# Prevention and treatment of hemostatic complications in liver disease

Bos, Sarah

*DOI:*
10.33612/diss.190914387

# Prevention and treatment of hemostatic complications in liver disease

Sarah Bos

Different parts of this thesis and PhD research were financially supported by the University of Groningen, Graduate School of Medical Science, Daiichi Sankyo, Ferring and Norgine.

Cover design and Lay-out: Publiss | www.publiss.nl
Print: Ridderprint | www.ridderprint.nl

rijksuniversiteit groningen

# Prevention and treatment of hemostatic complications in liver disease

**Proefschrift**

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

maandag 29 november 2021 om 9.00 uur

door

**Sarah Bos**

geboren op 11 maart 1984
te Groningen

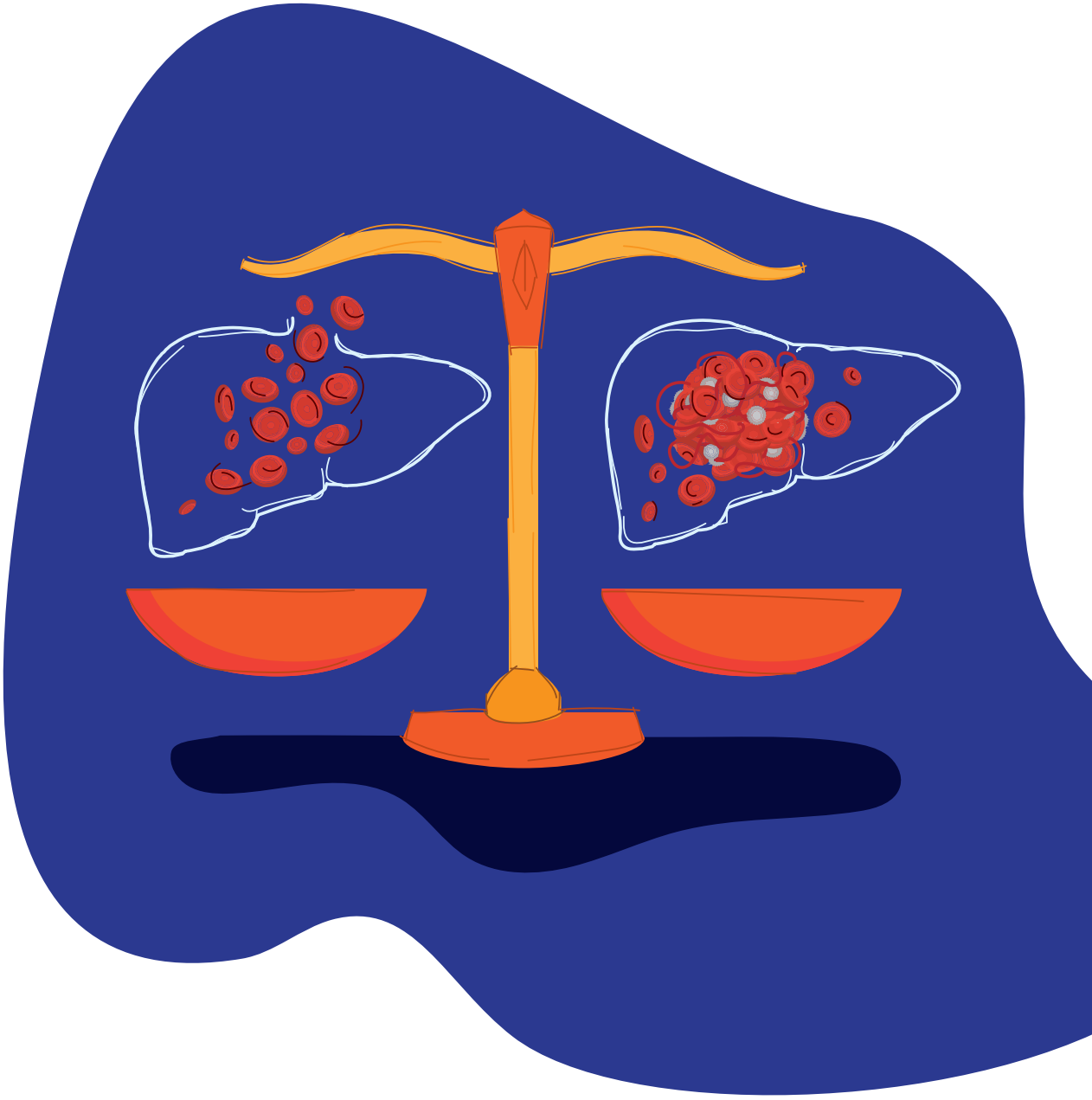
## Promotores
Prof. dr. J.A. Lisman
Prof. dr. T.P.W. Kamphuisen

## Copromotor
Dr. T.C.M.A. Schreuder

## Beoordelingscommissie
Prof. dr. H.J. Verkade
Prof. dr. T.M. Hackeng
Prof. dr. E. Villa

**Paranimfen**
Daan van Wessel
Robert Mousset

# Table of contents



# CHAPTER 1.

## General introduction

## Hemostasis

Hemostasis is a process to prevent and stop bleeding. It is a complex balance in which damage to the endothelium is managed and normal flow through the vessels is maintained. The interplay between the endothelium of the vessel wall, platelets, coagulation, and the fibrinolytic system (primary, secondary and tertiary hemostasis, respectively) normally ensure an adequate hemostatic balance. Disruption of this interplay can lead to thrombotic or hemorrhagic tendency.

## Primary hemostasis

Primary hemostasis entails the activation, adhesion and aggregation of platelets onto a damaged site in a vessel wall. The exposure of platelets to the damaged vessel wall initiates primary hemostasis. The exposed subendothelial elements further promote the recruitment of platelets in order to repair the defect. The presence of multimeric protein von Willebrand factor (VWF) at the site of injury is the key step in the mechanism of endothelial reparation. Circulating VWF will bind to the exposed subendothelial collagen and adhere to the platelet membrane. For further progression of plug formation, activation of feedback between nearby platelets is also necessary and mediated by agonists such as adenosine diphosphate and thromboxane A2.(1,2) Regulation of the multimeric VWF is essential for hemostasis; its main inhibitor is ADAMTS13 (a disintegrin an metalloproteinase with a thrombospondin type 1 motif, member 13).

## Coagulation

Coagulation or secondary hemostasis is a complex interplay of pro- and anticoagulant proteins which leads to the generation of thrombin. The widely known classic pathway of coagulation includes the intrinsic and extrinsic coagulation pathway and uses the sequential activation of coagulation factors leading to the formation of thrombin. The cell based model reflects in vivo hemostasis more accurately and does not present coagulation as a cascade but it suggests coagulation presents in overlapping stages.(3–5) Coagulation starts when tissue factor (TF) is exposed to the bloodstream.(2) Factor VII gets in contact with TF through exposed extravascular tissues. These two factors form a complex, activate factor IX and X, and serve as a bridge between extrinsic and intrinsic pathway. The complex of factor Xa and factor V that is formed activates factor II (prothrombin to thrombin). Next to multiple positive feedback loops, the negative feedback loop of thrombin generation is regulated by protein C, S and tissue factor pathway inhibitor (TFPI).(6)

## Fibrinolysis

Fibrinolysis is the process that ensures the breakdown of a clot formation. This process is triggered by tissue-type plasminogen activator (tPA), which converts plasminogen into plasmin. Plasmin degrades the fibrin mesh at various places, leading to the production of soluble fibrin degradation products. The conversion of plasminogen to plasmin is down regulated via plasminogen activator inhibitor-1 (PAI-1), an inhibitor of tPA, plasmin inhibitor (PI) and activated thrombin-activatable fibrinolysis inhibitor (TAFI). TAFI inhibits fibrinolysis by cleaving parts from partially degraded fibrin, thereby inhibiting t-PA–mediated plasminogen activation.(7)The balance in fibrinolysis prevents unwanted plasmin generation. Disruption of this balance may cause either hyper- or hypo-fibrinolysis.

## Hemostatic changes in liver disease

Cirrhosis is the end stage of chronic liver disease. Cirrhosis is defined as a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules.(8) The liver is the site of synthesis for many proteins involved in hemostasis. Consequently, patients with cirrhosis acquire multiple and complex alterations in their hemostatic system. Recent insights in consequences of the hemostatic changes in patients with cirrhosis have indicated a balanced, but unstable hemostatic system in these patients.(9,10)

Primary hemostasis in patients with cirrhosis is characterized by thrombocytopenia. The decrease in platelet count is mainly due to congestive splenomegaly and decreased production of thrombopoietin in the liver. In contrast, the decrease of ADAMTS13 in cirrhosis promotes primary hemostasis. ADAMTS13 inhibits the activity of von Willebrand factor (VWF), a large protein partly responsible for clot formation. Several studies showed the increase of VWF levels and decrease in levels of ADAMTS13.(11,12) These changes in protein activity appear to compensate for thrombocytopenia, leading to a new balance in primary hemostasis.

Patients with cirrhosis have a decreased synthesis of proteins involved in coagulation. The change in proteins promoting bleeding seems to be counterbalanced by the change in proteins that promote thrombosis. On one hand there is the decreased level of coagulation factors, II,V, VII, IX, X, XI, vitamin K deficiency and dysfibrinogenemia and on the other hand there is an elevated level of FVIII, decreased level of protein C, protein S, antithrombin, $\alpha$2-macroglobuline and heparin cofactor II.(9,10,13). The net effect of these changes in patients with cirrhosis result in a hypercoagulable state.(14,15)

The fibrinolytic system also seems to be in balance in patients with cirrhosis, due to the concomitant decline of antifi-brinolytics (antiplasmin, thrombin activatable fibrinolysis inhibitor), and plasminogen. However, current literature is not

consistent on whether there is a hyper- or hypofibrinolytic state in patients with cirrhosis.(7,16–18) These differences might be explained by the variety in and poor reproducibility of assays on the fibrinolytic status in patients with cirrhosis.

## Hemostatic testing in liver disease

Routine diagnostic tests of coagulation, such as prothrombin time (PT) and activated partial thromboplastin time (APTT), universally fail to test the hemostatic capacity of a blood sample, as these tests are only sensitive for plasma levels of procoagulant proteins. This is a particular concern in patients with liver diseases who have complex alterations in both pro- and anticoagulant pathways. The results of routine diagnostic tests of hemostasis, therefore have long been misinterpreted.

Thrombomodulin-modified thrombin generation gives an accurate representation of the balance between pro- and anticoagulant factors. It has been shown that thrombin generation in patients with cirrhosis is equal or even better than that in healthy individuals, which contrasts sharply with the results from PT and APTT tests which are prolonged in patients.(14,19–22)

## Hemostatic complications in liver disease

Patients with cirrhosis are vulnerable to both bleeding and thrombotic events. A relatively recent systematic review and meta-analysis showed in a cohort of 700.000 patients with cirrhosis and 1.5 million controls that patients with cirrhosis have a 1.7 fold increased risk of deep vein thrombosis and pulmonary embolism.(23) The prevalence of splanchnic vein thrombosis in patients with cirrhosis is 8 to 15%.(24,25) The incidence of venous thrombosis in cirrhosis correlates with the severity of the underlying disease.(26) Development of portal vein thrombosis increases the risk of disease deterioration and mortality.(26,27)

In patients undergoing hepatobiliary surgery there is a prolonged hypercoagulable state with an increased risk of developing venous thombo-embolisms.(28)

Bleeding complications in patients with cirrhosis are common, however many of the clinically relevant bleeds– i. e. variceal bleeds – are unrelated to hemostasis. These bleeding events are rather a signal of portal hypertension.(29)

## Anticoagulation in liver disease

Low-molecular weight heparins (LMWH) are the standard-of-care treatment for portal vein thrombosis in cirrhosis. Patients with cirrhosis have substantially decreased levels of antithrombin impeding correct dosing. There is increasing enthusiasm for the use of direct oral anticoagulants (DOACs). These anticoagulants

are the recommended treatment for venous thromboembolism and atrial fibrillation in the general population.(30,31) DOACs have several advantages over vitamin K antagonists (VKA) and LMWH. The administration is in tablet form, with no requirement of laboratory monitoring. The evidence on the use of DOACs in cirrhosis patients is still very limited as they were excluded from the large phase III trials that evaluated the efficacy and safety of DOACs. Currently DOACs are still contraindicated in patients with advanced liver disease accompanied with coagulopathy and clinically relevant bleeding risk. Even though study sizes are quite small, current pharmacokinetic and in vitro studies show encouraging safety data, providing basis for further studies on the use of DOACs in cirrhosis.(32–34)

## Procoagulants in liver disease

Treatment of bleeding complications in patients with cirrhosis traditionally are addressed as any other form of bleeding complication with the transfusion of packet cells, fresh frozen plasma (FFP), and sometimes even platelet concentrates guided by routine diagnostic test. The transfusion of large volumes may, in fact, be counterproductive as it leads to fluid overload and a increases portal venous pressure. Improving hemostasis guided via routine diagnostic potentially leads to the use of an overload of procoagulant products, which can lead to transfusion related complications.(35)

## Aim of this thesis

The main focus of this thesis is gaining insight in potential differences of the hemostatic balance of all etiologies of cirrhosis and presenting the effects of currently available drugs that are indicated for preventing or treating thrombosis and bleeding in patients with liver disease and in patients undergoing hepato-pancreatico-biliary surgery (HPB-surgery).

First this thesis will show an detailed study on the specific hemostatic status of cirrhosis and the different etiologies. Next the in vitro effect of the most commonly used anticoagulant and procoagulant drugs in patients with cirrhosis will be described. Furthermore the potency of one of the direct oral anticoagulant in cirrhosis will be described.

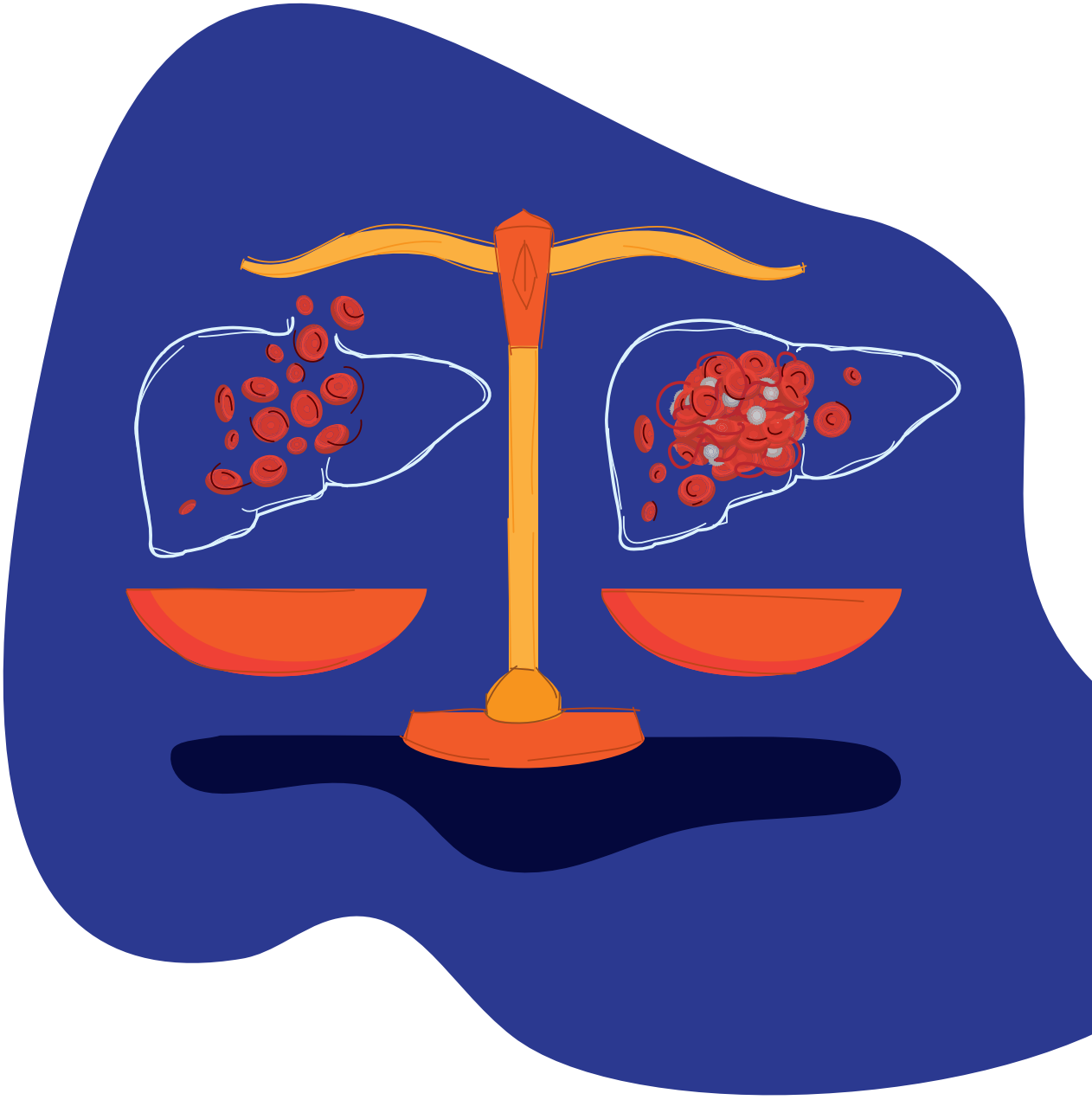
In the second part of this thesis the emphasis is gaining insight in the hemostasis in HPB surgery. At first a detailed overview of the incidence and prevalence of hemostatic complications and the possible therapeutic options will be given, This will be followed by a trial on the in vitro effect of the mainly used pro- and anticoagulant therapies in HPB surgery.

# References


1. Shen J, Sampietro S, Wu J, Tang J, Gupta S, Matzko CN, et al. Coordination of platelet agonist signaling during the hemostatic response in vivo. Blood Adv. 2017;1(27):2767–75.
2. Lisman T, Leebeek FWG, De Groot PG. Haemostatic abnormalities in patients with liver disease. J Hepatol. 2002;37(2):280–7.
3. Roberts HR, Hoffman M, Monroe DM. A cell-based model of thrombin generation. Semin Thromb Hemost. 2006;32(SUPPL. 1):32–8.
4. Hoffman M, Monroe DM. Rethinking the coagulation cascade. Curr Hematol Rep. 2005;4(5):391–6.
5. MacFarlane R. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. Nature. 1964;202:498–9.
6. Tripodi A, Mannucci PM. Abnormalities of hemostasis in chronic liver disease: Reappraisal of their clinical significance and need for clinical and laboratory research. J Hepatol. 2007;46(4):727–33.
7. Lisman T, Leebeek FWG, Mosnier LO, Bouma BN, Meijers JCM, Janssen HLA, et al. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. Gastroenterology. 2001;121(1):131–9.
8. Fukui H, Saito H, Ueno Y, Uto H, Obara K, Sakaida I, et al. Evidence-based clinical practice guidelines for liver cirrhosis 2015. J Gastroenterol. 2016;51(7):629–50.
9. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med. 2011;365(2):147–56.
10. Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease : evidence and clinical consequences. Blood. 2010;116(6):878–85.
11. Lisman T, Bongers TN, Adelmeijer J, Janssen HLA, De Maat MPM, De Groot PG, et al. Elevated levels of von Willebrand factor in cirrhosis support platelet adhesion despite reduced functional capacity. Hepatology. 2006;44(1):53–61.
12. Lisman T, Adelmeijer J, de Groot PG, Janssen HLA, Leebeek FWG. No evidence for an intrinsic platelet defect in patients with liver cirrhosis – studies under flow conditions. J Thromb Haemost. 2006;(4):2070–2.
13. Hollestelle MJ, Geertzen HGM, Straatsburg IH, Gulik TM Van, Mourik JA Van. Factor VIII expression in liver disease. Thromb Haemost. 2004;91(2):267–75.
14. Gatt A, Riddell A, Calvaruso V, Tuddenham EG, Makris M, Burroughs AK. Enhanced thrombin generation in patients with cirrhosis-induced coagulopathy. J Thromb Haemost. 2010;8(9):1994–2000.
15. Lisman T, Bakhtiari K, Pereboom ITA, Hendriks HGD, Meijers JCM, Porte RJ. Normal to increased thrombin generation in patients undergoing liver transplantation despite prolonged conventional coagulation tests. Journal of Hepatology. 2010. p. 355–61.
16. Bos S, Blokzijl H, van den Boom B, Lisman T, Adelmeijer J, Kamphuisen P, et al. Haemostatic Profiles are Similar across All Aetiologies of Cirrhosis. Thromb Haemost. 2019;119(02):246–53.
17. Colucci M, Binetti BM, Branca MG, Clerici C, Morelli A, Semeraro N, et al. Deficiency of Thrombin Activatable Fibrinolysis Inhibitor in Cirrhosis Is Associated With Increased Plasma Fibrinolysis. Hepatology. 2003;38(1):230–7.

18. Lisman T. Decreased Fibrinolytic Capacity in Cirrhosis and Liver Transplantation Outcomes. 2019;25(3):359–61.
19. Lebreton A, Sinegre T, Pereira B, Lamblin G, Duron C, Abergel A. Plasma hypercoagulability in the presence of thrombomodulin but not of activated protein C in patients with cirrhosis. J Gastroenterol Hepatol. 2017 Apr;32(4):916–24.
20. Groeneveld D, Porte RJ, Lisman T. Thrombomodulin-modified thrombin generation testing detects a hypercoagulable state in patients with cirrhosis regardless of the exact experimental conditions. Thromb Res. 2014;134(3):753–6.
21. Tripodi A, Salerno F, Chantarangkul V, Clerici M, Cazzaniga M, Primignani M, et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. Hepatology. 2005 Mar;41(3):553–8.
22. Youngwon N, Kim JE, Lim HS, Han KS, Kim HK. Coagulation proteins influencing global coagulation assays in cirrhosis: Hypercoagulability in cirrhosis assessed by thrombomodulin-induced thrombin generation assay. Biomed Res Int. 2013;2013.
23. Ambrosino P, Tarantino L, Di Minno G, Paternoster M, Graziano V, Petitto M, et al. The risk of venous thromboembolism in patients with cirrhosis. Thromb Haemost. 2016;117(1):139–48.
24. Ageno W, Squizzato A, Togna A, Magistrali F, Mangini M, Fugazzola C, et al. Incidental diagnosis of a deep vein thrombosis in consecutive patients undergoing a computed tomography scan of the abdomen: A retrospective cohort study. J Thromb Haemost. 2012;10(1):158–60.
25. Francoz C, Belghiti J, Vilgrain V, Sommacale D, Paradis V, Condat B, et al. Splanchnic vein thrombosis in candidates for liver transplantation: Usefulness of screening and anticoagulation. Gut. 2005;54(5):691–7.
26. Werner KT, Sando S, Carey EJ, Vargas HE, Byrne TJ, Douglas DD, et al. Portal vein thrombosis in patients with end stage liver disease awaiting liver transplantation: Outcome of anticoagulation. Dig Dis Sci. 2013;58(6):1776–80.
27. Stine JG, Shah PM, Cornella SL, Rudnick SR, Ghabril MS, Stukenborg GJ, et al. Portal vein thrombosis, mortality and hepatic decompensation in patients with cirrhosis: A meta-analysis. World J Hepatol. 2015;7(27):2774–80.
28. Arshad F, Adelmeijer J, Blokzijl H, van den Berg A, Porte R, Lisman T. Abnormal hemostatic function one year after orthotopic liver transplantation can be fully attributed to endothelial cell activation. F1000Research. 2014 Jul 30;3:1–18.
29. Garcia-Tsao G, Bosch J. Management of varices and variceal hemorrhage in cirrhosis. N Engl J Med. 2010;362(9):823.
30. Kearon C, Akl EA, Ornelas J, Blaivas A, Jimenez D, Bounameaux H, et al. Antithrombotic therapy for VTE disease: CHEST guideline and expert panel report. Chest. 2016;149(2):315–52.
31. You JJ, Singer DE, Howard PA, Lane DA, Eckman MH, Fang MC, et al. Antithrombotic therapy for atrial fibrillation: Antithrombotic therapy and prevention of thrombosis, 9th ed: American college of chest physicians evidence-based clinical practice guidelines. Chest. 2012;141(2 SUPPL.):e531S-e575S.
32. Mendell J, Johnson L, Chen S. An open-label, phase 1 study to evaluate the effects of hepatic impairment on edoxaban pharmacokinetics and pharmacodynamics. J Clin Pharmacol. 2015;55(12):1395–405.

33. Potze W, Adelmeijer J, Lisman T. Decreased in vitro anticoagulant potency of Rivaroxaban and Apixaban in plasma from patients with cirrhosis. Hepatology. 2015 Apr;61(4):1435–6.
34. Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Meijers JCM, et al. Differential In Vitro Inhibition of Thrombin Generation by Anticoagulant Drugs in Plasma from Patients with Cirrhosis. PLoS One. 2014 Feb;9(2):e88390.
35. de Boer MT, Christensen MC, Asmussen M, van der Hilst CS, Hendriks HGD, Slooff MJH, et al. The Impact of Intraoperative Transfusion of Platelets and Red Blood Cells on Survival After Liver Transplantation. Anesth Analg. 2008 Jan;106(1):32–44.



# CHAPTER 2.

## Hemostatic profiles are similar across all etiologies of cirrhosis.

Sarah Bos, Bente van den Boom, Pieter Willem Kamphuisen, Jelle Adelmeijer, Hans Blokzijl, Tim Schreuder, Ton Lisman

Thrombosis and Haemostasis 2019 Feb;119(2):246-253.

## Abstract

*Background:* patients with cirrhosis may acquire profound changes in hemostasis. Although hemostatic changes in cirrhosis have been extensively studied, most studies were performed in groups of patients with mixed etiology. As thrombotic events appear more common in some etiologies of disease, notably non-alcoholic steatohepatitis (NASH) and cholestatic disease, we hypothesized that hemostatic changes might be different across etiologies.

*Methods:* we studied 109 patients with cirrhosis (31 cholestatic liver disease, 23 NASH, 37 alcoholic liver disease (ALD), 18 viral hepatitis) and 44 healthy controls. Patients with malignancy were excluded. Routine diagnostic tests of hemostasis, thrombin generation assays, fibrin permeability assays, and a plasma-based fibrinolytic assay were performed.

*Results:* all patients had comparable severity of disease according to their MELD-score(9 [7-11]). Plasma levels of von Willebrand factor were substantially elevated across all etiologies, with ADAMTS13 levels comparable to controls. Thrombin generation capacity was elevated in all etiologies, most profoundly in ALD. Fibrin permeability was decreased in all etiologies, which was accompanied by elevated fibrinogen levels. Clot lysis times were prolonged in NASH and cholestatic disease. Plasma levels of individual proteins were similarly altered in all etiologies.

*Conclusion:* our in-depth hemostatic profiling of primary, secondary, and tertiary hemostasis in a group of patients with CPT A/B cirrhosis showed no large differences between etiologies, and was consistent with a general hypercoagulable profile in patients with mild cirrhosis. These results suggest that patients with cirrhosis have an increased risk of thrombosis, irrespective of their etiology.

## Introduction

Historically, patients with cirrhosis were considered to be prone to bleeding complications due to their acquired coagulopathy. The hemostatic profile in cirrhosis is characterized by decreased levels of most pro-coagulant factors. However, the decrease in plasma levels of procoagulant factors is paralleled by a decrease in plasma levels of most of the natural anticoagulants including antithrombin, protein C and protein S.(1) Similarly, decreased plasma levels of antifibrinolytic proteins are accompanied by decreased plasma levels of plasminogen.(2) Furthermore, the thrombocytopenia of cirrhosis appears to be counteracted by highly elevated levels of the platelet adhesive protein von Willebrand factor (VWF).(3) The net effect of all these changes in the pro- and antihemostatic pathways appears to be a reset of the hemostatic balance.(2) This restored hemostatic balance, however, is fragile and can easily be tipped towards a hypo- or hypercoagulable status.(2) Indeed, both bleeding and thrombotic events are common in cirrhosis, although it remains a challenge to predict which patient is at risk for thrombotic or hemorrhagic complications.

Recent studies have demonstrated that cirrhosis is actually a risk factor for development of venous thromboembolism.(4–8) Laboratory data in support of a maintained hemostatic balance in cirrhosis includes studies showing that thrombin generating capacity of patients with cirrhosis is normal or even enhanced compared to healthy individuals,(6–9) data that show a preserved fibrinolytic balance,(2) and studies showing that a highly elevated VWF levels compensate for cirrhotic thrombocytopenia.(3,10)

Most laboratory studies that assessed different aspects of hemostatic balance in cirrhosis have been performed using patient cohorts with mixed etiologies. However, clinically, there appear to be differences in hemostatic complications between different etiologies of disease. For example, it has been well established that patients with cholestatic disease display increased hypercoagulability by viscoelastic tests,(11–14), and anecdotally, patients with cholestatic disease appear to have decreased blood loss during liver transplantation. It has not been established whether patients with cholestatic disease are at increased risk for thrombotic complications. Two independent laboratory studies have demonstrated little differences between patients with cirrhosis due to NASH, alcohol or viral hepatitis, although specific hypercoagulable features of NASH were identified in one of these studies.(14,15) However, hospitalized patients with cirrhosis due to NASH were at increased risk for venous thrombosis compared to patients with cirrhosis of other etiologies.(16) In line with this clinical evidence for a (relative) hypercoagulable state in patients with NASH-cirrhosis, it has been demonstrated that portal vein thrombosis is more frequent in NASH-cirrhosis compared to cirrhosis of other etiologies.(17)

Given the paucity of clinical and laboratory data on the hemostatic status of cirrhosis of specific etiologies, we performed in depth hemostatic profiling of a large cohort of patients which we stratified according to etiology of disease. Better insight in etiology-specific hemostatic changes may facilitate prevention and/or treatment of bleeding and thrombotic events, and may lead to a more personalized approach to hemostatic management of the cirrhotic patient.

## Patients and methods

### *Patients*

One hundred and nine adult patients with cirrhosis were enrolled from the outpatient hepatology clinic of the University Medical Center Groningen, The Netherlands. In addition, 44 healthy volunteers were included to determine reference values for the various tests performed. Inclusion criteria for the patient group consisted of an age of 18 years or older, and a diagnosis of cirrhosis. The diagnosis of cirrhosis had to be confirmed via fibroscan suggestive for F4 cirrhosis, histology compatible with severe fibrosis (Metavir F4) or via imaging. Historical clinical diagnoses were used to define the etiology. Exclusion criteria were the presence of malignancy, a known hereditary bleeding disorder or current use of antithrombotic drugs. Additional exclusion criteria for the control group included the presence of auto-immune disease or known liver function abnormalities. The study protocol was approved by the local medical ethical committee (METC 2016/400) and informed consent was obtained from each subject before inclusion in the study.

### *Plasma samples*

Plasma samples for analyses were taken by venipuncture and collected into 3.2% citrate tubes. The blood was processed to platelet-poor plasma (PPP) by double centrifugation at 2000 g and 10,000 g respectively for 10 min. Plasma was stored at -80◦C until use. Pooled normal plasma, which was used to calibrate some of the tests, was a generous gift from Dr. J.C. Meijers (Sanquin and Academic Medical Center Amsterdam, Amsterdam, The Netherlands) and consisted of >200 healthy individuals.

### *Thrombin generation assay*

The thrombin generation assay (TGA) was performed in platelet-poor plasma (PPP) with the fluorimetric method described by Hemker, Calibrated Automated Thrombography® (CAT).(18) Coagulation was activated using commercially available reagents containing recombinant tissue factor (TF, final concentration 5 pM), phospholipids (final concentration 4 μM), in the presence of soluble thrombomodulin (TM, the concentration of which is not revealed by the

manufacturer). Reagents were purchased from Thrombinoscope BV, Maastricht, the Netherlands, and thrombin generation experiments were executed following protocols provided by Thrombinoscope. The following parameters were recorded: endogenous thrombin potential (ETP; which represents the total enzymatic work performed by thrombin during the time that it was active), peak, velocity index (slope between the end of lag time and peak thrombin), and lag time (time needed for thrombin concentration to reach 1/6th of the peak concentration).

### *Conventional coagulation assays*

The prothrombin time (PT) and activated partial thrombin time (APTT) were assessed on an automated coagulation analyzer (ACL 300 TOP) with reagents (Recombiplastin 2G for PT and Hemosil SynthaSil for APTT) and protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands).

### *Clot lysis time*

Lysis of a tissue factor-induced clot by exogenous tissue plasminogen activator (tPA) was studied by monitoring changes in turbidity during clot formation and subsequent lysis as described previously.(19) Clot lysis time was determined as the time from the midpoint of the clear to maximum turbid transition, which characterizes clot formation, to the midpoint of the maximum turbid to clear transition, which represents clot lysis.

### *Fibrin structure*

The average pore size of the fibrin clot was determined in permeation studies as described previously.(20,21) The permeability coefficient Ks was calculated following Darcy's Law. Plasma clots were generated as described previously.(22)

Levels of fibrinogen were assessed on an automated coagulation analyzer (ACL 300 TOP). We used QFA thrombin (Hemosil) and testing was performed according to protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands).

### *Plasma markers of primary hemostasis*

Soluble P-selectin (sP-selectin) levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Abingdon, UK). Levels VWF were assessed with in-house ELISA using commercially available polyclonal antibodies against VWF (DAKO, Glostrup, Denmark). Plasma activity of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) was measured using the FRETS-VWF73 assay (Peptanova, Sandhausen, Germany) which is described in detail previously.(23) Plasma samples

were pretreated with bilirubin oxidase (2.5 U/ml, Sigma-Aldrich, Zwijndrecht, The Netherlands) for 30 minutes at 37°C to avoid interference of bilirubin with the FRETS-VWF73 assay.(24) Levels of VWF and ADAMTS13 in pooled normal plasma were set at 100%, and values obtained in test plasmas were expressed as a percentage of pooled normal plasma.

### *Plasma markers of coagulation*

Levels of factor (F) VII, FVIII, prothrombin, antithrombin and protein C were assessed on an automated coagulation analyzer (ACL 300 TOP). We used factor deficient plasma for FII, FVII and FVIII, Hemosil Liquid Antithrombin for AT, and Hemosil protein C for protein C. Testing was performed according to protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands).

### *Plasma markers of fibrinolysis*

Plasminogen activator inhibitor-1 (PAI-1) levels were determined with an ELISA kit from R&D systems (Abingdon, UK). Levels of tPA were measured using an ELISA kit Nodia (Hyphen Biomed; Amsterdam, the Netherlands).

### *Statistical analysis*

Data are expressed as means (with standard deviations (SDs)), medians (with ranges), or numbers (with percentages) as appropriate. Means of two groups were compared by Student's t-test or distributions in the two groups by Mann-Whitney U test as appropriate. Multiple groups were compared using One-way ANOVA (with the Bonferroni posttest) or Kruskal-Wallis H test (with Dunn's posttest) as appropriate. Pearson's correlation coefficient was used to assess correlation between variables. P values of 0.05 or less were considered statistically significant. GraphPad Prism (San Diego, USA) and IBM SPSS Statistics 23 (New York, USA) were used for analyses.

## Results

### *Patient characteristics*

The selected group consisted of 153 patients and 44 healthy controls. We excluded 44 patients due to double diagnosis (n=10), no diagnosis (n=5), or a diagnosis which was too infrequent for further analysis (as for example Wilson's disease, auto-immune hepatitis, secondary biliary cirrhosis) (n= 29). Our analyses were performed in samples from 109 patients and 44 healthy controls. We stratified patients according to 4 etiologies of disease categories: cholestatic disease (PBC and PSC), NASH-related cirrhosis, alcohol-related disease (ALD), and viral hepatitis.

Baseline characteristics are reported in Table 1. In patients, the mean age across all etiologies was similar. BMI was highest in NASH patients, with a median [IQR] of 32.8 [28.3-36.8] kg/m2, and NASH patients had a substantially increased rate of diabetes compared to other etiologies. Model for end-stage liver disease (MELD) score was comparable across all etiologies. The majority of patients in the entire cohort (76%) had Childs Turcotte Pugh (CTP) A, with one single CTP C patient with ALD.

| | PSC/PBC (n =31) | NASH (n = 23) | ALD (n = 37) | Viral (n = 18) | Control (n = 44) |
|---|---|---|---|---|---|
| **Age** | 59 (51-65) [c] | 60 (54-64)[c] | 60 (55-65)[c] | 61 (53-65) [c] | 30 (27-42) |
| **Male** | 13 (42 ) | 12 (52 ) | 26 (70 ) | 16 (89) | 38 (51) |
| **BMI (kg/m2)** | 24.7 (22.1-28.1) | 32.8 (28.3-36.8)[c] | 28.0 (23.8-31.1)[b] | 28.1 (25.6-30.1)[c] | 23.7 (22.3-25.5) |
| **Diabetes** | 5 (16) | 18 (78) | 9 (24) | 3 (17) | - |
| **CVD** | 4 (13) | 10 (44) | 13 (35) | 2 (11) | - |
| **MELD** | 9 (7-12] | 8 (7-12] | 10 (7-13] | 8 (7-9] | - |
| **CTP A** | 20 (65) | 18 (78) | 27 (73) | 18 (100) | - |
| **B** | 11 (35) | 5 22) | 9 (24) | - | - |
| **C** | - | - | 1 (3) | - | - |
| **Ascites, n (%)** | 6 (19) | 8 (35) | 10 (27) | 2 (11) | - |
| **Encephalopathy** | 0 | 4 (17) | 5 (14) | 0 | - |
| **Platelet count (G/L)** | 122 (82-164) | 114 (88-164) | 111 (83-160) | 130 (74-159) | - |

**Table 1. Patient characteristics**

Abbreviations: ALD, alcoholic liver disease, viral comprises hepatitis B and hepatitis C; BMI, body mass index; CTP, Child–Turcotte–Pugh; CVD, cardiovascular disease; MELD, Model for End-Stage Liver Disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.
Note: Data are expressed as number (%), mean (standard deviation) or median (interquartile range).
[a]$p < 0.05$ vs. control, [b]$p < 0.01$ vs. control, [c]$p < 0.001$ vs. control.

## *Plasma levels of proteins involved in primary hemostasis*

VWF levels were significantly higher across all etiologies compared to controls (Figure 1). The highest VWF level was observed in ALD with a median of 347% [237-492]. Despite elevated VWF levels, which have been proposed to support platelet deposition, we found lower levels of the platelet activation marker sP-selectin in patients compared to controls. ADAMTS13, which decreases the reactivity of VWF towards platelets by reducing VWF multimer size, was decreased in patients with cholestatic disease, but significantly increased in NASH. (Figure 1).

