HHS Public Access

Author manuscript

Hepatology. Author manuscript; available in PMC 2021 December 15.

Published in final edited form as:

Hepatology. 2020 October ; 72(4): 1327–1340. doi:10.1002/hep.31443.

Acute Kidney Injury in Decompensated Cirrhosis Is Associated With Both Hypo-coagulable and Hyper-coagulable Features

Alberto Zanetto[1,2,3], **Henry M. Rinder**[4,5], **Elena Campello**[6], **Graziella Saggiorato**[6], **Yanhong Deng**[7], **Maria Ciarleglio**[7], **Francis P. Wilson**[8], **Marco Senzolo**[3], **Sabrina Gavasso**[6], **Cristiana Bulato**[6], **Paolo Simioni**[6], **Guadalupe Garcia-Tsao**[1,2]

[1]Digestive Disease Section, Internal Medicine, Yale School of Medicine, New Haven, CT

[2]VA-Connecticut Healthcare System, West Haven, CT

[3]Gastroenterology and Multivisceral Transplant Unit, Department of Surgery, Oncology, and Gastroenterology, Padova University Hospital, Padova, Italy

[4]Laboratory Medicine, Yale School of Medicine, New Haven, CT

[5]Hematology, Internal Medicine, Yale School of Medicine, New Haven, CT

[6]Thrombotic and Hemorrhagic Diseases Unit, General Internal Medicine, Padova University Hospital, Padova, Italy

[7]Yale Center for Analytical Sciences, Yale School of Public Health, New Haven, CT

[8]Program of Applied Translational Research, Yale School of Medicine, New Haven, CT.

Abstract

BACKGROUND AND AIMS: Recent evidence suggests that acute kidney injury (AKI) is the main predictor of postparacentesis bleeding in patients with cirrhosis. To assess the factors responsible for bleeding tendency in AKI, we performed a prospective study comparing all three aspects of hemostasis (platelets, coagulation, and fibrinolysis) in patients with decompensated cirrhosis with and without AKI.

APPROACH AND RESULTS: Primary hemostasis assessment included platelet aggregation and secretion (platelet function markers) and von Willebrand factor. Secondary hemostasis assessment included pro-coagulant (factor VIII and factor XIII) and anti-coagulant (protein C, protein S, and antithrombin) factors and thrombin generation. Tertiary hemostasis

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO: Paolo Simioni, M.D., Ph.D., Thrombotic and Haemorrhagic Diseases Unit, General Internal Medicine, Padova University Hospital, 35128 Padova, Italy, paolo.simioni@unipd.it, Tel.: +39-049-8212667; Guadalupe Garcia-Tsao, M.D., Digestive Diseases, VA Connecticut Healthcare System, Yale University School of Medicine, West Haven, CT 06516, guadalupe.garcia-tsao@yale.edu, Tel.: +1-203-737-6063.

Author Contributions: A.Z. was responsible for the research design and performance (patients' enrollment and laboratory work), interpretation of the data, and writing of the manuscript. H.M.R., P.S., and G.G.T. were responsible for the research design, interpretation of the data, critical revision, and final approval of the manuscript. E.C., G.S., S.G., and C.B. were responsible for the laboratory work. Y.D. and M.C. were responsible for the statistical analysis. F.P.W. was responsible for the data acquisition. M.S. was responsible for the research design and interpretation of the data.

Potential conflict of interest: Nothing to report.

Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.31443/suppinfo.

assessment included fibrinolytic factors and plasmin-antiplasmin complex. Eighty patients with decompensated cirrhosis were recruited (40 each with and without AKI). Severity of cirrhosis and platelet count were comparable between groups. Median serum creatinine was 1.8 mg/dL and 0.8 mg/dL in patients with and without AKI, respectively. At baseline, patients with cirrhosis and AKI had lower platelet aggregation and secretion, indicative of impaired platelet function (increased bleeding tendency), without differences in von Willebrand factor. Regarding coagulation factors, factor VIII was higher, whereas protein C, protein S, and antithrombin were all lower, which, together with increased thrombin generation, indicate hypercoagulability. In contrast, factor XIII was lower in AKI (increased bleeding tendency). Finally, while both hypofibrinolytic and hyperfibrinolytic changes were present in AKI, a higher plasmin-antiplasmin complex indicated a hyperfibrinolytic state. After AKI resolution (n = 23 of 40), platelet function and coagulation improved to levels observed in patients with cirrhosis patients without AKI; however, fibrinolysis remained hyperactivated.

CONCLUSIONS: In patients with decompensated cirrhosis, AKI is associated with both hypocoagulable and hypercoagulable features that can potentially increase the risk of both bleeding and thrombosis.

Patients with decompensated cirrhosis have multiple and complex alterations of hemostasis that predispose them to both bleeding and thrombotic complications[1]

Although patients with cirrhosis are traditionally considered to be at high risk of bleeding from procedures, data regarding the correlation between hemostatic alterations and risk of bleeding are relatively sparse.[2] In a recent study looking at predictors of postparacentesis bleeding, our group found that the only independent predictor of such bleeding was the presence of acute kidney injury (AKI).[3] This effect was independent of Model for End-Stage Liver Disease (MELD) score, sepsis, platelet count, and international normalized ratio (INR).[3]

Chronic kidney disease (CKD) and associated uremia are well-known risk factors for bleeding and thrombosis, and are respectively associated with platelet dysfunction, increased activation of coagulation, and impaired fibrinolysis.[4-6] Recently, similar hemostatic alterations have been described in patients with AKI without chronic liver disease.[7-10]

AKI is a relatively common complication of decompensated cirrhosis, occurring in approximately 20% of hospitalized patients,[11] and may be associated with an increased risk of bleeding.[3,12,13] However, the effect of AKI on the hemostatic system in patients with cirrhosis has not yet been fully investigated.

Understanding the factors responsible for the purported increased bleeding tendency in patients with cirrhosis and AKI, and investigating whether there may also be an increased clotting tendency, may have implications for the prevention and treatment of these potentially life-threatening complications. The goal of our prospective study was to assess all three aspects of hemostasis (platelet function, coagulation, and fibrinolysis) in hospitalized patients with decompensated cirrhosis and AKI.

Materials and Methods

PATIENT SELECTION

Adult (>18 years old) patients with decompensated cirrhosis admitted to the medical inpatient services of Yale New Haven Hospital from January 1, 2019, to September 1, 2019, were prospectively screened to determine eligibility to participate in the study.

The diagnosis of cirrhosis was confirmed with available data including histology, radiology, laboratory, and clinical assessment. Decompensation was defined by the presence or history of clinically evident decompensating events (ascites, variceal hemorrhage, and hepatic encephalopathy).[14]

Patients admitted for variceal hemorrhage or who experienced variceal hemorrhage and/or any other major bleeding[15] in the 30 days before admission, those with a diagnosis of acute on chronic liver failure (ACLF)[16] at time of screening, and those who were transferred from intensive care units to the medical services were not considered eligible.

Patients with ACLF were excluded because ACLF is a specific syndrome in which inflammation plays a predominant role and is associated with distinct hemostatic features.[16,17] Patients admitted to the intensive care unit were excluded because they are more severe and unstable and more likely to have ACLF compared with patients admitted to the medical floor, and because they are more frequently treated with drugs that interfere with hemostasis.

At screening, patients' medical records, past medical history, and laboratory data were reviewed for the following exclusion criteria: CKD, presence and/or history of portal vein thrombosis and/or venous thromboembolism, presence of extrahepatic tumors or known hematologic diseases, recent major surgery (within 1 month), human immunodeficiency virus infection, and history of any organ transplantation (including liver).

Patients on therapeutic anticoagulation and/or antithrombotic (acetylsalicylic acid or P2Y12 inhibitors) and/ or antifibrinolytic therapy, and those who received transfusion of platelets, cryoprecipitate, or fresh frozen plasma in the 3 days before screening were also excluded (these patients were excluded to avoid the potential interference of blood product transfusions on hemostasis testing).

