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ORIGINAL ARTICLE

Predicting bleeding after liver biopsy using comprehensive clinical and laboratory investigations: A prospective analysis of 302 procedures

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Abstract

Background: Liver biopsy carries a small risk of bleeding complications. No validated clinical or laboratory tool helps predict liver biopsy–related bleeding.

Objectives: To determine whether global hemostasis tests and/or a clinical questionnaire could identify patients at risk of liver biopsy–related bleeding.

Patients/Methods: Consecutive patients scheduled for liver biopsy with an overnight hospital stay were prospectively included. Before liver biopsy, routine hemostasis tests, Platelet Function Analyzer 100, thromboelastometry, thrombin generation assay, plasma clot lysis time, and a clinical questionnaire were performed. Bleeding was defined as a liver hematoma or new free fluid on a systematic ultrasound performed 24 h after liver biopsy or a decrease in hemoglobin level of 2 g/dL or more in patients with pre-existing free fluid in the abdominal cavity.

Results: Three hundred two patients were included: 173 underwent percutaneous and 129 transjugular liver biopsy. There were 21 bleeding episodes (7%); 20 based on ultrasonographic criteria, 1 on laboratory criteria. None of the hemostasis tests and no item of the clinical questionnaire were associated with liver biopsy–related bleeding in the overall study group. Same results were obtained in subgroup analyses focusing on patients who underwent percutaneous liver biopsy, transjugular liver biopsy, or on patients with cirrhosis. Pain 2 h after liver biopsy was more frequent in patients with liver biopsy–related bleeding (55% vs. 23% $p = .002$).

Conclusions: An extensive hemostasis workup, including global hemostasis assays, does not improve prediction of liver biopsy–related bleeding. Pain 2 h after liver biopsy should alert the clinician to the possibility of procedure-related bleeding.

Julien Bissonnette and Alix Riescher-Tuczkiewicz should be considered joint first authors.Emmanuelle de Raucourt and Pierre-Emmanuel Rautou should be considered joint senior authors.

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KEYWORDS
blood coagulation, diagnostic techniques and procedures, hemorrhage, liver cirrhosis, liver diseases

1 | INTRODUCTION

The liver is the main site of production of coagulation factors. Consequently, it plays a critical role in maintaining normal hemostasis. Chronic liver disease can result in various derangements of the three phases of hemostasis: primary hemostasis, coagulation, and fibrinolysis.[1,2] Clinically, patients with chronic liver disease may bleed following invasive procedures, but can also develop thrombotic events.[3–5]

Liver biopsy remains frequently needed in patients with chronic liver disease. Clinically detectable bleeding complications have a frequency ranging from 1 to 6 per 1000 biopsies.[6–8] When performing systematic ultrasound evaluation following biopsy, signs of bleeding, including liver hematoma or peritoneal fluid, are observed in 3%–23% of the patients.[9–12] Identifying patients at risk of liver biopsy–related bleeding remains an unmet need.

Routine hemostasis tests before liver biopsy usually include platelet count; prothrombin time (PT); and, in some institutions, activated partial thromboplastin time (APTT).[13] Despite being current practice, the predictive value of these tests is poor.[2] Moreover, each of these tests studies only one phase of hemostasis and does not take into account platelet function and fibrinolysis, two critical aspects of hemostasis that might be altered in chronic liver disease.[14]

Viscoelastic tests—that is, thromboelastography (TEG) and rotational thromboelastometry (ROTEM)—are global hemostasis tests that can assess the various phases of hemostasis. Four randomized controlled trials observed that the use of viscoelastic tests is associated with a decreased need for prophylactic blood transfusions.[15–19] These studies were a step forward in the management of invasive procedures in patients with liver diseases by demonstrating the necessity to reconsider the interest of blood products transfusion in these patients. However, these studies were not designed to determine whether viscoelastic tests can predict procedure-related bleedings. An attempt has been made in a previous study but suffered from a limited patient population and heterogeneous invasive procedures.[20]

Thrombin generation assay is another global hemostasis test that assesses the ability of the plasma to generate thrombin. Its use has helped us understand the complex coagulopathy of chronic liver disease, but its ability to predict bleeding complications following invasive procedures has not been tested in these patients.[2]

Standardized questionnaires have been validated to screen for primary defects of hemostasis, such as hemophilia or von Willebrand disease, in patients undergoing invasive procedures.[21,22] The use of such questionnaires has never been tested in the setting of chronic liver disease, but might help identify patients subject to bleeding after an invasive procedure.

Essentials

- No clinical or laboratory test has been validated to predict liver biopsy–related bleeding.
- All patients had a comprehensive hemostasis workup prior to liver biopsy.
- Extensive hemostasis workup does not improve the prediction of liver biopsy–related bleeding.
- Pain 2 h after liver biopsy was associated with the occurrence of biopsy-related bleeding.

This prospective observational study aimed at determining whether global hemostasis tests and/or a clinical questionnaire could identify patients at risk of bleeding complications after liver biopsy.

