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Comparison of Thromboelastography and Conventional Coagulation Tests in Patients With Severe Liver Disease

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Abstract

Objective: Thromboelastography (TEG) may provide rapid and clinically important coagulation information in acutely ill patients with chronic liver disease (CLD). Our objective was to describe the relationship between TEG and conventional coagulation tests (CCTs), which has not been previously explored in this population. **Methods:** In acutely ill patients with severe CLD (Child-Pugh score > 9, category C), we conducted a prospective observational study investigating coagulation assessment as measured by both CCTs and TEG. We used quantile regression to explore 30 associations between TEG parameters and corresponding CCTs. We compared TEG and CCT measures of coagulation initiation, clot formation, clot strength, and fibrinolysis. **Results:** We studied 34 patients on a total of 109 occasions. We observed inconsistent associations between TEG and CCT measures of coagulation initiation: TEG (citrated kaolin [CK] assay) standard reaction time and international normalized ratio: $R^2 = 0.117$ ($P = .044$). Conversely, there were strong and consistent associations between tests of clot formation: TEG (CK) kinetics time and fibrinogen: $R^2 = 0.202$ ($P < .0001$) and TEG (CK) α angle and fibrinogen 0.263 ($P < .0001$). We also observed strong associations between tests of clot strength, specifically TEG MA and conventional fibrinogen levels, across all TEG assays: MA (CK) and fibrinogen: $R^2 = 0.485$ ($P < .0001$). There were no associations between TEG and D-dimer levels. **Conclusions:** In acutely ill patients with CLD, there are strong and consistent associations between TEG measures of clot formation and clot strength and conventional fibrinogen levels. There are weak and/or inconsistent associations between TEG and all other conventional measures of coagulation.

Keywords

coagulopathy, coagulation, diagnosis, thrombosis

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Introduction

The liver is essential to the maintenance of hemostasis.[1] Patients with severe chronic liver disease (CLD) who become acutely unwell may demonstrate several coagulation abnormalities as assessed by conventional coagulation tests (CCTs). Traditionally, such coagulation abnormalities have been considered to predict a higher risk of bleeding. However, more recently it has been proposed that patients with CLD have a *rebalanced coagulation state,* whereby such patients are simultaneously at increased risk of thrombosis as well as bleeding.[2] In addition, a limitation of CCTs is that they are in vitro tests, which fail to capture the entirety of the in vivo coagulation system dysfunction in this population.[3,4] Despite such concerns however, CCTs are routinely performed in this patient group and are commonly used to guide clinical decisions such as blood product administration.

A potentially more physiologically relevant evaluation of coagulation status may be provided by thromboelastography (TEG).[5,6] Unlike CCTs, TEG uses the principle of viscoelasticity to measure coagulation initiation, clot formation, clot strength, and fibrinolysis, providing a global assessment of hemostatic function. Thromboelastography is a rapid, point-of-care test and is being increasingly used in cardiac surgery, trauma, and massive transfusions.[6,7]

We have previously demonstrated that among patients with decompensated CLD, TEG shows delayed coagulation initiation, weaker clot strength, and impaired fibrinogen function but also decreased clot lysis.[8] However, the relationship between TEG and CCTs in this population remains unknown. This is problematic because understanding the associations between these tests would help define which conventionally measured variable might be most informative about the coagulation process, identify areas of discordance between TEG and conventional measures, and help inform more appropriate coagulation management in this group.

Accordingly, we performed a prospective, observational study to describe the associations between CCTs and TEG in acutely ill patients with severe CLD. Specifically, we assessed the associations between CCT and TEG parameters in relation to coagulation initiation, clot formation, clot strength, and fibrinolysis.

Methods

Study Design, Environment, and Ethics Considerations

We conducted a prospective, observational study to investigate TEG and CCTs among adult (>18 years) acutely ill patients with severe CLD (Child-Pugh score >9, category C, duration >12 weeks). Patients were recruited from the intensive care unit (ICU) or the Victorian state liver transplant service ward between April and October 2015. The Human Research Ethics Committees at the Austin Hospital and Monash University approved this study and waived the need for informed participant consent (Austin HREC: LNR/15/Austin/70 February 2015, Monash University HREC: CF15/636—2015000291, March 2015).

