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Using microfluidic shear to assess transfusion requirements in trauma patients

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ABSTRACT

Background Viscoelastic assays have widely been used for evaluating coagulopathies but lack the addition of shear stress important to *in vivo* clot formation. Stasys technology subjects whole blood to shear forces over factor-coated surfaces. Microclot formation is analyzed to determine clot area (CA) and platelet contractile forces (PCFs). We hypothesize the CA and PCF from this novel assay will provide information that correlates with trauma-induced coagulopathy and transfusion requirements.

Methods Blood samples were collected on adult trauma patients from a single-institution prospective cohort study of high-level activations. Patient and injury characteristics, transfusion data, and outcomes were collected. Thromboelastography, coagulation studies, and Stasys assays were run on paired samples collected at admission. Stasys CA and PCFs were quantified as area under the curve calculations and maximum values. Normal ranges for Stasys assays were determined using healthy donors. Data were compared using Kruskal-Wallis tests and simple linear regression.

Results From March 2021 to January 2023, 108 samples were obtained. Median age was 37.5 (IQR 27.5–52) years; patients were 77% male. 71% suffered blunt trauma, 26% had an Injury Severity Score of ≥25. An elevated international normalized ratio significantly correlated with decreased cumulative PCF (p=0.05), maximum PCF (p=0.05) and CA (p=0.02). Lower cumulative PCF significantly correlated with transfusion of any products at 6 and 24 hours (p=0.04 and p=0.05) as well as packed red blood cells (pRBCs) at 6 and 24 hours (p=0.04 and p=0.03). A decreased maximum PCF showed significant correlation with receiving any transfusion at 6 (p=0.04) and 24 hours (p=0.02) as well as transfusion of pRBCs, fresh frozen plasma, and platelets in the first 6 hours (p=0.03, p=0.03, p=0.03, respectively).

Conclusions Assessing coagulopathy in real time remains challenging in trauma patients. In this pilot study, we demonstrated that microfluidic approaches incorporating shear stress could predict transfusion requirements at time of admission as well as requirements in the first 24 hours.

Level of evidence Level II.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ We know that early detection of traumatic coagulopathy is critical as coagulopathic patients have higher rates of mortality, and intervention on the derangements in coagulation with early transfusion can improve outcomes in trauma patients. Currently, viscoelastic assays, such as thromboelastography (TEG), are superior to prothrombin time/international normalized ratio and partial thromboplastin time in detecting coagulopathy and initiating transfusion protocols, but these assays come with multiple limitations.

WHAT THIS STUDY ADDS

⇒ Recent studies show the importance of shear forces in activating and sustaining *in vivo* clot formation. Stasys assay is the first diagnostic test to use microfluidic shear forces to create a much closer simulation of *in vivo* clotting as opposed to the completely artificial environment seen in TEG.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Once optimized, Stasys could allow for evaluation of *in vivo* clot formation and predict transfusion requirements in a point-of-care test that results in 3 minutes. This could provide earlier detection of coagulopathy and initiation of transfusion to improve outcomes in coagulopathic trauma patients.

BACKGROUND

Detection of post-traumatic coagulopathy and platelet dysfunction has been approached clinically and experimentally through standard coagulation studies (prothrombin time (PT) and partial thromboplastin time (PTT)), and viscoelastic tests that measure the functional properties of blood clotting (thromboelastography (TEG) or rotational thromboelastometry (ROTEM)). Studies have shown that timely recognition of acute traumatic coagulopathy is critical as these patients have a four times higher likelihood of death than those without coagulopathy.[1 2] Additionally, coagulopathy has been identified as an early marker of active hemorrhage and detection of derangements in coagulation has proven to be an effective trigger to initiate massive transfusion protocols and improve outcomes.[3 4] Recently, viscoelastic functional testing has been demonstrated to be equivalent, if not superior, to conventional coagulation tests in identifying these high-risk, coagulopathic trauma patients.[5 6]

Thus, at many centers, TEG and ROTEM have largely replaced conventional assays such as PT and PTT to guide resuscitation. Unfortunately, TEG and ROTEM have multiple limitations—including difficulty with standardization, high variability of results due to testing environments or user error, and limitations in describing platelet behavior—all of which have raised concern in the use of these tests as the 'gold-standard' assays for post-traumatic coagulopathy detection.[5 7] Further, the length of time to result, up to 30–60 minutes, limits their utility in directing transfusion in a high-acuity trauma.[8]

