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Rotational thromboelastometry in critical phase of dengue infection: Association with bleeding

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Abstract

Background: The critical phase of dengue carries a high risk of bleeding. Associations of coagulation test parameters and the risk of bleeding in the critical phase is unclear. This study examines the association of rotational thromboelastometry (ROTEM *delta* and ROTEM *platelet*) with bleeding risk of patients with dengue in the critical phase.

Methods: A total of 105 patients with confirmed dengue in the critical phase were recruited, with two subsequent prospective time point analyses of ROTEM parameters and platelet count within 24 and 48 hours from the onset of the critical phase. Conventional coagulation tests were performed only at the initial time point.

Results: Twenty of 105 patients developed bleeding after onset of the critical phase. Within the first 24 hours of critical-phase onset, platelet count, coagulation tests, and ROTEM *delta* were unable to differentiate patients with bleeding manifestations from those without ($P < .05$). Area under the curve of thrombin receptor activating peptide-6 assay of ROTEM *platelet* (TRAPTEM) discriminated patients with bleeding manifestations from those without, at a cutoff value of <12.5 Ω*min at a sensitivity and specificity of 73.7%, and 60.2%. In patients who developed bleeding, the maximum lysis of extrinsic pathway of ROTEM was significantly lower in patients with severe bleeding compared to those with mild to moderate bleeding. ($4.3 \pm 3.4\%$ vs $9.4 \pm 7.5\%$; $P = .01$).

Conclusion: An association with bleeding manifestations and TRAPTEM suggest a potential role for defective platelet aggregation in the pathogenesis of bleeding in the critical phase of dengue.

KEYWORDS
bleeding, coagulation, critical phase, dengue, rotational thromboelastometry

Essentials

- The exact pathogenesis of bleeding in dengue hemorrhagic fever is obscure.
- We conducted a cross-sectional study on 105 patients in the critical phase of dengue.
- Rotational thromboelastometry results seem to be more sensitive to coagulation derangements than conventional tests.
- Dysfunction of platelet aggregation contributes to bleeding in the critical phase of dengue.

1 | INTRODUCTION

Dengue is the world's most prevalent mosquito-borne viral infection, transmitted by the *Aedes aegypti* mosquito. Dengue infection has increased exponentially worldwide and has an incidence of 100 to 400 million cases each year.[1] Sri Lanka is an endemic country for dengue, where 31 162 suspected dengue cases were reported in 2020, and 7833 cases from January to June 2021.[2]

There are three defined phases in the natural history of dengue infection. In the febrile phase, patients present with nonspecific, constitutional symptoms and headache, backache, and general malaise. The critical phase of dengue infection is characterized by plasma leakage and carries the highest risk of hemorrhagic complications. Patients in the critical phase of dengue, with evidence of plasma leakage either ultrasonically or with a rise in the hematocrit from baseline with additional bleeding manifestations are classified as dengue hemorrhagic fever. Bleeding manifestations in patients with dengue hemorrhagic fever are heterogenous and range from mild mucocutaneous bleeding to more severe deep bleeding manifestations such as intracranial hemorrhage.[3] Bleeding in dengue is an important determinant of mortality.[4]

Platelet dysfunction, thrombocytopenia, derangement of coagulation, and fibrinolysis have been observed in dengue fever and dengue hemorrhagic fever.[5] In addition to the platelet count, conventional coagulation tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and serum fibrinogen level are commonly used to investigate hemostatic abnormalities in patients with dengue viral infection. However, several limitations of conventional coagulation tests such as the inability to determine fibrinolysis, clot stability, and hypercoagulability and to explain in vivo changes responsible for hemostatic dysfunction have made rotational thromboelastometry (ROTEM) *delta* and whole blood impedance aggregometry (ROTEM *platelet*) more widely used in the recent past.[6] ROTEM *delta* (TEM Innovations, Munich, Germany) has the ability to determine in vivo hemostasis during different phases of the coagulation cascade including clot formation, stability, and strength by different assays and provide real-time, graphic, and numerical results. A further advantage of ROTEM *delta* is the ability to detect evidence of fibrinolysis that cannot be identified by conventional coagulation tests. The ROTEM *delta* device analyzes extrinsic (EXTEM) and intrinsic (INTEM) pathways, fibrin polymerization (FIBTEM), and endogenous activation of hemostasis without coagulation activators (NATEM) using the parameters coagulation time (CT), clot formation time (CFT), amplitude at 10 minutes after CT (A10), maximum

clot firmness (MCF), and maximum lysis (ML).[7] ROTEM *platelet* (TEM Innovations, Munich, Germany) is an add-on module to using whole blood impedance aggregometry to determine overall platelet aggregation by the parameters maximum slope (MS), amplitude at run time of 6 minutes (A6), and area under the aggregation curve (AUC). ROTEM results in dengue fever have been reported, however, ROTEM results in patients with dengue hemorrhagic fever are scant.[8] Two studies performed on Brazilian and Sri Lankan patients with dengue fever have revealed that ROTEM viscoelastic testing has higher sensitivity than conventional coagulation tests in determining coagulation abnormalities in patients with dengue.[8,9] However, these studies did not evaluate the correlation of ROTEM abnormalities with clinically observed bleeding manifestations in patient with dengue infection. Nor did they focus on abnormalities of these tests in patients in the critical phase of illness when the risk of major bleeding is highest.[10]

