Digestive and Liver Disease 51 (2019) 1409–1415

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Acute kidney injury is associated with low factor XIII in decompensated cirrhosis

Nicolas M. Intagliata[a,*], Jessica P.E. Davis[a], Jeffery Lafond[a], Uta Erdbruegger[b], Charles S. Greenberg[c], Patrick G. Northup[a], Stephen H. Caldwell[a]

[a] University of Virginia, Division of Gastroenterology and Hepatology, Charlottesville, VA, United States
[b] University of Virginia, Division of Nephrology
[c] Medical University of South Carolina, Division of Hematology

ARTICLE INFO

Article history:
Received 8 February 2019
Accepted 13 March 2019
Available online 6 April 2019

Keywords:
Bleeding
Coagulopathy
Fibrinolysis
Viscoelastic

ABSTRACT

Background/aims: The coagulation system is known to be rebalanced but fragile in stable cirrhosis. Acute kidney injury (AKI) is common in these patients and associated with an increased bleeding risk. We aimed to assess coagulation parameters in this population.
Methods: We prospectively enrolled 43 hospitalized patients with decompensated cirrhosis with (n = 22) or without (n = 21) AKI. Coagulation factor levels, viscoelastic coagulation assay, and thrombin generation assay were performed and compared between these groups and a healthy reference group.
Results: Conventional markers of coagulation were not statistically different between patients with and without AKI. Factor XIII was significantly reduced in all patients with cirrhosis compared to healthy controls (p = <0.0001). In patients with AKI, factor XIII was significantly lower compared to patients without AKI (AKI 38% vs. non-AKI 60% p = 0.002). In patients with cirrhosis, factor XIII had a significantly positive correlation with EXTEM maximal clot firmness (r = 0.5440, p = 0.0002) and FIBTEM maximal clot firmness (r = 0.7397, p = <0.0001) and a negative correlation with EXTEM clot formation time (−0.413, p = 0.0065).
Conclusions: Factor XIII was significantly reduced in decompensated cirrhosis patients with AKI compared to decompensated patients without AKI. These findings suggest that exacerbation of factor XIII deficiency in AKI in decompensated cirrhosis may affect bleeding risk and warrants further study.

1. Introduction

A new paradigm in coagulopathy of liver disease has emerged over the last several years [1]. Where clinicians once believed patients with cirrhosis were "auto-anticoagulated," it is now recognized that the coagulation system is rebalanced in stable cirrhosis. Sepsis [2,3], hospitalization [4], and deteriorating hepatic function [5] may disrupt this balance resulting in thrombosis or bleeding.

Acutely decompensated (AD) liver disease and acute on chronic liver failure (ACLF) represent an important and distinct pathophysiology with high risk of morbidity and mortality [6]. These patients are particularly at risk to develop thrombosis or bleeding due to progressive multiorgan dysfunction, extended hospitalizations, and exposure to multiple invasive procedures [7]. One study examining patients with AD liver disease and ACLF demonstrated that thrombin generation remained intact even in severely decompensated hepatic disease states but displayed progressive instability in the most advanced patients [8]. Acute kidney injury (AKI) is common in patients with AD liver disease or ACLF. Clinical evidence now indicates hospitalized patients with decompensated cirrhosis, ascites, and AKI may be more prone to develop procedural-related bleeding than patients without AKI [9].

During hospitalization, patients with decompensated cirrhosis are at risk to develop bleeding and thrombosis and no current coagulation test adequately predicts these events. Global coagulation assays, such as thrombin generation assay (TGA) and viscoelastic tests (e.g. rotational thromboelastometry, ROTEM), have been studied in patients with stable and decompensated liver disease. While our understanding of the physiology and treatment of bleeding related to portal hypertension is well-established, bleeding related to other hemostatic mechanisms, including procedural-related bleeding, is not well-understood secondary to the complex pathophysiology [10].

* Corresponding author at: University of Virginia Medical Center, Division of Gastroenterology and Hepatology, PO Box 800708, Charlottesville, VA, 22908, United States.
E-mail address: intagliata@virginia.edu (N.M. Intagliata).

https://doi.org/10.1016/j.dld.2019.03.011
1590-8658/

Almost all coagulation factors are significantly altered in decompensated cirrhosis [11]. Factor XIII is an important protein in clot formation and has myriad effects, including the important role of stabilizing fibrin via cross-linking [12]. Deficiency of factor XIII is associated with increased bleeding tendency and this association has been recognized in cirrhosis for several years [13,14].

In this study we examine the coagulopathy of patients with decompensated cirrhosis with and without AKI admitted to the hospital using global coagulation assays and other markers of coagulation. We hypothesized that the development of AKI upsets the fragile rebalanced coagulation system in cirrhosis and increases hypocoagulability and overall bleeding risk.

2. Methods

2.1. Study design

This prospective study was performed at the University of Virginia and patients were selected during a study period of 2016–2018. Informed consent was obtained from each participant prior to enrollment. This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by University of Virginia internal research review board.

Hospitalized patients with decompensated cirrhosis (restricted to Child-Turcotte-Pugh (CTP) C cirrhosis) with or without AKI were screened for enrollment. Only adult patients (>18 years old) admitted to the inpatient hepatology service with decompensated cirrhosis were included. Prisoners, pregnant patients, and non-English speaking patients were excluded. Patients on therapeutic anticoagulation were excluded. Patients with a history end-stage renal disease were excluded.

The presence or absence of acute and chronic kidney disease was determined by reviewing the patient's medical record, past medical history, and previous laboratory values. The diagnosis of AKI was defined according to International Club of Ascites diagnostic criteria [15]. The baseline serum creatinine was obtained from the medical records within the prior 3 months and the most recent value available was used. Acute kidney injury was defined as an increase in serum creatinine of greater than or equal to 0.3 mg/dL within 48 h or a 50% increase in 7 days from baseline serum creatinine. Furthermore in all cases the patient had a diagnosis of AKI in the medical record at admission. Healthy controls without medical comorbidity, liver disease history, use of current medications, and without history of bleeding or thrombotic disorder were also enrolled.