In 2019, Ting *et al* developed an innovative approach to quantifying coagulopathy through a process more closely mimicking *in vivo* clotting. By exploiting microfluidics to generate shear forces on whole blood samples, the investigators were able to measure clot formation and properties via a prototype device, the Stasys platelet system (Stasys Medical Corporation, Seattle, WA).[9] The critical role of shear forces in clot formation was first described in 2017 by Drs. Ju and Chen's laboratory when they observed the conformational changes that took place during clot formation by using dynamic force spectroscopy.[10] Previously, it was thought that agonist diffusion drove clot formation, but this work demonstrated that shear forces are important in not only stretching out the von Willebrand factor (vWF) protein and its binding domain on glycoprotein Ib (GpIb), but also play a crucial role in facilitating the binding kinetics between vWF and GpIb.[10 11] The graded shear force and its effects on vWF and GpIb are what allow the platelets to bind and roll along injured endothelium until they adhere to the site of injury.[12 13]

Building off those findings, Ting *et al* later used the Stasys platelet system in a small cohort to assess coagulation properties in a point-of-care device that produces the shear forces shown to be essential in coagulation.[9 10] This device uses a method we now know to be much more similar to *in vivo* clotting to evaluate clot formation, instead of generating an artificial environment such as in TEG and ROTEM assays.[9 10 14] Importantly, Stasys can produce results in 3 minutes. Although a promising technology, its role in detecting post-traumatic coagulopathy and guiding resuscitation has not been investigated or externally validated. This independent feasibility study assesses the ability to correlate clot area (CA) and clot strength, or platelet contractile force (PCF), measured by the Stasys analyzer with transfusion requirements in trauma patients.

METHODS

Enrollment & sample collection

This study enrolled all adult trauma patients who presented as the highest-level trauma activations in a single, level 1 trauma center between March 2021 and January 2023 in an ongoing observational cohort study (PART—Precision Approaches to Resuscitation in Trauma). Blood samples were obtained at the time of placement of the initial intravenous line in the resuscitation bay (emergency department) as time zero samples. Any patient not having intravenous access established within 30 minutes of arrival, interfacility transfers, pregnant patients, and those in law enforcement custody were not eligible for enrollment. Patients were only enrolled when research coordinator staff were available for the initial trauma activations as assays of interest required fresh whole blood to be used (online supplemental file 1).

Patient demographics, injury characteristics, transfusion data, and outcomes were collected on all patients. Race and ethnicity were self-reported by the patient or family members, when possible. In a subset of the PART patients, simultaneously timed, matched blood samples were collected for standard coagulation studies (PT/international normalized ratio (INR), PTT), TEG (R time, maximum amplitude (MA) for citrated rapid TEG (CRT) and function fibrinogen (FF), citrated kaolin lysis (Ly30)), and Stasys (PCF, CA) assays.

Stasys measurements & calculations

The Stasys platelet system was developed by the Stasys Medical Corporation in 2019 as a potential alternative to existing analyzers of coagulopathy.[15] The analyzer uses whole blood samples which are injected into a cartridge that directs the blood to flow across a series of sensors coated in vWF and collagen (figure 1A,B). The flow rate of the blood through the cartridge has been shown to generate the shear force required to activate platelets, similar to in vivo coagulation (figure 1C).[9] As the clot forms along the downstream side of the sensors, the CA and strength of the clot, or PCF, can be measured as the post portion of the sensor is drawn towards the block portion (figure 1D).[9] These results are produced in near real time as the test is completed in 3 minutes.

Given the novelty of the Stasys analyzer, the raw data produced from the analyzer at the time of this study required additional analysis prior to providing a clinically useful result. Normal ranges for Stasys assays were determined using the Hoffmann method on healthy, uninjured subjects, after informed consent.[16 17] Cumulative Stasys CA and PCF were quantified as area under the curve calculations. The maximum value of each assay was also recorded to capture the samples with significant variations in positive and negative peaks. This would average to a normal cumulative result and potentially overlook substantial clotting dysfunction. For example, if the clot contracted down very quickly with a high PCF, or positive value, but then the clot weakened so the contractile force of the platelets was overcome by the dynamic flow of the blood, resulting in the post bending in the direction of flow, producing a negative value (online supplemental file 2). Additionally, each Stasys sample's confidence value was noted to exclude potentially less reliable results as this value represents the percentage of sensors in the cartridges that the analyzer deems are producing accurate results. This value can be affected by bubbles within the samples, bent sensors, or clots that became dislodged from the sensors during the test.

