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Published in:
Journal of Thrombosis and Haemostasis

DOI:
[10.1111/jth.15060](https://doi.org/10.1111/jth.15060)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Bos, S., van den Boom, B., Ow, T-W., Prachalias, A., Adelmeijer, J., Phoolchund, A., Dunsire, F., Milan, Z., Roest, M., Heaton, N., Bernal, W., & Lisman, T. (2020). Efficacy of pro- and anticoagulant strategies in plasma of patients undergoing hepatobiliary surgery. *Journal of Thrombosis and Haemostasis*, *18*(11), 2840-2851. <https://doi.org/10.1111/jth.15060>

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Efficacy of pro- and anticoagulant strategies in plasma of patients undergoing hepatobiliary surgery

Sarah Bos¹ | Bente van den Boom² | Tsai-Wing Ow³ | Andreas Prachalias⁴ |
 Jelle Adelmeijer² | Anju Phoolchund³ | Fraser Dunsire⁵ | Zoka Milan⁵ | Mark Roest⁶ |
 Nigel Heaton⁴ | William Bernal³ | Ton Lisman² 

¹Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

²Surgical Research Laboratory and Section of Hepatobiliary Surgery and Liver transplantation, Department of Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

³Liver Intensive Care Unit, Institute of Liver Studies, King College Hospital, London, UK

⁴Liver Transplant Surgery, Institute of Liver Studies, Kings College Hospital, London, UK

⁵Anesthetics Department, Institute of Liver studies, Kings College Hospital London, London, UK

⁶Synapse Research Institute, Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, Maastricht, The Netherlands

Correspondence

Ton Lisman, Department of Surgery, University Medical Center Groningen, BA33, Hanzplein 1, 9713 GZ Groningen, The Netherlands.
 Email: j.a.lisman@umcg.nl

Abstract

Background: In vitro efficacy of pro- and antihemostatic drugs is profoundly different in patients with compensated cirrhosis and in those who have cirrhosis and are critically ill.

Objectives: Here we assessed the efficacy of pro- and anticoagulant drugs in plasma of patients undergoing hepato-pancreato-biliary (HPB) surgery, which is associated with unique hemostatic changes.

Methods: We performed in vitro analyses on blood samples of 60 patients undergoing HPB surgery and liver transplantation: 20 orthotopic liver transplantations, 20 partial hepatectomies, and 20 pylorus-preserving pancreaticoduodenectomies. We performed thrombin generation experiments before and after in vitro addition of fresh frozen plasma (FFP), prothrombin complex concentrate (PCC), recombinant factor VIIa (rFVIIa), low molecular weight heparin (LMWH), unfractionated heparin, dabigatran, and rivaroxaban.

Results: We showed that patients undergoing HPB surgery are in a hypercoagulable state by thrombin generation testing. FFP and rFVIIa had minimal effects on thrombin generation, whereas PCC had a more pronounced procoagulant effect in patients compared with controls. Dabigatran showed a more pronounced anticoagulant effect in patients compared with controls, whereas rivaroxaban and LMWH had a decreased anticoagulant effect in patients.

Conclusion: We demonstrate profoundly altered in vitro efficacy of commonly used anticoagulants, in patients undergoing HPB surgery compared with healthy controls, which may have implications for anticoagulant dosing in the early postoperative period. In the correction of perioperative bleeding complications, PCCs appear much more potent than FFP or rFVIIa, and PCCs may require conservative dosing and caution in use in patients undergoing HPB surgery.

N.H., W.B., and T.L. are joint senior authors.

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KEYWORDS

anticoagulants, hepatectomy, liver transplantation, plasma, thrombin

1 | INTRODUCTION

Major hepato-pancreato-biliary (HPB) surgery is frequently associated with hemostatic complications including intraoperative bleeding and postoperative venous thrombosis, and these complications contribute to morbidity and mortality.¹ The pathogenesis of hemostatic events during or after HPB surgery is complex but is likely in part related to alterations in the hemostatic system that develop during surgery or are already present at baseline. For example, complex preoperative hemostatic abnormalities are frequently present in patients with liver disease.² In addition, hemostatic changes occur during and after partial hepatectomy or orthotopic liver transplantation (OLT) resulting from hemodilution, consumption, and decreased hepatic synthesis of pro- and anticoagulant factors. Although bleeding during partial hepatectomy may be largely due to surgical and anatomical factors, perioperative changes in the hemostatic system may also contribute.^{3,4} During OLT, the substantially altered hemostatic system may contribute to bleeding, although surgical factors and portal hypertension contribute significantly.^{5,6} The risk of deep vein thrombosis following HPB surgery is between 3% and 9%, even in patients receiving adequate thromboprophylaxis.⁷⁻¹¹ In addition, in liver transplant recipients, thrombotic complications of the hepatic artery or portal vein may occur, and may directly compromise graft function and vitality.¹²

Prediction of bleeding or thrombosis in this setting is difficult as routine tests of hemostasis, such as the prothrombin time or platelet count, do not appear to reflect actual hemostatic status.^{1,13} For example, routine hemostasis tests suggest a hypocoagulable state in patients with end-stage liver disease before OLT, but when tested with thrombin generation tests that take the balance between pro- and anticoagulant processes into account, patients appear in hemostatic balance, and even have hypercoagulable features.¹⁴⁻¹⁷ Indeed, centers now report that many of their liver transplant recipients can undergo the procedure without the use of any blood product transfusions, a clinical confirmation that patients are not overtly hypocoagulable.¹⁸ Similarly, although routine hemostatic tests may suggest a hypocoagulable state following OLT or partial hepatectomy, thrombin generation tests, or viscoelastic assays may show normo- to hypercoagulability.^{14,19-21} These laboratory data suggest that administration of prohemostatic products should be limited to actively bleeding patients, and suggest the need of a proactive approach to anticoagulant therapy. However, although this strategy has been disseminated in position papers,²² little clinical evidence on the efficacy and safety of clinically available pro- and anticoagulant drugs in these patient populations is available.

