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Minimal effects of acute liver injury/acute liver failure on hemostasis as assessed by thromboelastography

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Background & Aims: Patients with acute liver injury/failure (ALI/ALF) are assumed to have a bleeding diathesis on the basis of elevated INR; however, clinically significant bleeding is rare. We hypothesized that patients with ALI/ALF have normal hemostasis despite elevated INR.

Methods: Fifty-one patients with ALI/ALF were studied prospectively using thromboelastography (TEG), which measures the dynamics and physical properties of clot formation in whole blood. ALI was defined as an INR $\geqslant 1.5$ in a patient with no previous liver disease, and ALF as ALI with hepatic encephalopathy.

Results: Thirty-seven of 51 patients (73%) had ALF and 22 patients (43%) underwent liver transplantation or died. Despite a mean INR of 3.4 ± 1.7 (range 1.5–9.6), mean TEG parameters were normal, and 5 individual TEG parameters were normal in 32 (63%). Low maximum amplitude, the measure of ultimate clot strength, was confined to patients with platelet counts $<126 \times 10^9$/L. Maximum amplitude was higher in patients with ALF than ALI and correlated directly with venous ammonia concentrations and with increasing severity of liver injury assessed by elements of the systemic inflammatory response syndrome. All patients had markedly decreased procoagulant factor V and VII levels, which were proportional to decreases in anticoagulant proteins and inversely proportional to elevated factor VIII levels.

Conclusions: Despite elevated INR, most patients with ALI/ALF maintain normal hemostasis by TEG, the mechanisms of which include an increase in clot strength with increasing severity of liver injury, increased factor VIII levels, and a commensurate decline in pro- and anticoagulant proteins.

Introduction

Acute liver injury and acute liver failure (ALI/ALF) are syndromes defined by "coagulopathy" on the basis of increased prothrombin time (PT)/INR; ALF represents a more severe liver injury resulting in hepatic encephalopathy [1]. Thrombocytopenia frequently accompanies ALI/ALF, although its pathogenesis remains poorly defined [2]. Consequently, patients with ALI/ALF have been assumed to have a bleeding diathesis [3], even though most series report a low incidence of spontaneous, clinically significant bleeding [4]. Although invasive procedures such as intracranial pressure (ICP) monitor placement are also rarely associated with bleeding complications (<5% [5]), coagulation factor and platelet transfusion remain a routine practice despite potential adverse effects [6].

In patients with cirrhosis, who also have thrombocytopenia and elevated INR, a concept of "re-balanced hemostasis" has been proposed to explain the fact that patients rarely bleed outside of the consequences of portal hypertension [7]. As shown by Tripodi [8], thrombin generation is normal in patients with cirrhosis provided that thrombomodulin is added to the reaction mixture to activate the anticoagulant protein C system. These and other authors have explained the maintenance of hemostasis in patients with cirrhosis by the fact that decrements in procoagulant proteins are matched by decrements in anticoagulant proteins, such as proteins C and S, and antithrombin (AT) [9].

In contrast to conventional coagulation tests such as the PT/INR and the activated partial thromboplastin time (aPTT) which assay only clot formation time in a plasma environment, thromboelastography (TEG) assesses overall hemostasis, the cumulative effects of procoagulant and anticoagulant proteins, fibrinogen, platelets, and red blood cells. Component measurements of the

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Abbreviations: ALI, acute liver injury; ALF, acute liver failure; INR, international normalized ratio of prothrombin time; TEG, thromboelastogram/thromboelastography; PT, prothrombin time; ICP, intracranial pressure; AT, antithrombin; aPTT, activated partial thromboplastin time; R-time, reaction time of TEG; K-time, kinetic time of TEG; MA, maximum amplitude of TEG; SIRS, systemic inflammatory response syndrome; WBC, white blood cell count; LY-30, clot lysis in 30 min; CVVH, continuous veno-veno hemofiltration; BMI, body mass index; APAP, acetaminophen (paracetamol); MAP, mean arterial pressure; OLT, orthotopic liver transplantation; TFS, transplant-free survival; MELD, model for end-stage liver disease score; ADAMTS13, a disintegrin and metalloprotease with thrombospondin type-1 motifs 13; vWF, von Willebrand factor.