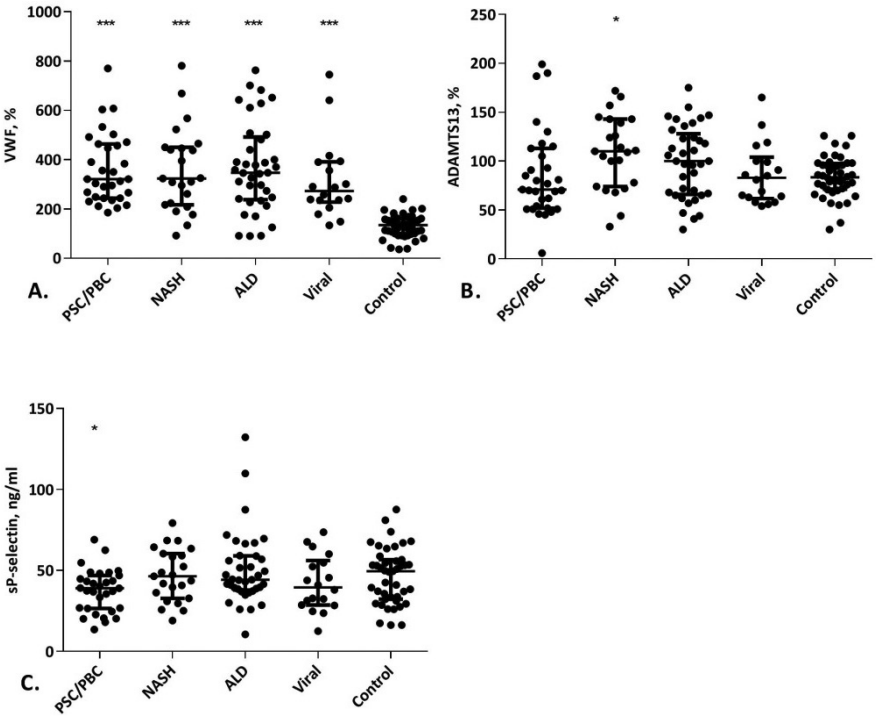



**Figure 1. Plasma levels of proteins involved in primary hemostasis.** Levels of VWF (A), ADAMTS13 (B) and sPselectin (C) were measured in patients with cirrhosis of varying etiologies and healthy controls. VWF, von Willebrand factor, ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13, sP-selectin, soluble P-selectin, PSC, primary sclerosing cholangitis, PBC, primary biliary cholangitis, NASH, non-alcoholic steatohepatitis, ALD, alcoholic liver disease, viral comprises hepatitis B and hepatitis C. Horizontal lines indicate median and IQR. *p<0.05, *** p<0.001 compared to controls.

### *Thrombin generation, PT, APTT*

All the parameters of thrombomodulin-modified TGA were increased across all etiologies compared to controls as depicted in Table 2 and Figure 2. The ETP for the patients varied from 719 [527-859] for ALD to 670 [381-786] for PSC/PBC, compared to 420 [305-581] in controls. The ETP variation between etiologies remained present when only patients with CTP A were analyzed. The PT was significantly higher in all etiologies compared to controls, with the highest levels in ALD (Table 2). The APTT was significantly increased in patients with cholestatic disease, but normal in NASH and alcohol-related cirrhosis and slightly elevated in viral cirrhosis.

| | PSC/PBC | NASH | ALD | Viral | Control |
|---|---|---|---|---|---|
| **TGA lagtime (min)** | 1.94 (1.3)[a] | 1.71 (0.4) | 1.67 (0.4) | 1.91 (0.4) | 1.53 (0.2) |
| **TGA ETP (nM IIa * min)** | 670 (381-786)[a] | 683 (528-940)[c] | 753 (609-944)[c] | 719 (527-859)[b] | 420 (305-581) |
| **TGA Peak (nM IIa)** | 160 (99-195) | 176 126-204)[b] | 177 (160-196)[c] | 156 (115-202) | 116 (88-164) |
| **TGA Velocity (nM/min)** | 88 (48-106) | 94 (69-120)[b] | 96 (82-113)[c] | 79 50-107) | 64 (46-96) |
| **PT (sec)** | 12.8 (1.4)[c] | 13.1 (1.8)[c] | 13.8 (2.3)[c] | 13.3 (2.0)[c] | 11.2 (0.7) |
| **APTT (sec)** | 37.5 (3.5)[a] | 34.1 (3.4) | 35.4 (4.3) | 36.8 (3.9) | 35.1 (2.9) |

**Table 2. Global coagulation tests**

Abbreviations: ALD, alcoholic liver disease, viral comprises hepatitis B and hepatitis C; aPTT, activated partial thromboplastin time; ETP, endogenous thrombin potential; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; PT, pro-thrombin time; TGA, thrombin generation assay.

Note: Data are expressed as mean (standard deviation), or median (interquartile range).

[a]$p < 0.05$ vs. control, [b]$p < 0.01$ vs. Control, [c]$p < 0.001$ vs. control.

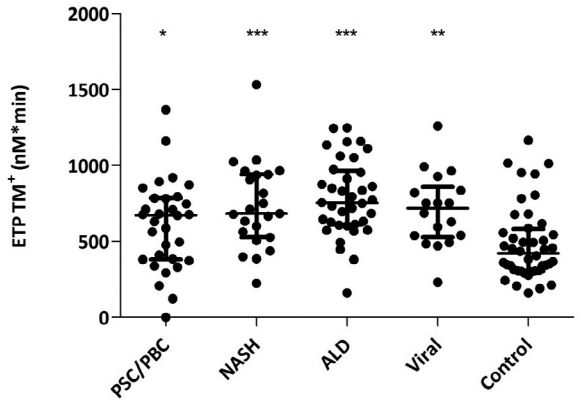



**Figure 2. Thrombin generation assay (TGA) in the presence of thrombomodulin (TM+).**

Thrombomodulin-modified thrombin generation was measured in patients with cirrhosis of varying etiologies and healthy controls. ETP, endogenous thrombin potential, PSC, primary sclerosing cholangitis, PBC, primary biliary cholangitis, NASH, non-alcoholic steatohepatitis, ALD, alcoholic liver disease, viral comprises hepatitis B and hepatitis C. Horizontal lines indicate median and IQR. *p<0.05, ** p<0.01, *** p<0.001 compared to controls.

### *Plasma levels of proteins involved in coagulation*

Plasma levels of both FII and FVII were significantly lower in all etiologies than the levels in controls, with the lowest levels in ALD, 59% [35-85] and 56% [35-85] for FII and FVII, respectively. In contrast, plasma levels of FVIII were significantly higher in all the etiologies with the highest levels in cholestatic disease (146% [115-174]). Plasma levels of the anticoagulant proteins AT and PC were significantly lower in all the etiologies of cirrhosis compared to controls, with no clear differences between etiologies (Table 3).

| | PSC/PBC | NASH | ALD | Viral | Control |
|---|---|---|---|---|---|
| **FII (%)** | 74 (59-89)[c] | 69 (60-89)[c] | 59 (35-85)[c] | 72 (57-84)[c] | 93 (86-104) |
| **FVII (%)** | 64 (48-86)[c] | 64 (42-88)[a] | 56 (35-85)[c] | 59 (49-84)[c] | 94 (75-113) |
| **FVIII (%)** | 146 (115-174)[c] | 131 (112-155)[c] | 129 (95-151)[c] | 111 (98-131)[c] | 87 (70-103) |
| **AT (%)** | 85 (72-107)[c] | 72 (58-91)[c] | 71 (45-94)[c] | 76 (53-90)[c] | 108 (101-115) |
| **PC (%)** | 77 (52-89)[c] | 79 (57-98)[c] | 79 (40-98)[c] | 84 (59-100)[c] | 112 (101-128) |

**Table 3. Plasma levels of proteins involved in coagulation**

Abbreviations: ALD, alcoholic liver disease, viral comprises hepatitis B and hepatitis C; AT, anti-thrombin; F–, factor–; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cholangitis; PC protein C; PSC, primary sclerosing cholangitis.

Note: Data are expressed as mean (standard deviation), or median (interquartile range).

[a]$p < 0.05$ vs. control, [b]$p < 0.01$ vs. Control, [c]$p < 0.001$ vs. control.

### *Fibrin structure*

The clot permeability was significantly lower in cirrhosis patients compared to controls in all etiologies (fig 3). Across etiologies the median values were comparable. Fibrinogen (Fg) levels were higher in patients compared to controls with no clear differences between etiologies. In all patients combined, fibrinogen levels were correlated with clot permeability ($r=-0.35$, $p<0.01$).

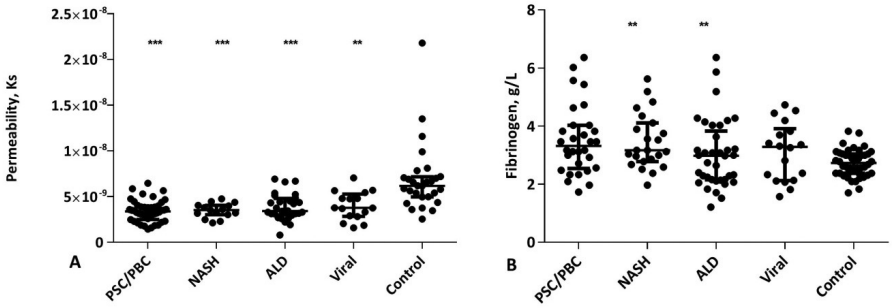



**Figure 3. Fibrin parameters.** The permeability coefficient (Ks, calculated following Darcy's Law) (A) and fibrinogen level (B) was measured in plasma of patients with cirrhosis of varying etiologies and healthy controls. PSC, primary sclerosing cholangitis, PBC, primary biliary cholangitis, NASH, non-alcoholic steatohepatitis, ALD, alcoholic liver disease, viral comprises hepatitis B and hepatitis C. Horizontal lines indicate median and IQR. ** p<0.01, *** p<0.001 compared to controls.

### *Fibrinolysis*

Clot lysis time was higher in patients with cholestatic disease, NASH and ALD compared to controls and only reached significance in the first two. In patients with cirrhosis due to viral disease the clot lysis time was similar compared to controls (fig 4). Plasma levels of PAI-1, an important determinant of clot lysis time, were significantly elevated in cholestatic disease and NASH, but not in alcohol- and viral-related cirrhosis. Plasma levels of tPA were significantly higher in all patient groups compared to controls (Table 4).

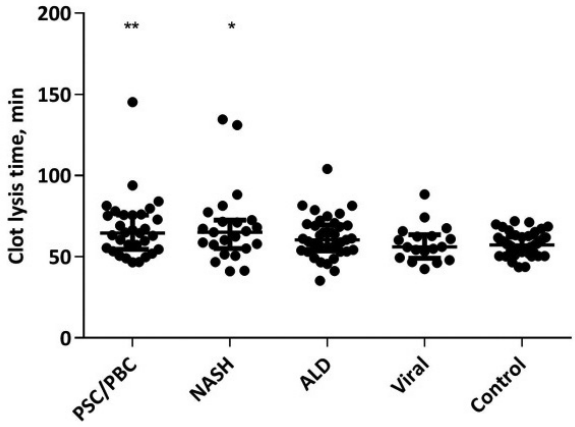



**Figure 4. Plasma fibrinolytic potential**. Clot lysis times were measured in plasma of patients with cirrhosis of varying etiologies and healthy controls. PSC, primary sclerosing cholangitis, PBC, primary biliary cholangitis, NASH, non-alcoholic steatohepatitis, ALD, alcoholic liver disease, viral comprises hepatitis B and hepatitis C. Horizontal lines indicate median and IQR. *p<0.05, ** p<0.01 compared to controls.

| | PSC/PBC | NASH | ALD | Viral | Controls |
|---|---|---|---|---|---|
| **PAI-1 (ng/ml)** | 3.5 (1.7-6.5)[b] | 2.6 (2.0-9.9)[b] | 1.6 (0.9-3.2) | 1.4 (0.5-1.8) | 1.8 (1.1-2.7) |
| **tPA (ng/ml)** | 13.4 (8.5-19.7)[c] | 14.1 (10.3-25.2)[c] | 17.1 (10.6-23.2)[c] | 11.4 (7.2-20.5)[c] | 2.8 (1.5-4.2) |

**Table 4. Plasma levels of proteins involved in fibrinolysis**

Abbreviations: ALD, alcoholic liver disease, viral comprises hepatitis B and hepatitis C; NASH, non-alcoholic steatohepatitis; PAI-1, plasminogen activator inhibitor-1; PBC, primary biliary cholangitis; tPA, tissue plasminogen activator.

Note: Data are expressed as median (interquartile range).

[a]$p < 0.05$ vs. control, [b]$p < 0.01$ vs. Control, [c]$p < 0.001$ vs. control.

## Discussion

We performed in-depth hemostatic profiling of primary hemostasis, secondary hemostasis and fibrinolysis and found that in general patients with cirrhosis have a hypercoagulable profile with no overt differences between etiologies of cirrhosis. Specifically, high levels of the platelet adhesive protein VWF, hypercoagulability by thrombomodulin-modified TGA, a prothrombotic fibrin structure, and hypofibrinolysis in our cohort of patients with predominantly CTP A/B cirrhosis were found. These results are in line with previous studies(6–8), with the exception of the hypofibrinolytic state. In summary, our results suggest that patients with CTP A/B cirrhosis, regardless of etiology, have a prohemostatic state that increases the risk for thrombotic complications, reinforcing the recent change in the historical dogma that cirrhosis is an acquired bleeding disorder. Indeed, it has now been firmly established that cirrhosis increases the risk for thrombotic events, notably venous thrombosis and portal vein thrombosis.(5,25,26)

Within our primary hemostasis analyses we found increased VWF levels in all etiologies, but slightly non-significantly decreased ADAMTS13 levels only in patients with cholestatic disease and viral cirrhosis. Although ADAMTS13 is synthesized in the liver,(28) and therefore levels are expected to decrease similar to levels of other hemostatic factors (e.g. those shown in Table 3), ADAMTS13 is synthesized also in stellate cells,(27,28) which may explain the mainly preserved ADAMTS13 levels in our patients. Although multiple studies have demonstrated decreased plasma levels of ADAMTS13 in cirrhosis, previous studies from our group found ADAMTS13 to be relatively preserved,(3,15) which is in line with our current data. The preserved levels of ADAMTS13 might also be related to the disease severity of our study population, which in mainly mild to moderate. Although the VWF/ADAMTS13 unbalance is potentially thrombogenic, we did not find evidence for ongoing platelet activation as evidenced by a lack of increase in the platelet activation marker P-selectin, which is also in line with previously published work.(15)

For secondary hemostasis testing our results of thrombin generation adds to growing literature showing that patients with cirrhosis have enhanced thrombin generating capacity when tested with assays that take plasma levels of many pro- and anticoagulant drivers into account (i.e., thrombomodulin-modified thrombin generation testing).(6,29–31) There appear to be subtle differences in thrombin generation capacity between etiologies of cirrhosis that are not explained by differences in the levels of pro- and anticoagulant proteins that we have measured. It may be that plasma factors other than the known pro- or anticoagulant proteins are responsible for these differences.

In contrast to the seminal paper by Tripodi et al. published in 2005,(7) who reported normal thrombin generation in patients with cirrhosis, it appears firmly established that patients with CTP A to critically ill patients are in fact characterized by enhanced thrombin generating capacity. These results stress that patients are not 'auto-anticoagulated' as suggested by their prothrombin time, and should encourage studies on optimal treatment modalities for prevention and treatment of thrombotic events in patients with cirrhosis.(32)

Interestingly, although the PT was prolonged in all etiologies, the aPTT was only prolonged in cholestatic disease and in viral hepatitis. These differences are not readily explained by plasma levels of individual coagulation factors, which were similarly decreased across etiologies. Even more, the cholestatic group had the highest levels of FVIII, which is an important determinant of the APTT, but paradoxically had the highest prolongation of the test. The prolonged APTT in the cholestatic group may therefore be related to the presence of an inhibitor, which may be related to the autoimmune component of cholestatic disease. Lupus anticoagulant activity, which is known to prolong the APTT, but is paradoxically also significantly associated with venous thrombosis(33,34), has been reported with high frequency in patients with cirrhosis.(33,34) Whether there is a difference between etiologies is not reported. Future studies should assess the role of such antibodies in prolongation of the APTT, and with thrombotic events in patients with cirrhosis.

Our analyses for fibrinolysis consisted of clot lysis time and clot density measurement together with fibrinogen levels. The prothrombotic nature of our in vitro generated fibrin clots is also in line with previous work. However, in the present cohort of patients with mild disease, plasma levels of fibrinogen were elevated compared to controls. Therefore, the elevated fibrinogen levels per se, rather than intrinsic changes in the fibrinogen molecule as we have previously reported, could explain the increase in fibrin clot thrombogenicity.

There is an ongoing debate on the fibrinolytic status of patients with cirrhosis, with most studies describing either hyper- or normofibrinolysis. In the present study, using a plasma-based assays that has been extensively validated,(35) we found a hypofibrinolytic state, particularly in cholestatic disease and also in NASH cirrhosis. These results suggest, that at least in the population of CTP A/B cirrhosis, decreased fibrinolysis could contribute to thrombotic events, which is somewhat counterintuitive given the concept of hyperfibrinolysis in cirrhosis that has already been proposed in 1914.(36)

It may be that hyperfibrinolysis only occurs in patients with more advanced disease, but it will be of considerable interest to assess whether there are differences between etiologies of disease in a population with advanced disease. Our current findings, however, are in line with studies finding no evidence of hyperfibrinolysis by thromboelastography in a large cohort of patients with well compensated cirrhosis.(14)

Despite the comprehensive analysis, this study has some limitations. Individual patient groups were still of moderate size and mainly consisted of patients with mild cirrhosis, which limits the possibility to draw firm conclusions. Nevertheless, many of the hemostatic changes seen in the present cohort, were also present in a cohort of critically ill patients we have recently analyzed.(37)

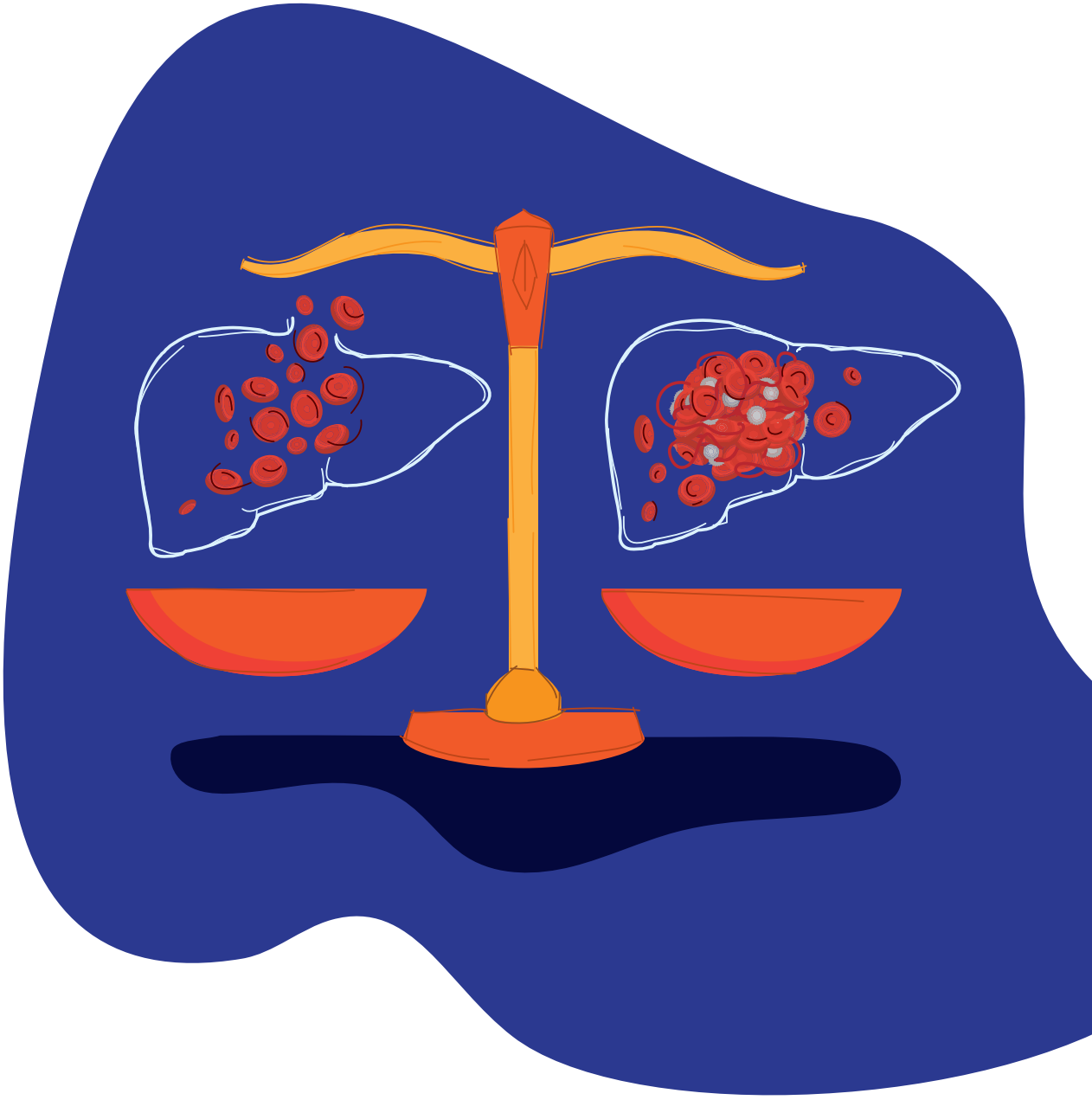
Our in-depth hemostatic profiling of primary, secondary, and tertiary hemostasis in patients with CTP A/B cirrhosis showed a hypercoagulable profile without large differences between etiologies. This reinforces the need for studies on anticoagulant therapy for prevention and treatment of thrombotic events. Our data do not provide an explanation for the difference in incidence of thrombotic events between etiologies, and future studies are required to address this issue.

## References


1. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med. 2011;365(2):147-156.
2. Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease : evidence and clinical consequences. Blood. 2010;116(6):878-885.
3. Lisman T, Bongers TN, Adelmeijer J, et al. Elevated levels of von Willebrand factor in cirrhosis support platelet adhesion despite reduced functional capacity. Hepatology. 2006;44(1):53-61.
4. Hernández Conde M, Llop Herrera E, de la Revilla Negro J, et al. Prevalence and outcome of portal thrombosis in a cohort of cirrhotic patients undergoing liver transplantation. Arthritis Care Res. 2016;108(11):716-720.
5. Aldawood A, Arabi Y, Aljumah A, et al. The incidence of venous thromboembolism and practice of deep venous thrombosis prophylaxis in hospitalized cirrhotic patients. Thromb J. 2011;9(1):1.
6. Groeneveld D, Porte RJ, Lisman T. Thrombomodulin-modified thrombin generation testing detects a hypercoagulable state in patients with cirrhosis regardless of the exact experimental conditions. Thromb Res. 2014;134(3):753-756.
7. Tripodi A, Salerno F, Chantarangkul V, et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. Hepatology. 2005;41(3):553-558.
8. Gatt A, Riddell A, Calvaruso V, Tuddenham EG, Makris M, Burroughs AK. Enhanced thrombin generation in patients with cirrhosis-induced coagulopathy. J Thromb Haemost. 2010;8(9):1994-2000.
9. Kleinegris MC, Habets CAM, Van De Sande AJP, et al. Liver cirrhosis is associated with hypercoagulability, decreased clot strength and normal fibrinolysis. J Thromb Haemost. 2013;11(May):49-50.
10. Vinholt PJ, Alnor AB, Nybo M, Hvas A-M. The primary hemostasis is more preserved in thrombocytopenic patients with liver cirrhosis than cancer. Blood Coagul Fibrinolysis. 2018;(29):307/313.
11. Krzanicki D, Sugavanam A, Mallett S. Intraoperative hypercoagulability during liver transplantation as demonstrated by thromboelastography. Liver Transplant. 2013;19(8):852-861.
12. Pihusch M, Hiller E, Beuers U, Pihusch R, Rank A, Go P. Platelet function rather than plasmatic coagulation explains hypercoagulable state in cholestatic liver disease. 2002;37:548-555.
13. Ben-Ari Z, Panagou M, Patch D, et al. Hypercoagulability in patients with primary biliary cirrhosis and primary sclerosing cholangitis evaluated by thrombelastography. J Hepatol. 1997;26(3):554-559.
14. Hugenholtz GCG, Lisman T, Stravitz RT. Thromboelastography does not predict outcome in different etiologies of cirrhosis. Res Pract Thromb Haemost. 2017;1(2):275-285.
15. Potze W, Siddiqui MS, Boyett SL, et al. Preserved hemostatic status in patients with non-alcoholic fatty liver disease. J Hepatol. 2016;65(5):980-987.

16. Stine JG, Niccum BA, Zimmet AN, et al. Increased risk of venous thromboembolism in hospitalized patients with cirrhosis due to non-alcoholic steatohepatitis. Clin Transl Gastroenterol. 2018;9(3):140.
17. Stine JG, Argo CK, Pelletier SJ, Maluf DG, Caldwell SH, Northup PG. Advanced non-alcoholic steatohepatitis cirrhosis: A high-risk population for pre-liver transplant portal vein thrombosis. World J Hepatol. 2017;9(3):139-146.
18. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiol Haemost Thromb. 2003;33(1):4-15.
19. Lisman T, Groot PG De, Meijers JCM, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. Blood. 2005;105(3):1102-1105.
20. Ariëns R a, Philippou H, Nagaswami C, Weisel JW, Lane D a, Grant PJ. The factor XIII V34L polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. Blood. 2000;96(3):988-995.
21. Lim BCB, Ariëns RAS, Carter AM, Weisel JW, Grant PJ. Genetic regulation of fibrin structure and function: Complex gene-environment interactions may modulate vascular risk. Lancet. 2003;361(9367):1424-1431.
22. Groeneveld DJ, Adelmeijer J, Hugenholtz GCG, Ariëns RAS, Porte RJ, Lisman T. Ex vivo addition of fibrinogen concentrate improves the fibrin network structure in plasma samples taken during liver transplantation. J Thromb Haemost. 2015;13(12):2192-2201.
23. Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. Br J Haematol. 2005;129(1):93-100.
24. Eckmann CM, de Laaf RTM, van Keulen JM, van Mourik JA, de Laat B. Bilirubin oxidase as a solution for the interference of hyperbilirubinemia with ADAMTS-13 activity measurement by FRETS-VWF73 assay. J Thromb Haemost. 2007;5(6):1330-1331.
25. Kuboki S, Shimizu H, Ohtsuka M, et al. Incidence, risk factors, and management options for portal vein thrombosis after hepatectomy: a 14-year, single-center experience. Am J Surg. 2015;210(5):878-885.e2.
26. Emuakhagbon V, Philips P, Agopian V, Kaldas FM, Jones CM. Incidence and risk factors for deep venous thrombosis and pulmonary embolus after liver transplantation. Am J Surg. 2016;211(4):768-771.
27. Zhou W, Inada M, Lee T-P, et al. ADAMTS13 is expressed in hepatic stellate cells. Lab Invest. 2005;85(6):780-788.
28. Uemura M, Tatsumi K, Matsumoto M, et al. Localization of ADAMTS13 to the stellate cells of human liver. Blood. 2005;106(3):922-924.
29. Russo FP, Zanetto A, Campello E, et al. Reversal of hypercoagulability in patients with HCV-related cirrhosis after treatment with direct-acting antivirals. Liver Int. 2018:1-9.
30. Sinegre T, Duron C, Lecompte T, et al. Increased factor VIII plays a significant role in plasma hypercoagulability phenotype of patients with cirrhosis. J Thromb Haemost. 2018;16:1-9.
31. Lebreton A, Sinegre T, Pereira B, Lamblin G, Duron C, Abergel A. Plasma hypercoagulability in the presence of thrombomodulin but not of activated protein C in patients with cirrhosis. J Gastroenterol Hepatol. 2017;32(4):916-924.
32. Lisman T, Kamphuisen PW, Northup PG, Porte RJ. Established and new-generation antithrombotic drugs in patients with cirrhosis - Possibilities and caveats. J Hepatol. 2013;59(2):358-366.

33. Violi F, Ferro D, Quintarelli C, et al. Dilute aPTT prolongation by antiphospholipid antibodies in patients with liver cirrhosis. Thromb Haemost. 1990;63(2):183-186.
34. Quintarelli C, Ferro D, Valesini G, Basili S, Tassone G, Violi F. Prevalence of lupus anticoagulant in patients with cirrhosis: relationship with beta-2-glycoprotein I plasma levels. J Hepatol. 1994;21(6):1086-1091.
35. Lisman T, Ariëns RAS. Alterations in Fibrin Structure in Patients with Liver Diseases. Semin Thromb Hemost. 2016;42(4):389-396.
36. Goodpasture EW. Fibrinolysis in chronic hepatic insufficiency. Johns Hopkins Hosp Bull. 1914;25:330-336.
37. Lisman T, Kleiss SF, Patel V, et al. In vitro efficacy of pro- and anticoagulant strategies in compensated and acutely ill patients with cirrhosis. Liver Int. 2018:1-9.



# CHAPTER 3.

## Changes of in vitro potency of anticoagulant drugs are similar between patients with cirrhosis due to alcohol or to non-alcoholic fatty liver disease

Sarah Bos, Wilma Potze, Mohammed Siddiqui, Sherry Boyett, Jelle Adelmeijer, Kalyani Daita, Ton Lisman T, Arun Sanyal

A shorter version of this chapter has been published as Letter: Thrombosis Research. 2017 Feb;150:41-43.

## Abstract

*Background:* cirrhosis is complicated by bleeding and thrombotic events. Using vitamin K antagonists (VKA) and low-molecular weight heparin (LMWH) in cirrhotic patients has major drawbacks. Direct oral anticoagulants (DOAC) might be promising for this specific group of patients. Little evidence exists on hemostatic effect in cirrhosis let alone on the different etiologies. The in vitro effect of DOAC will be compared to traditional anticoagulants in blood samples of patients with non-alcoholic steatohepatitis (NASH) versus alcoholic steatohepatitis (ASH) cirrhosis with blood samples from lean and obese volunteers as controls.

*Methods:* twenty-two blood samples of patients with NASH-cirrhosis and 15 samples of patients with ASH-cirrhosis were compared. Twenty samples of lean and obese subjects were included as well. The potency of LMWH, Dabigatran and Apixaban were examined by performing thrombin generation tests.

*Results:* similar results were seen between NASH and ASH-cirrhosis for LMWH and dabigatran. Apixaban had a significantly different endogenous thrombin potential (ETP) decrease compared between the samples of NASH and ASH cirrhosis. Similar results for apixaban are reported for the obese group when compared with NASH cirrhosis. However for dabigatran and LMWH there was a great variation in values of peak time and velocity between NASH-cirrhosis and the controls.

*Conclusions:* the in vitro effect of the different anticoagulant therapy in blood samples from patient with NASH and ASH cirrhosis shows no difference.

## Introduction

Cirrhosis may be associated with major changes in the hemostatic system. Although historically cirrhosis has been considered as the prototype of acquired bleeding disorders, it has become clear that the hemostatic balance in patients with cirrhosis is actually well preserved due to the concomitant decline in pro- and antihemostatic pathways.(1,2) The hemostatic balance in cirrhosis, however, is much more fragile compared to that of individuals with intact liver function. This likely explains why patients with cirrhosis may experience both bleeding and thrombotic complications.(1)



Prevention or treatment of thrombotic complications in patients with cirrhosis is complicated by multiple factors involving the coagulopathy of cirrhosis. For example, vitamin K antagonists are difficult to dose, as patients with cirrhosis frequently have an elevated international normalized ration (INR) at baseline. As the INR is used to dose vitamin K antagonists, the desired target range in these particular patients is unclear. We and others have recently demonstrated that the potency of low molecular weight heparin (LMWH) is enhanced in cirrhosis.(3,4) These findings may imply that dose-adjustments may be required. Unfortunately, monitoring of LMWH in cirrhosis is complicated as the anti-Xa assay underestimates LMWH mass in cirrhotic plasma.(5)

We also recently demonstrated that direct oral anticoagulants (DOACs) have an altered potency in cirrhotic plasma.(4,6) Confusingly, direct Xa inhibitors appear to have a decreased, and direct thrombin inhibitors an increased potency in cirrhosis. It is unknown whether the changes in potency of commonly used anticoagulant drugs differ according to the etiology of disease, as previous studies were performed with groups of patients of varying etiology.(3,4,6) One group of patients that appear to have an increased risk of thrombotic disease are patients with cirrhosis related to non-alcoholic fatty liver disease (NAFLD).(7) Hemostatic status in patients with NAFLD-related cirrhosis (i.e., cirrhosis as a result of non-alcoholic steatohepatitis [NASH]) may be governed by a combination of the hemostatic changes induced by cirrhosis and the hemostatic changes associated with obesity and the metabolic syndrome.(8) Given these findings, it is conceivable that the potency of anticoagulants is different in NASH-associated cirrhosis compared to cirrhosis of other etiologies. Insight in the effect of commonly use anticoagulant drugs in this growing patient population is of utmost importance for a more rational approach to prevention or treatment of thrombotic disease in this difficult-to-anticoagulate patient population.(9,10)Here, we will study the in vitro potency of three classes of anticoagulant drugs, previously tested by us in a group of patients with mixed cirrhosis (LMWH, the Xa inhibitor apixaban, and the thrombin inhibitor dabigatran). We will compare anticoagulant potency of these

drugs between healthy lean controls, healthy obese controls, patients with NASH-related cirrhosis, and patients with cirrhosis related to alcohol abuse.

## Methods

### *Patients*

Twenty-two patients with known and well-defined NASH-related cirrhosis were included. These patients were under routine control at the department of internal medicine of the Virginia Commonwealth University in Richmond, USA. The diagnosis of NAFLD-related cirrhosis was made through liver biopsy, which was graded according to the NASH Clinical Research Network (NASH CRN) scoring system, and the NAFLD activity score (NAS) was based on the unweighted sum of steatosis, lobular inflammation, and hepatocellular ballooning scores.(11) Exclusion criteria consisted of a documented history of congenital coagulation disorders, presence of active infection (<2 weeks), use of anticoagulant drugs in the past 10 days, pregnancy, human immunodeficiency virus (HIV) positivity and recent (7 days) transfusion with blood products. The results of the patients with NASH-related cirrhosis were compared to the results of fifteen patients with cirrhosis related to alcohol abuse (alcoholic steatohepatitis [ASH]-related cirrhosis), and to two control groups, which consist of lean (BMI<25kg/m2; n=20) and overweight (BMI > 25kg/m2; n=20) subjects with no evidence of liver disease or hepatic steatosis. The local medical ethical committee approved this study protocol and written informed consent was obtained from each subject before inclusion.

### *Thrombin generation testing*

Potency of three anticoagulants was tested in patients and controls by thrombin generation testing. The thrombin generation test was performed using platelet-poor plasma (PPP) with the fluorimetric method.(12) To activate coagulation, a commercially available reagent containing recombinant tissue factor (TF), phospholipids and soluble thrombomodulin was used (Thrombinoscope BV, Maastricht, The Netherlands). All procedures were according to the protocol suggested by Thrombinoscope B.V.

The potency of the anticoagulant drugs was expressed as the percentual change in various parameters of the thrombin generation curve (endogenous thrombin potential (ETP), peak, velocity index, and lag time) after the addition of anticoagulants. These percentages were compared between groups.

The following anticoagulants were tested

- LMWH (Clexane, Sanofi-Aventis BV, Gouda, the Netherlands), 0.2 U/ml
- Dabigatran (Alsachim, Illkirch Graffenstaden, France), 300 ng/ml
- Apixaban (Alsachim, Illkirch Graffenstaden, France), 25 ng/ml

### *Statistics*

Data are expressed as mean +/- standard deviation (SD) or as median with interquartile range (IQR) as appropriate. Frequencies and percentages are given for categorical variables. Groups were compared by One-way ANOVA (with the Bonferroni posttest) or Kruskal-Wallis H test (with Dunn's posttest) as appropriate. For statistical analyses, SPSS software was used (Statistical Package for the Social Sciences, version 23.0, Inc. Chicago, Illinois, USA). P values were considered to indicate a significant difference if $p < 0.05$.



## Results

### *Patient characteristics*

The baseline characteristics of the study population are reported in table 1. Twenty-two patients with biopsy-proven NASH-related cirrhosis, fifteen patients with ASH-related cirrhosis, twenty lean subjects (BMI<25kg/m2), and twenty overweight subjects (BMI>25kg/m2) were included. Patients with cirrhosis had an increased prothrombin time (PT) and activated partial thromboplastin time (APTT) compared to controls. Mean model of end-stage liver disease (MELD) scores were slightly different between patients with NASH- and ASH-related cirrhosis.

**Table 1. Baseline characteristics of the study population**

| Characteristics | Lean controls | Obese controls | NASH cirrhosis | ASH cirrhosis |
|---|---|---|---|---|
| | **n=20** | **n=20** | **n= 22** | **n=15** |
| **Age (yrs)** | 45.0 (13.3) | 40.0 (10.9) | 61.4 (10.1) | 53.9 (7.8) |
| **Gender (male)** | 6 (30) | 4 (20) | 9 (41) | 12 (80) |
| **BMI** | 22.9 (1.1) | 30.5 (4.1) | 35.2 (5.4) | 27.2 (5.7) |
| **PT** | 10.6 (9.4-12.1) | 10.6 (9.1-11.4) | 11.9 (10.2-17.2) | 12.7 (9.8-16.9) |
| **APTT** | 28.9 (25.6-36.7) | 30.1 (25.3-51.8) | 32.6 (27.4-39.0) | 34.5 (28.0-38.4) |
| **MELD** | | | 9.1 (3.0) | 11.3 (3.4) |

Abbreviations: BMI: Body mass index, PT: Prothrombine time, APTT: Activated partial thromboplastine time, MELD: model for end-stage liver disease. Data are expressed as numbers (%) mean (SD) or median (range).

Dabigatran addition decreased thrombin generation in both control and patient plasma, but the extend of inhibition was N2-fold greater in patient plasma, as expressed by the ETP (Fig. 1). Inhibition of thrombin generation by dabigatran appeared less in obese control subjects com- pared to lean controls. In addition, dabigatran prolonged the lag-time similarly in all patient groups (table 2). Furthermore, dabigatran inhibited peak thrombin generation and the velocity index in patients, whereas these were not inhibited in controls. The inhibition of thrombin generation by dabigatran appeared higher in patients with ASH-related cirrhosis compared to patients with NASH-related cirrhosis, which may be related to the higher MELD score in the ASH- cirrhosis group.

Addition of apixaban had virtually identical effects on thrombin generation parameters in all groups. Apixaban decreased ETP (Fig. 1), peak thrombin generation, and velocity index in all groups to a similar extent (lean peak, 28.3% (22–34); obese peak, 28.3% (24–30); NASH peak 28.7% (22–35); ASH peak, 23.4% (21–29); lean velocity, 26.6% (19–35); obese velocity, 25.9% (19–32); NASH velocity, 28.5% (22– 36); ASH velocity, 21.7% (16–33))whereas the lag-time was not affected (lean 0.0% (0–0); obese, 6.7% (0–12); NASH, 8.5% (7–15); ASH, 9.2% (0– 17)).

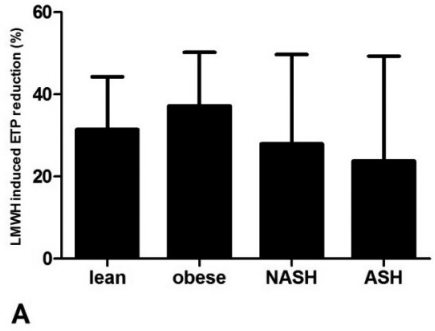
LMWH decreased ETP (Fig. 1), peak, velocity index, but not lag time in all groups (lean peak, 28.8% (23–33); obese peak, 36.4% (24– 42); NASH peak, 26% (17–32); ASH peak, 17.7% (13–25); lean velocity, 26.1 (21–26); obese velocity, 37.1% (24–47); NASH velocity, 27.6% (15–35); ASH velocity, 17.3% (11–28); lean lag-time, 0% (0–0); obese lag-time, 0% (0–0); NASH lag-time, 0% (–7.1–0); ASH lag-time, 0% (0–0)). The capacity of LMWH to inhibit thrombin generation appeared increased in obese compared to lean controls. In contrast, compared to controls, LMWH was less effective in reducing thrombin generation in patients with cirrhosis, in particular those with ASH-related cirrhosis.

