Coagulation status of critically ill patients with and without liver disease assessed using a novel thrombin generation analyzer.

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ORIGINAL ARTICLE

Coagulation status of critically ill patients with and without liver disease assessed using a novel thrombin generation analyzer

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Abstract

The liver synthesizes the majority of pro- and anti-coagulant and fibrinolytic proteins, and during liver dysfunction synthesis of these proteins is reduced. The end point of conventional hemostatic tests, such as the prothrombin time (PT), occurs when only 5% of thrombin generation (TG) has taken place and is not sensitive to the effects of natural anti-coagulants. The aim of this study was to determine whether TG in the presence of thrombomodulin (TM) provides more useful information about coagulation potential, in comparison to the PT. Analysis was performed on ST Genesia, a novel TG analyzer from Diagnostica Stago. TG was measured using STG-Thromboscreen, a reagent containing an intermediate concentration of human tissue factor (TF) ± rabbit TM to account for anti-coagulant protein C (PC) activity. Platelet-poor plasma (PPP) samples were from the Intensive Care Study of Coagulopathy-2 (ISOC-2), which recruited patients admitted to critical care with a prolonged PT (3 seconds above the reference range). Despite a prolonged PT, 48.0% and 60.7% of patients in the liver and non-liver groups had TG parameters within the normal range. Addition of TM reduced TG by 34.5% and 41.8% in the liver and non-liver groups, respectively. Interestingly, fresh frozen plasma (FFP) transfusion had no impact on TG. Measurement of TG with addition of TM provides a more informative assessment of coagulation capacity and indicates that hemostasis is balanced in patients with liver disease during critical illness, despite conventional tests suggesting that bleeding risk is increased.

KEYWORDS

critically ill, liver disease, thrombin, thrombomodulin, transfusion

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1 | INTRODUCTION

Thrombin generation (TG) is an essential part of normal hemostasis and contributes to an individual's risk of bleeding or thrombosis. Conventionally, TG is assessed using the prothrombin time (PT) and activated partial thromboplastin time (APTT). However, we now know that these basic coagulation tests provide limited assessment of thrombin generation in vivo and are poor at predicting clinical bleeding.[1-5] This is due to several factors: (a) the tests record clotting time when only 5% of TG has occurred; (b) the tests reflect only the pro-coagulant factors, and do not take in to account natural anti-coagulants;[5-8] and (c) they are insensitive to modest, but clinically relevant reductions in factor concentrations.

As a consequence, there remains a need for better hemostatic tests to reliably predict whether patients with (or without) prolonged PTs are at risk of bleeding and whether they obtain any benefit from plasma transfusion prior to traumatic procedures. This study uses samples obtained from the Intensive Care Study of Coagulopathy 2 (ISOC-2), a heterogeneous group of critically ill patients on intensive care units (ICUs) in which there was uncertainty with regard to bleeding risk and requirement for fresh frozen plasma (FFP) transfusion.[5,9] Introduction of a novel diagnostic assay to assess coagulation, such as TG, could avoid delays in interventional procedures, avoid complications of unnecessary plasma transfusion, and reduce bleeding. FFP is not without risk for the patient[9-16] and yet is frequently administered to non-bleeding patients with mild or moderate abnormalities of PT, for example, as prophylaxis prior to invasive procedures, although evidence indicates negligible effects on correction of any PT prolongation when conventional doses are used.[17-23]

A problem with the introduction of TG into clinical practice is that it is typically a research tool and performed retrospectively on batched blood samples. A novel TG analyzer (ST Genesia, Diagnostica Stago, Asnieres, France) provides the technology to deliver standardized and fully automated assays in vitro,[24] helping to regulate temperature and eliminate manual pipetting errors and reagent variation. The TG measurement is based on the fluorescence principle originally described by Hemker et al.[25] Quality control checks incorporate a reference plasma alongside a low, normal, or high TG plasma to allow validation of the patient results.

Three kits are available commercially: STG-BleedScreen (low TF) to evaluate bleeding risk in hemophilia patients; STG-DrugScreen (high TF) to monitor the effect of anti-thrombotic drugs, such as direct oral anti-coagulants (DOAC) and Vitamin K antagonists (VKA); and STG-ThromboScreen (intermediate TF) to evaluate thrombotic risk in patients with thrombophilia or recurrent deep vein thrombosis (DVT). In this study, we use the STG-ThromboScreen kit, which incorporates thrombomodulin (TM) into the TG test allowing activation of the potent PC anti-coagulant pathway and allowing assessment of both arms of hemostasis. Our earlier work on ISOC-2 identified that many patients had evidence of normal endogenous thrombin potential coagulopathy (ETP-coagulopathy), despite prolongation of PT, but did not incorporate TM in the TG assay.[5,9]

Essentials

- The end point of conventional hemostatic tests occurs when only 5% of thrombin generation has taken place and is insensitive to natural anti-coagulants.
- Thrombin generation performed on a fully automated analyzer in the presence of thrombomodulin may provide a more accurate interpretation of an individual's coagulation potential.
- Patients with liver disease have balanced hemostasis and are not significantly different from those without liver disease.
- Fresh frozen plasma transfusion did not alter a patients thrombin generation and may not be necessary for minor procedures.

TM is a transmembrane protein expressed on the surface of endothelial cells that forms a complex with thrombin, switching its function from pro- to anti-coagulant.[26-28] The thrombin-TM complex activates PC, which in turn downregulates coagulation by cleavage of activated factor V (FVa) and factor VIII (FVIIIa).[26-28] It also activates thrombin activatable fibrinolysis inhibitor (TAFI) to attenuate fibrinolysis.[29-31] Addition of TM enables the TG measurement to test these aspects of the coagulation system in patients with complex hemostatic alterations, including those with liver disease. In liver disease, coagulation factor synthesis is decreased because the liver is responsible for synthesizing many anti-coagulant and fibrinolytic factors, as well as pro-coagulant factors.[32] This group of patients is frequently regarded as hypocoagulable, although research has indicated that in fact hemostasis may be re-balanced in this group of patients.[33-36] Here, we discuss the use of a novel TG analyzer to monitor critically ill patients with liver disease, to compare findings with patients without liver disease, and to determine the effects of plasma transfusion on TG parameters.