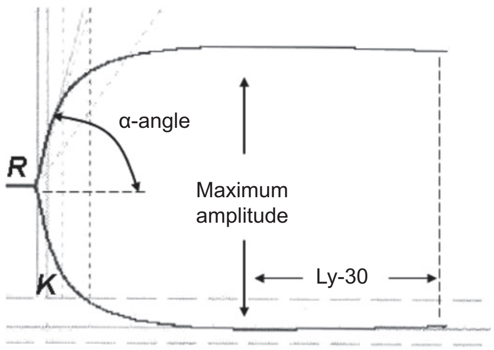

Fig. 1. Representative thromboelastogram from a patient with acute liver failure from acetaminophen. The tracing is normal in all of the 5 TEG parameters indicated despite an INR of 4.2: (R-time, 3.8 min; K-time, 1.1 min; α-angle, 73.8°; MA, 63.0 mm; lysis at 30 min, 0.3%). At the time of this TEG, the patient had grade 3 hepatic encephalopathy and venous ammonia of 120 μmol/L. Other coagulation components included: platelet count, 163×10^9/L; fibrinogen, 189 mg/dl; factor VII, 4% of normal; factor VIII, 558% of normal; protein C, 5% of normal.

TEG reflect specific phases of clot formation (Fig. 1) [10–12]. The reaction (R)-time reflects the latency of activation of the coagulation cascade, and correlates with the PT/INR and aPTT. The kinetic (K)-time reflects the rate of initial clot formation, and is proportional to fibrinogen concentrations and platelet count, as is the α-angle, which describes the rate of fibrin formation and cross-linking. Finally, the maximum amplitude (MA) reflects maximal clot strength, the culmination of all constituents of the clotting cascade. Although blood clot formation by TEG in non-infected patients with cirrhosis is generally preserved [13–15], patients with ALI/ALF, in whom INR is much more elevated, have not been extensively studied.

We hypothesized that, similar to patients with cirrhosis, most patients with ALI and ALF maintain normal overall hemostasis despite elevated INR. The aims of the current study were to assess overall hemostasis by TEG on admission to the hospital for ALI/ALF, to explore possible mechanisms of maintaining balanced hemostasis using TEG and conventional coagulation tests, and to explore possible relationships between TEG parameters, the severity of liver injury, and complications of the ALI/ALF syndrome.

Materials and methods

Patients

This study was an ancillary project of the Acute Liver Failure Study Group. Fifty-one consecutive patients with ALI/ALF were prospectively studied after admission to Virginia Commonwealth University Medical Center between October, 2008 and November, 2010. Informed consent was obtained from either the patient or their next-of-kin according to the patient's level of hepatic encephalopathy. Inclusion criteria included: (1) an INR of $\geqslant$1.5; (2) absence of a previous history of liver disease; and (3) illness $\leqslant$26 weeks duration. Patients with ALF were also required to have altered mentation ascribed to their liver injury (hepatic encephalopathy). Patients who received procoagulant treatments other than vitamin K prior to enrollment were excluded. Clinical and laboratory data were collected from the nearest time point to the performance of TEG, usually within 24 h of admission.

Definition of ALI/ALF complications

Components of the systemic inflammatory response syndrome (SIRS) were defined according to previously defined criteria [16]: while blood cell count (WBC) >12 or <4×10^9/L, temperature <36 or >38 °C, respiratory rate of >20/min, and pulse >90 beats/min. Complications of ALI/ALF were defined as follows: infection was defined as a positive urine culture in the presence of pyuria, presence a pulmonary infiltrate on chest X-ray consistent with infectious etiology, or a positive blood culture not felt to be a contaminant with a skin organism. More than one positive blood culture was required for bacteremia with commensal organisms. Renal failure was defined as persistent azotemia and oliguria after hydration requiring continuous veno-venous hemofiltration (CVVH). Thrombosis was defined as spontaneous occlusion of a native blood vessel or indwelling dialysis catheter. Bleeding was defined as the presence of blood per naso-gastric tube, blood per rectum or endotracheal tube, or bleeding at the site of invasive procedure.

Thromboelastography

TEG was performed on a single instrument (Thrombelastograph Haemostasis Analyzer 5000 [Haemonetics Corp., Haemoscope Division, Niles, IL]) by a single operator (A. I.). Briefly, 5 ml of citrated whole blood was subjected to TEG within 2 h of blood draw. Clotting was initiated at 37 °C by the addition of kaolin to 0.34 ml of re-calcified blood. Kinetic changes in clot formation and clot dissolution were measured for 30 min after reaching maximal clot firmness. Five parameters were recorded:

- R-time (in minutes): the latency of clot formation from the beginning of the clotting reaction to the initial formation of fibrin (defined as an amplitude of 2 mm).
- K-time (in minutes): the time from initial fibrin formation required to reach a specific clot firmness (defined as an amplitude of 20 mm).
- α-Angle (in degrees): the kinetics of clot formation, measuring the rate of fibrin formation and cross-linking on platelets.
- MA (in mm): measures the maximal clot strength.
- Lysis at 30 min (Lysis-30; in percent): clot dissolution 30 min after reaching maximum amplitude, a measure of fibrinolysis.