Following admission to the inpatient service and having determined eligibility, patients were categorized into cases (with AKI) and controls (without AKI).

AKI was defined according to the International Club of Ascites as an increase in serum creatinine of greater than or equal to 0.3 mg/dL within 48 hours or a 50% increase within 7 days from baseline serum creatinine.[18] For patients who were admitted with AKI, baseline serum creatinine was obtained from the medical records within the previous 3 months, and the most recent stable value was considered to be baseline; for patients who developed AKI during hospitalization, baseline serum creatinine was the one obtained at admission.

A third group of hospitalized patients with AKI but without liver disease was included as controls, and they were compared with a group of healthy subjects. The Yale University Research Joint Data Analytics Team provided the research team a daily list of all inpatients with a new diagnosis of AKI.[18] Patients' medical records, past medical history, and previous laboratory data were reviewed to apply the same exclusion criteria used in patients with cirrhosis plus the presence of any signs (clinical, biochemical, or imaging) and/or history of liver disease.

STUDY DESIGN

This was a prospective, single-center, cohort study, approved by the Yale Human Investigation Committee (#2000024288). The study was conducted in compliance with the Declaration of Helsinki, and all patients gave written informed consent before enrollment.

Patients with cirrhosis and AKI were recruited within 24 hours of the diagnosis of AKI, both when the AKI was present at admission and when the AKI developed during hospitalization. Evaluation of hemostasis was performed twice: at enrollment, and on the day after AKI resolution, as defined by a return of serum creatinine to a level within 0.3 mg/dL from baseline. If the patient was treated with renal replacement therapy, the second evaluation was not performed. In cases of transfusion of platelets, fresh frozen plasma, or cryoprecipitate, we waited 3 days before performing the second hemostasis study. In patients with cirrhosis without AKI, the evaluation of hemostasis was performed once, at enrollment. Patients with AKI but without liver disease were recruited within 24 hours of the diagnosis of AKI, and evaluation of hemostasis was performed once, at enrollment.

SAMPLE COLLECTION AND HEMOSTASIS ASSESSMENT

Blood Sampling—See Supporting Information.

Hemostasis Assessment—Hemostasis assessment included primary hemostasis, secondary hemostasis, and tertiary hemostasis.

Primary hemostasis (platelets) was assessed by measuring platelet aggregation and secretion (markers of platelet function) by whole-blood lumiaggregometry (Chronolog Model 700) and von Willebrand factor (platelet adhesive glycoprotein), both antigen (VWF:Ag) and function (ristocetin cofactor activity [VWF:RCo] and collagen binding activity [VWF:CB]).[19,20]

Secondary hemostasis (coagulation) was assessed by measuring procoagulant factor VIII (FVIII), fibrin-stabilizing factor XIII (FXIII), natural anticoagulants (protein C chromogenic and coagulometric [PC], protein S [PS], and antithrombin [AT]), as well as thrombin generation assay (TGA) with and without thrombomodulin (TM).[21,22] Thrombin-antithrombin complex (TAT) was determined as a marker of coagulation activation.

Tertiary hemostasis (fibrinolysis) was assessed by measuring plasminogen, tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), α2-antiplasmin (α2-AP), and activated-inactivated thrombin-activatable fibrinolytic inhibitor (TAFIa/ai).

TAFIa/ai represents the amount of TAFI that has been activated. Plasmin-antiplasmin complex (PAP) was determined as a marker of fibrinolysis activation.

See Supporting Information for more information.

DATA COLLECTION

Data collected from the medical records included causes for admission, patient demographics, presence or absence of infection at the time of AKI, and laboratory data. Thrombocytopenia was defined as platelet count $<150 \times 10^9$/L, and subclassified as mild (100×10^9/L to 150×10^9/L), moderate (50×10^9/L to 100×10^9/L), or severe ($<50 \times 10^9$/L).

DATA ANALYSIS

Study Objective—The primary objective of this study was to compare primary hemostasis, secondary hemostasis, and tertiary hemostasis in patients with decompensated cirrhosis with versus without AKI.

Because the measurement of platelet aggregation and secretion by whole-blood lumiaggregometry depends on platelet count, this comparison was performed at two levels: overall, and according to the severity of thrombocytopenia. This adjustment was performed to better ascertain the impact of AKI on platelet function.

Infections, a common precipitant of AKI, may also affect hemostasis; therefore, we performed a *post hoc* analysis looking only at patients whose AKI was not driven by infection.

Sample-Size Determination—See Supporting Information.

Statistical Analysis—See Supporting Information.

Results

DEMOGRAPHICS

Eighty patients with decompensated cirrhosis were recruited (40 each with and without AKI) (Fig. 1). Baseline demographics and severity of cirrhosis by means of Child-Pugh score were comparable between the two groups (Table 1). Abdominal pain or suspected infection, ascites, and hepatic encephalopathy accounted for 60%-85% of admissions. MELD score was significantly higher in patients with AKI than in those without AKI (25 vs. 18, respectively), due solely to differences in serum creatinine (1.8 vs. 0.8 mg/dL, respectively). Indeed, bilirubin (2.9 vs. 2.6 mg/dL) and INR (1.7 vs. 1.5) were comparable between the study groups. Bacterial infections were more frequent in patients with AKI (47% vs. 23%), and spontaneous bacterial peritonitis was the most common (30%) infection in both groups.

Prerenal azotemia was the most frequent (60%) cause of AKI, followed by hepatorenal syndrome (20%) and acute tubular necrosis (20%). Half of the patients had AKI stage II, with a median creatinine level of 1.8 mg/dL (interquartile range [IQR] 1.6-2.5).

Twenty-three (57.5%) patients with cirrhosis and AKI had repeat assessments at AKI resolution (Fig. 1). The median duration of AKI was 5 days (IQR 4-8), but it ranged widely according to AKI etiology (5 days [IQR 4-7] in prerenal azotemia, 16 days [IQR 12-19] in hepatorenal syndrome, and 8 days [IQR 6-13] in acute tubular necrosis). At AKI resolution, the median MELD score significantly decreased, such that it became comparable to patients with cirrhosis without AKI (17 vs. 18, respectively).

Ten inpatients with AKI but without liver disease were also recruited (male/female 3/7, median age 54 years [IQR: 45-69]) as controls. Reasons for admission were abdominal pain (n = 3), hypertensive crisis (n = 2), chronic obstructive pulmonary disease exacerbation (n = 3), bilateral leg edema (n = 1), and fall with shoulder trauma (n = 1). Median platelet count was 231×10^9/L (IQR: 191-303) in these patients. Prerenal azotemia and acute tubular necrosis were the etiology of AKI in 70% and 30% of the patients, respectively. Most patients (60%) had AKI stage II, with creatinine level similar to that of patients with cirrhosis (1.8 mg/dL [IQR 1.6-2]).

BASELINE SAMPLE COLLECTION

In patients with cirrhosis, baseline samples were collected at or near admission (median time 1.5 days [IQR 1-4] vs. 2 days [IQR 1-3] in patients with and without AKI, respectively [$P = 0.9$]). None of the patients received platelet transfusion before baseline sample collection. Among patients with AKI, 80% (32 of 40) had AKI at admission, while the remaining 20% developed AKI during hospitalization with a median time from admission to blood draw of 5 days (range 3-7). None of the patients with cirrhosis and AKI was on renal replacement therapy at the time of blood draw.

PRIMARY HEMOSTASIS IN PATIENTS WITH DECOMPENSATED CIRRHOSIS: AKI IS ASSOCIATED WITH REVERSIBLE PLATELET DYSFUNCTION AFFECTING BOTH AGGREGATION AND SECRETION

As indicated in Table 1, platelet count was comparable between patients with and without AKI (79×10^9/L vs. 66×10^9/L, respectively). Nearly all were thrombocytopenic, with most having moderate thrombocytopenia (Table 1).