2 | METHODS

2.1 | Plasma samples

Platelet-poor citrated plasma (PPP) samples were obtained from the ISOC-2 trial,[5] which recruited patients admitted to critical care with impaired coagulation. This was defined by a PT 3 seconds above the upper limit of the normal reference range within 48 hours of admission. Any patients with evidence of active clinical bleeding or receiving treatment-dose anti-coagulant therapy were excluded. Samples were not taken from lines used for heparin infusions or those blocked and flushed with fibrinolytic drugs. Anti-factor Xa

levels were performed in all samples to check for heparin contamination. One patient received prophylaxis with an anti-platelet agent (epoprostenol). Samples were taken upon admission, pre-plasma infusion, post-plasma infusion, and at the end of the study (5 days after entry). PPP samples were stored at −80°C since commencement of the original ISOC-2 study in 2014 and did not undergo any freeze-thaw cycles prior to the analysis performed in this substudy. This substudy focuses on patients with liver disease (n = 78) as assigned by the treating clinician, which are compared to those critically ill patients without liver disease (n = 94). Liver disease was defined by the referring clinician (Figure 1). Baseline clinical characteristics for both patient groups are described in Table 1. Normal reference ranges were calculated as mean ± 1.96 x standard deviation (SD) from 45 healthy volunteers.

2.2 | Thrombin generation

The ST Genesia incorporates a fully automated and standardized TG method.[24,37,38] On each day of testing a new calibration test, three levels of quality control (low, normal, and high TM resistance), and a reference plasma to normalize parameters are assessed. PPP samples were thawed at 37°C for 10 minutes before beginning the TG test, which was performed using the STG-Thromboscreen kit, which contains pro-coagulant phospholipids, an intermediate picomolar concentration of human recombinant TF ± rabbit lung TM. TG was initiated by addition of the fluorogenic substrate and calcium chloride. Lag time, peak height, time to peak, velocity index, start tail, and ET), were extracted from the Thrombograms.

2.3 | Data analysis

Access to the full ISOC-2 case report form (CRF) allowed comparison of TG parameters with conventional laboratory tests and incidence of bleeding. Bleeding was defined using the Hemorrhage Measurement (HEME) assessment tool. Results are represented as individual data points and display the mean ± SD. Statistical analysis was performed using Graph Pad Prism 8.0 and normality assessed using a D'agostino-Pearson omnibus test. A non-parametric Mann-Whitney t-test was used to analyze the data. $P < .05$ was considered significant.

3 | RESULTS

3.1 | TM-ETP

The ST Genesia was used to measure TG in two groups of patients from the ISOC-2 study: those with liver disease (n = 78) and non-liver disease patients (n = 94; Figure 1, Table 1). Patients recruited to the study had abnormal routine clotting test results, but despite a prolonged PT, many of the patients had TG parameters within the normal range.

The ETP is the most commonly reported TG parameter and has been taken to represent an individual's risk of bleeding or thrombosis. Despite a prolonged PT as a requirement for recruitment to the ISOC-2 study, 48% of patients in the liver group had normal ETP (912.4-1715.6 nmol/L/min), and the remaining 52% were below the normal limit (<912.4 nmol/L/min) (Figure 2A). In the non-liver group,

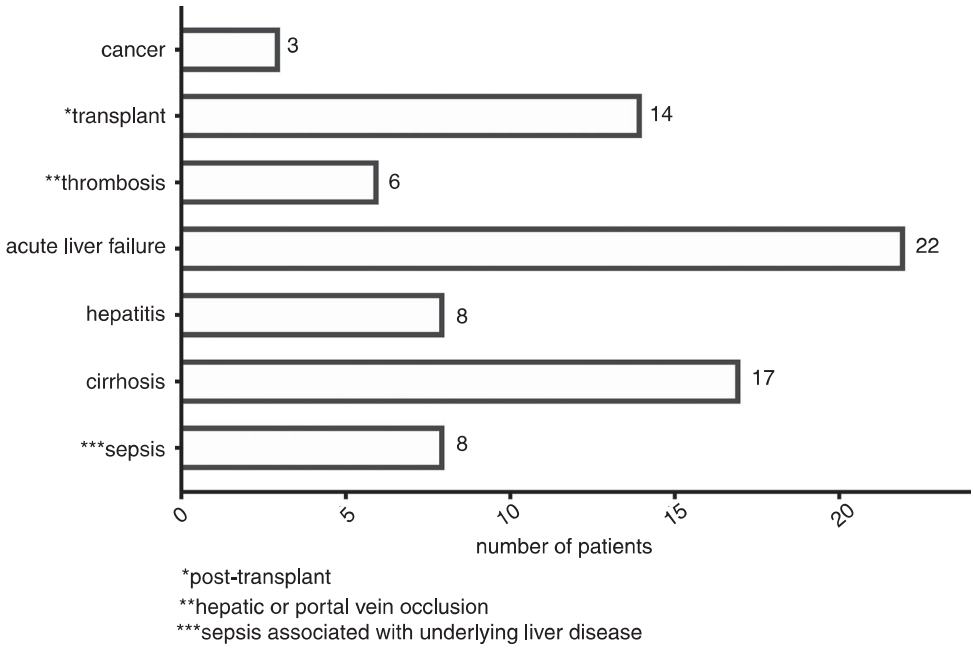

FIGURE 1 Etiology of liver disease. Bar chart illustrating the number of patients within the liver cohort and the diagnosis or cause of liver disease for each patient

TABLE 1 Baseline clinical characteristics for patients in the liver (n = 78) and non-liver (n = 94) cohorts

	Liver	Non-liver
Route of administration		
Age (years)	54 ± 13	64 ± 15
Sex (male)	50 (64)	50 (53.2)
Weight (kg)	79 ± 19	78 ± 28
PT on enrollment (s)	27 ± 13	24 ± 7
APTT (s)	41 ± 13	39 ± 10
Fibrinogen (g/L)	2.8 ± 2	3.9 ± 2
Platelet (x 10^9/L)	107 ± 71	161 ± 113
Emergency department	18 (23)	25 (26)
Ward	29 (37)	56 (59)
Hospital transfer	11 (14)	1 (1)
Operating room	20 (25)	12 (13)
ICNARC code		
Sepsis	0	69 (73)
Liver	78 (100)	0
Gastrointestinal	0	8 (9)
Respiratory	0	4 (4)
Cardiovascular	0	10 (11)
Neurological	0	1 (1)
Endocrine	0	1 (1)
Haemotologic	0	1 (1)

Note: This includes the mean age, sex, weight, and prothrombin time test result that triggered enrollment to the initial Intensive Care Study of Coagulopathy-2 study. Patients are assigned a pathway to intensive care unit admission: emergency department, ward, hospital transfer, or operating room. Patients with liver disease were identified by the referring clinician and given an intensive care national audit and research centre (ICNARC) liver category code. The remaining group of patients (non-liver) had varied ICNARC codes.

more variation was observed; 34%, 60.7%, and 5.3% of patients had low, normal, or elevated ETP, respectively (Figure 2A).

Addition of TM (TM-ETP) reduced thrombin generation in both groups, as well as normal controls (Figure 2B). In the liver group 42% of patients had normal TM-ETP, and 21.8% and 35.9% of patients had low and high TM-ETP, respectively (Figure 2B). In the non-liver group, 21.3% of patients were within the normal range for TM-ETP, and 28.7% and 50% had low and high TM-ETP, respectively (Figure 2B). The liver and non-liver groups were not significantly different in the presence of TM (Figure 2B).

