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Published in:
Journal of Thrombosis and Haemostasis

DOI:
10.1111/jth.15296

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

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

Publication date:
2021

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
van den Boom, B. P., von Meijenfeldt, F. A., Adelmeijer, J., Roberts, L. N., Bernal, W., & Lisman, T. (2021). Heparins have adequate ex vivo anticoagulant effects in hospitalized patients with cirrhosis. *Journal of Thrombosis and Haemostasis*, *19*(6), 1472-1482. https://doi.org/10.1111/jth.15296

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Download date: 29-10-2022

Check for updates

Received: 8 February 2021 | Accepted: 8 March 2021

DOI: 10.1111/jth.15296

ORIGINAL ARTICLE

Heparins have adequate ex vivo anticoagulant effects in hospitalized patients with cirrhosis

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Abstract

Background: Patients with cirrhosis are at risk of venous thromboembolism (VTE), but strategies for thromboprophylaxis have not been defined. Previous *in vitro* studies suggest an altered anticoagulant effect of heparins in patients with cirrhosis.

Objectives: To assess the anticoagulant effects of prophylactic low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) doses in patients with cirrhosis in a real-life clinical setting.

Methods: We studied patients with cirrhosis (n = 16) and acute-on-chronic liver failure (ACLF) (n = 14), and compared these with patients without underlying liver disease admitted to non-liver general medical wards (n = 18) and non-liver intensive care units (n = 14), respectively. Blood samples were taken before and 4 h after administration of the first dose of LMWH or UFH. We assessed hemostatic status using thrombin generation assays, thrombin-antithrombin complexes (TAT), and conventional coagulation assays, and included healthy controls (n = 20) to establish reference values. Anti-Xa activity was determined to estimate peak heparin levels.

Results: Baseline thrombin generation was similar among all cohorts and healthy controls despite alterations in conventional coagulation assays. On heparin, both absolute and proportional changes of thrombin generation were comparable between all four cohorts (−62% to −85%). TAT levels decreased in all cohorts apart from the ACLF cohort, but did not correlate with the proportional change in thrombin generation. Anti-Xa activity correlated with the proportional change in thrombin generation in patients receiving LMWH, but not in patients receiving UFH.

Conclusions: These data suggest that current prophylactic heparin doses have comparable anticoagulant effects in patients with cirrhosis compared with patients without underlying liver disease.

KEYWORDS
acute-on-chronic liver failure, liver cirrhosis, low-molecular-weight heparin, heparin, venous thromboembolism

Bente P. van den Boom and Fien A. von Meijenfeldt are Joint first authors and William Bernal and Ton Lisman are senior authors.

Manuscript handled by: Sabine Eichinger

Final decision: Sabine Eichinger, 08 March 2021

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Essentials

- Strategies on thromboprophylaxis in patients with cirrhosis have not yet been defined.
- We assessed the haemostatic status of patients with cirrhosis on thromboprophylaxis.
- Heparin doses had comparable anticoagulant effects in patients with and without cirrhosis.
- These findings add to an evidence-based approach on thromboprophylaxis in patients with cirrhosis.

1 | INTRODUCTION

Patients with cirrhosis often have complex alterations of their hemostatic system. Simultaneous changes in production and clearance of pro- and anticoagulant drivers by the liver result in a delicate "rebalanced hemostasis."[1,2] Because of the limited stability of the hemostatic balance, patients are at risk for both bleeding and thrombosis. Even though liver disease has historically been classified as a bleeding disorder, large meta-analyses have shown that patients with cirrhosis have a more than 2-fold increased risk of venous thromboembolism (VTE) compared with patients without underlying liver disease.[3] Although published data are scarce, VTE likely occur more often in sicker patients with systemic complications of their liver disease, as seen in acute decompensation of cirrhosis and acute-on-chronic liver failure (ACLF).[4] However, it is yet unclear which individual cirrhotic patient is at particular risk for VTE, and thus which patients might benefit from thromboprophylaxis.

Current guidelines on in-hospital thromboprophylaxis – often involving risk assessment models such as the Padua Prediction Score for Risk of VTE[5] were developed in hospitalized general medical patients,[6] and do not take the complex hemostatic alterations of patients with cirrhosis into account. Even though such risk assessment models might be useful in patients with cirrhosis,[7] large prospective studies are needed to validate these models in this specific patient group. Despite increasing awareness of the increased risk of VTE in patients with cirrhosis, current prescription rates of thromboprophylaxis with low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) in hospitalized patients are low, ranging from 9% to 52%.[8-13] This in part reflects poor adherence to protocols for thromboprophylaxis also seen in the general medical inpatient population,[14] but may also reflect the ongoing perception of clinicians that patients with cirrhosis are "autoanticoagulated" based on their elevated International Normalized Ratio (INR). Evidence-based guidelines are therefore needed to support clinicians in their decision to prescribe thromboprophylaxis in this patient group.