It is currently unclear why some patients in the critical phase of dengue go on to manifest bleeding and progress to dengue hemorrhagic fever. It is also currently unclear if these bleeding manifestations are primarily driven by platelet dysfunction or by coagulation disturbance. Furthermore, there is a paucity of reliable clinical and laboratory predictors to determine the risk and severity of bleeding complications in patients in the critical phase of dengue. This is an important therapeutic need in dengue management with implications for triage, monitoring of patients, and early identification of bleeding complications to limit the morbidity and mortality of the disease. To this end, we evaluated the utility of ROTEM *delta* and ROTEM *platelet* as a tool to differentiate patients with bleeding manifestations from those without in the critical phase of dengue infection.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a prospective study with two time point analyses within 24 and 48 hours from the onset of the critical phase. Patients were recruited from two hospitals, National Hospital of Sri Lanka and National Institute of Infectious Diseases in Western Province, from December 1, 2019, to March 31, 2021. These hospitals are responsible for management of most patients with dengue in the most populous province of the country. All recruited patients were followed up for the duration of the hospital stay to detect late onset of bleeding.

FIGURE 1 Process of recruiting patients and sample collection at two time points in the study. NS1, dengue virus nonstructural protein 1; ROTEM, rotational thromboelastometry
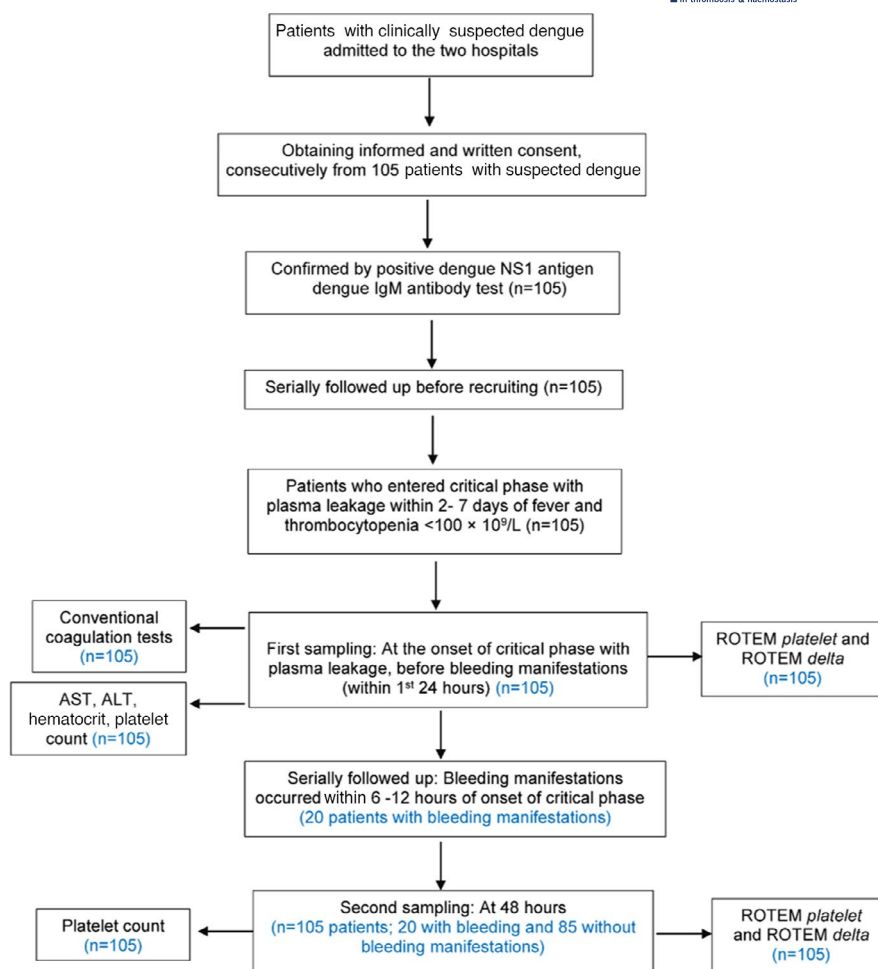
2.2 | Selection of the study population

Patients with dengue fever admitted to the above hospitals within the first 3 days of symptom onset were serially followed up and recruited soon after they entered the critical phase of dengue, which is characterized by plasma leakage.