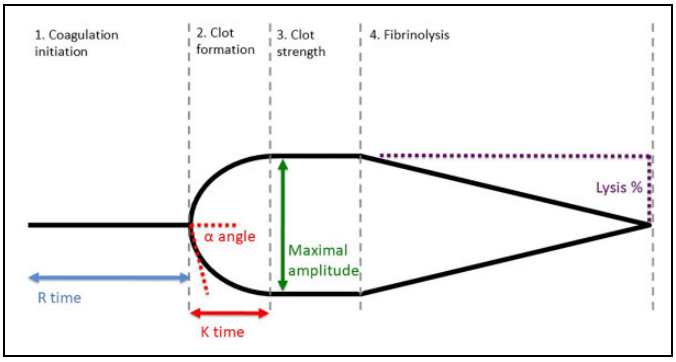

Figure 1. Thromboelastography (TEG) tracing, depicting rate of formation and degradation of clot, as well as the reaction time (R), kinetics time (K), α angle, maximum amplitude (MA) and lysis (LY30%). Note: LY30% is increased in this image.

Details of Data Collected

Patient characteristics, including disease severity (Child-Pugh criteria), reason for hospital/ICU admission, and ICU Acute Physiology and Chronic Health Evaluation (APACHE) III score, were recorded. Admission liver function tests, blood products, and pro/anticoagulant medications administered during the enrollment period were also recorded.

Sample Analysis

Initial TEG and CCTs were performed within 72 hours of hospital admission by a single investigator and were repeated at 24-hour increments for up to 7 days or until discharge. Samples were stored in 2 standard 3.2% citrate solution vials, with 1 vial used for TEG, analyzed by a single, calibrated TEG6S device (Haemonetics) and the other for CCTs. The TEG6S is the latest iteration of TEG viscoelastic testing methodology. The system exposes blood samples to a fixed vibration frequency. The degree of vibration (vertical motion) of the coagulating blood meniscus is measured by an infrared detector to determine coagulation parameters.[9,10] Conversely, the older TEG5000 model assesses coagulation by measuring shear elasticity of a coagulating blood sample mechanically using a torsion wire connected to a suspended pin within the sample.[11] The TEG6S uses greater automation, eliminating the need for manual sample preparation, minimal pipetting, and calibration, and allows for multiple assays to be performed simultaneously.

Each TEG sample was recalcified (addition of 20 µL calcium chloride 10%) and analyzed by a 4-channel TEG cartridge. These channels are comprised of:

1. Citrated kaolin (CK) assay: contains the coagulation activator, kaolin, providing standard reaction (R) time, kinetics (K) time, α angle, maximum amplitude (MA), and the percentage decrease in amplitude at 30-minute post-MA (lysis [LY30%]). An illustration of a TEG trace and these parameters is shown in Figure 1.
2. Rapid-TEG (RT) assay: contains tissue factor (TF) and kaolin, providing R time, K time, α angle, MA, LY30%,

and an activated clotting time (ACT) measuring the initiation of the clotting phase.
3. Heparinase kaolin (HK) assay: contains kaolin and a heparin-neutralizing enzyme, providing only R time, K time, α angle, and MA.
4. Functional fibrinogen (FF) assay: contains kaolin and a glycoprotein IIb/IIIa antagonist, providing MA based only on fibrinogen contribution to the thrombus, and functional fibrinogen level (FLEV), an estimation of plasma fibrinogen level.
5. Sampling technique and CCT analysis were performed as previously described (Appendix A).[8] Sampling was performed independent of blood product or pro/anticoagulant administration, with results not being used to influence treatment decisions.

Thromboelastography examines each variable simultaneously and in real time. Associations between TEG parameters and CCTs were grouped according to tests measuring (1) coagulation initiation (TEG R time, ACT, and conventional international normalized ratio (INR), prothrombin time (PT), and activated partial thromboplastin time (APTT); (2) clot formation (TEG K time, α angle, and conventional fibrinogen level); (3) clot strength (TEG MA and conventional fibrinogen level, platelet count); and (4) fibrinolysis (TEG LY30% and conventional D-dimer levels).

Statistical Analysis

Analyses were performed using STATA analytical software (Stata version SE, 13.0). Categorical data are presented as counts and proportions, and continuous data are presented as medians with interquartile ranges (IQRs). Nonparametric statistics were used given the small sample size of this exploratory analysis. The relationship between each individual TEG parameter and CCT was investigated using clustered quantile regression with 500 bootstrap samples, a technique superior to mean regression for nonparametric data. Clustered quartile regression deals robustly with outliers and skewing while controlling for the inflating effect of repeated measures.[12] Quantile regression also generates an association estimate with 95% confidence interval (ie, slope of the regression line), a pseudo-R^2 value (an estimate of the strength of the association between the variables under examination), and a P value. We defined a pseudo-R^2 value of >0.20 as indicating excellent goodness of fit, and by extension a "strong" association, as per the most conservative convention.[13,14] Significance was taken to be $P < .01$ to account for repeated measures.