Procoagulant strategies that are commonly used include fresh frozen plasma (FFP), prothrombin complex concentrate (PCC), and

Essentials

- Hepatobiliary surgery is frequently complicated by intraoperative bleeding and postoperative thrombosis.
- We added commonly used pro- and anticoagulant agents to samples taken during and after hepatobiliary surgery.
- Prothrombin complex concentrates had the most pronounced prohemostatic effect in vitro.
- In vitro anticoagulant effects of common drugs differed between patients and controls.

less often recombinant factor VIIa (rFVIIa). FFP is frequently used during partial hepatectomy and OLT to treat perceived coagulopathy or prevent bleeding. The disadvantage of the use of FFPs is that often large volumes are needed to achieve meaningful increases in factor levels. Moreover, the efficacy of FFP as a procoagulant agent continues to be debated both in the general population,^{23,24} and in patients with liver disease.^{25,26} The advantage of PCCs over FFP is the low volume and the potential to fully normalize factor levels, whereas the disadvantage is that PCCs do not contain all procoagulant factors. A strategy combining PCC with fibrinogen concentrate has been used as first-line hemostatic management during OLT.²⁷

Heparins are frequently used in anticoagulant management of patients undergoing HPB surgery. Importantly, monitoring of heparins in these patients who have decreased antithrombin levels is complicated by the underestimation of heparin levels when tested by an anti-Xa assays.²⁸ Although direct oral anticoagulants (DOACs) are not indicated in surgical settings beyond major hip or knee surgery, there are theoretical advantages of DOACs in the HPB surgical setting, as antithrombin levels can become very low in the early postoperative period, particularly following OLT and major partial hepatectomies.^{14,19} Of note, although the clinical use of DOACs in patients with liver disease is increasing,²⁹ DOACs have never been studied in clinical trials in this patient population. In addition, in package inserts, DOACs are contraindicated or advised to use cautiously in patients with advanced liver disease.

We have recently demonstrated that the in vitro efficacy of pro- and antihemostatic drugs is profoundly different in patients with compensated cirrhosis and in those who have cirrhosis and are critically ill.^{30,31} This likely relates to differences in the profound alterations in their hemostatic systems.

In this study, we aimed to assess the efficacy of both pro- and anticoagulant drugs in plasma of patients undergoing HPB surgery because hemostatic changes in these patients are also frequently substantial. Better understanding of the efficacy of commonly used pro- and anticoagulant approaches may inform future clinical

studies on optimizing use of pro- and anticoagulants in this patient population.

2 | METHODS

2.1 | Patients and setting

The study was performed at King's College Hospital, a 950-bed tertiary hospital in London, United Kingdom, from September 2017 until December 2017. Sixty consecutive adult patients who were scheduled for OLT, partial hepatectomy, or pylorus-preserving pancreaticoduodenectomy (PPPD), who had given written informed consent were included in this study. Twenty patients per group were recruited. Exclusion criteria were: age younger than 18 years, acute liver failure, hereditary thrombophilia or hemophilia, use of vitamin K antagonists, transfusion of blood products (<7 days), deep vein thrombosis (<30 days), pregnancy, and HIV positivity.

To establish reference values for the various laboratory tests used, blood samples of 42 healthy individuals were used. Exclusion criteria for healthy volunteers were similar to those applied in patients with addition of systemic diseases requiring clinical intervention or follow-up, the use of anticoagulant medications, history of venous thromboembolic events, and blood (product) transfusion up to 7 days before inclusion. The study was approved by NRES Committee London – Westminster, Study Number 17/LO/0527.

2.2 | Blood samples

Blood samples for analyses were taken into 3.2% sodium citrate tubes at the time points indicated. Samples were drawn by venipuncture from controls and in the postoperative period using a 21G needle using minimal stasis. Intraoperatively, blood was drawn from nonheparinized indwelling vascular catheters already placed by the anesthesiologist. The citrate tube was always taken after taking a serum or EDTA tube that was used for routine diagnostic testing. In those patients receiving postoperative thromboprophylaxis, postoperative samples were taken just before administration of low molecular weight heparin (LMWH).

| | Liver transplantation | Partial hepatectomy and PPPD |
|---|---|-------------------------------|
| 1 | After induction of anesthesia | After induction of anesthesia |
| 2 | 30 minutes after the start of the anhepatic phase | At the end of surgery |
| 3 | 30 minutes after reperfusion | Postoperative day 1 |
| 4 | At the end of surgery | Postoperative day 3 |
| 5 | Postoperative day 1 | Postoperative day 6 |
| 6 | Postoperative day 3 | |
| 7 | Postoperative day 6 | |

Blood samples were directly taken from the operating room or clinical ward to the laboratory by the clinical investigators (S.B.

and B.v.d.B.) who immediately processed the samples. Platelet-poor plasma was obtained by centrifuging blood samples at 18°C for 10 minutes at 2000g and subsequently for 10 minutes at 10 000g within 30 minutes after blood collection. Plasma samples were then stored at –80°C until use.

2.3 | In vitro addition of pro- and anticoagulants

We added the following agents to plasma samples of each patient and control:

- Pooled normal plasma (to mimic FFP transfusion – obtained by combining plasma from > 200 healthy volunteers, a generous gift from Dr JC Meijers, Academic Medical Center Amsterdam, The Netherlands) – final concentration 20% (v/v)
- Cofact (a four-factor PCC, Sanquin, Amsterdam, Netherlands) – final concentration 0.5 U/mL
- rFVIIa (Novo Nordisk, Bagsvaerd, Denmark) – final concentration 50 nmol/L
- The LMWH Clexane (Sanofi-Aventis BV, Gouda, the Netherlands) – final concentration 0.2 U/mL
- Unfractionated heparin (Leo Pharma, Denmark) – final concentration 0.1 U/mL
- Dabigatran (Alsachim, Illkirch Graffenstaden, France) – final concentration 300 ng/mL
- Rivaroxaban (Alsachim, Illkirch Graffenstaden, France) – final concentration 25 ng/mL

The final concentrations of the anticoagulant drugs were based on initial experiments in which drugs were added in various concentrations to pooled normal plasma, after which thrombin generation was performed as described in the next paragraph. Those drugs concentrations that gave appreciable (but not maximal) inhibition of thrombin generation in pooled normal plasma were selected so it would be possible to detect both increased and decreased drug effects in patients compared with controls. The final concentrations of the procoagulant drugs were chosen to mimic clinically relevant doses. All drugs were added in the same volume: thrombin generation tests are performed with 80 µL of plasma per well and, in all experiments, 3 µL of plasma was replaced by vehicle or drug. The exception was the pooled normal plasma addition, in which per well 16 µL of plasma was replaced by 16 µL of pooled normal plasma.

All pro- and anticoagulants were added to samples obtained after induction of anesthesia, whereas only procoagulants were added to intraoperative, and only anticoagulants were added to postoperative samples.