2 | MATERIALS AND METHODS

2.1 | Patients

Consecutive patients (women and men) undergoing liver biopsy with a scheduled hospital overnight stay, at our institution, between December 2014 and February 2016, were prospectively included after providing written informed consent. At our institution, liver biopsy is performed as an inpatient procedure, except for patients living close (<30 min) to the hospital, and not alone during the night following the liver biopsy, and with a preserved liver function (Child-Pugh A). This study was approved by the institutional review board (Comité d'Évaluation de l'Éthique des projets de Recherche Biomédicale de Paris Nord, IRB 00006477).

All patients had an abdominal imaging procedure performed within 1 month prior to liver biopsy.

Percutaneous biopsies of liver parenchyma and of liver nodules were performed under ultrasound guidance by radiologists, using 16 or 18G biopsy needles. Transjugular liver biopsies were performed under fluoroscopic guidance through right internal jugular vein access, by hepatologists, as described previously,[23] using Tru-Cut biopsy needle (Cook). Figure 1 illustrates how the decision between percutaneous and transjugular route was made. Patients routinely received premedication consisting of a single oral dose of 5 mg of morphine prior to liver biopsy, unless refused or contra-indicated by an altered mental status.

Antiplatelet agents and anticoagulation were considered maintained if patients received antiplatelet agent or vitamin K antagonists within 5 days prior to liver biopsy, direct oral anticoagulant within 3 days prior to liver biopsy, and heparin within 24 h prior to liver biopsy. Otherwise, treatments were considered discontinued.

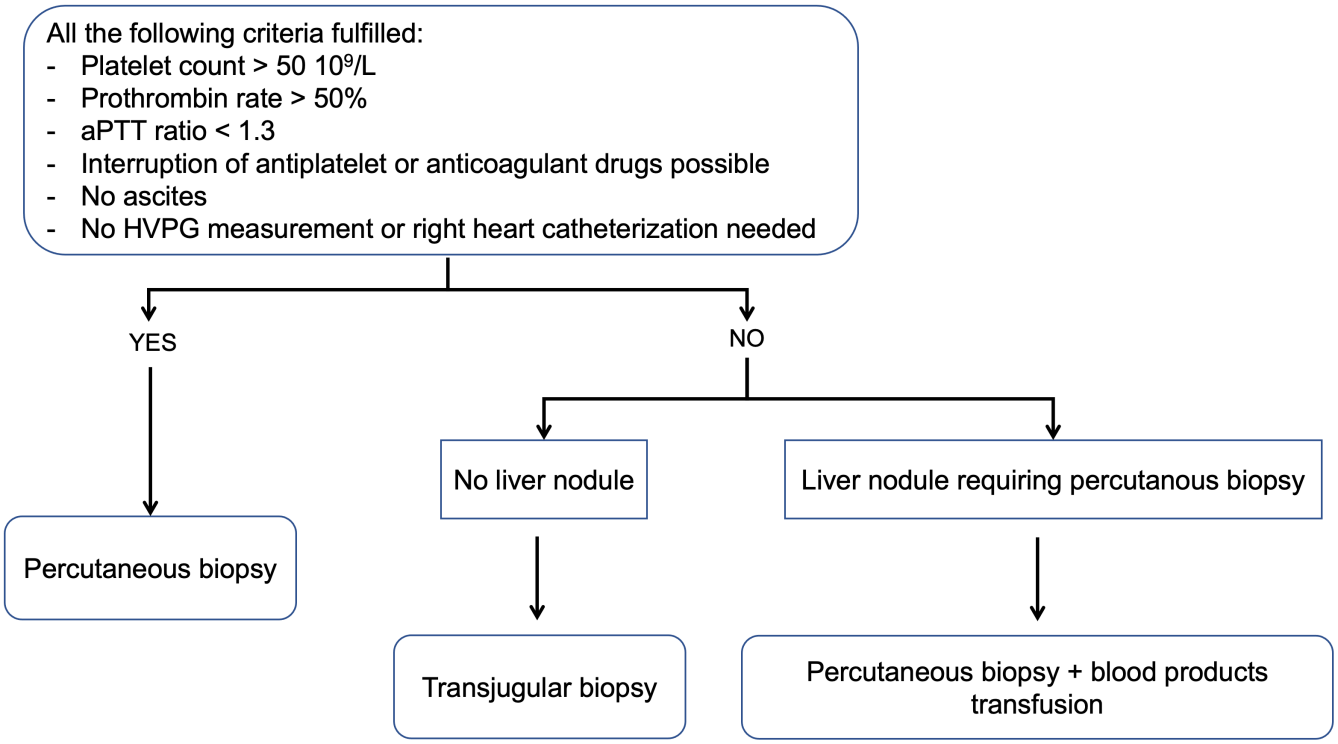

FIGURE 1 Decision tree for liver biopsy route. APTT, activated partial thromboplastin time; HVPG, hepatic venous pressure gradient.

2.2 | Clinical questionnaire

A clinical questionnaire was designed to assess the bleeding risk, based on equivalent tools validated to detect bleeding disorders (Table S1 in supporting information).[21,22] On the morning of the liver biopsy, patients were asked by a physician to answer this questionnaire based on their best recollection on events over the last 12 months to take into account the progression in time of chronic liver disease.