At baseline, patients with cirrhosis and AKI had a significantly more altered platelet function than patients with cirrhosis without AKI (Fig. 2). Collagen-induced aggregation, and all agonist-induced secretion, were significantly lower in patients with AKI. In contrast, adenosine diphosphate (ADP)–induced aggregation was comparable between the two groups (Table 2). The reduction of platelet function in the group of patients with cirrhosis and AKI was consistent with the overall findings noted previously in all three subclasses of thrombocytopenia (Supporting Table S1).

Baseline VWF:Ag, VWF:RCo, and VWF:CB were comparable between patients with and without AKI (Table 2).

Resolution of AKI was associated with significant improvement in both platelet aggregation and secretion, regardless of the severity of thrombocytopenia (Fig. 3). Indeed, platelet aggregation and secretion at resolution became comparable to baseline values in patients with cirrhosis without AKI at all severities of thrombocytopenia (Supporting Table S1).

In contrast, VWF:Ag, VWF:RCo, and VWF:CB remained unchanged (Supporting Table S2).

SECONDARY HEMOSTASIS IN PATIENTS WITH DECOMPENSATED CIRRHOSIS: AKI IS ASSOCIATED WITH HIGH FVIII, LOW PC, PS AND AT, INCREASED ENDOGENOUS THROMBIN POTENTIAL RATIO (HYPERCOAGULABLE FEATURES), AND WITH LOW FXIII (HYPOCOAGULABLE FEATURE)

At baseline, patients with cirrhosis and AKI had higher FVIII and lower FXIII, PC, PS, and AT, respectively, compared to patients with cirrhosis without AKI (Table 2).

Endogenous thrombin potential (ETP) by TGA was comparable among patients with cirrhosis with AKI, patients with cirrhosis without AKI, and healthy controls (Table 2 and Supporting Table S3). The addition of TM significantly reduced the ETP in healthy controls but not in patients with cirrhosis (Table 2 and Supporting Table S3). Indeed, the ETP ratio was significantly higher in patients with cirrhosis than in healthy controls (Fig. 4). Among patients with cirrhosis, the ETP ratio was significantly higher in those with versus without AKI ($P < 0.001$) (Fig. 4). Other TGA parameters were comparable between the two groups (Supporting Table S3).

After AKI resolution, FVIII decreased and FXIII, PC, PS, and AT all increased (Supporting Table S2). Indeed, both procoagulant and anticoagulant factors became comparable to values obtained in patients with cirrhosis without AKI.

Similarly, the ETP ratio tended to decrease and became comparable to that in patients with cirrhosis without AKI (Supporting Table S2).

Baseline TAT was higher in patients with cirrhosis with versus without AKI (Table 2). Resolution of AKI was associated with a significant decrease in TAT level, which became comparable to the baseline value in patients with cirrhosis without AKI (Fig. 5).

TERTIARY HEMOSTASIS IN PATIENTS WITH DECOMPENSATED CIRRHOSIS: AKI IS ASSOCIATED WITH MIXED HYPOFIBRINOLYTIC AND HYPERFIBRINOLYTIC ALTERATIONS

At baseline, patients with cirrhosis and AKI showed a significantly lower level of plasminogen and α2-AP than patients with cirrhosis without AKI. Conversely, t-PA and TAFIa/ai were significantly increased in patients with AKI. PAI-1 was not different between groups (Table 2).

After AKI resolution, plasminogen and α2-AP increased, TAFIa/ai decreased, and PAI-1 and t-PA remained unchanged (Supporting Table S2).

Baseline PAP was higher in patients with cirrhosis with versus without AKI (Table 2). Resolution of AKI was associated with a further increase in PAP level, which remained

significantly higher compared to baseline value in patients with cirrhosis without AKI (Fig. 5).

PRIMARY HEMOSTASIS IN PATIENTS WITH AKI WITHOUT LIVER DISEASE

Compared to healthy controls, patients with AKI without liver disease had lower platelet aggregation and secretion (Supporting Table S4). VWF:Ag, VWF:RCo, VWF:CB were higher than the normal reference range (Supporting Table S5).

SECONDARY HEMOSTASIS IN PATIENTS WITH AKI WITHOUT LIVER DISEASE

Compared to healthy controls, patients with AKI without liver disease had higher FVIII and PC. FXIII, PS, and AT were normal (Supporting Table S5). The ETP ratio was significantly higher compared with that in healthy subjects (Fig. 4).

Level of TAT was comparable between patients with AKI without liver disease and healthy subjects (2.5 ng/mL [IQR 2-2.7] vs. 2.5 ng/mL [IQR 1.9-3.3]; $P = 0.9$).

TERTIARY HEMOSTASIS IN PATIENTS WITH AKI WITHOUT LIVER DISEASE

Compared to healthy controls, patients with AKI without liver disease had higher levels of α2-AP, t-PA, and TAFIa/ai. No difference was found in plasminogen and PAI-1 (Supporting Table S5).

The level of PAP was significantly lower in patients with AKI without liver disease than in healthy subjects (39 ng/mL [37-40] vs. 48 ng/mL [42-62]; $P < 0.001$]).

POST HOC ANALYSES OF HEMOSTATIC ALTERATIONS IN PATIENTS WITH CIRRHOSIS WITHOUT BACTERIAL INFECTION

Similar to the overall analysis, noninfected patients with cirrhosis and AKI (n = 21) showed lower platelet aggregation and secretion than noninfected patients without AKI (n = 31) (Supporting Table S6). Resolution of AKI was similarly associated with a return to the level observed in patients with cirrhosis without AKI. Differences in secondary hemostasis and fibrinolysis between noninfected patients with and without AKI were similar to those observed in the overall analysis (Supporting Table S6).

Discussion

Hospitalized patients with decompensated cirrhosis and AKI may be at increased risk of bleeding,[3,12,13] but the hemostatic alterations that contribute to this purported increased bleeding tendency have not been thoroughly investigated. In patients without liver disease, platelet dysfunction has been proposed as the main factor for increased bleeding tendency in patients with uremia with CKD[4] and, although less well studied, perhaps also in patients with AKI.[9] While awaiting larger prospective studies to confirm the association between AKI and increased bleeding risk in patients with decompensated cirrhosis, we performed a study to investigate all aspects of hemostasis in this patient population. Because hemostasis in cirrhosis, particularly in the patients with decompensated cirrhosis, is complex

and multiple factors may be responsible for the bleeding tendency in AKI, we included determination not only of platelet function but also of coagulation and fibrinolysis.

This study demonstrates, in hospitalized patients with decompensated cirrhosis, that AKI is associated with a profound hemostatic derangement that includes both prohemorrhagic (platelet dysfunction, low FXIII, and hyperfibrinolytic alterations) and prothrombotic (high FVIII, low anticoagulants PC, PS and AT, and hypofibrinolytic defects) changes.

These AKI-driven hemostatic alterations may easily disrupt the precarious hemostatic status of patients with decompensated cirrhosis toward either hypocoagulability or hypercoagulability, thus increasing the risk of bleeding and/or thrombosis (Fig. 6).

The primary hemostasis assessment revealed that patients with cirrhosis and AKI have a significantly lower platelet aggregation and secretion compared to patients with cirrhosis and normal renal function. As platelet dysfunction is known to increase the risk of bleeding, these results support the association between AKI and postparacentesis bleeding in patients with cirrhosis.[3,12,13] The resolution of platelet abnormalities to levels observed in patients with decompensated cirrhosis and normal renal function is strong evidence that AKI is responsible for this platelet dysfunction and is akin to the observed platelet-dysfunction reversal observed in patients with CKD after dialysis.[23]

In vivo, in case of vessel injury, platelets adhere to subendothelial collagen, where they aggregate, leading to thrombus formation and the secretion of intraplatelet granules that contain substances (ADP, adenosine triphosphate) that bind to specific receptors on the platelet membrane and further enhance aggregation. Lumiaggregometry, the method we used to assess platelet aggregation and secretion, mimics *in vivo* blood conditions.

However, because this assessment depends on the platelet count, one cannot compare patients with thrombocytopenia to patients with normal platelet count.[24] To avoid this pitfall, we used a control group of patients with cirrhosis without AKI, and we matched cases and controls by severity of thrombocytopenia. Remarkably, we found that impaired platelet function occurred, not only in the overall group, but also at all levels of thrombocytopenia.