Statistical analysis

Demographic and clinical data are presented as means (SD), medians (IQR), or percentages. Univariate comparisons were made using Wilcoxon rank-sum or Kruskal-Wallis test for non-parametric data. Simple linear regression was performed to assess for associations between continuous values. Regression data were reported as the coefficient, or β-value, and correlating CI. An $\alpha \leq 0.05$ was considered significant. All analyses were performed using Stata V.18 (StataCorp, College Station, TX). The Strengthening the Reporting of Observational Studies in Epidemiology guideline was used to ensure proper reporting of methods, results, and discussion.[18]

RESULTS

Cohort demographics

Between March 2021 and January 2023, 262 patients were enrolled in the PART Study. Of the total cohort, 109 were excluded from statistical analysis due to missing paired sample results. All of the missing matched samples were due to reagent or cartridge shortages and represented a random selection of patients excluded. Patients on anticoagulation (n=34) were also

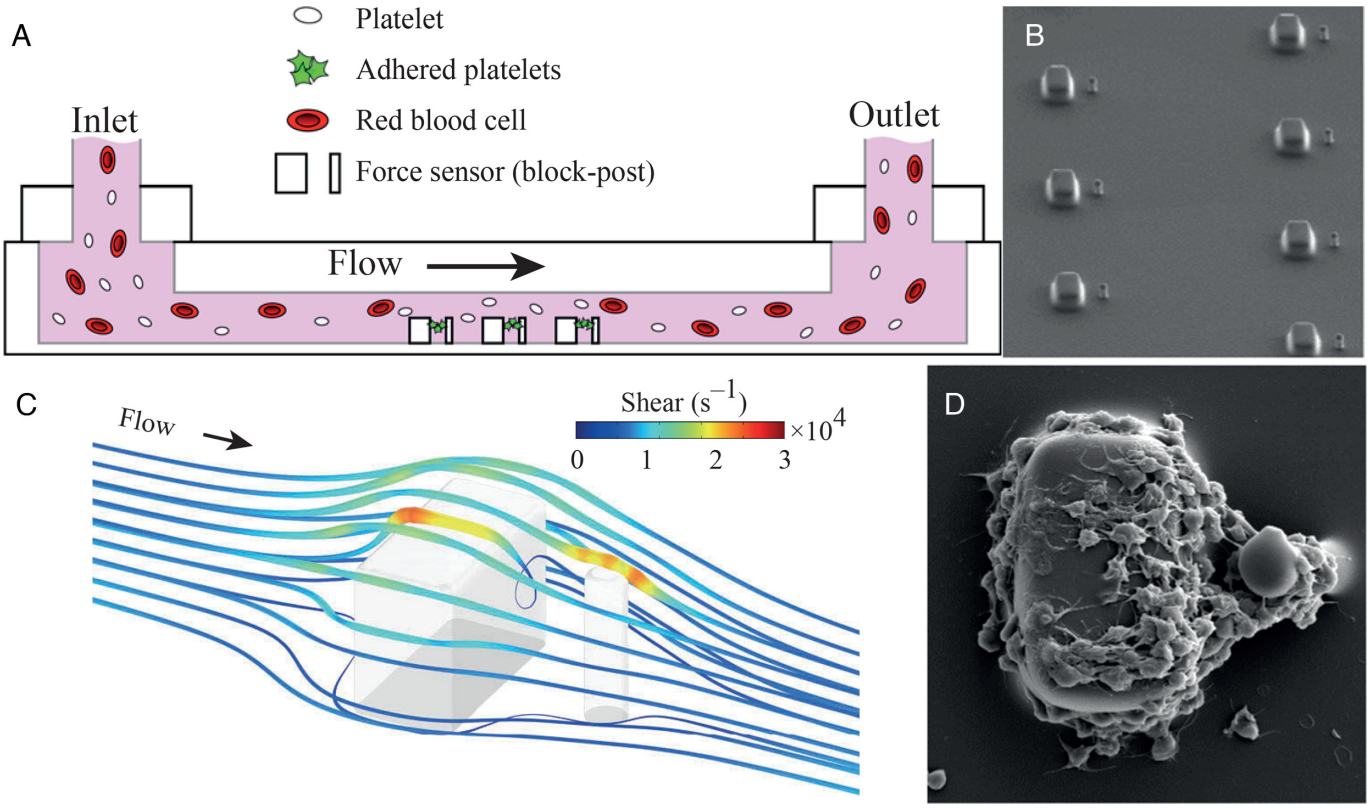

Figure 1 Schematic of Stasys platelet system. (A) Schematic of analyzer cartridge in which whole blood is injected at the inlet and flows across (B) arrays of microscale blocks and flexible posts for the measurement of platelet forces. (C) Simulation of shear forces created by microfluidics, including the high shear that platelets encounter as they flow over the blocks and posts. (D) Scanning electron microscopy micrograph of a platelet aggregate formed on a block and post after 45 seconds at 8000 s−1 shear force. Platelets have undergone shear-induced activation and shape change. Platelet contractile force is derived from the change in distance between the block and post over time as the platelet aggregate contracts and pulls the post upstream toward the block. Scale bar, 10 µm. Adapted from Ting *et al*.[9]