An abnormal TEG parameter was defined as an R- or K-time above the upper limit of normal, or an α-angle or MA below the lower limit of normal for our laboratory, which are similar to other series [17].

Coagulation tests

PT/INR, aPTT, and levels of pro- and anticoagulant factors were assayed by the Clinical Coagulation Laboratory at VCU. The PT/INR, aPTT, factor levels, and protein C/S levels were determined using the STA-R Evolution® clot detection system. The PT/INR was determined using re-calcified plasma and recombinant human tissue factor and synthetic thromboplastin (Dade® Innovin® Reagent; Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The aPTT was determined using the STA® – PTT Kit, protein C and S activities were assayed using the STA® – Staclot® Protein C and STA® – Staclot® Protein S Kits, respectively, and AT activity determined using the STA® – Stachrom® ATIII Kit according to manufacturer's (Diagnostica Stago, Asnières sur Seine, France) instructions. Factor V, VII, and VIII activity levels were performed using calibration curves generated from $\geqslant$6 dilutions of specific factor-deficient substrates.

Statistical analysis

Continuous data were tested for normality and expressed as mean ± SD or median [range], and analyzed by ANOVA or Wilcoxon/Kruskal–Wallis Rank Sums test, as appropriate. Categorical variables were analyzed by Chi Square test and correlation of continuous data by Pearson correlation (r value). All data were analyzed using JMP 8.0 software. Significance was defined as a p value $\leqslant$0.05.

Results

Demographic, clinical, and laboratory characteristics of study population

A total of 51 consecutive patients with ALI/ALF were enrolled, with a mean age of 43.1 ± 14.7 years and mean body mass index (BMI) of 28.2 ± 6.6 kg/m^2 (Table 1). Women and Caucasians predominated (61% and 65%, respectively). The etiology of ALI/ALF was acetaminophen (APAP) in 22 patients (43%), autoimmune

Table 1. Demographic, clinical and laboratory characteristics on hospital admission for ALI/ALF and subgroups according to outcome. (Mean ± SD or median [range])

Feature	Normal Range	Entire Group (n = 51)	Spontaneous Survivors (n = 29)	Death or OLT (n = 22)
Demographics:				
Age (years)		43.1 ± 14.7	40.3 ± 15.0	46.7 ± 13.8
Female Gender (%)		61	59	64
Caucasian Race (%)		65	66	64
BMI (Kg/m²)		28.2 ± 6.6	26.7 ± 5.1	30.3 ± 8.0
Clinical Characteristics:				
Etiology of ALI/ALF (N [%]):				
Acetaminophen		22 [43]	17 [59]	5 [23]**
Autoimmune hepatitis		7 [14]		
Hepatitis B		7 [14]		
Idiosyncratic drug		6 [12]		
Indeterminate		4 [8]		
Hepatic ischemia		2 [4]		
Mushroom poisoning		1 [2]		
Heat stroke		1 [2]		
Malignant infiltration		1 [2]		
Hepatic encephalopathy (ALF) (%)		73	55	96**
Number of SIRS		1.6 ± 1.2	1.2 ± 1.0	2.1 ± 1.3**
Pulse (beats/min)		95 ± 21	93 ± 21	97 ± 22
Mean arterial pressure (mmHg)		86 ± 14	87 ± 13	85 ± 16
Respiratory rate (breaths/min)		20 ± 6	18 ± 4	22 ± 8*
Temperature (°C)		36.7 ± 0.7	37.0 ± 0.7	36.3 ± 0.6***
Laboratory Data:				
White blood cell count (x 10^9/L)	3.9-11.7	11.8 ± 7.2	11.1 ± 6.7	12.5 ± 7.8
Creatinine (mg/dl)	0.5-1.0	1.0 [0.4-8.1]	0.9 [0.4-7.5]	1.5 [0.5-8.1]
Total bilirubin (mg/dl)	0.0-1.3	6.5 [0.3-44.2]	4.7 [0.9-29.4]	21.0 [0.3-44.2]**
Albumin (g/dl)	3.7-5.2	2.9 ± 0.5	3.0 ± 0.5	2.7 ± 0.4*
Venous ammonia (μmol/L)	0-35	80 ± 38	71 ± 36	91 ± 38
Lactate (mmol/L)	0.5-2.2	3.4 [0.4-21.4]	2.5 [0.4-6.6]	5.6 [0.7-21.4]**
Phosphate (mg/dl)	2.5-4.6	3.6 ± 2.4	2.8 ± 1.3	4.8 ± 3.1**
Fibrinogen (mg/dl)	200-450	195 ± 84	223 ± 55	154 ± 102**
PTT (sec)	25-36	49 ± 17	41 ± 10	59 ± 19****
INR		3.4 ± 1.7	3.0 ± 1.3	4.0 ± 1.9*
MELD score		31.3 ± 8.6	27.7 ± 7.1	36.2 ± 8.3***
TEG Parameters:				
R-time (min)	2.5-7.5	4.7 ± 1.9	4.1 ± 1.5	5.5 ± 2.2**
K-time (min)	0.8-2.8	1.7 [0.8-20.0]	1.9 [0.8-20.0]	1.7 [0.9-10.5]
α-Angle (degrees)	55.2-78.4	63.7 ± 12.2	63.6 ± 12.7	63.7 ± 11.8
Maximum Amplitude (mm)	50.6-69.4	55.0 ± 10.9	55.0 ± 11.2	55.1 ± 10.6
Lysis 30 (%)	0.0-7.5	0.0 [0.0-2.1]	0.0 [0.0-1.8]	0.0 [0.0-2.1]

