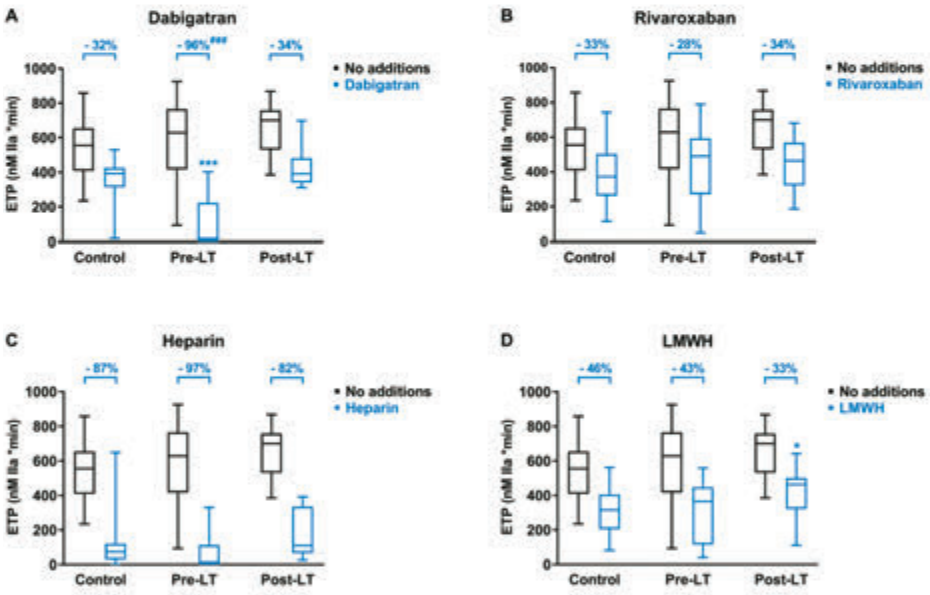

Figure 2 | In vitro effects of anticoagulant drugs on thrombin generation capacity in study patients and controls.
*Shown are ETP values in samples from healthy controls and in samples from patients prior to and 30 days after LT in absence (black) and presence (blue) of in vitro added anticoagulant agents. The values on top represent median percentual decreases in ETP after addition of the anticoagulant drug. *P < 0.05 and ***P < 0.001 when comparing ETP values of patients to that of healthy controls; #P < 0.05 and ###P < 0.001 comparing the percentual decrease in ETP between patients and controls. P-values using Kruskal-Wallis with Dunn's post-test. Abbreviations: ETP, endogenous thrombin potential; LT, liver transplantation; LMWH, low molecular weight heparin.*

DISCUSSION

This study shows the *in vitro* effects of commonly used pro- and anticoagulant drugs on endogenous thrombin generation capacity in blood samples derived from children with ESLD undergoing LT, compared to age-matched controls with intact liver function.

We found remarkable differences in the efficacy of common clinically used drugs between patients and controls. For procoagulant drugs, PNP, which mimics FFP transfusion, had no effect in samples derived from healthy controls or patients at start of transplantation, but did slightly improve coagulation during LT. The 4-factor PCC, on the other hand, was very potent in boosting thrombin generation capacity in both study and control group. For anticoagulant drugs, at the start of transplantation, both dabigatran and unfractionated heparin showed

higher anticoagulant potency in patients, whereas LWMH was slightly less effective in patients and the effect of rivaroxaban comparable to controls.

Based on the results of this study 4-factor PCC is a very effective drug to improve hemostatic function in children with ESLD, in contrast to PNP. As expected, addition of PNP to plasma samples of healthy controls had no effect on thrombin generation capacity, but neither did addition of PNP to samples taken pre-LT. Similar findings were previously reported in adult patients with ESLD.[14,15] Although FFP infusion improves factor levels and PT/INR, it does not improve hemostatic status as assessed by *in vitro* thrombin generation. Prophylactic FFP administration in patients with ESLD therefore appears ineffective, and might even do harm as it does increase portal hypertension and may result in fluid overload.[25,27]

PCCs are interesting and upcoming procoagulant drugs in the management of bleeding complications in adult LT, but no data on pediatric transplantation are available yet.[13] In our study, 4-factor PCC had a very pro-hemostatic effect in both controls and patients, with an enhanced effect during transplantation. This enhanced effect was related to a perioperative decrease in prohemostatic proteins. ETP levels were comparable between patients and controls, despite significantly lower levels of factor II and antithrombin in patients. Addition of 4-factor PCC to these plasma samples resulted in a more pronounced relative increase of these and related hemostatic factors, with a subsequent prominent increase in thrombin generation capacity. Indeed, we demonstrated a negative correlation between the procoagulant effect of PCC and baseline FII plasma levels. The enhanced procoagulant effect of 4-factor PCC in samples taken during transplantation suggests that dosing of PCCs should be conservative in liver transplant patients, to avoid overcorrection to a hypercoagulable and potentially prothrombotic hemostatic state.