Table 1. Patient Demographics, Baseline Liver Function Tests, CCTs and Blood Products, Vitamin K, and Anticoagulant Administration.[a]

Demographics		
Male	21 (62%)	
Age, years old	56 (48-65)	
Child-Pugh score	12 (11-12)	
Bilirubin	3 (2-3)	
Albumin	3 (2-3)	
International normalized ratio	2 (1-3)	
Ascites	3 (2-3)	
Encephalopathy	2 (1-2)	
Model end-stage liver disease score	26 (20-36)	
Previous liver transplant; total	3	
Intensive care unit patients; total	14 (41%)	
Ward patients	20 (59%)	
Liver function tests		Normal reference range
Bilirubin, mmol/L	77.5 (50-165)	<21
Alanine aminotransferase, units/L	41.5 (28-93)	5-35
Aspartate aminotransferase, units/L	127.5 (86-178.5)	<40
Gamma glutaminase, units/L	62.5 (37-124)	<60
Alkaline phosphatase, units/L	146.5 (85-223)	30-110
Albumin, g/L	26.5 (21-32)	35-50
Coagulation tests		
Prothrombin time, seconds	19.1 (16-25.6)	11.0-15.0
International normalized ratio	1.8 (1.5-2.5)	
Activated partial thromboplastin time, seconds	43.4 (35.2-50.8)	22-41
Fibrinogen, g/L	1.5 (0.8-3.1)	2.0-4.0
Platelets, $\times 10^9$ cell/L	61 (45.8-95.8)	150-400
D-dimer, mg/L	1.8 (0.8-3.1)	<0.5
Blood product	n (%)	Median (IQR) units
Cryoprecipitate	4 (12%)	10 (6.5-12)
Packed red blood cells	12 (35%)	2.5 (1-6)
Fresh-frozen plasma	7 (21%)	2 (2-7.5)
Platelets		
Standard units	5 (15%)	3 (1.5-4)
Pooled platelets	5 (15%)	1 (1-4)
Medication		
Vitamin K (10 mg IV/d)	21 (62%)	
Aspirin	1 (3%)	
Warfarin	3 (9%)	
Enoxaparin	14 (41%)	
Heparin		
Low (<10 000 units/d)	4 (12%)	
High (>10 000 units/d)	4 (12%)	
Dextran	1 (3%)	

Abbreviations: CCTs, conventional coagulation tests; IV, intravenous; IQR, interquartile range.
[a]Values Presented as median (IQR) or percentage, n = 34.

Results

Patient Characteristics

A total of 109 paired CCT and TEG samples were obtained from 34 acutely ill patients with CLD, with a median of 3 (IQR: 1-5) samples per patient. Fourteen (41%) participants were enrolled from the ICU and 20 (59%) participants from the acute hepatology wards (Table 1). Infection was the most common ICU admission diagnosis (n = 7, 50%).

On hospital admission, 34 (100%) patients had deranged liver functions tests, characterized by hyperbilirubinemia and

Table 2. Parameters of TEG in Chronic Liver Disease.[a]

TEG assay channel	Study cohort, median[b] (IQR)	Normal reference range
CK: Citrated kaolin		
R time, minutes	6.9 (6.3-8.3)	4.6-9.1
K time, minutes	2.9 (1.7-4)	0.8-2.1
α Angle, degrees	65 (58.7-70.2)	63-78
Maximum amplitude, mm	43.3 (30.4-53.3)	52-69
Lysis, %	0.1 (0-1.1)	0-2.6
RT: rapid TEG		
R time, minutes	1.1 (0.6-1.9)	0.3-1.1
K time, minutes	3.3 (1.5-4.9)	0.8-2.7
α angle, degrees	60.3 (48-73.5)	60-78
Maximum amplitude, mm	41.9 (33-54.1)	52-70
Lysis, %	0 (0-0.4)	0-2.2
Activated clotting time, seconds	157.1 (110.3-230.1)	82-152
Heparinase kaolin		
R time, minutes	6.4 (5.8-8)	4.3-8.3
K time, minutes	2.7 (1.5-3.6)	0.8-1.9
α angle, degrees	66 (60-72.8)	64.3-77.1
Maximum amplitude, mm	42.9 (35.5-54.1)	52.3-68.9
Functional fibrinogen		
Maximum amplitude, mm	12 (4.3-20.5)	15-32
Functional fibrinogen level, mg/dL	219.4 (122-387.7)	278-581