Given the complex alterations of the hemostatic system in patients with cirrhosis and the role of the liver and kidneys in clearance of commonly used anticoagulant drugs, it is plausible that the efficacy of thromboprophylaxis is altered. In patients without underlying liver disease, plasma activity of LMWH and UFH can be monitored using anti-Xa tests. It has been proposed that these tests are unreliable in patients with cirrhosis and that they underestimate the plasma levels of LMWH and UFH because of the reduced levels of antithrombin (AT) present in patients' plasma.[15,16] Also, anti-Xa tests are an indicator of heparin plasma levels, but do not necessarily correlate with anticoagulant activity. Functional tests, such as the thrombin generation assay (TGA),[17] might be better suited for monitoring the anticoagulant effects of LMWH and UFH in patients with cirrhosis. Using this technique, Senzolo et al. described an increased anticoagulant effect of LMWH in patients with cirrhosis after in vitro addition to patients' plasma.[18] Although a similar study performed by our group did not find an increased anticoagulant effect of LMWH or UFH in plasma of patients with stable cirrhosis,[19] *in vitro* addition of LMWH to plasma of patients with decompensated cirrhosis or ACLF did result in an increased anticoagulant effect compared with healthy controls.[20]

Even though these in vitro experiments have shown an altered response to LMWH and UFH in plasma of patients with cirrhosis, these experiments do not take *in vivo* changes in cirrhotic patients into account, such as the possibility of an altered clearance of these drugs by the liver. In this study, we therefore aimed to assess the effect of LMWH and UFH in patients with cirrhosis in a real-life clinical setting, by measuring thrombin generation before and after administration of a first dose of standard prophylactic LMWH or UFH.

2 | METHODS

2.1 | Patients

This prospective observational cohort study was performed at King's College Hospital London, UK, from June 2019 until December 2019. We recruited four different patient cohorts. These consisted of patients with compensated or decompensated cirrhosis admitted to liver wards, patients without preexistent liver disease admitted to non-liver general medical wards, patients with ACLF admitted to the liver-specific intensive care unit (ICU), and patients without pre-existing liver disease admitted to non-liver ICUs. A group of healthy controls was included to determine reference ranges for the tests performed. Inclusion criteria were an age of 18 years or older, signed informed consent (or, in case of incapacity, a consultee signed declaration of assent) and an indication for starting thromboprophylaxis, as prescribed by a physician of the direct clinical care team. Diagnosis of cirrhosis in the cirrhosis and ACLF cohorts was confirmed by biopsy or by radiology (FibroScan F4 and/or radiologic features suggestive of cirrhosis). Exclusion criteria for patients and healthy controls consisted of evidence of malignancy (with exception of nondisseminated hepatocellular carcinoma in the compensated/decompensated cirrhosis and ACLF patient cohorts), documented hereditary thrombophilia or hemophilia, HIV positivity,

pregnancy, and use of anticoagulant medications such as direct oral anticoagulants or vitamin K antagonists. Additional exclusion criteria for healthy controls were history of VTE and the use of oral contraceptives. The study was approved by Health Research Authority and Health Care and Research Wales, study number 19/WA/0168 and the local Research and Innovation Department of King's College Hospital.

2.2 | Intervention

Patients receiving prophylactic dosages of the following anticoagulants were investigated in this study:

- The LMWH enoxaparin (Sanofi), subcutaneous injection
- UFH (Wockhardt), subcutaneous injection

The dose for thromboprophylaxis was determined by the attending physician and was body weight–dependent, per thromboprophylaxis guidelines at King's College Hospital London based on previous research.[21] For patients receiving enoxaparin, the recommended dosages were: body weight <50 kg 20 mg daily; body weight 50 to 100 kg 40 mg daily; body weight 101 to 150 kg 40 mg twice daily or 80 mg once daily; or body weight >150 kg 60 mg twice daily or 120 mg once daily. For patients receiving UFH, recommended dosages were: body weight <100 kg 5000 units twice daily or >100 kg 5000 units three times a day. In this study, patients receiving enoxaparin or UFH were recruited before the first dose administered.

2.3 | Blood samples

Blood samples were collected before and 4 h after administration of the first dose of thromboprophylaxis. Blood was taken either by venipuncture or from indwelling nonheparinized vascular catheters (in which case a discard tube was used). Each blood sample consisted of 9 ml of blood collected into vacuum tubes containing 3.2% sodium citrate as an anticoagulant, at a blood to anticoagulant ratio of 9:1. The citrated blood was processed to platelet-poor plasma by double centrifugation at 2000*g* and 10 000*g*, respectively, for 10 min at 18°C. Plasma was stored at −80°C until use for analyses. Pooled normal plasma, used for calibrating some of the hemostatic tests performed in this study, was a generous gift from Dr. J.C. Meijers (Sanquin and Amsterdam University Medical Center, Amsterdam, The Netherlands) and consisted of plasma from >200 healthy individuals.

2.4 | Thrombin generation assay

Thrombomodulin-modified TGA was performed in platelet-poor plasma with the fluorimetric method described by Hemker et al.[17] 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 (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 thrombin, velocity index (slope between the end of lag time and peak thrombin), and lag time (time needed for thrombin concentration to reach one-sixth of the peak concentration). The anticoagulant effect of heparin administration was expressed as the percentage change of ETP after heparin administration. We calculated the percentage of change in ETP for each individual patient, and compared the median change in ETP between the different cohorts.

2.5 | Anti-Xa activity

Anti-Xa activity was measured on an automated coagulation analyzer (ACL 300 TOP) using Heparin LRT (Hyphen Biomed). Because the low AT levels in patients with cirrhosis could underestimate anti-Xa activity,[15] we measured anti-Xa activity in the presence and absence of 1 plasma equivalent unit (PEU) of AT (Hyphen Biomed, Amsterdam, The Netherlands) added to the test sample.