2.4 | Thrombin generation

The thrombin generation test was performed using platelet-poor plasma with calibrated automated thrombography in absence or presence of the previously mentioned agents. Coagulation was

activated using commercially available reagents containing recombinant tissue factor (final concentration 5 pmol/L), phospholipids (final concentration 4 μ mol/L), in the presence of soluble thrombomodulin (the concentration of which is not revealed by the manufacturer). These reagents were purchased from Thrombinoscope BV, Maastricht, The Netherlands. Thrombin Calibrator (Thrombinoscope BV) was added to calibrate the thrombin generation curves. For each plasma sample, we used a single calibration using plasma that was not spiked with pro- or anticoagulants. This calibrator was also used for the samples to which the various pro- and anticoagulants were added. A fluorogenic substrate with CaCl_2 (FluCa-kit, Thrombinoscope BV) was dispensed in each well to allow a continuous registration of thrombin generation. Fluorescence was read in time by a fluorometer, Fluoroskan Ascent (ThermoFisher Scientific). All procedures were undertaken according to the protocol suggested by Thrombinoscope BV.

The pro- or anticoagulant potency of the different agents was expressed as the percentual change of endogenous thrombin potential (ETP) after addition of the study agent. We calculated the percentage of change in ETP for each individual sample and compared the median change in ETP between patients and controls.

2.5 | Coagulation parameters

The international normalized ratio (INR) was assessed with commercially available methods on an automated coagulation analyzer (ACL 300 TOP) with reagents (Recombiplastin 2G) and protocols from the manufacturer (Instrumentation Laboratory).

Levels of fibrinogen and antithrombin were assessed on an automated coagulation analyser (ACL 300 TOP). We used QFA Thrombin (Hemosil) for fibrinogen and Liquid Antithrombin for antithrombin. Testing was performed according to the protocols from the manufacturer (Instrumentation Laboratory).

2.6 | Statistical analyses

Data are expressed as means (with standard deviations), medians (with interquartile ranges), or numbers (with percentages) as appropriate. Multiple groups were compared using one-way analysis of variance or Kruskal-Wallis H test as appropriate. *P* values of .05 or less were considered statistically significant. Statistical analyses were performed with Graph Pad Prism and IBM SPSS Statistics 23.0 (IBM).

3 | RESULTS

3.1 | Patient characteristics

Of the 60 included patients, 20 underwent OLT, 20 had a partial hepatectomy, and 20 underwent a PPPD. The main demographic and

clinical characteristics of the study population are shown in Table 1. Additional clinical characteristics of the patients undergoing OLT are shown in Table 2.

Almost all the patients had their blood drawn at the planned time points. For two OLT patients, it was not possible to get a blood sample on the third postoperative day. Eight of the patients who underwent a partial hepatectomy were already discharged before the measurement on day 6, and one of these eight patients declined sampling on day 3. Among the patients who underwent a PPPD, seven did not have their blood drawn at day 6.

3.2 | In vitro efficacy of pro- and antihemostatic agents in samples taken during and after OLT

We studied changes in routine hemostatic tests and total thrombin generation in samples taken during OLT. The INR was elevated compared with controls in patients at the start of OLT, and further prolonged during transplantation, with a normalization at postoperative day 6. In addition, plasma fibrinogen and antithrombin levels were lower in patients at the start of surgery, and decreased further during the procedure, with a postoperative normalization (Figure S1). In contrast, patients generated more thrombin compared with controls at each time point, in agreement with our previously published data (Figure S1).³¹

We next studied changes in total thrombin generation by in vitro addition of commonly used pro- and anticoagulant agents. Figure 1 shows absolute ETP values of controls and intraoperative plasma samples in absence or presence of procoagulant agents, and Figure 2 shows the absolute ETP values in the absence and presence of commonly used anticoagulants. Statistical differences indicated are differences in proportional change in ETP upon addition of pro- or anticoagulants between controls and patients. In other words, these comparisons show whether an agent has altered pro- or anticoagulant potency in patients compared with controls. Table S1 shows absolute ETP values and percentual differences between ETP values in the absence and presence of pro- or anticoagulant agents, with significance levels relative to the healthy control group.

When pooled normal plasma or rFVIIa was added to the plasma of controls or patients, there was very little change in total thrombin generation in patients and controls. In contrast, addition of PCC resulted in a substantial increase in the ETP in patients and controls. In patients, the increase in ETP was more pronounced with an exaggerated response particularly in samples after reperfusion and at the end of surgery.

Addition of LMWH resulted in a comparable decrease in ETP between patients and controls, but absolute ETP values in the presence of LMWH remained significantly higher in patients compared with controls at all postoperative days. Addition of unfractionated heparin (UFH) led to a more pronounced, but nonsignificant, decrease in thrombin generation in patients compared with controls, but the absolute ETP values in the presence of heparin were similar or even higher in patients compared with

TABLE 1 Patient characteristics

| | OLT (n = 20) | P-HEP (n = 20) | PPPD (n = 20) | Controls (n = 42) |
|---|----------------------|----------------------|-----------------------|-------------------|
| Age, y | 53 [41-59] | 60 [46-77] | 65 [60-73] | 29 [27-40] |
| Sex, male/female | 12/8 | 9/11 | 12/8 | 20/16 |
| Cirrhosis, n (%) | 20 (100) | — | 1 (5) | — |
| Laparoscopic, n (%) | — | 6 (30) | — | — |
| Hemoglobin, g/dL | 122 [104-128] | 133 [123-139] | 126 [118-136] | |
| Platelet count, G/L | 134 [69-163] | 238 [199-299] | 237 [208-312] | |
| WBC count, G/L | 5 [4-7.0] | 7 [5-8] | 7 [6-9] | |
| Sodium, mmol/L | 136 [132-140] | 140 [137-141] | 139 [136-140] | |
| Creatinine, μ mol/L | 72 [55-91] | 68 [57-86] | 72 [58-78] | |
| Alkaline phosphatase, U/L | 177 [108-278] | 115 [74-153] | 149 [100-349] | |
| AST, U/L | 65 [46-131] | 25 [21-36] | 30 [23-68] | |
| GGT, U/L | 100 [57-178] | 52 [31-129] | 127 [37-481] | |
| Bilirubin, μ mol/L | 36 [21-71] | 8 [6-10] | 15 [10-21] | |
| Length of surgery, min | 335 [271-377] | 263 [180-362] | 406 [343-530] | — |
| Estimated blood loss, mL | 3236 [2205-4000] | 625 [263-1825] | 145 [110-1163] | — |
| RBC transfusion, mL | 554 [271-1190] | 447 [0] | 570 [0] | — |
| FFP transfusion, mL | 1796 [1348-2650] | 600 [0] | — | — |
| Platelet transfusion, mL | 315 [304-576] | — | — | — |
| Length of hospital stay, d | 13 [10-16] | 8 [6-10] | 14 [9-18] | — |
| Thromboprophylaxis, n (%) | 18 (90) ^a | 18 (90) ^a | 20 (100) ^a | — |
| Postoperative VTE, n (%) | 1 (5) | 0 (0) | 1 (5) | |
| <i>Surgical indications</i> | | | | |
| Metastatic disease, n (%) | — | 7 (35) | | |
| Hepatocellular carcinoma, n (%) | — | 4 (20) | | |
| Neuroendocrine tumor, n (%) | — | 1 (5) | | |
| Cholangiocarcinoma, n (%) | — | 1 (5) | | |
| Epithelioid hemangioendothelioma, n (%) | — | 1 (5) | | |
| Benign tumor, n (%) | — | 6 (30) | | |
| Pancreatic carcinoma, n (%) | | | 12 (60) | |
| Ampullary adenoma, n (%) | | | 4 (20) | |
| Side branch IPMN, n (%) | | | 1 (5) | |
| Common bile duct stricture, n (%) | | | 2 (10) | |
| Insulinoma, n (%) | | | 1 (5) | |