*p <0.05, **p ⩽0.01, ***p <0.001, ****p ⩽0.0001 indicates significant difference between spontaneous survivors and those who died or underwent OLT.

hepatitis and hepatitis B in 7 patients (14%) each, idiosyncratic drug reactions in 6 (12%), indeterminate etiology in 4 patients (8%), ischemia in 2 patients (4%), and malignant infiltration of the liver, heat stroke, and mushroom (*Amanita*) poisoning in 1 (2%) case each. Hepatic encephalopathy of some degree (ALF) was present in 37 (73%) of patients. At the time of TEG measurement, the mean number of SIRS components was 1.6; 8 patients had a mean arterial pressure (MAP) of ⩽70 mm Hg. Fourteen patients (28%) died and 8 (16%) underwent orthotopic liver transplantation (OLT), yielding a transplant-free survival (TFS) of 29 patients (57%). Clinical features and laboratories predictive of poor outcome (death or OLT) included non-APAP etiology, the presence of hepatic encephalopathy, lower body temperature, albumin, and fibrinogen, and higher number of SIRS, respiratory rate, bilirubin, lactate, phosphate, aPTT, INR, and MELD score (Table 1).

Mean/median TEG parameter values were within normal limits for the entire study population (Table 1; mean R-time

4.7 min [normal range 2.5–7.5], median K-time 1.7 min [normal 0.8–2.8 min], mean α-angle 63.7° [normal 55.2–78.4], mean MA 55.0 mm [normal 50.6–69.4]). Thirty-two patients (63%) had completely normal studies; 8 (16%) had 1, 5 (10%) had 2, 4 (8%) had 3, and 2 (4%) had 4 TEG parameters in a hypocoagulable range. An MA below normal was confined to patients with platelets $<126 \times 10^9$. Four (8%) patients had TEG parameters in a hypercoagulable range. No differences in any TEG component were observed in patients with APAP-induced ALI/ALF compared to those with other etiologies (data not shown). Despite reports of fibrinolysis in patients with ALF, the lysis-30 component of the TEG was normal in all 51 patients. These data suggest that the dynamics of clot formation as assessed by TEG are generally well preserved in patients with ALI/ALF.

Correlation of TEG components with coagulation factor levels, SIRS components, and admission chemistries

Specific TEG components in patients with ALI/ALF reflected specific parameters of clot formation (Table 2). The R-time correlated directly with standard assays of coagulation (aPTT [r = 0.60, *p* <0.0001], INR [r = 0.32, *p* = 0.022]), and inversely with factor V levels (r = −0.30, *p* = 0.035). In contrast, the K-time correlated inversely with fibrinogen (r = −0.44, *p* = 0.001), platelet count (r = −0.50, *p* = 0.0002), and factor VIII levels (r = −0.31, *p* = 0.028), whereas the α-angle correlated directly with fibrinogen (r = 0.34, *p* = 0.014), platelet count (r = 0.58, *p* <0.0001), and factor VIII levels (r = 0.38, *p* = 0.006), as did the MA (fibrinogen [r = 0.58; *p* <0.001], platelet count [r = 0.73; *p* <0.0001], and factor VIII levels [r = 0.38; *p* = 0.006]). None of the TEG components correlated significantly with levels of anticoagulant proteins (protein C/S, AT).