The effect of TM was recorded as the % ETP inhibition, which was lower in both liver and non-liver patients (34.5 ± 2.5% and 41.8 ± 2.4%, respectively) compared to healthy volunteers (59.7% ± 2.9%; Figure 2C). After addition of TM, the inter-individual variation in ETP was reduced in both patient groups, which was expressed as the coefficient of variance of the ETP ± TM; 42.42% versus 66.52% in the non-liver group and 40.71% versus 36.2% in the liver group (Figure 2A,B).

3.2 | The effect of TM on TG parameters

The effect of TM was evaluated in the remaining TG parameters (Figure 3). Addition of TM significantly altered the lag time, peak height, and ETP in both groups of patients, and time to peak in non-liver patients (Figure 3A-C, Figure 1A,B, $P < .0001$). TM did not influence the velocity index or start tail (Figure 3D-E).

Despite a prolonged PT, 79.5% of the liver patients and 56.6% in the non-liver group had a normal TM-lag time (1.3-4.6 minutes; Figure 4A). The TM lag time in patients with liver disease was significantly shorter than those without (3.8 ± 1.6 versus 5.9 ± 2.8 minutes, respectively; Figure 4A).

The TM peak height was not significantly different between the two patient groups, again suggesting that patients with liver disease do not have unduly impaired coagulation (Figure 4B). Interestingly, the TM time to peak was significantly shorter in the liver group than in the non-liver group: 6 ± 2 versus 8.8 ± 3.6 minutes, respectively (Figure 4C). Additionally, 75.6% of liver patients had a TM time to peak within the normal range (3.3-6.9 minutes) in comparison to only 46.4% in the non-liver group (Figure 4C). The difference in TM time to peak between liver and non-liver patients may be due to increased PC activation in the non-liver group. Activated PC (APC) limits the concentration of FVa, thus prolonging the time to peak, and the lack of effect on the TM-peak height may be explained by reduction in other anti-coagulant factors.

3.3 | Effect of PC

Lower TG in the liver group compared to non-liver disease patients may be explained by decreased PC.[39,40] Therefore, to establish whether PC was also reduced in non-liver patients and responsible for low TG, PC activity was measured (Figure 4D); PC was recorded below the normal limit (0.7 IU/mL) in 98% of patients in both liver and non-liver groups (Figure 4D). The liver patients had significantly lower PC than the non-liver group (0.3 ± 0.2 versus 0.42 ± 0.2 IU/mL, $P < .01$).

3.4 | Correlation of TG parameters and conventional tests

Analysis of the remaining TG parameters revealed a strong positive correlation between TM peak height and TM-ETP ($r^2 = .6552$; Figure 5A). No correlation was observed between the other TG parameters, suggesting that each parameter describes a different aspect of coagulation.

Interestingly, no TG parameters correlated with conventional laboratory tests, including PT, APTT, von Willebrand factor (VWF), C-reactive protein (CRP), Clauss fibrinogen, or platelet count (Figure 5B). This was reflected in the r^2 correlation co-efficients with TM-ETP: .0036, .004, .0041, .00051, and .0061 for VWF, CRP, PT, platelet count, and fibrinogen, respectively (Figure 5B).

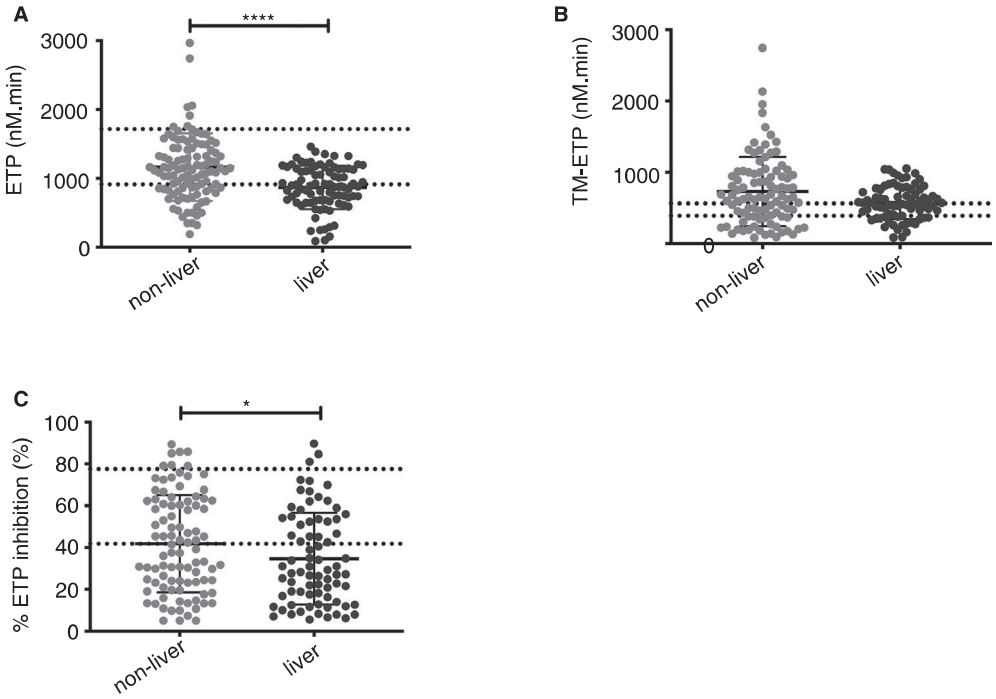

FIGURE 2 Thrombomodulin in thrombin generation (TG) provides a global assessment of pro- and anti-coagulant factors. TG was measured using ST Genesia and STG-Thromboscreen (STG-TBS) kits. STG-TBS initiates TG with an intermediate concentration of human recombinant tissue factor ± rabbit lung thrombomodulin (TM). Patients were split into two distinct groups: non-liver and liver patients. Normal reference ranges were calculated as mean ± 1.96 x standard deviation from 45 healthy volunteers (dotted line). A, Endogenous thrombin potential (ETP) in the absence and (B) presence of TM (TM-ETP). C, % ETP inhibition is calculated by the analyzer and represents the extent of activated protein C inhibition on TG. *P < .05, ****P < .0001

3.5 | Longitudinal changes

Samples taken upon enrolment to the study (Day 1) and at the end of the 5-day study period (Day 5) were not significantly different from one another when measuring TM-ETP (Figure 6). However, the PT measurement was significantly different between the two time points, showing a shortening of the PT over time (Figure 6). This may be explained by the contribution of anti-coagulant factors to the TG test, for which the PT test does not account.

Often patients are administered blood components or drug treatments based on routine coagulation test results. In our analysis, FFP transfusion had no identifiable effect on TG parameters (Figure 7A, P = .81).