As for anticoagulant drugs, in current clinical practice direct oral anticoagulants (DOACs), including rivaroxaban and dabigatran, are emerging in the treatment of venous thrombosis.[28] Although DOACs have been controversial in patients with liver dysfunction for a long time, due to an increased gastrointestinal bleeding risk and lack of reversal agents, they recently have been indicated as safe and efficacious alternatives to conventional anticoagulant drugs.[28,29] Advantages of DOACs are mode of administration, lack of monitoring requirements due to predictable pharmacokinetics and -dynamics, lack of drug and food interactions, and short half-life so they can be stopped shortly before surgery. These advantages are probably even more important for children.

A few studies examined the effects of DOACs in children, which indicated them to be safe and of similar potency as in adults.[30-32] Moreover, developmental hemostatic changes did not or only minimally influence the response to dabigatran in children, in contrast to unfractionated heparin and LWMH.[18,32,33] No studies on DOACs in children with liver disease are available yet. In the current study, we observed little differences in anticoagulant potency of rivaroxaban between patients and controls. In contrast, dabigatran had a profoundly enhanced (threefold)

anticoagulant effect in patients pre-LT. Our group previously demonstrated this enhanced anticoagulant potency of dabigatran in adults as well, which appeared to be directly related to the severity of liver disease.[14,22]

Unfractionated heparin is a frequently used anticoagulant drug in pediatric LT. Advantages of unfractionated heparin are its short half-life time and adjustable titration based on weight and APTT. However, drawbacks of heparin are its age-dependent response in children, and the unreliable monitoring by APTT and anti-Xa assays in patients with cirrhosis.[12,18,34] Thrombin generation tests might provide more reliable monitoring, but are relatively complex and not available in clinically practice yet, although whole blood thrombin generation tests are in development.[35] In our study, unfractionated heparin had a slightly enhanced anticoagulant effect pre-LT, which disappeared post-LT. This effect could partly be explained with the severity of liver disease.[34] For now, conservative dosing of unfractionated heparin is recommended in children with ESLD. More clinical studies are required to establish optimal dosing and dose guidance in pediatric LT.

The anticoagulant effect of LMWH, was comparable for controls and patients pre-LT, but seemed to decrease post-LT. This is in contrast to previous experiences in adults with cirrhosis, in whom a normal or enhanced anticoagulant activity of LMWH has been described.[22,36] Importantly, ETP after addition of LMWH to samples posttransplantation were comparable with ETPs in samples from controls without additions. This might suggest that dosing of LMWH after transplantation should be increased to obtain an optimal anticoagulant effect. Importantly, the risk of post-operative thrombotic complications after adult and pediatric LT is substantial, even in those patients receiving thromboprophylaxis with LMWH, suggesting that improvement of the thromboprophylactic regimen might be indicated.[1,17]

The increase in *in vitro* efficacy of unfractionated heparin, dabigatran, and Cofact reflect the profoundly altered hemostatic environment in patients with severe liver disease, with altered plasma concentrations of pro- and anticoagulant drugs. Whether these differences are clinically relevant requires further study as the drugs that we tested may also have altered pharmacokinetics in patients with ESLD.

Strengths of our study are that effects of various pro- and anticoagulant drugs were tested at several time-points and compared with age-matched controls. Limitations of this study are that effects of drugs were tested *in vitro* and samples from controls were taken at only one time point. Samples from patients were taken pre-LT, during LT and 30 days post-LT, whereas many complications occur in the first days after LT. However, in the first week after LT these children received unfractionated heparin according to clinical standard care, and we felt that additional addition of anticoagulant drugs to those samples would not yield meaningful results.

In conclusion, this is the first study in which the effects pro- and anticoagulant drugs were analyzed in children undergoing LT. Important differences were demonstrated in efficacy of commonly used pro- and anticoagulant drugs between children with ESLD undergoing LT and healthy controls. Dose adjustments of these drugs may be required and the present results may offer a platform for further exploration of these drugs in the pediatric liver transplant population. These new insights may be helpful in development of urgently needed protocols for strategies to prevent and treat bleeding and thrombotic complications in pediatric LT.

What is known on this topic

- Pro- and anticoagulant drugs are commonly used in pediatric liver transplantation to prevent and treat thrombotic and bleeding complications.
- The combination of baseline hemostatic changes in children with liver disease and additional changes induced by transplantation makes this very challenging.

What does this paper add

- This prospective study demonstrates important differences in *in vitro* efficacy of commonly used pro- and anticoagulant drugs between 20 children with end-stage liver disease undergoing liver transplantation and 30 healthy controls.
- Dose adjustments of these drugs may be required in children with end-stage liver disease.
- The results of our study may be helpful in development of urgently needed protocols for strategies to prevent and treat bleeding and thrombotic complications in pediatric liver transplantation.