excluded to limit confounding correlations. Lastly, samples with Stasys confidence values of ≤40% (n=11) were excluded, as this was deemed a reasonable cut-off after discussion with the manufacturer. The final cohort for analysis includes 108 patients who had time zero Stasys assays performed (online supplemental file 1). Although this is a smaller proportion of the original cohort, the excluded and included patients showed no significant difference in proportion of male patients (p=0.16), white race (p=0.67), those receiving transfusions at 6 hours (p=0.99) and 24 hours (p=0.87), or blunt mechanism of injury (p=0.75). The only significant differences noted between the excluded and included cohorts were the excluded patients were older with a median age of 48 years, as opposed to a median age of 37.5 years in the included patients, and the included cohort had a significantly higher proportion of patients with severe anatomic injury, indicated by Injury Severity Score (ISS) of ≥25 (26% of included patients, 14% of excluded patients, p=0.02).

The median age of the analyzed cohort was 37.5 years (IQR 27.5–52), with 77% male and 48% white. The majority (71%) suffered from blunt trauma and 26% with severe anatomic injury (table 1). Compared with the national average of 3% rate of massive transfusion,[1 19] a high proportion of the cohort (31%) received transfusion of any blood products within 24 hours of presentation. The majority of those (94%) were transfused in the first 6 hours of admission and 26% of those transfused received massive transfusion (8% of total cohort), as defined by receiving ≥10 units of packed red blood cells (pRBCs) in 24 hours.[20]

Comparison studies

To establish normal ranges for comparison, 14 healthy donors were assessed. Median age of healthy controls was 32 (IQR 29.5–36) years. The healthy donors were similar to the study population in race (50% white, p=0.88), but were only 55% male compared with the 77% male composition of the study cohort (p=0.04). Mean cumulative PCF was 53 521 with an SD of 28 598; thus, a normal range of 24 922–82 120 was established for healthy controls. Similarly, mean cumulative CA was found to be 47 with an SD of 23, giving a normal range of 24–70. Normal range for maximum PCF was 204–452 (mean 328, SD 124) and for maximum CA was 0.13–0.29 (mean 0.21, SD 0.08). These normal ranges were compared with the injured cohort (online supplemental file 3). The patients who received transfusions in the first 6 hours were found to have a median cumulative PCF of 25 194 (IQR −9495 to 42 008), a median maximum PCF of 206 (IQR 142–283), a median cumulative CA of 53 (IQR 45–72), and a maximum CA of 0.26 (IQR 0.18–0.34). Comparatively, the patients who did not receive transfusions during this time frame had a median cumulative PCF of 32 736 (IQR 4123–53 523), a median maximum PCF of 243 (IQR 141–351), a median cumulative CA of 58 (IQR 39–77), and a median maximum CA of 0.25 (IQR 0.18–0.34) (online supplemental file 4).

First, TEG and Stasys assays were compared to evaluate if there was a direct relationship in results between the two testing methods. There was no significant association noted between the TEG assays and Stasys PCF (R time β=911.51, p=0.90;

Table 1 Cohort demographics

	n=108
Age (median, IQR)	37.5 (27.5–52)
% 18–40 years	56
% 41–64 years	32
% ≥65 years	12
Male gender (%)	77
Race* (%)	
White	48
Black	19
Other	33
Hispanic ethnicity* (%)	24
Mechanism of injury (%)	
Blunt trauma	71
Penetrating trauma	29
Vitals at presentation (median, IQR)	
GCS	15 (13–15)
Systolic blood pressure	130 (115–143)
% ≤90 mm Hg	5
Heart rate	96 (84–112)
% ≥120 beats/min	17
Labs at presentation (median, IQR)	
PT (s)/INR	10 (9.5–10.6)/1.09 (1.04–1.17)
% INR >1.5	6
Platelet count (×10^9/L)	251 (209–300)
% <150×10^9/L	11
Base excess (mmol/L)	0 (−4 to 2)
% ≤−6 mmol/L	17
Injury Severity Score (ISS) (median, IQR)	10 (4–25)
% ISS 0–14	60
% ISS 15–24	14
% ISS ≥25	26
Transfused at 6 hours (%)	30
Transfused at 24 hours (%)	31

*As self-reported by patient or family where required.
GCS, Glasgow Coma Scale; INR, international normalized ratio; PT, prothrombin time.