A diagnosis of dengue was identified by the presence of a suggestive clinical syndrome including fever, nausea, rash, arthralgia, myalgia, leukopenia, and warning signs such as abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness, liver enlargement, and postural hypotension.[11] Informed and written consent was obtained from consecutive patients with suspected dengue infection. All patients who gave consent underwent dengue virus nonstructural protein 1 (NS1) and dengue IgM testing for confirmation of the diagnosis. A case of confirmed dengue was defined as a patient with a suggestive clinical syndrome with a positive dengue NS1 antigen test and/or positive dengue IgM antibody test using ELISA methodology.[11]

Plasma leakage and the onset of the critical phase of illness was diagnosed by (i) a >15% rise in hematocrit or leakage identified by ultrasound scan (U/S) or X-ray or (2) a >20% rise in hematocrit with or without U/S or X-ray evidence of leakage.[12] All patients thus selected (n = 105) were monitored for evidence of bleeding, with the

severity of bleeding categorized into moderate and severe based on the following criteria.

2.2.1 | Patients with mild to moderate bleeding

Patients with mild to moderate bleeding have either (i) no evidence of hemodynamic compromise with the need of intervention, (ii) bleeding at injection site, (iii) nose or gum bleeding, (iv) gastrointestinal tract bleeding without shock or hemodynamic instability with need of blood transfusion, (v) macroscopic hematuria, (vi) vaginal bleeding requiring hormonal therapy, or (vii) eye bleeding.[12]

2.2.2 | Patients with severe bleeding

Severe bleeding is any bleeding (i) at a critical organ, (ii) resulting in hemodynamic instability, (iii) resulting in death or disability, (iv) in need of transfusion, or (v) that persists after taking measures to stop bleeding.[12]

Patients with a prior diagnosis of a bleeding disorder, liver disease, or chronic kidney disease were excluded. Furthermore, those with an estimated glomerular filtration rate (eGFR) <90 mL/min/1.73 m^2

TABLE 1 Baseline characteristics and routine laboratory parameters in patients with and without bleeding manifestations

	Patients with bleeding manifestations	Patients without bleeding manifestations	P value
n (%) (N = 105)	20 (19)	85 (81)	
Sex			
Male	11 (55)	63 (75)	.07
Female	9 (45)	22 (25)	
Age, y	36.5 ± 12.9 (19-60)	31.7 ± 12.4 (18-84)	.12
AST, U/L, first time point	152 ± 14 (20-655)	179 ± 19 (32-1271)	.92
ALT, U/L first time point	149 ± 20 (26-378)	173 ± 24 (40-398)	.89
Hematocrit, %	40.7 ± 1.5 (17.2-51.0)	42 ± 0 (25.5-53.6)	.47
Platelets × 10^9/L (day 1)	32 ± 5 (9-98)	38 ± 2 (4-98)	.26
Platelets × 10^9/L (day 2)	28 ± 3 (5-62)	37.0 ± 3 (4-105)	.09

Note: Data are *n* (%) and mean ± standard deviation (range).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

$P < .05$; statistically significant in differentiating patients with bleeding manifestations from without.

or criteria fulfilling acute kidney injury[13] at baseline or at the stage of blood sampling for coagulation studies and ROTEM were excluded.

A flowchart demonstrating patient recruitment is presented in Figure 1.

2.3 | Data collection

Samples were collected at two time points: (i) within 24 hours of onset of plasma leakage at the critical phase and before the onset of bleeding manifestations and (ii) at 48 hours. All patients recruited were followed up for the duration of the hospital stay to detect late onset of bleeding.

At both time points, 1.6 mL of blood was collected to hirudin tubes for ROTEM *platelet* (TRAPTEM assay) and 1.8 mL of blood into 3.2% sodium citrate for ROTEM *delta* (EXTEM, INTEM, FIBTEM, NATEM). In addition, 1.8 mL of blood was taken into a citrated tube at the first time point for conventional coagulation tests by semi-automated coagulation analyzer Coatron M1 (PT, APTT, TT, Clauss fibrinogen test). Both instruments were calibrated, and procedures were quality controlled before analysis. The run time of ROTEM *delta* was 70 minutes. ROTEM analysis was performed within 1 hour of sample collection.

Platelet count, hematocrit, serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT) were obtained from the patient's clinical case records at the first time point (within 24 hours). At the second time point (48 hours), only a platelet count result was obtained. Serum creatinine and eGFR values were obtained before recruitment and patients were excluded from the study if they had an eGFR <90 mL/min/1.73 m^2.