Regarding platelet aggregation, significant differences were found mostly in its response to collagen (being lower in patients with AKI), but not as much in its response to ADP. Common to all platelet stimuli is their binding to specific receptors on cell surface. The reduction in collagen-induced aggregation in the presence of an unaffected ADP-induced aggregation suggests that AKI selectively interferes with the collagen pathway.

On the other hand, platelet secretion was significantly lower in patients with AKI, independent of the agonist used. This suggests that AKI does not interfere with a single pathway, but is acting downstream in the secretion process. It has been proposed that platelets in cirrhosis appear to have less granules.[25] Therefore, with AKI it may be that the effects on secretion itself may be compounded on an already low content of granules, making the defects in secretion more profound and uniform.

The main function of VWF, a glycoprotein released by endothelial cells, is to facilitate the adhesion of platelets to subendothelial collagen. In cirrhosis, VWF is elevated due to shear stress and inflammation.[26] Previous data suggested that the high VWF in cirrhosis might be enough to support primary hemostasis, despite a low platelet count.[27] In our cohort, VWF was comparable between patients with and without AKI and did not change after AKI resolution. Interestingly, the evolution of platelet function with AKI (impaired at baseline and improved with resolution) indicates that the deleterious effect of AKI on platelet function in cirrhosis occurs independently of VWF.

Opposite to our findings regarding platelet function, our secondary hemostasis assessment demonstrates that, in patients with decompensated cirrhosis and AKI, there is activation of coagulation and prothrombotic alterations. The main AKI-driven hyper-coagulable changes were (1) increased FVIII and (2) decreased PC, PS, and AT. As the severity of cirrhosis was comparable between patients with and without AKI, these differences likely reflect an acute phase reaction to AKI.[28-30] Another potential explanation for the lower levels of PC, PS, and AT in patients with AKI may be a transient worsening of liver synthetic function.

The thrombomodulin-modified thrombin generation assay that we used is a well-established research tool in evaluating the clotting process in cirrhosis.[22,31] Previous data show that patients with cirrhosis maintain the capacity of generating thrombin (i.e., clotting) despite a low level of procoagulant factors, as reflected by a high ETP.[32,33] Current theory posits that the decreased level of procoagulant factors is rebalanced by the decreased hepatic production of anticoagulant factors (PC, PS, and AT) and by an increased level of procoagulant factors synthesized outside the liver (FVIII).[34] In fact, we show that thrombin-generating capacity is preserved in patients with cirrhosis and is not reduced by the addition of thrombomodulin (a protein C activator). Interestingly, when comparing patients with cirrhosis with versus without AKI, we found an increased ETP ratio in those with AKI, possibly indicative of a more pronounced hypercoagulable state.[35] With resolution of AKI, we observed a tendency for FVIII to decrease and for PC, PS, and AT to increase, but not quite to the levels observed in patients without AKI. The coagulation re-assessment at AKI resolution was performed on the very first day after normalization of creatinine, which is probably too short a time for a complete resolution of an acute phase reaction. However, the fact that with resolution of AKI, the ETP ratio decreased and became comparable to that in patients with cirrhosis and normal renal function is further evidence that AKI is responsible for the observed alterations in secondary hemostasis.

Further prospective studies are needed to validate the hypothesis that AKI-driven hypercoagulable changes, as detected in our study, are risk factors for thromboembolic complications in hospitalized patients with decompensated cirrhosis.

In the only previous study that evaluated coagulation in patients with cirrhosis with versus without AKI, the only significant difference was a lower FXIII in AKI,[36] which we confirm in our cohort. The mechanisms leading to lower FXIII in AKI may encompass transient worsening of liver function, accelerated catabolism of FXIII, a presumed effect of AKI on platelets/megakaryocytes, or a combination of these factors. Indeed, we observed a trend toward improvement in FXIII with AKI resolution. On the other hand, and in contrast

to our results, FVIII and PC were not different between patients with and without AKI.[36] While Intagliata et al included both patients with decompensated cirrhosis and ACLF, we excluded those with ACLF. Because ACLF is an inflammatory syndrome with specific hemostatic characteristics,[16,17] it may be that the procoagulant effect of AKI in patients with ACLF is less profound than in patients with decompensated cirrhosis. To test this hypothesis, further studies should look specifically at the impact of AKI in patients with ACLF.

For fibrinolysis analysis, our data demonstrate that AKI in patients with decompensated cirrhosis is associated with mixed hypofibrinolytic (low plasminogen and increased TAFIa/ai) and hyperfibrinolytic (low antiplasmin and increased t-PA) changes.

In patients without liver disease, AKI has been associated with a hypofibrinolytic status.[9,10,37,38] This is confirmed by our findings in hospitalized patients with AKI without liver disease, in whom the level of PAP, a marker of fibrinolysis activation, was significantly lower than in healthy subjects. As decompensated cirrhosis is associated with profound alterations in the fibrinolytic system,[39-41] it may be that the superimposed effect of AKI in patients with cirrhosis is less uniform than that in patients without liver disease, thus explaining the coexistence of hypofibrinolytic and hyperfibrinolytic changes.

However, the increased level of PAP in patients with cirrhosis and AKI compared to controls with cirrhosis and normal renal function would suggest that the overall effect of AKI-driven fibrinolytic changes is a relatively hyperfibrinolytic status. Because our analysis of single protein levels overlooks regulatory interactions and cellular contributions, this hypothesis needs to be validated by global fibrinolytic assays. Interestingly, and contrary to our findings in primary and secondary hemostasis, resolution of AKI was associated with a further increase in PAP level, indicating worsening of this hyperfibrinolytic state beyond normalization of renal function.

To analyze whether the effect of AKI on hemostasis was specific to cirrhosis, we included a control group of patients with AKI but without liver disease. Compared with healthy controls, these patients showed lower platelet function, increased thrombin-generating capacity, and lower fibrinolysis activation, in line with previous findings.[9,10] Although these groups are not completely comparable (hospitalized patients vs. healthy subjects), these results confirm that AKI interferes with hemostasis in any setting, and not only in patients with cirrhosis.

In our study, infections were more frequent in patients with AKI, and the association between bacterial infections and alterations in hemostasis in cirrhosis is well known.[42,43] By analyzing separately patients without infections (with and without AKI) and finding the same abnormalities, we could demonstrate that the effect of AKI on hemostasis is independent of infection. However, this requires further validation, as the number of patients in this subanalysis was relatively low.

Despite this comprehensive analysis, our study has some limitations. As in any study of hemostasis, the lack of interplay between blood components and the vessel wall is unavoidable. Drugs could have interfered with hemostasis, but this effect should have been

balanced by the use of the same inclusion criteria for both groups and the exclusion of other confounders such as ACLF, CKD, bleeding, and thrombosis.

In conclusion, in a prospective study of hospitalized patients with decompensated cirrhosis, we demonstrate that AKI is associated with complex hemostatic changes, including both prohemorrhagic and prothrombotic features. On the one hand, AKI was associated with platelet dysfunction, low FXIII, and hyperfibrinolytic alterations (increased bleeding tendency), while on the other hand AKI was associated with increased FVIII, reduced anticoagulants, and hypofibrinolytic defects (increased thrombotic tendency). Optimization of renal function in patients with decompensated cirrhosis and AKI may help restore the hemostatic rebalance and mitigate such risks. However, part of the AKI-driven hemostatic alterations appear to persist after normalization of serum creatinine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by the American Association for the Study of the Liver Foundation Clinical & Translation Research Fellowship Award (to A.Z.), Yale Liver Center National Institutes of Health grant (P30 DK34989 to G.G.T.), and a research grant from the Italian Ministry of Education, University and Research (to P.S.).