Table 2 Association of coagulopathy and injury severity with Stasys values

	Coefficient (β)	CI	P value
International normalized ratio			
Cumulative platelet contractile force	−942.38	−1620.09 to −246.46	0.05*
Maximum platelet contractile force	−176.74	−351.34 to −2.13	0.05*
Cumulative clot area	−0.21	−0.54 to 0.12	0.21
Maximum clot area	−0.11	−0.21 to −0.02	0.02*
Injury Severity Score			
Cumulative platelet contractile force	−19266.21	−75543.1 to −18485.20	0.01*
Maximum platelet contractile force	−2.03	−4.21 to 0.15	0.07
Cumulative clot area	−25.22	−51.36 to −0.92	0.06
Maximum clot area	0.01	−0.01 to 0.01	0.38

Data were compared uing simple linear regression and were reported with the corresponding coefficient, or β-value, CI, and p value.
*Indicates statistically significant findings of p≤0.05.

CRT-MA β=783.09, p=0.91; FF-MA β=−12.98, p=0.65; Ly30 β=−9114.68, p=0.38) or CA (R time β=−2.69, p=0.14; CRT-MA β=0.59, p=0.97; FF-MA β=0.34, p=0.12; Ly30 β=0.24, p=0.30).

We then evaluated if Stasys assays were associated with an elevated INR and found that a decrease in all Stasys markers except for cumulative CA were significantly associated with an increase in INR (cumulative PCF β=−942.28, p=0.05; maximum PCF β=−176.74, p=0.05; cumulative CA β=−0.21, p=0.21; maximum CA β=−0.11, p=0.02) (table 2). Stasys assays were also evaluated for association with injury severity. It was found that a decreased cumulative PCF was associated with an ISS of ≥15 (β=−19266.21, p=0.01), whereas a decreased maximum PCF and decreased cumulative CA trended with an elevated ISS (maximum PCF β=−2.03, p=0.07; cumulative CA β=−25.22, p=0.06) (table 2). Interestingly, platelet count did not significantly correlate with any of the Stasys values (cumulative PCF (p=0.13), maximum PCF (p=0.54), cumulative CA (p=0.50), maximum CA (p=0.12)).

The Stasys results of those patients not requiring any transfusion were then compared with those patients receiving transfusions at 0–6 and 0–24 hours after injury, with a statistically significant difference noted in cumulative PCF for patients who received transfusion of any products at 6 and 24 hours (p=0.04 and p=0.05) as well as pRBCs at 6 and 24 hours (p=0.04 and p=0.03). Additionally, there was a statistically significant difference in the maximum PCF for the patients who received transfusion of any products at 6 hours (p=0.04) as well as platelets at 6 hours (p=0.03) compared with patients who did not receive a transfusion. Further, there was also a significant difference in maximum PCF when comparing patients who received any products at 24 hours (p=0.02), as well as pRBCs (p=0.03) and fresh frozen plasma (FFP, p=0.03) at 24 hours. There was no significant difference noted in cumulative or maximum CA values between the transfused and non-transfused groups at 6 or 24 hours after injury (table 3).

Simple linear regression was performed evaluating the relationship between the number of units of blood product transfused to the Stasys CA or PCF. There was a significant correlation between decreased cumulative PCF, or weaker clot strength, and number of pRBC transfusions from 0 to 6 hours (β=−3119.42, p=0.05). A trend of decreased cumulative PCF with the number of FFP units (β=−2148.12, p=0.07) and platelet units (β=−10051.02, p=0.08) transfused from 0 to 6 hours was also noted. The number of products transfused at later time intervals (6–12 or 12–24 hours after injury) was not found to be related to cumulative PCF (table 4).

Maximum PCF was then evaluated to determine if this was a stronger predictor of early (≤6 hours) transfusion needs. Decreased maximum PCF was found to be significantly associated with the number of individual components transfused in the first 6 hours after injury: pRBC (β=−11.47, p=0.03), FFP (β=−10.93, p=0.03), and platelets (β=−39.75, p=0.03). Similarly, to cumulative PCF, a decreased maximum PCF was not associated with an increase in units of any products transfused at later time points (6–12 or 12–24 hours) after injury (table 4).