**Table 2. In vitro inhibition of thrombin generation in plasma after addition of several anticoagulant drugs**

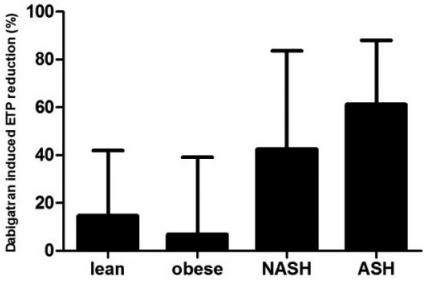
| | **Dabigatran** | | | |
|---|---|---|---|---|
| | **ETP** | **Peak** | **Lag-time** | **Velindex** |
| Lean | 14.7 (-19.1-27.3) | -1.0 (-35.8-18.1) + | 196.6 (131.3-209.8) | -30.4 (-60.9- 5.5) |
| Obese | 6.9 (-32.4-12.7) + | -6.7 (-61.7- -3.0) + | 161.9 (137.7.-218.8) | -40.7 (-115.1 - -14.2) + |
| NASH | 42.2 (-1.1-65.9) | 40.7 (-14.4-72.5) * | 206.6 (181.3-271.2) | 32.1 (-35.0-70.7) |
| ASH | 61.2 (37.5-75.7) * | 62.9 (36.3-82.9) * | 195.1 (139.5-233.5) | 56.4 (24.4-82.8) * |
| | **Apixaban** | | | |
| | **ETP** | **Peak** | **Lag-time** | **Velindex** |
| Lean | 30.7 (25.2-36.6) | 28.3 (22.0-33.5) | 0.0 (0.0-0.0) + | 26.6 (19.0-35.2) |
| Obese | 32.3 (28.5-34.1) | 28.3 (24.2-30.1) | 6.7 (0.0-12.4) | 25.9 (18.6-32.1) |
| NASH | 31.0 (26.0-36.0) | 28.7 (22.3-34.7) | 8.5 (6.6-15.1) * | 28.5 (21.9-36.1) |
| ASH | 28.1 (22.9-31.1) | 23.4 (21.3-28.8) | 9.2 (0.0-16.5) * | 21.7 (15.7-32.7) |
| | **LMWH** | | | |
| | **ETP** | **Peak** | **Lag-time** | **Velindex** |
| Lean | 31.4 (24.1-33.9) | 28.8 (23.4-33.1) | 0.0 (0.0-0.0) | 26.1 (20.5-26.1) |
| Obese | 37.2 (24.6-42.4) | 36.4 (24.1-41.6) | 0.0 (0.0-0.0) | 37.1 (24.1-47.1) + |
| NASH | 28.0 (22.6-32.2) | 26.1 (17.2-31.5) | 0.0 (-7.1-0.0) | 27.6 (14.5-34.8) |
| ASH | 23.8 (20.2-28.7) | 17.7 (13.1-24.6) | 0.0 (0.0-0.0) | 17.3 (11.1-28.3) |

Abbreviations: ETP: endogenous thrombin potential, Velindex: velocity index, LMWH: low molecular weight heparin.

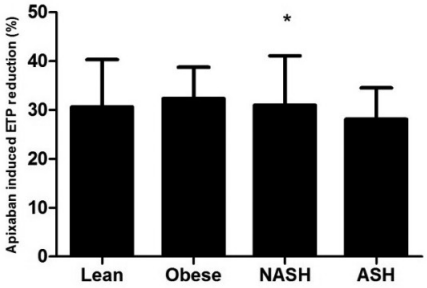
The percentual inhibition of the ETP, peak, or velocity and percentual increase in lagtime are shown. Data are expressed as median percentages with interquartile range. * = $p<0.05$ compared to lean controls, + =$p<0.05$ compared to NASH-cirrhosis




A




B




C

**Figure 1. Reduction in ETP after the addition of several anticoagulant drugs.**
ETP reduction after (A) LMWH, (B) Dabigatran and (C) Apixaban in healthy lean and obese subjects and in patients with NASH and ASH cirrhosis. The bars indicate medians and the error bars interquartile ranges. *=P<0.05 compared to ASH cirrhosis

## Discussion

In this study we assessed the anticoagulant potency of 3 classes of anticoagulant drugs in patients with mild cirrhosis due to NASH or ASH. In line with our previous study,(4) we found a profoundly increased anticoagulant response of dabigatran in patients with cirrhosis. No clear differences in anticoagulant response between controls and patients were found for apixaban and LMWH, which is also in line with our previous study, in which we only found an enhanced anticoagulant effect of a direct Xa inhibitor in patients with more advanced disease and only found an enhanced anticoagulant effect of LMWH in cirrhosis when we used thrombin generation in the absence of thrombomodulin.(4)



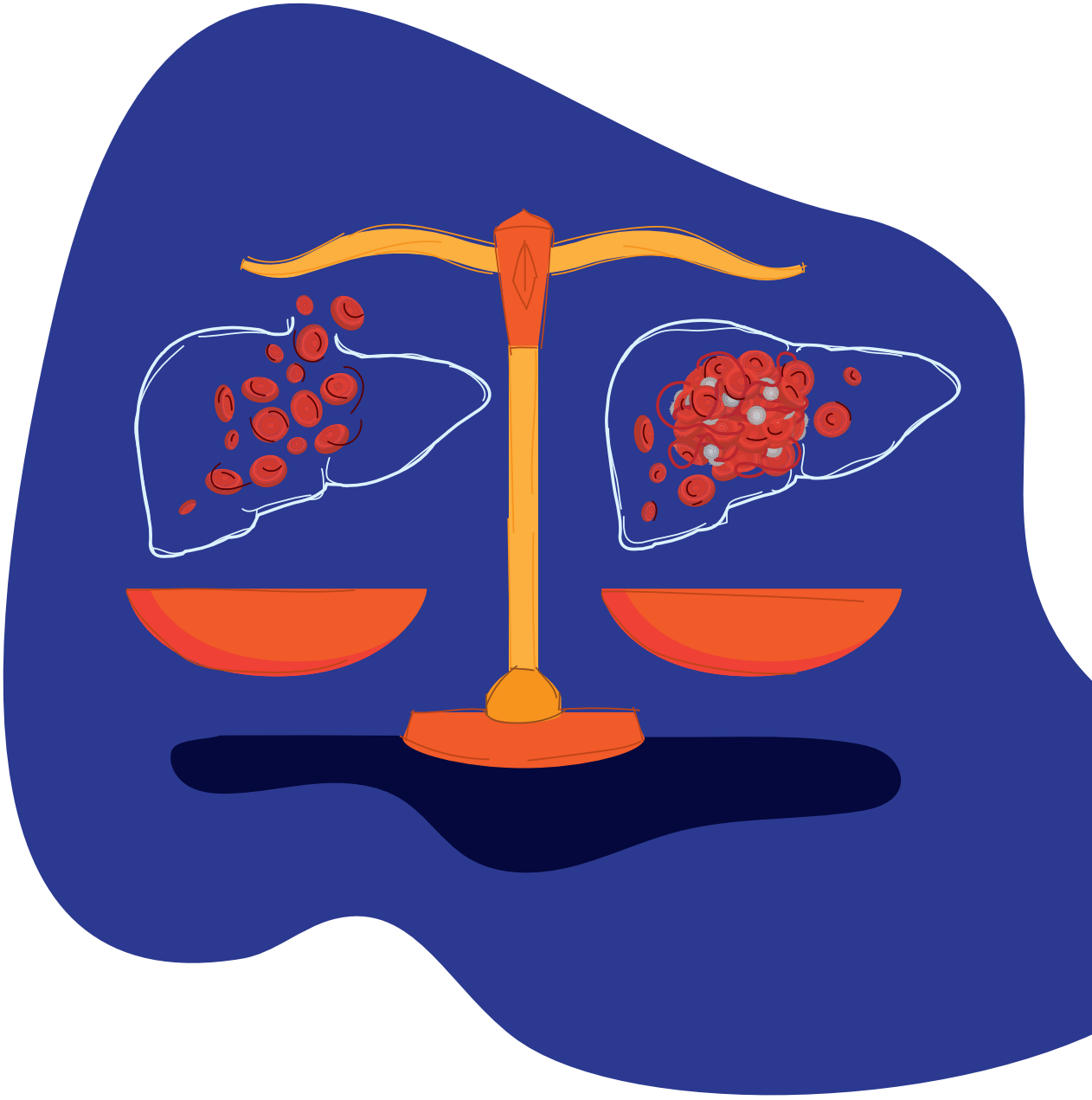
Thus, in patients with mild disease, the anticoagulant potency of LMWH or apixaban is not different from that in controls, regardless of the etiology of disease. This is in line with increasing clinical studies on the safety of LMWH and direct Xa inhibitors in patients with mild to moderate cirrhosis.(13–15) The profoundly enhanced anticoagulant effect of dabigatran, however, suggests the need for further study of this drug, even in patients with mild hepatic impairment.

We did not observe an altered in vitro anticoagulant potency of anticoagulants between patients with NASH or ASH-related cirrhosis. However, dabigatran did appear to exert a larger anticoagulant effect in plasma from patients with ASH-related cirrhosis compared to NASH-related cirrhosis. As this difference may be explained by the slightly higher MELD score in patients with ASH-related cirrhosis, this warrants further study. Notably, a comprehensive profile of the hemostatic status of these patients revealed very few differences between patients with NASH- and ASH-related cirrhosis.(16) These findings contrast the concept that NASH-related cirrhosis is associated with an hypercoagulable profile compared to liver diseases of other etiologies. Rather, our results are consistent with the notion that the surplus of thrombotic events in NASH-related cirrhosis is caused by cardiometabolic risk factors and features of the metabolic syndrome, and not directly by a hypercoagulable state.(7,8,16)

In conclusion, this study confirms previous findings on an enhanced in vitro potency of dabigatran in patients with cirrhosis and a similar *in vitro* anticoagulant potency of apixaban and enoxaparin in patients with mild cirrhotic disease. Our study does not suggest differences in *in vitro* anticoagulant potency of these commonly used classes of drugs between patients with different etiologies of disease. Collectively, the results emphasize the need for further assessment of *in vivo* potency and safety of anticoagulation in cirrhosis

## References


1. Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease : evidence and clinical consequences. Blood. 2010;116(6):878–85.
2. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med. 2011;365(2):147–56.
3. Senzolo M, Rodriguez-Castro KI, Rossetto V, Radu C, Gavasso S, Carraro P, et al. Increased anticoagulant response to low-molecular-weight heparin in plasma from patients with advanced cirrhosis. J Thromb Haemost. 2012;10(9):1823–9.
4. Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Meijers JCM, et al. Differential In Vitro Inhibition of Thrombin Generation by Anticoagulant Drugs in Plasma from Patients with Cirrhosis. PLoS One. 2014 Feb;9(2):e88390.
5. Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Porte RJ, et al. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. Br J Haematol. 2013;163(5):666–73.
6. Potze W, Adelmeijer J, Lisman T. Decreased in vitro anticoagulant potency of Rivaroxaban and Apixaban in plasma from patients with cirrhosis. Hepatology. 2015 Apr;61(4):1435–6.
7. Potze W, Siddiqui MS, Sanyal AJ. Vascular Disease in Patients with Nonalcoholic Fatty Liver Disease. Semin Thromb Hemost. 2015;41(5):488–93.
8. Targher G, Chonchol M, Miele L, Zoppini G, Pichiri I, Muggeo M. Nonalcoholic fatty liver disease as a contributor to hypercoagulation and thrombophilia in the metabolic syndrome. Semin Thromb Hemost. 2009;35(3):277–87.
9. Lisman T, Kamphuisen PW, Northup PG, Porte RJ. Established and new-generation antithrombotic drugs in patients with cirrhosis - Possibilities and caveats. J Hepatol. 2013;59(2):358–66.
10. Hugenholtz GCG, Northup PG, Porte RJ, Lisman T. Is there a rationale for treatment of chronic liver disease with antithrombotic therapy? Blood Rev. 2015;29(2):127–36.
11. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313–21.
12. Hemker HC, Giesen P, Al Dieri R, Regnault V, De Smedt E, Wagenvoord R, et al. Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiol Haemost Thromb. 2003;33(1):4–15.
13. Amitrano L, Guardascione MA, Menchise A, Martino R, Scaglione M, Giovine S, et al. Safety and efficacy of anticoagulation therapy with low molecular weight heparin for portal vein thrombosis in patients with liver cirrhosis. J Clin Gastroenterol. 2010;44(6):448–51.
14. Intagliata NM, Maitland H, Caldwell SH. Direct Oral Anticoagulants in Cirrhosis. Curr Treat Options Gastroenterol. 2016;14(2):247–56.
15. De Gottardi A, Trebicka J, Klinger C, Plessier A, Seijo S, Terziroli B, et al. Antithrombotic treatment with direct-acting oral anticoagulants (DOACs) in patients with splanchnic vein thrombosis and cirrhosis. Liver Int. 2016;
16. Potze W, Siddiqui MS, Boyett SL, Adelmeijer J, Daita K, Sanyal AJ, et al. Preserved hemostatic status in patients with non-alcoholic fatty liver disease. J Hepatol. 2016;65(5):980–7.



# CHAPTER 4.

## Anticoagulant activity of edoxaban in patients with cirrhosis

Sarah Bos, Tim Schreuder, Hans Blokzijl, Jelle Adelmeijer, Ton Lisman, Pieter Willem Kamphuisen

A shorter version of this chapter has been published as Letter:
Blood 2020 Sep 24;136(13):1561-1564

## Abstract

*Background:* patients with cirrhosis are at risk for bleeding and thrombosis. In management of thromboses, direct oral anticoagulants (DOAC) have clear advantages over traditional anticoagulants, since they are orally administered without the need for monitoring. The safety and efficacy of DOACs in cirrhosis has not been extensively studied, and the optimal dose in these patients is unknown.

*Methods:* we administered a therapeutic dose of edoxaban (60 mg, once daily) for one week to 16 patients with cirrhosis (15 with Child A, and 1 with Child B cirrhosis) and 16 healthy controls, and drew blood at various time points. We studied calibrated automated thrombinography, edoxaban-calibrated anti-Xa levels, and D-dimer levels at baseline, and at peak level on day 1, 3 and 7.

*Results:* at baseline, the median endogenous thrombin potential (ETP) of patients was higher compared to healthy subjects. On day 1, 3, and 7, the ETP in both patients and healthy subjects was substantially lower compared to baseline, but remained significantly higher in patients. In addition, D-dimer levels decreased over time in controls, but not in patients. Edoxaban plasma levels were similar in patients and controls.

*Conclusion:* therapeutic dose of the DOAC edoxaban strongly decreases ETP in patients with cirrhosis and in controls, but hemostatic activity remains higher in patients. Whether this difference translates into a higher risk of thrombosis and necessitates dose-adjustments in patients with cirrhosis should be further assessed.

## Introduction

The liver is the site of synthesis for many proteins involved in hemostasis. Consequently, patients with end-stage chronic liver disease (i.e., cirrhosis) acquire multiple and complex alterations in their hemostatic system. Recent insights in the hemostatic changes in patients with cirrhosis have indicated a balanced, but unstable hemostatic system in these patients with a risk for both bleeding and thrombotic complications including venous thromboembolism (VTE) and portal vein thrombosis.(1,2) Prevention and treatment of thrombotic events are a challenge due to a frequently prolonged baseline international normalized ratio (INR) and substantially decreased levels of antithrombin, impeding correct dosing and monitoring of vitamin K antagonists (VKA) and heparins, respectively.(3,4) There is very limited clinical experience with the new-generation direct oral anticoagulants (DOAC) in patients with cirrhosis, as these patients were excluded from all clinical trials with these new agents. However, DOACs have potential advantages over VKAs and heparins, such as the oral route of administration, the lack of requirement of laboratory monitoring, their mechanism of action, and the wider therapeutic window,(4) which has resulted in increasing interest from the hepatology community.



Currently four DOACs are registered in Europe and the US for prevention or treatment of VTE and the prevention of ischemic stroke in patients with non-valvular atrial fibrillation. Edoxaban was found to be non-inferior to warfarin in the prevention of stroke in atrial fibrillation and VTE.(5,6) In addition, edoxaban showed a favourable risk profile with 2.75% incidence of major bleeding compared to 3.43% in patients treated with warfarin.(6)

DOACs are contraindicated in patients with advanced liver disease accompanied with coagulopathy and clinically relevant bleeding risk. Nevertheless, a growing number of patients with cirrhosis are treated with these new drugs, despite an absence of clinical information on safety and efficacy. Intagliata et al. were the first to describe their experience with the use of DOACs in patients with cirrhosis. They reported a retrospective cohort in which 20 cirrhotic patients were safely treated with factor Xa inhibitors. The number of bleeding events was similar between patients treated with DOACs or VKAs.(7) A number of studies, including a large study from Taiwan that reported on 1438 patients with cirrhosis treated with a DOAC underline the findings of Intagliata.(8–12) DOACs have been used in attempts to treat portal vein thrombosis and venous thrombosis, and in patients with atrial fibrillation. Although the available safety data are encouraging, most studies were underpowered for efficacy endpoints.

Cautious use of the DOACs in patients with advancing cirrhosis is recommended by the manufacturers. Since all the DOACs are cleared by the liver and kidney, drug accumulation with a potentially increased bleeding risk is a main concern. In addition, in vitro studies have indicated altered anticoagulant effects of all registered DOACs in plasma from patients with cirrhosis. In vitro studies showed an altered anticoagulant effect of both the traditional anticoagulants and DOACs in patients with cirrhosis.(13) The Xa inhibiting DOACs were shown to be less potent in patients with cirrhosis, whereas IIa inhibitors showed an increased potency.(13) In a small group of patients with Child Pugh A and B cirrhosis, a single gift of edoxaban 15 mg resulted in a comparable pharmacokinetic effect with healthy matched controls(10). The route of clearance of Edoxaban is mainly renal (50%). The remainder of the drug is cleared through the hepatobiliary route.(14) Despite the contribution of the hepatobiliary system to clearance, the overall exposure of edoxaban was similar between the patients with Child Pugh A or B cirrhosis and their matched healthy controls, suggesting the drug might be safely used in patients with mild to moderate cirrhosis. However, altered potency of edoxaban as identified in our previous in vitro studies was not taken into account.

Whether prolonged treatment with a DOAC, such as edoxaban, leads to accumulation of the drug in patients with cirrhosis is unknown. However, the clinical importance of such a question is obvious. We therefore monitored the drug levels and analysed the ex-vivo anticoagulant effects during a one week therapeutic dose of edoxaban (60 mg once daily) in cirrhotic patients and healthy subjects.

## Methods

### *Study design*

We performed a prospective case-controlled mono center intervention study to analyze the ex-vivo potency of edoxaban in patients with Child Pugh A and B cirrhosis.

### *Patients*

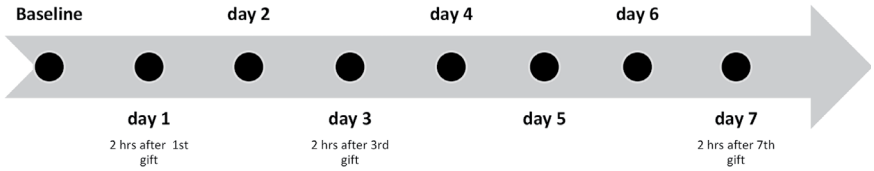
Sixteen adult patients with an established diagnosis of cirrhosis were enrolled from the outpatient hepatology clinic of the University Medical Center Groningen, The Netherlands. In addition, 16 healthy volunteers were included as a control group. Cirrhosis had to be confirmed via fibroscan suggestive for F4 fibrosis, histology compatible with cirrhosis, or via diagnostic imaging. Exclusion criteria were the presence of malignancy, renal failure requiring intervention with drugs or dialysis, weight under 60 kg, active infection, a known hereditary bleeding disorder, use of anticoagulant drugs in the past 10 days, use of cyclosporine, dronedarone, erythromycin or ketoconazol, history of thrombotic disease, recent variceal bleeding or known varices grade 2 or 3, pregnancy, or HIV positivity.

Additional exclusion criteria for the control group included the presence of known liver disease and a history of clinically relevant bleeding complications. The study protocol was approved by the local medical ethical committee (METc 2016/226), and was registered at Netherlands Trial Register (NTR6397). Written informed consent was obtained from each subject before inclusion in the study.

### *Plasma Samples*

Blood samples were drawn by venipuncture twice at day 1 (baseline and 2 hrs after the first dose) and once on day 3 and day 7 at peak level, two hours after ingestion of edoxaban. Specifically, 18 ml of blood in tubes containing 3.2% sodium citrate was drawn at the following time points:






The blood was processed to platelet-poor plasma (PPP) by double centrifugation at 2000 g and 10,000 g respectively for 10 min. Plasma was stored at -80◦C until use.

### *Thrombin generation assay*

The thrombin generation assay (TGA) was performed in platelet-poor plasma (PPP) with the fluorimetric method described by Hemker, Calibrated Automated Thrombography® (CAT).(19) Coagulation was activated using commercially available reagents containing recombinant tissue factor (TF, final concentration 5 pM), phospholipids (final concentration 4 μM), in the presence of soluble thrombomodulin (TM, the concentration of which is not revealed by the manufacturer). Reagents were purchased from Thrombinoscope BV, Maastricht, the Netherlands, and thrombin generation experiments were executed following protocols provided by Thrombinoscope. The endogenous thrombin potential (ETP; which represents the total enzymatic work performed by thrombin during the time that it was active) was calculated from the thrombin generation curve using the Thrombinoscope software, and the percentual decrease in ETP following edoxaban administration was calculated.

### *Conventional coagulation assays*

The prothrombin time (PT), activated partial thrombin time (APTT), and levels of prothrombin (FII), fibrinogen (FI) and D-dimer were assessed on an automated coagulation analyzer (ACL 300 TOP) with reagents (Recombiplastin 2G for PT, Hemosil

SynthaSil for APTT, factor deficient plasma for FII and QFA thrombin for FI) and protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands).

### *Edoxaban-calibrated Anti-Xa levels*

We estimated edoxaban plasma levels by edoxaban-calibrated anti-Xa assays. The anti-Xa assay was assessed on the ACL 300 TOP (Werfen, Breda, the Netherlands) with BIOPHEN heparin (LRT) purchased from Nodia (Amsterdam, the Netherlands). This was calibrated with the BIOPHEN Edoxaban calibrator from Nodia (Amsterdam, the Netherlands).

### *Statistical analysis*

A difference of 20% in the reduction of thrombin generation following edoxaban between patients and controls was considered relevant. This difference could be detected with an alpha of 0.05, a standardized power of 0.80, and a two-sided sigma of 20% in both groups using 16 subjects per group.

Data are expressed as means (with standard deviations (SDs)), medians (with ranges), or numbers (with percentages) as appropriate. Two groups were compared by Student's t-test or Mann-Whitney U test as appropriate. Multiple groups were compared using One-way ANOVA (with the Bonferroni posttest) or Kruskal-Wallis H test (with Dunn's posttest) as appropriate. The Wilcoxon signed rank test was used to assess differences between time points within a single group. P values of 0.05 or less were considered statistically significant. GraphPad Prism (San Diego, USA) and IBM SPSS Statistics 23 (New York, USA) were used for analyses.

## Results

### *Patient characteristics*

Sixteen patients and 16 healthy subjects were included. The median age of patients was 60 [48-65] years versus 49 [42-58] years for the healthy subjects. Mean BMI was similar between patients and healthy subjects as shown in table 1. Fifteen patients had Child Pugh A cirrhosis and one had Child Pugh B cirrhosis. None of the patients was decompensated. Etiologies of cirrhosis were distributed as follows: 4 cholestatic liver disease (1 primary biliary cholangitis, 2 primary sclerosing cholangitis (PSC), 1 small duct PSC), 2 non-alcoholic steatohepatitis (NASH), 4 alcoholic steatohepatitis (ASH), 2 auto-immune hepatitis (AIH), 1 hepatitis C virus (HCV), 1 hemochromatosis and 2 overlap syndromes (1 PSC/AIH and 1 NASH/ASH/AIH).

**Table 1. Baseline characteristics**

| | | Patients (n=16) | Controls (n=16) |
|---|---|---|---|
| **Age** | | 60 [48-65] | 49 [42-58]* |
| **Female** | | 6 (38) | 7 (44) |
| **BMI (kg/m2)** | | 28.7 [5] | 26.4 [4.4] |
| **Diabetes** | | 4 (25) | 1 (6) |
| **CVD** | | 5 (31) | 2 (13) |
| **CTP** | **A** | 15 (94) | - |
| | **B** | 1 (6) | - |
| **Ascites** | | 2 (13) | - |
| **Encephalopathy** | | 0 | - |

BMI, body mass index, CVD, cardiovascular disease, CTP, Child Turcotte Pugh. Data are expressed as number (%), mean [SD], or median [IQR]. *p<0.05



### *Baseline conventional coagulation assays*

At baseline, patients and healthy controls had similar fibrinogen plasma levels, 2.8 [0.9] g/l and 3.1 [0.7] g/L respectively and a comparable APTT, 34.4 [4.2] sec and 32.2 [2.7] sec. The PT, INR, prothrombin levels and d-dimer level were all significantly lower in the healthy controls compared to the patients, table 2.

**Table 2. Baseline coagulation parameters**

| | Patients n =16 | Controls n=16 |
|---|---|---|
| **APTT, sec** | 34.4 [4.2] | 32.2 [2.7] |
| **PT, sec** | 12.3 [2.4] | 11.0 [0.7]* |
| **INR** | 1.1 [0.2] | 1.0 [0.1]* |
| **Fibrinogen, g/L** | 2.8 [0.9] | 3.1 [0.7] |
| **Prothrombin, %** | 65 [15] | 102 [11]* |
| **D-dimer, ng/ml** | 517 [308-1049] | 185 [124-276]* |

APTT, activated partial thromboplastin time, PT, prothrombin time, INR, international normalized ratio. Data are expressed as mean [SD], or median [IQR]. * = p<0.05

### *Hemostatic activity*

The ETP of patients was substantially higher at baseline, 707 [482-789] nM IIa*min, compared to controls, 403 [304-506] nM IIa*min (p<0.05). On day 1, 3, and 7, ETP decreased in both patients and controls, but remained significantly and substantially higher in patients compared to the healthy subjects (P<0.05). The percentual decrease of the ETP after edoxaban was significantly less in patients compared to controls at all time points (P< 0.05) (figure 1).

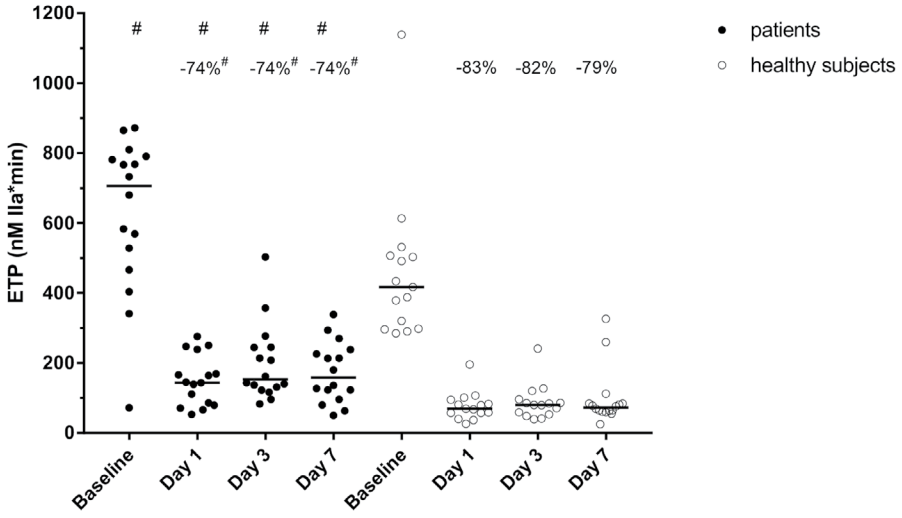



**Figure 1.** Endogenous thrombin potential (ETP) at baseline and in samples taken at 3 time points during administration of 60 mg edoxaban once daily to 16 patients with cirrhosis and 16 healthy controls. The median percentual decrease is indicated and horizontal lines represent medians. # $p<0.05$ vs controls

At baseline, D-dimer levels were substantially higher in patients compared to controls. On day 3 and 7, D-dimer levels decreased significantly in controls compared to baseline ($p<0.05$), but remained similar compared to baseline in patients (figure 2).

### *Edoxaban plasma levels*

In patients and controls alike, anti Xa levels as a proxy for edoxaban plasma levels remained relatively constant during the week of administration, with median plasma levels around 200 ng/ml (figure 3). At day 3, two of the patients had particularly low edoxaban plasma levels, which may have been caused by lack of compliance.

### *Adverse events*

Two patients experienced a minor nosebleed. One patient decided not to participate any further after this event. Mild bruising mainly at the site of venipuncture was present in 4 patients and 5 healthy controls. No further adverse events occurred.

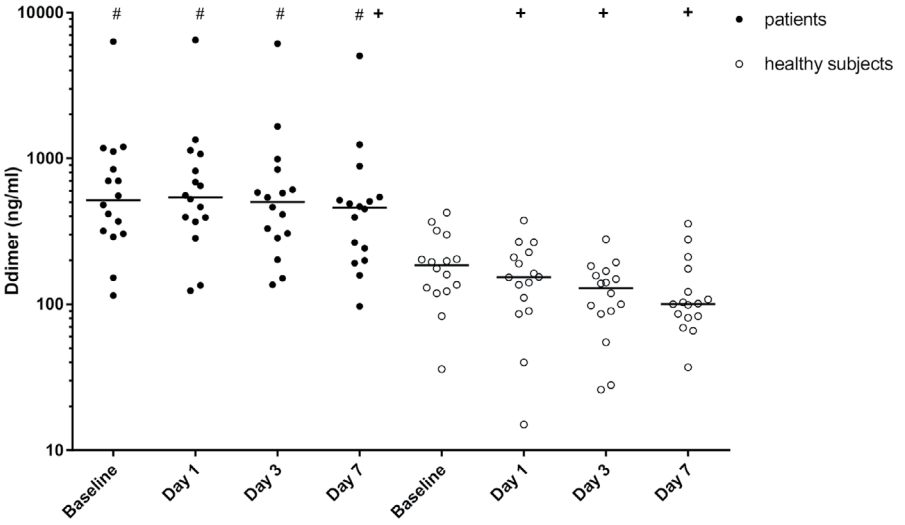



**Figure 2.** D-dimer levels at baseline and in samples taken at 3 time points during administration of 60 mg edoxaban once daily to 16 patients with cirrhosis and 16 healthy controls. Horizontal lines represent medians. # p<0.05 vs controls + p<0.05 versus baseline

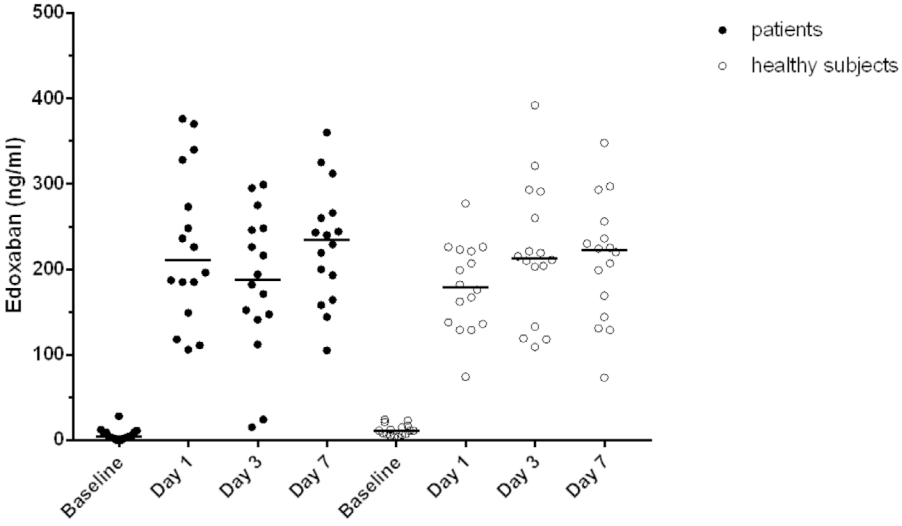



**Figure 3.** Anti-Xa assay calibrated for edoxaban at at baseline and in samples taken at 3 time points during administration of 60 mg edoxaban once daily to 16 patients with cirrhosis and 16 healthy controls. Horizontal lines indicate medians. # p<0.05 vs controls

## Discussion

This is the first study to monitor the effect of repeated exposure to edoxaban in a therapeutic dose in patients with cirrhosis. We chose to study a 7 day-exposure time to ensure we would reach steady state, which is achieved after 3 days of dosing in healthy individuals. We found that edoxaban reduces ex vivo hemostatic potential and in vivo activation of coagulation less efficiently in patients compared to controls, despite similar plasma levels. Specifically, the relative decrease of the ETP was lower in patients compared to controls. In addition, the absolute on-drug ETP level was two-fold higher in patients, suggesting insufficient anticoagulant activity in patients. Indeed, d-dimer levels clearly decreased over time in controls, but remained similar in patients. These results are in line with in vitro studies that showed Xa-inhibiting DOACs to have decreased anticoagulant effects in plasma from patients with cirrhosis.(13,15) However, although we find evidence of a less effective downregulation of coagulation by edoxaban in patients with cirrhosis, clinical studies are required to assess whether this translates to a less effective antithrombotic effect in patients in both a prophylactic and a treatment setting.(3)

Our data show that edoxaban accumulation due to decreased clearance does not occur in patients with mild cirrhosis. Whether drug accumulation occurs in patients with more advanced cirrhosis, or in patients with cirrhosis combined with poor renal function requires further study. DOACs are cleared in part by metabolic inactivation in the liver, and in part by renal excretion. The route of elimination of edoxaban is approximately 50% hepatic and 50% renal, which is roughly comparable to that of rivaroxaban (65 vs 35%) and apixaban (75 vs 25%), whereas dabigatran is cleared primarily by the kidneys (20% vs 80%). Future studies will be required to examine whether the slightly increased role of the liver in clearing rivaroxaban and apixaban results in drug accumulation at lower levels of hepatic failure. Based on our data, plasma levels of edoxaban should be as high or perhaps somewhat higher in patients with cirrhosis compared to patients with intact liver function to obtain optimal anticoagulant effects. Although therapeutic drug levels of edoxaban have not yet been firmly established, peak levels between 120 ng/ml and 250 ng/ml have been found in patients considered to be adequately treated with 60 mg once daily.(16) As edoxaban clearance may change when patients develop worsening liver disease, we propose to monitor drug levels in patients with cirrhosis using edoxaban-calibrated anti-Xa assays, that previously has been shown to be suitable for DOAC monitoring of patients with cirrhosis to obtain values considered therapeutic or slightly supratherapeutic in the general population.(3,17,18)

In published studies, Xa-directed DOACs dosing tends to be conservative with reduced or low doses in a large proportion of patients.(8,11,19,20) Also in the ongoing Cirroxaban trial (clinicaltrials.gov NCT02643212), which is a placebo-controlled trial on Rivaroxaban in cirrhosis, a reduced dose was chosen. We have only studied patients with mild cirrhosis, and future studies should explore pharmacokinetics and -dynamics of DOACs in patients with more advanced disease that are at increased risk for both thrombotic and bleeding complications. Nevertheless, given the hypercoagulable state of patients with cirrhosis of all severities,(21) our results argue for a reevaluation of the conservative dosing regimens that have been used so far. However, although more stringent anticoagulation might lead to a better efficacy, it might also increase bleeding risk in these patients with a known complex and vulnerable hemostatic status.(1,2)



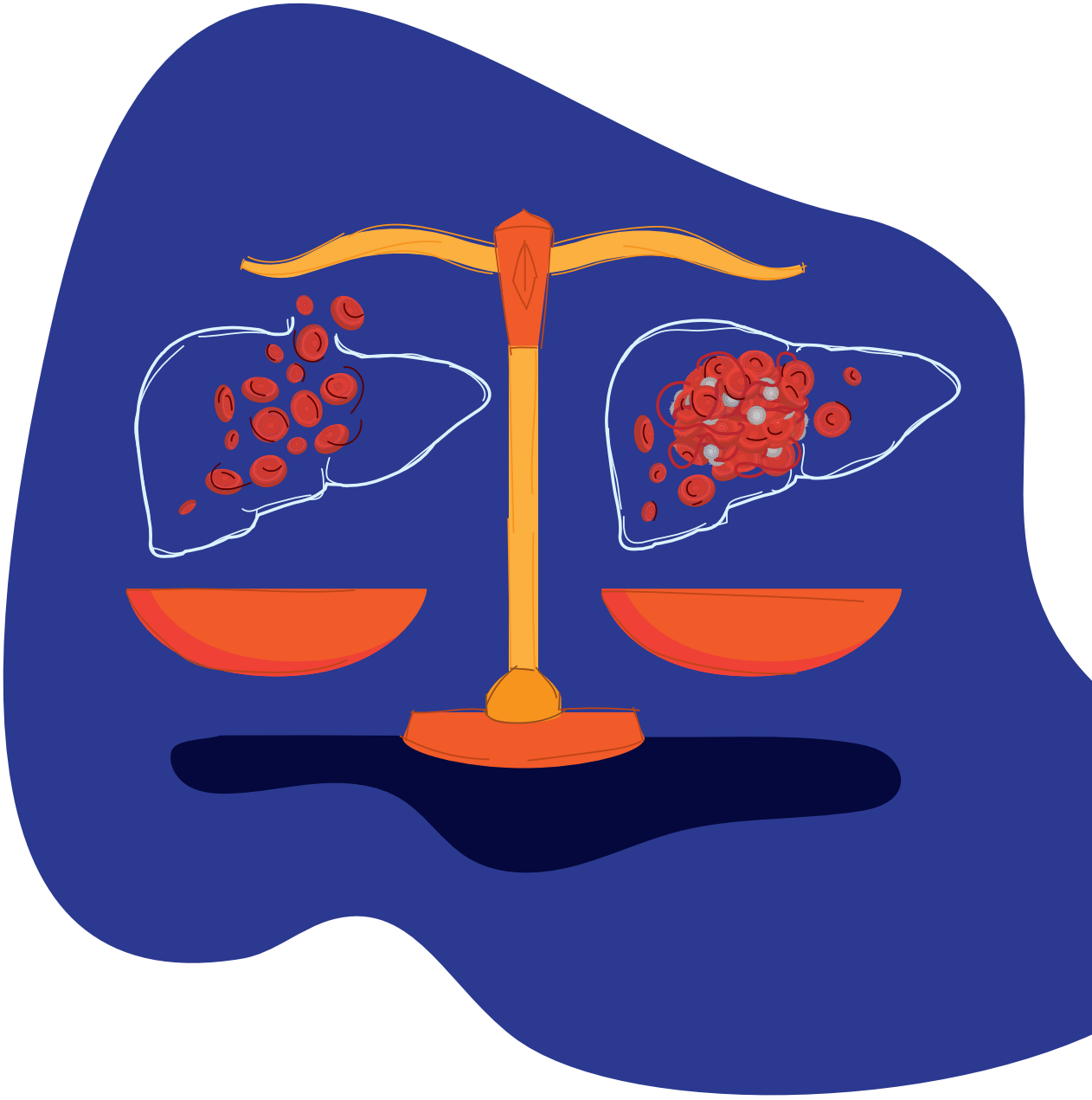
Taken together, edoxaban plasma levels are similar between patients with cirrhosis and healthy controls after 7 days of treatment. However, edoxaban less efficiently reduces ex vivo hemostatic potential and in vivo activation of coagulation. These data suggest that reduced dosing is not necessary and would lead to undertreatment which is not desirable in these already hypercoagulable patients. Whether dose escalations are warranted requires further study.