2.6 | Conventional coagulation assays

The INR, activated partial thromboplastin time (APTT), coagulation factor II, factor X, and AT levels were assessed using the STA Compact Max3 analyzer with reagents and protocols from the manufacturer (Stago). Clauss fibrinogen was measured on an automated coagulation analyzer (ACL 300 TOP) with reagents and protocols from the manufacturer (Werfen). Levels of von Willebrand factor (VWF) were assessed with in house enzyme-linked immunosorbent assay using commercially available polyclonal antibodies against VWF (DAKO). Levels of thrombin-antithrombin (TAT) complexes were measured to assess *in vivo* thrombin generation using the Enzygnost TAT micro kit (Siemens Healthcare Diagnostics).

2.7 | Routine laboratory tests

Serum levels of sodium, potassium, creatinine, albumin, bilirubin, alkaline phosphatase, aspartate transaminase, gamma-glutamyltransferase, and C-reactive protein, and a whole blood cell count (white blood cell count, red blood cell count, platelet count, hemoglobin level, mean corpuscular volume) were measured in the Blood Sciences Laboratory of King's College Hospital London as part of routine clinical care.

2.8 | Statistical analysis

Data are expressed as medians (with interquartile ranges) or numbers (with percentages) as appropriate. Comparisons were made between patient cohorts and healthy controls, and between liver and non-liver patient cohorts (compensated/decompensated cirrhosis vs. patients without underlying liver disease admitted to non-liver general medical wards, and ACLF vs. patients without underlying liver disease admitted to non-liver ICUs). Multiple cohorts were compared using Kruskal-Wallis *H* test (with Dunn's posttest). Spearman's correlation coefficient was used to assess the association between continuous variables. *p* values of .05 or smaller were considered statistically significant. Statistical analyses were performed using GraphPad Prism v7 and SPSS Statistics 23 (IBM).

3 | RESULTS

3.1 | Patients

In total, 65 patients met the inclusion criteria and were consecutively included in this study: 16 with compensated or decompensated cirrhosis (5 with compensated and 11 with decompensated disease, from here on referred to as cirrhosis), 18 without underlying liver disease admitted to non-liver general medical wards, 14 with ACLF, and 14 without underlying liver disease admitted to non-liver ICUs. In addition, 20 healthy controls were included. Table 1 summarizes demographics and clinical and laboratory data of patients and controls. Details of etiology and severity of liver disease in the cirrhosis and ACLF cohorts are depicted in Table 2.

3.2 | Baseline conventional coagulation assays show abnormalities in patients with cirrhosis

We measured the INR and APTT and determined plasma levels of factor II, factor X, AT, fibrinogen, and VWF at baseline (Table 3). The INR was higher in all cohorts except for the non-liver general medical ward cohort compared with healthy controls, with the most pronounced elevation found in the ACLF cohort. The APTT was similar in all cohorts, with the exception of the ACLF cohort, in which the APTT was significantly prolonged compared with both healthy controls and the non-liver ICU cohort.

Levels of factor II, AT, and factor X were lower in the cirrhosis and ACLF cohorts compared with healthy controls, with milder reductions in the non-liver cohorts. Fibrinogen levels were elevated in all cohorts, except in the ACLF cohort, in which fibrinogen levels were decreased compared with healthy controls. VWF levels were significantly increased across cohorts, particularly in ACLF, where levels were approximately 6-fold higher than in healthy controls.

3.3 | Change in thrombin generation after administration of prophylactic heparin is similar across cohorts

Baseline ETP among all cohorts were similar (Table 3). We found no statistically significant differences in ETP between patient cohorts and healthy controls, or between liver and non-liver patient cohorts (cirrhosis vs. non-liver general medical ward patients and ACLF vs. patients on non-liver ICUs). However, both baseline peak thrombin and velocity index were lower in the ACLF cohort compared to healthy controls. Baseline lag times of the ACLF and non-liver ICU cohorts were similar, but prolonged compared with those of healthy controls.

After administration of prophylactic heparins, changes in thrombin generation were comparable across all cohorts (Figure 1). On heparin, the ETP decreased by 85% [80–100] in the cirrhosis cohort, by 70% [51–88] in the non-liver general medical ward cohort, by 62% [41–100] in the ACLF cohort, and by 83% [50–100] in the non-liver ICU cohort. Complete inhibition of thrombin generation occurred in seven patients of the cirrhosis cohort (44%), three of the non-liver general medical ward cohort (17%), six of the ACLF cohort (43%), and four of the non-liver ICU cohort (29%) ($p = .287$). The absolute ETP after administration of heparin did not differ among cohorts, nor did the proportional change in ETP.

The findings were similar across other TGA parameters after administration of prophylactic heparins (Table 3). On heparin, peak, lag time and velocity indices were similar across all cohorts. The proportional change of peak and velocity indices did not differ among cohorts, nor did proportional change of lag times.