Stasys CA was then evaluated. Decreased cumulative CA was correlated with the number of pRBC units transfused at 12–24 hours (β=−6.03, p=0.04) and there were trends noted in decreased cumulative CA with number of FFP units transfused at 12–24 hours (β=−4.11, p=0.09). Cumulative CA did not show significant correlation with the number of blood product units

Table 3 Association of Stasys assays and patients receiving transfusions

	Transfusions at 0–6 hours				Transfusions at 0–24 hours			
	Any products (n=32; 30%)	pRBCs (n=31; 29%)	FFP (n=27; 25%)	PLT (n=14; 11%)	Any products (n=34; 31%)	pRBCs (n=33; 30%)	FFP (n=29; 27%)	PLT (n=17; 14%)
Platelet contractile force								
Cumulative	25 593 (0.04*)	25 193 (0.04*)	25 832 (0.12)	24 815 (0.18)	29 570 (0.02*)	33 308 (0.03*)	33 301 (0.09)	33 569 (0.37)
Maximum	203.27 (0.04*)	206.40 (0.06)	200.13 (0.06)	174.32 (0.03*)	207.26 (0.02*)	208.11 (0.03*)	205.88 (0.03*)	198.50 (0.09)
Clot area								
Cumulative	55.40 (0.99)	52.52 (0.95)	49.74 (0.51)	49.40 (0.99)	56.42 (0.57)	51.59 (0.54)	49.74 (0.22)	49.46 (0.71)
Maximum	0.26 (0.72)	0.26 (0.83)	0.24 (0.69)	0.23 (0.89)	0.26 (0.80)	0.26 (0.92)	0.26 (0.62)	0.22 (0.81)

Data presented as median and p values for comparison of transfused versus non-transfused patients, using Kruskal-Wallis test listed above. Portion of cohort receiving transfusion reported as absolute value and percentage.
*Indicates statistically significant findings of p≤0.05.
FFP, fresh frozen plasma; PLT, platelets; pRBCs, packed red blood cells.

transfused in the remaining time frames. A decreased maximum CA did not show significant correlation with the number of blood products transfused at any time in the first 24 hours of admission (table 5).

Table 4 Change in platelet contractile force by number of units of product transfused

	Coefficient (β)	CI	P value
Cumulative platelet contractile force			
Number of units transfused at 0–6 hours			
pRBCs 0–6 hours	−3119.42	−6275.61 to −36.78	0.05*
FFP 0–6 hours	−2148.12	−5199.05 to 902.82	0.07
PLT 0–6 hours	−10051.02	−21385.31 to 1283.26	0.08
Number of units transfused at 6–12 hours			
pRBCs 6–12 hours	−3539.41	−20374.41 to 13 295.58	0.68
FFP 6–12 hours	−3331.80	−23195.48 to 16 531.89	0.74
PLT 6–12 hours	6681.87	−51178.01 to 64 541.78	0.82
Number of units transfused at 12–24 hours			
pRBCs 12–24 hours	−8566.44	−33510.66 to 16 377.77	0.50
FFP 12–24 hours	496.70	−26724.59 to 27 717.99	0.97
PLT 12–24 hours	11 359.83	−23548.52 to 46 298.18	0.52
Maximum platelet contractile force			
Number of units transfused at 0–6 hours			
pRBCs 0–6 hours	−11.47	−21.56 to −1.37	0.03*
FFP 0–6 hours	−10.93	−20.61 to −1.26	0.03*
PLT 0–6 hours	−39.75	−75.93 to −3.57	0.03*
Number of units transfused at 6–12 hours			
pRBCs 6–12 hours	−25.99	−79.96 to 27.98	0.34
FFP 6–12 hours	−23.94	−87.71 to 39.83	0.46
PLT 6–12 hours	−77.19	−262.78 to 108.39	0.41
Number of units transfused at 12–24 hours			
pRBCs 12–24 hours	−71.47	−149.89 to 6.96	0.07
FFP 12–24 hours	−61.17	−147.10 to 24.76	0.16
PLT 12–24 hours	−16.82	−128.25 to 94.61	0.77

Data were compared using simple linear regression and were reported with the corresponding coefficient, or β-value, CI, and p value.
*Indicates statistically significant findings of p≤0.05.
FFP, fresh frozen plasma; PLT, platelets; pRBCs, packed red blood cells.

DISCUSSION

Several studies have shown that early identification and correction of coagulopathy in trauma patients lead to better outcomes.[21–23] Previously, it was thought that hemodilution from resuscitation, hypothermia, and acidemia were the main driving factors responsible for triggering trauma-induced coagulopathy