Abbreviations: IQR, interquartile range; K, kinetics; R, reaction; TEG, thromboelastography.
[a] n = 34.
[b] Study cohort median = population median, derived from the mean of each patient, across all patient samples, in order to appropriately measure central tendency.

hypoalbuminemia. The median Child-Pugh score was 12 (IQR: 11-12). The median model for end-stage liver disease score was 26 (IQR: 20-34). The median APACHE III illness severity score for the 14 ICU patients was 84 (IQR: 60-90; Table 1).

Blood Product and Pro/Anticoagulant Administration

Most patients received vitamin K (n = 21, 62%), and most patients (n = 25, 74%) received some form of anticoagulant therapy (warfarin: n = 3 [9%]; prophylactic enoxaparin: n = 14 [41%]; heparin: n = 8 [24%]). A summary of procoagulant and anticoagulant medications administered is summarized in Table 1. Blood products were administered to 15 (44%) patients during the study. Packed red blood cells were the most commonly administered product, administered to 12 (35%) patients (median 3 units/patient; Table 1).

Conventional Coagulation Tests Results

Conventional coagulation tests were abnormal in our patient cohort, characterized by elevated median PT, INR, APTT, and D-dimer levels and reduced median fibrinogen levels and platelet counts versus normal reference ranges (Table 1).

Thromboelastography Results

Comparing TEG results in the CLD patient cohort versus normal manufacturer reference ranges, we observed normal clot initiation (demonstrated by normal R time), poor clot formation (demonstrated by longer K time and low-normal alpha angles), decreased clot strength (demonstrated by reduced MA), and low normal fibrinolysis (measured by LY30%). These findings were consistent across all TEG assays (Table 2).

Relationship Between TEG and CCTs

We investigated 30 associations between TEG and CCTs in total. This included 12 associations between tests measuring coagulation initiation, 6 between tests measuring clot formation, 4 between tests measuring clot strength, and 2 between tests measuring fibrinolysis: A strong association (R^2 value >0.2) was demonstrated in 5 of the 12 associations assessed between TEG and CCT markers of coagulation initiation (TEG R time, ACT, and conventional INR, PT, APTT; Table 3). Similarly, a strong association (R^2 value >0.2) was demonstrated in all associations between TEG and CCT markers of clot formation (TEG K time, α angle, and conventional fibrinogen level; Table 4). We observed a consistent association pattern between TEG markers of clot strength and CCTs with 5 of the 10 associations being strong (TEG MA and conventional fibrinogen level, platelet count; Table 5). Consistently, all strong associations were between TEG parameters and fibrinogen level, with no strong associations between TEG parameters and platelet count. Finally, there were no strong associations between TEG and CCT markers of fibrinolysis (TEG LY30% and conventional D-dimer levels; Table 6).

Outcomes

The median (IQR) length of ICU stay was 16 days ($^{3\text{-}40}$); 12 (86%) of 14 patients who required an ICU admission survived. Median (IQR) length of hospital admission was 22 days (5-91); 29 (85%) patients survived with an overall in-hospital mortality of 15%.

Discussion

Key Findings

We performed a prospective observational study describing the relationship between CCTs and TEG in acutely ill patients with severe CLD. In this cohort of patients, the strongest associations between TEG and CCTs occurred when measuring clot formation and clot strength. Strong associations were consistently seen between TEG markers of both clot formation (K time and α angle) and TEG markers of clot strength (MA), and conventional fibrinogen levels. Conversely, TEG and CCTs demonstrated weak and inconsistent associations across all other parameters, including tests of coagulation initiation, platelet count, and fibrinolysis. Finally, both TEG and CCTs

Table 3. Association Between TEG and Conventional Coagulation Tests for Measurement of Coagulation Initiation.[a,b]