## References


1. Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease : evidence and clinical consequences. Blood. 2010;116(6):878–85.
2. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med. 2011;365(2):147–56.
3. Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Porte RJ, et al. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. Br J Haematol. 2013;163(5):666–73.
4. Lisman T, Kamphuisen PW, Northup PG, Porte RJ. Established and new-generation antithrombotic drugs in patients with cirrhosis - Possibilities and caveats. J Hepatol. 2013;59(2):358–66.
5. Investigators TH-V. Edoxaban versus Warfarin for the Treatment of Symptomatic Venous Thromboembolism. N Engl J Med. 2013 Oct 10;369(15):1406–15.
6. Giugliano RP, Ruff CT, Braunwald E, Murphy, Sabina A, Wiviott, Stephen D, Halperin, Jonathan L, et al. Edoxaban versus warfarin in patients with atrial fibrillation. N Engl J Med. 2013;369(22):2093–104.
7. Intagliata NM, Henry ZH, Maitland H, Shah NL, Argo CK, Northup PG, et al. Direct Oral Anticoagulants in Cirrhosis Patients Pose Similar Risks of Bleeding When Compared to Traditional Anticoagulation. Dig Dis Sci. 2016;61(6):1721–7.
8. Hoolwerf EW, Kraaijpoel N, Büller HR, van Es N. Direct oral anticoagulants in patients with liver cirrhosis: A systematic review. Thromb Res. 2018;170(July):102–8.
9. Martinez M, Tandra A, Vuppalanchi R. Treatment of acute portal vein thrombosis by nontraditional anticoagulation. Hepatology. 2014;60(1):425–6.
10. Mendell J, Johnson L, Chen S. An open-label, phase 1 study to evaluate the effects of hepatic impairment on edoxaban pharmacokinetics and pharmacodynamics. J Clin Pharmacol. 2015;55(12):1395–405.
11. Lee HF, Chan YH, Chang SH, Tu HT, Chen SW, Yeh YH, et al. Effectiveness and Safety of Non–Vitamin K Antagonist Oral Anticoagulant and Warfarin in Cirrhotic Patients With Nonvalvular Atrial Fibrillation. J Am Heart Assoc. 2019;8(5).
12. Nagaoki Y, Aikata H, Daijyo K, Teraoka Y, Shinohara F, Nakamura Y, et al. Efficacy and safety of edoxaban for treatment of portal vein thrombosis following danaparoid sodium in patients with liver cirrhosis. Hepatol Res. 2018;48(1):51–8.
13. Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Meijers JCM, et al. Differential In Vitro Inhibition of Thrombin Generation by Anticoagulant Drugs in Plasma from Patients with Cirrhosis. PLoS One. 2014 Feb;9(2):e88390.
14. Padrini R. Clinical Pharmacokinetics and Pharmacodynamics of Direct Oral Anticoagulants in Patients with Renal Failure. Eur J Drug Metab Pharmacokinet. 2019;44(1):1–12.
15. Potze W, Adelmeijer J, Lisman T. Decreased in vitro anticoagulant potency of Rivaroxaban and Apixaban in plasma from patients with cirrhosis. Hepatology. 2015 Apr;61(4):1435–6.
16. Weitz JI, Connolly SJ, Patel I, Salazar D, Rohatagi S, Mendell J, et al. Randomised, parallel-group, multicentre, multinational phase 2 study comparing edoxaban, an oral factor Xa

inhibitor, with warfarin for stroke prevention in patients with atrial fibrillation. Thromb Haemost. 2010;104(3):633–41.

17. Gosselin R, Grant RP, Adcock DM. Comparison of the effect of the anti-Xa direct oral anticoagulants apixaban, edoxaban, and rivaroxaban on coagulation assays. Int J Lab Hematol. 2016;38(5):505–13.
18. Lip GYHH, Agnelli G. Edoxaban: A focused review of its clinical pharmacology. Eur Heart J. 2014;35(28):1844–55.
19. Weinberg EM, Palecki J, Reddy KR. Direct-Acting Oral Anticoagulants (DOACs) in Cirrhosis and Cirrhosis-Associated Portal Vein Thrombosis. Semin Liver Dis. 2019;39(2):195–208.
20. Intagliata NM, Maitland H, Caldwell SH. Direct Oral Anticoagulants in Cirrhosis. Curr Treat Options Gastroenterol. 2016;14(2):247–56.
21. Lebreton A, Sinegre T, Pereira B, Lamblin G, Duron C, Abergel A. Plasma hypercoagulability in the presence of thrombomodulin but not of activated protein C in patients with cirrhosis. J Gastroenterol Hepatol. 2017 Apr;32(4):916–24.



# CHAPTER 5.

## Hemostatic complications in hepatobiliary surgery.

Sarah Bos, William Bernal, Robert Porte, Ton Lisman

Seminars in Thrombosis and Hemostasis
2017 Oct;43(7):732-741

## Abstract

Hepatobiliary surgery is a well-known risk factor for thrombotic complications but is also associated with substantial perioperative blood loss. Given the central role of the liver in hemostasis, hepatobiliary surgery is frequently accompanied by complex changes in the hemostatic system. Increasing knowledge of these changes has resulted in an improved understanding of the etiology of some of the hemostatic complications.

In the early postoperative period a prolongation of conventional coagulation test times, such as the prothrombin time, is frequently seen. Together with a decreased platelet count, this suggests a hypocoagulable state. The concomitant decline of anticoagulant factors and development of a von Willebrand factor/ ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) imbalance, however, suggest a hypercoagulable state, potentially contributing to the risk of thromboembolism.

Postoperative thromboprophylaxis should be initiated early to avoid thrombosis, and intensified prophylaxis might benefit high- risk patients. The risk of hemorrhagic complications during hepatobiliary surgery has diminished over time, mainly due to improved surgical and anesthesiological techniques. However, bleeding can still be profound in individual patients and is difficult to predict using (global) hemostasis tests. A restrictive transfusion and fluid infusion policy to maintain a low central venous pressure is crucial in prevention of perioperative bleeding. However, when active bleeding occurs, proactive prohemostatic management is required.

## Introduction

Hepatobiliary surgery is associated with a substantial risk of bleeding and thrombotic complications. Given the central role of the liver in hemostasis, it is not surprising that hemostatic changes occur during and after partial hepatectomy or liver transplantation. Also, preoperative hemostatic abnormalities are frequently present in patients with the (end-stage) liver disease.(1)

Bleeding during partial hepatectomy may be largely due to surgical and anatomical factors, but perioperative changes in the hemostatic system may also contribute.(2,3) During liver transplant surgery, the substantially altered hemostatic system may contribute to bleeding, although surgical and anesthesiological factors and portal hypertension contribute significantly.(4,5) The risk of deep vein thrombosis following hepatobiliary surgery is not negligible, even in patients receiving adequate thromboprophylaxis.(6) In liver transplant recipients, thrombotic complications of the hepatic artery or portal vein may occur, and may directly compromise graft function and vitality.(7,8) Prevention and treatment of bleeding and thrombosis, therefore, are essential in the management of patients undergoing hepatobiliary surgery.



In the past decade, clinical and laboratory studies have led to a better understanding of the status of the hemostatic system of the patient undergoing hepatobiliary surgery. These new insights are significant to further optimize clinical management.(9,10)

In this article, we will provide an overview of the new insights in hemostatic changes during hepatobiliary surgery. Also, developments in understanding risk factors and the possible predictors of hemostatic complications during the perioperative period of hepatobiliary surgery will be discussed. Finally, strategies for prevention and treatment of bleeding and thrombotic complications will be summarized.

## Hemostatic changes during and after hepatobiliary surgery

Patients undergoing hepatobiliary surgery may have an intact hemostatic system before the procedure, for example, patients requiring a partial hepatectomy for metastasized colon cancer, or patients with a metabolic disorder requiring liver transplantation. However, frequently the hemostatic function is already substantially compromised, such as patients with cirrhosis requiring partial hepatectomy or liver transplantation. The hemostatic changes of patients with cirrhosis have been reviewed extensively elsewhere.(11–13) In short, despite alterations in routine indices of hemostasis such as the platelet count and the prothrombin time (PT), patients with cirrhosis appear to be in hemostatic balance due to a concomitant decline in pro- and antihemostatic drivers.(13) During hepatobiliary surgery, substantial (additional) changes in the hemostatic system occur. These are likely due to a combination of factors. On the one hand, there is consumption induced by surgical damage and reperfusion injury during liver transplantation, hemodilution, decreased or absent synthesis of liver-derived hemostatic components following partial hepatectomy or during the anhepatic phase of liver transplantation.(14,15) On the other hand, there is a decreased or absent clearance of activated hemostatic proteins when functional liver volume becomes compromised.(14,15) Such changes lead to further abnormalities in routine diagnostic tests of hemostasis. We will summarize new insights into the development of hemostatic abnorm- alities during partial hepatectomy and liver transplantation below.

### *Primary Hemostasis*

The platelet count decreases during and after partial hepatectomy and liver transplantation, reaching a nadir around day 3, after which it rapidly increases to supraphysiological levels.(16–18) An imbalance in the von Willebrand factor (VWF)/ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) axis has been suggested to compensate (in part) for the thrombocy- topenia of cirrhosis and acute liver failure.(19,20) A similar mechanism likely acts during and after partial hepatectomy and liver transplantation, as high VWF, low ADAMTS13, and enhanced VWF-dependent in vitro platelet adhesion have been observed in plasma samples taken during and after both procedures. (21–23) High levels of VWF likely relate to endothelial cell activation, whereas decreased ADAMTS13 is likely due to a combination of hemodilution, consumption, and decreased hepatic synthesis. The imbalanced VWF/ ADAMTS13 axis may not only compensate for the thrombocytopenia during these procedures but may contribute to thrombotic risk.(24) Indeed, imbalanced VWF/ADAMTS13 has been shown to be a risk factor for arterial thrombosis in the general population. (25,26) Interestingly, a VWF/ADAMTS13 imbalance also develops during pancreas resection,(22) although to a lesser extent as compared with the imbalance

developing during partial hepatectomy, indicating that the decrease in ADAMTS13 following partial hepatectomy is only partly related to decreased synthetic capacity of the remnant liver.

Although the function of the primary hemostatic system may be much better preserved during hepatobiliary surgery than suggested by the platelet count, the developing thrombocytopenia may affect the outcome. Platelets not only are critical in hemostasis but also appear to play a role in liver injury and regeneration. Animal studies have demonstrated that platelets contribute substantially to liver regeneration following partial hepatectomy,(27–30) although the mechanisms involved are incompletely understood.(31) In humans, it has been demonstrated that a low postoperative platelet count is associated with delayed liver function recovery after partial hepatectomy, which suggests that platelets play a critical role in liver regeneration after hepatectomy also in humans.(16,17,32,33) Also, a recent study in living donor transplant recipients demonstrated that in those recipients that did not receive intraoperative platelet transfusions, the intraoperative platelet count was positively associated with graft regeneration as assessed by graft volume measurements by computed tomography.(34)



### *Secondary Hemostasis*

During partial hepatectomy and liver transplantation, plasma levels of coagulation factors and inhibitors decrease, which is likely related to a combination of hemodilution, consumption, and defective hepatic synthesis.(35,36) In patients with an uncomplicated postoperative course, nadir levels are reached within 24 hours, and coagulation proteins recover to normal levels in the first postoperative weeks.(37,38) The reduction in levels of procoagulant proteins results in a further prolongation in the PT, which suggests a hypocoagulable state.(38,39) In some samples taken during a liver transplant, the PT even becomes immeasurably high.(39) However, the reduction in procoagulants is accompanied by a reduction in natural anticoagulant proteins.(38,39) As the PT is only sensitive to plasma levels of procoagulant proteins; the test does not assess the net effect of concomitant alterations in levels of pro- and anticoagulant proteins. In addition, plasma levels of procoagulants appear to recover more quickly as compared with levels of anticoagulant proteins.(40) More advanced hemostatic tests including thrombomodulin-modified thrombin generation or thromboelastography, therefore, indicate normo- to hypercoagulability in these patients, despite prolongations in the PT.(39,41–46) Interestingly, one study has shown that a hypercoagulable TEG (Haemonetics Corp, Massachusetts, United States) as defined by a shortened r-time developed in as much as 30% of patients during the anhepatic phase of liver transplantation.(45)

Plasma fibrinogen levels decrease during partial hepatectomy and liver transplantation,(40,47) and recover over time to supraphysiological levels.(48) In patients with cirrhosis, plasma fibrinogen has both hypo- and hypercoagulable features. Specifically, hypersialation impairs fibrin polymerization and thus delays clot formation.(49) However, the ultimately formed fibrin clot has a decreased permeability as compared with clots generated from healthy individuals.(50) As fibrin clot permeability is considered the "gold standard" of fibrin clot quality, we previously concluded that the fibrin clot of patients with cirrhosis has a net prothrombotic nature.(47,50) During liver transplantation, the permeability of the plasma clot increases and the quality of fibrin clot during transplant becomes substantially impaired.(51) Fibrin clot structure, to our knowledge, has not been studied in samples taken during partial hepatectomy.

### *Fibrinolysis*

During partial hepatectomy and liver transplantation, plasma levels of liver-derived fibrinolytic proteins (i.e., plasminogen, antiplasmin, thrombin-activatable fibrinolysis inhibitor) decreases, whereas levels of endothelial-derived fibrinolytic proteins (i.e., tissue-type plasminogen activator [tPA] and plasminogen activator inhibitor type 1 [PAI-1]) increases.(36,52) The net effect of the complex changes in the fibrinolytic system during and after hepatobiliary surgery is an intraoperative hyperfibrinolytic status in part of the patients, likely because release of t-PA overwhelms the circulating and acutely released PAI-1.(53,54) Following any surgery, a temporary hypofibrinolytic state occurs due to a temporary elevation of PAI-1 (the postoperative fibrinolytic shutdown).(55)

Following partial hepatectomy, one study has shown normalization of plasma fibrinolytic potential at day 1, with a "second wave" of hypofibrinolysis between days 3 and 7.(36) Interestingly, a strikingly similar "two wave" hypo- fibrinolytic state was observed following pancreas resection, indicating that the sustained hypofibrinolytic state is at least in part unrelated to decreased synthetic function of the liver.(36) Following liver transplantation, plasma fibrinolytic potential slowly normalizes over time.(56) Plasma hypofibrinolysis thus characterizes the early postoperative period of hepatobiliary surgery.

*Summary of Hemostatic Status during and after Hepatobiliary Surgery* Table 1 summarizes changes in the hemostatic system during and after partial liver resection and liver transplantation. Maintained hemostatic balance characterizes the hemostatic function of patients during and after hepatobiliary surgery despite intra- operative decreases in plasma levels of hemostatic proteins, decreasing platelet count, and increasing PT. Intra- operatively, the hemostatic balance has distinct hypo- and hypercoagulable features. Specifically, hypofibrinogenemia and

hyperfibrinolysis impair hemostasis and might contribute to bleeding, whereas the VWF/ADAMTS13 imbalance and increased thrombin generation capacity support hemostasis and perhaps contribute to thrombosis. Postoperatively, hepatobiliary surgery is characterized by hypercoagulability, which includes VWF/ADAMTS13 imbalance, enhanced thrombin generating capacity, and sustained hypofibrinolysis.(22,36,38) As all these factors have been shown to form a risk factor for thrombotic events in the general population, it is fair to hypothesize that the hypercoagulable status following hepatobiliary surgery may contribute to post-operative thrombotic events, but formal evidence for this is lacking.

| | Preoperative | | Intraoperative | | Postoperative | |
|---|---|---|---|---|---|---|
| | **PH** | **LT** | **PH** | **LT** | **PH** | **LT** |
| **Platelet count** | Normal | Decreased | Decreased | | Following an initial decrease, normalization at day 10-14 with no thrombocytosis | |
| **VWF/ADAMTS13** | - High VWF<br>- Slightly decreased ADAMTS13 | - High VWF<br>- Low ADAMTS13 | - VWF no change<br>- ADAMTS13 further decrease | - VWF slight decrease<br>- ADAMTS13 substantial decrease, <5% in some patients | Day 30:<br>- VWF remains elevated<br>- ADAMTS13 decreased | Day 10:<br>- VWF remains elevated<br>- ADAMTS13 decreased |
| **FVIII** | Slightly elevated | Substantially elevated | Slight increase | Slight decrease | Slightly elevated at day 30 | Substantially elevated at day 10 |
| **Pro- and anticoagulant factors (except FVIII)** | Normal | Substantially decreased | Decreased | | Normalization at day 30 | Normalization at day 10 |
| **Pro- and antifibrinolytic factors (except tPA and PAI-1)** | Normal | Substantially decreased | Decreased | | Normalization at day 30 | Normalization at day 10 |
| **tPA/PAI-1** | Normal | Increased | - tPA unknown<br>- PAI-1 high at the end of surgery | - tPA peak during anhepatic and/or reperfusion phases<br>- PAI-1 high at the end of surgery | Normalization at day 30 | Normalization at day 10 |
| **Prothrombin time** | Normal | Prolonged | Prolonged | | Normalization after 5-7 days | |
| **Thrombin generation** | Normal | Hypercoagulable | Hypercoagulable | Normo- to hypercoagulable | Sustained hypercoagulability until day 30 | Sustained hypercoagulability until day 10 |
| **Plasma fibrinolytic potential** | Normal | Slight hyperfibrinolysis | Hypofibrinolysis at the end of surgery | Hyperfibrinolysis during anhepatic and reperfusion phases, hypofibrinolysis at the end of surgery | Second peak of hypofibrinolysis at day 3, normalization at day 30 | Normalization at day 3-5 |
| **Thromboelastography** | Normal | Hypo-, normo-, or hypercoagulable, dependent on etiology | Normal | Hypo-, normo- or hypercoagulable | Increased fib-tem at day 5 | Unknown |

**Table 1** Changes in the hemostatic system prior to, during, and after partial hepatectomy in non-cirrhotic and liver transplantation in patients with cirrhotic livers
Abbreviations: PH, partial hepatectomy; LT, liver transplantation; VWF, von Willebrand factor; tPA, tissue plasminogen activator; PA-1, plasminogen activator inhibitor-1. Data in this table are based on published studies of our laboratory [16,19,20,33,35,36,53] and others [37,42].

## Hemorrhagic Complications

Bleeding may complicate hepatobiliary surgery. Clinically relevant bleeding rates vary widely between centers, but blood loss requiring blood product transfusion is not uncommon. In a published series from our center, one-third of patients undergoing partial hepatectomy required red blood cell transfusions,(32) and mean red blood cell requirements during liver transplantation were eight units. (57) Although the improvements in surgical and anesthesiologic techniques have contributed to a substantial decrease in blood loss and transfusion requirements over time,(4) profound blood loss may occur in individual patients. The main causes of blood loss in hepatobiliary surgery consist of surgical and patient-related factors, which includes altered hemostasis in those patients with the end-stage liver disease.(5) Factors that may contribute to bleeding during partial hepatectomy include the quality of liver tissue to be transected, the method of parenchymal transection, and the central venous pressure.(2,3,58,59) In liver transplantation, factors that may con- tribute to perioperative blood loss are severity and etiology of liver disease, severity of portal hypertension, nutritional state, concomitant renal failure, length of the cold ischemia time, previous surgical procedures, and the type of surgical technique used (vena cava replacement vs. piggyback technique).(4,60,61)



Although blood loss is manageable in the vast majority of patients by surgical repair and transfusion of blood products, there are multiple reasons to limit blood loss as much as possible. Blood loss and blood product requirements have been dose-dependently linked to adverse outcomes including mortality in patients undergoing partial hepatectomy or liver transplant surgery.(4,62) Although mechanisms that may be involved in deleterious effects of blood product transfusion in patients undergoing hepatobiliary surgery are in- completely understood, they include general transfusion reactions including transfusion-related acute lung injury, which appears more prevalent in patients undergoing hepatobiliary surgery as compared with patients transfused in other contexts.(63) Also, transfusion-associated circulatory overload may contribute to exacerbation of bleeding as it increases portal hypertension.

## Thrombotic Complications

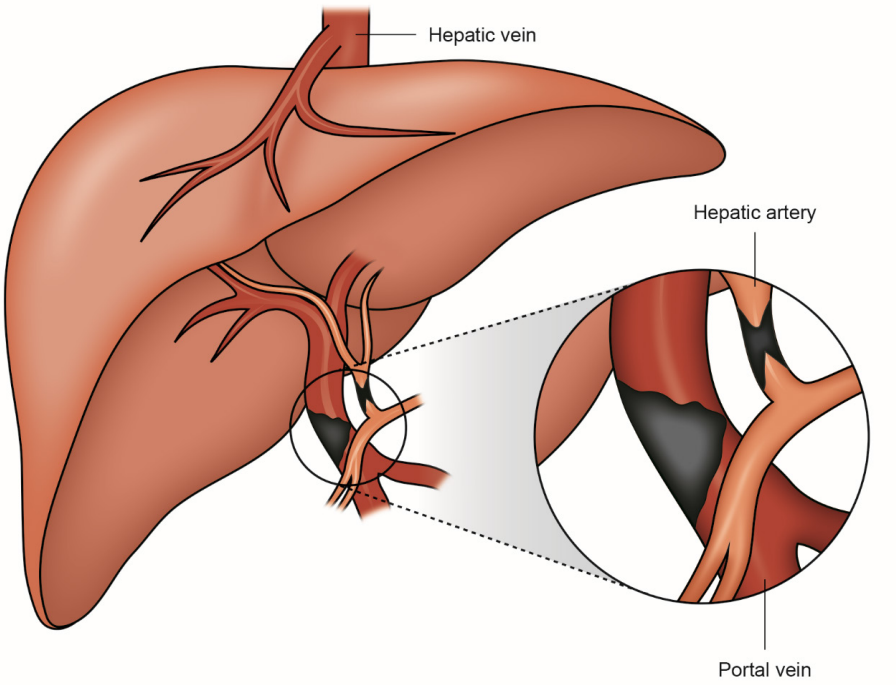
Besides an acquired hypercoagulable state related to hepatobiliary surgery, multiple additional risk factors for post- operative thrombotic events may be present in these patients, including (preoperative) cancer, local vascular abnormalities, local abnormalities in blood flow, presence of indwelling catheters, and prolonged postoperative immobilization.(64,65)

### *Partial Hepatectomy*

Venous thromboembolism (VTE) occurs after partial hepatectomy, with a reported incidence varying from 2.9 to 4.8%.6.(64–66) In one of the larger retrospective studies, VTE was found to be directly proportional to the magnitude of hepatectomy.(64) Major hepatectomy was associated with a threefold increase in the risk of VTE (1 VTE per 17 patients) compared with minor hepatectomy (1 VTE per 48 patients).(64)

Besides deep vein thrombosis and pulmonary embolism, portal vein thrombosis (PVT, Fig. 1) is a frequent complication after hepatectomy, with a reported incidence varying from 2.1 to 9.1%.(67,68) As portal venous flow is an important determinant of liver regeneration,(69–71) it is possible that reduced portal venous flow due to PVT results in delayed liver regeneration.

Figure1. Hepatic vessel thrombosis



### *Liver Transplantation*

Thrombotic events occurring after liver transplantation can be divided into local hepatic vessel thrombosis (Fig. 1) and systemic thrombotic complications. Hepatic vessel thrombosis poses a threat to both patient and graft survival. The incidence of hepatic artery thrombosis (HAT) is approximately 3 to 7%.(72–75) HAT may occur early (within 2–3 months) after transplantation, but may also occur years after the procedure.(75) Early HAT may result in necrosis of the bile ducts and eventually graft loss if the arterial flow is not restored in time.(73) In comparison with early HAT, late HAT might not be life threatening or even have clinical consequences because of the formation of collateral arterial circulation before total obstruction.(73) Preoperative hyper- coagulability, assessed by thromboelastography, has been shown to indicate an increased risk for postoperative HAT.(76) In addition, it has been shown that preoperative PVT is a risk factor for development of postoperative HAT, again suggesting that a relative hypercoagulable state predisposes to HAT.(77)



PVT complicates around 2 to 3.1% of liver transplantations.(7,72,78) Notably, the incidence of preexisting PVT discovered during surgery is considerably higher, 4.9 to 14%, with an even higher incidence in specific subgroups, such as patients with a malignancy.(7,79) The risk of PVT after liver transplantation is related to technical difficulties during surgery, prior PVT, a pediatric recipient, splenectomy, the use of venous conduits, and small portal vein size.(54) Early postoperative PVT can cause acute clinical deterioration because of ischemia, ascites, and increased portal vein pressure.(7,54) Early PVT is associated with an increased mortality compared with liver transplant recipients who do not develop a PVT.(8,72)

Systemic thrombotic complications may occur in the perioperative period, but also years after transplantation. Recently, several cohort studies have reported on the overall incidence of VTE after liver transplantation. These reports showed incidences varying between 4.5 and 8.6%.(80–84) Notably, the study that reported an incidence of 8.6% only considered the number of deep vein thrombosis,(82) an even higher incidence would likely have been found if pulmonary embolisms had been taken into account. Importantly, none of the patients in this study received pharmacological thromboprophylaxis.

Although less common, intraoperative thrombosis is of significant relevance due to the association with an increased morbidity and mortality.(45) Intraoperatively, acute intracardiac thrombosis or pulmonary embolism may occur, with an estimated incidence between 0.4 and 6.2%.(85–87) These complications are potentially fatal and appear to be more frequent in liver transplant recipients than in other surgical patients.(88) As previously argued; the current literature shows that a large proportion of the patients undergoing liver transplantation

develop a hypercoagulable state during surgery, which may contribute to the development of intraoperative or early postoperative thrombotic complications.(45) Intraoperative hypercoagulability was particularly frequent in patients with cholestatic disease, acute liver failure, and nonalcoholic steatohepatitis.(45)

## Prevention and Treatment of Bleeding

Hepatic resection is often accompanied by intraoperative blood loss primarily occurring during parenchymal transection or tumor resection. Similarly, liver transplantation may also cause excessive blood loss during surgery, which may lead to increased postoperative morbidity and mortality.(89) There are several approaches available to attempt to reduce intraoperative blood loss, as will be outlined below.

Central to our strategy to minimize blood loss is a restrictive fluid infusion policy. Multiple studies have demonstrated that maintenance of a low central venous pressure (CVP) and even a preoperative reduction of CVP by phlebotomy is a beneficial strategy in minimizing blood loss during hepatectomy or liver transplantation.(58,59,90,91) Our fluid restriction management includes the absence of routine prophylactic correction of abnormal coagulation tests (PT or point-of-care). Indeed, routine correction of abnormal coagulation tests with an infusion of fresh frozen plasma (FFP) is not effective in reducing intraoperative blood loss.(5,90) Moreover, preoperative coagulation tests have proven to be very poor predictors of intraoperative bleeding as reviewed in detail by Larsen et al in this issue.(92)

Treatment of bleeding during liver surgery traditionally consists of transfusion of FFP, fibrinogen concentrate or cryoprecipitate, and platelet concentrates guided by routine diagnostic test or point-of-care testing. Transfusion of large amounts of FFP may, in fact, be counterproductive as it leads to fluid overload and a subsequent increase in the central and portal venous pressure, which is already elevated in many cirrhotic patients. Prothrombin complex concentrate (PCC) may be used as an alternative to FFP. PCC is a low-volume plasma product that contains selected procoagulant proteins and the anticoagulant proteins S and C. The advantage of PCCs over FFP is the low volume and the potential to fully normalize factor levels, while the disadvantage is that PCCs do not contain all procoagulant factors. A recent single-center retrospective study of liver transplant recipients showed that a ROTEM (Tem International GmbH, Munich, Germany)-based approach to administering PCCs and/or fibrinogen concentrate was safe and effective as compared with an FFP/platelet concentrate-based approach.(93) Another low-volume prohemostatic, recombinant factor VIIa (rFVIIa) has been trialed in liver transplantation. A meta-analysis on the use of prophylactic rFVIIa during hepatic surgery, however, did not show efficacy on perioperative bleeding.(94) Although in this meta-analysis rFVIIa did not show an increase in the risk for thromboembolic events, the thrombotic risk

is of concern.(94) Position on the use of rFVIIa as a possible rescue agent in patients with intractable bleeding has yet to be defined.(95)

Whereas the evidence for the benefits of blood products in perioperative medicine is low, the supporting evidence for transfusion-related complications including transfusion associated lung injury, transfusion-associated circulatory overload, and infectious complications are increasingly acknowledged.(62) As in other types of surgery,(96,97) transfusion of blood products during liver surgery and liver transplantation has been associated with increased morbidity and mortality.(4) Our current practice is in general one of wait-and-see approach to start blood product transfusion only in actively bleeding patients with evidence of hemostatic abnormalities. Point- of-care testing by thromboelastography is used to guide blood product transfusion.

In the past two decades, improvements in surgical techniques have had an important impact in improving outcome after liver transplantation. Mainly the introduction of the piggyback technique (liver transplantation with preservation of the recipient vena cava) resulted in lower blood transfusion requirements compared with patients transplanted using the 'classical' technique.(98,99)



Although the cause of blood loss during liver transplantation is multifactorial, as noted earlier hyperfibrinolysis has been identified as an important component of the hemostatic dysfunction during this procedure. This has provided a scientific basis for the use of antifibrinolytic drugs, in an attempt to restore the balance between coagulation and fibrinolysis and to reduce blood loss. Tranexamic acid and aprotinin have been shown to reduce blood transfusion requirements by approximately 30% during liver transplantation by well-designed, placebo-controlled, randomized trials.(100–102)No increased risk of thromboembolic complications has been shown in any of the randomized controlled trials.

## Prevention and Treatment of Thrombosis

As the hemostatic system following liver surgery is balanced into a hypercoagulable state, with a corresponding risk of thrombotic events, a proactive approach to anticoagulant management after liver surgery appears warranted. Importantly, thromboprophylaxis should not be withheld from patients with a prolonged PT or low platelet count, as these factors unjustifiably suggest a hypocoagulable state and increased bleeding risk.

Following partial hepatectomy, pharmacological thromboprophylaxis has been shown to reduce the incidence of postoperative VTE.(6,64,66,103) However, since the risk of thrombotic events, is still appreciable even in those patients receiving optimal thromboprophylaxis, studies on safety and efficacy of more aggressive

thromboprophylactic strategies appear warranted. Notably, the current clinical practice appears suboptimal as a recent survey in the United States showed that although the vast majority of hepatobiliary surgeons would use thromboprophylaxis, many would delay heparin administration in patients with thrombocytopenia or a prolonged PT.(104) Also, only 14% of surveyed surgeons would continue prophylaxis after discharge. Nonetheless, awareness of the need for adequate postoperative pharmacologic thromboprophylaxis is increasing, leading to adaptations in clinical guidelines.(105)

Similarly, optimal prevention of thrombotic events following liver transplant surgery requires clinical studies. Whereas we know that a prolonged PT, in this particular study displayed as an increase in international normalized ration (INR), following liver transplantation does not protect from thrombotic disease, it was recently shown that those patients that developed a venous thrombosis following liver transplantation had a significantly higher INR at day 7 after transplantation compared with those that did not develop a thrombotic event.(80) These results suggest that delayed liver function recovery forms a risk for VTE following liver transplantation, and reinforce the notion that thrombo- prophylaxis should not be withheld from patients with a prolonged PT.

In liver transplantation, there is only one study that reports on the efficacy of pharmacological thromboprophylaxis to prevent systemic thrombosis using subcutaneous unfractionated heparin every 8 hours. The incidence of VTE in the non-heparin group was 3.5 versus 1% in the treated group.(84)In two other cohorts assessing incidence of VTE following liver transplantation, numbers on the use of prophylaxis were absent or only given when patients received anticoagulant treatment before surgery or when an intraoperative thrombectomy was performed.(80,81)

Pharmacological thromboprophylaxis may also help to prevent early PVT or HAT, but to our knowledge, the effect of routine thromboprophylaxis on PVT or HAT has never been assessed in the post livertransplant population. Thromboprophylaxis has been shown to reduce the risk for PVT following partial hepatectomy,(106) suggesting a role for anticoagulation in post-transplant hepatic vessel thrombosis. HAT is traditionally believed to be a surgical complication,(78,107,108) although patient- and graft-related factors such as prior liver transplantation, prolonged cold ischemic time, prolonged operating time, low recipient weight, acute rejection, hemodynamic, infectious and immunological factors, have also been reported to contribute.(74,76,109) Nevertheless, there is increasing amounts of data suggesting that changes in the hemostatic system may contribute to the development of HAT as well.(54,76) Endothelial damage and activation of the hemostatic system can be the result of cytomegalovirus infection (CMV). Supported by the reported

association of CMV with an increased risk of HAT,(109,110) screening, and early thromboprophylaxis and/or antiviral treatment should be considered.

Another possibility in the prevention of HAT is treatment with platelet inhibitors. Two independent studies have shown a significant incidence reduction of HAT by aspirin.(111,112) One of the studies reported a reduction in the overall incidence of HAT from 4.6 to 3.0%,(111)the second study reported an incidence reduction of late HAT from 3.6 to 0.6%.(112) Even though these studies are limited by their retrospective design and study populations are heterogeneous; there was a clear benefit of antiplatelet use without an increase in bleeding events. A further well-designed randomized study to explore safety and efficacy of aspirin to prevent HAT would be indicated. Next, to prophylaxis, early detection of HAT via screening with the regular use of Doppler ultrasound or contrast enhanced ultrasound could be considered.(73,75,78,113)



## Conclusion

Laboratory studies and clinical observations have changed the insights in the hemostatic status during and following hepatobiliary surgery. Whereas conventional hemostasis tests (platelet count, PT/INR) are suggestive of a perioperative- bleeding tendency, more advanced hemostatic tests indicate a balanced hemostasis with hypercoagulable features. The concept of maintenance of hemostatic balance with hypercoagulable features is reflected in the thrombotic risk following liver surgery. Nevertheless, intraoperative bleeding remains a concern, and further refinements in hemostatic management are required to decrease (excessive) blood loss in individual patients. Our management strategies include avoidance of prophylactic correction of abnormal hemostasis tests since they do not predict bleeding events. Blood loss can be minimized through surgical techniques and anesthesiological interventions including a restrictive fluid infusion policy. We advise to use blood products wisely and preferably only when active bleeding occurs. The use of blood products should be guided by conventional hemostasis tests or point-of-care testing, based on the local experience.

Because of a hypercoagulable postoperative state following liver surgery, we suggest initiating pharmacological thromboprophylaxis with low molecular weight heparin as soon as possible. We routinely start thromboprophylaxis at 6 hours after surgery unless active bleeding occurs. It is plausible that a higher dosage of postoperative thromboprophylaxis is needed for specific patient populations with increased risk of thrombotic complications. The prevalence of VTE in hepatobiliary surgery patients, even in those that receive early thromboprophylaxis, stresses the need for further research to optimize thromboprophylaxis in these patients.

## References


1. Lisman T, Leebeek FWG, De Groot PG. Haemostatic abnormalities in patients with liver disease. J Hepatol. 2002;37(2):280–7.
2. Romano F, Garancini M, Uggeri F, Degrate L, Nespoli L, Gianotti L, et al. Bleeding in hepatic surgery: Sorting through methods to prevent it. HPB Surg. 2012;2012.
3. Bismuth H, Castaing D, Garden JO. Major hepatic resection under total vascular exclusion. Ann Surg. 1989;(07):13–9.
4. De Boer MT, Molenaar IQ, Hendriks HGD, Slooff MJH, Porte RJ. Minimizing blood loss in liver transplantation: Progress through research and evolution of techniques. Dig Surg. 2005;22(4):265–75.
5. Westerkamp AC, Lisman T, Porte RJ. How to minimize blood loss during liver surgery in patient with cirrhosis. Hpb. 2009;11(6):453–8.
6. Reddy SK, Turley RS, Barbas AS, Steel JL, Tsung A, Marsh JW, et al. Post-Operative Pharmacologic Thromboprophylaxis after Major Hepatectomy. J Gastrointest Surg. 2011;15(9):1602–10.
7. Davidson BR, Gibson M, Dick R, Burroughs A, Rolles K. Incidence, risk factors, management, and outcome of portal vein abnormalities at orthotopic liver transplantation. Transplantation. 1994 Apr 27;57(8):1174–7.
8. Ghabril M, Agarwal S, Lacerda M, Chalasani N, Kwo P, Tector AJ. Portal Vein Thrombosis Is a Risk Factor for Poor Early Outcomes After Liver Transplantation: Analysis of Risk Factors and Outcomes for Portal Vein Thrombosis in Waitlisted Patients. Transplantation. 2016;100(1):126–33.
9. Lisman T, Porte RJ. Value of Preoperative Hemostasis Testing in Patients with Liver Disease for Perioperative Hemostatic Management. Anesthesiology. 2017;126(2):338–44.
10. Hugenholtz GCG, Northup PG, Porte RJ, Lisman T. Is there a rationale for treatment of chronic liver disease with antithrombotic therapy? Blood Rev. 2015;29(2):127–36.
11. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med. 2011;365(2):147–56.
12. Tripodi A. Liver Disease and Hemostatic (Dys) function. Semin Thromb Hemost. 2015;41(5):462–7.
13. Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease : evidence and clinical consequences. Blood. 2010;116(6):878–85.
14. Feltracco P, Barbieri S, Cillo U, Zanus G, Senzolo M, Ori C. Perioperative thrombotic complications in liver transplantation. World J Gastroenterol. 2015;8004–13.
15. Hartmann M, Szalai C. Hemostasis in liver transplantation: Pathophysiology, monitoring , and treatment. World J Gastroenterol. 2016;
16. Yoshizumi T, Itoh S, Imai D, Ikegami T, Ninomiya M, Iguchi T, et al. Impact of platelets and serotonin on liver regeneration after living donor hepatectomy. Transplant Proc. 2015;47(3):683–5.
17. Lesurtel M, Raptis DA, Melloul E, Schlegel A, Oberkofler C, El-Badry AM, et al. Low platelet counts after liver transplantation predict early posttransplant survival: the 60-5 criterion. Liver Transpl. 2014;20(2):147–55.