2.3 | Blood and plasma collection

Fifteen milliliters of whole blood were collected from patients on the morning of the liver biopsy, using ethylenediaminetetraacetic acid tubes for blood count and trisodium citrate-containing Vacutainer® tubes (1 volume trisodium citrate 0.109 M to 9 volumes blood) for coagulation assays. Plasma was prepared by double centrifugation at 2500 *g* for 15 min at 18°C. The obtained platelet-poor plasma (PPP) was either directly analyzed or immediately aliquoted and stored at −80°C until use.

2.4 | Coagulation and fibrinolysis assays

Coagulation tests including PT, APPT, factor II (FII), factor V (FV), factor VII (FVII), factor X (FX), factor VIII (FVIII), factor IX (FIX), factor XI (FXI), and fibrinogen were determined using a Behring Coagulation System automaton (Siemens). Plasma von Willebrand factor (VWF) activity and antigen were measured on Behring Coagulation System analyzer using immunoturbidimetric assays (INNOVANCE® VWF Ac and VWF ag; Siemens Healthcare Diagnostics).

Fibrinolysis was studied using the plasma clot lysis time. Euglobulin fraction was prepared by adding acetic acid solution to PPP. The euglobulin fraction was resuspended in borate solution. Clotting was then activated by adding calcium chloride at 37°C. Fibrinolysis was determined by observing the clot within test tube every 10 min for 3 h.

2.5 | Thrombin generation assay

Thrombin generation assay was performed using the Calibrated Automated Thrombogram assay (CAT®, Diagnostica Stago) according to the manufacturer's instructions. Briefly, in a 96-well microplate, 80 µl of PPP were mixed with 20 µl of triggering reagent containing tissue factor at 1 pmol/L and phospholipids at 4 µmol/L (PPP Reagent low [Stago]) with or without thrombomodulin (TM). TM was used at the final concentration of 2 nmol/L (Thrombomodulin Rabbit Lung Sekisui). After incubation for 10 min at 37°C, 20 µl of a mixture of the fluorogenic substrate (Z-Gly-Gly-Arg-AMC, Stago; final concentration 417 µmol/L) and $CaCl_2$ (final concentration 16.7 nmol/L) was distributed automatically to the test system. Measurements were carried out in triplicate for each plasma every 20 s for 60 min.

2.6 | Thromboelastometry

Global hemostasis was evaluated by thromboelastometry. Rotational thromboelastometry was carried out using a ROTEM Delta (Tem Innovations GmbH) device according to the manufacturer's instructions. We analyzed the following features: clotting time (in seconds), that is, time from the beginning of the test to a trace's amplitude of 2 mm; clot formation time (in seconds), that is, time from an amplitude of 2 mm to an amplitude of 20 mm; and maximum clot firmness (in mm), that is, the maximal amplitude reached before the lysis of the clot begins. Clotting time, clot formation time, and maximum clot firmness were measured using EXTEM, INTEM, FIBTEM, and APTEM. EXTEM reflects the functionality of the extrinsic and common pathways of coagulation, whereas INTEM reflects that of intrinsic and common pathways. FIBTEM describes the role of fibrinogen in clot formation. APTEM enables the detection of hyperfibrinolysis. A prolonged INTEM/EXTEM clotting time indicates a deficiency of coagulation factors. A prolonged clot formation time or reduced maximum clot firmness in INTEM/EXTEM suggests a fibrinogen deficiency or a fibrin polymerization disorder when associated with a reduced maximum clot firmness in FIBTEM, or a low platelet count or severe platelet dysfunction when FIBTEM maximum clot firmness is normal. In EXTEM, hemostasis is triggered by tissue factor, in INTEM by ellagic acid, in FIBTEM by ellagic acid and cytochalasin D (inhibiting platelet function), and in APTEM by tissue factor combined with aprotinin to inhibit fibrinolysis.

2.7 | PFA-100

The PFA-100 (Platelet Function Analyzer 100) system creates an artificial vessel consisting of a sample reservoir, a capillary, and a biologically active membrane with a central aperture, coated with collagen plus adenosine diphosphate (C-ADP) or collagen plus epinephrine (C-EPI). The application of a constant negative pressure aspirates the anticoagulated blood of the sample from the reservoir through the capillary (mimicking the resistance of a small artery) and the aperture (mimicking the injured part of the vessel wall). As a result, platelets form plugs that gradually occlude the aperture; consequently, the blood flow through the aperture gradually decreases and eventually stops. The time required to interrupt blood flow ("closure time") was recorded.

2.8 | Follow-up after liver biopsy

Pain was systematically evaluated 2 h and 24 h after liver biopsy, using a visual analog scale ranging from 1 to 10 and prospectively collected.

Twenty-four hours after liver biopsy, all patients had a blood count to identify any change in hemoglobin level, and an ultrasound evaluation of the liver to screen for bleeding complications.

One week after liver biopsy, all patients were contacted by phone to search for any late bleeding complication.

2.9 | Endpoints

The primary endpoint was the occurrence of liver biopsy–related bleeding a priori defined as one or several of the following events: (1) ultrasound evaluation of the liver at 24 h after liver biopsy showing parenchymal or subcapsular hematoma or new-onset peritoneal free fluid in patients devoid of it prior to liver biopsy; (2) decrease in hemoglobin concentration of 2 g/dL or more in patients with pre-existing free fluid in the abdominal cavity.