TEG components also correlated with the presence of the SIRS and laboratory parameters with prognostic importance in ALF (Table 2). Generally, increasing liver injury was associated with more hypocoagulable R-time but more hypercoagulable K-time, α-angle and MA. Longer R-time was associated with higher respiratory rate (r = 0.46, *p* = 0.0007), WBC count (r = 0.29, *p* = 0.043), serum lactate (r = 0.58, *p* <0.0001), and serum phosphate concentrations (r = 0.47, *p* = 0.0005), which portend poor prognosis [18–20]. In contrast, shorter K-time (more hypercoagulable) was associated with higher pulse (r = −0.43, *p* = 0.002), respirations (r = −0.34, *p* = 0.015), and venous ammonia (r = −0.37, *p* = 0.007), while higher MA (more hypercoagulable) was directly associated with higher pulse (r = 0.44, *p* = 0.001), respirations (r = 0.31, *p* = 0.028), WBC (r = 0.30, *p* = 0.034), and venous ammonia (r = 0.38, *p* = 0.006). The increase in clot MA according to increasing numbers of SIRS is depicted in Fig. 2 (*p* = 0.024).

Relationship between TEG parameters with complications and outcome of ALI/ALF

The association of TEG profiles with complications and outcomes of patients with ALI/ALF are shown in Table 3. Patients with ALF were clearly more ill than those with ALI, with all but one death or liver transplant occurring in the former group (*p* = 0.001), a higher number of SIRS (1.8 ± 1.2 *vs.* 1.1 ± 1.0, respectively; *p* = 0.056), higher ammonia (92 ± 33 *vs.* 37 ± 18 μmol/L, respectively; *p* <0.0001), and higher MELD scores (34 ± 8 *vs.* 26 ± 8, respectively; *p* = 0.003) (data not shown). Interestingly, patients with hepatic encephalopathy were relatively hypercoagulable compared to those without encephalopathy, with shorter K-time (median 1.6 *vs.* 3.3 min, *p* <0.01), higher α-angle (66 ± 10 *vs.* 57 ± 15°, *p* = 0.01), higher MA (57 ± 10 *vs.* 50 ± 13 mm, *p* = 0.05), and fewer number of TEG abnormalities (0.5 ± 1.1 *vs.* 1.4 ± 1.2, *p* = 0.02). No difference was observed in R-time between patients with and without hepatic encephalopathy. These observations agree with the correlations of TEG parameters, ammonia, and severity of disease as assessed by SIRS, noted above (Table 3).

In contrast to hepatic encephalopathy, other complications of the ALI/ALF syndrome and poor outcomes were associated with longer (more hypocoagulable) R-time (Table 4). Patients with infection had significantly longer R-time than those without (6.0 ± 3.0 *vs.* 4.3 ± 1.2 min, respectively; *p* = 0.007), as did those who had renal failure requiring CVVH (5.8 ± 2.3 *vs.* 3.9 ± 1.1 min, respectively; *p* = 0.0004). Patients with thrombosis or bleeding also had longer R-times than those without these complications (5.7 ± 3.0 *vs.* 4.5 ± 1.5 min for those with and without thrombosis, respectively [*p* = 0.07], and 6.4 ± 3.5 *vs.* 4.5 ± 1.6 min for those with and without bleeding, respectively [*p* = 0.02]). Finally, R-time was also longer in patients with poor outcome (5.5 ± 2.2 for those who underwent OLT or died *vs.* 4.1 ± 1.5 min for spontaneous survivors [*p* = 0.013; Table 1]; and 5.9 ± 2.6 for those who died overall *vs.* 4.3 ± 1.4 min for those alive at discharge from the hospital [*p* = 0.005; Table 4]). There were no differences in the K-time, α-angle, or MA in patients with and without complications other than encephalopathy, including patients who died or underwent OLT.

Anticoagulant proteins decline in concert with procoagulant proteins in patients with ALI/ALF

Similar to patients with cirrhosis, preserved hemostasis in patients with ALI/ALF may also be maintained by a commensurate decline in liver-derived, pro- and anticoagulant proteins (Table 4). Levels of both pro- and anticoagulant proteins were markedly reduced (25%, 6%, 5%, 16%, and 37% of normal for factor V, factor VII, protein C, protein S, and AT, respectively). Levels of the anticoagulant proteins were decreased in direct proportion to those of liver-derived procoagulant proteins and fibrinogen concentrations (protein C/factor V: r = 0.42, *p* <0.01; protein C/factor VII: r = 0.62, *p* <0.001; protein C/fibrinogen: r = 0.53, *p* <0.001; protein S/factor V: r = 0.49, *p* <0.001; protein S/factor VII: r = 0.37, *p* <0.01; protein S/fibrinogen: r = 0.37, *p* <0.01; AT/factor VII: r = 0.43, *p* <0.01; AT/fibrinogen: r = 0.51, *p* <0.001). In contrast, anticoagulant proteins C and S were weakly inversely correlated with factor VIII levels, which were increased to a mean of 445 ± 232% of normal, reflecting endothelial activation/injury. The correlation of protein C and factor VIII was significant (r = −0.32, *p* = 0.024).