18. Richards EM, Alexander GJ, Calne RY, Baglin TP. Thrombocytopenia following liver transplantation is associated with platelet consumption and thrombin generation. Br J Haematol. 1997;98:315–21.
19. Lisman T, Bongers TN, Adelmeijer J, Janssen HLA, De Maat MPM, De Groot PG, et al. Elevated levels of von Willebrand factor in cirrhosis support platelet adhesion despite reduced functional capacity. Hepatology. 2006;44(1):53–61.
20. Hugenholtz GCG, Adelmeijer J, Meijers JCM, Porte RJ, Stravitz RT, Lisman T. An unbalance between von Willebrand factor and ADAMTS13 in acute liver failure: Implications for hemostasis and clinical outcome. Vol. 58, Hepatology. 2013. p. 752–61.
21. Kobayashi T, Wada H, Usui M, Sakurai H, Matsumoto T, Nobori T, et al. Decreased ADAMTS13 Levels in Patients after Living Donor Liver Transplantation. Thromb Res. 2009;124(5):541–5.
22. Groeneveld DJ, Alkozai EM, Adelmeijer J, Porte RJ, Lisman T. Balance between von Willebrand factor and ADAMTS13 following major partial hepatectomy. Br J Surg. 2016;103(6):735–43.
23. Pereboom ITA, Adelmeijer J, Van Leeuwen Y, Hendriks HGD, Porte RJ, Lisman T. Development of a severe von willebrand factor/adamts13 dysbalance during orthotopic liver transplantation. Am J Transplant. 2009;9(5):1189–96.
24. Kobayashi S-I, Yokoyama Y, Matsushita T, Kainuma M, Ebata T, Igami T, et al. Increased von Willebrand Factor to ADAMTS13 ratio as a predictor of thrombotic complications following a major hepatectomy. Arch Surg. 2012;147(10):909–17.
25. Chion CKNK, Doggen CJM, Crawley JTB, Lane DA, Rosendaal FR. ADAMTS13 and von Willebrand factor and the risk of myocardial infarction in men. Blood. 2007 Mar 1;109(5):1998–2000.
26. Andersson HM, Siegerink B, Luken BM, Crawley JTB, Algra A, Lane DA, et al. High VWF, low ADAMTS13, and oral contraceptives increase the risk of ischemic stroke and myocardial infarction in young women. Blood. 2012 Feb 9;119(6):1555–60.
27. Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, et al. Platelet-derived serotonin mediates liver regeneration. Science. 2006 Apr;312(5770):104–7.
28. López ML, Kieling CO, Uribe Cruz C, Osvaldt A, Ochs de Muñoz G, Meurer L, et al. Platelet increases survival in a model of 90% hepatectomy in rats. Liver Int. 2014;34(7):1049–56.
29. Murata S, Ohkohchi N, Matsuo R, Ikeda O, Myronovych A, Hoshi R. Platelets promote liver regeneration in early period after hepatectomy in mice. World J Surg. 2007;31(4):808–16.
30. Helmy A, Kishta S, Khaled E. Interrelation between peripheral platelet count and platelet activation during and after liver surgery in pigs. Blood Coagul Fibrinolysis. 2010 Apr;21(3):237–41.
31. Lisman T, Porte RJ. Mechanisms of platelet-mediated liver regeneration. Blood. 2016;128(5): 625–30.
32. Alkozai EM, Nijsten MW, de Jong KP, de Boer MT, Peeters PMJG, Slooff MJ, et al. Immediate Postoperative Low Platelet Count is Associated With Delayed Liver Function Recovery After Partial Liver Resection. Ann Surg. 2010 Feb;251(2):300–6.
33. Wang HQ, Yang J, Yang JY, Wang WT, Yan LN. Low immediate postoperative platelet count is associated with hepatic insufficiency after hepatectomy. World J Gastroenterol. 2014;20(33):11871–7.

34. Han S, Park H-W, Song JH, Gwak MS, Lee WJ, Kim G, et al. Association Between Intraoperative Platelet Transfusion and Early Graft Regeneration in Living Donor Liver Transplantation. Ann Surg. 2015 Dec;264(6):1065–72.
35. Marietta M, Facchini L, Pedrazzi P, Busani S, Torelli G. Pathophysiology of Bleeding in Surgery. Transplant Proc. 2006;38(3):812–4.
36. Kleiss SF, Adelmeijer J, Meijers JCM, Porte RJ, Lisman T. A sustained decrease in plasma fibrinolytic potential following partial liver resection or pancreas resection. Thromb Res. 2016;140:36–40.
37. Stahl RL, Duncan A, Hooks MA, Henderson MJ, Millikan WJ, Warren WD. A Hypercoagulable State Follows Orthotopic Liver Transplantation. Hepatology. 1990 Sep;12(3):553–8.
38. Potze W, Alkozai EM, Adelmeijer J, Porte RJ, Lisman T. Hypercoagulability following major partial liver resection - detected by thrombomodulin-modified thrombin generation testing. Aliment Pharmacol Ther. 2015 Jan;41(2):189–98.
39. Lisman T, Bakhtiari K, Pereboom ITA, Hendriks HGD, Meijers JCM, Porte RJ. Normal to increased thrombin generation in patients undergoing liver transplantation despite prolonged conventional coagulation tests. Journal of Hepatology. 2010. p. 355–61.
40. Mallett S V., Sugavanam A, Krzanicki DA, Patel S, Broomhead RH, Davidson BR, et al. Alterations in coagulation following major liver resection. Anaesthesia. 2016 Jun;71(6):657–68.
41. Barton JS, Riha GM, Differding JA, Underwood SJ, Curren JL, Sheppard BC, et al. Coagulopathy after a liver resection: Is it over diagnosed and over treated? Hpb. 2013;15(11):865–71.
42. De Pietri L, Montalti R, Begliomini B, Scaglioni G, Marconi G, Reggiani A, et al. Thromboelastographic changes in liver and pancreatic cancer surgery: hypercoagulability, hypocoagulability or normocoagulability? Eur J Anaesthesiol. 2010;27:608–16.
43. Cerutti E, Stratta C, Romagnoli R, Schellino MM, Skurzak S, Rizzetto M, et al. Thromboelastogram monitoring in the perioperative period of hepatectomy for adult living liver donation. Liver Transplant. 2004;10(2):289–94.
44. Louis SG, Barton JS, Riha GM, Orloff SL, Sheppard BC, Pommier RF, et al. The international normalized ratio overestimates coagulopathy in patients after major hepatectomy. Am J Surg. 2014;207(5):723–7.
45. Krzanicki D, Sugavanam A, Mallett S. Intraoperative hypercoagulability during liver transplantation as demonstrated by thromboelastography. Liver Transplant. 2013 Aug;19(8):852–61.
46. Weinberg L, Scurrah N, Parker FC, Dauer R, Marshall J, McCall P, et al. Markers of coagulation activation after hepatic resection for cancer: evidence of sustained upregulation of coagulation. Anaesth Intensive Care. 2011 Sep;39(5):847–53.
47. Lisman T, Ariëns RAS. Alterations in Fibrin Structure in Patients with Liver Diseases. Semin Thromb Hemost. 2016;42(4):389–96.
48. Tanaka KA, Key NS, Levy JH. Blood coagulation: Hemostasis and thrombin regulation. Anesth Analg. 2009;108(5):1433–46.
49. Martinez J, Palascak JE, Kwasniak D. Abnormal sialic acid content of the dysfibrinogenemia associated with liver disease. J Clin Invest. 1978;61(2):535–8.
50. Hugenholtz GCG, Macrae F, Adelmeijer J, Dulfer S, Porte RJ, Lisman T, et al. Procoagulant changes in fibrin clot structure in patients with cirrhosis are associated with oxidative

modifications of fibrinogen. J Thromb Haemost. 2016 May;14(5):1054–66.

51. Groeneveld DJ, Adelmeijer J, Hugenholtz GCG, Ariëns RAS, Porte RJ, Lisman T. Ex vivo addition of fibrinogen concentrate improves the fibrin network structure in plasma samples taken during liver transplantation. Vol. 13, J Thromb Haemost. 2015. p. 2192–201.
52. Arshad F, Adelmeijer J, Blokzijl H, van den Berg A, Porte R, Lisman T. Abnormal hemostatic function one year after orthotopic liver transplantation can be fully attributed to endothelial cell activation. F1000Research. 2014 Jul 30;3:1–18.
53. Porte RJ, Bontempo FA, Knot EAR, Lewis JH, Kang YG, Starzl TE. Tissue-Type-Plasminogen-Activator-Associated Fibrinolysis in Othotopic liver transplantation. Transpl Proc. 1989;21(3):3542.
54. Arshad F, Lisman T, Porte RJ. Hypercoagulability as a contributor to thrombotic complications in the liver transplant recipient. Liver Int. 2013;33(6):820–7.
55. Kassis J, Hirsh J, Podor TJJ. Evidence that postoperative fibrinolytic shutdown is mediated by plasma factors that stimulate endothelial cell type I plasminogen activator inhibitor biosynthesis. Blood. 1992;80(7):1758–64.
56. Ruitenbeek K, Meijers JCM, Adelmeijer J, Hendriks HGD, Porte RJ, Lisman T. Intact thrombomodulin-mediated regulation of fibrinolysis during and after liver transplantation, despite a profoundly defective thrombomodulin-mediated regulation of coagulation. J Thromb Haemost. 2010 Apr 16;8(7):1646–9.
57. Arshad F, Ickx B, van Beem RT, Polak W, Grüne F, Nevens F, et al. Prothrombin complex concentrate in the reduction of blood loss during orthotopic liver transplantation: PROTON-trial. BMC Surg. 2013;13(1):22.
58. Smyrniotis V, Kostopanagiotou G, Theodoraki K, Tsantoulas D, Contis JC. The role of central venous pressure and type of vascular control in blood loss during major liver resections. Am J Surg. 2004;187(3):398–402.
59. Jones RM, Moulton CE, Hardy KJ. Central venous pressure and its effect on blood loss during liver resection. Br J Surg. 1998;85(8):1058–60.
60. Cleland S, Corredor C, Ye JJ, Srinivas C, Mccluskey SA. Massive haemorrhage in liver transplantation: Consequences, prediction and management. World J Transpl. 2016;6(2):291–305.
61. Ricci P, Therneau TM, Malinchoc M, Benson JT, Petz JL, Klintmalm GB, et al. A prognostic model for the outcome of liver transplantation in patients with cholestatic liver disease. Hepatology. 1997;25(3):672–7.
62. de Boer MT, Christensen MC, Asmussen M, van der Hilst CS, Hendriks HGD, Slooff MJH, et al. The Impact of Intraoperative Transfusion of Platelets and Red Blood Cells on Survival After Liver Transplantation. Anesth Analg. 2008 Jan;106(1):32–44.
63. Pereboom ITA, De Boer MT, Haagsma EB, Hendriks HGD, Lisman T, Porte RJ. Platelet transfusion during liver transplantation is associated with increased postoperative mortality due to acute lung injury. Vol. 108, Anesthesia and Analgesia. 2009. p. 1083–91.
64. Tzeng C-WD, Katz MHG, Fleming JB, Pisters PWT, Lee JE, Abdalla EK, et al. Risk of venous thromboembolism outweighs post-hepatectomy bleeding complications: analysis of 5651 National Surgical Quality Improvement Program patients. HPB. 2012 Aug;14(8):506–13.

65. Turley RS, Reddy SK, Shortell CK, Clary BM, Scarborough JE. Venous Thromboembolism After Hepatic Resection: Analysis of 5,706 Patients. J Gastrointest Surg. 2012;16(9):1705–14.
66. Hayashi H, Morikawa T, Yoshida H, Motoi F, Okada T, Nakagawa K, et al. Safety of postoperative thromboprophylaxis after major hepatobiliary- pancreatic surgery in Japanese patients. Surg Today. 2014;44(9):1660–8.
67. Yoshiya S, Shirabe K, Nakagawara H, Soejima Y, Yoshizumi T, Ikegami T, et al. Portal vein thrombosis after hepatectomy. World J Surg. 2014;38(6):1491–7.
68. Kuboki S, Shimizu H, Ohtsuka M, Kato A, Yoshitomi H, Furukawa K, et al. Incidence, risk factors, and management options for portal vein thrombosis after hepatectomy: a 14-year, single-center experience. Am J Surg. 2015;210(5):878–885.e2.
69. Marubashi S, Sakon M, Nagano H, Gotoh K, Hashimoto K, Kubota M, et al. Effect of portal hemodynamics on liver regeneration studied in a novel portohepatic shunt rat model. Surgery. 2004;136(5):1028–37.
70. Abshagen K, Eipel C, Vollmar B. A critical appraisal of the hemodynamic signal driving liver regeneration. Langenbeck's Arch Surg. 2012;397(4):579–90.
71. Wu T-J, Dahiya D, Lee C-S, Lee C-F, Chou H-S, Chan K-M, et al. Impact of portal venous hemodynamics on indices of liver function and graft regeneration after right lobe living donor liver transplantation. Liver Transplant. 2011 Mar;13(3):1035–45.
72. Duffy JP, Hong JC, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, et al. Vascular Complications of Orthotopic Liver Transplantation: Experience in More than 4,200 Patients. J Am Coll Surg. 2009;208(5):896–903.
73. Ma L, Lu Q, Luo Y. Vascular complications after adult living donor liver transplantation: Evaluation with ultrasonography. World J Gastroenterol. 2016;22(4):1617–26.
74. Bekker J, Ploem S, De Jong KP. Early hepatic artery thrombosis after liver transplantation: A systematic review of the incidence, outcome and risk factors. Vol. 9, American Journal of Transplantation. 2009. p. 746–57.
75. Mourad MM, Liossis C, Gunson BK, Mergental H, Isaac J, Muiesan P, et al. Etiology and management of hepatic artery thrombosis after adult liver transplantation. Liver Transplant. 2014 Jun;20(6):713–23.
76. Eldeen FZ, Roll GR, Derosas C, Rao R, Khan MS, Gunson BK, et al. Preoperative thromboelastography as a sensitive tool predicting those at risk of developing early hepatic artery thrombosis following adult liver transplantation. Transplantation. 2016;100(11):2382–90.
77. Stine JG, Pelletier SJ, Schmitt TM, Porte RJ, Northup PG. Pre-transplant portal vein thrombosis is an independent risk factor for graft loss due to hepatic artery thrombosis in liver transplant recipients. Int Hepato-Pancreato-Biliary Assoc. 2016;18(3):279–86.
78. Jia Y, Lu Q, Gong S, Ma B, Wen X, Peng Y, et al. Postoperative complications in patients with portal vein thrombosis after liver transplantation : Evaluation with Doppler ultrasonography. World J Gastroenterol. 2007;13(34):4636–40.
79. Werner KT, Sando S, Carey EJ, Vargas HE, Byrne TJ, Douglas DD, et al. Portal vein thrombosis in patients with end stage liver disease awaiting liver transplantation: Outcome of anticoagulation. Dig Dis Sci. 2013;58(6):1776–80.
80. Emuakhagbon V, Philips P, Agopian V, Kaldas FM, Jones CM. Incidence and risk factors for deep venous thrombosis and pulmonary embolus after liver transplantation. Am J Surg. 2016;211(4):768–71.

81. Blasi A, Hernandez V, Fernandez J, Colmenero J, Beltran J, Garcia-Valdecasas JC, et al. Venous Thrombotic Events After Liver Transplantation. Clin Appl Thromb. 2016 Nov 28;(Epub ahead of print):107602961668047.
82. Annamalai A, Kim I, Sundaram V, Klein A. Incidence and risk factors of deep vein thrombosis after liver transplantation. Vol. 46, Transplantation Proceedings. 2014. p. 3564–9.
83. Salami A, Qureshi W, Kuriakose P, Moonka D, Yoshida A, Abouljoud M. Frequency and predictors of venous thromboembolism in orthotopic liver transplant recipients: A single-center retrospective review. Vol. 45, Transplantation Proceedings. 2013. p. 315–9.
84. Yip J, Bruno DA, Burmeister C, Kazimi M, Yoshida A, Abouljoud MS, et al. Deep Vein Thrombosis and Pulmonary Embolism in Liver Transplant Patients: Risks and Prevention. Transplant direct. 2016;2(4):e68.
85. Peiris P, Pai S-L, Aniskevich S, Crawford CC, Torp KD, Ladlie BL, et al. Intracardiac thrombosis during liver transplant: A 17-year single-institution study. Liver Transplant. 2015 Oct;21(10):1280–5.
86. Xia VW, Ho JK, Nourmand H, Wray C, Busuttil RW, Steadman RH. Incidental intracardiac thromboemboli during liver transplantation: Incidence, risk factors, and management. Liver Transplant. 2010;16(12):1421–7.
87. Sakai T, Matsusaki T, Dai F, Tanaka KA, Donaldson JB, Hilmi IA, et al. Pulmonary thromboembolism during adult liver transplantation: incidence, clinical presentation, outcome, risk factors, and diagnostic predictors. Br J Anaesth. 2012 Mar 1;108(3):469–77.
88. Warnaar N, Molenaar IQ, Colquhoun SD, Slooff MJH, Sherwani S, De Wolf AM, et al. Intraoperative pulmonary embolism and intracardiac thrombosis complicating liver transplantation: A systematic review. J Thromb Haemost. 2008;6(2):297–302.
89. Kappa SF, Gorden DL, Davidson MA, Wright JK, Guillamondegui OD. Intraoperative blood loss predicts hemorrhage-related reoperation after orthotopic liver transplantation. Am Surg. 2010 Sep;76(9):969–73.
90. Massicotte L, Lenis S, Thibeault L, Sassine M-P, Seal RF, Roy A. Effect of low central venous pressure and phlebotomy on blood product transfusion requirements during liver transplantations. Liver Transplant. 2006 Jan;12(1):117–23.
91. Li Z, Sun YM, Wu FX, Yang LQ, Lu ZJ, Yu WF. Controlled low central venous pressure reduces blood loss and transfusion requirements in hepatectomy. World J Gastroenterol. 2014;20(1):303–9.
92. Larsen JB, Hvas AM. Predictive value of whole blood and plasma coagulation tests for intra- and postoperative bleeding risk: A systematic review. Semin Thromb Hemost. 2017;43(7):772–805.
93. Kirchner C, Dirkmann D, Treckmann JW, Paul A, Hartmann M, Saner FH, et al. Coagulation management with factor concentrates in liver transplantation: A single-center experience. Transfusion. 2014;54(1):2760–8.
94. Chavez-Tapia NC, Alfaro-Lara R, Tellez-Avila F, Barrientos-Gutiérrez T, González-Chon O, Mendez-Sanchez N, et al. Prophylactic activated recombinant factor VII in liver resection and liver transplantation: Systematic review and meta-analysis. PLoS One. 2011;6(7):1–8.
95. Lisman T, Porte RJ. Recombinant factor VIIa to treat severe bleeding in patients with liver disease: Pitfalls and possibilities. J Hepatol. 2011;55(4):950–1.

96. Spiess BD. Transfusion of blood products affects outcome in cardiac surgery. Semin Cardiothorac Vasc Anesth. 2004 Dec;8(4):267–81.

97. Jia Q, Brown MJ, Clifford L, Wilson GA, Truty MJ, Stubbs JR, et al. Prophylactic plasma transfusion for surgical patients with abnormal preoperative coagulation tests: a single-institution propensity-adjusted cohort study. Lancet Haematol. 2016 Mar;3(3):e139–48.

98. Miyamoto S, Polak WG, Geuken E, Peeters PMJG, Jong KP, Porte RJ, et al. Liver transplantation with preservation of the inferior vena cava. A comparison of conventional and piggyback techniques in adults. Clin Transplant. 2004 Dec;18(6):686–93.

99. Jovine E, Mazziotti A, Grazi GL, Ercolani G, Masetti M, Morganti M, et al. Piggy-back versus conventional technique in liver transplantation: Report of a randomized trial. Vol. 10, Transplant International. 1997. p. 109–12.

100. Porte RJ, Molenaar IQ, Begliomini B, Groenland TH, Januszkiewicz A, Lindgren L, et al. Aprotinin and transfusion requirements in orthotopic liver transplantation: a multicentre randomised double-blind study. EMSALT Study Group. Lancet. 2000 Apr 15;355(9212):1303–9.

101. Findlay JY, Rettke SR, Ereth MH, Plevak DJ, Krom RAF, Kufner RP. Aprotinin reduces red blood cell transfusion in orthotopic liver transplantation: A prospective, randomized, double-blind study. Liver Transplant. 2001 Sep;7(9):802–7.

102. Dalmau A, Sabate A, Koo M, Bartolome C, Rafecas A, Figueras J, et al. The prophylactic use of tranexamic acid and aprotinin in orthotopic liver transplantation: a comparative study. Vol. 10, Liver Transpl. 2004. p. 279–84.

103. Ejaz A, Spolverato G, Kim Y, Lucas DL, Lau B, Weiss M, et al. Defining Incidence and Risk Factors of Venous Thromboemolism after Hepatectomy. J Gastrointest Surg. 2014 Jun 13;18(6):1116–24.

104. Weiss MJ, Kim Y, Ejaz A, Spolverato G, Haut ER, Hirose K, et al. Venous thromboembolic prophylaxis after a hepatic resection: Patterns of care among liver surgeons. Vol. 16, Hpb. 2014. p. 892–8.

105. Aloia TA, Geerts WH, Clary BM, Day RW, Hemming AW, D'Albuquerque LC, et al. Venous Thromboembolism Prophylaxis in Liver Surgery. J Gastrointest Surg. 2016 Jan 21;20(1):221–9.

106. Yamashita Y-I, Bekki Y, Imai D, Ikegami T, Yoshizumi T, Ikeda T, et al. Efficacy of postoperative anticoagulation therapy with enoxaparin for portal vein thrombosis after hepatic resection in patients with liver cancer. Thromb Res. 2014;134(4):826–31.

107. Tiao GM, Alonso M, Bezerra J, Yazigi N, Heubi J, Balistreri W, et al. Liver transplantation in children younger than 1 year-the Cincinnati experience. J Pediatr Surg. 2005;40(1):268–73.

108. Zhao J-C, Lu S-C, Yan L-N, Li B, Wen T-F, Zeng Y, et al. Incidence and treatment of hepatic artery complications after orthotopic liver transplantation. World J Gastroenterol. 2003;9(12):2853–5.

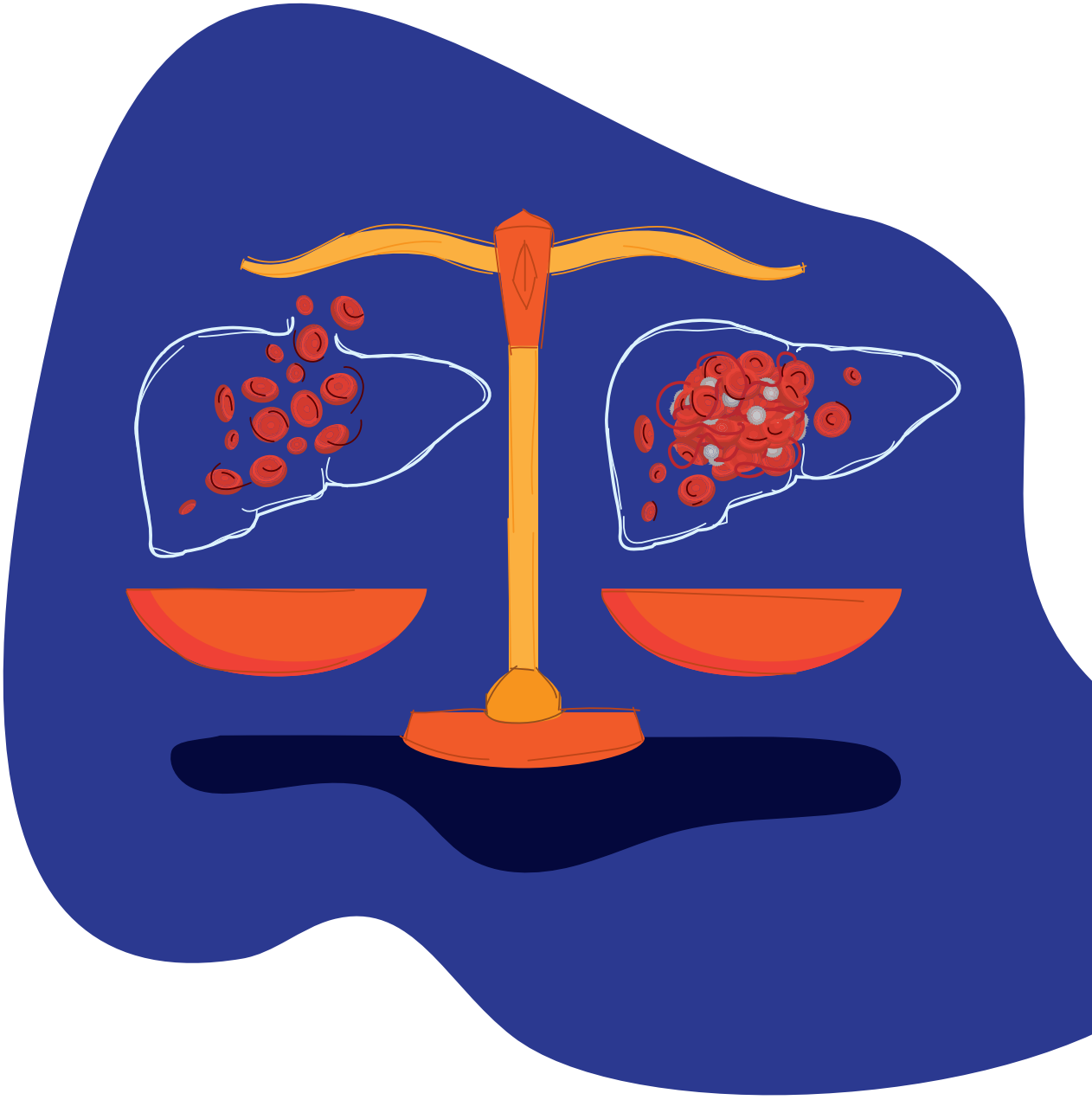
109. Silva MA, Jambulingam PS, Gunson BK, Mayer D, Buckels JAC, Mirza DF, et al. Hepatic artery thrombosis following orthotopic liver transplantation: A 10-year experience from a single centre in the United Kingdom. Liver Transplant. 2006 Jan;12(1):146–51.

110. Madalosso C, de Souza NF, Ilstrup DM, Wiesner RH, Krom RA. Cytomegalovirus and its association with hepatic artery thrombosis after liver transplantation. Transplantation. 1998 Aug 15;66(3):294–7.

111. Shay R, Taber D, Pilch N, Meadows H, Tischer S, McGillicuddy J, et al. Early Aspirin Therapy May Reduce Hepatic Artery Thrombosis in Liver Transplantation. Transplant Proc. 2013 Jan;45(1):330–4.

112. Vivarelli M, La Barba G, Cucchetti A, Lauro A, Del Gaudio M, Ravaioli M, et al. Can antiplatelet prophylaxis reduce the incidence of hepatic artery thrombosis after liver transplantation? Liver Transplant. 2007 May;13(5):651–4.

113. Hsiao CY, Ho CM, Wu YM, Ho MC, Hu RH, Lee PH. Management of early hepatic artery occlusion after liver transplantation with failed rescue. World J Gastroenterol. 2015;21(44):12729–34.



# CHAPTER 6.

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

Sarah Bos, Bente van den Boom, Tsai-Wing Ow, Andreas Prachialis, Jelle Adelmeijer, Anju Phoolchund, Zoka Milan, Mark Roest, Nigel Heaton, William Bernal, Ton Lisman

Journal of Thrombosis and Haemostasis
2020 Nov;18(11):2840-2851

## Abstract

*Background*: in vitro efficacy of pro- and anti-hemostatic 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 transplantation (OLT), 20 partial hepatectomies and 20 pylorus-preserving pancreaticoduodenectomies (PPPD). 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 to controls. Dabigatran showed a more pronounced anticoagulant effect in patients compared to controls while 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 as compared to 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.

## Introduction

Major hepato-pancreatico-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) due to 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 prior to 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 less often recombinant factor VIIa (rFVIIa). Fresh frozen plasma 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, while 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 in 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 anti-hemostatic 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, as 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.

## Methods

### *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 pancreatico-duodenectomy (PPPD), who had given written informed consent were included in this study. Twenty patients per group were recruited. Exclusion criteria were: age below 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 employed, blood samples of 42 healthy individuals were utilised. 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 seven days prior to inclusion. The study was approved by NRES Committee London – Westminster, Study Number 17/LO/0527.

### *Blood samples*

Blood samples for analyses were taken into 3.2% sodium citrate tubes at the time points indicated below. Samples were drawn by venepuncture from controls and in the post-operative period using a 21G needle using minimal stasis. Intraoperatively, blood was drawn from non-heparinised indwelling vascular catheters already placed by the anaesthesiologist. The citrate tube was always taken after taking a serum or EDTA tube that was used for routine diagnostic testing. In those patients receiving post-operative thromboprophylaxis, post-operative samples were taken just before administration of 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 | Post-operative day 1 |
| 4 | At the end of surgery | Post-operative day 3 |
| 5 | Post-operative day 1 | Post-operative day 6 |
| 6 | Post-operative day 3 | |
| 7 | Post-operative day 6 | |

*pylorus preserving pancreatico-duodenectomy

Blood samples were directly taken from the operating theatre or clinical ward to the laboratory by the clinical investigators (SB and BvdB) who immediately processed the samples. Platelet-poor plasma was obtained by centrifuging blood samples at 18°C for 10 minutes at 2,000g and subsequently for 10 minutes at 10,000g within 30 minutes after blood collection. Plasma samples were then

stored at -80°C until use.

### *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 fresh frozen plasma [FFP] transfusion – obtained by combining plasma from >200 healthy volunteers, a generous gift from Dr. J.C. Meijers, Academic Medical Center Amsterdam, The Netherlands) – final concentration 20% (v/v)
- Cofact (a 4-factor prothrombin complex concentrate (PCC), Sanquin, Amsterdam, Netherlands) – final concentration 0.5 U/ml
- Recombinant factor VIIa (Novo Nordisk, Bagsvaerd, Denmark) – final concentration 50 nM
- The low molecular weight heparin (LMWH) Clexane (Sanofi-Aventis BV, Gouda, the Netherlands) – final concentration 0.2 U/ml
- Unfractionated heparin (UFH, 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 which 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 to 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.

### *Thrombin generation*

The thrombin generation test was performed using platelet-poor plasma with Calibrated Automated Thrombography® in absence or presence of the above-

mentioned agents. Coagulation was activated using commercially available reagents containing recombinant tissue factor (final concentration 5 pM), phospholipids (final concentration 4 μM), in the presence of soluble thrombomodulin (TM, 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 CaCl2 (FluCa-kit, Thrombinoscope BV, Maastricht, The Netherlands) 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, Helsinki, Finland). All procedures were undertaken according to the protocol suggested by Thrombinoscope B.V.

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.



### *Coagulation parameters.*

The INR was assessed with commercially available methods on an automated coagulation analyser (ACL 300 TOP) with reagents (Recombiplastin 2G) and protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands).

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

### *Statistical analyses*

Data are expressed as means (with standard deviations [SDs]), medians [with interquartile ranges], or numbers (with percentages) as appropriate. Multiple groups were compared using One-way ANOVA or Kruskal-Wallis H test as appropriate. P values of 0.05 or less were considered statistically significant. Statistical analyses were performed with Graph Pad Prism (San Diego, USA) and IBM SPSS Statistics 23.0 (IBM, Chicago, USA).

# Results

### *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 2 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, 7 did not have their blood drawn at day 6.

### *In vitro efficacy of pro- and anti-hemostatic 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 to controls in patients at the start of OLT, and further prolonged during transplantation, with a normalization at post-operative 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 post-operative normalization (supplementary figure 1). In contrast, patients generated more thrombin as compared to controls at each time point, in agreement with our previously published data (supplementary figure 1).(31)

We next studied changes in total thrombin generation by in vitro addition of commonly used pro- and anti-coagulant agents. Figure 1 shows absolute ETP values of controls and intraoperative plasma samples in absence or presence of procoagulant agents, and figure 2 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 to controls. Supplementary table 1 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.

**Table 1. Patient characteristics**

| | OLT (n=20) | P-HEP (n=20) | PPPD (n=20) | Controls (n=42) |
|---|---|---|---|---|
| Age, years | 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] | |
| Creatinin, umol/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, µmol/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, days | 13 [10-16] | 8 [6-10] | 14 [9-18] | - |
| Thromboprophylaxis, n (%) | 18 (90)* | 18 (90) * | 20 (100) * | - |
| Postoperative VTE, n (%) | 1 (5) | 0 (0) | 1 (5) | |
| *Surgical indications* | | | | |
| Metastatic disease, n (%) | - | 7 (35) | | |
| Hepatocellular carcinoma, n (%) | - | 4 (20) | | |
| Neuroendocrine tumor, n (%) | - | 1 (5) | | |
| Cholangiocarcinoma, n (%) | - | 1 (5) | | |
| Epitelioid 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) | |

OLT, orthotopic liver transplantation; PPPD, pylorus-preserving pancreaticoduodenectomy; P-HEP, partial hepatectomy; WBC, white blood cell; RBC, red blood cell; FFP fresh frozen plasma; numbers are represented as median [IQR], mean [SD] or numbers (%).* thromboprophylaxis 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.

**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) |
| Auto-immune | | 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) |

MELD, model for end-stage liver disease, CTP, Child Turcotte Pugh, PBC, primary biliary cholangitis, PSC, primary sclerosing cholangitis, NASH, non-alcoholic steatohepatitis, ALD, alcoholic liver disease, HBV, hepatitis B virus, HCV, hepatitis C virus, HCC, hepatocellular carcinoma, CIT, cold ischemia time, WIT, warmth ischemia time. Numbers are represented as median [IQR] or numbers (%)

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 to controls at all post-operative days. Addition of UFH led to a more pronounced, but non-significant, decrease in thrombin generation in patients compared to controls, but the absolute ETP values in the presence of heparin were similar or even higher in patients compared to controls (figure 2).

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

### *In vitro efficacy of pro- and anti-hemostatic 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 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 (supplementary figure 2). Patients generated more thrombin as compared to controls throughout the procedure, which is in line with our previously published data(19), except for elevated thrombin generation at baseline (Supplementary table 2).



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. Supplementary table 2 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 recombinant factor VIIa 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 due to 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 post-operative samples compared to controls. Dabigatran showed a larger relative decrease of ETP in patients compared to controls, but absolute values in the presence of dabigatran were comparable or even higher in patients. Rivaroxaban was much less effective in patients compared to controls, with ETP values in the presence of drug exceeding ETP values in controls in absence of drug.

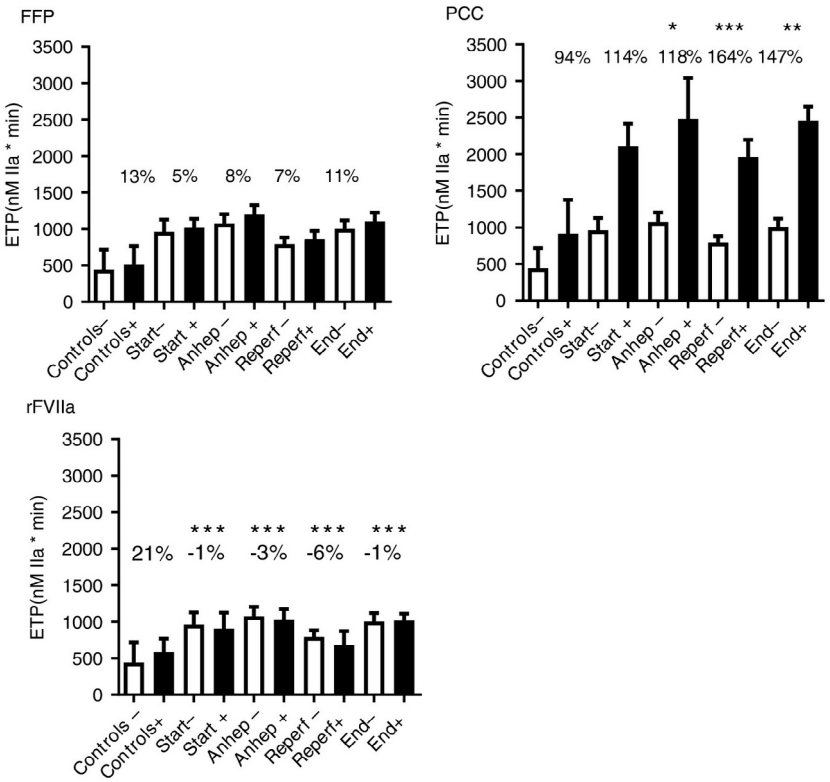



**Figure 1.** Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients during OLT prior to (-) and after (+) in vitro addition of prohemostatic agents. Start is after induction of anesthesia, anhep is 30 minutes after the start of the anhepatic phase, reperf is 30 minutes after reperfusion, 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<0.05 vs controls, ** P<0.01 vs controls, *** P<0.001 vs controls. FFP = fresh frozen plasma, PCC = prothrombin complex concentrate, rFVIIa = recombinant factor VIIa.

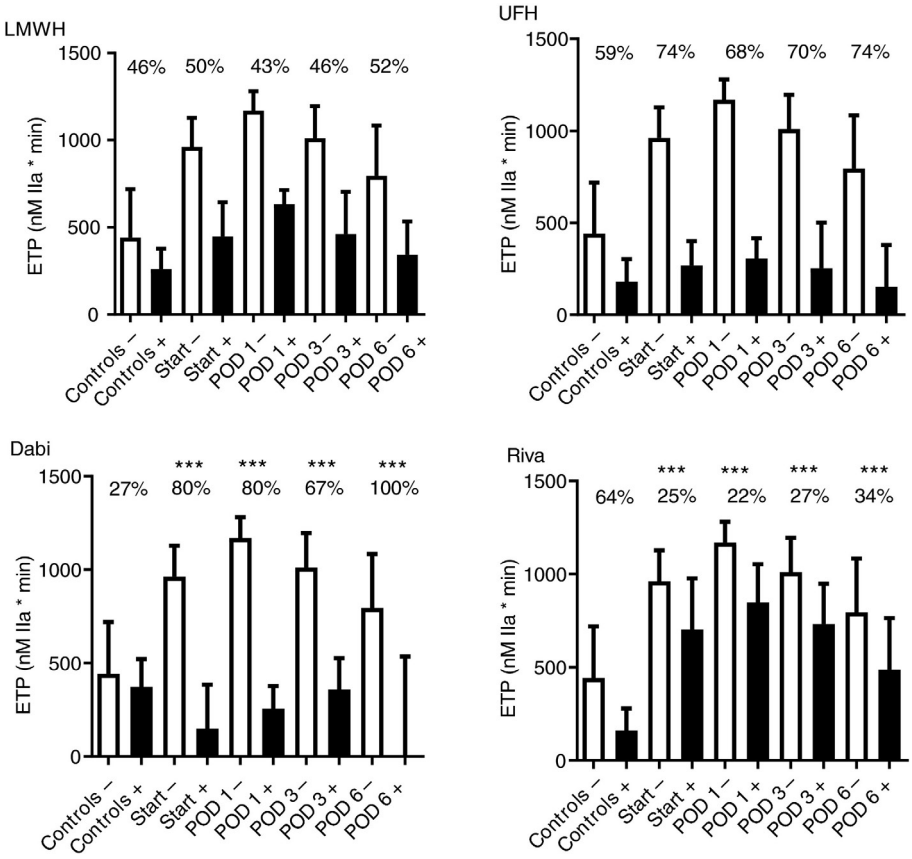



**Figure 2.** Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients after OLT prior to (-) and after (+) in vitro addition of anticoagulants. Start is after induction of anesthesia, POD = postoperative day. Shown are medians with error bars indicating 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<0.001 vs controls. LMWH = low molecular weight heparin, UFH = unfractionated heparin, Dabi = dabigatran, Riva = rivaroxaban.