The medical record was reviewed prior to enrollment and the diagnosis of cirrhosis was confirmed with available data including histology (if available), radiology, laboratory abnormalities, and clinical stigmata. Data were collected from the medical record including patient demographics, biochemical data, and in-hospital clinical events. Patients admitted to the inpatient hospital with cirrhosis were considered be acutely decompensated according to previous definitions and the main etiology to decompensation was defined (Table 1) [16]. Patients with cirrhosis, acute decompensation, and organ failure were considered to have ACLF. On-line calculators were used to determine patient scores of CLIF-ACLF (Chronic Liver Failure Consortium) score (https://www.clifresearch.com/ToolsCalculators.aspx) and CTP score. Model for end-stage liver disease (MELD) was calculated based on biochemical values from the day of enrollment. In-hospital major bleeding was defined according to International Society of Thrombosis and Haemostasis guidelines [17]. Procedures were also defined and recorded (Supplementary Table 4). Procedural related bleeding was determined to be a direct result of the procedure and related in time.

2.2. Sample collection and preparation

Peripheral blood was collected via standard venipuncture technique in citrate-containing vacutainer tubes from patients and healthy controls. All samples were collected in a similar manner by phlebotomy technicians using at least 21 g needles and tourniquet. Immediate viscoelastic testing (ROTEM) was performed on whole blood by trained technicians. Thereafter the remainder of the samples were processed to obtain platelet poor plasma (PPP) via centrifugation at 5000 *g* for 15 min at room temperature and snap frozen in liquid nitrogen in separate 1 mL aliquots and stored at −80 C.

2.3. Coagulation assays

Standard coagulation assays, including prothrombin time, INR, and factor level assays were performed by the special coagulation laboratory at the University of Virginia according to established standard techniques. Factor XIII antigen assay was performed according to manufacture instructions by the special coagulation laboratory (HemosIL, Bedford MA, USA). The assay is an automated latex enhanced immunoassay that detects the A subunit of factor XIII in plasma. ROTEM (TEM Innovations, Munich Germany) was performed on fresh whole blood immediately after collection in a standardized fashion according to manufacturer's instructions by trained technicians. Tests obtained included EXTEM, INTEM, FIBTEM and APTEM and were performed with reagents specific to each test as defined by manufacture protocol. EXTEM measures the "extrinsic system" and uses tissue factor to trigger coagulation. INTEM measures the "intrinsic system" and uses ellagic acid to trigger coagulation. FIBTEM measures the contribution of fibrinogen to clot formation by inhibiting platelets with addition of cytochalasin D. APTEM measures fibrinolysis and is used to compare with EXTEM after addition of aprotonin (inhibitor of fibrinolysis). Clotting time (CT), Clot Formation Time (CFT), Maximal Clot Firmness (MCF) and maximum lysis (ML) were recorded and each sample was performed for greater than 60 min.

Thrombin generation assay was performed as previously described Hemker et al. [18] using Calibrated Automated Thrombogram System and the Flouroskan Ascent Analyzer (Diagnostica Stago, Olive, NJ USA). Trigger reagent with and without thrombomodulin (TM) was used (Diagnostica Stago, Olive, NJ USA) as TM is located on endothelial cells and activates protein C which serves to inhibit coagulation. Experiments were performed at 37 °C in triplicate with and without the addition of thrombomodulin. 20 ul of PPP reagent (5 pM tissue factor and phospholipids) was added to each well and 20ul of PPP + TM (5 pM tissue factor and phospholipids and TM) was added to each well (Diagnostica Stago, Olive, NJ USA). Thereafter platelet poor plasma was thawed in 37 C water bath and centrifuged again at 10,000 *g* for 10 min and the supernatant was removed for use. Next, 80 ul of plasma was added to each well. The Fluoroscan Ascent Analyzer automatically distributed the fluorochrome substrate and buffer (Fluo-Substrate and Fluo-Buffer (with calcium chloride) Diagnostica Stago Olive, NJ USA) and dedicated software (version 3.0.0.29 Thrombinoscope) records thrombin activity as a function of time.

2.4. Statistical analysis

General demographics and subject characteristics were analyzed using summary statistics. Categorical statistical comparisons were performed using the Chi-square test or the Fisher's exact test as appropriate. Due to non-parametric data distributions and sample size constraints, continuous comparisons were performed using either the Student t-test, Mann–Whitney/Wilcoxon sum-rank, or ANOVA as appropriate with means with 95% confidence intervals

Table 1
Cohortcharacteristics.