2.4 | Statistical analysis

Analysis was performed with all 105 patients. No missing data were found. Descriptive data were reported as mean ± standard deviation

and/or range. All data were statistically analyzed using SPSS 23.0.0.0 (IBM Statistics for Windows version 23; IBM Corp., Armonk, NY, USA) under 95% confidence interval. Association of ROTEM parameters between patients with severe and mild to moderate bleeding manifestations was analyzed using chi-square testing. Mean comparison between patients with bleeding manifestations and those without bleeding (control group) was analyzed by independent sample *t* test and between the 2 days by paired *t* test. $P < .05$ was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was obtained for platelet count and ROTEM parameters. Laboratory-derived reference ranges were used for PT (11.3-16.2 seconds), aPTT (26.9-38.7 seconds), TT (16.5-23.7 seconds), and fibrinogen (214-335 mg/dL). Standard values were used for AST, ALT, platelet count, and ROTEM.[7,14]

2.5 | Ethical approval

Approval was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo (EC-19-033).

3 | RESULTS

3.1 | Baseline and routine laboratory results are uniform across patients with and without bleeding manifestations

The outcome of bleeding occurred in 20 patients (19%). Samples were drawn as described above, and this timing corresponded to 4 to 7 days from the onset of fever (mean, 5 ± 1 days). Bleeding occurred within 6 to 12 hours of the onset of the critical phase. The mean age of the population was 32.6 ± 12.5 years. No deaths were reported. The majority were men (70%). Elevated ALT and AST was seen in 96.9% and 89.6%, respectively. Each patient with bleeding had one or more

bleeding manifestations: hematuria (n = 6), gum bleeding (n = 3), melena (n = 5), hematemesis (n = 1), and hemoptysis (n = 2), and eight patients who had no overt bleeding but red cell transfusion was done (Table S1). Of all 20 patients with bleeding manifestations, 16 patients received red blood cell transfusions. No significant difference was observed in platelet count, hematocrit, AST, and ALT between patients with bleeding manifestations and patients without bleeding manifestations (Table 1). No thrombotic complications were reported.

3.2 | Conventional coagulation test parameters were similar between patients with bleeding manifestations and patients without bleeding manifestations

Increased PT, aPTT, and TT was observed in 2.8%, 27.6%, and 37.1%, respectively, and reduced fibrinogen in only 8.6% of patients (Table 2) at the first 24 hours. None of the patients with bleeding manifestations had significant derangements in PT. There was no significant difference in mean recorded values of the above conventional coagulation tests between groups ($P > .05$).

3.3 | ROTEM *delta* parameters are abnormal in most patients in the critical phase of dengue

Within 24 hours of onset of the critical phase, ROTEM *delta* derangements were shown by 99% (103/104) in at least one or combined EXTEM and INTEM parameters. A total of 63.5% (66/104) showed derangements in FIBTEM-CT, but FIBTEM-MCF was abnormal in only 13.4% (14/104). NATEM abnormalities were seen in 88.2% (90/102). However, it is notable that we did not observe a significant mean difference in the above stated ROTEM *delta* parameters between patients with bleeding manifestations and patients with nonbleeding manifestations within the first 24 hours of plasma leakage (Table 2). Only two patients with bleeding and one nonbleeding patient showed evidence of hyperfibrinolysis (high EXTEM ML). However, the ROTEM *delta* analysis after 24 hours demonstrated a significant mean difference in the INTEM and EXTEM CFT (Table S2).

3.4 | Variations in ROTEM *delta* parameters at 24- and 48-hour time points

We then sought to examine if there was any variation of the ROTEM *delta* parameters at two time points. MCF of INTEM, EXTEM, and NATEM and A10 of INTEM and EXTEM showed significant mean difference between the two time points. Overall, with regard to the ROTEM *delta* parameters that showed significant difference at the time points of 24 and 48 hours, ≈6% of the results in 48-hour samples returned to normal, whereas 30%

of them became further decreased. Around 50% of the 48-hour samples showed marginal improvement, but still were below the normal range (Table S3).

3.5 | ROTEM platelet detects differences in platelet aggregation in patients with bleeding

TRAPTEM in the ROTEM *platelet* module measures platelet aggregation activated by the agonist thrombin receptor activating peptide-6.[7] Although ROTEM *platelet* parameters are affected by platelet count,[7] this effect was alleviated by our recruitment criteria, ensuring that platelet counts were uniform across the groups. Overall platelet aggregation as assessed by AUC in ROTEM *platelet* was abnormally low in all patients with bleeding manifestations (100%) and in 98.7% (82/83) of patients without bleeding manifestations. Furthermore, AUC was zero in 31.5% (6/19) of patients with bleeding manifestations and 13.3% (11/83) of patients without bleeding manifestations. In considering mean values of the ROTEM *platelet* parameters A6, AUC, and MS, all were notably low in patients with bleeding, with the difference in AUC reaching statistical significance ($P = .04$) (Table 2). (see Table S4 for percentage derangement of ROTEM parameters between the patients with and without bleeding manifestations).