3.6 | Assessment of bleeding events using TG parameters

As discussed in our earlier manuscript,[5] 16 major bleeds, defined as blood loss >300 mL, were recorded and 4.9% of these patients were treated with FFP transfusion (Figure 7B). The majority of major bleeds (84%) were within the liver group. A further 28 bleeding events were recorded as minor bleeds of whom 3.8% received FFP (Figure 7B). Interestingly, only 42% of patients who received FFP transfusion had a prothrombin ratio > 2% and 75% had normal TG, defined as TM-ETP within 387.32 - 561.88 nmol/L/min.

Thrombin generation parameters were split into three categories: low, normal, and elevated TM-ETP. This was defined as < 387.3, 387.3 - 561.9, and > 561.9 nmol/L/min (Figure S1 in supporting information). Patients were then categorized as having low or high platelets (< or > 100 x 10^9 plts/L) and low or high fibrinogen (< or > 2 g/L), and bleeding events analyzed in each category (Figure S1). Interestingly, bleeding events were identified across all categories, including patients with elevated TM-ETP, fibrinogen, or platelets (Figure S1).

4 | DISCUSSION

It was previously thought that liver patients were hypocoagulable due to their decreased levels of measured coagulation factors and prolonged clotting in conventional tests. Our results support evidence[33,41-48] that the reduction of pro-coagulant factors is balanced by the simultaneous reduction of anti-coagulant factors in liver disease. In the literature, PC is described as the key factor responsible for re-balancing hemostasis; however, it is important to note the contribution of other pro- and anti-coagulant factors.[32,49-51] It has recently been shown that FVIII and VWF synthesis is increased,[35,52,53] whereas antithrombin and tissue factor pathway inhibitor (TFPI)-protein S levels are decreased[54] in liver disease. This provides a rationale for the addition of TM in the TG assay and our findings for longitudinal monitoring suggest

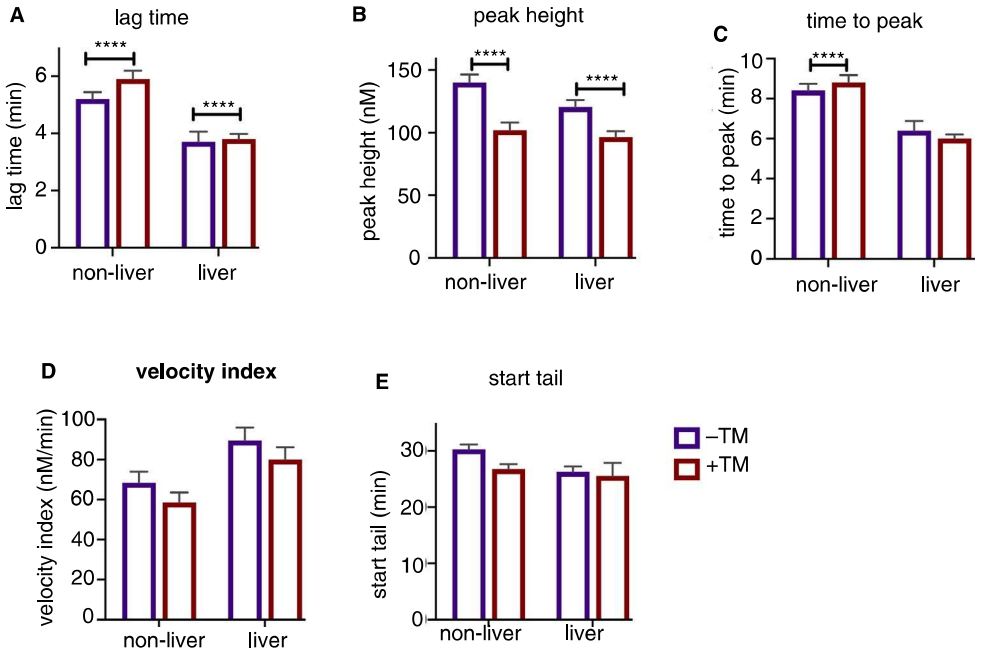

FIGURE 3 The effect of thrombomodulin (TM) on thrombin generation parameters. Thrombin generation (TG) was measured using ST Genesia and STG-Thromboscreen (STG-TBS) kits. STG-TBS initiates TG with an intermediate concentration of human recombinant tissue factor ± rabbit lung TM. Patients were split into two distinct groups: non-liver and liver patients. Lag time (A), peak height (B), time to peak (C), velocity index (D), and start tail (E) were measured. Normal reference ranges were calculated as mean ± 1.96 x standard deviation from 45 healthy volunteers (dotted line). **** $P < .0001$

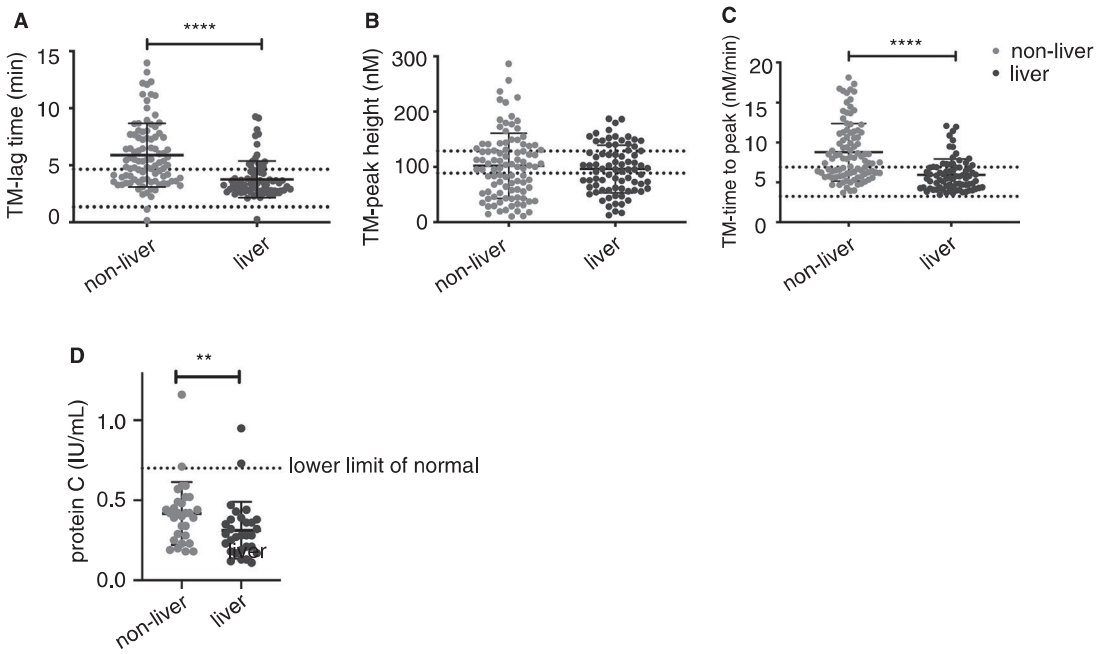

FIGURE 4THROMBOMODULIN(TM) peak height is strongly correlated with TM- endogenous thrombin potential (ETP). Thrombin generation (TG) was measured using ST Genesia and STG-Thromboscreen (STG-TBS) kits. STG-TBS initiates TG with an intermediate concentration of human recombinant tissue factor in presence of rabbit lung TM. Patients were split into two distinct groups: non-liver and liver patients. Normal reference ranges were calculated as mean ± 1.96 x standard deviation from 45 healthy volunteers (dotted line). Lag time (A), peak height (B), and time to peak (C) were measured in both liver and non-liver patients. **** $P < .0001$ (D) Protein C antigen was measured using Berichrom Protein C kit (Siemens) and Sysmex CS-5100 haemostasis analyzer. ** $P < .01$