Note: Numbers are represented as median [IQR], mean [standard deviation], or numbers (%).

Abbreviations: FFP, fresh frozen plasma; IPMN, intraductal papillary mucinous neoplasms; OLT, orthotopic liver transplantation; P-HEP, partial hepatectomy; PPPD, pylorus-preserving pancreaticoduodenectomy; RBC, red blood cell; WBC, white blood cell.

^aThromboprophylaxis was started at day 1, the first postoperative sample was drawn before the first dose, whereas other samples were drawn just before the next dose.

controls (Figure 2). Dabigatran was much more effective in inhibiting thrombin generation in patients compared with controls, with lower ETP values in the presence of dabigatran in patients compared with controls. In contrast, rivaroxaban was much less effective in patients compared with controls; consequently, the absolute ETP values in the presence of rivaroxaban were substantially higher in patients compared with controls, with ETP values in the presence of rivaroxaban in patients approximating ETP values in absence of rivaroxaban in controls.

3.3 | In vitro efficacy of pro- and antihemostatic agents in samples taken during and after partial hepatectomy

We next studied changes in routine hemostatic tests and total thrombin generation in samples taken during and after partial hepatectomy. The INR increased during and after surgery and started to normalize at day 3. Plasma fibrinogen was slightly elevated at the start of surgery, decreased during surgery, and was substantially

TABLE 2 Clinical characteristics of the 20 orthotopic liver transplant patients

| Characteristics | |
|--|---------------|
| MELD | 13 [9-17] |
| CTP, n (%) | |
| A | 3 (15) |
| B | 12 (60) |
| C | 5 (25) |
| Etiology of cirrhosis, n (%) | |
| PBC | 1 (5) |
| PSC | 6 (30) |
| NASH | 1 (5) |
| ALD | 5 (25) |
| Autoimmune | 1 (5) |
| Other | 6 (30) |
| HBV | 3 (15) |
| HCV | 1 (5) |
| HCC | 3 (15) |
| Ascites | 8 (40) |
| Encephalopathy | 4 (20) |
| CIT, min | 464 [403-522] |
| WIT, min | 36 [34-47] |
| Donation after brain death (vs donation after cardiac death) | 13 (65) |

Note: Numbers are represented as median [IQR] or numbers (%).

Abbreviations: ALD, alcoholic liver disease; CIT, cold ischemia time; CTP, Child Turcotte Pugh; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; MELD, model for end-stage liver disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; WIT, warmth ischemia time.

elevated thereafter until day 6. Antithrombin levels were normal in patients at the start of surgery, but decreased during surgery and did not normalize until day 6 (Figure S2). Patients generated more thrombin compared with controls throughout the procedure, which

is in line with our previously published data,¹⁹ except for elevated thrombin generation at baseline (Table S2).

In the samples taken from patients during and after partial hepatectomy, changes in thrombin generation after addition of pro- and antihemostatic drugs were also tested. Figure 3 shows absolute ETP values of patients and controls in absence or presence of procoagulant agents, with ETP values in absence or presence of anticoagulant agents shown in Figure 4. Statistical differences indicated are differences in proportional change in ETP upon addition of pro- or anticoagulants between controls and patients. Table S2 shows absolute ETP values and percentual differences between ETP values in absence and presence of pro- or anticoagulant agents, with significance levels relative to the healthy control group.

Addition of pooled normal plasma and rFVIIa resulted in very little change in thrombin generation in patients and controls. In contrast, PCCs substantially increased thrombin generation in patients and controls, and although the magnitude of the increase was similar between patients and controls, absolute ETP values were substantially higher in patients because of higher thrombin generation in the absence of PCC, particular at the end of surgery.

LWMH had a similar anticoagulant effect in patients and controls, although absolute ETP values in the presence of LMWH were very high in patients, with values even higher than control values in the absence of LMWH. At start of surgery, anticoagulant activity of LWMH was clearly higher in patients. With the addition of UFH, the relative decrease of the ETP in patients was comparable to the controls in all postoperative samples, but a more extensive anticoagulant effect was seen at baseline. Absolute thrombin generation in the presence of UFH was substantially higher in postoperative samples compared with controls. Dabigatran showed a larger relative decrease of ETP in patients compared with controls, but absolute values in the presence of dabigatran were comparable or even higher in patients. Rivaroxaban was much less effective in patients