	AKI (22)	No AKI (21)	Controls (10)	Group comparison[a]	AKI vs. non-AKI[b]
Age (years)	55 (50–63)	49 (40–58)	33 (31–35)	<0.0001	0.029
Male (%)	11 (50%)	11 (52%)	5 (50%)	–	0.999
Etiology cirrhosis					
Alcohol	9 (41%)	11 (53%)	–		
NASH	8 (36%)	3 (14%)	–	–	0.425
Hepatitis C	2 (9%)	3 (14%)	–		
Other	3 (14%)	4 (19%)	–		
Etiology of acute kidney injury (n)					
Prerenal azotemia	10 (45%)	–	–	–	–
Hepatorenal syndrome	7 (32%)	–	–	–	–
Acute tubular necrosis	5 (23%)	–	–	–	–
Sodium (mmol/L)	133 (131–134)	130 (127–133)	138 (137–139)	<0.0001	0.11
BUN (mg/dL)	45 (31– 54)	13 (10–18)	14 (11–17)	<0.0001	<0.0001
Creatinine (mg/dL)	2.2 (1.7–3.4)	0.8 (0.6–0.9)	0.8 (0.7–0.9)	<0.0001	<0.0001
Total bilirubin (mg/dL)	4.2 (2.1–16.2)	7.7 (3.3–13.4)	0.8 (0.5–0.9)	0.009	0.337
Albumin (g/dL)	3.1 (2.7–3.4)	2.5 (2.1–2.9)	4.4 (4.1–4.5)	<0.0001	0.002
Aspartate aminotransferase (U/L)	65 (38- 116)	92 (49- 127)	23 (22- 30)	0.037	0.206
Alanine aminotransferase (U/L)	33 (14–46)	28 (20–74)	21 (16–26)	0.012	0.552
MELD at admission	28 (21–33)	23 (19–27)	–	–	0.010
Child-Turcotte-Pugh	11 (10–12)	12 (10–12)	–	–	0.437
ACLF Grade 0	4 (18%)	10 (48%)	–	–	
ACLF Grade 1	12 (55%)	7 (33%)	–	–	0.119
ACLF Grade 2	6 (27%)	4 (19%)	–	–	
CLIF Organ Failure Score	9 (7–10)	9 (8–10)			0.941
CLIF-C ACLF Score	51 (41–56)	50 (45–55)			0.551
Infection present	7 (32%)	8 (38%)	–	–	0.666
SBP present	2 (9%)	4 (19%)	–	–	0.346
PVT at enrollment	3 (14%)	2 (10%)	–	–	0.674
Prior venous thromboembolism	2 (9%)	0	–	–	0.157
No previous decompensation	3 (14%)	3 (14%)	–	–	–
Previous type of decompensation			–	–	–
Ascites/effusion	18 (82%)	16 (76%)			
Hepatic encephalopathy	16 (73%)	16 (76%)			
Gastrointestinal hemorrhage	5 (23%)	7 (33%)			
Site of hospitalization at enrollment			–	–	–
Intensive care unit	2 (1%)	0			
Ward	20 (91%)	21 (100%)			
Principle reason for admission			–	–	–
Related to ascites/volume	11 (50%)	8 (38%)			
Hepatic encephalopathy	2 (10%)	4 (19%)			
Infection	3 (14%)	4 (19%)			
Gastrointestinal hemorrhage	0	0			
Other	6 (27%)	5 (24%)			
Principle acute decompensation precipitant			–	–	–
Infection	3 (14%)	5 (24%)			
Alcohol	2 (1%)	2 (1%)			
Volume/dehydration	11 (50%)	8 (38%)			
Other/unknown	6 (27%)	6 (29%)			

For continuous variables: median values reported with 25th and 75th percentile values in parenthesis. For categorical variables: n value reported with total percentage in parenthesis.
Abbreviations: AKI: acute kidney injury, NASH: non-alcohol steatohepatitis, MELD: model for end-stage liver disease, ACLF: acute on chronic liver failure, CLIF: Chronic liver failure consortium, SBP: spontaneous bacterial peritonitis, PVT: portal vein thrombosis, BUN: blood urea nitrogen.
[a] Group comparison ANOVA test with p-value.
[b] Wilcoxon rank sum test with p-value.

and medians with 25% and 75% quartile ranges reported. Pearson correlation tests were also performed. All statistical tests were two-sided and the level for type-one error reaching statistical significance was set at $P \leq 0.05$. All data analysis and manipulation were performed using SAS, version 9.4 (Cary, NC, USA).

3. Results

3.1. Patient demographics

A total of 43 patients hospitalized with acutely decompensated CTP C cirrhosis with and without AKI were enrolled in the study (Table 1). Samples were taken from patients at or near the day of admission with the median time from admission equal in both groups (1 day (IQR 1–2 days) AKI vs. 1 day (IQR 1–1 days) non-AKI). General demographics and disease characteristics were similar between patient groups. MELD score was significantly higher in the AKI cohort, likely reflective of the significant differences in creatinine (MELD 28 (IQR 21–33) AKI vs. MELD 23 (IQR 19–27) non-AKI, p = 0.010). Other components of MELD score including total bilirubin and INR were similar between groups. Other disease classification measures such as CTP score, CLIF Organ Failure score, ACLF Grade and cirrhosis etiology were similar between groups (Table 1). For comparison, samples from ten healthy controls were also analyzed.

Hospital length of stay was significantly longer in patients with AKI (median 9 days (IQR 5–15 days) in AKI vs. 4 days (IQR 3–6 days) in non-AKI, p = 0.01). In general, patients with AKI underwent more total procedures overall and had more bleeding events in the hospital (Supplementary Table 4). There were 4 in-hospital bleeding events (2 major bleeds) in AKI cohort compared to 2 bleeding events (1 major bleed) in the non-AKI group. Two bleeding events