Discussion

The purpose of the present study was to assess hemostasis in patients with ALI/ALF in order to determine the validity of "coagulopathy," implied by an elevated INR. A paradox has long been

Table 2. Correlation of TEG parameters with coagulation parameters, SIRS components, and admission laboratories in patients with ALI/ALF. Data represent correlation coefficients (Pearson r values), with negative numbers denoting inverse correlation. All laboratory and clinical data were collected at the time of TEG.

Feature	R time	K time	α-Angle	Maximum amplitude
Coagulation Parameters:				
INR	0.32*	0.17	-0.24	-0.23
PTT	0.60****	0.07	-0.07	-0.06
Platelets	0.01	-0.50***	0.58****	0.73****
Fibrinogen	-0.22	-0.44**	0.34*	0.57****
Factor V	-0.30*	-0.25	0.24	0.34*
Factor VII	0.08	-0.02	-0.06	0.17
Factor VIII	-0.27	-0.31*	0.38**	0.33*
Protein C Activity	-0.19	0.02	-0.14	-0.04
Protein S Activity	-0.14	0.11	-0.20	-0.14
Antithrombin Activity	-0.14	-0.02	-0.06	0.17
SIRS Components:				
Pulse	0.18	-0.43**	0.29*	0.44**
Respirations	0.46***	-0.34*	0.15	0.31*
Temperature	-0.20	0.03	0.02	-0.06
WBC	0.29*	-0.13	0.19	0.30*
Chemistries:				
Lactate	0.58****	0.16	-0.23	0.01
Ammonia (venous)	0.13	-0.37**	0.38**	0.38**
Phosphate	0.47***	-0.04	0.00	0.25
Creatinine	0.11	-0.05	0.13	0.23
Total Bilirubin	-0.14	-0.16	0.22	0.10

*p <0.05, **p <0.01, ***p <0.001, ****p ⩽0.0001 indicates significant correlation.

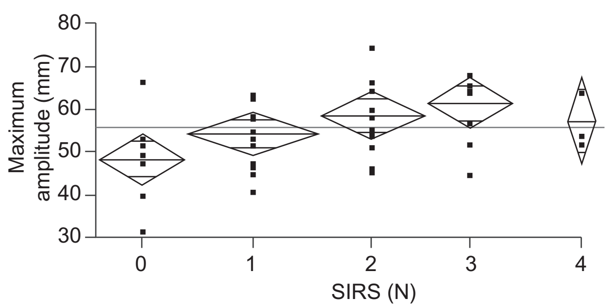

Fig. 2. Maximum amplitude of clot formation according to the number of SIRS concurrently determined in patients with acute liver injury/failure on admission to study. Data represent mean values with lower/higher 95%; width of columns reflect number of patients with indicated number of SIRS (p = 0.024 for trend).

recognized in patients with ALF: despite elevated INR, they rarely bleed. Nevertheless, the management of such patients often includes repletion of coagulation factors and platelets, introducing the possibility of adverse effects (volume overload, transfusion-associated lung injury, thrombosis), and added expense. Our results clearly demonstrate that most patients with ALI/ALF have normal clot formation by TEG despite admission INR ranging from 1.5 to 9.6, levels which would likely prompt coagulation factor repletion prior to an invasive procedure.

TEG parameters in patients with ALI/ALF reflect specific phases of blood clot formation and are associated with specific aspects of the clinical syndrome. The R-time mirrors activation of the coagulation cascades and procoagulant factor levels. Consistent with the well-recognized importance of the INR and factor V in predicting outcome in patients with ALF [21,22], the R-time directly correlated with the SIRS, specific laboratories which predict poor outcome (lactate and phosphate), complications of the ALI/ALF syndrome other than hepatic encephalopathy, and poor outcome. R-time was significantly higher in patients with infection, those requiring CVVH, and in those with bleeding complications. The TEG was, in fact, more sensitive than the INR for predicting bleeding, since the INR was not significantly different in those who bled and those who did not (p = 0.14). This observation supports those of Chau *et al.* [23], who noted that variceal rebleeding in patients with cirrhosis was predicted by TEG but not INR. Similarly, the platelet count, factor levels, and fibrinogen concentration were similar in patients who bled and those who did not, although the aPTT was significantly longer in the former (65 *vs.* 47 s; p = 0.016). Thus, monitoring the R-time by TEG or the aPTT would seem a more appropriate assessment of bleeding risk in patients with ALI/ALF than the INR.