TEG parameter	CCT	Pseudo-R^2 value	*P* value[c]	Gradient of slope "association estimate" (95% CI)
R time				
Citrated Kaolin assay	APTT	0.289	.004	5.42 (1.72 to 9.13)
	PT	0.001	.934	0.05 (−1.08 to1.18)
	INR	0.000	1.000	0.00 (−0.10 to 0.10)
Rapid TEG assay	APTT	0.048	.327	4.23 (−4.23 to 12.69)
	PT	0.267	.020	4.33 (0.69 to 7.98)
	INR	0.229	.016	0.40 (0.07 to 0.73)
Heparinase assay	APTT	0.143	.001	5.32 (2.13 to 8.52)
	PT	0.116	.005	2.13 (0.66 to 3.60)
	INR	0.098	.006	0.19 (0.05 to 0.33)
Rapid TEG ACT	APTT	0.048	.309	0.05 (−0.04 to 0.13)
	PT	0.267	.020	0.05 (0.01 to 0.09)
	INR	0.229	.019	0.00 (0.00 to 0.01)

Abbreviations: APTT, activated partial thromboplastin time; ACT, activated clotting time; CCT, conventional coagulation test; INR, international normalized ratio; PT, prothrombin time; R, reaction; TEG, thromboelastography.
[a] n = 34.
[b] Expressed as correlation coefficient, 95% CI, *P* value, and pseudo-R^2 value. All values within 95% CI with ±1.96 standard deviations.
[c] *P* value = Derived from regression analysis. Significance taken to be $P < .01$: *P* value and pseudo-R^2 rounded to 3 decimal places, other data to 2 decimal places.

Table 4. Association Between TEG and Conventional Coagulation Tests for Measurement of Clot Formation.[a,b]

TEG parameter	CCT	Pseudo-R^2 value	*P* value[c]	Gradient of slope "association estimate" (95% CI)
K time				
Citrated Kaolin assay	Fib	0.202	.000	−0.20 (−0.29 to −0.09)
Rapid TEG assay	Fib	0.281	.000	−0.17 (−0.25 to −0.08)
Heparinase assay	Fib	0.262	.000	−0.20 (−0.28 to −0.11)
α Angle				
Citrated Kaolin assay	Fib	0.263	.000	0.06 (0.04 to 0.09)
Rapid TEG assay	Fib	0.406	.000	0.06 (0.05 to 0.07)
Heparinase assay	Fib	0.313	.000	0.07 (0.05 to 0.09)

Abbreviations: CCT, conventional coagulation test; Fib, fibrinogen; K, kinetics; TEG, thromboelastography.
[a] n = 34.
[b] Expressed as correlation coefficient, 95% CI, *P* value, and pseudo-R^2 value. All values within 95% CI with ±1.96 standard deviations.
[c] *P* value = Derived from regression analysis. Significance taken to be $P < .01$: *P* value and pseudo-R^2 rounded to 3 decimal places, other data to 2 decimal places.

Table 5. Association Between TEG and Conventional Coagulation Tests for Measurement of Clot Strength.[a,b]

TEG parameter	CCT	Pseudo-R^2 value	*P* value[c]	Gradient of slope "association estimate" (95% CI)
Maximum amplitude				
Citrated Kaolin assay	Plt	0.148	.006	1.95 (0.57 to 3.33)
	Fib	0.485	.000	0.06 (0.05 to 0.73)
Rapid TEG assay	Plt	0.173	.005	1.97 (0.60 to 3.34)
	Fib	0.544	.000	0.06 (0.05 to 0.07)
Heparinase assay	Plt	0.158	.006	1.95 (0.56 to 3.34)
	Fib	0.517	.000	0.06 (0.05 to 0.07)
Functional fibrinogen assay	Plt	0.070	.150	1.71 (−0.62 to 4.04)
	Fib	0.391	.000	0.06 (0.03 to 0.09)
Estimated fibrinogen level				
Functional fibrinogen assay	Plt	0.070	.181	0.10 (−0.05 to 0.24)
	Fib	0.412	.000	0.00 (0.00 to 0.01)

Abbreviations: CCT, conventional coagulation test; Fib, fibrinogen; Plt, platelet; TEG, thromboelastography.
[a] n = 34.
[b] Expressed as correlation coefficient, 95% CI, *P* value, and pseudo-R^2 value. All values within 95% CI with ±1.96 standard deviations.
[c] *P* value = Derived from regression analysis. Significance taken to be $P < .01$: *P* value and pseudo-R^2 rounded to 3 decimal places, other data to 2 decimal places.