Abbreviations:

ACLF	acute on chronic liver failure
ADP	adenosine diphosphate
AKI	acute kidney injury
AT	antithrombin
CKD	chronic kidney disease
ETP	endogenous thrombin potential
FVIII	factor VIII
FXIII	factor XIII
INR	international normalized ratio
IQR	interquartile range
MELD	Model for End-Stage Liver Disease
PAI-1	plasminogen activator inhibitor-1
PAP	plasmin-antiplasmin complex
PC	protein C

PS	protein S
t-PA	tissue-type plasminogen activator
TAFIa/ai	activated-inactivated thrombin activatable fibrinolysis inhibitor
TAT	thrombin-antithrombin complex
TGA	thrombin generation assay
TM	thrombomodulin
VWF:Ag	von Willebrand factor antigen
VWF:CB	von Willebrand factor collagen binding activity
VWF:RCo	von Willebrand factor ristocetin cofactor activity
α2-AP	α2-antiplasmin

REFERENCES

1). Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med 2011;365:147–156. [PubMed: 21751907]

2). Intagliata NM, Argo CK, Stine JG, Lisman T, Caldwell SH, Violi F. Concepts and controversies in haemostasis and thrombosis associated with liver disease: proceedings of the 7th International Coagulation in Liver Disease Conference. Thromb Haemost 2018;118:1491–1506. [PubMed: 30060258]

3). 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;38:1437–1441. [PubMed: 29393567]

4). Gackler A, Rohn H, Lisman T, Benkö T, Witzke O, Kribben A, et al. Evaluation of hemostasis in patients with end-stage renal disease. PLoS One 2019;14:e0212237. [PubMed: 30785941]

5). Mortberg J, Blomback M, Wallen A, He S, Jacobson SH, Spaak J. Increased fibrin formation and impaired fibrinolytic capacity in severe chronic kidney disease. Blood Coagul Fibrinolysis 2016;27:401–407. [PubMed: 26650459]

6). Rabelink TJ, Zwaginga JJ, Koomans HA, Sixma JJ. Thrombosis and hemostasis in renal disease. Kidney Int 1994;46:287–296. [PubMed: 7967339]

7). 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;84:158–163. [PubMed: 23515053]

8). Huang MJ, Wei RB, Su TY, Wang Y, Li QP, Yang Xi, et al. Impact of acute kidney injury on coagulation in adult minimal change nephropathy. Medicine (Baltimore) 2016;95:e5366. [PubMed: 27861367]

9). Malyszko J, Malyszko JS, Pawlak D, Pawlak K, Buczko W, Mysliwiec M. Hemostasis, platelet function and serotonin in acute and chronic renal failure. Thromb Res 1996;83:351–361. [PubMed: 8873344]

10). Larsson SO, Hedner U, Nilsson IM. On coagulation and fibrinolysis in acute renal insufficiency. Acta Med Scand 1971;189:443–451. [PubMed: 5113860]

11). Garcia-Tsao G, Parikh CR, Viola A. Acute kidney injury in cirrhosis. HEPATOLOGY 2008;48:2064–2077. [PubMed: 19003880]

12). McVay PA, Toy PT. Lack of increased bleeding after paracentesis and thoracentesis in patients with mild coagulation abnormalities. Transfusion 1991;31:164–171. [PubMed: 1996485]

13). De Pietri L, Bianchini M, Montalti R, De Maria N, Di Maira T, Begliomini B, et al. Thrombelastography-guided blood product use before invasive procedures in cirrhosis with

severe coagulopathy: a randomized, controlled trial. HEPATOLOGY 2016;63:566–573. [PubMed: 26340411]

14). Garcia-Tsao G, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. HEPATOLOGY 2017;65:310–335. [PubMed: 27786365]

15). Schulman S, Kearon C, Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost 2005;3:692–694. [PubMed: 15842354]

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;144:1426–1437, e1421–1429. [PubMed: 23474284]

17). 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. [PubMed: 28843665]

18). 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;64:531–537. [PubMed: 25631669]

19). Dyszkiewicz-Korpanty AM, Frenkel EP, Sarode R. Approach to the assessment of platelet function: comparison between optical-based platelet-rich plasma and impedance-based whole blood platelet aggregation methods. Clin Appl Thromb Hemost 2005;11:25–35. [PubMed: 15678270]

20). Verfaillie CJ, De Witte E, Devreese KM. Validation of a new panel of automated chemiluminescence assays for von Willebrand factor antigen and activity in the screening for von Willebrand disease. Int J Lab Hematol 2013;35:555–565. [PubMed: 23551532]

21). Campello E, Zabeo E, Radu CM, Spiezia L, Gavasso S, Fadin M, et al. Hypercoagulability in overweight and obese subjects who are asymptomatic for thrombotic events. Thromb Haemost 2015;113:85–96. [PubMed: 25318550]

22). Russo FP, Zanetto A, Campello E, Bulato C, Shalaby S, Spiezia L, et al. Reversal of hypercoagulability in patients with HCV-related cirrhosis after treatment with direct-acting antivirals. Liver Int 2018;38:2210–2218. [PubMed: 29738632]

23). Arends JP, Krediet RT, Boeschoten EW, van der Lelie J, Veenhof CH, von dem Borne AE, et al. Improvement of bleeding time, platelet aggregation and platelet count during CAPD treatment. Proc Eur Dial Transplant Assoc 1981;18:280–285. [PubMed: 7329972]

24). Violi F, Basili S, Raparelli V, Chowdary P, Gatt A, Burroughs AK. Patients with liver cirrhosis suffer from primary haemostatic defects? Fact or fiction? J Hepatol 2011;55:1415–1427. [PubMed: 21718668]

25). Laffi G, Marra F, Gresele P, Romagnoli P, Palermo A, Bartolini O, et al. Evidence for a storage pool defect in platelets from cirrhotic patients with defective aggregation. Gastroenterology 1992;103:641–646. [PubMed: 1386051]

26). La Mura V, Reverter JC, Flores-Arroyo A, Raffa S, Reverter E, Seijo S, et al. Von Willebrand factor levels predict clinical outcome in patients with cirrhosis and portal hypertension. Gut 2011;60:1133–1138. [PubMed: 21427197]

27). Lisman T, Bongers TN, Adelmeijer J, Janssen HLA, de Maat MPM, de Groot PG, et al. Elevated levels of von Willebrand factor in cirrhosis support platelet adhesion despite reduced functional capacity. HEPATOLOGY 2006;44:53–61. [PubMed: 16799972]

28). Begbie M, Notley C, Tinlin S, Sawyer L, Lillicrap D. The factor VIII acute phase response requires the participation of NFkappaB and C/EBP. Thromb Haemost 2000;84:216–222. [PubMed: 10959692]

29). Niessen RW, Lamping RJ, Jansen PM, Prins MH, Peters M, Taylor FB, et al. Antithrombin acts as a negative acute phase protein as established with studies on HepG2 cells and in baboons. Thromb Haemost 1997;78:1088–1092. [PubMed: 9308758]

30). Vigano S, Mannucci PM, D'Angelo A, Gelfi C, Gensini GF, Rostagno C, et al. Protein C antigen is not an acute phase reactant and is often high in ischemic heart disease and diabetes. Thromb Haemost 1984;52:263–266. [PubMed: 6549416]

31). Senzolo M, Rodriguez-Castro KI, Rossetto V, Radu C, Gavasso S, Carraro P, et al. Increased anticoagulant response to low-molecular-weight heparin in plasma from patients with advanced cirrhosis. J Thromb Haemost 2012;10:1823–1829. [PubMed: 22712870]

32). Lisman T, Bakhtiari K, Pereboom IT, Hendriks HGD, Meijers JCM, Porte RJ. Normal to increased thrombin generation in patients undergoing liver transplantation despite prolonged conventional coagulation tests. J Hepatol 2010;52:355–361. [PubMed: 20132999]

33). 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;41:553–558. [PubMed: 15726661]

34). Zermatten MG, Fraga M, Moradpour D, Bertaggia Calderara D, Aliotta A, Stirnimann G, et al. Haemostatic alterations in cirrhotic patients: from primary haemostasis to fibrinolysis. HEPATOLOGY 2020;71:2135–2148. [PubMed: 32090357]