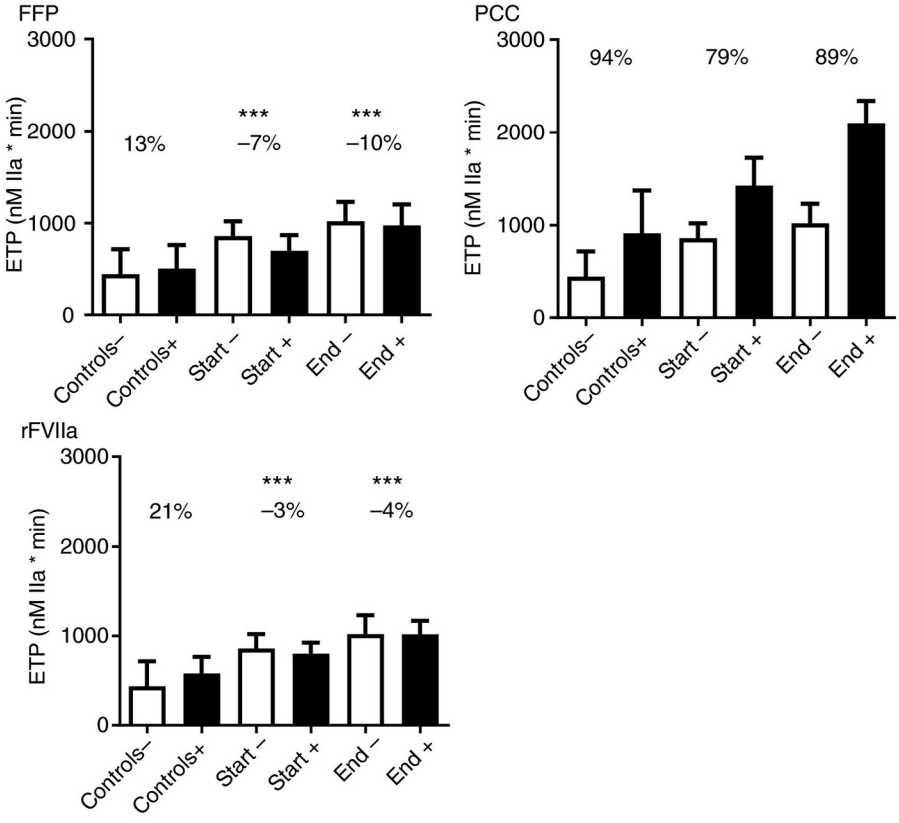



**Figure 3.** Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients during partial hepatectomy prior to (-) and after (+) in vitro addition of prohemostatic agents. Start is after induction of anesthesia, end is at the end of surgery. Shown are 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<0.001 vs controls. FFP = fresh frozen plasma, PCC = prothrombin complex concentrate, rFVIIa = recombinant factor VIIa.

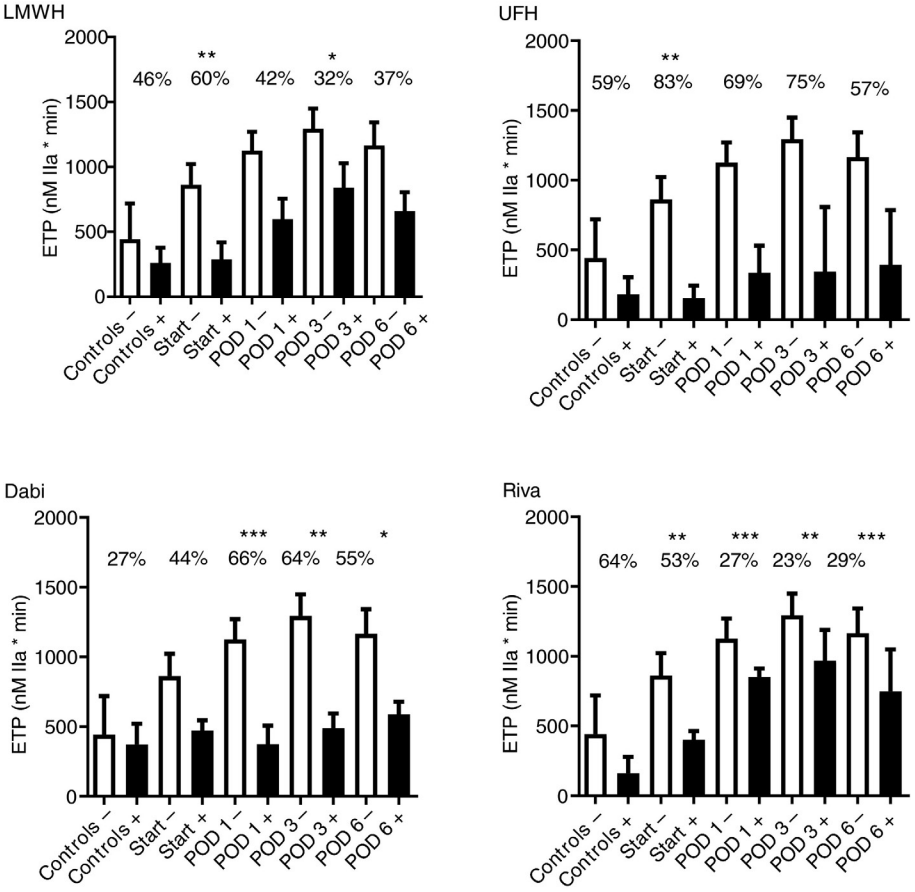



**Figure 4.** Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients after partial hepatectomy prior to (-) and after (+) in vitro addition of anticoagulants. Start is after induction of anesthesia, POD = postoperative day. Shown are medians with 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<0.05 vs controls, ** P<0.01 vs controls, *** P<0.001 vs controls. LMWH = low molecular weight heparin, UFH = unfractionated heparin, Dabi = dabigatran, Riva = rivaroxaban.

### *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 (supplementary figure 3). Patients generated more thrombin as compared to controls throughout the procedure, which is in contrast with our previously published data(19), in which we reported normal thrombin generation (Supplementary table 3).

The pro-hemostatic 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 figure 5 and 6. Statistical differences indicated are differences in proportional change in ETP upon addition of pro- or anticoagulants between controls and patients. Supplementary table 3 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 to 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 to controls, but with higher absolute ETPs in presence of drug in patients, particularly with LMWH. Rivaroxaban had very poor anticoagulant effects both in relative and absolute terms.

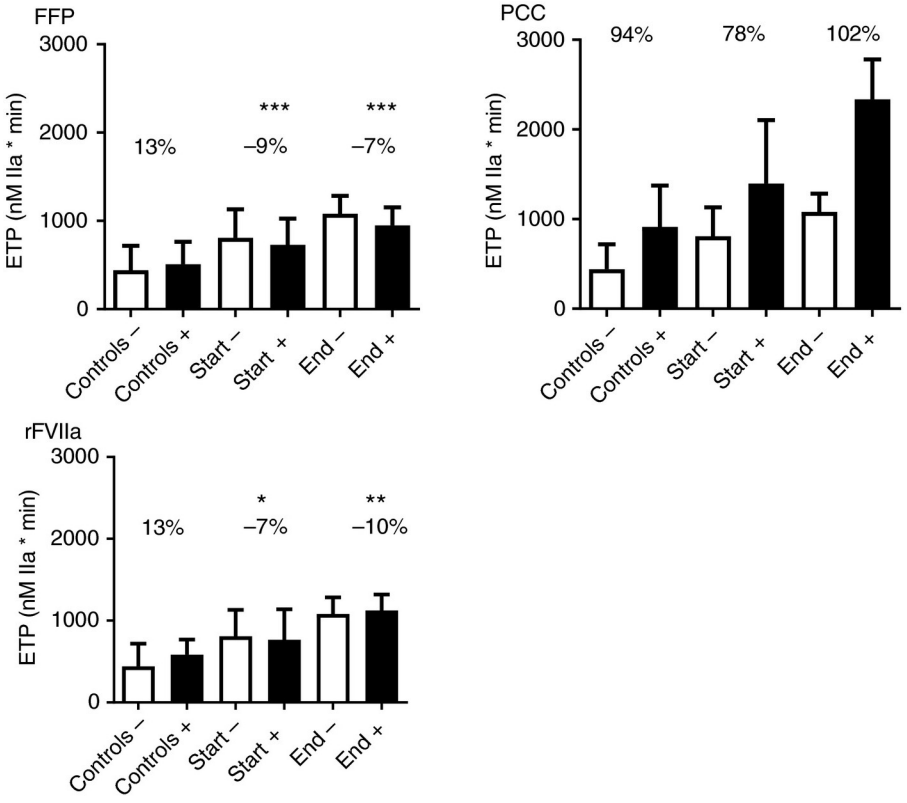



**Figure 5.** Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients after PPPD prior to (-) and after (+) in vitro addition of prohemostatic agents. Start is after induction of anesthesia, end is at the end of surgery. Shown are medians with 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<0.05 vs controls, ** P<0.01 vs controls, *** P<0.001 vs controls. FFP = fresh frozen plasma, PCC = prothrombin complex concentrate, rFVIIa = recombinant factor VIIa.

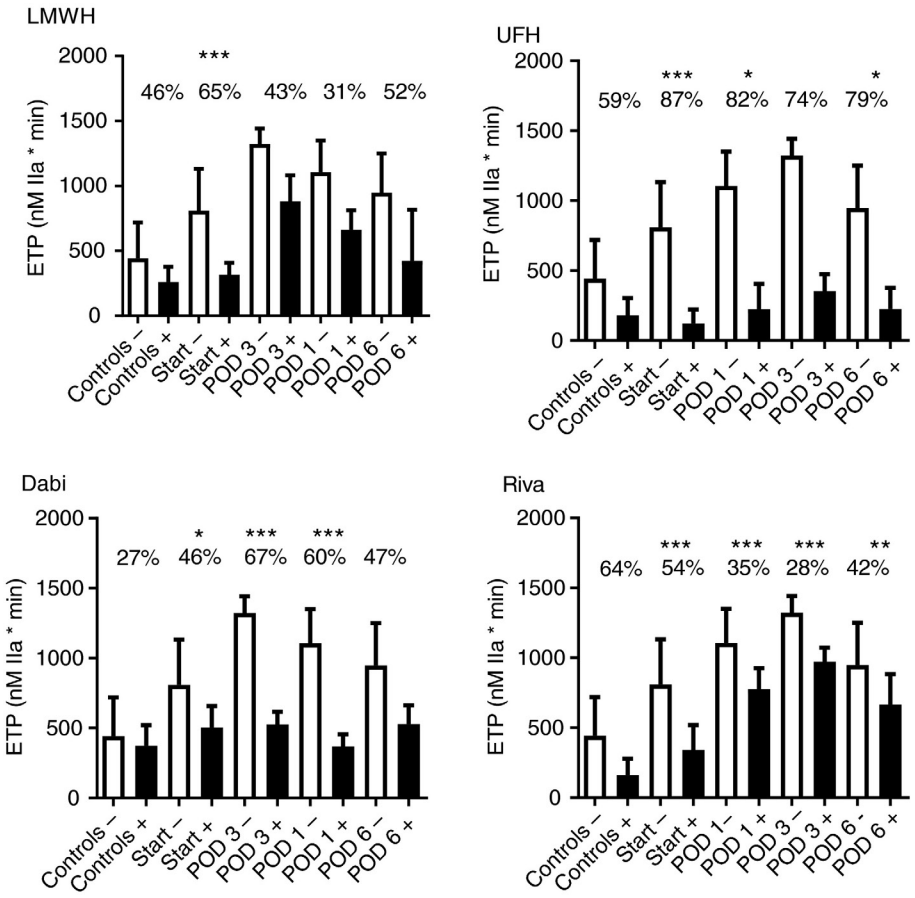



**Figure 6**. Absolute ETP levels from thrombomodulin modified thrombin generation testing in plasma of controls, and patients after PPPD prior to (-) and after (+) in vitro addition of anticoagulants. Start is after induction of anesthesia, POD = postoperative day. Shown are medians with error bars 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<0.05 vs controls, ** P<0.01 vs controls, *** P<0.001 vs controls. LMWH = low molecular weight heparin, UFH = unfractionated heparin, Dabi = dabigatran, Riva = rivaroxaban.

## 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 with 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 to controls, particularly in case of LMWH. The anticoagulant effect of rivaroxaban was substantially lower in patients compared to 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 to 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 LWMH(7,8,11) and our current data, it may be justified to increase the LMWH dose early after HPB surgery, although clinical studies are requires 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 hypofibrinolysis(35) and a VWF/ADAMTS13 unbalance(36,37), which further contribute to the hypercoagulable state of these patients.

Direct oral anticoagulants (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 is 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(40), and with increasing data arguing against liberal use of FFP

in OLT(18,22), and cirrhosis(26,41). In vitro and ex vivo studies have demonstrated little to no prohemostatic effect of FFP by thrombin generation tests in patients with cirrhosis(25,42). 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 importantly, 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 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, as 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 as compared to an FFP/platelet concentrate-based approach(43). 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(44).

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. In addition, it is unknown which level of ETP represents the optimal pro- or anticoagulant status, and 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 heterogenous cohorts. Our OLT cohort contains a large proportion of patients with cholestatic liver disease, which are known to be more hypercoagulable compared to 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.

## References


1 Bos S, Bernal W, Porte RJ, Lisman T. Hemostatic Complications in Hepatobiliary Surgery. Semin Thromb Hemost 2017; 43: 732–41.

2 Lisman T, Porte RJ. Pathogenesis, prevention, and management of bleeding and thrombosis in patients with liver diseases. Res Pract Thromb Haemost Wiley; 2017; 1: 150–61.

3 Romano F, Garancini M, Uggeri F, Degrate L, Nespoli L, Gianotti L, Nespoli A, Uggeri F. Bleeding in hepatic surgery: Sorting through methods to prevent it. HPB Surgery. 2012;2012: 169351.

4 Alkozai EM, Lisman T, Porte RJ. Bleeding in Liver Surgery: Prevention and Treatment. Clinics in Liver Disease. 2009; 13:145-154.

5 De Boer MT, Molenaar IQ, Hendriks HGD, Slooff MJH, Porte RJ. Minimizing blood loss in liver transplantation: Progress through research and evolution of techniques. Dig Surg 2005; 22: 265–75.

6 Westerkamp ACAC, Lisman T, Porte RJRJ. How to minimize blood loss during liver surgery in patient with cirrhosis. 2009; 11: 453–8.

7 Turley RS, Reddy SK, Shortell CK, Clary BM, Scarborough JE. Venous Thromboembolism After Hepatic Resection: Analysis of 5,706 Patients. J Gastrointest Surg 2012; 16: 1705–14.

8 Tzeng C-WD, Katz MHG, Fleming JB, Pisters PWT, Lee JE, Abdalla EK, Curley SA, Vauthey J-N, Aloia TA. Risk of venous thromboembolism outweighs post-hepatectomy bleeding complications: analysis of 5651 National Surgical Quality Improvement Program patients. HPB 2012; 14: 506–13.

9 Annamalai A, Kim I, Sundaram V, Klein A. Incidence and risk factors of deep vein thrombosis after liver transplantation. Transplant Proc; 2014; 46: 3564–9.

10 Yip J, Bruno DA, Burmeister C, Kazimi M, Yoshida A, Abouljoud MS, Schnickel GT. Deep Vein Thrombosis and Pulmonary Embolism in Liver Transplant Patients. Transplant Direct; 2016; 2: e68.

11 Ejaz A, Spolverato G, Kim Y, Lucas DL, Lau B, Weiss M, Johnston FM, Kheng M, Hirose K, Wolfgang CL, Haut E, Pawlik TM. Defining Incidence and Risk Factors of Venous Thromboemolism after Hepatectomy. J Gastrointest Surg 2014; 18: 1116–24.

12 Duffy JP, Hong JC, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, Busuttil RW. Vascular Complications of Orthotopic Liver Transplantation: Experience in More than 4,200 Patients. J Am Coll Surg 2009; 208: 896–903.

13 Lisman T, Porte RJ. Value of Preoperative Hemostasis Testing in Patients with Liver Disease for Perioperative Hemostatic Management. Anesthesiology. 2017; 126:338-344.

14 Lisman T, Bakhtiari K, Pereboom ITAA, Hendriks HGDD, Meijers JCMM, Porte RJ. Normal to increased thrombin generation in patients undergoing liver transplantation despite prolonged conventional coagulation tests. J Hepatol 2010; 52: 355–61.

15 Werner MJM, de Meijer VE, Adelmeijer J, de Kleine RHJ, Scheenstra R, Bontemps STH, Reyntjens KMEM, Hulscher JBF, Lisman T, Porte RJ. Evidence for a rebalanced hemostatic system in pediatric liver transplantation: A prospective cohort study. Am J Transplant 2020;20:1384-1392

16 Tripodi A, Salerno F, Chantarangkul V, Clerici M, Cazzaniga M, Primignani M, Mannucci PM. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. Hepatology 2005; 41: 553–8.

17 Lebreton A, Sinegre T, Pereira B, Lamblin G, Duron C, Abergel A. Plasma hypercoagulability in the presence of thrombomodulin but not of activated protein C in patients with cirrhosis. J Gastroenterol Hepatol; 2017; 32: 916–24.

18 Massicotte L, Thibeault L, Roy A. Classical Notions of Coagulation Revisited in Relation with Blood Losses, Transfusion Rate for 700 Consecutive Liver Transplantations. Semin Thromb Hemost 2015; 41: 538–46.

19 Potze W, Alkozai EM, Adelmeijer J, Porte RJ, Lisman T. Hypercoagulability following major partial liver resection - Detected by thrombomodulin-modified thrombin generation testing. Aliment Pharmacol Ther 2015; 41: 189–98.

20 Mallett S V., Sugavanam A, Krzanicki DA, Patel S, Broomhead RH, Davidson BR, Riddell A, Gatt A, Chowdary P. Alterations in coagulation following major liver resection. Anaesthesia; 2016; 71: 657–68.

21 De Pietri L, Montalti R, Begliomini B, Scaglioni G, Marconi G, Reggiani A, Di Benedetto F, Aiello S, Pasetto A, Rompianesi G, Gerunda GE. Thromboelastographic changes in liver and pancreatic cancer surgery: Hypercoagulability, hypocoagulability or normocoagulability? Eur J Anaesthesiol 2010; 27: 608–16.

22 Biancofiore G, Blasi A, De Boer MT, Franchini M, Hartmann M, Lisman T, Liumbruno GM, Porte RJ, Saner F, Senzolo M, Werner MJ. Perioperative hemostatic management in the cirrhotic patient: A position paper on behalf of the Liver Intensive Care Group of Europe (LICAGE). Minerva Anestesiologica. 2019;85:782-798.

23 Stanworth SJ, Brunskill SJ, Hyde CJ, McClelland DBL, Murphy MF. Is fresh frozen plasma clinically effective? A systematic review of randomized controlled trials. Br J Haematol 2004; 126: 139–52.

24 Huber J, Stanworth SJ, Doree C, Fortin PM, Trivella M, Brunskill SJ, Hopewell S, Wilkinson KL, Estcourt LJ. Prophylactic plasma transfusion for patients without inherited bleeding disorders or anticoagulant use undergoing non-cardiac surgery or invasive procedures. Cochrane Database of Systematic Reviews. 2019;11:CD012745

25 Rassi AB, D'Amico EA, Tripodi A, da Rocha TRF, Migita BY, Ferreira CM, Carrilho FJ, Farias AQ. Fresh frozen plasma transfusion in patients with cirrhosis and coagulopathy: Effect on conventional coagulation tests and thrombomodulin-modified thrombin generation. J Hepatol 2020;72:85-94

26 Bernal W, Caldwell SH, Lisman T. Nails in the coffin of fresh frozen plasma to prevent or treat bleeding in cirrhosis? Journal of Hepatology. 2020;72:12-13.

27 Hartmann M, Walde C, Dirkmann D, Saner FH. Safety of coagulation factor concentrates guided by ROTEMTM-analyses in liver transplantation: Results from 372 procedures. BMC Anesthesiol; 2019; 19: 97.

28 Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Porte RJ, Lisman T. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. Br J Haematol 2013; 163: 666–73.

29 Hoolwerf EW, Kraaijpoel N, Büller HR, van Es N. Direct oral anticoagulants in patients with liver cirrhosis: A systematic review. Thrombosis Research 2018; 170:102–8.

30 Potze W, Arshad F, Adelmeijer J, Blokzijl H, Van Den Berg AP, Meijers JCM, Porte RJ, Lisman T. Differential in vitro inhibition of thrombin generation by anticoagulant drugs in plasma from patients with cirrhosis. PLoS One 2014; 9: e88390.

31 Lisman T, Kleiss S, Patel VCVC, Fisher C, Adelmeijer J, Bos S, Singanayagam A, Stoy SHSH, Shawcross DLDL, Bernal W. In vitro efficacy of pro- and anticoagulant strategies in compensated and acutely ill patients with cirrhosis. Liver Int 2018; 38: 1988–96.

32 Yoshiya S, Shirabe K, Nakagawara H, Soejima Y, Yoshizumi T, Ikegami T, Yamashita YI, Harimoto N, Nishie A, Yamanaka T, Maehara Y. Portal vein thrombosis after hepatectomy. World J Surg 2014; 38: 1491–7.

33 Machlus KR, Cardenas JC, Church FC, Wolberg AS. Causal relationship between hyperfibrinogenemia, thrombosis, and resistance to thrombolysis in mice. Blood 2011; 117: 4953–63.

34 van Hylckama Vlieg A, Rosendaal FR. High levels of fibrinogen are associated with the risk of deep venous thrombosis mainly in the elderly. J Thromb Haemost 2003; 1: 2677–8.

35 Kleiss SF, Adelmeijer J, Meijers JCM, Porte RJ, Lisman T. A sustained decrease in plasma fibrinolytic potential following partial liver resection or pancreas resection. Thromb Res 2016; 140: 36-40.

36 Groeneveld DJ, Alkozai EM, Adelmeijer J, Porte RJ, Lisman T. Balance between von Willebrand factor and ADAMTS13 following major partial hepatectomy. Br J Surg 2016; 103: 735-743.

37 Pereboom ITA, Adelmeijer J, Van Leeuwen Y, Hendriks HGD, Porte RJ, Lisman T. Development of a severe von willebrand factor/adamts13 dysbalance during orthotopic liver transplantation. Am J Transplant 2009; 9: 1189-96.

38 Morrow GB, Beavis J, Harper S, Baker P, Desbourough MJR, Curry N, Stanworth SJ, Laffan MA. Coagulation status of critically ill patients with and without liver disease assessed using a novel thrombin generation analyser. J Thromb Haemost 2020, in press.

39 Wan J, Roberts LN, Hendrix W, Konings J, Ow TW, Rabinowich L, Barbouti O, de Laat B, Arya R, Patel VC, Roest M, Lisman T, Bernal W. Whole blood thrombin generation profiles of patients with cirrhosis explored with a near patient assay. J Thromb Haemost 2020; 18: 834-843.

40 Chavez-Tapia NC, Alfaro-Lara R, Tellez-Avila F, Barrientos-Gutiérrez T, González-Chon O, Mendez-Sanchez N, Uribe M. Prophylactic activated recombinant factor VII in liver resection and liver transplantation: Systematic review and meta-analysis. PLoS One 2011; 6: 1–8.

41 Weeder PD, Porte RJ, Lisman T. Hemostasis in liver disease: Implications of new concepts for perioperative management. Transfusion Medicine Reviews. 2014; 28:107–13.

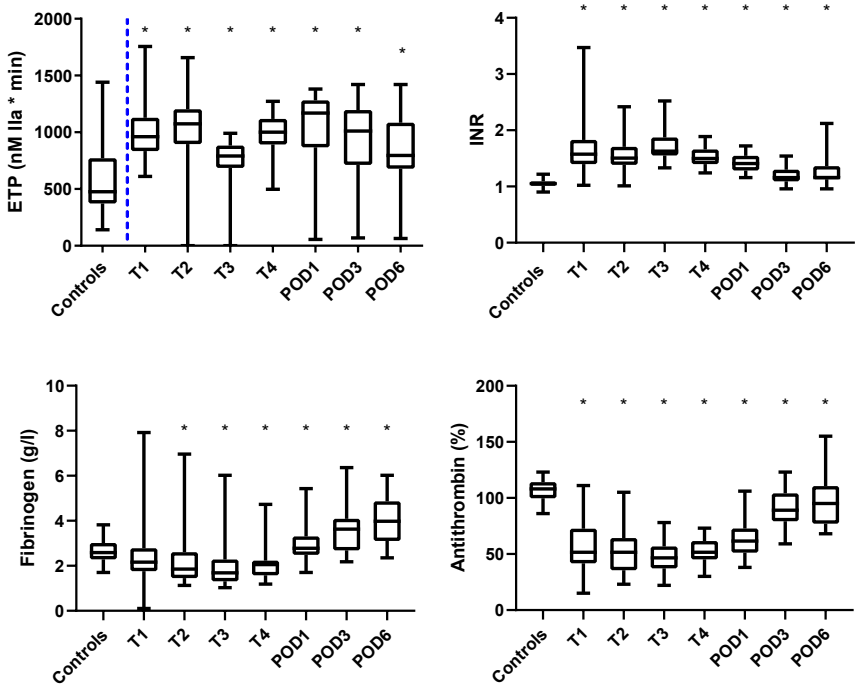
42 Tripodi A, Chantarangkul V, Primignani M, Clerici M, Dell'Era A, Aghemo A, Mannucci PM. Thrombin generation in plasma from patients with cirrhosis supplemented with normal plasma: Considerations on the efficacy of treatment with fresh-frozen plasma. Intern Emerg Med 2012; 7: 139–44.

43 Bezinover D, Dirkmann D, Findlay J, Guta C, Hartmann M, Nicolau-Raducu R, Mukhtar AM, Moguilevitch M, Pivalizza E, Rosenfeld D, Saner F, Wray C, Wagener G, West J. Perioperative coagulation management in liver transplant recipients. Transplantation 2018; 102: 578–92.

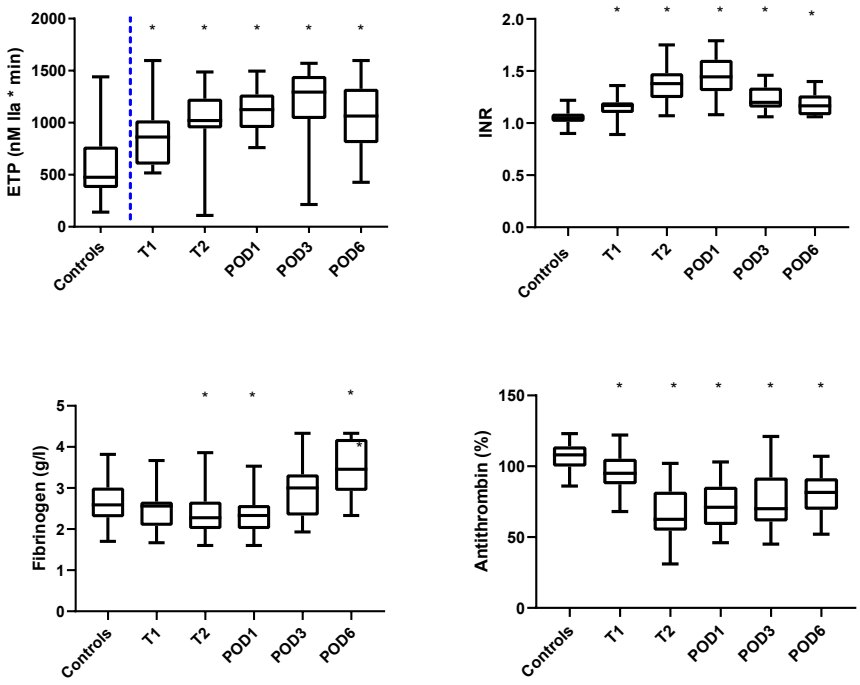
44 Abuelkasem E, Hasan S, Mazzeffi MA, Planinsic RM, Sakai T, Tanaka KA. Reduced Requirement for Prothrombin Complex Concentrate for the Restoration of Thrombin Generation in Plasma From Liver Transplant Recipients. Anesth Analg 2017; 125: 609–15.

45 Krzanicki D, Sugavanam A, Mallett S. Intraoperative hypercoagulability during liver transplantation as demonstrated by thromboelastography. Liver Transplant 2013; 19: 852–61.

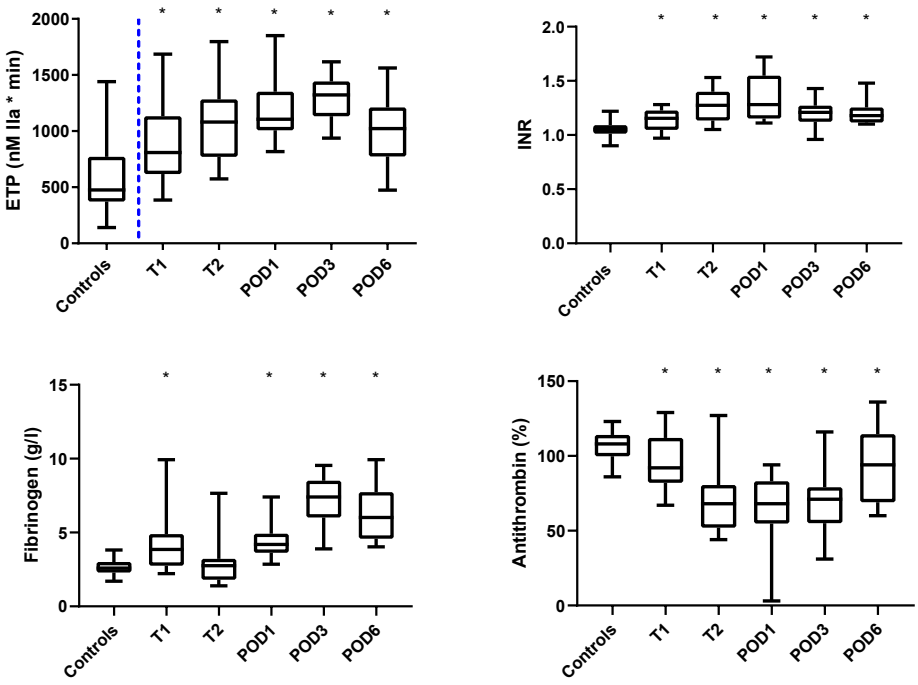
# Supplementary data




**Supplementary figure 1.** ETP levels from thrombomodulin modified thrombin generation testing, INR, antithrombin and fibrinogen levels in plasma of controls, and patients during and after orthotopic liver transplantation. T1 is after induction of anesthesia, T2 is 30 minutes after the start of the anhepatic phase, T3 is 30 minutes after reperfusion, T4 is at the end of surgery, Shown are medians with error bars indicating interquartile ranges. * P< 0.05




**Supplementary figure 2**. ETP levels from thrombomodulin modified thrombin generation testing, INR, antithrombin and fibrinogen levels in plasma of controls, and patients during and after partial hepatectomy. T1 is after induction of anesthesia, T2 is at the end of surgery. Shown are medians with error bars indicating interquartile ranges. * P< 0.05




**Supplementary figure 3**. ETP levels from thrombomodulin modified thrombin generation testing, INR, antithrombin and fibrinogen levels in plasma of controls, and patients during and after pylorus preserving partial duodenectomy (PPPD). T1 is after induction of anesthesia, T2 is at the end of surgery. Shown are medians with error bars indicating interquartile ranges .* P< 0.05

**Supplementary table 1.** Endogenous thrombin potential in absence or presence of in vitro addition of pro- and anticoagulant drugs in plasma of patients undergoing orthotopic liver transplantation

| OLT | Sample | Absolute ETP in absence of drug | P values vs control | Absolute ETP in presence of drug | P values vs control | % difference in ETP | P values vs control |
|---|---|---|---|---|---|---|---|
| **FFP** | Control | 442 [349-720] | | 508 [414-765] | | 13 [1.3-21] | |
| | T1 | 962 [834-1128] | <0.001 | 1016 [918-1140] | <0.001 | 5.1 [-3.1-15] | ns |
| | T2 | 1075 [895-1203] | <0.001 | 1198 [985-1326] | <0.001 | 7.6 [0.1-21] | ns |
| | T3 | 792 [685-882] | 0.017 | 864 [670-973] | 0.018 | 7.2 [3.4-16] | ns |
| | T4 | 1001 [893-1118] | <0.001 | 1105 [1007-1223] | <0.001 | 11 [1.8-22] | ns |
| **PCC** | Control | 442 [349-720] | | 915 [745-1374] | | 94 [66-123] | |
| | T1 | 962 [834-1128] | <0.001 | 2111 [1693-2421] | <0.001 | 114 [88-146] | ns |
| | T2 | 1075 [895-1203] | <0.001 | 2481 [2011-3040] | <0.001 | 118 [94-161] | 0.02 |
| | T3 | 792 [685-882] | 0.017 | 1962 [1712-2198] | <0.001 | 164 [129-199] | <0.001 |
| | T4 | 1001 [893-1118] | <0.001 | 2459 [2001-2651] | <0.001 | 147 [109-192] | 0.003 |
| **rFVIIa** | Control | 442 [349-720] | | 585 [484-770] | | 21 [8.6-43] | |
| | T1 | 962 [834-1128] | <0.001 | 906 [787-125] | <0.001 | -1.6 [-8.5-0.2] | <0.001 |
| | T2 | 1075 [895-1203] | <0.001 | 1032 [861-1174] | <0.001 | -3.4 [-8.1 - -0.1] | <0.001 |
| | T3 | 792 [685-882] | 0.017 | 685 [590-871] | 0.153 | -6.1 [-15.1- 3.6] | <0.001 |
| | T4 | 1001 [893-1118] | <0.001 | 1016 [857-1109] | <0.001 | -0.8 [-4.7-1.1] | <0.001 |
| **LMWH** | Control | 442 [349-720] | | 261 [191-378] | | 46 [33-59] | |
| | T1 | 962 [834-1128] | <0.001 | 327 [215-464] | 0.001 | 50 [44-57] | ns |
| | POD1 | 1170 [867-1281] | <0.001 | 634 [455-715] | <0.001 | 43 [35-53] | ns |
| | POD3 | 1011 [713-1195] | 0.003 | 462 [304-705] | 0.017 | 46 [36-56] | ns |
| | POD6 | 796 [680-1084] | 0.006 | 343 [254-535] | 0.043 | 52 [46-56] | ns |
| **UFH** | Control | 442 [349-720] | | 180 [128-304] | | 59 [40-76] | |
| | T1 | 962 [834-1128] | <0.001 | 265 [122-401] | ns | 74 [60-84] | ns |
| | POD1 | 1170 [867-1281] | <0.001 | 307 [144-417] | ns | 68 [58-85] | ns |
| | POD3 | 1011 [713-1195] | 0.003 | 253 [172-502] | ns | 70 [55-76] | ns |
| | POD6 | 796 [680-1084] | 0.006 | 153 [56-379] | ns | 74 [51-87] | ns |
| **Dabigatran** | Control | 442 [349-720] | | 372 [272-522] | | 27 [15-38] | |
| | T1 | 962 [834-1128] | <0.001 | 148 [117-384] | 0.002 | 80 [68-87] | <0.001 |
| | POD1 | 1170 [867-1281] | <0.001 | 256 [87-377] | 0.002 | 80 [69-92] | <0.001 |
| | POD3 | 1011 [713-1195] | 0.003 | 358 [0-527] | ns | 67 [53-100] | <0.001 |
| | POD6 | 796 [680-1084] | 0.006 | 0 [0-536] | 0.013 | 100 [54-100] | <0.001 |

| OLT | Sample | Absolute ETP in absence of drug | P values vs control | Absolute ETP in presence of drug | P values vs control | % difference in ETP | P values vs control |
|---|---|---|---|---|---|---|---|
| **Rivaroxaban** | Control | 442 [349-720] | | 160 [112-280] | | 64 [58-69] | |
| | T1 | 962 [834-1128] | <0.001 | 703 [585-977] | <0.001 | 25 [13-34] | <0.001 |
| | POD1 | 1170 [867-1281] | <0.001 | 848 [578-1053] | <0.001 | 22 [18-33] | <0.001 |
| | POD3 | 1011 [713-1195] | 0.003 | 732 [442-948] | <0.001 | 27 [21-35] | <0.001 |
| | POD6 | 796 [680-1084] | 0.006 | 487 [407-765] | <0.001 | 34 [26-43] | <0.001 |

Shown are medians (interquartile ranges). OLT, orthotopic liver transplantation; FFP, fresh frozen plama; PCC, prothrombin complex concentrate; rFVIIa, recombinant factor VIIa; LMWH, low-molecular weight heparin; UFH, unfractionated heparin; ETP, endogenous thrombin potential. T1 is after induction of anesthesia, T2 is 30 minutes after the start of the anhepatic phase, T3 is 30 minutes after reperfusion, T4 is at the end of surgery; POD, post-operative day.