In contrast to the R-time, the K-time, α-angle, and MA correspond to fibrin formation and platelet activation, and were associated with different complications of ALI/ALF. These parameters become more "hypercoagulable" with increasing SIRS, venous ammonia, and the presence of hepatic encephalopathy. The find-

Table 3. TEG parameters in patients with and without specific complications of the ALI/ALF syndrome. (Mean ± SD or median [range].) See Materials and methods for definition of specific complications. Types of infection included pneumonia in 7, urinary tract in 4, bacteremia in 1, and ascitic fluid infection in 1 patient. Bleeding was from gastric aspirate coffee grounds in 4, oozing from cutaneous puncture sites in 1, and hemobilia after transjugular liver biopsy in 1 patient. Thrombosis included dialysis catheter thrombosis in 8, peripheral vascular thrombosis in 2, and portal venous thrombosis in 1 patient.

Complication		N	R Time (min)	K Time (min)	α-Angle (degrees)	Maximum Amplitude (mm)
Encephalopathy	ALI	14	4.2 ± 1.1	3.3 [0.9-20.0]	56.6 ± 14.5	50.4 ± 12.8
	ALF	37	4.9 ± 2.2	1.6 [0.8-10.5]**	66.3 ± 10.2**	56.8 ± 9.7*
Infection	Absent	38	4.3 ± 1.2	1.8 [0.9-20.0]	64.1 ± 11.5	54.1 ± 10.6
	Present	13	6.0 ± 3.0**	1.6 [0.8-10.5]	62.4 ± 14.6	57.7 ± 11.8
Renal Failure	No CVVH	30	3.9 ± 1.1	2.0 [0.9-7.6]	64.8 ± 7.9	53.6 ± 8.6
	CVVH	21	5.8 ± 2.3***	1.5 [0.8-20.0]	62.0 ± 16.7	57.0 ± 13.4
Thrombosis	Absent	40	4.5 ± 1.5	1.8 [0.8-20.0]	64.2 ± 11.7	54.7 ± 10.5
	Present	11	5.7 ± 3.0	1.5 [1.1-10.5]	61.6 ± 14.4	56.3 ± 12.5
Bleeding	Absent	45	4.5 ± 1.6	1.8 [0.8-20.0]	63.2 ± 12.7	54.8 ± 11.1
	Present	6	6.4 ± 3.5*	1.6 [1.9-3.3]	67.1 ± 7.7	56.8 ± 9.7
Overall Survival	Alive	37	4.3 ± 1.4	1.8 [0.8-20.0]	64.7 ± 11.8	55.2 ± 10.8
	Dead	14	5.9 ± 2.6**	1.7 [1.0-10.5]	61.0 ± 13.2	54.7 ± 11.5

*p <0.05, **p ⩽0.01, ***p <0.001 *vs.* comparator group.

Table 4. Correlation or pro- and anticoagulant factors in patients with ALI/ALF. Levels reflect mean ± SD or median [range]. Other data represent correlation coefficients (Pearson r values), with negative numbers denoting inverse correlation.

Procoagulant Proteins		Anticoagulant Proteins		
		Protein C activity	Protein S activity	Antithrombin activity
	Level	5.0 [5.0-50.0]%	15.9 ± 11.6%	36.8 ± 13.4%
Factor V activity	25.0 ± 20.1%	0.42**	0.49***	0.21
Factor VII activity	6.0 [0.5-45.0]%	0.62***	0.37**	0.43**
Factor VIII activity	445 ± 232%	-0.32*	-0.22	-0.05
Fibrinogen	195 ± 84 mg/dl	0.53***	0.37**	0.51***

*p <0.05, **p <0.01, ***p <0.001 indicates significant correlation.

ing of increased hemostatic potential in patients with more severe liver injury is similar to patients with sepsis [24,25]. Plausible explanations include increased factor VIII levels due to increased endothelial cell activation/injury, decreased ADAMTS13 activity, or increased levels of platelets and fibrinogen as "acute phase reactants." In fact, platelet counts tended to be higher in patients with ALF compared to ALI (200 *vs.* 150 × 10^9/L, respectively; $p = 0.09$) and correlated directly with ammonia ($r = 0.30$; $p = 0.031$). ADAMTS13, a protease synthesized in hepatic stellate cells, degrades highly active, large multimers of von Willebrand factor (vWF) [26], and is decreased in proportion to the severity of chronic liver failure [27]. Conceivably, patients with ALF may be deficient in ADAMTS13, leading to an accumulation of large vWF multimers, and consequently to an increase in TEG parameters which reflect platelet function.