3.6 | ROC curve analysis for TRAPTEM AUC provides potential cutoff values to predict bleeding and bleeding severity

ROC curve analysis showed that TRAPTEM AUC is useful in diagnosing the disease state at significant levels under maximum possible sensitivity and specificity. A cutoff value of <12.5 Ω*min could be used to discriminate patients with and without bleeding manifestations at a sensitivity of 73.7% and specificity of 60.2% (AUC = 0.646, $P = .04$) (Figure 2A). A cutoff value of <7.5 Ω*min could be used to discriminate patients with severe bleeding manifestations from others (patients with mild bleeding or without bleeding manifestations) at a sensitivity of 61.5% and specificity of 65.2% (AUC = 0.672, $P = .04$) (Figure 2B). None of the other ROTEM parameters demonstrated significance between patients with bleeding manifestations and those without (see Table S5).

3.7 | Association of bleeding severity with ROTEM parameters

Patients with bleeding manifestations were categorized into (i) severe bleeding and (ii) mild to moderate bleeding, as described earlier. Significant association of bleeding severity as defined above was seen only with the EXTEM ML ($P = 0.01$) (Table 3). (See Table S6 for the association of all ROTEM parameters with type of bleeding.)

TABLE 2 Means of conventional coagulation tests, ROTEM *delta* and ROTEM *platelet* parameters among patients with and without bleeding manifestations at first time point (24 h)

	Patients with bleeding manifestations	Patients without bleeding manifestations	P value
Conventional coagulation tests			
PT, s	13.2 ± 0.3 (11.4-16.2)	12.9 ± 0.1 (10.9-16.7)	.26
APTT, s	35.4 ± 1.04 (28.7-44.8)	34.8 ± 0.7 (21.7-55.1)	.74
TT, s	23.7± 0.9 (17.2-34.1)	23.4 ± 0.4 (16.5-36.9)	.77
Fibrinogen, mg/dL	343 ± 18.0 (151-498)	349 ± 12 (158-753)	.83
ROTEM *delta* parameters			
INTEM CT, s	222 ± 42 (116-288)	228 ± 38 (171-351)	.79
INTEM CFT, s	449 ± 267 (172-1315)	446 ± 283 (130-1526)	.97
INTEM MCF, mm	35.8 ± 5.1 (24-45)	35.8 ± 7.7 (23-53)	.94
INTEM A10, mm	24.9 ± 5.0 (15-35)	26.4 ± 7.4 (14-44)	.56
INTEM ML, %	5.4 ± 4.2 (0-16)	6.0 ± 3.9 (0-17)	.55
EXTEM CT, s	67.8 ± 14.5 (46-110)	71.7 ± 15.9 (47-132)	.78
EXTEM CFT, s	406 ± 285 (45-1047)	378 ± 236 (99-1589)	.98
EXTEM MCF, mm	36.5 ± 6.6 (24-48)	38.3 ± 7.4 (21-58)	.35
EXTEM A10, mm	26.5 ± 6.3 (16-38)	27.7 ± 7.1 (15-49)	.49
EXTEM ML, %	5.8 ± 5.4 (0-18)	6.7 ± 3.8 (0-16)	.66
FIBTEM MCF, mm	13.1 ± 3.6 (5-18)	13.1 ± 3.9 (3-25)	.80
FIBTEM A10, mm	11.8 ± 3.4 (5-17)	11.7 ± 3.0 (3-20)	.62
FIBTEM ML, %	3.4 ± 4.5 (0-17)	6.0 ± 13.5 (0-93)	.40
NATEM CT, s	752 ± 312 (215-1571)	719 ± 308 (9-1701)	.67
NATEM MCF, mm	27.5 ± 9.5 (7-44)	27.3 ± 11.2 (4-48)	.96
NATEM ML, %	9.8 ± 23.7 (0-100)	9.9 ± 23.3 (0-100)	.99
ROTEM *platelet* parameters			
TRAPTEM A6, Ω	3.2 ± 2.5 (0-9)	5.1 ± 4.3 (0-19)	.06
TRAPTEM AUC, Ω*min	9.9 ± 9.3 (0-37)	17.9 ± 16.4 (0-67)	.04*
TRAPTEM MS, Ω/min	1.05 ± 0.9 (0-3)	1.6 ± 1.3 (0-5)	.09

Note: Data are mean ± standard deviation (range).