**Supplementary table 2.** Endogenous thrombin potential in absence or presence of in vitro addition of pro- and anticoagulant drugs in plasma of patients undergoing partial hepatectomy

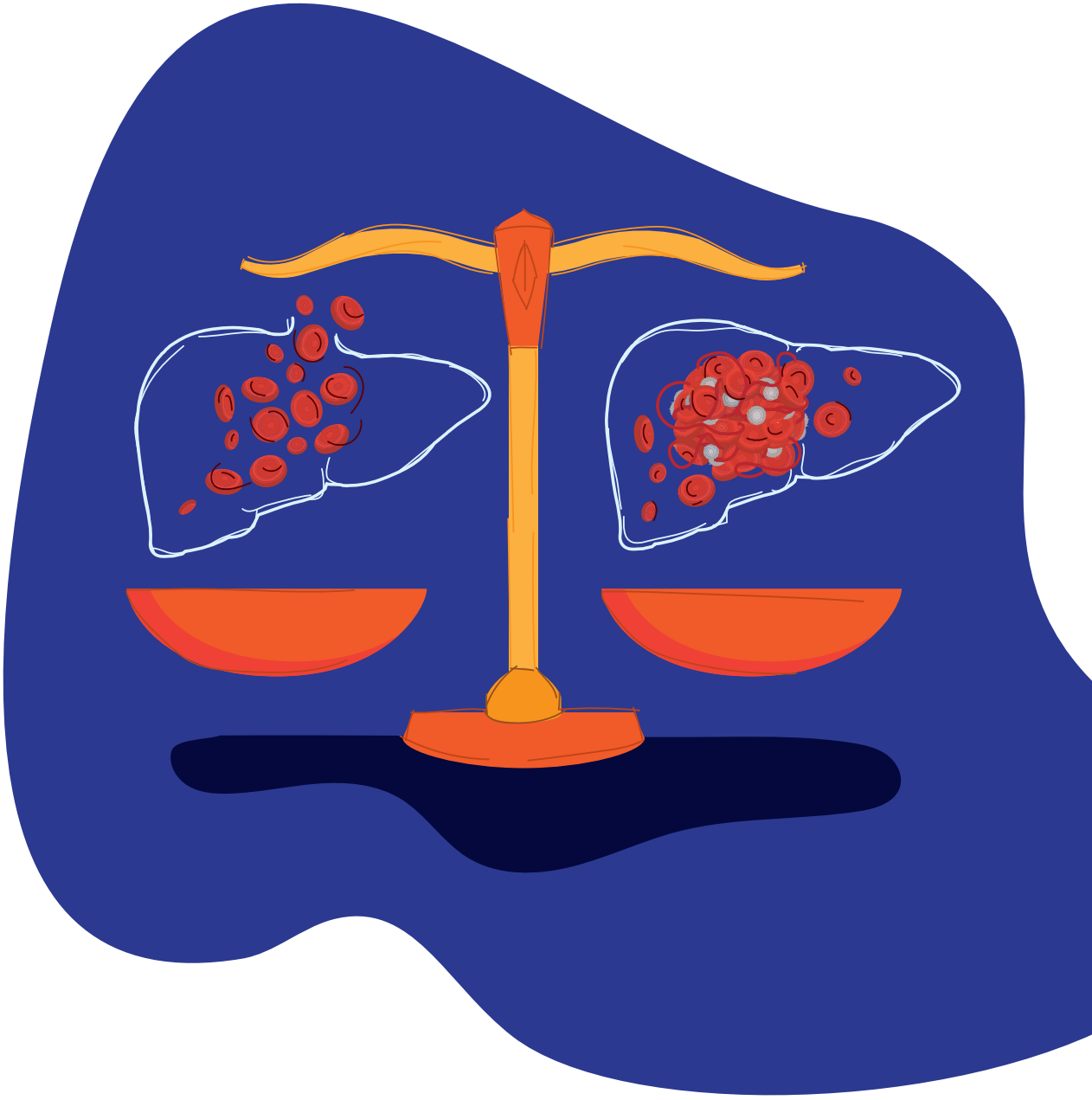
| PHEP | Sample | Absolute ETP in absence of drug | P values vs control | Absolute ETP in presence of drug | P values vs control | % difference in ETP | P values vs control |
|---|---|---|---|---|---|---|---|
| FFP | Control | 442 [349-720] | | 508 [414-765] | | 13 [1.3-21] | |
| | T1 | 862 [597-1022] | 0.001 | 702 [594-872] | 0.01 | -6.9 [-20- 2.0] | <0.001 |
| | T2 | 1022 [945-1230] | <0.001 | 976 [803-1204] | <0.001 | -9.8 [-14 - -1.6] | <0.001 |
| PCC | Control | 442 [349-720] | | 915 [745-1374] | | 94 [66-123] | |
| | T1 | 862 [597-1022] | 0.001 | 1425 [1101-1725] | 0.001 | 79 [66-93] | ns |
| | T2 | 1022 [945-1230] | <0.001 | 2100 [1902-2341] | <0.001 | 89 [82-113] | ns |
| rFVIIa | Control | 442 [349-720] | | 585 [484-770] | | 21 [8.6-43] | |
| | T1 | 862 [597-1022] | 0.001 | 803 [615-926] | 0.02 | -2.7 [-14-6.2] | <0.001 |
| | T2 | 1022 [945-1230] | <0.001 | 1018 [[869-1168] | <0.001 | -4.1 [-7.9-4.2] | <0.001 |
| LMWH | Control | 442 [349-720] | | 180 [128-304] | | 46 [33-59] | |
| | T1 | 862 [597-1022] | 0.001 | 289 [234-419] | ns | 60 [52-71] | 0.003 |
| | POD1 | 1126 [949-1271] | <0.001 | 598 [472-756] | <0.001 | 42 [38-49] | ns |
| | POD3 | 1294 [1036-1449] | <0.001 | 840 [647-1027] | <0.001 | 32 [22-41] | 0.03 |
| | POD6 | 1166 [885-1342] | <0.001 | 659 [523-805] | <0.001 | 37 [31-50] | ns |
| UFH | Control | 442 [349-720] | | 180 [128-304] | | 59 [40-76] | |
| | T1 | 862 [597-1022] | 0.001 | 154 [93-244] | ns | 83 [70-89] | 0.003 |
| | POD1 | 1126 [949-1271] | <0.001 | 335 [204-530] | 0.003 | 69 [51-82] | ns |
| | POD3 | 1294 [1036-1449 | <0.001 | 344 [190-808] | 0.005 | 75 [46-85] | ns |
| | POD6 | 1166 [885-1342] | <0.001 | 392 [137-785] | 0.03 | 57 [47-80] | ns |
| Dabigatran | Control | 442 [349-720] | | 372 [272-522] | | 27 [15-38] | |
| | T1 | 862 [597-1022] | 0.001 | 472 [428-547] | ns | 44 [24-54] | ns |
| | POD1 | 1126 [949-1271] | <0.001 | 374 [225-508] | ns | 66 [52-79] | <0.001 |
| | POD3 | 1294 [1036-1449 | <0.001 | 489 [330-594] | ns | 64 [52-73] | 0.001 |
| | POD6 | 1166 [885-1342] | <0.001 | 588 [39-680] | ns | 55 [37-67] | 0.01 |
| Rivaroxaban | Control | 442 [349-720] | | 160 [112-280] | | 64 [58-69] | |
| | T1 | 862 [597-1022] | 0.001 | 401 [274-464] | <0.001 | 53 [44-60] | 0.003 |
| | POD1 | 1126 [949-1271] | <0.001 | 850 [693-912] | <0.001 | 27 [20-38] | <0.001 |
| | POD3 | 1294 [1036-1449 | <0.001 | 968 [833-1189] | <0.001 | 23 [12-36] | 0.001 |
| | POD6 | 1166 [885-1342] | <0.001 | 747 [603-1049] | <0.001 | 29 [19-41] | <0.001 |

Shown are medians (interquartile ranges). PHEP, partial hepatectomy; FFP, fresh frozen plama; PCC, prothrombin complex concentrate; rFVIIa, recombinant factor VIIa; LMWH, low-molecular weight heparin; UFH, unfractionated heparin, ETP, endogenous thrombin potential. T1 is after induction of anesthesia, T2 is at the end of surgery; POD, post-operative day.

**Supplementary table 3.** Endogenous thrombin potential of pro- and anticoagulant drugs in plasma of patients undergoing pyloric preserving pancreaticoduodenectomy

| PPPD | Sample | Absolute ETP in absence of drug | P values vs control | Absolute ETP in presence of drug | P values vs control | % difference in ETP | P values vs control |
|---|---|---|---|---|---|---|---|
| FFP | Control | 442 [349-720] | | 508 [414-765] | | 13 [1.3-21] | |
| | T1 | 810 [618-1132] | 0.001 | 730 [566-1026] | 0.009 | -8.7 [-12- -3.6} | <0.001 |
| | T2 | 1081 [770-1283] | <0.001 | 949 [790-1153] | <0.001 | -6.8 [-17- -0.4] | <0.001 |
| PCC | Control | 442 [349-720] | | 915 [745-1374] | | 94 [66-123] | |
| | T1 | 810 [618-1132] | 0.001 | 1396 [1103-2104] | 0.002 | 78 [65-95} | ns |
| | T2 | 1081 [770-1283] | <0.001 | 2337 [1461-2782] | <0.001 | 102 [81-132] | ns |
| rFVIIa | Control | 442 [349-720] | | 585 [484-770] | | 21 [8.6-43] | |
| | T1 | 810 [618-1132] | 0.001 | 767 [699-1139] | 0.005 | 0.3 [-8- 15] | 0.01 |
| | T2 | 1081 [770-1283] | <0.001 | 1124 [893-1318] | <0.001 | 2.1 [-1.3 -13} | 0.003 |
| LMWH | Control | 442 [349-720] | | 180 [128-304] | | 46 [33-59] | |
| | T1 | 810 [618-1132] | 0.001 | 316 [158-408] | ns | 65 [59-75] | <0.001 |
| | POD1 | 1105 [1007-1350] | <0.001 | 663 [478-812] | <0.001 | 43 [32-57] | ns |
| | POD3 | 1323 [1132-1442] | <0.001 | 883 [644-1083] | <0.001 | 31 [24-46] | ns |
| | POD6 | 948 [721 -1250] | 0.001 | 422 [256-818] | 0.017 | 52 [31-67] | ns |
| UFH | Control | 442 [349-720] | | 180 [128-304] | | 59 [40-76] | |
| | T1 | 810 [618-1132] | 0.001 | 121 [61-223] | 0.018 | 87 [83-92] | <0.001 |
| | POD1 | 1105 [1007-1350] | <0.001 | 225 [135-405] | ns | 82 [69-88] | 0.014 |
| | POD3 | 1323 [1132-1442] | <0.001 | 354 [241-475] | 0.01 | 74 [64-80] | ns |
| | POD6 | 948 [721 -1250] | 0.001 | 225 [102-378] | ns | 79 [65-86] | 0.05 |
| Dabigatran | Control | 442 [349-720] | | 372 [272-522] | | 27 [15-38] | |
| | T1 | 810 [618-1132] | 0.001 | 504 [370-658] | ns | 46 [25-58] | 0.05 |
| | POD1 | 1105 [1007-1350] | <0.001 | 369 [286-456] | ns | 67 [58-78] | <0.001 |
| | POD3 | 1323 [1132-1442] | <0.001 | 525 [435-616] | 0.024 | 60 [51-69] | <0.001 |
| | POD6 | 948 [721 -1250] | 0.001 | 527 [432-663] | 0.03 | 47 [20-63] | ns |
| Rivaroxaban | Control | 442 [349-720] | | 160 [112-280] | | 64 [58-69] | |
| | T1 | 810 [618-1132] | 0.001 | 341 [274-519] | <0.001 | 54 [49-58] | <0.001 |
| | POD1 | 1105 [1007-1350] | <0.001 | 775 [532-926] | <0.001 | 35 [26-44] | <0.001 |
| | POD3 | 1323 [1132-1442] | <0.001 | 973 [687-1073] | <0.001 | 28 [23-31] | <0.001 |
| | POD6 | 948 [721 -1250] | 0.001 | 666 [355-884] | <0.001 | 42 [34-51] | 0.002 |

Shown are medians (interquartile ranges). PPPD, pyloric preserving pancreaticoduodenectomy; FFP, fresh frozen plama; PCC, prothrombin complex concentrate; rFVIIa, recombinant factor VIIa; LMWH, low-molecular weight heparin; UFH, unfractionated heparin; ETP, endogenous thrombin potential. T1 is after induction of anesthesia, T2 is at the end of surgery; POD, post-operative day.



# CHAPTER 7

## General discussion

In this thesis, the hemostatic system of patients with cirrhosis has been studied in detail. Next to this, in vitro and in vivo studies of anticoagulation in cirrhosis have been performed. Finally, in collaboration with Kings College Hospital in London, in vitro studies on the effect of pro-and anticoagulant therapies have been performed in the plasma of liver transplant, and hepato-pancreatico-biliary (HPB) surgery patients.

Patients with cirrhosis are at risk for both bleeding and thrombotic complications, and these complications may either occur spontaneously or associated with invasive procedures. In clinical practice, physicians often struggle with the indication for and dosages of both pro- and anticoagulant therapy in patients with cirrhosis to prevent or treat bleeding or thrombosis. Although high quality evidence is lacking, there is a need for clinical guidance for prevention and treatment of bleeding and thrombosis. Such guidance includes indications for treatment, drug of choice, and adequate dosing of these drugs. Importantly, treatment advice is complicated by possible alterations in drug efficacy and alterations in clearance of drugs. To come to a rational treatment advice, we first need to know whether the heterogenous group of patients with cirrhosis have uniform alterations on a hemostatic level. In chapter 2, an in-depth hemostatic profiling of primary hemostasis, secondary hemostasis and fibrinolysis was performed in a group of patients with mild cirrhosis associated with various underlying etiologies.

## Hemostatic profiles in cirrhosis

The results in chapter 2 suggest that patients with Child-Turcotte-Pugh (CTP) A/B cirrhosis have an increased risk for thrombotic complications indicated by their prohemostatic state. These results are regardless of their etiology and once again this reinforces the altered balance in hemostasis in patients with cirrhosis.(1–3) Increasing evidence emerges that confirm that patients with cirrhosis have an increased risk for thrombotic events, notably venous thrombosis and portal vein thrombosis.(4–6)

Literature on thrombin generation in cirrhosis varies. Whereas some studies show normal thrombin generating capacity, others reported increased levels of thrombin generation. One of the first papers to address the altered balance in cirrhosis is the key paper of Tripodi, which showed normal levels of thrombin generation in patients with cirrhosis.(7) Follow-up studies from other laboratories showed slightly different results. In these studies patients with cirrhosis regardless of their disease severity, expressed by MELD or CTP-score, are all characterized by increased thrombin generating capacity.(5,8–12) These results stress that patients with cirrhosis are not solely at risk for bleeding complications because of the often prolonged prothrombin time (PT) and low platelet levels .

There are multiple other results from this study cohort that are of interest. The thrombomodulin modified thrombin generation that was used, comprises all major pro- and anticoagulant proteins. Our cohort showed, in agreement with the latest literature, an enhanced thrombin generating capacity.(5,6,9,11) There seems to be some slight differences in the endogenous thrombin potential between some of the etiologies of cirrhosis. More detailed testing of levels of pro- and anticoagulant proteins did not explain these slight differences. Possibly other unknown factors that influence hemostasis explain the possible differences between etiologies.

Even though the PT and all the individual plasma levels of coagulation factors were similar between all etiologies, there was an increase in the activated partial thromboplastin time (aPTT) in patients with cholestatic disease and viral hepatitis. None of the levels of the individual procoagulant or anticoagulant proteins could explain these differences in aPTT.

## Effect of in vitro anticoagulants in patients with cirrhosis

Since patients with cirrhosis have profound and complex changes in their hemostatic system, it is conceivable that pro- and anticoagulant drugs have altered pro- or anticoagulant effects in these patients. A first step in the assessment of the efficacy of anticoagulant drugs in patients with cirrhosis are in vitro studies. In chapter 3 the anticoagulant potency of 3 classes of anticoagulant drugs in patients with mild cirrhosis due to non-alcoholic steatohepatitis (NASH) or alcoholic steatohepatitis (ASH) were studied. We found that in patients with CTP A/B cirrhosis, the anticoagulant potency of low molecular weight heparin (LMWH) or apixaban in NASH cirrhosis and ASH cirrhosis is not different from that in controls. These findings are supported by increasing evidence of clinical studies on the efficacy and safety effect of LMWH and direct Xa-inhibitors in patients with CTP A/B cirrhosis.(13–16)

In contrast to the similar effect of LMWH and apixaban in both NASH and ASH cirrhosis, dabigatran had a stronger in vitro anticoagulant effect in the plasma of patients with ASH cirrhosis compared to NASH-related cirrhosis. The only clinical difference between these groups that might influence these results, is the difference in MELD-score which is higher in the group of patients with ASH related cirrhosis. As was shown in chapter 2, on a detailed hemostatic level there are no significant differences between NASH and ASH cirrhosis. An earlier study which also tested the hemostatic status of NASH and ASH cirrhosis patients in detail supported the findings of chapter 2.(17) In contrast to these results, a recent retrospective cohort study among nearly 9000 patients with NASH or ASH cirrhosis showed an increased incidence of portal vein thrombosis (PVT) in NASH cirrhosis

patients versus patients with ASH cirrhosis, 14.8% versus 9.2% respectively.(18) The combined findings of these studies support the notion that patients with NASH cirrhosis have an increased risk of thrombotic events which is not readily explained on a hemostatic level. Possibly features of the metabolic syndrome as well as other cardiometabolic risk factors contribute to the increased risk of thrombosis.(17,19,20)

## From in vitro to in vivo

As described in chapter one, there has been a shift from using vitamin K antagonists and LMWH as a therapy for patients with venous thrombo-embolism (VTE) and in patients with atrial fibrillation, in prevention of ischemic stroke. The convenience of dosing direct oral anticoagulants (DOACs) and route of administration together with less major side effects would propagate a suitable alternative for all patients. Unfortunately all the large phase 3 trials excluded patients with liver disease.(21–24) Therefore clinicians were left with many gaps in their knowledge on the use of DOACs in patients with cirrhosis. In the last few years more information on the use of DOACS in patients with cirrhosis is emerging.

Several in vitro studies on the effect of anticoagulation including DOACS in cirrhosis have been performed.(16,25,26). These studies provide essential information for new clinical trials. A rational first step would be to assess ex-vivo anticoagulant effects of drugs administered to patients without acute thrombotic disease. Such studies will reveal whether altered in vitro potency of anticoagulant drugs are also relevant in vivo. In addition, such studies are important to examine whether the pharmacokinetics of various anticoagulant drugs in patients with cirrhosis are similar to patients without liver disease.

One small, single dose studies reported on the clearance of Xa-inhibitor edoxaban in patients with hepatic impairment.(27) Another small study reported on the pharmacokinetics and pharmacodynamics of a single dose of apixaban in a similar group.(28) When patients with CTP A cirrhosis are compared with healthy controls the clearance of a low dose of edoxaban is similar. The pharmacokinetics and pharmacodynamics of apixaban were not altered in the group with CTP A cirrhosis compared to healthy subject. Whether prolonged treatment of a Xa-inhibitor in cirrhosis leads to comparable drug concentrations, efficacy and safety, is not readily explained by these small studies.

In chapter 4 we have shown the results of the first study to monitor the effect of prolonged edoxaban treatment in patients with CTP A/B cirrhosis. To reach steady state, a therapeutic dose of edoxaban for a full week was given. Normally steady state is reached after 3 days of edoxaban in healthy individuals.

We used ex-vivo thrombin generation tests and d-dimer levels to assess the anticoagulant potency of edoxaban. Specifically, we used thrombomodulin-modified thrombin generation tests, which gives an accurate balance between pro- and anticoagulant mechanisms, and confirmed enhanced thrombin generating capacity in patients. Several clinical characteristics, however, may affect the thrombin generation test. For example, it is known that an age related increase in thrombin generation exists which might be a indicator of the increase in thromboembolic events with higher age.(29–31) Our patients and controls had a mean age difference of approximately 10 years, but the difference in ETP between patients and controls far exceeds the increase in thrombin generating capacity due to ageing. Importantly, we have assessed both absolute and relative effects of edoxaban exposure on thrombin generation, and found impaired anticoagulant capacity in patients in both approaches.

Although plasma levels of edoxaban were similar in patients and in controls, the inhibition of coagulation was less effective in patients compared to healthy controls. The absolute level of ETP potential of patients on anticoagulation was twice as high compared to healthy controls. This might suggest an insufficient anticoagulant effect. However, there are no standardized levels of endogenous thrombin potential (ETP) of patients on anticoagulation which should be targeted to achieve optimal anticoagulant treatment.



Another finding which might support an insufficient anticoagulant effect is that the d-dimer levels, an indirect reflection of coagulation, of patients did not alter during the study period whereas the d-dimer levels of healthy controls slowly decreased over time. Earlier in vitro studies corroborate these findings as they showed that the Xa inhibitors rivaroxaban and apixaban also exert a decreased anticoagulant effect in plasma of patients with CTP A/B/C cirrhosis.(16,25)

The data shown in chapter 4 clearly shows no evidence of accumulation of edoxaban due to decreased hepatic clearance in patient with CTP A cirrhosis. Since our study group mainly consisted of patients CTP A cirrhosis, we have no data on the clearance in CTP B/C cirrhosis. Edoxaban has a 30-50% renal clearance. The remainder of the drug is cleared by biliary/intestinal excretion with a minimal through metabolism (<4%) by CYP450 enzymes.(27,32) Theoretically, patients with cirrhosis and impaired renal function are more prone for drug accumulation since DOACs are partially cleared by metabolic inactivation in the liver, and in part by renal excretion. Extrapolation of our results to the other Xa inhibitors, rivaroxaban and apixaban, should be done carefully. Even though the clearance of both these drugs are somewhat similar to edoxaban (65% hepatic and 35% renal for rivaroxaban, 75% hepatic and 25% renal for apixaban) studies must determine whether accumulation occurs with these drugs in patients with CTP A cirrhosis.

Based on plasma levels of edoxaban an optimal anticoagulant effect of edoxaban is reached in patients with cirrhosis with normal therapeutic dosages. However, the on-drug ETP and d-dimer levels might suggest that an even higher, supratherapeutic dosage is needed for adequate anticoagulant effect. When testing such escalated dose regiments, monitoring of drug levels with edoxaban calibrated anti-Xa assays might be a helpful tool to check and possible adjust dosages in patient with cirrhosis. Drug level monitoring is particularly relevant with prolonged use since cirrhosis is prone for deterioration over time and decreased hepatic and or renal clearance might occur.(25)

However, it is unclear whether the ETP findings have clinical consequences, i.e., that a therapeutic dose of edoxaban is less effective to prevent thrombosis in patients with cirrhosis compared to other patients. It is far too early to advocate higher therapeutic doses before a better assessment of the efficacy in patients with cirrhosis and thrombosis has been performed. Furthermore, the value of anti-Xa assessment to target DOAC treatment is uncertain. Whether this is useful in patients with cirrhosis remains to be established.

There are several retrospective clinical studies that used different DOACs in different dosages without a clear dosing rational. Many of these studies use reduced dosages for patients with cirrhosis.(8,11,16,17) Some of these studies compared DOACs with traditional anticoagulants. Although these studies did not show a difference in adverse events between the two regimens (DOACs and traditional anticoagulants), it is unclear whether DOACs were as effective as traditional anticoagulants.(33–38)

Hanafy et al. were one of the first to publish on a full dose treatment of rivaroxaban for portal vein thrombosis in hepatitis C cirrhosis.(39) The treatment regimen consisted of rivaroxaban 10 mg twice daily. Compared to warfarin, thrombus resolution rates on ultrasound of the group receiving rivaroxaban were higher with less side effects. Before initiation of the treatment, both groups had similar MELD-scores. A significant decrease in MELD score was seen in the group treated with rivaroxaban compared to the warfarin group. These results are in line with the study of Villa in which a low dose of enoxaparin delayed the occurrence of decompensated cirrhosis and improved survival.(40) The results of this study are promising and hopefully lead to the expansion of our therapeutic options for the treatment of thrombosis in patients with cirrhosis.

To date, our study is the only study which provides information on the anticoagulant effect of edoxaban in patient with CTP A cirrhosis. All patients with cirrhosis regardless of their disease severity, are hypercoagulable.(6) This might argue for a reassessment of the careful dosing regimens that currently are used.

The downside of a more liberal dosing regimen is an increase in bleeding risk in patients with an already complex and fragile hemostatic balance.(41,42)

## Hemostasis in liver transplant and HPB-surgery

Not only in liver disease, but also in HPB-surgery and liver transplant surgery changes occur in the hemostatic balance. Since the liver has a pivotal role in hemostasis, it is not surprising that during and after liver surgery (transplantation or partial hepatectomy) hemostatic changes occur. Pre-operative hemostatic changes are also often seen in patients with chronic liver disease.(43) Although conventional diagnostic tests of hemostasis in this population (platelet count, prothrombin time, fibrinogen level) are suggestive of a pre-operative bleeding tendency, it is now widely accepted that these tests do not reflect true hemostatic capacity in this population. These conventional diagnostic tests are also unable to predict bleeding events. Pre-emptive correction of the hemostatic status of the patient therefore does not lead to decreased bleeding events and can even provoke thrombotic complications.(44)

Although there is a hypercoagulable profile in patients with chronic liver disease, it is well known that this renewed hemostatic balance is fragile and bleeding complications also still occur. Surgical and anesthesiologic improvements have led to a substantial decrease in blood loss.(45) Management of intraoperative bleeding still needs refinements in order to further decrease blood loss. Peri-operative blood loss is associated with portal hypertension and seems to be more relevant than the hemostatic status of the patient.(46)



The increased incidence of VTE after liver surgery indicates a more hypercoagulable profile in this specific patient population.(47–50) Strategies to reduce VTE can still be optimized. Nowadays almost all patients receive pharmacological thromboprophylaxis with LMWH as soon as possible after surgery. There is some rational for a higher dose of postoperative thromboprophylaxis for specific patient populations with increased risk of thrombotic complications such as obesity and cancer. Even in HPB-surgery patients treated with thromboprophylaxis VTE still occurs. Whether a higher dose or an alternative therapy is indicated is unclear and further research on this topic is needed.(47,51)

## Pro- and anticoagulant therapy in liver transplant and HPB-surgery

As described in chapter 5 and 6 there is an increased risk of DVT following HPB-surgery. About 3-9% of the patients develop DVT despite sufficient thromboprophylaxis.(47,49,52–54) In patients after liver transplantation the development of portal vein thrombosis or hepatic artery thrombosis is a direct threat to graft function.(55,56) Given the peri-operative hemostatic changes

in patients undergoing HPB-surgery, dosing of pro- or anticoagulant therapy is complicated. Better understanding of the efficacy of commonly used pro- and anticoagulant drugs is therefore warranted. In chapter 6 we assessed the efficacy of both pro- and anticoagulant drugs in plasma of patients undergoing HPB-surgery. (57) Patients undergoing HPB-surgery are hypercoagulable when assessed with thrombomodulin-modified thrombin generation testing. Assessment of the risk of bleeding or thrombosis in patients undergoing HPB-surgery is as difficult as in patients with cirrhosis. Although PT, platelet count, and an INR during or after the procedure may suggest a bleeding tendency, they do not appear to reflect the actual hemostatic status. As for patients with cirrhosis, patients undergoing HPB-surgery also have a higher risk of thrombosis with lower incidences of bleeding complications.(47,49,52,53,58,59)

The commonly used anticoagulant drugs (LMWH, unfractionated heparin (UFH)) show slightly altered potencies in patients that undergo HPB-surgery. Especially of interest is the finding that standard dosages of LMWH and rivaroxaban suggest an insufficient anticoagulant effect, shown by an increased ETP compared to controls, in patients that undergo HPB-surgery.

Next to enhanced thrombin generation it is also shown in chapter 6 that patients that underwent HPB-surgery more often have elevated levels of fibrinogen. Postoperative hypofibrinolysis (10) and a VWF/ADAMTS13 unbalance (60,61) may also contribute to the hypercoagulable state of these patients.

Despite standard dosing of thromboprophylaxis the risk of VTE after partial hepatectomy is still increased.(47,49,54) Together with the data in this thesis, it might be suggested to increase the dose of thromboprophylaxis postoperatively. Increased dosages have been suggested in earlier studies, however, safety and efficacy of such an approach requires further study.(54,55)

In specific clinical settings, such as orthopedic surgery, DOACs can be used as an alternative for LMWH as thromboprophylaxis. To date, the use of DOACs as thromboprophylaxis have not been extensively explored in general surgery, HPB-surgery or even in liver transplantation. A small phase 1 study showed that the concentration of rivaroxaban after bariatric surgery does not alter compared to values pre-operatively.(62) Clinical outcomes of rivaroxaban in this group are unfortunately not available. As described earlier, the main advantage of DOACs over LMWH is the mode of administration. DOACs also do not need to be monitored as for VKA and in some clinical settings LMWH as well. Besides, monitoring LMWH is troublesome after OLT and partial hepatectomy since antithrombin levels are frequently low with the risk of overdosing.(3,50,63) This thesis and previous in vitro studies show a substantially altered anticoagulant

effect of several DOACs.(16,64) Before any clinical application of these drugs in the prevention or treatment of VTE's can be recommended, further efficacy, safety and dose finding studies are warranted.

Data on procoagulant therapy in HPB-surgery patients is quite clear, there is no increase in coagulation potential, assessed by thrombin generation tests, after the administration of fresh frozen plasma (FFP) and recombinant factor VIIa (rFVIIa) in patients and controls. There is however a significant procoagulant effect of prothrombin complex concentrate (PCC) on thrombin generation in patients undergoing liver transplantation.(65,66) A previous study on the use of rFVIIa in HPB-surgery patients show similar results.(67) There is surmounting data against the use of FFP in patients with cirrhosis and in patients undergoing OLT.(68–71)

There is almost no prohemostatic effect of FFP shown by thrombin generation in patients with cirrhosis.(72,73) Although it may lead to the normalization of routine coagulation parameters such as the INR, the actual effect of FFP on hemostasis is questionable. It has to be noted that the administration of FFP can have possible side effects such as volume overload leading to an increased portal pressure and an increased risk of bleeding events. The clinical effect of FFP on hemostasis has little to no evidence, in this light alternative prohemostatic options should be explored.(74,75)



PCC might be a suitable option as an alternative to FFP. The data of chapter 6 shows that PCC is effective in improving hemostatic capacity in HPB-surgery patients. Additionally PCC has a highly concentrated amount of coagulation factors in a small volume. The exaggerated in vitro response of PCC seen in chapter 6 might warrant careful use and dosing of these drugs. Earlier thrombin generation testing supports the use of PCC versus FFP in samples taken from patients during OLT (66).

The in vitro approach in chapter 6 limits extrapolation of our results to the clinical in vivo setting. Thrombin generation is only used for research settings. There is no data on which level of ETP represents the optimal value for anticoagulation or procoagulation in a patient. Bed side testing of hemostasis would further facilitate implementation of new anti- and procoagulant strategies. To date there is only one study on whole blood bed side testing and one study on automated thrombin generation.(76,77) Clinical implications of these findings need to be explored in further larger studies.

## Concluding remarks

The intricate balance of hemostasis in patients with cirrhosis and in patients undergoing HPB-surgery is difficult to depict in an overall hemostasis test. For all parts of hemostasis there are various tests available, all with their own advantages and disadvantages. Targeting therapy on a test that does not provide an accurate reflection of hemostasis is troublesome. Since there is no gold standard to test hemostasis, local preferences occur and pro-and anticoagulant strategies might differ between countries, hospitals, and even inside hospitals itself.

This thesis is a step forward in providing rationale for further clinical studies on the use of pro-and anticoagulant therapies in patients with cirrhosis and in patients undergoing HPB-surgery.

## Future perspectives

Future studies will have to address whether enhanced thrombin generation in patients with cirrhosis contributes to the elevated risk of important clinical outcomes, such as venous thrombosis and/or PVT, and what steps should be taken to safely decrease the hypercoagulable state to avoid thrombotic events in this patient population.

To establish which of the currently available anticoagulants is the best option in the treatment of any kind of thrombosis in cirrhosis, more long term safety and efficacy data is required. Long-term cohort studies on the use of anticoagulants in this patient population will lead to better answers.

Peri-operative hemostatic management still needs further optimizing. This thesis provides solid in vitro results to further explore the options of in vivo hemostatic management.

## References


1. Bos S, Blokzijl H, van den Boom B, Lisman T, Adelmeijer J, Kamphuisen P, et al. Haemostatic Profiles are Similar across All Aetiologies of Cirrhosis. Thromb Haemost. 2019;119(02):246–53.
2. Tripodi A, Anstee QM, Sogaard KK, Primignani M, Valla DC. Hypercoagulability in cirrhosis: Causes and consequences. J Thromb Haemost. 2011;9(9):1713–23.
3. Lisman T, Bakhtiari K, Pereboom ITA, Hendriks HGD, Meijers JCM, Porte RJ. Normal to increased thrombin generation in patients undergoing liver transplantation despite prolonged conventional coagulation tests. Journal of Hepatology. 2010. p. 355–61.
4. Vinholt PJ, Alnor AB, Nybo M, Hvas A-M. The primary haemostasis is more preserved in thrombocytopenic patients with liver cirrhosis than cancer. Blood Coagul Fibrinolysis. 2018;(29):307/313.
5. Sinegre T, Duron C, Lecompte T, Pereira B, Massoulier S, Lamblin G, et al. Increased factor VIII plays a significant role in plasma hypercoagulability phenotype of patients with cirrhosis. J Thromb Haemost. 2018;16:1–9.
6. Lebreton A, Sinegre T, Pereira B, Lamblin G, Duron C, Abergel A. Plasma hypercoagulability in the presence of thrombomodulin but not of activated protein C in patients with cirrhosis. J Gastroenterol Hepatol. 2017 Apr;32(4):916–24.
7. Tripodi A, Salerno F, Chantarangkul V, Clerici M, Cazzaniga M, Primignani M, et al. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. Hepatology. 2005 Mar;41(3):553–8.
8. Gatt A, Riddell A, Calvaruso V, Tuddenham EG, Makris M, Burroughs AK. Enhanced thrombin generation in patients with cirrhosis-induced coagulopathy. J Thromb Haemost. 2010;8(9):1994–2000.
9. Groeneveld D, Porte RJ, Lisman T. Thrombomodulin-modified thrombin generation testing detects a hypercoagulable state in patients with cirrhosis regardless of the exact experimental conditions. Thromb Res. 2014;134(3):753–6.
10. Kleiss SF, Adelmeijer J, Meijers JCM, Porte RJ, Lisman T. A sustained decrease in plasma fibrinolytic potential following partial liver resection or pancreas resection. Thromb Res. 2016;140:36–40.
11. Russo FP, Zanetto A, Campello E, Bulato C, Shalaby S, Spiezia L, et al. Reversal of hypercoagulability in patients with HCV-related cirrhosis after treatment with direct-acting antivirals. Liver Int. 2018;1–9.
12. Kleinegris MC, Habets CAM, Van De Sande AJP, Henskens YMC, Ten Cate-Hoek AJ, Van Deursen C, et al. Liver cirrhosis is associated with hypercoagulability, decreased clot strength and normal fibrinolysis. J Thromb Haemost. 2013;11(May):49–50.
13. Amitrano L, Guardascione MA, Menchise A, Martino R, Scaglione M, Giovine S, et al. Safety and efficacy of anticoagulation therapy with low molecular weight heparin for portal vein thrombosis in patients with liver cirrhosis. J Clin Gastroenterol. 2010;44(6):448–51.
14. Intagliata NM, Maitland H, Caldwell SH. Direct Oral Anticoagulants in Cirrhosis. Curr Treat Options Gastroenterol. 2016;14(2):247–56.
15. De Gottardi A, Trebicka J, Klinger C, Plessier A, Seijo S, Terziroli B, et al. Antithrombotic treatment with direct-acting oral anticoagulants (DOACs) in patients with splanchnic vein thrombosis and cirrhosis. Liver Int. 2017;37(5):694–9.

16. Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Meijers JCM, et al. Differential In Vitro Inhibition of Thrombin Generation by Anticoagulant Drugs in Plasma from Patients with Cirrhosis. PLoS One. 2014 Feb;9(2):e88390.
17. Potze W, Siddiqui MS, Boyett SL, Adelmeijer J, Daita K, Sanyal AJ, et al. Preserved hemostatic status in patients with non-alcoholic fatty liver disease. J Hepatol. 2016;65(5):980–7.
18. Molinari M, Carrillo CF, Dongling D, Jorgensen D, Clemente-Sanchez A, Dharmayan S, et al. Portal Vein Thrombosis and Renal Dysfunction: A National Comparative Study of Liver Transplant Recipients for NAFLD versus Alcoholic Cirrhosis. Transpl Int. 2021;epub ahead of print.
19. Potze W, Siddiqui MS, Sanyal AJ. Vascular Disease in Patients with Nonalcoholic Fatty Liver Disease. Semin Thromb Hemost. 2015;41(5):488–93.
20. Targher G, Chonchol M, Miele L, Zoppini G, Pichiri I, Muggeo M. Nonalcoholic fatty liver disease as a contributor to hypercoagulation and thrombophilia in the metabolic syndrome. Semin Thromb Hemost. 2009;35(3):277–87.
21. Hylek EM, Hanna M, Al-khalidi HR, Ph D, Ansell J, Atar D, et al. Apixaban versus Warfarin in Patients with Atrial Fibrillation. N Engl J Med. 2011;365(11):981–92.
22. Giugliano RP, Ruff CT, Braunwald E, Murphy, Sabina A, Wiviott, Stephen D, Halperin, Jonathan L, et al. Edoxaban versus warfarin in patients with atrial fibrillation. N Engl J Med. 2013;369(22):2093–104.
23. Hacke W, Ph D, Breithardt G, Halperin JL, Hankey GJ, Piccini JP, et al. Rivaroxaban versus Warfarin in Nonvalvular Atrial Fibrillation. N Engl J Med. 2011;365(10):883–91.
24. Eikelboom J, Oldgren J, Ph D, Parekh A, Pogue J, Sc M, et al. Dabigatran versus Warfarin in Patients with Atrial Fibrillation Stuart. N Engl J Med. 2009;361(12):1139–51.
25. Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Porte RJ, et al. Routine coagulation assays underestimate levels of antithrombin-dependent drugs but not of direct anticoagulant drugs in plasma from patients with cirrhosis. Br J Haematol. 2013;163(5):666–73.
26. Bos S, Potze W, Siddiqui MS, Boyett SL, Adelmeijer J, Daita K, et al. Changes of in vitro potency of anticoagulant drugs are similar between patients with cirrhosis due to alcohol or non-alcoholic fatty liver disease. Thromb Res. 2017;150:41–3.
27. Mendell J, Johnson L, Chen S. An open-label, phase 1 study to evaluate the effects of hepatic impairment on edoxaban pharmacokinetics and pharmacodynamics. J Clin Pharmacol. 2015;55(12):1395–405.
28. Frost C, Yu Z, Wang J, Li T, Zeigler C, Schuster A, et al. Single-dose safety and pharmacokinetics of apixaban in subjects with mild or moderate hepatic impairment. Clin Pharmacol Ther. 2009;85(Suppl.1):S34 (PI-84).
29. Haidl H, Cimenti C, Leschnik B, Zach D, Muntean W. Age-dependency of thrombingeneration measured by means of calibrated automated thrombography(CAT). Thromb Haemost. 2006;95:772–5.
30. Wu J, Zhao HR, Zhang HY, Ge YL, Qiu S, Zhao JJZ, et al. Thrombin generation increasing with age and decreasing with use of heparin indicated by calibrated automated thrombogram conducted in Chinese. Biomed Environ Sci. 2014;27(5):378–84.
31. Schneider T, Siegemund T, Siegemund R, Petros S. Thrombin generation and rotational thromboelastometry in the healthy adult population. Hamostaseologie. 2015;35(2):181–6.