3.4 | TAT levels decrease on heparin, but not in the ACLF cohort

At baseline, we found increased plasma levels of TAT in all cohorts compared with those in healthy controls, with a particular increase in the ACLF and non-liver ICU cohorts (Table 3). After administration of heparin, median TAT levels decreased only in the non-liver cohorts (Table 3). However, TAT levels did decrease substantially in individual patients (Figure 2), and considering the proportional changes in TAT levels for individual patients, TAT responses were observed in the cirrhosis, but not ACLF cohorts. On heparin, TAT levels decreased by 21% (8–48) in the cirrhosis cohort, by 20% (6–39) in the non-liver general medical ward cohort, by 14% (–25 to 40) in the ACLF cohort, and by 30% (15–59) in the non-liver ICU cohort.

The proportional change in TAT correlated with the proportional change in the ETP in the cirrhosis cohort ($r = 0.632$, $p = .012$). However, in the other cohorts the proportional change in TAT did not correlate to the proportional change in ETP.

TABLE 1 Patient characteristics

Variable	Healthy Controls (*n* = 20)	Cirrhosis (*n* = 16)	Non-liver General Medical Ward (*n* = 18)	ACLF (*n* = 14)	Non-liver ICU (*n* = 14)
Demographics					
Age, y	44 [39.0–52.5]	59 [50–65]	52 [45–63]	50 [38–63]	59 [43–75]
Female	9 (45%)	8 (50%)	11 (61%)	4 (29%)	7 (50%)
Body mass index, kg/m^2	24.1 [20.3–27.4]	27.9 [24.2–33.5]	27.8 [24.9–36.3]	24.8 [22.3–33.5]	24.9 [22.9–29.5]
Diabetes mellitus, yes	1 (5%)	6 (38%)	3 (17%)	5 (36%)	2 (14%)
Cardiovascular disease, yes	0	6 (38%)	10 (56%)	5 (36%)	8 (57%)
Infection present	0	3 (19%)	13 (72%)	12 (86%)	7 (50%)
Sepsis present	0	1 (6%)	5 (28%)	8 (57%)	7 (50%)
On vasopressors, yes	n/d	0	0	11 (79%)	7 (50%)
Glasgow Coma Scale	n/d	15 [15–15]	15 [15–15]	10 [3–14]	8 [5–15]
Mechanical ventilation, yes	n/d	0	0	7 (50%)	8 (57%)
Renal replacement, yes	n/d	0	0	13 (93%)	1 (7%)
SOFA Score	n/d	n/d	n/d	15 [12–18]	6 [4–7]
Reasons for admission	n/a	Decompensation (*n* = 7) Post minor procedure (*n* = 6) Investigations (*n* = 2) Sepsis (n = 1)	COPD/asthma (*n* = 6) Infection (*n* = 10) Minor trauma (*n* = 1) Post minor procedure (*n* = 1)	ACLF–1 (*n* = 1) ACLF–2 (*n* = 3) ACLF–3 (*n* = 10)	Neurological event (*n* = 4) Sepsis (*n* = 4) Cardiac arrest (*n* = 2) Post minor procedure (*n* = 3) Trauma (*n* = 1)
Type of prophylactic treatment					
LMWH	n/a	11 (69%)	18 (100%)	0	12 (86%)
Units per kilogram	n/a	49.6 [47.3–54.4]	50.6 [42.0–66.5]	n/a	59.3 [53.9–62.8]
UFH	n/a	5 (31%)	0	14 (100%)	2 (14%)
Units per kilogram	n/a	50.0 [47.0–61.2]	n/a	62.5 [51.4–75.5]	73.7 [72.0–75.3]
Laboratory parameters					
WBC count, ×10^9/L	5.88 [5.26–7.93]	7.49 [4.85–11.48]	9.79 [7.83–12.99]	18.35 [13.64–24.44]	11.59 [8.38–15.08]
RBC count, ×10^9/L	4.74 [4.35–5.08]	3.82 [3.25–4.04]	4.20 [4.04–4.65]	2.45 [2.27–2.89]	3.69 [3.07–4.13]
Hemoglobin, g/L	143 [128–151]	124 [97–131]	129 [114–141]	86 [80–102]	110 [96–118]
Mean corpuscular volume, fL	91.6 [89.1–94.0]	94.7 [92.9–100.4]	93.1 [89.7–96.3]	105.1 [101.1–107.3]	92.7 [90.7–95.8]
Platelet count, ×10^9/L	244 [184–323]	157 [105–197]	190 [164–250]	89 [69–104]	261 [184–447]
Sodium, mmol/L	n/d	137 [132–139]	140 [136–142]	136 [136–137]	140 [138–141]
Creatinine, µmol/L	n/d	66 [53–89]	72 [59–92]	120 [67–223]	86 [55–129]
Albumin, g/L	n/d	34 [29–39]	41 [39–45]	27 [26–32]	37 [29–38]

(Continues)

TABLE 1 (Continued)

Variable	Healthy Controls (*n* = 20)	Cirrhosis (*n* = 16)	Non-liver General Medical Ward (*n* = 18)	ACLF (*n* = 14)	Non-liver ICU (*n* = 14)
Total bilirubin, μmol/L	n/d	31 [15–89]	10 [5–17]	390 [165–538]	8 [6–10]
Alkaline phosphatase, IU/L	n/d	152 [89–217]	67 [57–83]	151 [102–240]	77 [63–92]
Aspartate transaminase, IU/L	n/d	72 [41–122]	23 [19–29]	113 [72–215]	43 [18–56]
Gamma-glutamyl transferas, IU/L	n/d	107 [53–282]	32 [18–51]	133 [70–234]	30 [20–93]
C-reactive protein, mg/L	n/d	11.9 [5.1–55.6]	130.6 [8.6–232.3]	66.2 [35.8–97.5]	38.4 [4.7–158.3]

The results are presented as median [interquartile range] for continuous variables, and number (percentage) for categorical variables.