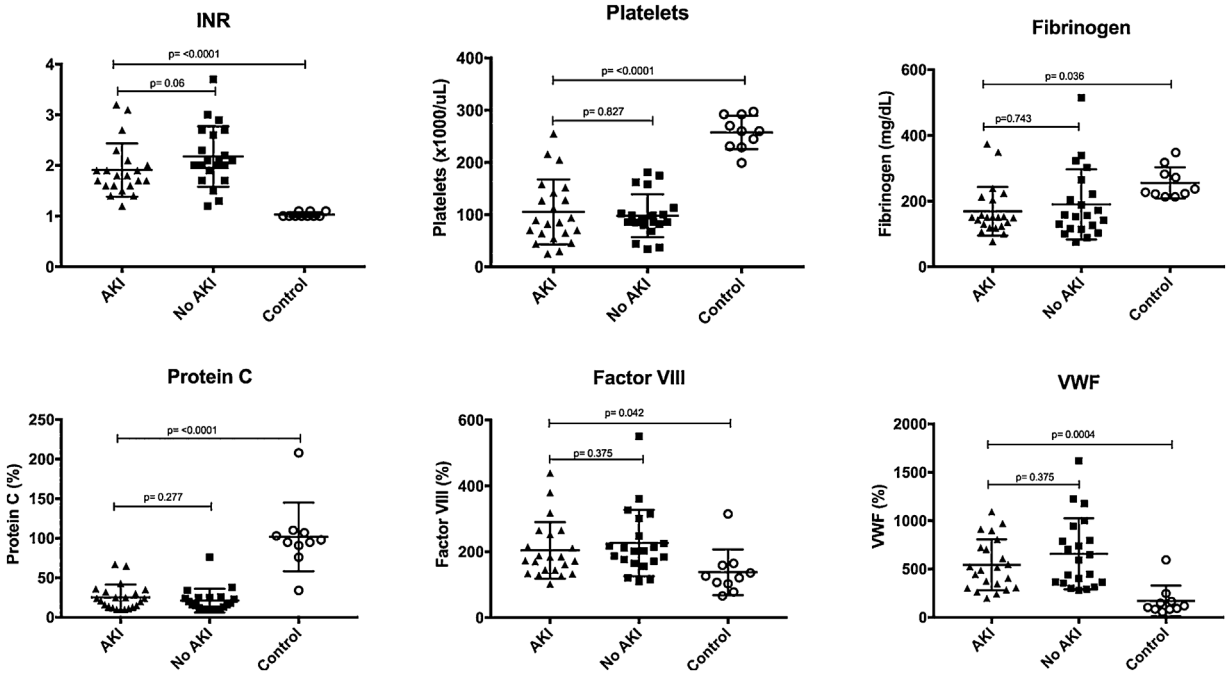

Fig. 1. Conventional coagulation studies and factor levels in patients and healthy controls. Median values with 25th and 75th percentile bars. AKI: acute kidney injury.

were related to procedures in the AKI group (both post-paracentesis intrabdominal hemorrhage). One patient without AKI received the majority of transfusions in this group (8 units of packed red blood cells and 13 units of cryoprecipitate). Excluding this patient, the cohort with AKI overall received more blood transfusions.

3.2. Coagulation studies

When comparing the AKI and non-AKI groups, all conventional markers of coagulation including platelets (87,000/uL (IQR 64,000–142,000/uL) AKI vs 89,000/uL (IQR 82,000–102,000/uL) non-AKI, p = 0.827), INR 1.8 (IQR 1.6–2.0) vs. 2.1 (IQR 1.9–2.6), p = 0.06), and fibrinogen (151 mg/dL (IQR 124–204 mg/dL) vs 156 mg/dL (IQR 116–221 mg/dL), p = 0.743) were similar. Coagulation factors produced in either liver sinusoidal and vascular endothelial cells, including factor VIII and von Willebrand Factor (VWF), were both significantly elevated in AKI and non-AKI compared to healthy controls, in accordance with previously published studies (Fig. 1) [11,19]. Protein C, an anticoagulant protein which is activated by endothelial derived TM, was significantly reduced in AKI and non-AKI patients compared to controls, but without significant differences between the two groups (Table 2, Fig. 1). Factor XIII was significantly reduced in the overall cohort of patients with cirrhosis compared to healthy controls (Fig. 2). In patients with AKI, factor XIII was significantly lower compared to patients without AKI (AKI 38% (IQR 28–58%) vs. non-AKI 60% (IQR 48–98%), p = 0.0002).

Thrombin generation assay parameters that were evaluated included endogenous thrombin potential (ETP), peak velocity, and peak thrombin (Supplementary Table 3). Median ETP with and without TM between groups are displayed in Fig. 3. Endogenous thrombin potential with TM was significantly elevated in patients with cirrhosis compared to healthy controls. With addition of TM, values did not significantly change in both AKI and non-AKI cohorts (ETP ratio = ETP without TM/ETP with TM) 0.92 in AKI and 0.93 in non-AKI, p = 0.183), however in healthy controls ETP was significantly reduced after addition of TM (ETP ratio 0.56, p = 0.0001) consistent with prior studies. Values for endogenous thrombin potential, total peak thrombin production, and velocity of thrombin production trended lower in patients with AKI compared to patients without AKI but were not significant.

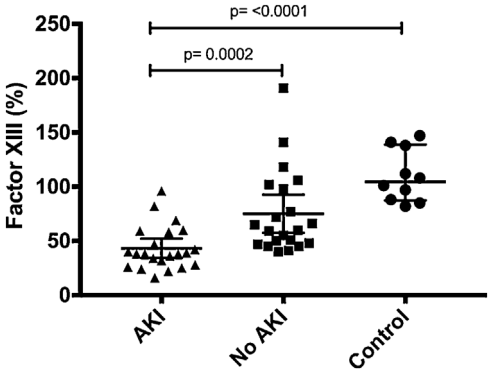

Fig. 2. Factor XIII levels in patients and healthy controls. Median value with 25th and 75th percentile bars. AKI: acute kidney injury.

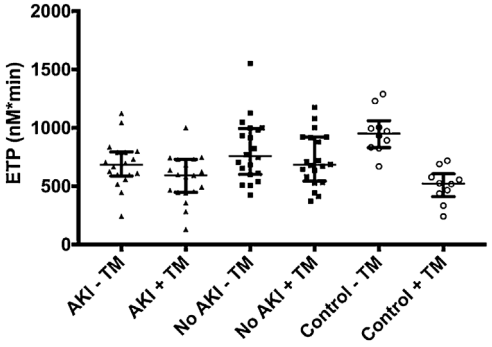

Fig. 3. Endogenous thrombin potential with and without thrombomodulin in patients with and without acute kidney injury and healthy controls. AKI: acute kidney injury.