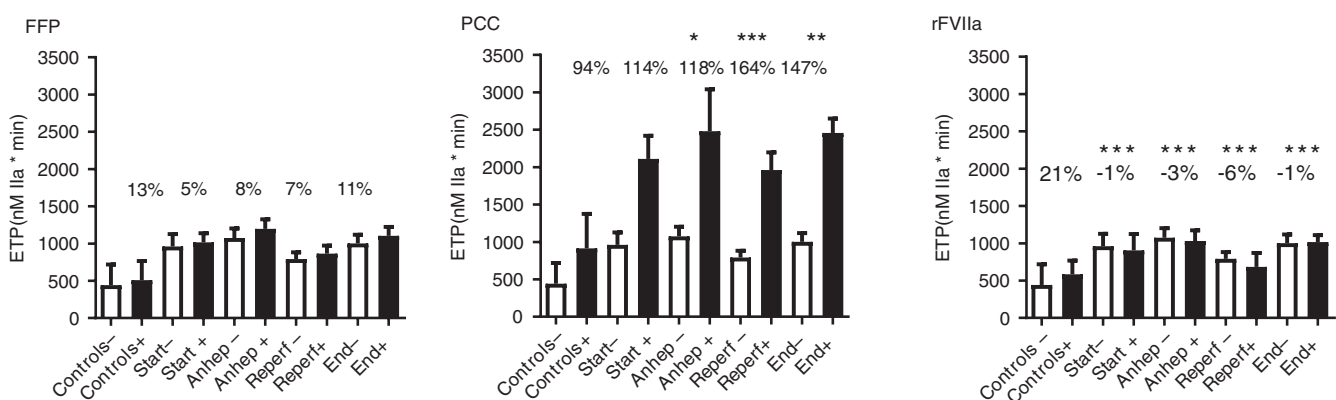


FIGURE 1 Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients during OLT before (-) and after (+) in vitro addition of prohemostatic agents. Start is after induction of anesthesia, anhep is 30 minutes after the start of the anhepatic phase, reperf is 30 minutes after reperfusion, and end is at the end of surgery. Shown are medians with error bars indicating interquartile ranges, and the proportional difference in ETP upon addition of procoagulants. Statistical differences indicated are differences in proportional change in ETP upon addition of procoagulants between controls and patients. * $P < .05$ vs controls, ** $P < .01$ vs controls, *** $P < .001$ vs controls. FFP, fresh frozen plasma; PCC, prothrombin complex concentrate; rFVIIa, recombinant factor VIIa

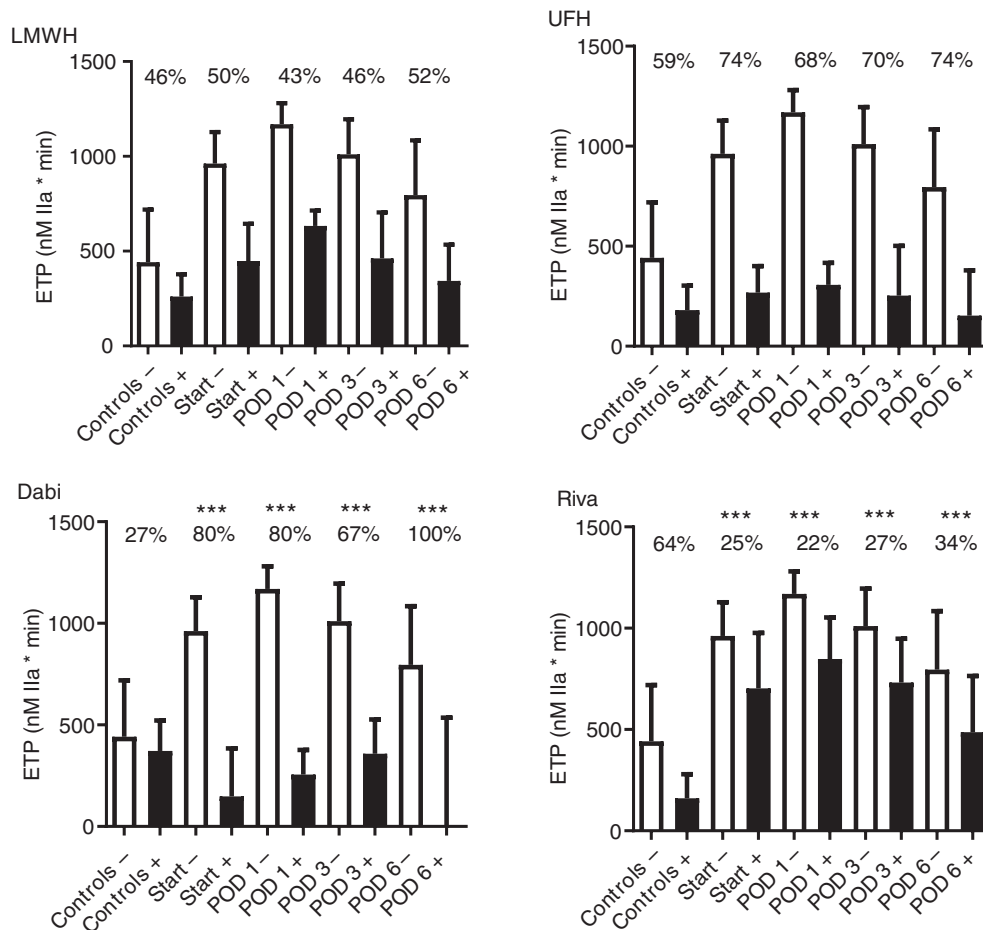


FIGURE 2 Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients after OLT before (-) and after (+) in vitro addition of anticoagulants. Start is after induction of anesthesia, POD, postoperative day. Shown are medians with error bars indicating interquartile ranges, and the proportional difference in ETP upon addition of anticoagulants. Statistical differences indicated are differences in proportional change in ETP upon addition of anticoagulants between controls and patients. *** $P < .001$ vs controls. Dabi, dabigatran; LMWH, low molecular weight heparin; Riva, rivaroxaban; UFH, unfractionated heparin

compared with controls, with ETP values in the presence of drug exceeding ETP values in controls in absence of drug.

3.4 | In vitro efficacy of pro- and antihemostatic agents in samples taken during and after PPPD

We studied changes in routine hemostatic tests and total thrombin generation in samples taken during and after PPPD. The INR increased during and after surgery, and began to normalize at day 3. Plasma fibrinogen was slightly elevated at the start of surgery, decreased during surgery, and was substantially elevated thereafter until day 6. Antithrombin levels were normal in patients at the start of surgery, but decreased during surgery and did not fully normalize until day 6 (Figure S3). Patients generated more thrombin compared with controls throughout the procedure, which is in contrast with our previously published data,¹⁹ in which we reported normal thrombin generation (Table S3).

The prohemostatic drugs and anticoagulants that were tested in the samples from patients undergoing OLT and partial

hepatectomy were also added to the samples taken from patients during and after PPPD. Absolute ETP levels in absence and presence of the pro- and anticoagulant drugs are shown in Figures 5 and 6. Statistical differences indicated are differences in proportional change in ETP upon addition of pro- or anticoagulants between controls and patients. Table S3 shows absolute ETP values and percentual differences between ETP values in absence and presence of pro- or anticoagulant agents, with significance levels relative to the healthy control group.