Abbreviations: ACLF, acute-on-chronic liver failure; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; LMWH, low-molecular-weight heparin; n/a, not applicable; n/d, not determined; RBC, red blood cell; SOFA, Sequential Organ Failure Assessment; UFH, unfractionated heparin; WBC, white blood cell.

TABLE 2 Liver disease characteristics

Variable	Cirrhosis (*n* = 16)	ACLF (*n* = 14)
Etiology of liver disease		
Alcoholic steatohepatitis	7	6
Nonalcoholic steatohepatitis	3	3
Hepatitis C virus	2	1
Primary biliary cholangitis	1	0
Primary sclerosing cholangitis	2	0
Cryptogenic	0	2
Combined	1[a]	2[b]
MELD score	13 [10–20]	38 [34–40]
Child-Pugh Score	8 [6–10]	12 [12–13]
Child-Pugh Group		
A	5	0
B	7	0
C	4	14
EF CLIF-AD score	49 [45–58]	n/a
EF CLIF-ACLF score	n/a	65 [61–71]
EF CLIF-SOFA score	n/a	15 [13–16]
Ascites		
No	7	1
Slight	3	4
Moderate	4	3
Severe	2	6
Encephalopathy		
No	13	1
Grade 1	3	4
Grade 2	0	5
Grade 3	0	2
Grade 4	0	2
Hepatocellular carcinoma		
No	0	0
Solitary	1	0
Treated	2	0

The results are presented as median [interquartile range] for continuous variables, and number for categorical variables.

Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; EF CLIF, the European Foundation for the study of Chronic Liver Failure; MELD, Model for End-stage Liver Disease; n/a, not applicable; SOFA, Sequential Organ Failure Assessment.

[a]Combination of autoimmune hepatitis and alcoholic steatohepatitis.

[b]One patient with combined alcoholic steatohepatitis and hepatitis E, one patient with combined alcoholic steatohepatitis and nonalcoholic steatohepatitis.

3.5 | Anti-Xa activity does not correlate with thrombin generation in patients with cirrhosis

In samples taken on heparin, we measured anti-Xa activity in absence and presence of 1 PEU of AT (Table 4). Before addition

TABLE 3 Coagulation assays

Variable	Healthy Controls (*n* = 20)	Cirrhosis (*n* = 16)	Non-liver General Medical Ward (*n* = 18)	ACLF (*n* = 14)	Non-liver ICU (*n* = 14)
Baseline coagulation tests					
INR	1.01 [0.99–1.05]	1.25 [1.07–1.40]*	1.15 [1.09–1.25]	1.91 [1.59–2.12]**	1.20 [1.06–1.39]*
APTT, s	33.4 [31.4–35.0]	33.6 [29.1–36.0]	32.7 [30.4–35.2]	57.4 [42.7–63.4]**	31.1 [29.1–36.9]
Factor II, %	130 [113–144]	79 [61–108]	118 [98–133]	41 [29–56]**	81 [72–110]*
AT, %	113 [104–126]	85 [46–108]*	100 [92–109]	28 [17–40]**	106 [92–131]
Factor X, %	122 [108–134]	82 [60–99]**	94 [83–109]*	74 [52–94]**	110 [92–138]
Fibrinogen, g/L	3.00 [2.77–3.50]	3.37 [2.47–4.48]‡	5.01 [3.65–7.03]*	2.10 [1.56–3.05]*	4.74 [3.55–6.73]*
VWF, %	132 [96–176]	339 [214–544]**	229 [152–328]*	839 [646–1020]**	253 [214–361]*
TGA					
Baseline					
ETP, nM × min	495 [342–660]	432 [348–727]	508 [351–772]	365 [240–559]	441 [201–594]
Peak, nM	81 [60–128]	82 [54–120]	101 [62–146]	44 [19–82]*	84 [45–118]
Lag time, min	2.4 [2.0–3.1]	2.8 [2.2–4.3]	3.3 [2.3–4.5]	3.9 [3.0–6.7]*	4.1 [3.1–5.8]*
Velindex, nM/min	29 [19–48]	30 [16–45]	37 [19–61]	11 [3–27]*	29 [12–48]
On anticoagulant					
ETP, nM ×min	n/a	65 [0–144]	128 [40–337]	161 [0–238]	66 [0–283]
Peak, nM	n/a	7 [0–20]	19 [3–60]	15 [0–27]	10 [0–47]
Lag time, min	n/a	3.8 [3.3–5]	3.3 [3.2–7.0]	5.1 [4–7.8]	4.7 [3.9–8.6]
Velindex, nM/min	n/a	1 [0–5]	5 [0–19]	3 [0–7]	3 [0–12]
TAT levels					
Baseline, µg/ml	1.56 [1.35–2.24]	3.89 [2.75–19.64]**	3.86 [2.29–6.27]*	10.17 [6.79–21.38]**	8.33 [5.89–15.26]**
On anticoagulant, µg/ml	n/a	3.73 [2.53–6.63]	2.41 [2.10–4.18]	10.44 [5.66–14.81]	5.53 [3.32–11.96]

The results are presented as median [interquartile range]. Abbreviations: ACLF, acute-on-chronic liver failure; AT, antithrombin; ETP, endogenous thrombin potential; ICU, intensive care unit; INR, International Normalized Ratio; n/a, not applicable; TAT, thrombin-antithrombin complex; TGA, thrombin generation assay; Velindex, velocity index; VWF, von Willebrand factor.