many patients have a stable profile of TG during admission, despite many changes in treatment and condition. A limitation of our study is the heterogenous nature of the liver patient group, which includes post-transplant patients, sepsis associated with underlying liver disease, and cirrhosis; all which have different disease etiology (Figure 1).

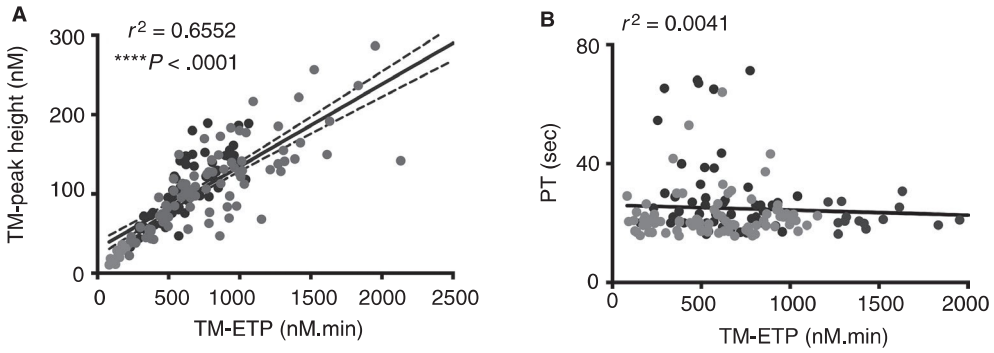

FIGURE 5 There is no correlation between standard laboratory tests and thrombomodulin endogenous thrombin potential (TM-ETP). Thrombin generation (TG) was measured using ST Genesia and STG-Thromboscreen (STG-TBS) kits. STG-TBS initiates TG with an intermediate concentration of human recombinant tissue factor in the presence rabbit lung TM. Patients were split into two distinct groups: non-liver and liver patients. A, Scatter plot deciphering a strong positive correlation between TM-peak height and TM-ETP (r^2 = .6552). B, prothrombin time (PT) was measured at sample collection during the Intensive Care Study of Coagulopathy-2 study and obtained from the case report form. The result was plotted against TM-ETP and correlation assessed using linear regression

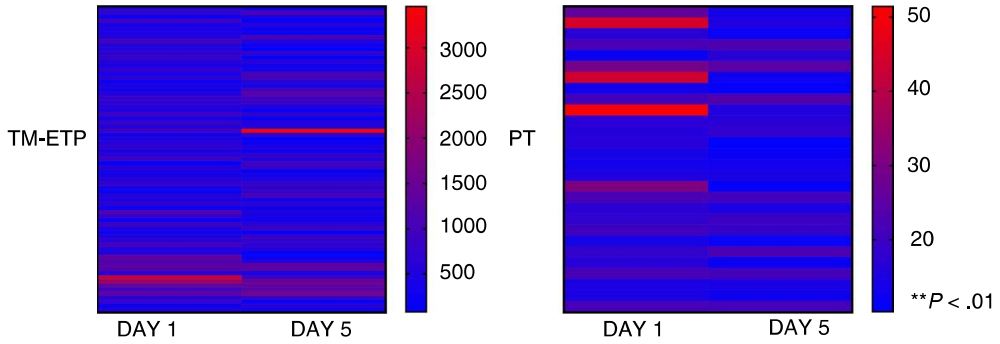

FIGURE 6 Comparison of thrombomodulin endogenous thrombin potential (TM-ETP) and prothrombin time (PT) over the study time period. TM-ETP and PT were measured at Day 1 and Day 5 in liver and non-liver patients. Thrombin generation (TG) was measured using ST Genesia and STG-Thromboscreen (STG-TBS) kits. STG-TBS initiates TG with an intermediate concentration of human recombinant tissue factor in the presence of rabbit lung TM. PT was measured at sample collection during the Intensive Care Study of Coagulopathy-2 study and obtained from the case report form. **P < .01

This study explored whether an algorithm could better predict bleeding in patients admitted to critical care with the aim to reduce unnecessary FFP transfusion. We were unable to develop a clear relationship between any of the coagulation parameters and bleeding risk (Figure S1), suggesting bleeding is multi-factorial, ie, low platelets and high factor VIII or high VWF and low fibrinogen. Other reasons might include the limited number of bleeding events recorded, additional factors contributing to bleeding in an individual patient, and the circumstances around bleeding. It remains possible that any coagulation test, including TM-ETP, is insensitive to bleeding risk prediction and this may argue against emphasizing the need for coagulation testing in many patients, such as prior to invasive procedures. A limitation to our study was the absence of precise details of the bleeding events, and it is important to recognize that many of the bleeds may be associated with surgical or mechanical interventions, and not with hemostatic failure per se. The site of the bleed may be another factor determining outcomes, for example intracranial compared to wound-related or non-traumatic intra-articular bleeds.

A recent study carried out by the European Society of Intensive Care Medicine (ESICM) surveyed transfusion practice in the non-bleeding critically ill.[55] The study found practice in plasma and platelet transfusion is heterogenous and local transfusion guidelines were lacking in the majority (71%) of ICUs.[55] Our results indicate the inefficacy of FFP transfusion on TG and continues to provide reassurance to clinicians that it is not necessary for patients within near normal, conventional coagulation tests. Samples taken upon enrolment (Day 1) and at the end (Day 5) of the study were not significantly different when measuring TM-ETP (Figure 6). We were unable to determine the effect of prophylactic plasma transfusion on PT in this cohort; however, other studies have addressed this question and found no effect.[17-19,21,23]

Although TG provides a different assessment of an individual's hemostatic status than conventional coagulation tests, and is a more representative test of what occurs in vivo, it remains incompletely physiological in some aspects. These include replacement of platelets with synthetic phospholipids,[56,57] absence of cell and vessel wall components including endothelial cells (which is where TM is