Table 6. Association Between TEG and Conventional Coagulation Tests for the Measurement of Fibrinolysis.[a,b]

TEG parameter	CCT	Pseudo-R^2 value	*P* value[c]	Gradient of slope "association estimate" (95% CI)
Lysis %				
Citrate Kaolin assay	D-dimer	0.064	0.200	−0.38 (−0.97 to 0.20)
Rapid TEG assay	D-dimer	0.043	0.257	−0.59 (−1.60 to 0.43)

Abbreviations: CCT, conventional coagulation test; TEG, thromboelastography.
[a] $n = 34$.
[b] Expressed as correlation coefficient, 95% CI, *P* value, and pseudo-R^2 value. All values within 95% CI with ± 1.96 standard deviations.
[c] *P* value = Derived from regression analysis. Significance taken to be $P < .01$: *P* value and pseudo-R^2 rounded to 3 decimal places, other data to 2 decimal places.

demonstrated a generally hypocoagulable picture in this group, characterized by slow clot formation and reduced clot strength. The TEG and CCT markers of fibrinolysis were inconsistent, with D-dimer being elevated, while TEG demonstrated low normal fibrinolysis in this patient group.

Relationship With Existing Evidence

Evidence suggests CCTs fail to predict the risk of bleeding following invasive procedures in patients with CLD.[15,16] The risk of postprocedural bleeding has been repeatedly demonstrated to be independent of platelet count and INR/PT in this cohort.[17,18] Moreover, there is no strong evidence that PT or INR predicts spontaneous bleeding in this group.[19] Additionally, a 2018 study examining 280 patients with cirrhosis concluded that platelet count does not predict unprovoked major or minor bleeding in this group.[20] Conversely, a 2016 study on over 1400 patients concluded that hypofibrinogenemia (defined as fibrinogen levels <200 mg/dL) was associated with an approximately 6-fold increase in the risk of major spontaneous bleeding compared to patients with fibrinogen levels $\geq$200 mg/dL.[21] As such, the available evidence suggests that tests measuring clot formation and strength (ie, fibrinogen) may have greater predictive value for bleeding events than tests of coagulation initiation/coagulation speed.

When examining the differences in associations between various TEG assays and CCTs, RT generated different associations with CCTs for clotting initiation. Also, the FF assay performed differently when measuring clotting strength.

There were weak associations between TEG R time and conventional PT and INR in both standard CK and HK assays. Conversely, we observed strong associations between both PT and INR and TEG R time in the RT assay. We also noted strong associations between both PT and INR and RT ACT. This trend was not continued between RT R time and APTT. These data imply that testing via RT generates a stronger association with conventional PT and INR than other TEG assays. This may be due to the presence of TF as a reagent in the RT assay. Emerging evidence suggests that TF is a critical mediator of coagulation in liver disease.[22] Moreover, in patients with severe cirrhosis (Child-Pugh C), observational studies have demonstrated an increase in circulating TF without a counterbalancing increase in TF pathway inhibitor.[23] The presence of extrinsic TF in the RT assay may exaggerate this imbalance and subsequently more closely relate to PT/INR tests which are also dependent on the presence of extrinsically added TF.[24] Whether the stronger associations between RT and PT/INR are related to a closer reflection of in vivo coagulation or a result of the presence of TF as a reagent requires further research. It should be acknowledged that the pseudo-R^2 of the associations between both PT/INR and both RT R time and ACT were only slightly >0.2.

While all associations between TEG MA and conventional fibrinogen levels were strong, the association between functional fibrinogen TEG MA and conventional fibrinogen was weaker than other TEG MA and fibrinogen associations examined. Additionally, the association between FLEV and conventional fibrinogen was also weaker than TEG MA and fibrinogen associations examined. We observed that FF FLEV estimated higher fibrinogen levels compared to conventional testing; conventional fibrinogen = 1.5 g/L (0.8-3.1g/L) compared to FLEV = 2.2 (1.2-3.9g/L; Tables 1 and 2). This difference in absolute fibrinogen value is insufficient to explain the weaker association between conventional fibrinogen and FF TEG tests. This unexpected finding has not been demonstrated by other work to our knowledge and requires further investigation.