35). Tripodi A Detection of procoagulant imbalance. Modified endogenous thrombin potential with results expressed as ratio of values with-to-without thrombomodulin. Thromb Haemost 2017;117:830–836. [PubMed: 28229158]

36). Intagliata NM, Davis JPE, Lafond J, Erdbruegger U, Greenberg CS, Northup PG, et al. Acute kidney injury is associated with low factor XIII in decompensated cirrhosis. Dig Liver Dis 2019;51:1409–1415. [PubMed: 30967339]

37). Kanfer A, Vandewalle A, Beaufils M, Delarue F, Sraer JD. Enhanced antiplasmin activity in acute renal failure. Br Med J 1975;4:195–197. [PubMed: 1191995]

38). Seitz R, Karges HE, Wolf M, Egbring R. Reduced fibrinolytic capacity and its restoration by plasminogen substitution in acute renal failure. Int J Tissue React 1989;11:39–46. [PubMed: 2530187]

39). Lisman T, Leebeek FW, Mosnier LO, Bouma BN, Meijers JCM, Janssen HLA, et al. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. Gastroenterology 2001;121:131–139. [PubMed: 11438502]

40). Rijken DC, Kock EL, Guimaraes AH, Talens S, Murad SD, Janssen HLA, et al. Evidence for an enhanced fibrinolytic capacity in cirrhosis as measured with two different global fibrinolysis tests. J Thromb Haemost 2012;10:2116–2122. [PubMed: 22906184]

41). Bos S, van den Boom B, Kamphuisen PW, Adelmeijer J, Blokzijl H, Schreuder T, et al. Haemostatic profiles are similar across all aetiologies of cirrhosis. Thromb Haemost 2019;119:246–253. [PubMed: 30609442]

42). 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;65:571–581. [PubMed: 27641757]

43). Montalto P, Vlachogiannakos J, Cox DJ, Pastacaldi S, Patch D, Burroughs AK. Bacterial infection in cirrhosis impairs coagulation by a heparin effect: a prospective study. J Hepatol 2002;37:463–470. [PubMed: 12217599]

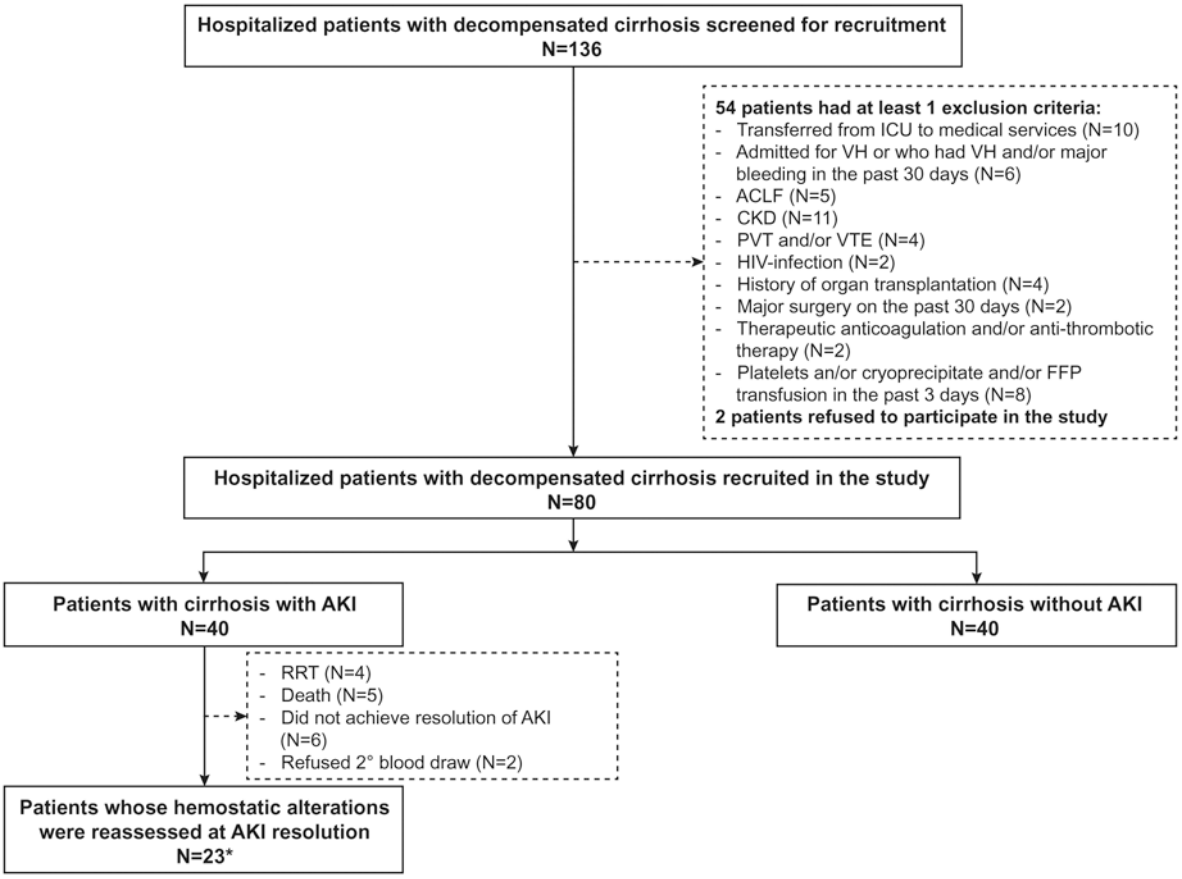

FIG. 1.

Flow chart of the study. *Three patients received platelet transfusion (6, 8, and 9 days) before repeat testing. Abbreviations: FFP, fresh frozen plasma; HIV, human immunodeficiency virus; ICU, intensive care unit; PVT, portal vein thrombosis; RRT, renal replacement therapy; VH, variceal hemorrhage; VTE, venous thromboembolism.