Abbreviations: A10, amplitude at 10 min after CT; A6, amplitude at 6 min run time; APTT, activated partial thromboplastin time; AUC, area under the aggregation curve; CFT, clot formation time; CT, clotting time; EXTEM, extrinsically activated ROTEM assay; FIBTEM, ROTEM assay assessing fibrin contribution to clot firmness; INTEM, intrinsically activated ROTEM assay; MCF, maximum clot firmness; ML, maximum lysis; MS, maximum slope; NATEM, nonactivated ROTEM assay; PT, prothrombin time; ROTEM, rotational thromboelastometry; TRAPTEM, thrombin receptor activating peptide-6 thromboelastometry; TT, thrombin time.

*$P < .05$; statistically significant in differentiating patients with bleeding manifestations from without.

3.8 | NATEM versus EXTEM in detecting fibrinolysis

NATEM is a more sensitive method in detecting true endogenous coagulopathy than EXTEM. NATEM does not use any activators except for recalcification while EXTEM is an activated method.[15] Accordingly, good correlation between the MCF of NATEM and EXTEM ($r = .69$, $R^2 = .48$, $P = .0004$) and a weak correlation between the ML of NATEM and EXTEM ($r = .15$, $R^2 = .02$, $P = .002$) was observed. NATEM ML was >80% in six of seven (one patient with bleeding manifestations and five patients without bleeding manifestations), indicating fibrinolysis. Moreover, of the nine patients who showed hyperfibrinolysis by NATEM, only two showed abnormal EXTEM ML. The other seven patients had a normal EXTEM ML. Fair correlation was observed between EXTEM ML and TRAPTEM AUC ($r = .21$, $R^2 = .05$, $P = .03$), but not with NATEM ML.

4 | DISCUSSION

To our knowledge, this is the first viscoelastometry and impedance aggregometry study done on patients who are in the critical phase of dengue comparing those with bleeding and those without.

FIGURE 2 ROC curves for TRAPTEM AUC. Diagonal line indicates the reference line. Fluctuated line indicates the respective ROC curve for the TRAPTEM AUC. (A) ROC for differentiating patients who have bleeding manifestations from those without bleeding. (B) ROC curve for differentiating patients with severe bleeding from those with mild to moderate bleeding and without bleeding manifestations together. ROC, receiver operating curve; TRAPTEM, thrombin receptor activating peptide-6 thromboelastometry
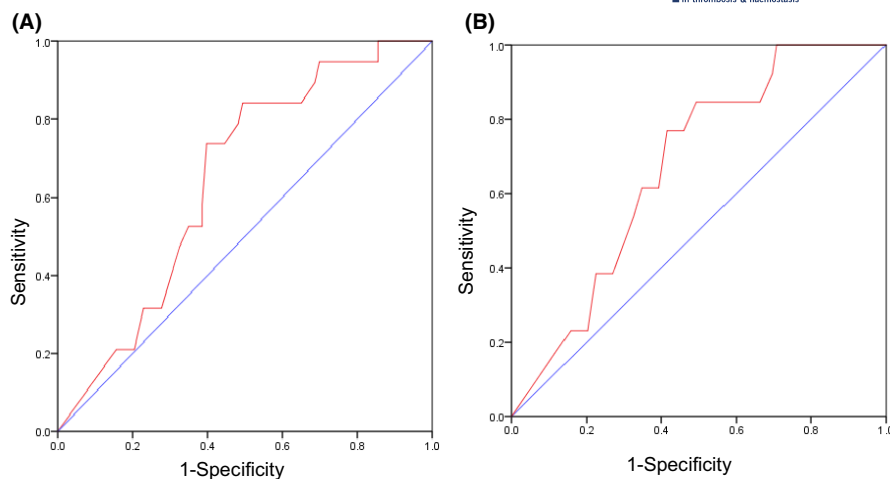
TABLE 3 Association of EXTEM and FIBTEM ML, NATEM CT, ML, and MCF with the type of bleeding (24-h time point)

		Severe bleeding (n=15)	Mild to moderate bleeding (n=5)	P value
EXTEM ML, %	Normal	15	3	.01*
	Abnormal (high)	0	2	
	Mean	4.3 ± 3.4	9.4 ± 7.5	
FIBTEM ML (%)	Normal	13	5	.53
	Abnormal (high)	1	0	
	Mean	4.2 ± 5.0	5.0 ± 1.0	
NATEM CT, s	Normal	10	4	.57
	Abnormal	5	1	
	Mean	778 ± 340	677 ± 234	
NATEM MCF, mm	Normal	1	0	.55
	Abnormal	14	5	
	Mean	27.5 ± 10.5	27.3 ± 2.9	
NATEM ML, %	Normal	14	3	.07
	Abnormal	1	2	
	Mean	9.1 ± 26.2	13.3 ± 5.5	

Note: Data are *n* (count) and mean (range).

Abbreviations: CT, clotting time; EXTEM, extrinsically activated ROTEM *delta* assay; MCF, maximum clot firmness; ML, maximum lysis; NATEM, nonactivated ROTEM assay.