To our knowledge, there is only one other study investigating the relationship between viscoelastic testing and CCTs in patients with severe CLD. This study also demonstrated an association between TEG measures of clot formation and fibrinogen estimates and other coagulation studies, however this study examined the relationship between viscoelastic measures and specific factor levels and examined a less unwell cohort (patients with cirrhosis not in ICU) and used rotational thromboelastometry (ROTEM) rather than TEG.[25]

Our study agrees with analysis of TEG and CCTs performed in other patient groups, which also demonstrated a strong relationship between TEG MA and conventional fibrinogen level.[26,27] An association between TEG MA and both conventional fibrinogen level and platelet count has also been demonstrated postpediatric cardiac surgery.[28] To our knowledge, there has been no study comparing TEG and CCTs in patients with CLD, with the only similar studies in patients undergoing liver transplantation.[29,30]

Implications of Study Findings

Firstly, the finding of strong and consistent association between TEG parameters and traditional fibrinogen level (clot

formation and clot strength) is important given that fibrinogen deficit or dysfunction is related to both increased bleeding risk and mortality in this patient cohort.[2,31] Hypersialylation of the fibrinogen molecule in this setting may also lead to defective fibrinogen-to-fibrin conversion possibly underpinning some of the observed coagulopathy on TEG.[32] We demonstrated that TEG has weak or inconsistent relationships with all conventional tests, with the exception of fibrinogen. This implies that the TEG FF assay can be used to guide the clinical management of patients with CLD, given its strong association with conventional fibrinogen levels. To our knowledge, our study is the first to demonstrate this in this population group.

The poor associations between TEG and CCTs measuring coagulation initiation imply that such measures in this cohort remain difficult to interpret and should be used cautiously to inform clinical decisions, adding to available evidence that these tests are poorly predictive of bleeding risk, and poorly reliable in this cohort.[33]

We demonstrated poor association between TEG and conventional measures of fibrinolysis (characterized by low normal TEG LY30% and elevated D-dimer levels), which to our knowledge is the first time this has been demonstrated in this patient group. D-dimer is a nonspecific marker of fibrin degradation, with elevated levels present in a number of disease states including thromboembolism, neoplasia, nonspecific inflammation, or sepsis, with a mild rise in keeping with a multitude of pathophysiological processes in a critically ill population.[34] While evidence suggests that elevated D-dimer in the presence of other plasma abnormalities (ie, elevated tissue plasminogen activator) indicates hyperfibrinolysis and can predict gastrointestinal bleeding in this population, elevated D-dimer alone provides limited information regarding an individual's fibrinolytic state.[35,36] Thromboelastography has been suggested to be superior to conventional methods of detecting clot lysis in other patient groups.[37] Our findings imply that TEG may provide more information than conventional tests in detecting fibrinolysis abnormalities in patients with CLD. Emerging evidence suggests primary hyperfibrinolysis is an increasingly important pathophysiological process in CLD resulting in an increased risk of variceal bleeding, having potentially important implications for coagulation monitoring in this group.[38,39]

Our findings may also be partly explained by the role of factor XIII (FXIII) and its role in clot stabilization. Factor XIII is being increasingly understood to be an important mediator in the coagulopathy of liver disease, with low FXIII associated with more bleeding events and a higher mortality in patients with severe liver disease.[40] Viscoelastic tests, including ROTEM and TEG, have previously been used to demonstrate reduced FXIII activity in patients with severe liver disease, characterized by reduced MA and increased lysis.[41,42] The observed reduced MA and increased clot lysis support existing literature that suggests low FXIII activity, in addition to abnormal fibrinogen level and platelet count, may have an important role in mediating coagulation in this patient group. Finally, our data imply that patients with severe CLD demonstrate a generally hypocoagulable picture, characterized by slower clot formation and reduced clot strength, and conflicting, difficult to interpret fibrinolysis results. By illustrating that prolonged PT/INR/APTT was not generally associated with a prolonged reaction (R) time but that reduced fibrinogen level was consistently associated with reduced clot strength, our study demonstrates how TEG may provide additional coagulation information to clinicians in this patient group, including for use in guiding appropriate blood product administration.[43]

Strengths and Limitations

This study provides a robust, contemporary and comprehensive assessment of the relationship between CCTs and TEG in a cohort of acutely ill patients with CLD. We assessed coagulation across multiple modern TEG assays using modern TEG6S technology, which unlike the previous model, generates 17 parameters across 4 simultaneously executed assay types, as opposed to 6 parameters generated from a single assay, as in the previous models (TEG5000, Haemonetics). Additionally, this is the first study comparing coagulation assessment modalities (TEG and conventional methods) in this patient group (severe CLD) using repeated measures, via daily sampling, thus providing dynamic data in each patient. Finally, these findings have clinical and pathophysiological implications and provide novel information for coagulation management.