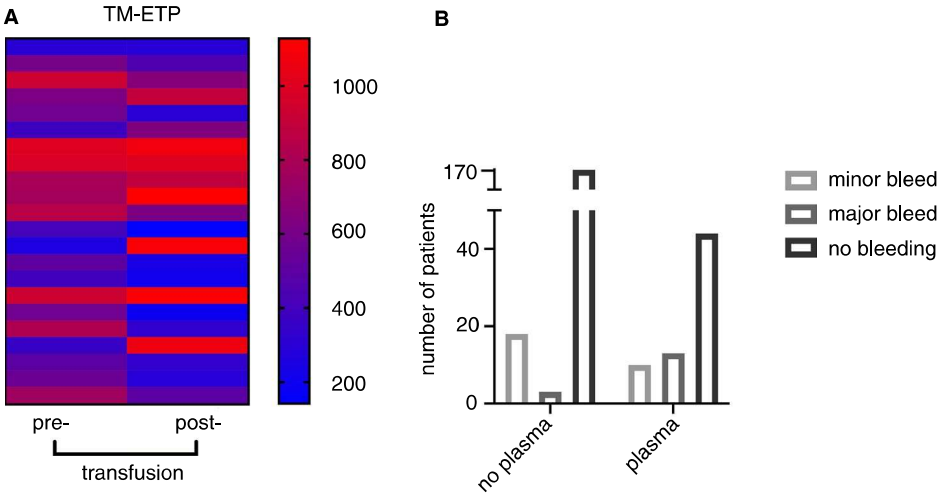

FIGURE 7 Fresh frozen plasma (FFP) transfusion was independent of bleeding events. A, Patients who received FFP had samples taken before and after transfusion. Thrombin generation (TG) was measured using ST Genesia and STG-Thromboscreen (STG-TBS) kits. STG-TBS initiates TG with an intermediate concentration of human recombinant tissue factor in the presence of rabbit lung thrombomodulin (TM). B, Bleeding was characterized according to the Hemorrhage Measurement (HEME) assessment tool, and recorded as a minor or major bleed. Data shows bleeding events in patients who did and did not receive FFP transfusion

expressed),[56,57] and as this is a static system, the impact of flow, which removes activated factors, and alters thrombus formation and subsequently, structure.[58-60] A disadvantage of using TM-ETP is that it cannot be calculated until TG has come to an end, ie, when all thrombin in the sample has been inhibited by anti-thrombin. In critically ill patients, this can take up to 120 minutes (Figure 4C). Thus, TM peak height may be beneficial as a predictor of TM-ETP and for use in clinical monitoring of critically ill patients. The lack of correlation of TM-ETP with conventional laboratory tests, such as VWF, CRP, platelet count, or fibrinogen, supports our hypothesis that current hemostatic tests provide only a limited assessment of hemostatic capacity.

Our data support previous observations that measurement of TG in the presence of TM provides a global assessment of pro- and anti-coagulant factors. Second, comparison of TM-ETP and ETP indicates that hemostasis is balanced in critically ill patients with liver disease, and that this results from their decreased levels of PC (Figure 2 A,B; Figure 4D). In summary, our results support the need for a novel diagnostic strategy based on TG, and the ST Genesia should be considered for future use in clinics to identify critically ill patients who do not require FFP transfusion.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

Diagnostica Stago had no role in the design, conduct, analysis, or write-up of the study. MJRD received consultancy fees from Takeda. GBM, JB, SH, PB, NC, SJS, and MAL have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

GBM performed the research, analyzed the data, and wrote the manuscript; JB, SH, and PB performed the research; MJRD analyzed the data; NC, SJS, and ML supervised the research, analyzed the data, and wrote the manuscript.

REFERENCES

1. Mann KG. Thrombin formation. *Chest*. 2003;124(3 Suppl):4S-10S.
2. Mann KG, Brummel K, Butenas S. What is all that thrombin for? *J Thromb Haemost*. 2003;1(7):1504-1514.
3. Dahlback B. Progress in the understanding of the protein C anticoagulant pathway. *Int J Hematol*. 2004;79(2):109-116.
4. Tripodi A, Chantarangkul V, Mannucci PM. Acquired coagulation disorders: revisited using global coagulation/anticoagulation testing. *Br J Haematol*. 2009;147(1):77-82.
5. Stanworth SJ, Desborough MJR, Simons G, *et al*. Clinical bleeding and thrombin generation in admissions to critical care with prolonged prothrombin time: an exploratory study. *Transfusion*. 2018;58(6):1388-1398.
6. Tripodi A. Thrombin generation assay and its application in the clinical laboratory. *Clin Chem*. 2016;62(5):699-707.
7. Deitcher SR. Interpretation of the international normalised ratio in patients with liver disease. *Lancet*. 2002;359(9300):47-48.
8. Burns ER, Goldberg SN, Wenz B. Paradoxic effect of multiple mild coagulation factor deficiencies on the prothrombin time and activated partial thromboplastin time. *Am J Clin Pathol*. 1993;100(2):94-98.
9. Stanworth SJ, Walsh TS, Prescott RJ, *et al*. A national study of plasma use in critical care: clinical indications, dose and effect on prothrombin time. *Crit Care*. 2011;15(2):R108.
10. Stainsby D,Walsh TS, Prescott RJ, *et al*. Serious hazards of transfusion: a decade of hemovigilance in the UK. *Transfus Med Rev*. 2006;20(4):273-282.
11. Holness L, Knippen M, Simmons L, Lachenbruch P. Fatalities caused by TRALI. *Transfus Med Rev*. 2004;18(3):184-188.