Addition of pooled normal plasma or rFVIIa had very little effect on total thrombin generation in patients and controls. In contrast, the addition of PCC increased thrombin generation to a similar extent in patients and controls, but total thrombin generation in the presence of PCC was much higher in patients as a result of elevated thrombin generation compared with controls in the absence of drug. The effects of the anticoagulants in the plasma of patients after PPPD are similar to the effect described in the patients after partial hepatectomy, with heparins and dabigatran exerting similar to increased anticoagulant potency in patients compared with controls, but with higher absolute ETPs in presence of drug in patients,

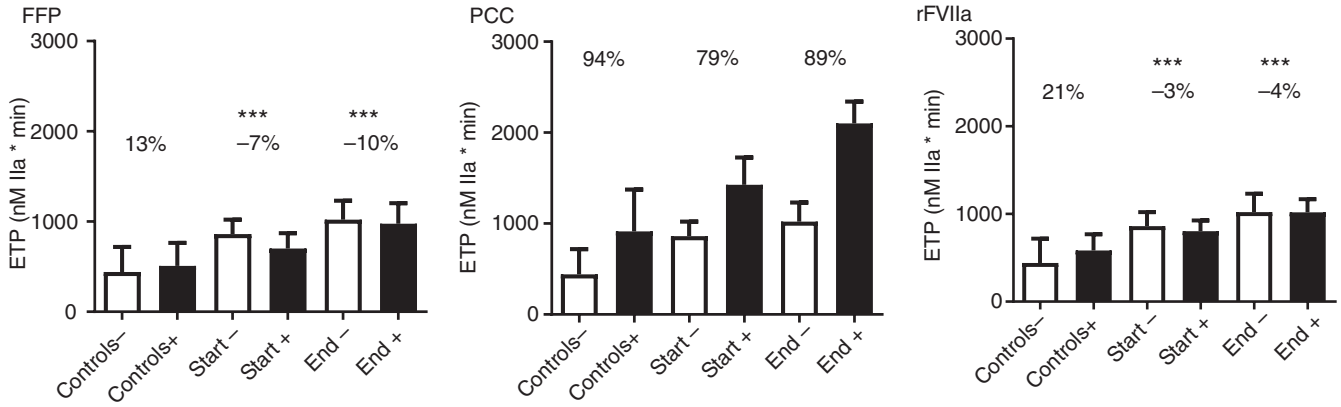


FIGURE 3 Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients during partial hepatectomy before (-) and after (+) in vitro addition of prohemostatic agents. Start is after induction of anesthesia, end is at the end of surgery. Shown are medians with error bars indicating interquartile ranges, and the proportional difference in ETP upon addition of procoagulants. Statistical differences indicated are differences in proportional change in ETP upon addition of procoagulants between controls and patients. *** $P < .001$ vs controls. FFP, fresh frozen plasma; PCC, prothrombin complex concentrate; rFVIIa, recombinant factor VIIa

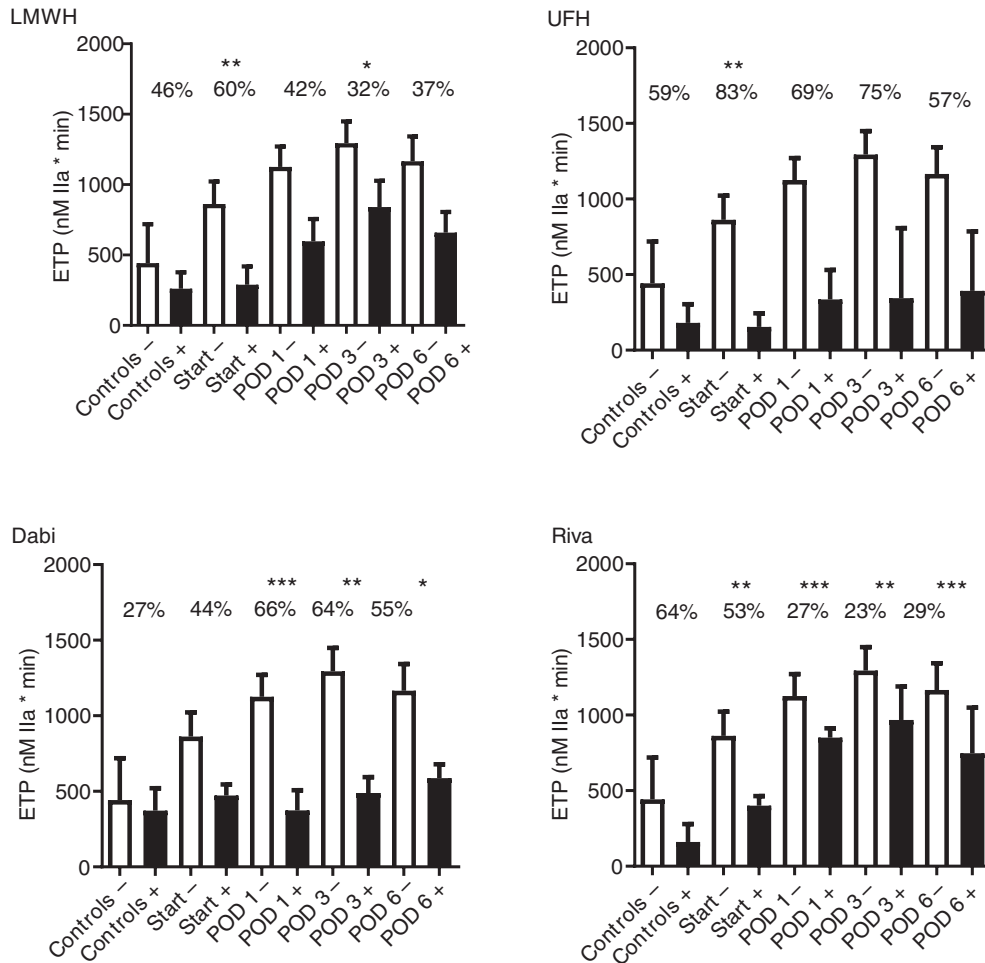


FIGURE 4 Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients after partial hepatectomy before (-) and after (+) in vitro addition of anticoagulants. Start is after induction of anesthesia, POD, postoperative day. Shown are medians with error bars indicating interquartile ranges, and the proportional difference in ETP upon addition of anticoagulants. Statistical differences indicated are differences in proportional change in ETP upon addition of anticoagulants between controls and patients. * $P < .05$ vs controls, ** $P < .01$ vs controls, *** $P < .001$ vs controls. Dabi, dabigatran; LMWH, low molecular weight heparin; Riva, rivaroxaban; UFH, unfractionated heparin

particularly with LMWH. Rivaroxaban had very poor anticoagulant effects both in relative and absolute terms.

