SUPPLEMENTAL MATERIAL

Methods

This study was approved by the local institutional review board. Patient consent was obtained from the patient, or a surrogate if appropriate.

Study design and population

The study was a prospective observational study that included patients admitted to a tertiary center with a dedicated Neuroscience Intensive Care Unit. The enrollment period was from January 2018 to August 2021. Inclusion criteria were patients presenting age 18 or older with SAH secondary to a ruptured aneurysm. SAH was diagnosed by initial computed tomography (CT) scan or by xanthochromia in the cerebrospinal fluid if the CT was not diagnostic. CTA (computerized tomographic angiography) or conventional diagnostic angiogram was used to confirm the presence of a culprit aneurysm. SAH from secondary etiology such as trauma, dissection, vascular malformation, admission after 72 hours from ictus, and patients with known coagulopathy or history of anticoagulants were excluded.

Assessment of clinical parameters

Data collected included basic demographic data including age, sex, race, past medical history, medication history. Clinical severity was assessed by the Hunt-Hess score (HH) and radiological scans were graded using the Fisher scale. Patients were classified as having DCI based on accepted published standards.[1] The definition of DCI used in the study: "*The occurrence of focal neurological impairment (such as hemiparesis, aphasia, apraxia, hemianopia, or neglect), or a decrease of at least 2 points on the Glasgow Coma Scale (either on the total score or on one of its individual components [eye, motor on either side, verbal]). This should last for at least 1 hour, is not apparent immediately after aneurysm occlusion, and cannot be attributed to other causes by means of clinical assessment, CT or MRI scanning of the brain, and appropriate laboratory studies*". Initial laboratory findings collected included complete blood count, basic metabolic profile, standard coagulation profile (INR, PT/PTT) and TEG. Modified Rankin Scale (mRS) score at 3 months was used to assess the outcome. Good clinical outcomes were defined as a mRS 0-2, and poor clinical outcomes were defined as a mRS of 3-6. All patients underwent treatment of the aneurysm within 24 hours. Protocolized standard of care was applied to all patients. All patients were allowed permissive hypertension and patients with DCI underwent augmented blood pressure and angiography with treatment if necessary. All patients received deep venous thrombosis (DVT) prophylaxis within 24 hours of treatment. Only the first time point of TEG was obtained prior to DVT prophylaxis initiation in all patients.

Thromboelastography (TEG) data collection

To assess the coagulation profile trends over time, TEG was performed at 5 predetermined time points: at <24 hours(T_1), at 24-48 hours (T_2), at 3-5 days (T_3), 6-9 days (T_4), and after 10 days (T_5) of admission. Each blood sample was analyzed by trained personal for TEG analysis within an hour of draw. Serial TEG tests were performed on the same computerized TEG coagulation analyzer (Haemonetics Corp, Model 5000; Braintree, Mass).

TEG parameters included R (time to clot initiation), K (time to thrombin-platelet interaction, 20mm clot strength), MA (maximum clot strength), G value (calculate value of clot strength), alpha angle (rate of fibrin cross linking) and LY30 (measure of clot lysis). The parameters are described in table S1 and figure S1.

Statistical Analysis

Continuous variables were presented as mean and standard deviation (SD). Categorical variables were presented as frequencies and percentages. The two-tailed Student's t test and Fisher's exact test were used for comparisons of demographics, initial severity of SAH (HH, GCS), and TEG values.

Multivariable logistic regression model was performed for comparing outcome groups while adjusting for potential confounders detected on univariate analysis. Bar graphs with error bars were plotted while the lower and upper ends of the error bars stand for mean - standard error and mean + standard error, respectively. Area under the receiver operating characteristic curve (AUC) was used to quantify the discriminative power of each single TEG parameter, and heatmaps were plotted to visualize the results. Statistically significant difference was defined as P value <0.05. All analyses were conducted using R version 4.0.5.

Results

Patient population and baseline TEG

During the study period, 201 patients met the inclusion criteria and were enrolled in the study and underwent serial TEG measurements. The mean age at presentation was 53.6 (range: 22-84). Of those patients, 69.7% were female. DCI was seen in 70 patients (34.8%). 144 patients had outcome data at 3 months. Poor mRS was seen in 63 (44%) of patients. Classical parameters associated with poor outcomes included older age ($p<0.001$), higher Hunt Hess grade on arrival ($p<0.001$) and lower Glasgow Coma Scale ($p<0.001$). There was no difference in outcomes for clipped versus coiled patients. On univariate analysis, G value on admission was significantly higher in patients with higher HH grade (**12.5** vs **11.1**, ***p= 0.009***). K and angle also demonstrated hypercoagulability in higher grades (***p= 0.04 for both***). Patients' characteristics and demographics are summarized tables S2 and S3.