*$P < .05$; statistically significant association.

4.1 | Dysfunction of platelet aggregation in the critical phase of dengue with plasma leakage as a driver of bleeding manifestations

Based on our findings, despite the platelet count being uniform across the groups, the TRAPTEM AUC was significantly lower in those with bleeding manifestations. The TRAPTEM AUC assesses platelet aggregation activated by the agonist thrombin receptor activating peptide-6. This indicates a potential role for dysfunctional platelet aggregation in patients with dengue with bleeding manifestations.[16] A study using multiple-electrode aggregometry in patients with dengue to assess platelet function reports that thrombocyte aggregation induced by adenosine diphosphate, collagen, and thrombin receptor activating peptide-6 is impaired in patients with dengue infection compared with healthy controls and patients with other causes of fever. Interestingly, the investigators of the above study did not find any correlation between platelet count and platelet aggregation/AUC. Further clinical correlation demonstrated that lower AUC may indicate the severity of the disease and may correlate with the duration of the hospital stay.[17] In the context of other viral hemorrhagic fevers such as Crimean-Congo hemorrhagic fever, Lassa fever, and Argentinian hemorrhagic fever, platelet aggregometry studies have suggested that the coagulopathy is clearly due to defects in platelet function and aggregation.[18,19] The findings of the study on Crimean-Congo hemorrhagic fever found that mucosal bleeding was not due to thrombocytopenia but rather due to platelet dysfunction in aggregation.[18]

The observation that bleeding occurs in patients with dengue fever with normal platelet counts also provides evidence that alteration in platelet function, activation, and aggregation is probably the key factor in the pathogenesis of bleeding in dengue.[20]

Furthermore, we failed to demonstrate statistically significant differences in the mean values of INTEM, EXTEM, and FIBTEM in patients on the first time point with bleeding manifestations compared to those without bleeding manifestations. This further reinforces the hypothesis of the potential role of platelet aggregation abnormalities in the pathogenesis of bleeding. We propose further studies to clarify these associations and to establish the role of ROTEM *platelet* assays as a biomarker predictive of bleeding in patients with dengue infection.

Proposed mechanisms for reduced platelet aggregation in dengue hemorrhagic fever are the presence of dengue-specific immune complexes and platelet destruction.[21] Protein disulfide isomerase on the surface of platelets has been found to cross react with antibodies against dengue virus nonstructural protein 1 (NS1) in patients with dengue hemorrhagic fever.[22,23] These interactions ultimately result in inhibition of platelet adhesion and aggregation, coagulation defects, and endothelial cell damage.[24,25] Moreover, platelet destruction due to the entry of virus into platelets and absorption of immune complexes to the membrane of the platelet may contribute to reduction in platelet aggregation.[16,25,26]

As there was a significant difference in TRAPTEM AUC, the proposed cutoff values would be beneficial to use as screening parameters for identifying those with bleeding manifestations from those without bleeding and patients with severe bleeding manifestations from those with mild and without bleeding manifestations in routine clinical practice, with modest sensitivity and specificity. Further prospective validation of these cutoff values is recommended.

4.2 | Derangements in ROTEM *delta* profiles EXTEM, INTEM, NATEM, and FIBTEM do not relate to clinical bleeding manifestations in 24-hour samples

ROTEM *delta* profile, EXTEM, INTEM, NATEM, and FIBTEM exhibited >60% derangement. Considering the extrinsic pathway, 20% of patients with bleeding manifestations exhibited derangements in EXTEM CT, while PT was normal. Derangements in the intrinsic pathway were exhibited by 25% of patients with bleeding manifestations by aPTT and 35% by INTEM CT. It is evident from our findings that the ROTEM *delta* detected more abnormalities in coagulation in patients with dengue hemorrhagic fever compared to conventional coagulation tests. These findings are consistent with other studies using ROTEM *delta* to interrogate coagulation in patients with dengue. Conventional coagulation tests and ROTEM analysis were performed on Brazilian patients with dengue fever with thrombocytopenia (<100 × 10^9/L). This was the first viscoelastic study in dengue, but their sample size was small (n = 53) and the disease severity was not considered. INTEM and EXTEM derangements were seen in 71.7% and 54.7%, respectively, and 94.3% showed normal FIBTEM in

dengue fever.[8] An Indian study reported that thromboelastography parameters are more effective than conventional coagulation tests in determining coagulation abnormalities and further showed that factor deficiency, platelet dysfunction, and primary fibrinolysis are major abnormalities among patients with dengue fever.[27] However, in our study, widespread derangement in coagulation detected by ROTEM *delta* did not necessarily translate to clinically evident bleeding manifestations. This important finding indicates that the primary pathogenic mechanisms for clinically significant bleeding in dengue may not be due to abnormalities in coagulation. It further raises the question as to how to interpret ROTEM *delta* abnormalities in the clinical setting in patients with dengue with plasma leakage.