This study also carries several limitations. It was a noninterventional, observational study and did not have the capacity to allow outcome-based comparisons to determine the clinical value of TEG over CCTs or assess TEG as a predictor of bleeding or thrombosis. Administration of blood products and anticoagulant medications were confounders in this study. However, clinicians administered blood products in an attempt to restore normal coagulation and despite such product administration, patients with CLD remained coagulopathic. Similarly, around half the patients in this study received anticoagulant drugs; however, this was controlled for by sensitivity analysis. We acknowledge various treatments (including but not limited to various types and quantities of blood product administration, various doses of various pro- and anticoagulant medication, continuous renal replacement therapy, surgery, and/or crystalloid administration) may influence TEG and conventional coagulation results. However, understanding the effect of such therapies on the associations between TEG and CCTs would require significant multivariate analyses and a large sample size and is unfortunately beyond the scope of this observational study. We recognize that TEG is not gold standard for the assessment of coagulation in this patient group; however a number of studies have shown that TEG may be superior to CCTs in assessing bleeding and clotting risk in other patient groups.[43-45] Finally, this study examined all patients presenting with severe CLD and did not assess the differential impact of any specific underlying etiology. However, our study aimed to assess the coagulation state of a heterogeneous population of acutely ill patients with CLD.

Conclusion

In acutely ill patients with CLD, strong associations were observed between TEG measures of both clot formation and clot strength, and conventional fibrinogen level tests. However, weak or inconsistent associations were observed between TEG/CCTs measuring coagulation initiation (ie, TEG R time and INR/APTT/PT), TEG and conventional platelet count, and measures of fibrinolysis (TEG LY30% and conventional D-dimer). This supports available evidence that measures of clotting initiation and speed are difficult to interpret in this cohort, while TEG MA and conventional fibrinogen may be more reliable. Additionally, we observed that both TEG and CCTs demonstrated a hypocoagulable picture in this patient group, characterized by slower clot formation and reduced clot strength. Our findings provide novel information on the relationship between TEG and CCTs and provides rationale for further studies investigating the role of TEG in predicting bleeding and guiding clinical decision-making in this in this coagulopathy-prone, high-risk population.

Appendix A

Method of Conventional Coagulation Test Sample Analysis

Activated partial thromboplastin time was measured via a clot-based assay using purified phospholipid and micronized silica activator (Triniclot HS reagent, TCOAG). Prothrombin time and international normalized ratio (INR) were measured using platelet poor plasma collected in 3.2% citrate anticoagulant, analyzed by a clot-based assay using recombinant TF relipidated in a synthetic phospholipid blend combined with calcium chloride (PT-Recombiplastin reagent, Instrumentation Laboratory). From this, INR was generated using the following equation:

$$\text{INR} = (\text{PT}/\text{mean PT}) \times \text{ISI}$$

PT: prothrombin time; ISI: International Sensitivity Index, provided by Instrumentation Laboratory (IL), manufacturers of the PT reagent, PT Recombiplastin. The ISI is referenced by IL to an international standard. The mean PT and ISI values currently used at Austin Pathology for PT-Recombiplastin lot number N0741093 are 10.6 (seconds) and 0.97, respectively. Fibrinogen level was estimated using derived fibrinogen, from absorbance during the PT clot-based assay relative to a calibrator (PT-Recombiplastin reagent, Instrumentation Laboratory). When derived fibrinogen yielded a result less than 2.0 g/L, a Clauss fibrinogen was measured, which is more accurate below this level. The 2 results show strong correlation within the normal reference interval. D-dimer was measured using latex-enhanced immunoassay (D-dimer HS reagent, Instrumentation Laboratory). Finally, platelet count was taken from the last available routine full blood count recorded as per standard care, rather than at time of sampling. This was in order to both minimize the number of additional blood vials collected, making the study more ethically amenable and to simultaneously negate the impact of citrate on platelet function, known to unpredictably underestimate platelet count. For this purpose, routine full blood count was performed using a Sysmex XE5000 analyser (Sysmex corporation), which utilizes impedance methodology using hydrodynamic focusing.

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Ethics Approval

Ethical approval for this study was obtained from the Human Research Ethics Committees at the Austin Hospital and Monash University (Austin HREC: LNR/15/Austin/70 February 2015, Monash University HREC: CF15/636—2015000291, March 2015).

Informed Consent

Informed consent for patient information to be published in this article was not obtained as this was waived by the above Human Research Ethics Committees, as samples were deemed as part of standard care.

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