TEG and clinical outcomes

mRS was dichotomized into good (0-2) versus poor (3-6). 144 patients had outcomes data at 3 months. TEG parameters across the different timepoints were compared between the two groups. While several TEG parameters did show a significant difference at some point along the timeline on univariate analysis, the MA and G-value were the only two indices to maintain significance with multivariate analysis. MA was significantly elevated in patients with poor mRS compared to those with good mRS at T_1 and T_5 (**p= 0.019 for T_1 and 0.043 for T_5**). The G-value (calculated clot strength) was also significantly higher in patients with poor outcomes on T_5 (**p= 0.017**). These findings were consistent with findings in other studies.[2] Figure 1A demonstrates these two indices across different timepoints in relation to mRS.

TEG and delayed cerebral ischemia

Patients was dichotomized into a group with DCI (n=70) and one without (n=131). Of the TEG parameters, R (***p= 0.03***), Angle (***p=0.023***) and K (***p= 0.04***) showed a significant difference between the two groups on multivariate analysis, however it was only evident on T4 (time point at occurrence of DCI).

TEG is not predictive of neither outcomes nor development of delayed cerebral ischemia

The different TEG parameters were tested as a predictive biomarker for poor outcomes. A receiver operating characteristic (ROC) curve was generated for each parameter at each time point. None of the generated curves demonstrated an acceptable area under the curve (AUC) for clinical use. Similarly, we conducted this analysis in relation to DCI. Each individual time point (until occurrence of DCI) of each parameter was plotted for an ROC curve to predict DCI. No parameter timepoint was successful. Figure 1B summarizes such findings in a heat map.

TEG	Definition	Reference	Units
R	Reaction time to clot formation, thrombin & fibrin formation	3 - 8	minutes
K	Time from end of R until clot reaches 20 mm, speed of fibrin crosslinking & platelet interaction	1 - 3	minutes
Angle (α)	Tangent of the curve made reaching K, fibrin cross linking	55 - 78	degrees
MA	Maximum strength of clot, platelet-fibrin interaction	51 - 69	mm
G	Log-derivation of MA, platelet-fibrin interaction	5 - 12	Dynes/sec
LY30	% lysis 30 minutes after MA, clot stability	0 - 8	%

Table S1. TEG parameters and definitions

	No DCI (N=131)	DCI (N=70)	Total (N=201)	p-value
Age				**0.256**
Mean (SD)	52.7 (14.3)	55.1 (13.6)	53.6 (14)	
Range	22.0 - 81.0	26.0 - 84.0	22.0 - 84.0	
Gender				**0.199**
Female	87 (66.4%)	53 (75.7%)	140 (69.7%)	
Race				**0.575**
Black	23 (17.6%)	16 (22.9%)	39 (19.4%)	
White	99 (75.6%)	51 (68.9%)	150 (74.6%)	
Asian	9 (6.9%)	3 (4.3%)	12 (6.0%)	
HTN				**0.363**
Yes	76 (58.0%)	46 (65.7%)	122 (60.7%)	
Aspirin				**1.000**
No	107 (81.7%)	57 (81.4%)	164 (81.16%)	
Clopidogrel				**1.000**
No	124 (94.7%)	67 (95.7%)	191 (95.0%)	
Intervention				**0.546**
Clipped	53 (40.5%)	25 (35.7%)	78 (38.8%)	
Coiled	78 (59.5%)	45 (64.3%)	123 (61.2%)	
GCS				**0.103**
Mean (SD)	11 (3.9)	10.1 (4.0)	10.7 (4.0)	
Range	3.0 - 15.0	3.0 - 15.0	3.0 - 15.0	
Hunt Hess on arrival (1-5)				**0.009**
Mean (SD)	2.9 (0.9)	3.2 (0.9)	3.0 (0.9)	
Range	1.0 - 5.0	2.0 - 5.0	1.0 - 5.0	
Fisher scale				**1.000**
1-2	4 (3.1%)	2 (2.9%)	6 (3.0%)	
3-4	127 (96.9%)	68 (97.1%)	195 (97.0%)	
Platelet, Mean (SD)	241.3 (64.7)	238.5 (72.4)	240.3 (67.4)	**0.776**

PTT, Mean (SD)	29.5 (17.2)	28.6 (7.0)	29.1 (14.4)	**0.698**
INR, Mean (SD)	1.0 (0.1)	1.1 (0.5)	1.0 (0.3)	**0.118**