When compared to healthy controls, there were several significant differences with evidence of weaker and delayed clot formation in patients with cirrhosis (Supplementary Table 3). There

Table 2
Coagulation studies.

	AKI (22)	No AKI (21)	Controls (10)	Group comparison[a]	AKI vs. non-AKI[b]
Hemoglobin (g/dL)	8.8 (7.5–10.2)	9.5 (8.6–10.9)	13.8 (12.9–15.3)	<0.0001	0.192
Platelets (*1000/uL)	87 (64–142)	89 (82–102)	260 (231–292)	<0.0001	0.827
INR	1.8 (1.6–2)	2.1 (1.9–2.6)	1 (1–1.1)	<0.0001	0.060
Prothrombin time (s)	20.4 (17.8–23.2)	23.2 (20.1–27.5)	11.3 (10.8–12.0)	<0.0001	0.126
Fibrinogen (mg/dL)	151 (124–204)	156 (116–221)	232 (222–282)	0.036	0.743
Factor VIII (%)	179 (144–252)	203 (171–248)	124 (103–159)	0.042	0.375
Factor XIII (%)	38 (28–58)	60 (48–98)	105 (88–138)	<0.0001	0.0002
Protein C (%)	22.5 (13–32)	16 (10–25)	97 (91–107)	<0.0001	0.277
vWF (%)	503 (307–723)	595 (364–796)	112 (85–166)	0.0004	0.375
vWF-coFactor (%)	445 (312–618)	512 (292–623)	101 (80–162)	<0.0001	0.799

Median values reported with 25th and 75th percentile values in parenthesis.
[a] Group comparison ANOVA test with p-value.
[b] Wilcoxon rank sum test with p-value.

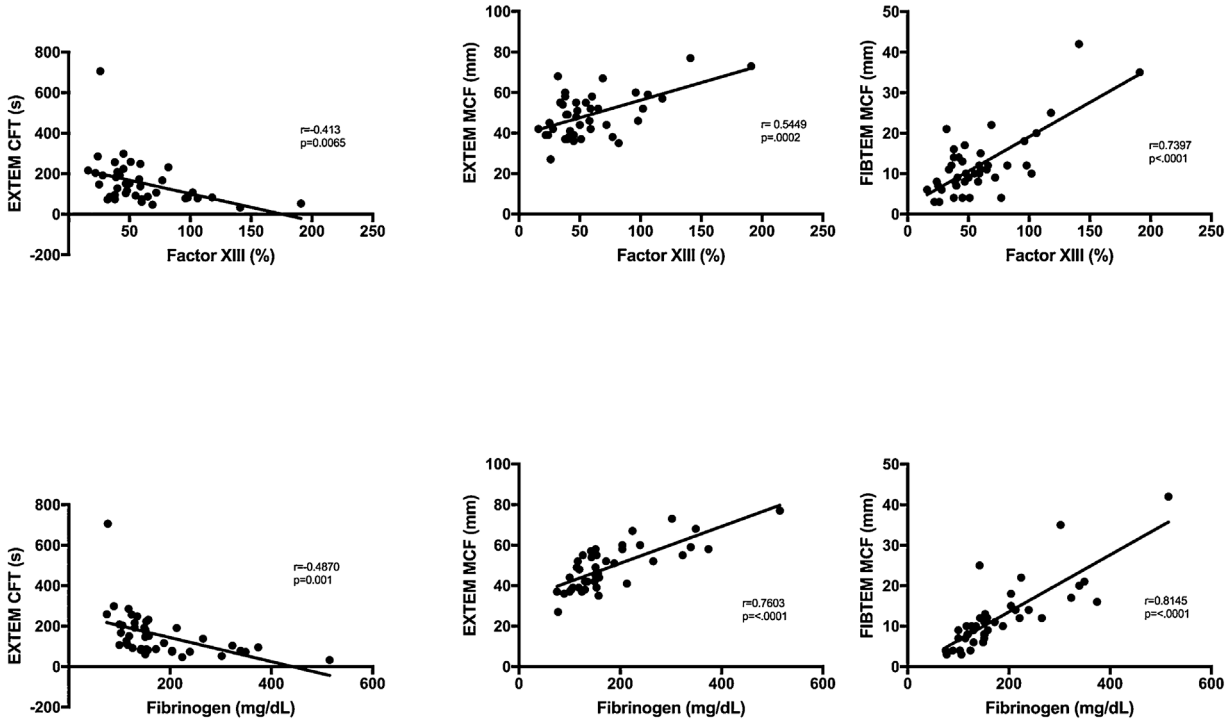

Fig. 4. ROTEM values correlated with factor XIII and fibrinogen.

were no significant differences between patient groups in ROTEM variables (Supplementary Table 3). However, there were trends toward decreased clot strength in EXTEM, INTEM and FIBTEM as determined by clot formation time and maximum clot firmness. There was no evidence of hyperfibrinolysis between groups with similar values in maximum clot lysis in EXTEM and APTEM. Factor XIII level and fibrinogen level were strongly correlated with measures of clot strength on ROTEM (Fig. 4). In patients with cirrhosis, factor XIII level had a positive linear correlation with EXTEM MCF (r = 0.5440, p = .0002) and FIBTEM MCF (r = 0.7397, p=<.0001) and a negative linear correlation with EXTEM CFT (−0.413, p = .0065). Fibrinogen level also had similar correlations indicating the importance of these factors in the speed of clot formation and the strength and integrity of the clot.