2.10 | Statistical analyses

Sample size was calculated for the primary endpoint. Previous data reported that the area under the receiver operating characteristic (ROC) curve of platelet count for prediction of liver biopsy–related bleeding in patients with liver disease was 0.62.[24] Assuming a 10% rate of liver biopsy–related bleeding according to previous reports,[9–12] the sample size was calculated to detect an improvement of the area under the ROC curve of 0.15, with an estimated correlation coefficient of 0.6, a two-sided type I error of 0.05, and statistical power of 0.80. The calculated required sample size was 310.

Quantitative data are presented as median (interquartile range [IQR]). Categorical variables are presented as absolute and relative frequencies. Comparisons between patients with and without biopsy-related bleeding were performed using the Mann–Whitney test for quantitative variables and the chi-square test or Fisher's exact test, as appropriate, for qualitative variables. Sensitivity analyses of patients with advanced fibrosis or cirrhosis, and patients with transjugular or percutaneous biopsy were also performed. Principal component analysis including results of all hemostasis tests was also performed. Statistical significance was set at $p < .05$. Statistical analyses were performed using the SPSS statistical package 20.0 software (SPSS Inc.).

3 | RESULTS

3.1 | Patient characteristics

The study included 306 patients. Of those patients, one patient received prophylactic platelet transfusion prior to a liver biopsy targeting a nodule because his platelet count was 45.10^9/L and 3 patients did not have an ultrasound evaluation after liver biopsy. Those 4 patients have not been included in further analyses. None of the patients received fresh frozen plasma, clotting factor concentrate, tranexamic acid, or fibrinogen concentrate. Characteristics of the 302 patients and of the liver biopsy procedures are presented in Table 1. One hundred seventy-three patients (57%) patients had a percutaneous liver biopsy,

TABLE 1 Patients' characteristics ($n = 302$)

-	*n*	Percutaneous biopsy	*n*	Transjugular biopsy	*n*	All
Clinical features						
Age (years)	173	58 (50–67)	129	52 (46–61)	302	56 (48–65)
Gender (male:female)	173	108:65	129	76:53	302	184:118
Indication for liver biopsy	173		129			
Nodule (diagnostic, prior to treatment)		108 (62%)		4 (3%)		109 (36%)
Abnormal liver blood tests		23 (13%)		37 (29%)		61 (20%)
Prior to liver transplant		0		33 (26%)	302	33 (11%)
Transplanted patient		22 (13%)		9 (7%)		31 (10%)
Cause or diagnosis of cirrhosis		15 (9%)		21 (16%)		38 (13%)
Others		5 (3%)		25 (19%)		30 (10%)
Prior liver transplantation	173	34 (20%)	129	12 (9%)	302	45 (15%)
Body mass index (kg/m^2)	171	25 (23–28)	127	25 (22–30)	298	25 (23–29)
Laboratory features						
Serum AST (IU/L)	169	45 (29–70)	129	58 (38–115)	298	50 (33–88)
Serum ALT (IU/L)	171	40 (23–73)	128	34 (21–84)	299	37 (22–76)
Serum alkaline phosphatase (IU/L)	170	103 (70–169)	129	132 (93–170)	299	114 (77–180)
Serum bilirubin (μmol/L)	172	12 (8–23)	129	40 (21–91)	301	20 (10–43)
Serum albumin (g/L)	159	37 (33–40)	126	28 (23–33)	285	34 (28–38)
Serum creatinine (μmol/L)	173	71 (64–89)	129	65 (56–78)	302	69 (60–85)
Hemoglobin (g/dL)	171	14 (13–15)	129	11 (10–13)	300	13 (11–14)
Platelet count (x 10^9/L)	171	177 (118–243)	132	104 (72–187)	300	148 (91–226)
Prothrombin rate (%)	172	88 (71–101)	129	56 (40–76)	301	76 (56–96)
Fibrosis stage						
No fibrosis	173	63 (36%)	129	37 (29%)	302	100 (33%)
Portal fibrosis		13 (8%)		5 (4%)		18 (6%)
Portal fibrosis with few septa		23 (13%)		9 (7%)		32 (11%)
Bridging fibrosis		13 (8%)		8 (6%)		21 (7%)
Cirrhosis		57 (33%)		68 (53%)		125 (41%)
Undetermined		4 (2%)		2 (1%)		6 (2%)[a]
Treatment						
Anti-platelet agent						
- Discontinued	173	44 (25%)	129	7 (5%)	302	51 (17%)
- Maintained		2 (1%)		12 (9%)		14 (5%)
Heparin						
- Discontinued	171	8 (5%)	127	21 (17%)	298	29 (10%)
- Maintained		0		1 (1%)		1 (0%)
Vitamin K antagonist						
- Discontinued	173	6 (4%)	127	5 (4%)	300	11 (4%)
- Maintained		0		5 (4%)		5 (2%)
Direct oral anticoagulant						
- Discontinued	173	1 (1%)	127	1 (1%)	300	2 (1%)
- Maintained		0		0		0

Note: Results are presented as median (interquartile range) or absolute (relative frequencies).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[a]Fibrosis could not be graded in six patients because of a fragmented specimen or the biopsy targeting only nodular lesions or malignant infiltration of the liver.