12. Dara SI, Rana R, Afessa B, *et al*. Fresh frozen plasma transfusion in critically ill medical patients with coagulopathy. *Crit Care Med*. 2005;33(11):2667-2671.
13. Gajic O, Dzik WH, Toy P. Fresh frozen plasma and platelet transfusion for nonbleeding patients in the intensive care unit: benefit or harm? *Crit Care Med*. 2006;34(5 Suppl):S170-S173.
14. Khan H, Belsher J, Yilmaz M, *et al*. Fresh-frozen plasma and platelet transfusions are associated with development of acute lung injury in critically ill medical patients. *Chest*. 2007;131(5):1308-1314.
15. Rana R, Fernandez-Perez ER, Khan SA, *et al*. Transfusion-related acute lung injury and pulmonary edema in critically ill patients: a retrospective study. *Transfusion*. 2006;46(9):1478-1483.
16. Popovsky MA. Transfusion-associated circulatory overload: the plot thickens. *Transfusion*. 2009;49(1):2-4.
17. Cahill C, Blumber N, Sharma A, *et al*. Is prophylactic plasma transfusion prior to interventional radiology procedures effective in correcting INR. *Transfusion* 2017. Conference: AABB annual meeting 2017. United states. 57:176A.
18. Cahill C, Gore E, Sharma A, *et al*. Plasma transfusion for INR correction before interventional radiology procedures. *Anesth Analg*. 2017. Conference: 2017 annual meeting of the society for the advancement of blood management, SABM 2017. United states, 125: 14-15.
19. Hoang NS, Kothary N, Saharan S, *et al*. Administering blood products before selected interventional radiology procedures: developing, applying, and monitoring a standardized protocol. *J Am Coll Radiol*. 2017;14(11):1438-1443.
20. Patel MD, Joshi SD. Abnormal preprocedural international normalized ratio and platelet counts are not associated with increased bleeding complications after ultrasound-guided thoracentesis. *AJR Am J Roentgenol*. 2011;197(1):W164-W168.
21. Muller MC, Arbous MS, Spoelstra-de Man AM, *et al*. Transfusion of fresh-frozen plasma in critically ill patients with a coagulopathy before invasive procedures: a randomized clinical trial (CME). *Transfusion*. 2015;55(1):pp. 26–35; quiz 25.
22. Puchalski JT, Argento AC, Murphy TE, Araujo KLB, Pisani MA. The safety of thoracentesis in patients with uncorrected bleeding risk. *Ann Am Thorac Soc*. 2013;10(4):336-341.
23. Rassi AB, d'Amico EA, Tripodi A, *et al*. Fresh frozen plasma transfusion in patients with cirrhosis and coagulopathy: effect on conventional coagulation tests and thrombomodulin-modified thrombin generation. *J Hepatol*. 2020;72(1):85-94.
24. Douxfils J, MorimontL, BouvyC, *et al*. Assessment of the analytical performances and sample stability on ST Genesia system using the STG-DrugScreen application. *J Thromb Haemost*. 2019;17(8):1273-1287.
25. Hemker HC, Willems GM, Beguin S. A computer assisted method to obtain the prothrombin activation velocity in whole plasma independent of thrombin decay processes. *Thromb Haemost*. 1986;56(1):9-17.
26. Davenport RA, Guerreiro M, Frith D, *et al*. Activated protein C drives the hyperfibrinolysis of acute traumatic coagulopathy. *Anesthesiology*. 2017;126(1):115-127.
27. Esmon CT. The roles of protein C and thrombomodulin in the regulation of blood coagulation. *J Biol Chem*. 1989;264(9):4743-4746.
28. Esmon CT. The protein C pathway. *Chest*. 2003;124(3 Suppl):26S-32S.
29. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. *J Biol Chem*. 1996;271(28):16603-16608.
30. Mutch NJ, Thomas L, Moore NR, Lisiak KM, Booth NA. TAFIa, PAI-1 and alpha-antiplasmin: complementary roles in regulating lysis of thrombi and plasma clots. *J Thromb Haemost*. 2007;5(4):812-817.
31. Nesheim M, Wang W, Boffa M, *et al*. Thrombin, thrombomodulin and TAFI in the molecular link between coagulation and fibrinolysis. *Thromb Haemost*. 1997;78(1):386-391.
32. Lisman T, Porte RJ. Pathogenesis, prevention, and management of bleeding and thrombosis in patients with liver diseases. *Res Pract Thromb Haemost*. 2017;1(2):150-161.
33. Hugenholtz GC, Adelmeijer J, Meijers JCM, *et al*. An unbalance between von Willebrand factor and ADAMTS13 in acute liver failure: implications for hemostasis and clinical outcome. *Hepatology*. 2013;58(2):752-761.
34. Lisman T, Bakhtiari K, Adelmeijer J, *et al*. Intact thrombin generation and decreased fibrinolytic capacity in patients with acute liver injury or acute liver failure. *J Thromb Haemost*. 2012;10(7):1312-1319.
35. Lisman T, Bongers TN, Adelmeijer J, *et al*. Elevated levels of von Willebrand Factor in cirrhosis support platelet adhesion despite reduced functional capacity. *Hepatology*. 2006;44(1):53-61.
36. Lisman T, Leebeek FWG, Mosnier LO, *et al*. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. *Gastroenterology*. 2001;121(1):131-139.
37. Calzavarini S, Brodard J, Quarroz C, *et al*. Thrombin generation measurement using the ST Genesia thrombin generation system in a cohort of healthy adults: normal values and variability. *Res Pract Thromb Haemost*. 2019;3(4):758-768.
38. Siguret V,Abdoul J, Delavenne X, *et al*. Rivaroxaban pharmacodynamics in healthy volunteers evaluated with thrombin generation and the active protein C system: Modeling and assessing interindividual variability. *J Thromb Haemost*. 2019;17(10):1670-1682.
39. Bell H, Abdoul J, Delavenne X. Protein C in patients with alcoholic cirrhosis and other liver diseases. *J Hepatol*. 1992;14(2–3):163-167.
40. Saray A, Mesihovic R, Vanis N, Amila M. Protein C deficiency in chronic hepatitis C: correlation with histological extent of liver fibrosis. *Clin Appl Thromb Hemost*. 2017;23(1):72-77.
41. Tripodi A, Primignani M, Chantarangkul V, *et al*. Thrombin generation in patients with cirrhosis: the role of platelets. *Hepatology*. 2006;44(2):440-445.
42. Tripodi A, Salerno F, Chantarangkul V, *et al*. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. *Hepatology*. 2005;41(3):553-558.
43. Groeneveld D, Porte RJ, Lisman T. Thrombomodulin-modified thrombin generation testing detects a hypercoagulable state in patients with cirrhosis regardless of the exact experimental conditions. *Thromb Res*. 2014;134(3):753-756.
44. Gatt A, Riddell A, Calvaruso V, *et al*. Enhanced thrombin generation in patients with cirrhosis-induced coagulopathy. *J Thromb Haemost*. 2010;8(9):1994-2000.
45. Youngwon N, Kim J-E, Lim HS, Han K-S, Kim HK. Coagulation proteins influencing global coagulation assays in cirrhosis: hypercoagulability in cirrhosis assessed by thrombomodulin-induced thrombin generation assay. *Biomed Res Int*. 2013;2013:856754.
46. Kremers RMW, Kleinegris M-C, Ninivaggi M, *et al*. Decreased prothrombin conversion and reduced thrombin inactivation explain rebalanced thrombin generation in liver cirrhosis. *PLoS ONE*. 2017;12(5):e0177020.
47. Lebreton A, Sinegre T, Pereira B, *et al*. Plasma hypercoagulability in the presence of thrombomodulin but not of activated protein C in patients with cirrhosis. *J Gastroenterol Hepatol*. 2017;32(4): 916-924.
48. Raparelli V,Basili S, Carnevale R, *et al*. Low-grade endotoxemia and platelet activation in cirrhosis. *Hepatology*. 2017;65(2):571-581.
49. Lisman T, Caldwell SH, Burroughs AK, *et al*. Hemostasis and thrombosis in patients with liver disease: the ups and downs. *J Hepatol*. 2010;53(2):362-371.
50. Caldwell SH, Hoffman M, Lisman T, *et al*. Coagulation disorders and hemostasis in liver disease: pathophysiology and critical