*$p < .05$ versus healthy controls. **$p < .001$ versus healthy controls. †$p < .05$ versus non-liver ICU. ††$p < .001$ versus non-liver ICU. ‡$p < .05$ versus non-liver general medical ward.

of AT, anti-Xa activity on LMWH was similar in patients with cirrhosis and patients without underlying liver disease admitted to non-liver general medical wards. As expected, the anti-Xa activity in samples of non-liver cohorts, with normal levels of AT, did not change after addition of AT. We also did not find altered anti-Xa activity after addition of AT in the cirrhosis cohort. In contrast, in the ACLF cohort, with relatively low AT levels compared with the other cohorts, the anti-Xa activity increased significantly when we added of 1 PEU of AT to the sample. In further analyses, we therefore used the anti-Xa activity measured after addition of 1 PEU of AT.

We found a significant positive correlation between anti-Xa activity and the proportional ETP change in patients receiving LMWH in both non-liver general medical ward and ICU cohorts. In the cirrhosis cohort a positive correlation between anti-Xa activity and proportional ETP change was found in the patients receiving LMWH. However, no correlation was found between anti-Xa activity and proportional ETP change in patients on UFH in both cirrhosis and ACLF cohorts (Figure 3).

4 | DISCUSSION

In this study, we found that the anticoagulant effect of a prophylactic dose of LMWH and UFH administered in a real-life clinical setting is similar in patients with cirrhosis and ACLF compared to patients without liver disease. Specifically, we did not find any differences in either absolute or proportional ETP change across all four cohorts after administration of heparin. These data suggest that the prophylactic heparin doses as currently used in patients without underlying liver disease might equally be suitable for patients with cirrhosis.

Although the anticoagulant effect as demonstrated by TGA should conceptually be largely reflected by a decrease in plasma levels of the *in vivo* marker of activation of coagulation TAT, we did not find a clear correlation between the two. In addition, in some patients, TAT levels increased after heparin, so that median TAT levels did not change in the liver cohorts (Table 3), but when examined as an individual proportional decrease, TAT levels did decrease in the cirrhosis cohort (Figure 2). Notably, neither absolute nor proportional TAT levels decreased in the ACLF cohort, in which all patients

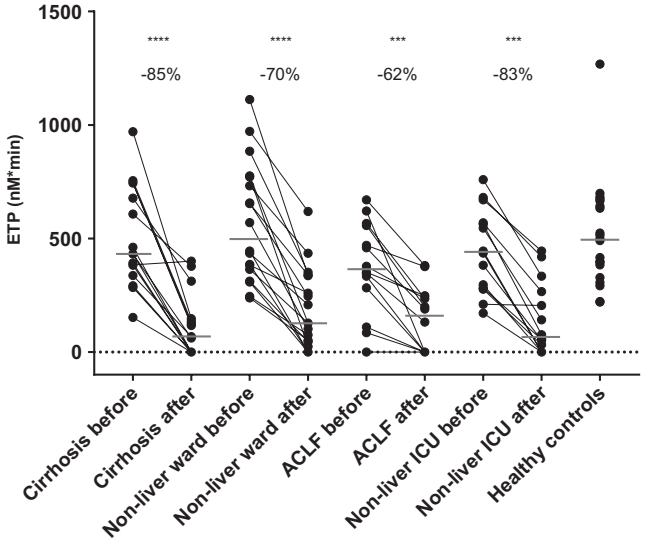

FIGURE 1 Thrombin generation before and after administration of prophylactic heparin doses. The endogenous thrombin potential was determined in plasma samples from healthy controls and from patients before and 4 h after administration of heparin. Dots representing individual patient data before and after heparin are connected by a line. Horizontal lines indicate medians. ***$p < .001$, ****$p < .0001$. Abbreviations: ACLF, acute-on-chronic liver failure; ETP, endogenous thrombin potential; ICU, intensive care unit

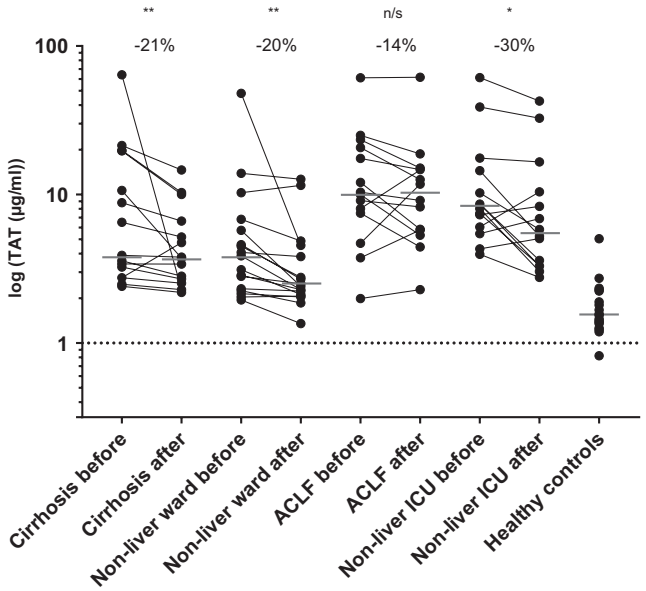

FIGURE 2 Thrombin-antithrombin levels before and after administration of prophylactic heparin doses. Dots representing individual patient data before and after heparin are connected by a line. Horizontal lines indicate medians. *$p < .05$, **$p < .01$. Abbreviations: ACLF, acute-on-chronic liver failure; ICU, intensive care unit; n/s, not significant; TAT, thrombin-antithrombin