including 108 (62%) a biopsy targeting a nodule (36 benign, 71 malignant, and 1 non-contributive), and 129 (43%) patients had a transjugular liver biopsy. Biopsy was performed while antiplatelet agent was maintained in 14 patients (including 12 transjugular route) and while anticoagulation was maintained in 6 patients (all transjugular route). Of the 148 patients with advanced liver fibrosis or cirrhosis, 53 (36%) had ascites and median Model for End-Stage Liver Disease score was 12 (IQR 9–16). Cause of liver disease was excessive alcohol consumption in 72 patients (24%), metabolic syndrome in 51 patients (17%), chronic viral hepatitis in 66 patients (22%), and other in 139 patients (46%; 26 patients had several causes of liver disease).

3.2 | Features associated with liver biopsy–related bleeding

Liver biopsy–related bleeding occurred in 21 patients (7%), including 20 with parenchymal or subcapsular hematoma or new-onset peritoneal free fluid and 1 with a decrease in hemoglobin concentration of 2 g/dL or more. Two patients underwent arterial embolization to control bleeding and one had red blood cell transfusion (Table S2 in supporting information). No additional bleeding complication was retrieved during the phone calls performed 1 week after liver biopsy.

There was no significant difference in liver biopsy–related bleeding frequency according to the route used for biopsy (Table 2). In the overall cohort, none of the clinical features or the laboratory tests was significantly associated with bleeding episodes, as presented in Tables 2 and 3, and Figures S1–S6 in supporting information. Identical results were obtained when analyzing separately patients undergoing percutaneous or transjugular liver biopsy (Tables S4 and S5 in supporting information). Cause of liver disease was not associated with the occurrence of liver biopsy–related bleeding (data not shown). Seven patients had underlying blood disease (i.e., essential thrombocytemia, polycythemia vera, or nocturnal paroxystic hemoglobinuria) and two patients had liver metastatic cancer; none of them had liver biopsy–related bleeding.

Specific cutoffs for hemostasis tests (i.e., platelet count $<50.10^9$/L, international normalized ratio [INR] < 1.8, fibrinogen <1.2 g/L) were neither associated with a higher risk of bleeding in the whole cohort (Table S6 in supporting information), nor in the subgroups of patients who underwent a transjugular liver biopsy or a percutaneous liver biopsy, or a percutaneous liver biopsy targeting a nodule (data not shown).

Individual items of the clinical questionnaire or the sum of these items were neither associated with liver biopsy–related bleeding in the overall cohort (Table S3 in supporting information) nor in groups of patients who underwent transjugular or percutaneous liver biopsy (data not shown).

Sensitivity analyses focusing on the 148 patients with advanced liver fibrosis or cirrhosis found the same results (Table S7 in supporting information).

Principal component analysis was conducted to summarize the information contained in all hemostasis tests performed. As shown in Figure 2, patients who had a liver biopsy–related bleeding were not separated from those who had not, reinforcing the view that hemostasis tests, even taken together, do not identify patients at risk of bleeding.

3.3 | Occurrence of pain and need for analgesics

Details on pain after liver biopsy are provided in Table 4. Pain 2 h after liver biopsy was more frequent following percutaneous than transjugular liver biopsy (33% vs. 15%; $p < .001$) and was twice as common in patients with liver biopsy–related bleeding than in those without (55% vs. 23% $p = .002$). Out of all patients with pain 2 h after liver biopsy ($n = 71$), 15% had a liver biopsy-related bleeding, while out of those without pain 2 h after liver biopsy ($n = 209$), 4% had a liver biopsy–related bleeding ($p = .002$). Patients with severe pain 2 h after liver biopsy (i.e., pain score ≥8) were more prone to have liver biopsy–related bleeding compared to patients with no pain or with moderate pain (i.e., pain score <7; 31% vs. 6%, $p = .001$). Pain 2 h after liver biopsy had a 55% (95% CI 0.35–0.73) sensitivity, 75% (95% CI 0.70–0.80) specificity, and 96% (95% CI 0.93–0.97) negative predictive value for liver biopsy–related bleeding. Nine patients (45%) with liver biopsy–related bleeding did not have pain at 2 h.

4 | DISCUSSION

The ability of hemostasis tests to predict liver biopsy–related bleeding is unclear. This study addresses this gap in knowledge, with two main strengths: (1) we prospectively collected data from 302 patients who had a liver biopsy and a comprehensive hemostasis workup, including quantitative and functional tests, namely PFA-100, thrombin generation assay, thromboelastometry, and plasma clot lysis time, and a clinical questionnaire; (2) we used a clinical endpoint, namely signs of bleeding at systematic ultrasound evaluation following biopsy and/or a decrease in hemoglobin concentration, as a surrogate marker of severe bleeding.