Table S2. Baseline characteristics and demographics for enrolled patients

	Poor mRS (N=63)	Good mRS (N=81)	Total (N=144)	p-value
Age				< 0.001
Mean (SD)	58.7 (12.7)	49.0 (13.9)	53.3 (14.1)	
Range	26.0 - 84.0	22.0 - 77.0	22.0 - 84.0	
Gender				0.583
Female	46 (73.0%)	55 (67.9%)	101 (70.1%)	
Race				0.854
Black	13 (20.6%)	13 (16.0%)	26 (18.1%)	
White	47 (74.6%)	64 (79.0%)	111 (77.1%)	
Asian	3 (4.8%)	4 (4.9%)	7 (4.9%)	
HTN				0.025
Yes	46 (73.0%)	44 (54.3%)	90 (62.5%)	
Aspirin				1.000
No	52 (82.5%)	66 (81.5%)	118 (81.9%)	
Plavix (Clopidogrel)				1.000
No	60 (95.2%)	78 (96.3%)	138 (95.8%)	
Intervention				0.597
Clipped	20 (31.7%)	30 (37.0%)	50 (34.7%)	
Coiled	43 (68.3%)	51 (63.0%)	94 (65.3%)	
GCS				< 0.001
Mean (SD)	8.6 (4.2)	11.7 (3.5)	10.3 (4.1)	
Range	3.0 - 15.0	3.0 - 15.0	3.0 - 15.0	
Hunt Hess on arrival (1-5)				< 0.001
Mean (SD)	3.4 (1.0)	2.6 (0.8)	3.0 (0.9)	
Range	1.0 - 5.0	1.0 - 5.0	1.0 - 5.0	
Fisher scale				0.504
1-2	0 (0.0%)	2 (2.5%)	2 (1.4%)	
3-4	63 (100.0%)	79 (97.5%)	142 (98.6%)	
Platelet, mean (SD)	230.6 (69.2)	245.0 (64.1)	238.7 (66.5)	0.199
PTT, mean (SD)	30.8 (23.5)	28.7 (6.9)	29.6 (16.4)	0.484
INR, mean (SD)	1.0 (0.1)	1.1 (0.5)	1.1 (0.4)	0.458

Table S3. Baseline characteristics and demographics for enrolled patients with 3 months follow up

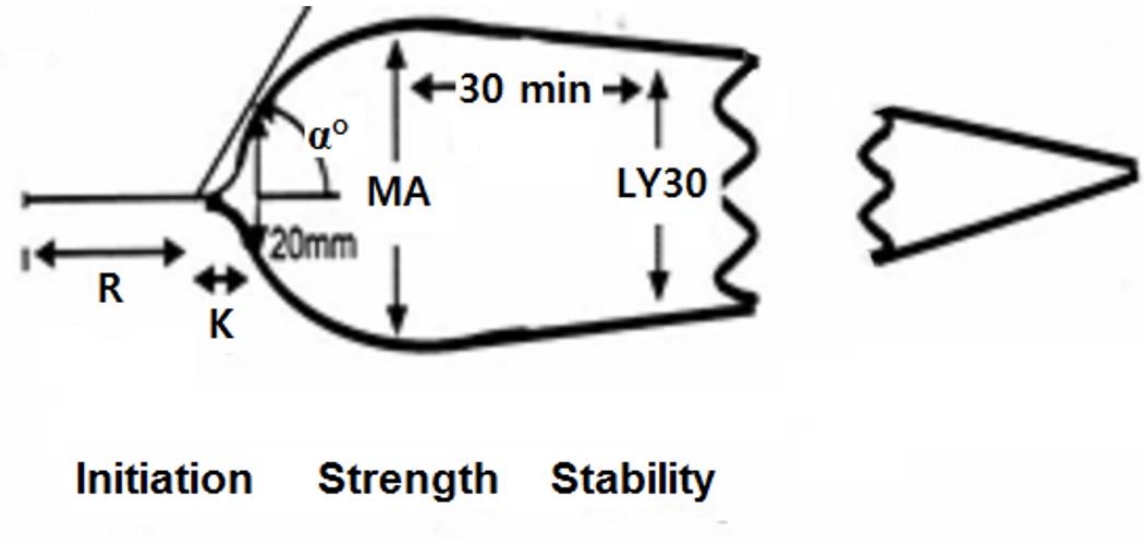

Figure S1. Definition and reference range of parameter of thromboelastography (TEG). Schematic trace of TEG shows change of each TEG parameters from initiation to fibrinolysis of clot. Definitions and reference ranges of each parameter are explained in table S1.

1. Vergouwen MD, Vermeulen M, van Gijn J, et al. Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: proposal of a multidisciplinary research group. *Stroke.* 2010;41(10):2391-2395.
2. Ramchand P, Nyirjesy S, Frangos S, et al. Thromboelastography Parameter Predicts Outcome After Subarachnoid Hemorrhage: An Exploratory Analysis. *World Neurosurg.* 2016.