FIBTEM excludes the contribution of platelets in clot formation. Since FIBTEM-MCF derangements were only 13.4% (2 bleeding and 11 nonbleeding patients), which suggests that the pathogenesis of bleeding in patients with dengue in the critical phase was mainly due to platelet function abnormalities.

The ROTEM *delta* analysis after 48 hours exhibited a significant mean difference in MCF and A10 of both INTEM and EXTEM and NATEM MCF compared to the 24-hour sample. However, the change in directionality of the parameters assessed in 48 hours varies. A large cohort study would be required to predict an exact underlying reason for this observation.

4.3 | Lower fibrinolytic activity in patients in the critical phase with severe bleeding manifestations may be a result of a compensatory response

Furthermore, in classifying patients based on clinical severity, patients with severe bleeding manifestations had a significantly lower EXTEM ML, which indicates lower fibrinolytic activity/fibrinolysis shutdown. Previous studies show that increased EXTEM ML is associated with severity of bleeding in other clinical settings[7,28] ML>15% indicates hyperfibrinolysis and increases with the severity of bleeding.[29] The fact that this parameter is low in patients with severe bleeding manifestations in our study may be indicative of fibrinolysis shutdown in some patients with dengue hemorrhagic fever as shown in patients with bacterial sepsis and severe COVID-19.[30,31] However, this finding should be interpreted cautiously and replicated in further studies given the small sample size of patients with severe bleeding manifestations. Though we found NATEM to be a better predictor of fibrinolysis than EXTEM, we did not find a similar association with NATEM ML as EXTEM ML with bleeding severity. It is likely that NATEM is more sensitive for assessing fibrinolysis than EXTEM in a bleeding patient.[28] However, higher EXTEM and NATEM ML in mild to moderate bleeding compared to severe bleeding can also be based on platelet-mediated clot retraction.[32] Moreover, the association between EXTEM ML and TRAPTEM AUC also supports platelet-mediated clot retraction. This may reflect better preserved platelet function in patients with mild to moderate bleeding manifestations. Accordingly, future studies should focus on the relationship between platelet function,

on the one hand, and fibrinolysis and platelet-mediated clot retraction, on the other hand. Recent studies demonstrated a cross talk between both systems, for example, the release of plasmin activator inhibitor-1 by activated platelets.[33]

4.4 | Limitations

Though this was an explorative study, we report a new finding, which is an association of platelet dysfunction and bleeding manifestations the first 24 hours of the critical phase. It is important to note that this association does not prove causality, as it is derived from single-time-point analyses and limited by sample size. To confirm a predictive biomarker from ROTEM, a large cohort study is required with sufficient population from both with bleeding and without bleeding groups, followed serially with multiple-time-point analyses leading up to the outcome of bleeding. Even though we noted a significant mean difference in MCF and A10 in both INTEM and EXTEM and NATEM MCF in the 24-hour sample compared to the 48-hour time point, corresponding renal and liver function tests are not available to adjust for confounding effects on the coagulation indices at this time point. Furthermore, race/ethnicity of the study participants was not collected and, hence, the social determinants of health in relation to propensity of bleeding manifestations could not be assessed.

5 | CONCLUSION

ROTEM *delta* parameters are more sensitive to changes in coagulation in patients with dengue in the critical phase with plasma leakage than conventional coagulation tests. However, they could not differentiate those with bleeding manifestations from those without bleeding manifestations in the initial part of the critical phase. The only ROTEM parameter that was able to discriminate those with bleeding manifestations from those without was TRAPTEM AUC on ROTEM *platelet*. This association suggests that platelet function may contribute to bleeding in patients with dengue with plasma leakage more than thrombocytopenia and coagulation derangements. NATEM was more sensitive in detecting fibrinolysis than EXTEM, but there was no clear evidence suggesting that fibrinolysis has an impact on bleeding in these patients with thrombocytopenia. Future studies should focus on the cross talk between platelet function and the fibrinolytic system in both critical phase and febrile phase and include a larger number of patients with bleeding manifestations to provide more robust results.

AUTHOR CONTRIBUTIONS
KG conceptualized the study and provided resources. LG was involved in project conceptualization and administration. LG and PW were involved in funding acquisition. WW was involved in data collection, investigation, formal analysis, and methodology. TP collected data and investigated. PK and AW supervised the project. YA was involved in data curation and writing of the original draft. SJ, SR, RA, and all other authors were involved in reviewing and editing of the manuscript. All the authors viewed the manuscript and provided approval for submission of this article.

DATA AVAILABILITY STATEMENT
Relevant data will be made available on request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.