The first major finding of this study is that adding global coagulation assays (thromboelastometry, thrombin generation assay) or tests assessing specific phases of hemostasis (PFA-100, plasma clot lysis time) to routine hemostasis tests prior to liver biopsy did not improve the prediction of liver biopsy–related bleeding. Indeed, none of the results of the hemostasis tests were associated with the occurrence of liver biopsy–related bleeding. A limitation to these results is that the population included was heterogeneous including patients with and without cirrhosis and patients undergoing percutaneous and transjugular liver biopsy. However, sensitivity analyses focusing on patients with cirrhosis or on a specific route for liver biopsy did not show any trend toward an association between hemostasis tests and liver biopsy–related bleeding either. Of note, patients who underwent transjugular biopsy had a more impaired

TABLE 2 Occurrence of liver biopsy-related bleeding according to clinical and laboratory characteristics in 302 patients who underwent liver biopsy

-	*n*	No liver biopsy–related bleeding	*n*	Liver biopsy–related bleeding	*p*-value
Clinical features					
Age (years)	281	56 (48–65)	21	57 (47–63)	.86
Gender: male/female	281	174/107	21	10/11	.20
Route for liver biopsy					
- Percutaneous	281	159 (56)	21	14 (68)	.37
- Transjugular		122 (44)		7 (31)	
Percutaneous liver biopsy targeting a nodule	281	100 (36)	21	8 (38)	.82
Prior liver transplantation	281	40 (14)	21	5 (22)	.24
Body mass index (kg/m^2)	277	25 (23–29)	21	25 (22–29)	.62
Laboratory features					
Serum AST (IU/L)	278	50 (33–89)	20	48 (27–110)	.89
Serum ALT (IU/L)	278	37 (22–73)	21	37 (19–91)	.80
Serum alkaline phosphatase (IU/L)	279	113 (77–180)	20	125 (76–204)	.86
Serum bilirubin (μmol/L)	280	20 (10–43)	21	18 (8–47)	.63
Serum albumin (g/L)	264	34 (27–38)	21	35 (32–39)	.33
Serum creatinine (μmol/L)	281	69 (60–85)	21	67 (59–85)	.68
Hemoglobin (g/dL)	280	13 (11–14)	20	13 (12–14)	.62
Platelet count (x10^{9}L)	280	147 (90–231)	20	172 (95–220)	.98
Prothrombin rate (%)	280	76 (55–96)	21	71 (59–97)	.75
Fibrosis stage					
No fibrosis	281	90 (32%)	21	10 (48%)	.14
Portal fibrosis		18 (6%)		0	.23
Portal fibrosis with few septa		29 (10%)		3 (14%)	.57
Bridging fibrosis		20 (7%)		1(5%)	.68
Cirrhosis		119 (43%)		6 (29%)	.22
Undetermined		5 (2%)		1 (4%)	.17
Treatment					
Anti-platelet agent					
- Discontinued	281	47 (16%)	21	4 (18%)	.78
- Maintained		14 (5%)		0	.30
Heparin					
- Discontinued	277	29 (10%)	21	0	.12
- Maintained		1 (0%)		0	.78
Vitamin K antagonist					
- Discontinued	279	11 (4%)	21	0	.35
- Maintained		5 (2%)		0	.5
Direct oral anticoagulant					
- Discontinued	279	2 (1%)	21	0	.70
- Maintained		0		0	-

Note: Results are presented as median (interquartile range) or absolute (relative frequencies).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

hemostasis workup but did not have more bleeding than patients who underwent percutaneous liver biopsy. Although this study was not designed to establish the safety profile of transjugular biopsy, these data suggest that transjugular liver biopsy is a safe procedure, even in patients with impaired coagulation status.[25] This study was not designed to assess whether routine hemostasis tests (i.e., platelet count, APTT, prothrombin rate) are necessary and useful before liver biopsy. Nevertheless, the design of the present study, using a

TABLE 3 Occurrence of liver biopsy-related bleeding according to hemostasis tests in 302 patients who underwent liver biopsy