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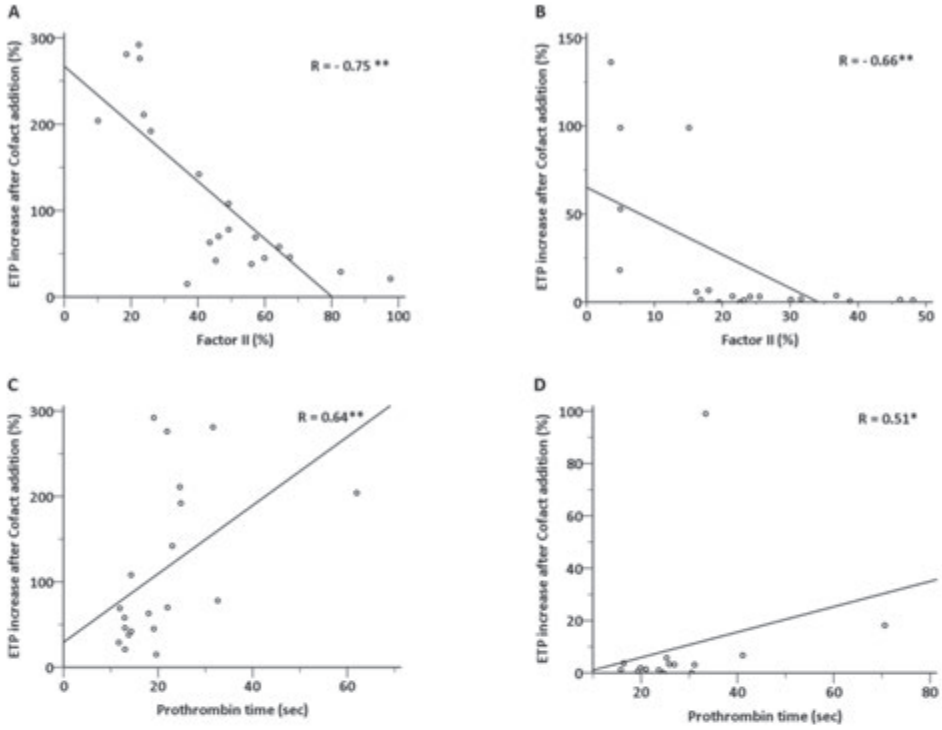

Supplementary Figure 1 | Correlations between percentual increase in ETP and Factor II levels and percentual increase in ETP and prothrombin times after addition of Cofact in samples derived from children before (A&C) and during (B&D) liver transplantation.
**P < 0.05; **P < 0.01. Abbreviations: ETP, endogenous thrombin potential. R, Spearman's correlation coefficient.*

Supplementary Table 1 | Demographic and clinical characteristics study patients.

Recipient characteristics	**(n=20)**	
Gender, female	11	(55%)
Age, yr	2.3	(0.7-6.0 ; 0.4-16.2)
Weight, kg	14	(8-21 ; 7-45)
Length, cm	90	(68-114 ; 61-165)
Indication for transplantation		
Biliary atresia	9	(45%)
Congenital cholestasis	6	(30%)
Metabolic	4	(20%)
Malignancy	1	(5%)
PELD score	28	(28-30 ; 25-40)
Lab MELD score	15	(6-22 ; 6-29)
High urgency	3	(15%)
Previous portal vein thrombosis	2	(10%)
Donor characteristics		
Gender, female	8	(40%)
Age, yr	33	(23-43 ; 1-62)
Weight, kg	76	(65-83; 10-96)
Length, cm	174	(165-182 ; 80-196)
Donor type		
Living donor	13	(65%)
Brain death	5	(25%)
Circulatory death	2	(10%)
Graft type		
Left lateral segments	15	(75%)
Left lobe	1	(5%)
Full-size	4	(20%)
Graft weight, g	316	(254-413 ; 184-522)
Graft/recipient weight ratio	2.3	(1.7-3.4 ; 0.9-5.9)
Transplantation characteristics		
Cold ischemia time, min	61	(47-404 ; 26-569)
Warm ischemia time, min	34	(29-39 ; 23-52)
Operation time, min	472	(426-526 ; 398-643)
Blood loss, mL/kg	67	(35-116 ; 18-233)
Blood transfusion, mL/kg	32	(13-68 ; 0-109)

Data presented as median (IQR ; range) or number (%) where appropriate. *P*-value using two sample Mann-Whitney U test and Pearson chi-square tests. Abbreviations: PELD score, pediatric end-stage liver disease score; MELD score, Model for end-stage liver disease score.