A commensurate decline in anticoagulant proteins with procoagulant proteins by the failing liver may represent another mechanism by which hemostasis is maintained in patients with ALI/ALF. As shown by Tripodi *et al.* in patients with cirrhosis [8], the parallel decline in liver-derived pro- and anticoagulant proteins results in normal thrombin generation in the presence of thrombomodulin, which allows activation of protein C. Indeed, levels of anticoagulant proteins were markedly reduced in patients with ALI/ALF, and strongly correlated with reductions in procoagulant proteins including fibrinogen, except for factor VIII.

The observations that hemostasis by TEG is usually preserved in patients with ALI/ALF contrasts with a recent study of 10 ALF patients undergoing OLT [28]. Senzolo *et al.* noted that R-time, K-time, and α-angle were significantly abnormal in patients with ALF, and more hypocoagulable than in a control group of 10 patients with cirrhosis. These findings were ascribed to the presence of endogenous heparinoids, as abnormalities in TEG were ameliorated by the addition of heparinase to the reaction mixture. Although the discrepancy may be explained by significant clinical and methodological differences [29,30], their study was similar to the present in that MA and a surrogate marker for thrombin generation were not only comparable to cirrhotic controls but also to normal controls. Thus, although endogenous heparinoids may slow the velocity of initial clot formation, they do not affect ultimate clot strength in patients with ALF. These and other observations in patients with cirrhosis [14,31] provide a plausible link between the SIRS and R-time in the present study: cytokine-induced SIRS is likely accompanied by endothelial release of glycosaminoglycans (heparinoids), resulting in prolongation of the R-time. Endogenous heparinoids may also mediate the correlation of infection and prolongation of the R-time in our study population.

Since bleeding complications were uncommon in the current series, we are not able to make firm recommendations for coagulation factor repletion in patients with ALI/ALF. However, in multi-variable analysis, the single most important component determining clot strength in TEG is the platelet count, followed by fibrinogen concentration, and last by procoagulant factor levels. Therefore, repletion of platelets rather than the administration of cryoprecipitate or plasma seems of primary importance prior to invasive procedures. A study examining the effects of factor repletion on TEG in patients with liver disease concluded similarly, that only transfusion of platelets significantly improved TEG parameters [32]. Another recent study concluded that platelet count, but not coagulation defects, were associated with procedure-related bleeding in patients with cirrhosis [33]. In the present study, MA was within normal limits in all patients with platelet counts of $>126 \times 10^9$/L. Conversely, MA was low in 10 of 11 patients with platelet counts of $<83 \times 10^9$/L. These observations suggest that platelet transfusion before invasive procedures should be considered in patients with platelet counts of (roughly) $<100 \times 10^9$/L.

The current study also has implications for thrombosis in patients with ALI/ALF. Indeed, thrombotic complications were more common than bleeding complications, and required heparin infusions in all patients in the current series, which were well-tolerated. Ganey *et al.* [34] have shown that intrasinusoidal activation of hemostasis and subsequent formation of microthrombi within the liver may perpetuate liver injury in animal models of ALF. Clearly, this observation argues against the routine use of procoagulants including recombinant factor VIIa prior to invasive procedures, as the practice may exacerbate this process and impair hepatic regeneration after ALI/ALF, and has been associated with other thrombotic complications [35].

Several limitations of our observations must be acknowledged. First, although more a measure of overall hemostasis than the INR or aPTT, the TEG does not reflect the role of endothelial cells, which are markedly activated/injured in patients with ALF, and have important effects on the balance of hemostasis in patients with ALF [36,37]. Second, and related to the first limitation, TEG assays in this study were performed without exogenous thrombomodulin, an endothelial receptor not present in plasma but required for activation of the protein C system. The omission of thrombomodulin may explain the absence of correlation between protein C levels and TEG parameters. Third, the potential effects of endogenous heparinoids were not assessed in TEG assays, and may explain the relationship of R-time with more severe liver injury. Finally, kaolin activation of the clotting reaction in our TEG assays may have introduced a non-physiological variable with potential effects on our results [30].

In conclusion, the present study demonstrates that hemostasis assessed by TEG in patients with ALI/ALF is generally preserved, providing an explanation for the scarcity of clinically significant bleeding despite elevated INR. Potential mechanisms of maintained hemostasis include relatively normal platelet counts and fibrinogen, and the fact that platelets generally increase with increased severity of liver injury as part of the acute phase reaction, contributing to increased clot strength. Increased factor VIII release from injured epithelium may also compensate for deficiencies in other procoagulant proteins. Finally, similar to patients with cirrhosis, liver-derived anticoagulant proteins are markedly deficient in patients with ALI/ALF, and may also compensate for deficient procoagulant proteins. The INR predicts the bleeding diathesis of ALI/ALF poorly, and should not guide procoagulant factor repletion; nevertheless, the INR remains a valid indicator of prognosis.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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