Laboratory	*n*	No liver biopsy–related bleeding	*n*	Liver biopsy–related bleeding	*p*-value
Laboratory features					
Activated partial thromboplastin time ratio	280	1.1 (1.0–1.3)	21	1.1 (0.9–1.1)	.37
Fibrinogen (g/L)	279	2.8 (2.2–3.7)	21	3.3 (2.3–3.7)	.44
Factor II (%)	281	94 (66–118)	21	90 (74–124)	.59
Factor V (%)	281	98 (73–119)	21	101 (78–126)	.61
Factor VII (%)	281	80 (50–100)	21	73 (60–95)	.96
Factor VIII (%)	266	154 (133–197)	21	155 (139–194)	.99
Factor IX (%)	264	89 (63–108)	21	89 (72–117)	.61
Factor X (%)	281	91 (67–115)	21	92 (75–114)	.80
Factor XI (%)	264	78 (56–101)	21	79 (58–104)	.85
VWF antigen (%)	256	261 (187–391)	21	251 (161–379)	.51
PFA-100 ADP (sec)	266	101 (80–131)	20	104 (94–131)	.40
PFA-100 Epinephrin (sec)	263	139 (111–183)	20	131 (119–154)	.57
Clot lysis time (min)					
- >180	278	267	20	19	.82
- ≤180		11		1	
Thromboelastometry					
EXTEM Clotting time (sec)	203	69 (60–79)	15	70 (59–79)	.75
EXTEM Clot formation time (sec)	203	99 (74–137)	15	100 (77–177)	.74
EXTEM Clot firmness (mm)	203	60 (53–66)	15	59 (46–66)	.50
INTEM Clotting time (sec)	199	183 (165–202)	15	172 (166–182)	.09
INTEM Clot formation time (sec)	199	90 (66–125)	15	73 (65–155)	.81
INTEM Clot firmness (mm)	199	57 (50–64)	15	61 (49–65)	.85
FIBTEM Clot firmness (mm)	202	16 (12–21)	15	12 (10–23)	.57
APTEM Clotting time (sec)	201	65 (58–77)	15	64 (55–71)	.50
APTEM Clot firmness (mm)	201	59 (51–65)	14	60 (44–65)	.72
Thrombin generation assay					
In the absence of thrombomodulin					
- Lag time (min)	261	4.4 (3.9–5.4)	18	4.7 (4.2–5.1)	.85
- Time to peak (min)	261	7.5 (6.7–8.8)	18	7.4 (6.8–8.4)	.78
- Peak height (nM)	261	184 (136–232)	18	189 (148–246)	.47
- ETP (nM*min)	257	1047 (858–1257)	18	989 (817–1499)	.88
- Velocity index (nM/min)	260	61 (42–86)	18	60 (42–100)	.61
- Time to tail (min)	257	24 (22–26)	18	22 (21–27)	.19
In the presence of thrombomodulin					
- Lag time (min)	238	5.8 (4.9–8)	16	6.6 (5.8–7.7)	.29
- Time to peak (min)	239	8.6 (7.4–10.9)	16	9.1 (7.8–10.5)	.55
- Peak height (nM)	239	112 (63–157)	16	93 (73–132)	.85
- ETP (nM*min)	238	504 (283–708)	16	430 (312–651)	.66
- Velocity index (nM/min)	238	46 (24–70)	16	43 (24–59)	.84
- Time to tail (min)	236	23 (21–26)	16	23 (21–26)	.47

Note: Results are presented as median (interquartile range) or absolute (relative frequencies).

Thromboelastometry was available in 218 patients (not performed in 84 patients for technical reasons) and thrombin generation assay was available in 279 patients (not performed in 23 patients because of insufficient amount of plasma).

Abbreviations: ETP, endogenous thrombin potential; PFA-100, Platelet Function Analyzer 100; VWF, von Willebrand factor.

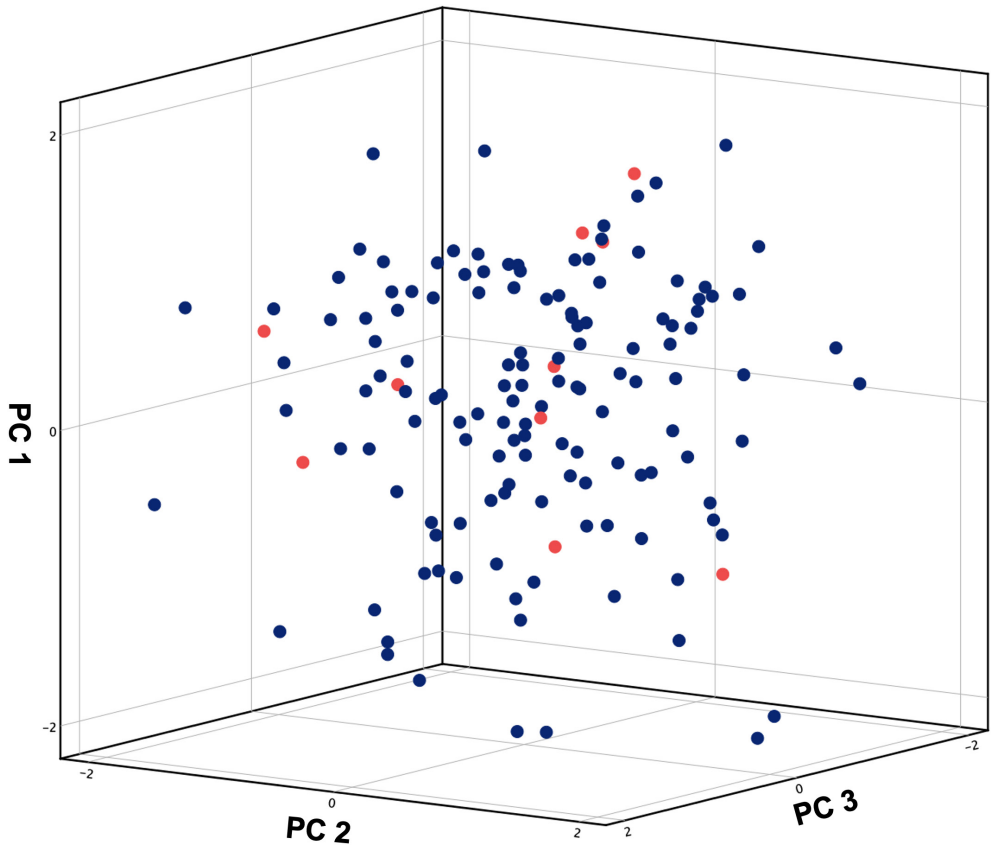

FIGURE 2 Principal component analyses including results of all hemostasis tests performed in patients undergoing liver biopsy. Principal component analysis is a technique for synthetizing a large number of data and variables (i.e., hemostasis data), increasing interpretability but at the same time minimizing information loss. The three principal components (PC) used contain most of the information. Each dot represents a patient. Red dots represent patients with liver biopsy–related bleeding and blue dots represent patients without liver biopsy–related bleeding. Patients who had liver biopsy–related bleeding were not separated from those who were not in the subgroup of 158 patients with complete hemostasis workup including thromboelastometry and thrombin generation assay.