4 | DISCUSSION

In this study, we found that patients undergoing HPB surgery are hypercoagulable when assessed with thrombomodulin-modified thrombin generation testing. Although an elevated INR during or after the procedure may suggest a bleeding tendency, the actual hemostatic status appears prothrombotic, which is in line with clinical observations on mild bleeding in many patients, even those with preexisting liver failure,¹⁸ and confirms thrombotic risk following HPB surgery.^{7-11,32} We also demonstrated altered potency of commonly used anticoagulant drugs comparable to enhanced anticoagulant effects for UFH, LMWH, and dabigatran, and profoundly decreased anticoagulant effects of rivaroxaban. Despite the increased anticoagulant effects of dabigatran and heparins, absolute on-drug thrombin generation was higher in patients compared with controls, particularly in case of LMWH. The anticoagulant effect of rivaroxaban was substantially lower in patients compared with controls with on-drug thrombin generation levels that substantially exceeded off-drug thrombin generation in controls. Our results therefore suggest an insufficient anticoagulant effect of standard dosages of LMWH and rivaroxaban in patients that undergo HPB surgery. Finally, we found no appreciable procoagulant effects of FFP and rFVIIa in patients and controls, but significant procoagulant activity of PCCs. The relative prohemostatic effect of PCCs appeared to be higher in liver transplant recipients compared with controls.

We also found elevated fibrinogen levels, mainly after oncological surgery, which could be considered as an additional thrombotic risk factor.^{33,34} In light of the published data on increased risk of VTE after partial hepatectomy in the presence of optimal thrombosis

prophylaxis with LMWH^{7,8,11} and our current data, it may be justified to increase the LMWH dose early after HPB surgery, although clinical studies are required to assess safety and efficacy of such an approach. Dose adjustments have been previously proposed for patients undergoing partial hepatectomy,^{1,11} but no clinical studies have yet assessed this approach. Besides enhanced thrombin generation and hyperfibrinogenemia, patients that underwent HPB surgery are characterized by a persistent postoperative hypofibrinolysis³⁵ and a VWF/ADAMTS13 unbalance,^{36,37} which further contribute to the hypercoagulable state of these patients.

DOACs are replacing LMWH in thromboprophylaxis after orthopedic surgery, but use of DOACs in other surgical settings has not been extensively explored. The major advantage of DOACs over LMWH is the mode of administration, and an additional advantage in the HPB surgery setting being the independence of antithrombin, which is frequently low after OLT and major partial hepatectomy. However, given the substantially altered anticoagulant effects of the Xa-directed DOAC rivaroxaban, and the IIa-directed DOAC dabigatran, careful use is warranted in clinical application of these drugs in the surgical HPB setting, preferably guided by well-designed clinical studies.

Our data on prohemostatic strategies show that rFVIIa and FFP have little to no in vitro prohemostatic effect. These results are in line with clinical data on the use of rFVIIa in HPB surgery,³⁸ and with increasing data arguing against liberal use of FFP in OLT^{18,22} and cirrhosis.^{26,39} In vitro and ex vivo studies have demonstrated little to no prohemostatic effect of FFP by thrombin generation tests in patients with cirrhosis.^{25,40} Although prophylactic administration of FFP in HPB surgery is common and leads to improvement of routine laboratory parameters such as the INR, the actual prohemostatic effect of FFP is questionable. More important, FFP can lead to circulating volume overload, which may increase bleeding risk by increasing portal and central venous pressure. Given the poor evidence that FFP is clinically effective in prophylactic and treatment settings,^{23,24} a search for alternative prohemostatic options would be wise. Our data

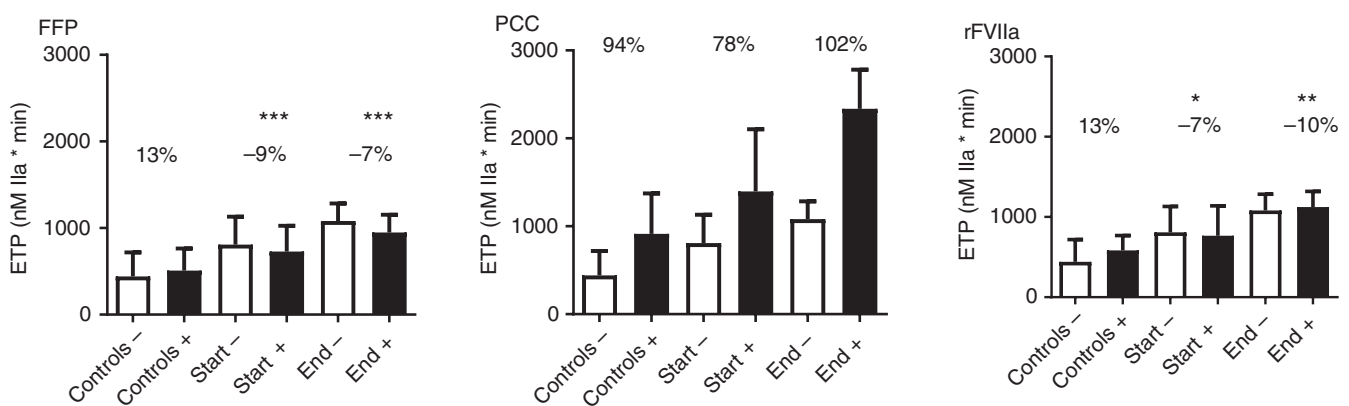
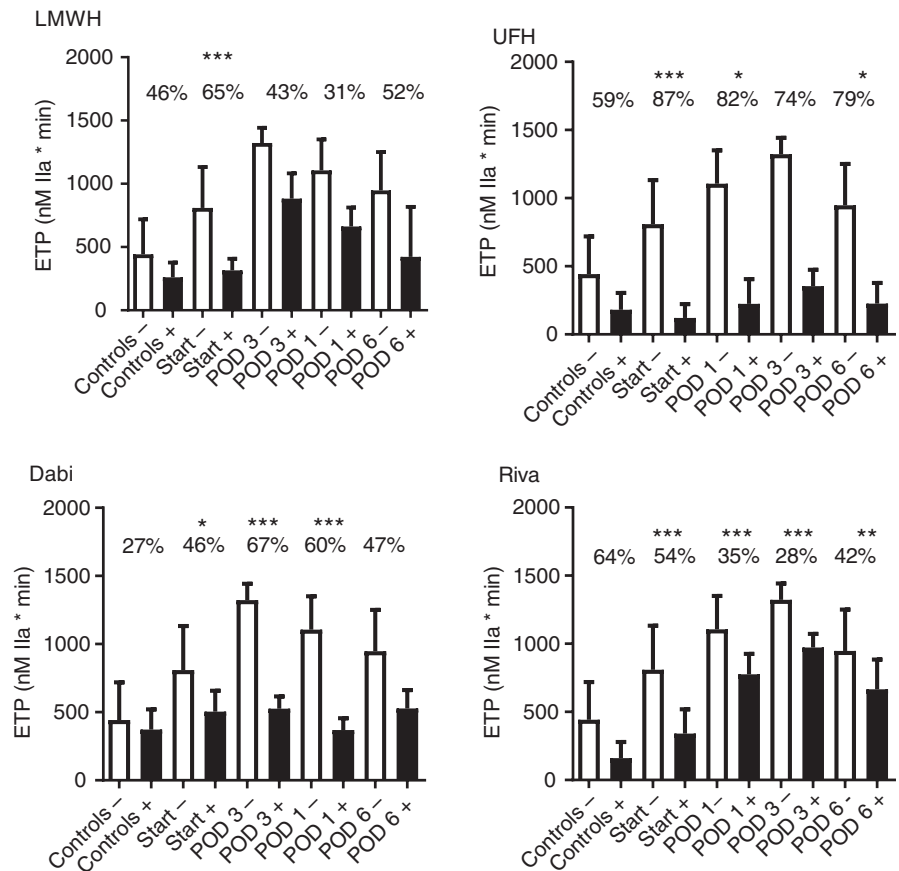