4. Discussion

Hospitalized patients with decompensated cirrhosis and AKI are at risk for significant morbidity and mortality which is often related to bleeding. While uremia in chronic kidney disease has long been considered a risk factor for bleeding through platelet dysfunction [20,21], only recently has this association been described in patients with liver disease and acute kidney injury [9,22]. In our cohort we found an association between features of hypocoagulability in patients with cirrhosis and AKI, which may be explained, in part, by diminished factor XIII. The results support the paradigm of a rebalanced coagulation system in patients with cirrhosis that is vulnerable to exogenous disruption such as AKI. Although this study does not prove causality, these results suggest that changes in factor XIII are a significant consideration in decompensated cirrhosis when associated with AKI.

Delayed bleeding is common in cirrhosis and hyperfibrinolysis is often implicated. The diagnosis of hyperfibrinolysis in cirrhosis is often challenging and remains controversial [23,24]. Similar to other components of the coagulation system, the fibrinolytic system in cirrhosis is thought to be dramatically altered, but also rebalanced. Factor XIII is a protransglutaminase protein with myriad functions located in cells, such as platelets, and in plasma [12]. In plasma, factor XIII zymogen circulates as two A and two B subunits bound to fibrinogen and is activated via thrombin during coagulation whereby cross-linking of fibrin occurs, significantly strengthening the clot and increasing resistance to fibrinolysis [25].

The B subunit of factor XIII is produced in the liver and the A subunit is produced primarily in the bone marrow. Megakaryocytes also produce factor XIII A subunit and platelets may uptake some through plasma. Once activated, factor XIII acts to cross-link fibrin, and individual fibers in the clot are compacted together and overall pore size is decreased. Severe deficiencies in factor XIII lead to life-threatening spontaneous bleeding, impaired wound healing, and spontaneous abortion [26]. Studies using viscoelastic testing, such as ROTEM, indicate that factor XIII has an important role in maintaining clot integrity and low levels of factor XIII are associated with decreased clot firmness [27–29].

Factor XIII is low in patients with cirrhosis and has been associated with decreased clot strength as determined by ROTEM [30], decreased survival, and overall increased bleeding risk [12,13,31]. In one study in patients without cirrhosis, factor XIII was significantly lower in patients with AKI compared to patients with chronic kidney disease [32]. Our study similarly showed factor XIII levels were significantly reduced in patients with AKI compared to patients without AKI and this was directly correlated with clot strength as measured by ROTEM. Factor XIII levels were negatively correlated with EXTEM clot formation time, indicating an association with delay in clot formation in patients with reduced factor XIII. As indicated in Fig. 4, fibrinogen plays an important role clot formation and hence significantly affects ROTEM curve output. Therefore it is important to interpret these data cautiously as the relationship between thrombin, fibrinogen, and factor XIII is confounded by interdependent interactions of these proteins in plasma [33]. Interestingly, there was no evidence using ROTEM of early clot dissolution which can be measured in maximum clot lysis in APTEM. The mechanism to explain lower levels of factor XIII in AKI is not known, however this association has been reported in patients with AKI without cirrhosis [32]. As patient characteristics regarding hepatic dysfunction were similar between groups, we hypothesize that decreased hepatic production does not necessarily explain our findings. Uremia contributes to hemostatic defects on platelets [21] and is also associated with modification of fibrinogen which may explain other defects in hemostasis [34]. As platelets and megakaryocytes play a role in factor XIII storage and uptake a possible link may exist between uremia, platelets, and factor XIII level, but further study is needed to explore these relationships.

ROTEM has also been studied in patients with cirrhosis mainly in the liver transplant population [35], but with some studies in patients with compensated and decompensated cirrhosis [36–38]. Prior studies have shown no clear association with prediction of clinical bleeding risk with viscoelastic tests. Rather associations with 'hypocoagulable profiles' and conventional coagulation parameters are most often reported. However, a recent study examining coagulopathy in patients with ACLF found parameters in TEG to be predictive of major bleeding and mortality [39]. In this present study there were significant differences in patients with cirrhosis compared to healthy controls in several parameters similar to previous reports. When comparing patients with and without AKI there were non-significant trends toward slower clot development and weaker clot formation in EXTEM, INTEM, and FIBTEM in patients with AKI. The relationship, dependency, and individual effects of fibrinogen and factor XIII in clot formation and strength are challenging to uncouple with available coagulation testing. However, studies examining the effects of clot kinetics and strength as measured by ROTEM with ex vivo supplementation of fibrinogen and factor XIII with ROTEM report synergistic effects of these two important factors for improving clot kinetics and strength [40,41].

Thrombin generation assay is now a well-established research tool in the study of coagulopathy of liver disease and early seminal studies established significant foundations in the field [42–44]. It is now known patients with cirrhosis maintain the ability to generate thrombin despite significantly reduced synthesis of coagulation proteins [42,43,45,46]. Current theory posits that this rebalanced system is maintained by elevated factors synthesized outside of hepatocytes, decreased production of inhibitors of coagulation, and multiple other mechanisms [47,48]. In this cohort, thrombin generation was preserved in patients with decompensated cirrhosis compared to healthy controls in accordance with previously published studies (Fig. 2). A trend of decreased thrombin production was observed in patients with AKI and without AKI indicating a potential risk of hypocoagulability in this cohort. Fibrinogen, protein C activity, and factor VIII activity were not significantly different between these two groups (Fig. 1). Our data confirm and extend results from a previous study examining TGA in patients with AD cirrhosis and ACLF [8]. While the authors do not elaborate specifically on AKI in subgroup comparison, overall patients with ACLF appear to have a decreased ETP with TM compared to patients with AD cirrhosis.