received UFH. One explanation for the unaffected TAT levels might be an altered clearance of these complexes by the liver,[22] which should be further explored in future research. Even though our TGA results suggest otherwise, another explanation for the unaffected TAT levels might be the so-called "heparin resistance" resulting from low AT levels in these patients.[23,24] If this is indeed the case, we have to question the use of UFH in patients with ACLF altogether, and investigate whether alternative thromboprophylactic strategies might be a better fit for patients with ACLF.

Contrary to other studies,[15,16,18] we found comparable anti-Xa activity in patients with cirrhosis compared with patients without underlying liver disease after administration of the LMWH enoxaparin. A possible explanation for this discrepancy could be the relative preservation of AT in this cirrhosis cohort, levels of which seem to be similar to those of patients with Child-Pugh A cirrhosis.[15,18,25] The decreased anti-Xa activity in the ACLF cohort might therefore be a result of the much lower AT levels in this cohort. Although anti-Xa activity after addition of AT and proportional change of ETP are positively correlated in patients receiving LMWH in the cirrhosis cohort, we did not find a correlation in patients receiving UFH in the ACLF cohort.

In line with previous studies,[26-28] thrombin generation at baseline was preserved in patients with liver disease, even in those that were very ill and required organ support. However, despite the general preservation of thrombin generation, some individual patients generated very little thrombin as measured by TGA, specifically in the ACLF cohort. In these patients, very low levels of anti-Xa corresponded with full inhibition of thrombin generation. Possibly, these very low levels of anti-Xa activity, with concomitant anti-IIa activity might therefore result in sufficient anticoagulant activity to completely suppress thrombin generation in these individuals. Why certain patients had clearly decreased thrombin generation at baseline is unclear. Future studies should be performed to assess whether those patients with low baseline thrombin generation are at decreased risk for thrombotic events, and whether thromboprophylaxis would be indicated and safe.

To our knowledge, this is the first report on the anticoagulant effect of thromboprophylaxis of LMWH and UFH in patients with cirrhosis and ACLF in a real-life clinical setting. Previous *in vitro* laboratory studies have suggested an altered efficacy of LMWH and UFH in patients with cirrhosis.[18–20] Moreover, a recent study on the direct oral anticoagulant edoxaban, performed in a similar real-life setting, suggests that the anticoagulant effect of this drug is decreased in patients with cirrhosis.[29] Interestingly, the results of this study do not reflect those of previous studies, but rather suggest a similar anticoagulant effect of LMWH and UFH in patients with cirrhosis. That other clinical studies have suggested thromboprophylaxis is effective in preventing VTE and safe in terms of bleeding risk in these patients[12,30–33] further supports this concept.

By studying thrombin generation before and after in vivo administration of prophylactic doses of LMWH and UFH, we were able to create a more realistic overview of the anticoagulant effect of these therapies in patients with cirrhosis and ACLF. Limitations of the study include the sample size and the evaluation of the anticoagulant effects following only a single dose of thromboprophylaxis. For enoxaparin, steady state is usually reached after 3 doses (4–5 half-lives)[34,35] and as accumulation is recognized in renal (and

TABLE 4 Anti-Xa activity in absence and presence of 1 plasma equivalent unit of antithrombin

	On LMWH		On UFH	
	Anti-Xa − AT (U/ml)	Anti-Xa + AT (U/ml)	Anti-Xa − AT (U/ml)	Anti-Xa + AT (U/ml)
Cirrhosis	0.28 [0.18–0.36]	0.25 [0.22–0.32]	0.03 [0–0.07]	0.10 [0.09–0.11]
Non-liver general medical ward	0.24 [0.17–0.36]	0.24 [0.18–0.31]	n/a	n/a
ACLF	n/a	n/a	0.02 [0–0.03]	0.08 [0.03–0.11]*
Non-liver ICU	0.33 [0.24–0.44]	0.31 [0.20–0.38]*	0.01 [0–0.1]	0.03 [0.02–0.04]

The results are presented as median [interquartile range]. Abbreviations: ACLF, acute-on-chronic liver failure; anti-Xa, anti-activated factor X; AT, antithrombin; ICU, intensive care unit; LMWH, low-molecular-weight heparin; UFH, unfractionated heparin.

*p < .01 versus absence of AT.