assessment of current management. *Hepatology*. 2006;44(4):1039-1046.
51. Roberts LN, Patel RK, Arya R. Haemostasis and thrombosis in liver disease. *Br J Haematol*. 2010;148(4):507-521.
52. Sinegre T,Duron C, Lecompte T, *et al*. Increased factor VIII plays a significant role in plasma hypercoagulability phenotype of patients with cirrhosis. *J Thromb Haemost*. 2018;16(6):1132-1140.
53. Hollestelle MJ, Geertzen H, Straatsburg I, *et al*. Factor VIII expression in liver disease. *Thromb Haemost*. 2004;91(2):267-275.
54. Tripodi A, Primignani M, Chantarangkul V, *et al*. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology*. 2009;137(6):2105-2111.
55. de Bruin S, Scheeren TWL, Bakker J, *et al*. Transfusion practice in the non-bleeding critically ill: an international online survey-the TRACE survey. *Crit Care*. 2019;23(1):309.
56. Aldea GS,Soltow LO, Chandler WL, *et al*. Limitation of thrombin generation, platelet activation, and inflammation by elimination of cardiotomy suction in patients undergoing coronary artery bypass grafting treated with heparin-bonded circuits. *J Thorac Cardiovasc Surg*. 2002;123(4):742-755.
57. Duarte RCF, Ferreira CN, Rios DRA, *et al*. Thrombin generation assays for global evaluation of the hemostatic system: perspectives and limitations. *Rev Bras Hematol Hemoter*. 2017;39(3):259-265.
58. Swieringa F, Baaten CCFMJ, Verdoold R, *et al*. Platelet control of fibrin distribution and microelasticity in thrombus formation under flow. *Arterioscler Thromb Vasc Biol*. 2016;36(4):692-699.
59. Weiss HJ, Turitto VT, Baumgartner HR. Role of shear rate and platelets in promoting fibrin formation on rabbit subendothelium. Studies utilizing patients with quantitative and qualitative platelet defects. *J Clin Invest*. 1986;78(4):1072-1082.
60. Sakariassen KS, Orning L, Turitto VT. The impact of blood shear rate on arterial thrombus formation. *Future Sci OA*. 2015;1(4):FSO30.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX

ISOC-2 STUDY GROUP HOSPITAL SITES AND INDIVIDUALS

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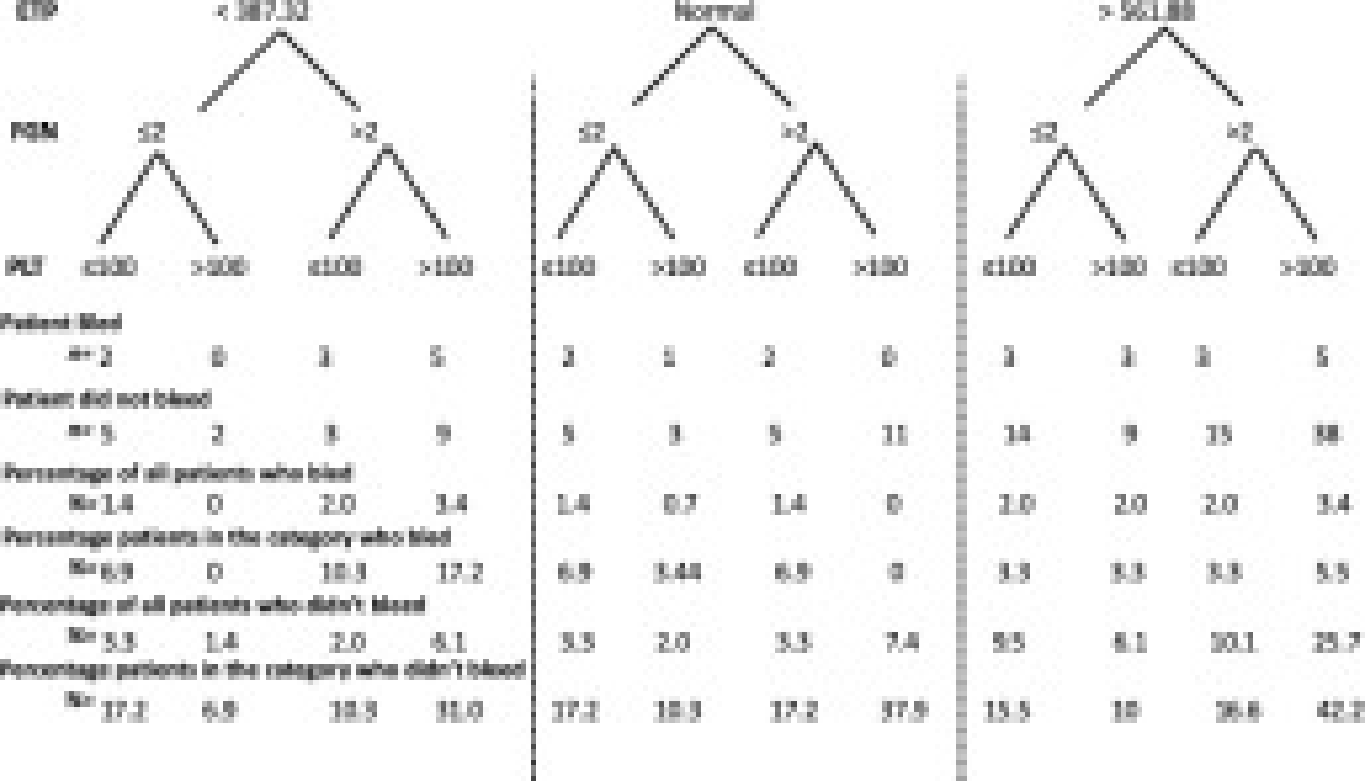
ETP
< 387.33
Normal
> 561.88
PSN
≤2
>2
≤2
>2
≤2
>2
PLT
≤100
>100
≤100
>100
≤100
>100
≤100
>100
≤100
>100
≤100
>100
Patient Bled
No 2 0 3 5 3 1 2 0 3 3 3 5
Patient did not bleed
No 5 2 8 9 5 3 5 11 14 9 15 38
Percentage of all patients who bled
No 1.4 0 2.0 3.4 1.4 0.7 1.4 0 2.0 2.0 2.0 3.4
Percentage patients in the category who bled
No 6.9 0 10.3 17.2 6.9 3.44 6.9 0 3.3 3.3 3.3 5.5
Percentage of all patients who didn't bleed
No 3.5 1.4 2.0 6.1 3.5 2.0 3.5 7.4 9.5 6.1 10.1 25.7
Percentage patients in the category who didn't bleed
No 17.2 6.9 10.3 31.0 17.2 10.3 17.2 37.9 15.5 10 16.6 42.2