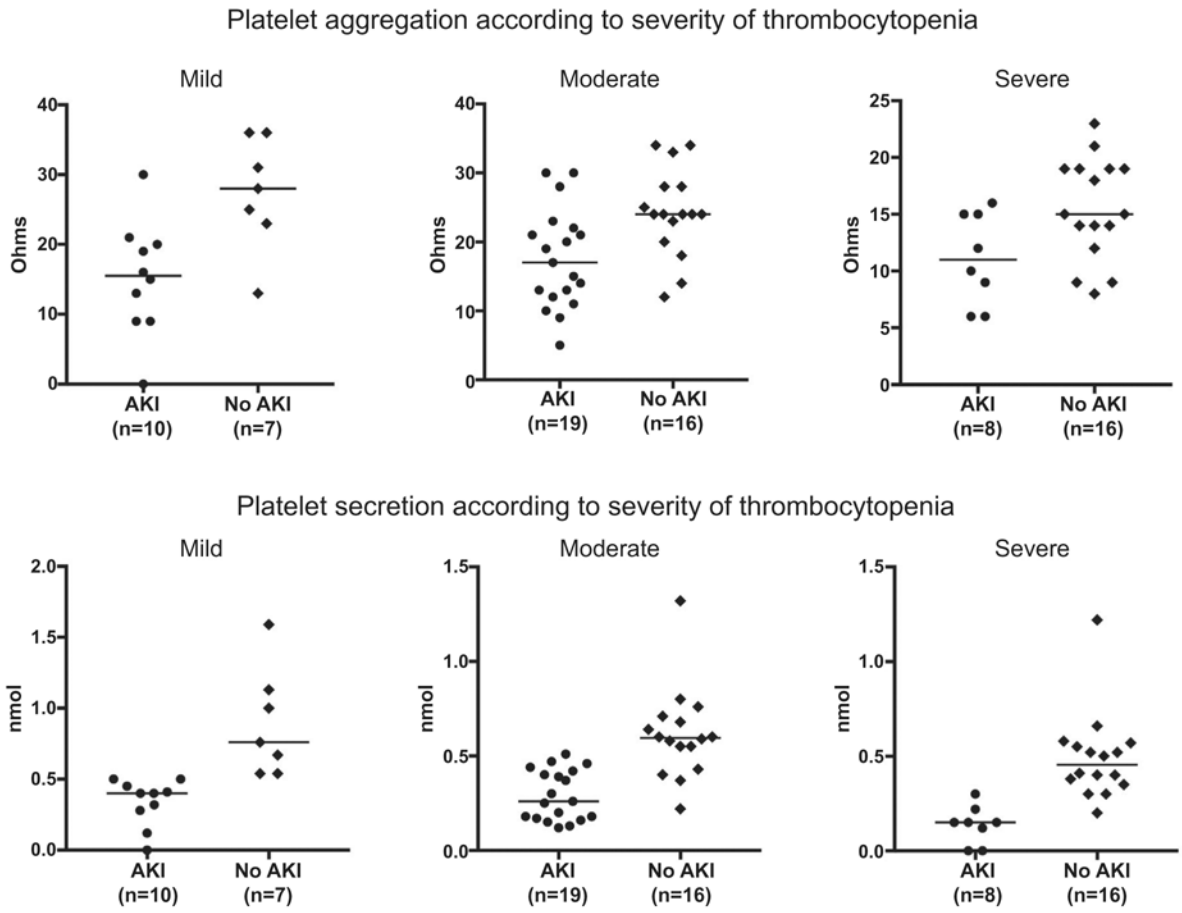

FIG. 2.

According to the severity of thrombocytopenia (mild: 100×10^9/L to 150×10^9/L; moderate: 50×10^9/L to 100×10^9/L; severe: $<50 \times 10^9$/L), both collagen-induced platelet aggregation and thrombin-induced platelet secretion are more altered in patients with cirrhosis with AKI compared to those without AKI. For numerical values, refer to Supporting Table S1.

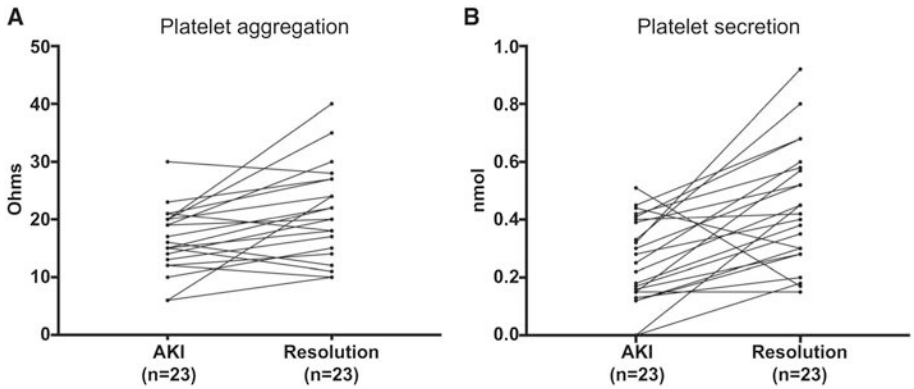

FIG. 3.

After resolution of AKI, platelet function (both platelet aggregation and platelet secretion) improves in patients with cirrhosis and AKI. (A) Collagen-induced aggregation ($P = 0.001$). (B) Thrombin-induced secretion ($P = 0.0002$). Resolution of AKI was defined by a return of serum creatinine to a level within 0.3 mg/dL from baseline.

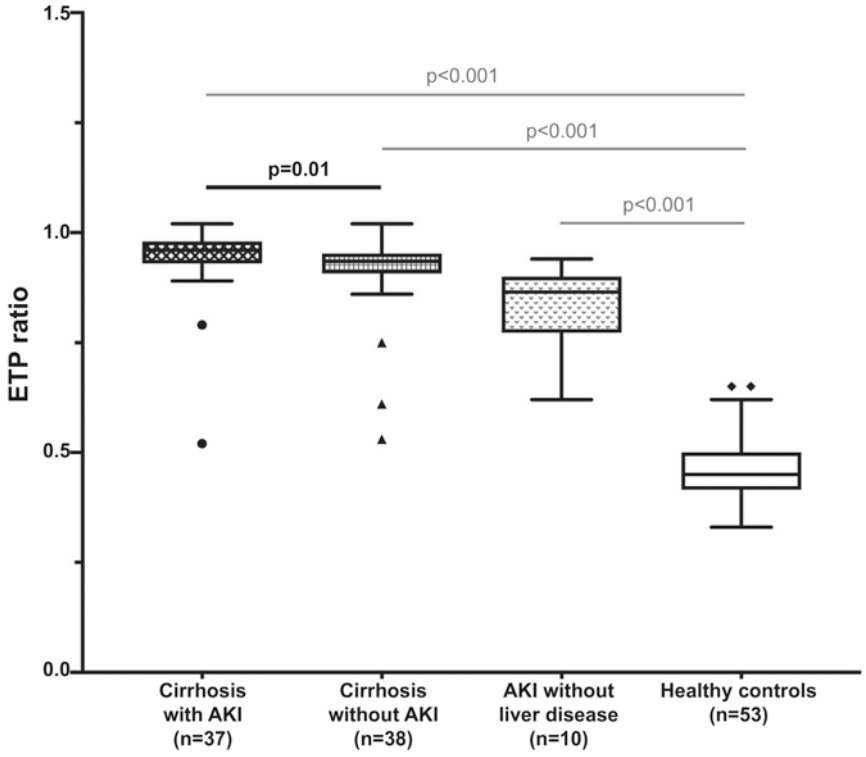

FIG. 4.

Secondary hemostasis, as evidenced by the ETP ratio, was significantly more altered (prothrombotic) in patients with cirrhosis and AKI compared to patients without AKI, AKI without liver disease, and healthy controls.

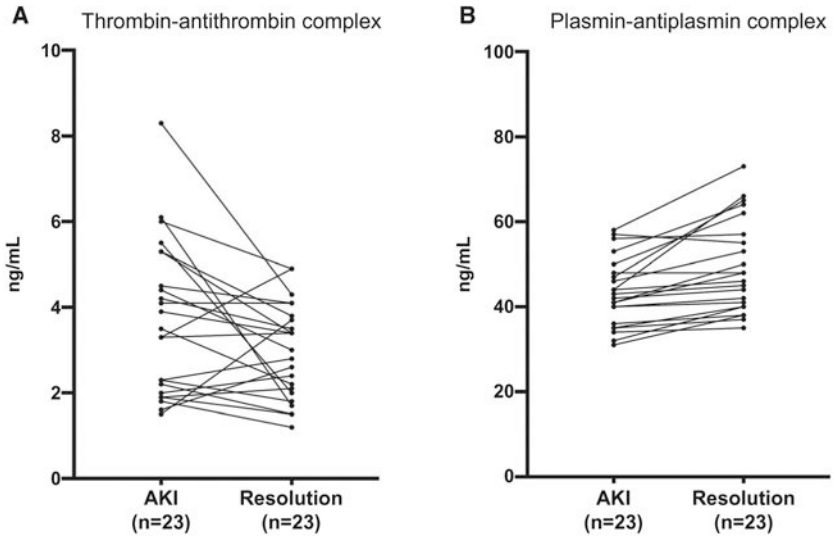

FIG. 5.

(A) Resolution of AKI was associated with decreased activation of coagulation, as evidenced by a decrease in thrombin-antithrombin complex ($P = 0.03$). (B) Resolution of AKI was associated with further fibrinolysis activation, as evidenced by an increase in plasmin-antiplasmin complex ($P < 0.001$). Resolution of AKI was defined by a return of serum creatinine to a level within 0.3 mg/dL from baseline.