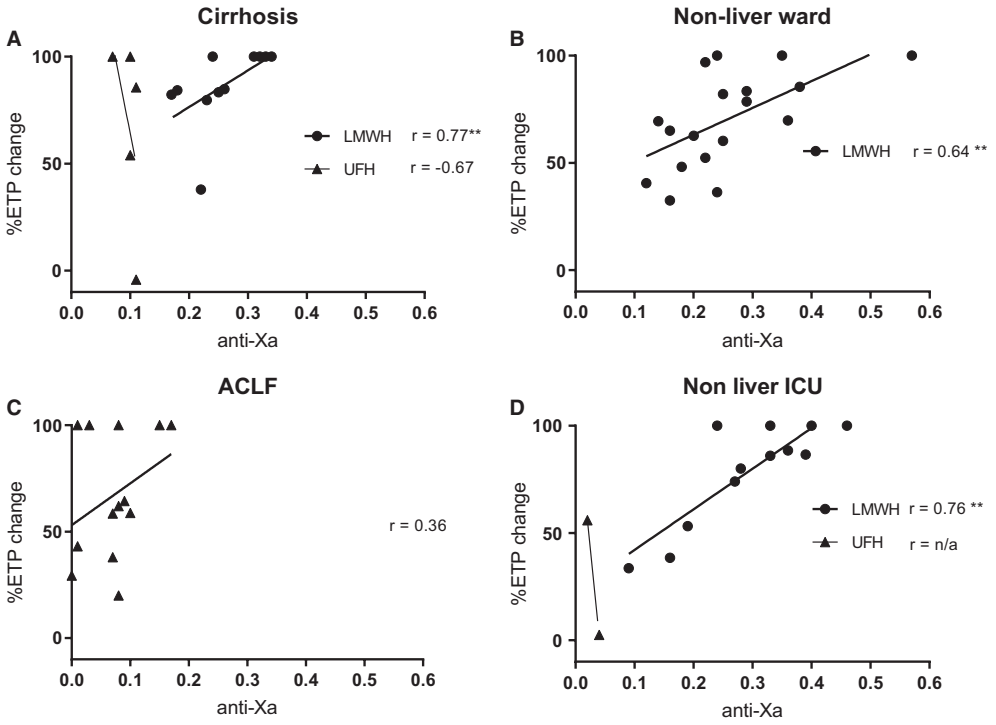

FIGURE 3 Correlation between proportional ETP change and levels of anti-Xa determined in the presence of 1 plasma equivalent unit of antithrombin. Correlations were assessed in the (A) cirrhosis cohort, (B) non-liver general medical ward cohort, (C) ACLF cohort, and (D) non-liver ICU cohort with Pearson correlation coefficients. **p < .01. Abbreviations: ACLF, acute-on-chronic liver failure; ETP, endogenous thrombin potential; ICU, intensive care unit; LMWH, low-molecular-weight heparin; UFH, unfractionated heparin

possibly hepatic) impairment,[36] future longitudinal studies should be performed to further assess the pharmacokinetics in patients with cirrhosis. Also, patients in the ACLF cohort were generally sicker than the patients in the non-liver ICU cohort, often requiring more organ support. In contrast to patients without underlying liver disease admitted to non-liver ICUs, almost all ACLF patients were on renal replacement therapy (7% vs. 93%, respectively), which is why they were administered UFH rather than LMWH. It would have been preferable to match these cohorts better in terms of severity of disease and type of thromboprophylaxis. On the other hand, the strength of the observational design of this study is that it reflects the effect of thromboprophylactic strategies in the current clinical setting, accepting the heterogeneity of this difficult patient population. Future studies, preferably randomized clinical trials, could further explore the efficacy of these strategies in a more controlled setting. Moreover, further research should be conducted on the application of point-of-care hemostatic testing (such as thromboelastography or rotational thromboelastometry or the newly developed whole blood TGA[37]) on the evaluation of heparin efficacy because these tests could be used in a clinical setting more easily and might have value in monitoring heparin therapy in a clinical setting.

Taken together, our results suggest a similar response to thromboprophylaxis in patients with cirrhosis and ACLF. In keeping with previous studies, patients with cirrhosis and ACLF appear to have

a preserved thrombin generation at baseline despite abnormal conventional laboratory coagulation tests. Moreover, our data suggest that standard prophylactic dosages of LMWH and UFH result in a comparable change in thrombin generation in both patients with and without liver disease. This study is a first step in evaluating current thromboprophylactic strategies and may serve as a stepping stone for the development of evidence-based guidelines on such strategies in patients with cirrhosis and ACLF.

ACKNOWLEDGMENTS

B.P.v.d.B. and F.A.v.M. acknowledge Francesca Trovato for her help during our stay at King's College Hospital. The authors all acknowledge the King's College Hospital clinical staff (especially from the liver wards and liver intensive care unit) and laboratory staff (from the Institute of Liver Studies), and specifically Debbie Shawcross, Vishal Patel, Brian Hogan, and Mark McPhail.

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Concept and design: Bente P. van den Boom, Fien A. von Meijenfeldt, Lara N. Roberts, Ton Lisman, William Bernal; data acquisition: Bente P. van den Boom, Fien A. von Meijenfeldt, Jelle Adelmeijer; data interpretation: all; manuscript drafting: BenteP. van den Boom, Ton Lisman, William Bernal; revision of manuscript: all; and study supervision: Ton Lisman, William Bernal.

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How to cite this article: van den Boom BP, von Meijenfeldt FA, Adelmeijer J, Roberts LN, Bernal W, Lisman T. Heparins have adequate ex vivo anticoagulant effects in hospitalized patients with cirrhosis. *J Thromb Haemost*. 2021;19:1472–1482. https://doi.org/10.1111/jth.15296