FIGURE 5 Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients after PPPD before (-) and after (+) in vitro addition of prohemostatic agents. Start is after induction of anesthesia, end is at the end of surgery. Shown are medians with error bars indicating interquartile ranges, and the proportional difference in ETP upon addition of procoagulants. Statistical differences indicated are differences in proportional change in ETP upon addition of procoagulants between controls and patients. * $P < .05$ vs controls, ** $P < .01$ vs controls, *** $P < .001$ vs controls. FFP, fresh frozen plasma; PCC, prothrombin complex concentrate; rFVIIa, recombinant factor VIIa

FIGURE 6 Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients after PPPD before (-) and after (+) in vitro addition of anticoagulants. Start is after induction of anesthesia. POD, postoperative day. Shown are medians with error bars indicating interquartile ranges, and the proportional difference in ETP upon addition of anticoagulants. Statistical differences indicated are differences in proportional change in ETP upon addition of anticoagulants between controls and patients. * $P < .05$ vs controls, ** $P < .01$ vs controls, *** $P < .001$ vs controls. Dabi, dabigatran; LMWH, low molecular weight heparin; Riva, rivaroxaban; UFH, unfractionated heparin



suggest PCCs to be effective in improving hemostatic capacity during HPB surgery, although the exaggerated responses in our in vitro test may warrant careful dosing. The advantage of PCCs over FFP is that PCCs lead to a much more robust increase in coagulation factor levels because PCCs contain highly concentrated coagulation factors in a small volume. Our results are in line with a single-center retrospective study of liver transplant recipients, which showed that the administration of PCCs and/or fibrinogen concentrate guided via bedside hemostatic testing was safe and effective compared with an FFP/platelet concentrate-based approach.⁴¹ In addition, an in vitro study in which plasma samples taken during OLT were supplemented with PCC or FFP showed a better improvement of thrombin generating capacity by PCCs as assessed by modern thrombin generation testing.⁴²

Although our data indicate a possible requirement for dose adjustments of commonly used pro- and anticoagulant strategies in the HPB patient, we acknowledge the limitations of our in vitro approach. Thrombin generation is thus far only used in a research setting. It is a relatively cumbersome test and not yet ready for clinical use, although the automated test (Genesia) has been launched and whole blood thrombin generation tests that may be suitable as a point-of-care test are in development.^{43,44} In addition, it is unknown which level of ETP represents the optimal pro- or anticoagulant status; therefore, we do not have ETP target levels for management of thrombosis or bleeding. To incorporate dose adjustments in further studies, we would need such information to be able to adjust the dosing of pro- or anticoagulants in this specific population.

Our study is limited by a relatively low sample size and heterogeneous cohorts. Our OLT cohort contains a large proportion of patients with cholestatic liver disease, which are known to be more hypercoagulable compared with patients with cirrhosis of other etiologies.⁴⁵ In addition, our partial hepatectomy cohort consists of patients with and without an underlying malignancy, and these patients also differ in their baseline hemostatic status. Although we did not detect obvious differences between patients in responses to pro- or anticoagulant agents in these subgroups, we note our cohorts are too small for meaningful subgroup analyses.

In conclusion, our data confirm a hypercoagulable profile of patients with cirrhosis and patients with HPB cancer, which remains present during and after major surgical procedures. We demonstrate profoundly altered in vitro efficacy of commonly used anticoagulants, with indications that LMWH and rivaroxaban require higher dosing in patients that underwent HPB surgery compared to the general population requiring these anticoagulants. We also demonstrate that in case of a perioperative bleeding complication, PCCs are much more potent than FFP or rFVIIa. Our results should be seen as a starting point for clinical studies aimed at improved pharmacological hemostatic management of patients undergoing HPB surgery.

CONFLICT OF INTEREST

None of the authors have a conflict of interest to report.

AUTHOR CONTRIBUTIONS

Sarah Bos: study design, patient inclusion, laboratory analyses, interpretation, drafting of manuscript; Bente van den Boom: patient inclusion, interpretation, revision of manuscript; Tsai-Wing Ow: patient inclusion, interpretation, revision of manuscript; Andreas Prachalias: patient inclusion, interpretation, revision of manuscript; Jelle Adelmeijer: laboratory analyses, interpretation, drafting of manuscript; Anju Phoolchund: patient inclusion, interpretation, revision of manuscript; Fraser Dunsire: patient inclusion, interpretation, revision of manuscript; Zoka Milan: patient inclusion, interpretation, revision of manuscript; Nigel Heaton: patient inclusion, interpretation, revision of manuscript; William Bernal: study design, supervision, patient inclusion, interpretation, revision of manuscript; Ton Lisman: study design, supervision, interpretation, drafting of manuscript.

ORCID

Ton Lisman  <https://orcid.org/0000-0002-3503-7140>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Bos S, van den Boom B, Ow T-W, et al. Efficacy of pro- and anticoagulant strategies in plasma of patients undergoing hepatobiliary surgery. *J Thromb Haemost.* 2020;18:2840-2851. <https://doi.org/10.1111/jth.15060>