Table 5 Change in clot area by number of units of product transfused

	Coefficient (β)	CI	P value
Cumulative clot area			
Number of units transfused at 0–6 hours			
pRBCs 0–6 hours	−0.12	−1.64 to 1.39	0.87
FFP 0–6 hours	−0.26	−1.72 to 1.18	0.71
PLT 0–6 hours	−0.08	−5.51 to 5.34	0.98
Number of units transfused at 6–12 hours			
pRBCs 6–12 hours	−4.67	−12.56 to 3.22	0.24
FFP 6–12 hours	−5.24	−14.56 to 4.08	0.27
PLT 6–12 hours	−4.36	−31.66 to 22.92	0.75
Number of units transfused at 12–24 hours			
pRBCs 12–24 hours	−6.03	−17.76 to −5.71	0.04*
FFP 12–24 hours	−4.11	−16.92 to 0.71	0.09
PLT 12–24 hours	−9.36	−25.76 to 7.04	0.26
Maximum clot area			
Number of units transfused at 0–6 hours			
pRBCs 0–6 hours	−0.01	−0.01 to 0.01	0.67
FFP 0–6 hours	−0.01	−0.01 to 0.01	0.54
PLT 0–6 hours	−0.01	−0.03 to 0.03	0.79
Number of units transfused at 6–12 hours			
pRBCs 6–12 hours	−0.03	−0.07 to 0.02	0.24
FFP 6–12 hours	−0.03	−0.06 to 0.01	0.16
PLT 6–12 hours	−0.04	−0.19 to 0.11	0.60
Number of units transfused at 12–24 hours			
pRBCs 12–24 hours	−0.04	−0.07 to 0.02	0.20
FFP 12–24 hours	−0.03	−0.10 to 0.04	0.37
PLT 12–24 hours	−0.05	−0.14 to 0.03	0.23

Data were compared using simple linear regression and were reported with the corresponding coefficient, or β-value, CI, and p value.
*Indicates statistically significant findings of p≤0.05.
FFP, fresh frozen plasma; PLT, platelets; pRBCs, packed red blood cells.

(TIC). Recent studies have uncovered that TIC develops early in trauma, before medical intervention or development of hypothermia and acidemia.[24–27] These findings, in addition to studies noting improved survival with early balanced resuscitation,[28–30] stress the importance of earlier detection of coagulopathy to allow transfusion protocols to be more rapidly activated.

Although traditional viscoelastic studies such as TEG and ROTEM have been found to be useful in guiding transfusion in trauma patients, the lack of standardization and correlation with coagulopathies, in addition to the extensive time it takes to get results, have left trauma teams without a 'gold standard' that can be used in the fast-paced trauma bay.[5 21 31 32] Although this study was not designed as a head-to-head comparison of the utility of Stasys versus viscoelastic assays, the findings of the current study combined with the rapidity of results support the promise of this technology in clinical care. The device uses an approach that more closely simulates in vivo clot formation and gives Stasys a strong potential of providing a better approach for assessing overall clotting dysfunction.[9]

Overall, our study showed that maximum PCF was the most predictive of number of products transfused and transfusion requirements, but we do acknowledge that not all Stasys parameters demonstrated strong correlation. Previously, the consumption or inhibition of coagulation factors was thought to be the major contributor of post-traumatic coagulopathy, but recent studies have now recognized that platelet dysfunction also plays a significant role.[24 33–35] These findings could provide an explanation for the lack of correlation seen with CA, which incorporates clotting factors as well as platelets. Additionally, the complex cytoskeleton interaction of platelets has been investigated and shown the degree of contractility a platelet is able to generate largely affects the hemostatic ability of clot formation.[36–38] We are encouraged that our findings of the predictive value of PCF support and build on previous research.

To better define the ability of Stasys to predict coagulopathy on presentation, we investigated the correlations with need for transfusion in the first 24 hours of care. We found that a decreased cumulative PCF correlated with transfusion of any products at 6 and 24 hours after presentation. More specifically, a decreased cumulative PCF was found to be predictive of number of pRBC units transfused at 6 and 24 hours. Surprisingly, a decreased maximum PCF, as opposed to the cumulative PCF, was a more significant predictor of transfusion needs of any products by 6 and 24 hours after admission. The maximum PCF also correlated not only with transfusion of platelets at both time points, but also pRBCs and FFP. We initially thought the area under the curve calculations, or cumulative PCF, would provide a better overall evaluation of clot strength, but these findings suggest that a decreased maximum PCF might provide an early indication of clotting dysfunction. A possible explanation could be the cumulative PCF would appear adequate if the platelet contraction is maintained at a low level for an extended period, but the maximum PCF would be decreased and the only indication of coagulopathy.