Potential limitations of the study include cohort heterogeneity and limited sample sizes of the selected cohorts. While characteristics were overall similar between cohorts, certain features such as the presence of thromboprophylaxis, portal vein thrombosis, and prior history of malignancy may alter coagulation profiles. Furthermore, patients with AKI are more likely to have longer, complicated hospitalizations which may bias toward increased bleeding events. In any study of coagulation it is important to relate observed laboratory testing to clinical outcomes of bleeding and thrombosis. In this study bleeding events were rare and therefore statistical analyses were limited. Second, there are limitations in any study of the coagulation system inherent to reliance on ex vivo measurement. Loss of interplay between the vascular environment and the multitude of factors in the coagulation system (known and unknown) are unavoidable in coagulation assays and when choosing specific factors to evaluate. Several factors were not measured in this study, including proteins in the fibrinolytic system, which may also limit these findings. A lack of potential key effectors of coagulation in testing limits conclusion to associative and therefore the results may not reflect causal relationships.

5. Conclusion

We have prospectively examined the underlying mechanisms in coagulation in patients with cirrhosis and AKI using conventional coagulation tests and more advanced measures of the hemostatic cascade. These data support the possibility that patients with AKI and cirrhosis may be at a higher risk of bleeding. As factor XIII deficiency is known to increase the risk of bleeding, the association identified here represents a previously unrecognized risk factor for bleeding in patients with AKI. The clinical association with AKI in patients with cirrhosis and increased risk of post-paracentesis hemorrhage is potentially supported by this study [9]. Larger prospective and comprehensive studies are needed to further elaborate the complex behavior of the coagulation system in decompensated cirrhosis and to assess the effects of more directed factor XIII replacement.

Conflict of interest

None to declare: JPED, JL, UE, CSG, PGN. NMI: research grant support Eisai, Vital therapies; SHC receives research grant support from Gilead, GEnfit, Conatus, Mallinkrad and Vital Therapy, Galmed, NGM, BMS, Dova and Shinogi. Intellectual property rights Halyard.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.dld.2019.03.011.