32. Ogata K, Mendell-Harary J, Tachibana M, Masumoto H, Oguma T, Kojima M, et al. Clinical safety, tolerability, pharmacokinetics, and pharmacodynamics of the novel factor Xa inhibitor edoxaban in healthy volunteers. J Clin Pharmacol. 2010;50(7):743–53.
33. Nagaoki Y, Aikata H, Daijyo K, Teraoka Y, Shinohara F, Nakamura Y, et al. Efficacy and safety of edoxaban for treatment of portal vein thrombosis following danaparoid sodium in patients with liver cirrhosis. Hepatol Res. 2018;48(1):51–8.
34. Intagliata NM, Henry ZH, Maitland H, Shah NL, Argo CK, Northup PG, et al. Direct Oral Anticoagulants in Cirrhosis Patients Pose Similar Risks of Bleeding When Compared to Traditional Anticoagulation. Dig Dis Sci. 2016;61(6):1721–7.
35. De Gottardi A, Seijo S, Plessier A, Schouten J, Trebicka J, Terziroli B, et al. Use of direct oral anticoagulants (DOACs) in patients with splanchnic vein thrombosis and/or cirrhosis. J Hepatol. 2015;62:S229.
36. De Gottardi A, Trebicka J, Klinger C, Plessier A, Seijo S, Terziroli B, et al. Antithrombotic treatment with direct-acting oral anticoagulants in patients with splanchnic vein thrombosis and cirrhosis. Liver Int. 2017;37(5):694–9.
37. Lee HF, Chan YH, Chang SH, Tu HT, Chen SW, Yeh YH, et al. Effectiveness and Safety of Non–Vitamin K Antagonist Oral Anticoagulant and Warfarin in Cirrhotic Patients With Nonvalvular Atrial Fibrillation. J Am Heart Assoc. 2019;8(5).
38. Pastori D, Lip GYH, Farcomeni A, Del Sole F, Sciacqua A, Perticone F, et al. Incidence of bleeding in patients with atrial fibrillation and advanced liver fibrosis on treatment with vitamin K or non-vitamin K antagonist oral anticoagulants. Int J Cardiol. 2018;264:58–63.
39. Hanafy AS, Abd-Elsalam S, Dawoud MM. Randomized controlled trial of rivaroxaban versus warfarin in the management of acute non-neoplastic portal vein thrombosis. Vascul Pharmacol. 2019;113(March 2018):86–91.
40. Villa E, Cammà C, Marietta M, Luongo M, Critelli R, Colopi S, et al. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. Gastroenterology. 2012;143(5):1253-1260.e4.
41. Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease : evidence and clinical consequences. Blood. 2010;116(6):878–85.
42. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med. 2011;365(2):147–56.
43. Lisman T, Leebeek FWG, De Groot PG. Haemostatic abnormalities in patients with liver disease. J Hepatol. 2002;37(2):280–7.
44. von Meijenfeldt FA, van den Boom BP, Adelmeijer J, Roberts LN, Lisman T, Bernal W. Prophylactic fresh frozen plasma and platelet transfusion have a prothrombotic effect in patients with liver disease. J Thromb Haemost. 2020;(July):1–13.
45. De Boer MT, Molenaar IQ, Hendriks HGD, Slooff MJH, Porte RJ. Minimizing blood loss in liver transplantation: Progress through research and evolution of techniques. Dig Surg. 2005;22(4):265–75.
46. Arshad F, Lisman T, Porte RJ. Blood Markers of Portal Hypertension Are Associated with Blood Loss and Transfusion Requirements during Orthotopic Liver Transplantation. Semin Thromb Hemost. 2020;46(6):751–6.
47. Turley RS, Reddy SK, Shortell CK, Clary BM, Scarborough JE. Venous Thromboembolism After Hepatic Resection: Analysis of 5,706 Patients. J Gastrointest Surg. 2012;16(9):1705–14.

48. Potze W, Alkozai EM, Adelmeijer J, Porte RJ, Lisman T. Hypercoagulability following major partial liver resection - detected by thrombomodulin-modified thrombin generation testing. Aliment Pharmacol Ther. 2015 Jan;41(2):189–98.

49. Tzeng C-WD, Katz MHG, Fleming JB, Pisters PWT, Lee JE, Abdalla EK, et al. Risk of venous thromboembolism outweighs post-hepatectomy bleeding complications: analysis of 5651 National Surgical Quality Improvement Program patients. HPB. 2012 Aug;14(8):506–13.

50. Stahl RL, Duncan A, Hooks MA, Henderson MJ, Millikan WJ, Warren WD. A Hypercoagulable State Follows Orthotopic Liver Transplantation. Hepatology. 1990 Sep;12(3):553–8.

51. Reddy SK, Turley RS, Barbas AS, Steel JL, Tsung A, Marsh JW, et al. Post-Operative Pharmacologic Thromboprophylaxis after Major Hepatectomy. J Gastrointest Surg. 2011;15(9):1602–10.

52. Annamalai A, Kim I, Sundaram V, Klein A. Incidence and risk factors of deep vein thrombosis after liver transplantation. Vol. 46, Transplantation Proceedings. 2014. p. 3564–9.

53. Yip J, Bruno DA, Burmeister C, Kazimi M, Yoshida A, Abouljoud MS, et al. Deep Vein Thrombosis and Pulmonary Embolism in Liver Transplant Patients: Risks and Prevention. Transplant direct. 2016;2(4):e68.

54. Ejaz A, Spolverato G, Kim Y, Lucas DL, Lau B, Weiss M, et al. Defining Incidence and Risk Factors of Venous Thromboemolism after Hepatectomy. J Gastrointest Surg. 2014 Jun 13;18(6):1116–24.

55. Bos S, Bernal W, Porte RJ, Lisman T. Hemostatic Complications in Hepatobiliary Surgery. Semin Thromb Hemost. 2017;43(7):732–41.

56. Lisman T, Porte RJ. Value of Preoperative Hemostasis Testing in Patients with Liver Disease for Perioperative Hemostatic Management. Anesthesiology. 2017;126(2):338–44.

57. Duffy JP, Hong JC, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, et al. Vascular Complications of Orthotopic Liver Transplantation: Experience in More than 4,200 Patients. J Am Coll Surg. 2009;208(5):896–903.

58. Weiss MJ, Kim Y, Ejaz A, Spolverato G, Haut ER, Hirose K, et al. Venous thromboembolic prophylaxis after a hepatic resection: Patterns of care among liver surgeons. Vol. 16, Hpb. 2014. p. 892–8.

59. Yoshiya S, Shirabe K, Nakagawara H, Soejima Y, Yoshizumi T, Ikegami T, et al. Portal vein thrombosis after hepatectomy. World J Surg. 2014;38(6):1491–7.

60. Groeneveld DJ, Alkozai EM, Adelmeijer J, Porte RJ, Lisman T. Balance between von Willebrand factor and ADAMTS13 following major partial hepatectomy. Br J Surg. 2016;103(6):735–43.

61. Pereboom ITA, Adelmeijer J, Van Leeuwen Y, Hendriks HGD, Porte RJ, Lisman T. Development of a severe von willebrand factor/adamts13 dysbalance during orthotopic liver transplantation. Am J Transplant. 2009;9(5):1189–96.

62. Kröll D, Nett PC, Borbély YM, Schädelin S, Bertaggia Calderara D, Alberio L, et al. The effect of bariatric surgery on the direct oral anticoagulant rivaroxaban: the extension study. Surg Obes Relat Dis [Internet]. 2018;14(12):1890–6. Available from: https://doi.org/10.1016/j.soard.2018.08.025

63. Mallett S V., Sugavanam A, Krzanicki DA, Patel S, Broomhead RH, Davidson BR, et al. Alterations in coagulation following major liver resection. Anaesthesia. 2016 Jun;71(6):657–68.

64. Potze W, Adelmeijer J, Lisman T. Decreased in vitro anticoagulant potency of Rivaroxaban and Apixaban in plasma from patients with cirrhosis. Hepatology. 2015 Apr;61(4):1435–6.

65. Bezinover D, Dirkmann D, Findlay J, Guta C, Hartmann M, Nicolau-Raducu R, et al. Perioperative coagulation management in liver transplant recipients. Transplantation. 2018;102(4):578–92.

66. Abuelkasem E, Hasan S, Mazzeffi MA, Planinsic RM, Sakai T, Tanaka KA. Reduced Requirement for Prothrombin Complex Concentrate for the Restoration of Thrombin Generation in Plasma From Liver Transplant Recipients. Anesth Analg. 2017;125(2):609–15.

67. Chavez-Tapia NC, Alfaro-Lara R, Tellez-Avila F, Barrientos-Gutiérrez T, González-Chon O, Mendez-Sanchez N, et al. Prophylactic activated recombinant factor VII in liver resection and liver transplantation: Systematic review and meta-analysis. PLoS One. 2011;6(7):1–7.

68. Bernal W, Caldwell SH, Lisman T. Nails in the coffin of fresh frozen plasma to prevent or treat bleeding in cirrhosis? J Hepatol. 2020;72(1):12–3.

69. Weeder PD, Porte RJ, Lisman T. Hemostasis in Liver Disease : Implications of New Concepts for Perioperative Management. Transfus Med Rev. 2014;28(3):107–13.

70. Massicotte L, Thibeault L, Roy A. Classical Notions of Coagulation Revisited in Relation with Blood Losses, Transfusion Rate for 700 Consecutive Liver Transplantations. Semin Thromb Hemost. 2015;41(05):538–46.

71. Biancofiore G, Blasi A, Boer MTDE, Franchini M, Hartmann M, Lisman T, et al. Perioperative hemostatic management in the cirrhotic patient : a position paper on behalf of the Liver Intensive Care Group of Europe ( LICAGE ). Minerva Anesthesiol. 2019;85(July):782–98.

72. Rassi AB, D'Amico EA, Tripodi A, Rocha TRF da, Migita BY, Ferreira CM, et al. Fresh frozen plasma transfusion in patients with cirrhosis and coagulopathy: effect on conventional coagulation tests and thrombomodulin-modified thrombin generation. J Hepatol. 2019;72(1):85–94.

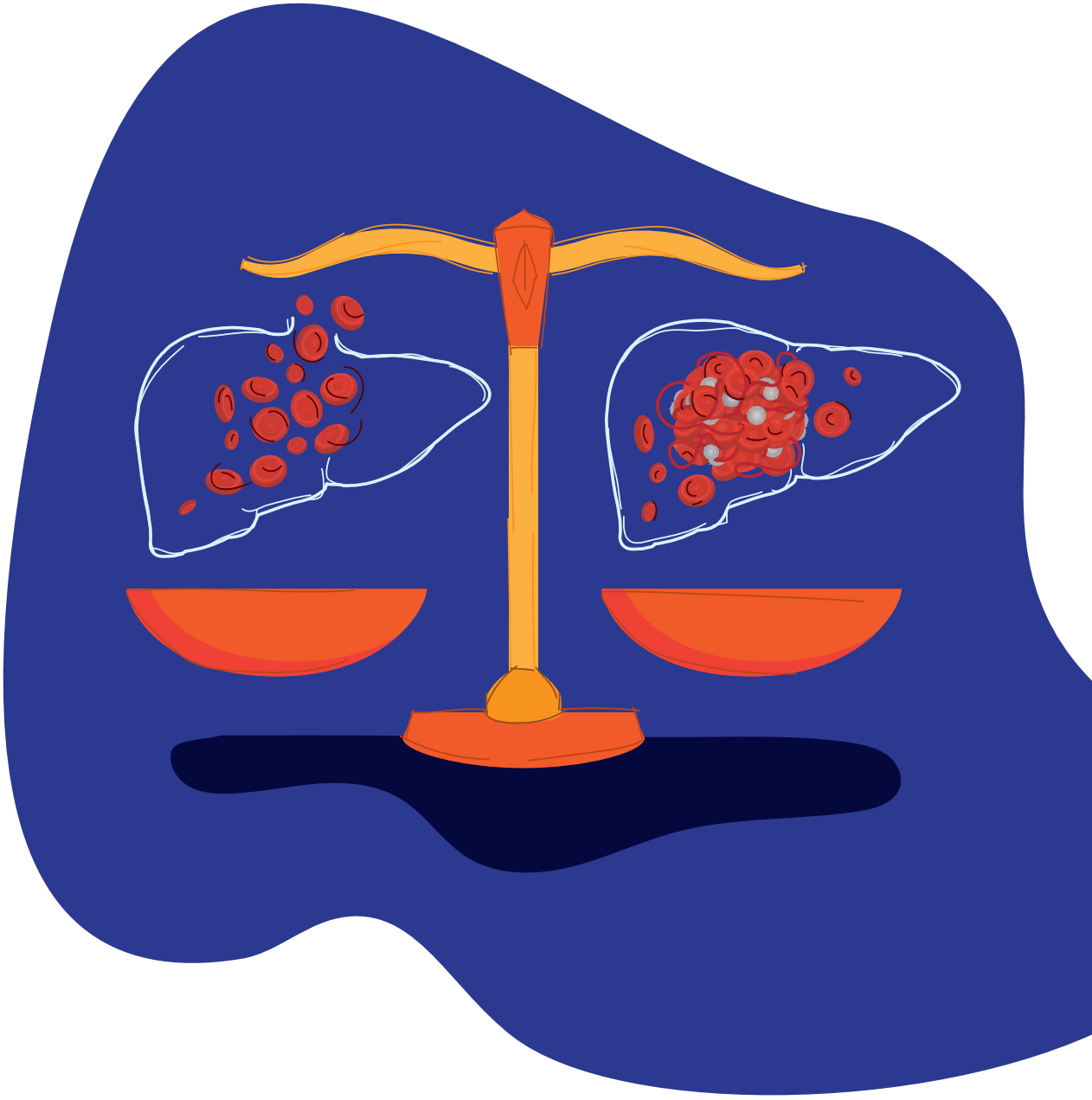
73. Tripodi A, Chantarangkul V, Primignani M, Mannucci PM. Thrombin generation in plasma from patients with cirrhosis supplemented with normal plasma: considerations on the efficacy of treatment with fresh-frozen plasma. 2012;139–44.

74. Stanworth SJ, Brunskill SJ, Hyde CJ, McClelland DBL, Murphy MF. Is fresh frozen plasma clinically effective? A systematic review of randomized controlled trials. Br J Haematol. 2004;126(1):139–52.

75. Bö H. Prothrombin Complex Concentrate Substitution during Liver Transplantation. Thromb Res. 1999;95:71–4.

76. Morrow GB, Beavis J, Harper S, Baker P, Desborough MJR, Curry N, et al. Coagulation status of critically ill patients with and without liver disease assessed using a novel thrombin generation analyzer. J Thromb Haemost. 2020;18(7):1576–85.

77. Wan J, Roberts LN, Hendrix W, Konings J, Ow TW, Rabinowich L, et al. Whole blood thrombin generation profiles of patients with cirrhosis explored with a near patient assay. J Thromb Haemost. 2020;18(4):834–43.



# APPENDICES

# Nederlandse samenvatting

## Introductie

In dit proefschrift wordt het bloedstollingsysteem (hemostase) van patiënten met leverziekten in detail bestudeerd en omschreven. Hierbij is er zowel laboratorium als patiëntgebonden onderzoek verricht. Ook is er in samenwerking met Kings College Hospital in London onderzoek gedaan naar het effect van stollings-en antistollingsmedicijnen in het bloed van patiënten die of een grote lever operatie, een alvleesklier operatie of een levertransplantatie ondergingen.

Patiënten met een gevorderde leverziekte waarbij litteken vorming van de lever optreedt (cirrose) hebben zowel een grotere kans op bloedingen als op trombose. Deze complicaties kunnen spontaan ontstaan of als gevolg van medische ingrepen. In de kliniek is het voor de behandelend arts soms lastig om voor de individuele patiënt het juiste middel in de juiste dosering te kiezen om stolsels dan wel bloedingen te behandelen of te voorkomen. Er is duidelijke vraag naar helderheid op dit vlak, helaas is er weinig onderzoek dat alle vragen hieromtrent goed kan beantwoorden. Een richtlijn waarbij indicatiestelling, keuze van medicament en adequate dosering aan bod komen is zeker gewenst.

Bij patiënten met cirrose hebben we ook nog te maken met de veranderde klaring van het medicijn door gedeeltelijke klaring en of omzetting van medicijnen door de lever zelf. Om tot een gedegen advies te komen, moeten we eerst weten of de groep patiënten met cirrose onafhankelijk van de oorzaak van hun cirrose vergelijkbare veranderingen hebben in hun hemostase.

In **hoofdstuk 2** wordt in detail het hemostatische profiel beschreven van patiënten die door verschillende ziekten cirrose hebben ontwikkelt. Van patiënten die cirrose hebben door vervetting van de lever (NASH) en galwegzieken is het bekend dat zij vaker trombose ontwikkelen dan patiënten met cirrose door andere oorzaken. Om dit te begrijpen hebben we een groep van 109 patiënten met cirrose door verschillende oorzaken onderzocht en tevens 44 gezonde personen. In deze groep hadden 23 patiënten NASH, 31 galwegziekten, 37 alcoholische leverziekte (ASH) en 18 virale leverziekte. Patiënten met een kwaadaardigheid werden uitgesloten omdat deze patiënten door de kwaadaardigheid al een verhoogde stollingsneiging hebben.

Er zijn enkele laboratoriumtesten gedaan die veel in de kliniek gebruikt worden, maar ook meerdere uitgebreidere laboratoriumtesten die de hemostase grondiger en meer accuraat reflecteren.

Als we het hebben over hemostase delen we dat vaak in drie onderdelen in, namelijk de primaire hemostase, secundaire hemostase en de tertiaire hemostase.

Dit laatste wordt ook wel de fibrinolyse (afbraak en opruiming van het stolsel) genoemd. Elk genoemd onderdeel heeft eigen testen om dit goed weer te geven. Een groot deel van de gemeten stoffen worden in de lever aangemaakt. Het is dan ook goed te begrijpen dat bij patiënten met cirrose de testen andere uitslagen geven dan bij gezonde mensen. We weten nu sinds enige tijd dat zowel de stoffen die nodig zijn om de hemostase aan te jagen maar ook de stoffen om deze af te remmen in mindere mate aanwezig zijn. Aangezien deze allemaal verlaagd zijn is er een nieuwe balans die echter wel kwetsbaar is en bij bijkomende ziekte gemakkelijk kan leiden tot een verandering in de hemostase met als gevolg een bloeding of trombose.

In hoofdstuk twee blijkt dat ongeacht de oorzaak van de cirrose alle patiënten vergelijkbare veranderingen hadden in hun hemostase op primair, secundair en tertiair niveau. Het is dan nog goed om te benoemen dat alle patiënten een milde mate van cirrose hadden. Het is namelijk zo dat als de mate van cirrose toeneemt of deze meer complicaties geeft aan andere organen (decompenseert) de afwijkingen in de hemostase meer uitgesproken worden. Met dit onderzoek kunnen we alleen wat zeggen over groep patiënten met een matige cirrose.

Gezien de grotere kans op trombose bij de groep patiënten met cirrose hebben we in **hoofdstuk 3** beoordeeld in welke mate verschillende antistollingsmiddelen, namelijk low-molecular weight heparin (LMWH), dabigatran en apixaban, inwerken op de hemostase van deze patiënten. De behandeling voor trombose is van oudsher met vitamine K antagonisten (VKA) via de trombosedienst. Ruim tien jaar terug zijn er nieuwe antistollingsmiddelen op de markt gekomen, de directe trombine remmers en de Xa antagonisten (DOAC's). Deze groep heeft als voordeel dat ze niet in het bloed gecontroleerd hoeven te worden zoals de VKA's en nog wel oraal toe te dienen zijn in tegenstelling tot de LMWH die middels een onderhuidse injectie moeten worden gegeven. In de eerste grote studies met DOAC's zijn echter alle lever patienten geëxcludeerd vanwege de fragiele stollingsbalans en de mogelijke veranderde werking van de medicijnen. Er is weinig literatuur over de effecten van DOAC's op de hemostase bij patienten met cirrose. In het onderzoek van hoofdstuk 3 hebben we bloed van patiënten met NASH cirrose en ASH cirrose in vergelijking met obese en slanke controles onderzocht. De eerdere genoemde medicamenten zijn aan het bloed toegevoegd en we hebben nadien een trombose generatie test verricht. Dit is een test waarin wordt beoordeeld in welke mate het bloed nog in staat is om een stolsel aan te maken. Uit deze test kwam naar voren dat patiënten met NASH en ASH op dezelfde wijze reageerden op de nieuwe en oude antistollingsmiddelen. De Xa-remmer apixaban gaf ook vergelijkbare waarden tussen NASH patiënten en obese controles. De andere medicijnen waren niet vergelijkbaar met patiënten en controles.

Zoals eerder al aangegeven is het ook de vraag of de antistollingsmedicatie bij patiënten met cirrose op dezelfde wijze door het lichaam wordt verwerkt. Hierom hebben we in **hoofdstuk 4** gekeken hoe 1 van de Xa-remmers, edoxaban, na 7 dagen behandeling verwerkt wordt door het lichaam van cirrose patiënten vergeleken met gezonde controles. Alle patiënten kregen de volle therapeutische dosering van 60 mg eenmaal daags. De groep bestond uit 16 patiënten met cirrose waarvan de meeste milde ziekte hadden en 1 matig ernstig ziekte had. Dit wordt ook wel weergegeven met de Child Pugh score, die in mate van ernst van A tot en met C loopt. Om te kijken hoe het medicijn zich gedroeg hebben we op meerdere momenten bloed afgenomen en verschillende stollings- en medicatie concentratie testen gedaan. Deze afnames gebeurden op dag 1, 3 en 7.

Voor de start van het onderzoek hadden de patiënten een sterkere stollingsneiging (gemeten met trombose generatie) in vergelijking met de gezonde controles. Op dag 1, 3 en 7 was de stollinsneiging van beide groepen zoals verwacht, afgenomen. Bij de patiënten groep was de remming op de stolling duidelijk minder sterk dan bij de gezonde controles. Hierbij viel op dat de edoxaban concentratie in het bloed wel gelijk was bij beide groepen. Dus dan hebben we ondanks een zelfde gemeten aanwezigheid van het medicijn wel een minder krachtige uitwerking op de stolling in de patiënten groep. Of deze gegevens ook direct vertalen naar een hoger trombose risico dan wel aanpassingen in dosering noodzakelijk zijn, moet verder worden uitgezicht.

In **hoofdstuk 5** is een overzicht gegeven van de op dit moment beschikbare literatuur over veranderde stolling rondom hepato-pancreatico-biliaire chirurgie (HPB-chirurgie). HPB-chirurgie is geassocieerd met een substantieel risico op bloedingen en trombotische complicaties. Gezien de lever een centrale rol heeft in de hemostase is het niet verassend dat er verschillende veranderingen optreden in de hemostase rondom HPB-chirurgie.

Bloedingen tijdens leverchirurgie komen voornamelijk door chirurgische en anatomische factoren, echter speelt de hemostase zeker ook een rol. Zo is dit ook het geval bij levertransplantatie. Hier speelt echter ook nog mee dat patiënten met cirrose, het merendeel van de groep patiënten die voor levertransplantatie in aanmerking komen, ook een verhoogde bloeddruk in de buik hebben (portale hypertensie). Na de operatie is er een verhoogd risico op een trombosebeen of trombose van een van de bloedvaten in de buik. Het ontstaan van trombose in een van de grote bloedvaten in de buik bij een levertransplantatie kan zeer ernstige gevolgen hebben. Dit kan bijvoorbeeld leiden tot verlies van functie van de donorlever. Het voorkomen en behandelen van bloedingen en trombose zijn dan ook essentieel in de management van patiënten die dergelijke operaties ondergaan. In de afgelopen 10 jaar zijn er verscheidene studies geweest die

hebben gezorgd voor een beter begrip in de veranderingen van de hemostase rondom HPB-chirurgie.

In **hoofdstuk 6** is bestudeerd op welke wijze medicatie, die de hemostase beïnvloedt, werkt voor tijdens en na HPB-chirurgie. Rondom deze operaties treden unieke veranderingen in de hemostase op. We hebben een analyse gedaan op het bloed van 60 patiënten en bij een aantal gezonde vrijwilligers (controles). In alle bloedmonsters zijn stollings- en antistollingmedicatie toegevoegd. Voor en na deze toevoegingen hebben we stollingstesten verricht. De medicijnen die we hebben gebruikt zijn fresh frozen plasma (FFP), prothrombin complex concentrate (PCC), recombinant factor VIIa (rFVIIa), low-molecular weight heparin (LMWH), unfractionated heparin, dabigatran, and rivaroxaban. De resultaten lieten zien dat patiënten die HPB-chirurgie ondergaan een verhoogde stollingsneiging hebben. FFP and rFVIIa hadden minimaal effect op trombine generatie, waar PCC een meer uitgesproken invloed had op het aanjagen van de stolling bij patiënten in vergelijking met controles. Dabigatran liet een uitgesproken antistollend effect zien bij patiënten terwijl rivaroxaban en LMWH bij patiënten een minder sterk antistollend gaf in vergelijking met de controle groep.

De fragiele balans in de hemostase bij patiënten met cirrose en patiënten die HPB-chirurgie ondergaan is lastig weer te geven in een overkoepelende hemostase test. Voor alle onderdelen van de hemostase zijn andere testen met hun eigen voor- en nadelen. Medicatie instellen of starten op geleide van een test die niet een adequate reflectie geeft van de stolling is lastig. Omdat er geen gouden standard is, zijn er regionaal, lokaal maar soms ook binnen de ziekenhuizen verschillende voorkeuren. Dit proefschrift is een stap voorwaarts in het geven van een rationale in het gebruik van medicatie die de hemostase beïnvloedt bij patiënten met cirrose en patiënten die HPB-chirurgie ondergaan.

## Toekomstperspectief

Nieuwe studies zullen moeten aangeven of een toegenomen trombine generatie in patiënten met cirrose ook daadwerkelijk een verhoogd risico geeft op belangrijke klinische uitkomsten zoals veneuze trombose. Maar ook zal moeten blijken welke stappen moeten worden gezet om veilig de verhoogde trombose neiging te verlagen in deze patiënten groep.

Om goed te kunnen beoordelen welke van de huidig beschikbare medicijnen die de stolling afremmen en aanjagen we moeten gebruiken, zullen we meer gegevens over de veiligheid nodig hebben. Lange termijn studies over het gebruik van deze medicijnen bij patiënten met cirrose zullen hierover meer informatie geven.

# List of publications


1. **Bos S**, Grobbee DE, Boer JM, Verschuren WM, Beulens JW. Alchol consumption and risk of cardiovascular disease among hypertensive women. Eur J Cardiovasc Prev Rehabil. 2010 Feb;17(1):119-26.
2. Amraoui F, **Bos S**, Vogt L, van den Born BJ. Long-term renal outcome in patients with malignant hypertension: a retrospective cohort study. BMC Nefrology. 2012 July 30;13:71.
3. **Bos S**, Nap R.R.H., Wouters R.S.M.E., van der Kleij F.G.H. Hypocitraturia: a common but not well-known cause of nephrolithiasis. Neth J of Med. 2014 Dec, vol. 72, no 10
4. **Bos S**, Potze W, Siddiqui MS, Boyett SL, Adelmeijer J, Daita K, Lisman T, Sanyal AJ. Changes of in vitro potency of anticoagulant drugs are similar between patients with cirrhosis due to alcohol or non-alcoholic fatty liver disease. Thromb Res. 2017 Feb; 150:41-43
5. **Bos S**, Bernal W, Porte R, Lisman T. Hemostatic complications in hepatobiliary surgery. Semin Thromb Hemost. 2017 Oct;43(7):732-741
6. van Miert JHA, **Bos S**, Veeger NJGM, Meijer K. Clinical usefulness of the SAMe-TT2R2 score: A systematic review and simulation meta-analysis. PLoS One. 2018 Mar 13;13(3):e0194208
7. Lisman T, **Bos S**, Intagliata NM. Mechanisms of enhanced thrombin-generating capacity in patients with cirrhosis. J Thromb and Haemost 2018 Jun;16(6):1128-1131
8. Lisman T, Kleiss S, Patel VC, Fisher C, Adelmeijer J, **Bos S**, Singanayagam A, Stoy SH, Shawcross DL, Bernal W. In vitro efficacy of pro- and anticoagulant strategies in compensated and acutely ill patients with cirrhosis. Liver International 2018
9. Burlage LC, **Bos S**, Adelmeijer J, Sakai T, Porte RJ, Lisman T. Plasma from Patients undergoing Liver Transplantation is Resistant to Anticoagulant Activity of Soluble Thrombomodulin (ART-123). Liver Transpl. 2018 Aug 1
10. von Meijenfeldt FA, Burlage LC, **Bos S**, Adelmeijer J, Porte RJ, Lisman T. Elevated plasma levels of cell-free DNA during orthotopic liver transplantation are associated with activation of coagulation. Liver Transplantation 2018 Aug 31.
11. **Bos S**, van den Boom B, Kamphuisen PW, Adelmeijer J, Blokzijl H, Schreuder T, Lisman T. Haemostatic Profiles are Similar across All Aetiologies of Cirrhosis. Thromb Haemost. 2019 Feb;119(2):246-253.
12. **Bos S**, van den Boom B, Ow TW, Prachalias A, Adelmeijer J, Phoolchund A, Dunsire F, Milan Z, Roest M, Heaton N, Bernal W, Lisman T. Efficacy of pro- and anticoagulant strategies in plasma of patients undergoing hepatobiliary surgery. J Thromb Haemost. 2020 Nov;18(11):2840-2851.
13. **Bos S**, Schreuder T, Blokzijl H, Adelmeijer J, Lisman T, Kamphuisen PW. Anticoagulant activity of edoxaban in patients with cirrhosis. Blood. 2020 Sep 24;136(13):1561-1564.

## Curriculum vitae

Sarah Bos werd geboren op 11 maart 1984 te Groningen. In 2003 haalde zij haar Gymnasium diploma aan het Maartens College te Haren. Na initieel gestart te zijn met International Economics and Business aan de Universiteit van Groningen, werd zij het jaar erop ingeloot voor geneeskunde aan de Universiteit van Amsterdam. Haar oudste coschap deed zij bij de interne geneeskunde in Almere en haar keuze coschap werd gevolgd op de Intensive Care in het Slotervaart ziekenhuis in Amsterdam, alwaar zij in 2011 ook haar eerste baan als anios kreeg. Tijdens haar coschappen deed zij onderzoek bij de vasculaire geneeskunde in het AMC onder begeleiding van dr. B.J. van den Born.

In 2012 is zij met haar gezin terug gegaan naar het noorden. Na hier kortstondig de huisartsenopleiding te hebben gedaan bleek zij toch meer aangetrokken door de uitdagingen die komen kijken bij het werken in het ziekenhuis en is zij in 2014 gestart als AIOS Interne Geneeskunde vanuit het UMCG bij de Treant Zorggroep in Emmen. Vanuit hier is zij een AGIKO traject opgestart samen met professor Kamphuisen, professor Lisman en dr. Schreuder. Via dit onderzoek kwam ze meer in contact met de Maag-, darm, en leverziekten wat in 2016 leidde tot een transitie naar dit specialisme. Eind november 2021 rond zij haar opleiding tot maag-, darm-leverarts af en is haar proefschrift in de tussentijd succesvol afgerond.

# Dankwoord

Het verrichten van wetenschappelijk onderzoek is bij uitstek een team effort. Ik wilde bij deze dan ook gebruik maken van de gelegenheid om de personen die mijn onderzoek tot een succes hebben gebracht te bedanken.

**Prof. Dr. T. Lisman**, beste Ton, zonder jouw hulp en ondersteuning was dit proefschrift mij hoogstwaarschijnlijk niet gelukt. Ik heb in de afgelopen vijf jaar veel van je geleerd. Je bent ongelofelijk snel en je schrijft jaloersmakend makkelijk. Wat fijn is aan jou is dat geen idee te gek was. Als er buitenland plannen zijn heb jij zo je contacten aangehaald en binnen een paar maanden een buitenlandse METC geregeld. Zo kreeg mijn traject een leuke wending en hebben we een mooi stuk afgeleverd met Kings. Bedankt voor de ruimte en mogelijkheden die je me hebt gegeven.

**Prof. Dr. P.W. Kamphuisen**, beste Pieter Willem. Al tijdens mijn eerste jaar als geneeskunde student hebben we elkaar leren kennen. Destijds was je mijn werkgroep begeleider. In het hele beloop van mijn studie heb ik jou vaak als mentor kunnen benaderen. Zo was er bij mij ook geen twijfel dat ik bij jou onderzoek wilde doen in Groningen. Binnen no-time had je een vlot en enthousiast team verzameld. De funding voor mijn onderzoek was ook iets wat je zo voor elkaar had. Ik ben je hier enorm dankbaar voor. Dank je wel voor je enthousiasme en je altijd positieve benadering van mijn onderzoek en werk!

**Dr. T.C.M.A. Schreuder**, beste Tim vanuit het onderzoek hebben we elkaar leren kennen. Je enthousiasme voor het werk en onderzoek werkt enorm aanstekelijk. Het was altijd een plezier om met jou te overleggen, niets was teveel. Je hebt je enorm ingezet voor het promoten van mijn studies en het includeren van patiënten. Ik ben je hier uiteraard erg dankbaar voor. Ondanks dat je rol in het beloop van mijn promotie iets meer op de achtergrond is geweest is jouw aandeel zeer waardevol!

Highly learned opponents of the assessment committee; **prof. H.J. Verkade**, **prof. E. Villa**, **prof. T. Hackeng**, thank you for reading and evaluating my thesis.

Tijdens mijn promotie kwam ik veelvuldig bij Tim op de kamer voor het bespreken van potentiele inclusies. Niet alleen Tim maar ook zijn kamergenoot **dr. H. Blokzijl**/ **Hans** was betrokken, enthousiast en positief over het onderzoek. De samenwerking was wederzijds erg prettig. Dit heeft er dan ook toe geleid dat op een gegeven moment de vraag kwam of ik niet beter zou passen bij de MDL. Hans, ik ben je bijzonder dankbaar voor het bijsturen van de richting van mijn carrière!

Prof. K. Meijer, beste **Karina**, graag wil ik je bedanken dat ik mocht aanhaken bij de stolling ook al viel ik daar niet officieel onder. Een werkplek kreeg ik ook toebedeeld en voor raad en advies kon ik altijd bij je terecht. Veel dank daarvoor!

Mijn dank gaat ook uit naar de **hepatologen** voor het helpen met het informeren en includeren van patiënten voor mijn studies.

Lieve **Lyda**, wat heb ik veel aan je gehad. Je kon ad hoc voor mij patiënten benaderen, prikken en samples afdraaien. Jouw onderzoeksvriezer was een mooie buffer voor mijn samples. Ook heb je voor de ondersteuning van mijn onderzoek extra tijd geïnvesteerd om weer bloedafnames te kunnen doen.

**Jelle**, wat heb je ongelofelijk veel werk verzet. Zeker je afgezonderde dagen in Maastricht met alle samples van Kings zijn een monster sessie geweest. Ontzettend veel dank voor je precieze en vlotte hulp hierbij. Zonder deze data geen proefschrift!

Alle collega's van het chirurgisch onderzoekslab, in het bijzonder **Dafna**, **Fien** en **Susanne,** dank voor de leuke tijd op en buiten het lab!

Onderzoeksmaatjes, **Hilde**, **Jaime**, **Vladimir** , **Soeraya** en **Tessa** dank jullie wel voor de leuke tijd bij de interne en op de Y3 onderzoekskamer. **Jasper**, onze samenwerking was altijd erg gezellig. Jouw verontwaardiging over de opzet van bepaald onderzoek stak je niet onder stoelen of banken. Meestal had je ook wel gelijk als er statistisch toch iets niet helemaal klopte. Door het samenwerken met jou heb ik veel over statistiek geleerd. Onze projecten hadden iets overlap wat maakte dat we samen een project hebben kunnen afronden tot een mooi manuscript.

Voor een stage wetenschap kwam je bij mijn onderzoek terecht, **Bente**. Wat is hier een leuke samenwerking uit ontstaan. Je hebt me enorm geholpen in Londen. Na een korte prikcursus van de lokale phlebotomist en een sessie afdraaien en stickeren had je jouw draai zo gevonden in Kings. Ik vind het erg leuk dat je nu vervolg hebt gegeven aan je onderzoek in een eigen promotie traject bij Ton.

Prof. W. Bernall, dear **Will** it was great to work with you in London. Many thanks for your quick replies and great help with the ethical committee and politics in Kings.

Voor mijn onderzoek in London ben ik ben ik veel dank verschuldigd aan de collega's daar ter plaatse. Many thanks to the **scrub nurses**, **transplant coordinators**, **HPB surgeons** and **anesthesiologists**. Special thanks to **Tsai Wing O** for assisting me in the lab and helping me in and around Kings!

Het merendeel van mijn promotie vond plaats tijdens mijn opleidingsjaren bij de MDL. Ik zou dan ook mijn **collega's in UMCG en Isala** willen bedanken voor de leuke opleidingsjaren. Er is altijd ruimte geweest om even wat tijd te nemen voor mijn onderzoek waar ik jullie zeer dankbaar voor ben.

Mijn paranimfen/carpool buddy's **Robert** en **Daan**. Wat ontzettend leuk dat jullie mijn paranimfen willen zijn. We kennen elkaar door onze vele uren in de auto van en naar Zwolle ondertussen behoorlijk goed. Ik heb altijd veel lol met jullie samen, maar is altijd ook ruimte voor de serieuzere gesprekken. Ik kon mij geen beter tweetal bedenken om deze mooie dag samen mee voor te bereiden!

Lieve **Conny** en **Frans** graag zou ik jullie ook willen bedanken. Vooral voor de ondersteuning die jullie ons als gezin hebben gegeven de afgelopen jaren. Jullie staan voor ons (en de hele familie) klaar. Dit voelt vertrouwd en hier ben ik jullie zeer dankbaar voor. Lieve **Maartje**, **Jan**, **Neeltje** en **Christiaan**, **Zoë** en **Ralph**, **Denise** en **Arjen** de afgelopen jaren heb we samen vele leuke momenten gedeeld. Dit is zeer waardevol en hopelijk kunnen we de komen jaren nog meer mooie dagen en vakanties samen doorbrengen.

Lieve **Naomi** we hebben altijd ons eigen pad bewandeld maar zijn uiteindelijk, net als onze ouders beide in de zorg terecht gekomen. Allebei zoeken we naast ons werk de verdere uitdaging en ontwikkeling. Ook al hebben onze uitdagingen en ontwikkelingen niet op alle fronten overlap ik kan altijd bij je terecht om te kletsen (of te klagen). Ik ben je zeer dankbaar voor je oprechte interesse. **Zita,** ondanks ons leeftijds verschil lijkt het alsof we de afgelopen jaren meer en meer raakvlak hebben gekregen in ons werk. Ik vind het dan ook leuk om met je te sparren over onderzoek en daarbij te merken dat we tegen dezelfde problemen aanlopen. Dank je wel voor je steun de afgelopen jaren! Ook zou ik **Robert** en **Mark** willen bedanken voor de interesse in mijn onderzoek en afleiding waar nodig.

Lieve **Hyke** wie had gedacht tijdens onze eerste jaren bij Nereus dat we allebei een promotie traject zouden bewandelen? Dichtbij maar ook op afstand heb ik veel aan jou en **Floris** gehad. Er is vanuit jou altijd een luisterend oor, maar een muurtje verven in Zuidlaren is ook geen probleem. We hebben samen veel mooie en soms ook verdrietige momenten meegemaakt. Soms zien we elkaar iets minder frequent maar het voelt altijd direct weer vertrouwd. Er zullen vast nog meer mooie momenten komen die we kunnen delen!

**Papa** en **mama**, wat zou ik zonder jullie moeten. Jullie zijn onze steun en toeverlaat. We kunnen heerlijk ontspannen samen, thuis of op vakantie. Als er werk aan de winkel is wordt er altijd hulp aangeboden. Ook jullie hulp bij de meiden heeft mij veel rust en mogelijkheden gegeven om mijn werk de invulling te geven die het nu

heeft. Ik ben jullie allebei dan ook zeer dankbaar voor jullie sturing, ondersteuning en ruimte voor ontwikkeling

Lieve **Noëlle**, **Felice** en **Florine**, wat een heerlijke meiden zijn jullie. Als ik thuiskom van een drukke dag ben ik zo alles kwijt door jullie gezellige geklets. Ik hou ontzettend van jullie. Ik vind het erg leuk dat deze dag met jullie kan delen.

Allerliefste **Karel-Jan**, onze tijd samen hebben we naast ons mooie leven met de meiden, professioneel een vergelijkbaar pad bewandeld. Het fijne hieraan is dat ik altijd met alles bij je terecht kan. Je gaf de aanmoedigingen waar nodig en voor een kritische blik op teksten of praktische problemen kon ik altijd op je hulp rekenen. Het allerbelangrijkste is denk ik wel dat we samen een mooie balans hebben gecreëerd in werk en privé wat onze promoties zeker tot een succes heeft gebracht. Op naar de volgende mooie jaren samen!

Tot slot zou ik graag alle patiënten willen bedanken voor hun deelname aan de onderzoeken beschreven in dit proefschrift