surrogate clinical endpoint occurring in ≈ 10% of the patients, might be valuable in future large studies to overcome the issue of statistical power related to the rarity of severe liver biopsy–related bleedings. Another novelty of the present study lies in elaborating a clinical questionnaire derived and adapted from those shown to improve the evaluation of patients with suspected bleeding disorder.[21,22] Such a questionnaire does not exist in the setting of chronic liver disease. The questionnaire we used was, however, not associated with the occurrence of liver biopsy–related bleeding, possibly because coagulation alterations change over time in chronic liver diseases.

The second major finding of this study is that pain 2 h after liver biopsy was twice as frequent in patients who had liver biopsy–related bleeding than in those without bleeding. The absence of pain had a good predictive value (96%) to rule out a liver biopsy–related bleeding episode, but it must be highlighted that 85% of the patients with pain did not have a bleeding episode. Therefore, pain should only increase awareness about the possibility of bleeding, but without being alarming.[26] Moreover, 45% of the patients with liver biopsy–related bleeding did not have pain at 2 h. Although we are here using a surrogate marker of bleeding, these results point to the need to improve early detection of liver biopsy–related bleeding, particularly when considering the current trend to decrease the duration of hospital stay after liver biopsy.[13,27,28] Future studies might test a duration of hospital stay after liver biopsy adjusted on the results of liver ultrasonography: patients with signs of bleeding at ultrasonography (≈ 10% of the patients) might be hospitalized overnight, while those without such signs be discharged after 1 h. Such studies should take into account pharmacokinetics of the preemptive analgesia protocols used, as they vary across centers (Table S8 in supporting information) and might influence the results.

In conclusion, in this large prospective study, an extensive hemostasis laboratory and clinical workup did not improve liver biopsy–related bleeding prediction. Therefore, PFA-100, thrombin generation assay, thromboelastometry, plasma clot lysis time, and clinical questionnaire are useless in patients undergoing percutaneous liver biopsy when platelet count is >50 10^9/L, prothrombin index >50%, and APTT ratio <1.3 or in patients undergoing transjugular liver biopsy. Whether these tests are helpful to predict bleeding in patients undergoing percutaneous liver biopsy and having coagulation changes beyond the above-mentioned thresholds will deserve further studies. Pain in the hours after liver biopsy should increase awareness on the possibility of liver biopsy–related bleeding.

TABLE 4 Occurrence of biopsy-related bleeding according to the occurrence of pain

Laboratory	*n*	No liver biopsy–related bleeding	*n*	Liver biopsy–related bleeding	*p*-value
Pain score at 2 h	260	0 (0–0)	20	2 (0–6)	.001
- Score = 0		200 (77)		9 (45)	.0003
- Score 1–4		33 (13)		5 (25)	
- Score 5–7		18 (7)		2 (10)	
- Score 8–10		9 (3)		4 (20)	
Any pain at 2 h	260	60 (23)	20	11 (55)	.002
Pain score at 24 h	270	0 (0–0)	20	0 (0–4)	<.0001
- Score = 0		246 (91)		11 (55)	<.0001
- Score 1–4		15 (6)		5 (25)	
- Score 5–7		7 (3)		2 (10)	
- Score 8–10		2 (1)		1 (5)	
Any pain at 24 h	266	25 (9)	19	8 (42)	<.0001
Pain requiring analgesics within 24 h after liver biopsy	276	55 (20)	21	12 (57)	<.001

Note: Results are presented as median (interquartile range) or absolute (relative frequencies). Pain was evaluated 2 h and 24 h after liver biopsy, using a visual analog scale ranging from 1 to 10. Out of the 302 patients, pain could not be completely assessed in 12 patients for organizational reasons.

AUTHOR CONTRIBUTIONS

J. Bissonnette designed and performed research, was responsible for patient recruitment, analyzed data, and wrote the paper. A. Riescher-Tuczkiewicz analyzed the data and wrote the paper. E. Gigante and H. Soliman helped with patient recruitment and provided expertise and critical evaluation. C. Bourdin performed hemostasis tests. L. Boudaoud performed hemostasis tests and provided expertise and critical evaluation. D. Valla, M. Ronot, and F. Durand provided expertise and critical evaluation. V. Vilgrain provided expertise and critical evaluation and performed the ultrasonography evaluation. E. de Raucourt designed the research, performed hemostasis tests, and provided expertise and critical evaluation. P. E. Rautou designed and supervised research and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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CONFLICTS OF INTERESTS

Authors' declarations of personal interests: P. E. Rautou has received research funding from Terrafirma and acted as consultant for Hemostod, Mursla, and Abbelight; provided training sessions for Cook; and received speaker fees from Tillots pharma.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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