References

[1] Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med 2011;365:147–56.
[2] Violi F, Ferro D, Basili S, Saliola M, Quintarelli C, Alessandri C, et al. Association between low-grade disseminated intravascular coagulation and endotoxemia in patients with liver cirrhosis. Gastroenterology 1995;109:531–9.
[3] Raparelli V, Basili S, Carnevale R, Napoleone L, Del Ben M, Nocella C, et al. Low-grade endotoxemia and platelet activation in cirrhosis. Hepatology 2017;65:571–81.
[4] Intagliata NM, Henry ZH, Shah N, Lisman T, Caldwell SH, Northup PG. Prophylactic anticoagulation for venous thromboembolism in hospitalized cirrhosis patients is not associated with high rates of gastrointestinal bleeding. Liver Int 2014;34:26–32.
[5] Stine JG, Wang J, Shah PM, Argo CK, Intagliata N, Uflacker A, et al. Decreased portal vein velocity is predictive of the development of portal vein thrombosis: a matched case-control study. Liver Int 2018;38:94–101.
[6] Bernal W, Jalan R, Quaglia A, Simpson K, Wendon J, Burroughs A. Acute-on-chronic liver failure. Lancet 2015;386:1576–87.
[7] Drolz A, Horvatits T, Roedl K, Rutter K, Staufer K, Kneidinger N, et al. Coagulation parameters and major bleeding in critically ill patients with cirrhosis. Hepatology 2016;64:556–68.
[8] Fisher C, Patel VC, Stoy SH, Singanayagam A, Adelmeijer J, Wendon J, et al. Balanced haemostasis with both hypo- and hyper-coagulable features in critically ill patients with acute-on-chronic-liver failure. J Crit Care 2018;43:54–60.
[9] Hung A, Garcia-Tsao G. Acute kidney injury, but not sepsis, is associated with higher procedure-related bleeding in patients with decompensated cirrhosis. Liver Int 2018;38:1437–41.
[10] Schepis F, Turco L, Bianchini M, Villa E. Prevention and management of bleeding risk related to invasive procedures in cirrhosis. Semin Liver Dis 2018;38(3):215–29.
[11] Tripodi A, Primignani M, Chantarangkul V, Dell'Era A, Clerici M, de Franchis R, et al. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. Gastroenterology 2009;137:2105–11.
[12] Schroeder V, Kohler HP. Factor XIII: structure and function. Semin Thromb Hemost 2016;42(4):422–8.
[13] Biland L, Duckert F, Prisender S, Nyman D. Quantitative estimation of coagulation factors in liver disease. The diagnostic and prognostic value of factor XIII, factor V and plasminogen. Thromb Haemost 1978;39:646–56.
[14] Tacke F, Fiedler K, von Depka M, Luedde T, Hecker H, Manns MP, et al. Clinical and prognostic role of plasma coagulation factor XIII activity for bleeding disorders and 6-year survival in patients with chronic liver disease. Liver Int 2006;26:173–81.
[15] Angeli P, Gines P, Wong F, Bernardi M, Boyer TD, Gerbes A, et al. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. Gut 2015;64:531–7.
[16] Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. Gastroenterology 2013;144:1426–37, 37 e1-9.
[17] Schulman S, Kearon C. Subcommittee on control of anticoagulation of the S, standardization committee of the international society on T, haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost 2005;3:692–4.
[18] Hemker HC, Giesen P, AlDieri R, Regnault V, de Smed E, Wagenvoord R, et al. The calibrated automated thrombogram (CAT): a universal routine test for hyper- and hypo-coagulability. Pathophysiol Haemost Thromb 2002;32:249–53.
[19] Lisman T, Bongers TN, Adelmeijer J, Janssen HL, de Maat MP, de Groot PG, et al. Elevated levels of von Willebrand Factor in cirrhosis support platelet adhesion despite reduced functional capacity. Hepatology 2006;44:53–61.
[20] Mannucci PM, Remuzzi G, Pusineri F, Lombardi R, Valsecchi C, Mecca G, et al. Deamino-8-D-arginine vasopressin shortens the bleeding time in uremia. N Engl J Med 1983;308:8–12.
[21] Mannucci PM, Tripodi A. Hemostatic defects in liver and renal dysfunction. Hematol Am Soc Hematol Educ Program 2012;2012:168–73.
[22] Agarwal B, Gatt A, Riddell A, Wright G, Chowdary P, Jalan R, et al. Hemostasis in patients with acute kidney injury secondary to acute liver failure. Kidney Int 2013;84:158–63.
[23] Lisman T, Leebeek FW, Mosnier LO, Bouma BN, Meijers JC, Janssen HL, et al. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. Gastroenterology 2001;121:131–9.
[24] Rijken DC, Kock EL, Guimaraes AH, Talens S, Darwish Murad S, Janssen HL, et al. Evidence for an enhanced fibrinolytic capacity in cirrhosis as measured with two different global fibrinolysis tests. J Thromb Haemost 2012;10:2116–22.
[25] Byrnes JR, Wolberg AS. Newly-recognized roles of factor XIII in thrombosis. Semin Thromb Hemost 2016;42:445–54.
[26] Muszbek L, Katona E. Diagnosis and management of congenital and acquired FXIII deficiencies. Semin Thromb Hemost 2016;42:429–39.
[27] Nielsen VG, Gurley Jr WQ, Burch TM. The impact of factor XIII on coagulation kinetics and clot strength determined by thrombelastography. Anesth Analg 2004;99:120–3.
[28] Wettstein P, Haeberli A, Stutz M, Rohner M, Corbetta C, Gabi K, et al. Decreased factor XIII availability for thrombin and early loss of clot firmness in patients with unexplained intraoperative bleeding. Anesth Analg 2004;99:1564–9, table of contents.
[29] von Rappard S, Hinnen C, Lussmann R, Rechsteiner M, Korte W. Factor XIII deficiency and thrombocytopenia are frequent modulators of postoperative clot firmness in a surgical intensive care unit. Transfus Med Hemother 2017;44:85–92.
[30] Bedreli S, Sowa JP, Malek S, Blomeyer S, Katsounas A, Gerken G, et al. Rotational thromboelastometry can detect factor XIII deficiency and bleeding diathesis in patients with cirrhosis. Liver Int 2017;37:562–8.
[31] Ballerini G, Guerra S, Rodeghiero F, Castaman G. A contribution to the pathology of acquired plasma factor XIII deficiency. Semin Thromb Hemost 1985;11:357–61.
[32] Carmassi F, Mariani G, Palla R, Fusani L, Bionda A, Molea N, et al. Coagulation factor XIII in patients with acute and chronic renal disease. Nephron 1980;25:179–83.
[33] Greenberg CS, Achyuthan KE, Fenton 2nd JW. Factor XIIIa formation promoted by complexing of alpha-thrombin, fibrin, and plasma factor XIII. Blood 1987;69:867–71.
[34] Binder V, Bergum B, Jaisson S, Gillery P, Scavenius C, Spriet E, et al. Impact of fibrinogen carbamylation on fibrin clot formation and stability. Thromb Haemost 2017;117:899–910.
[35] Stancheva A, Spassov L, Tzatchev K. Correlation between rotation thrombelastometry ROTEM analysis and standard haemostatic parameters during liver transplantation. Clin Lab 2011;57:407–13.
[36] Davis JPE, Northup PG, Caldwell SH, Intagliata NM. Viscoelastic testing in liver disease. Ann Hepatol 2018;17:205–13.
[37] Tripodi A, Primignani M, Chantarangkul V, Viscardi Y, Dell'Era A, Fabris FM, et al. The coagulopathy of cirrhosis assessed by thromboelastometry and its correlation with conventional coagulation parameters. Thromb Res 2009;124:132–6.
[38] Kleinegris MC, Bos MH, Roest M, Henskens Y, Ten Cate-Hoek A, Van Deursen C, et al. Cirrhosis patients have a coagulopathy that is associated with decreased clot formation capacity. J Thromb Haemost 2014;12:1647–57.
[39] Premkumar M, Saxena P, Rangegowda D, Baweja S, Mirza R, Jain P, et al. Coagulation failure is associated with bleeding events and clinical outcome during systemic inflammatory response and sepsis in acute-on-chronic liver failure: an observational cohort study. Liver Int 2019;39(4):694–704.
[40] Hanna J, Winstedt D, Schott U. Fibrinogen and FXIII dose response effects on albumin-induced coagulopathy. Scand J Clin Lab Invest 2013;73:553–62.
[41] Shams Hakimi C, Carling MS, Hansson EC, Brisby H, Hesse C, Radulovic V, et al. The effect of ex vivo factor XIII supplementation on clot formation in blood samples from cardiac and scoliosis surgery patients. Clin Appl Thromb Hemost 2018;24:677–83.
[42] 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;41:553–8.
[43] Lisman T, Bakhtiari K, Pereboom IT, Hendriks HG, Meijers JC, Porte RJ. Normal to increased thrombin generation in patients undergoing liver transplantation despite prolonged conventional coagulation tests. J Hepatol 2010;52:355–61.
[44] Tripodi A. Thrombin generation assay and its application in the clinical laboratory. Clin Chem 2016;62:699–707.
[45] Tripodi A, Primignani M, Lemma L, Chantarangkul V, Dell'Era A, Iannuzzi F, et al. Detection of the imbalance of procoagulant versus anticoagulant factors in cirrhosis by a simple laboratory method. Hepatology 2010;52:249–55.
[46] 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;8:1994–2000.
[47] Lisman T, Bos S, Intagliata NM. Mechanisms of enhanced thrombin-generating capacity in patients with cirrhosis. J Thromb Haemost 2018;16:1128–31.
[48] Sinegre T, Duron C, Lecompte T, 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;16:1132–40.