In this study, we also demonstrated that decreased Stasys PCF and CA correlate with an abnormal PT/INR, suggesting that Stasys detects coagulopathy as benchmarked against standard 'coagulation' markers like PT/INR. We also found that a decreased PCF is related to injury severity, suggesting additional potential utility as an early marker of degree of injury burden. Although we were surprised the Stasys assays did not correlate with the platelet count at the time of admission, this likely just emphasizes that absolute count of the platelets is not as significant as the platelet's ability to form a clot and apply the contractile forces needed to maintain a clot, which is partly what Stasys uniquely evaluates. We also investigated the correlation between Stasys and viscoelastic testing regarding detection of coagulopathy. Theoretically, Stasys more closely mimics in vivo clotting formation and thus, we anticipated the two testing methodologies may not yield the same results. As expected, we demonstrated that Stasys assays were not found to correlate strongly with TEG results. This likely represents the differences of testing methodology rather than a lack of coagulopathy detection.

Despite the high rate of transfusion in this cohort (one-third of patients), there was also a sizeable number of non-injured or minimally injured (ISS <9) patients included. This broad composition in degree of injury in the cohort, ranging from mildly injured to severe injury, is a strength of this study, as it demonstrated the ability of Stasys to differentiate between transfusion needs across a wide spectrum of patients, promising for eventual clinical implementation in a heterogeneous trauma population. The cohort composition of the patients who were included in this study is similar to other level 1 trauma center patient populations with regard to median age, gender, mechanism of injury and ISS.[39] Further, the Stasys group compared with the PART patients not having a Stasys assay appears to represent a random selection of patients, or in statistical terms, the data appear to be missing at random. Thus, the study is unlikely to be biased by the selection criteria of who received a Stasys assay. This is consistent with the study protocol which stipulated a stratified prioritization for assays with the traditional clinical laboratory parameters given preference for use of the blood volume obtained over assessment of this new, largely unexplored technology. Thus, if limited blood was available due to access issues, other assays were prioritized first. This contributed to the lower number of patients receiving Stasys compared with the total enrollment in the PART cohort.

Limitations

This study was conducted as a convenience sample when research coordinators were available. Although this has risk of sampling bias, the patients enrolled reflect a similar degree of injury for our highest-level trauma activations regardless of time of arrival. Additionally, this study focuses on demonstrating the utility of new technology for the detection of abnormalities and not for comparing patient populations. Thus, even if sampling bias exists, it would not change the results of this study. During early phases of this study, as our laboratory was working with the Stasys group to identify problems associated with developing a new analyzer, it was noted that there was a higher number of samples that were excluded due to low Stasys confidence values. As the study progressed, these issues were addressed, the analysis of samples became more standardized and the confidence values were seen to improve. However, the higher number of excluded samples and possible sample bias should be acknowledged as a limitation of this study and would need to be addressed in further validation studies. As this was the first independent study of Stasys, there was also a need to establish normal ranges and develop a technique to report the results in a clinically useful manner. Healthy, uninjured controls were recruited to establish preliminary normal ranges for Stasys assays for this initial comparison study. We acknowledge that a limitation of the study is the small number of healthy controls analyzed. The small cohort of healthy controls may have contributed to the larger SDs which subsequently caused an overlap of the normal values with those values seen in the transfused population. As this was a feasibility study, future validation studies should consider using a larger group of healthy controls. The utilization of healthy controls adds rigor to our establishment

of normal ranges compared with relying on the patients found to be non-injured but presenting as traumas. Healthy controls were selected instead of using the uninjured trauma patients because it was not known how the stress response of being in a trauma would alter the baseline measurements in this new technology. Our work has demonstrated that this technology is able to distinguish need for transfusion between those who are injured and are not/mildly injured who do not demonstrate coagulopathy. Thus, our 'normal' ranges established appear to hold promise for accurately reflecting clinical utility but should be validated in a larger group of healthy controls given the small sample size in this work. We also acknowledge that it is difficult to account for all the confounding factors in the current sample size that could be affecting the results of the study, so further investigations would be required.

Future directions

As is the case with new assays, the clinical significance of results requires further investigation to fully elucidate the utility of this technology. Conceptually, this analyzer has demonstrated significant promise in a relatively broad trauma patient population for the detection of coagulopathy. However, paired comparison of Stasys results with viscoelastic tests performed simultaneously would add rigor to establishing the equivalence or potential superiority of Stasys for being the optimum method for detecting post-traumatic coagulopathy. Understanding the sensitivity and specificity of Stasys compared with viscoelastic assays for predicting transfusion need in trauma patients is an important future endeavor to better characterize the strengths and potential pitfalls of this new technology.

CONCLUSIONS

Quickly assessing coagulopathy in trauma patients remains challenging, but imperative. Our study demonstrated that the Stasys assays, using shear stress-inducing microfluidics, appear to allow for a near real-time evaluation of in vivo clot formation and predict transfusion requirements at time of admission as well as requirements in the first 24 hours.

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