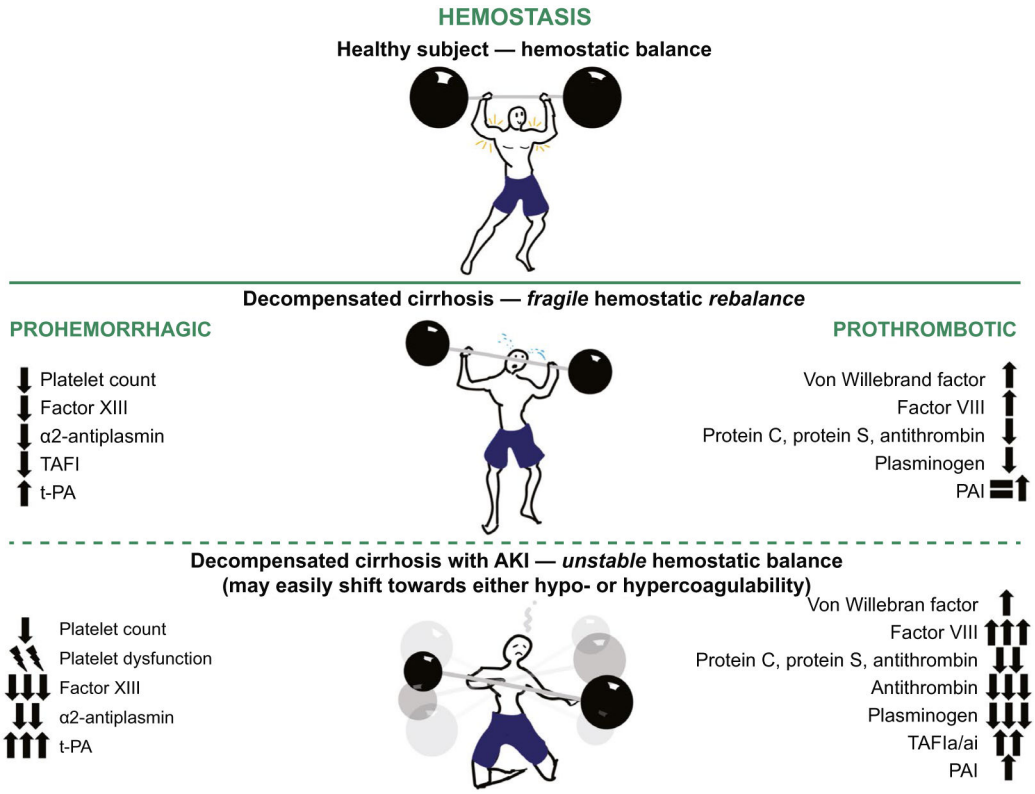

FIG. 6.

Effect of AKI on the hemostatic balance in hospitalized patients with decompensated cirrhosis.

TABLE 1.

Baseline Characteristics in Patients With Decompensated Cirrhosis

	AKI (n = 40)	No AKI (n = 40)
Age, years	56 (52-65)	57 (52-64)
Male gender, %	60	73
Etiology of cirrhosis, %		
Alcohol	53	58
Alcohol + HCV	12 *	7 *
NASH	12	15
HCV	3	15
Other	20	5
Child-Pugh score †	10 (7-13)	10 (7-12)
MELD score	25 (20-29)	18 (11-26)
Ascites, %	83	85
Reason for admission, %		
Abdominal pain/suspected infection	20	25
Ascites	23	30
AMS or HE	20	30
AKI	12	0
Trauma	15	0
Other	10	15
Bacterial infection ‡, %	48	23
VTE prophylaxis, %	47.5	45
Etiology of AKI, %		
Prerenal/HRS/ATN	60/20/20	–
AKI stage, %		
1/2/3	30/50/20	–
Hepatocellular carcinoma, %	10	13
Total bilirubin, mg/dL	2.9 (1.9-4.7)	2.6 (1.5-5.8)
INR	1.7 (1.4-1.8)	1.5 (1.3-1.8)
Albumin, g/dL	3.2 (2.6-3.5)	2.9 (2.4-3.3)
Hemoglobin, g/dL	8 (7.3-9.2)	9.2 (8-11)
Platelet count, 10^9/L	79 (61-127)	66 (48-96)
Thrombocytopenia, %		
Present	93	98
Mild 100-150 × 10^9/L	27	18
Moderate 50-100 × 10^9/L	51	41
Severe <50 × 10^9/L	22	41
BUN, mg/dL	34 (27-46)	10 (8-16)
Creatinine, mg/dL	1.8 (1.6-2.5)	0.8 (0.7-0.9)
Sodium, mmol/L	134 (132-138)	136 (130-138)

	AKI (n = 40)	No AKI (n = 40)
Potassium, mmol/L	4.2 (3.9-4.6)	3.9 (3.6-4.3)
AST, U/L	39 (31-61)	52 (35-65)
ALT, U/L	20 (15-33)	31 (23-45)

Note: Median values are reported with 25th and 75th percentile values in parenthesis.

*Among patients with HCV-related cirrhosis, 2 patients in the AKI group and 3 patients in the no AKI group had positive HCV-RNA.

†Median (range).

‡Bacterial infections included spontaneous bacterial peritonitis (33%), pneumonia (17%), urinary tract infection (6%), sepsis (22%), and other (22%) in the AKI group, and spontaneous bacterial peritonitis (30%), pneumonia (20%), urinary tract infection (10%), sepsis (20%), and other (20%) in the no AKI group, respectively.

Abbreviations: ALT, alanine aminotransferase; AMS, altered mental status; AST, aspartate aminotransferase; ATN, acute tubular necrosis; BUN, blood urea nitrogen; HCV, hepatitis C virus; HE, hepatic encephalopathy; HRS, hepatorenal syndrome; NASH, nonalcoholic steatohepatitis; VTE, venous thromboembolism.

TABLE 2.

Hemostatic Alterations in Patients With Cirrhosis With Versus Without AKI at Baseline

	AKI (n = 40)	No AKI (n = 40)	*P* Value
Primary hemostasis (platelets)			
Platelet aggregation, Ω			
Collagen 1	12 (7-18)	16 (10-22)	0.03
Collagen 5	15 (11-20)	22 (15-27)	0.002
ADP	14 (9-18)	12 (9-18)	0.8
Platelet secretion, nmol			
Thrombin	0.28 (0.20-0.40)	0.57 (0.40-0.70)	<0.001
Collagen 1	0.12 (0.00-0.24)	0.34 (0.21-0.41)	<0.001
Collagen 5	0.22 (0.14-0.33)	0.48 (0.32-0.63)	<0.001
ADP	0.20 (0.10-0.27)	0.38 (0.26-0.61)	<0.001
VWF:Ag, %	375 (314-587)	347 (242-491)	0.1
VWF:RCo, %	355 (275-464)	357 (250-412)	0.7
VWF:CB, %	327 (164-485)	361 (190-429)	0.9
Secondary hemostasis (coagulation)			
FVIII, %	225 (193-266)	181 (155-245)	0.01
FXIII, %	41 (29-59)	54 (41-68)	0.01
PC coagulometric, %	25 (19-41)	30 (22-46)	0.05
PC chromogenic, %	27 (20-44)	31 (23-47)	0.1
PS, %	49 (42-57)	55 (44-64)	0.07
AT, %	28 (23-48)	41 (30-51)	0.005
ETP*, nmol/L *minutes	947.9 (792.8-1134.6)	950.4 (850.3-1157.2)	0.5
ETP + TM*, nmol/L *minutes	891.8 (781.5-1064.4)	915.7 (791.7-1029.0)	0.8
ETP ratio*	0.96 (0.93-0.98)	0.93 (0.90-0.95)	<0.001
TAT, ng/mL	3.4 (2.2-4.5)	2.4 (1.8-3.8)	0.04
Tertiary hemostasis (fibrinolysis)			
Plasminogen, %	25 (20-37)	41 (27-48)	0.001
t-PA, ng/mL	31 (22-44)	22 (15-31)	0.001
PAI-1, ng/mL	24 (17-39)	28 (14-48)	0.7

	AKI (n = 40)	No AKI (n = 40)	*P* Value
α2-AP %	57 (48-74)	74 (58-81)	0.002
TAFIa/ai, ng/mL	27 (20-42)	20 (17-25)	0.001
PAP, ng/mL	44 (39-56)	39 (35-47)	0.04

Note: Median values are reported with 25th and 75th percentile values in parenthesis.

*Thrombin generation was undetectable in 3 patients with AKI and in 2 patients without AKI.