Characterization of platelet dysfunction after trauma

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BACKGROUND: The increased morbidity and mortality associated with coagulopathy and thrombocytopenia after trauma are well described. However, few studies have assessed platelet function after injury.

METHODS: Blood samples were prospectively collected from 101 patients with critical injury and trauma on arrival to the emergency department and serially after admission to a Level I urban trauma intensive care unit from November 2010 to October 2011 and functionally assayed for responsiveness to adenosine diphosphate, thrombin receptor-activating peptide, arachidonic acid (AA), and collagen using multiple electrode impedance aggregometry.

RESULTS: Of the 101 enrolled patients, 46 (45.5%) had below-normal platelet response to at least one agonist ("platelet hypofunction") at admission, and 92 patients (91.1%) had platelet hypofunction some time during their intensive care unit stay. Admission platelet hypofunction was associated with low Glasgow Coma Scale score and a nearly 10-fold higher early mortality. Logistic regression identified admission Glasgow Coma Scale (odds ratio, 0.819; $p = 0.008$) and base deficit (odds ratio, 0.872; $p = 0.033$) as independent predictors of platelet hypofunction. Admission AA and collagen responsiveness were significantly lower for patients who died ($p < 0.01$), whereas admission platelet counts were similar ($p = 0.278$); Cox regression confirmed thrombin receptor-activating peptide, AA, and collagen responsiveness as independent predictors of in-hospital mortality ($p < 0.05$). Receiver operating characteristic analysis identified admission AA and collagen responsiveness as negative predictors of both 24-hour (AA area under the curve [AUC], 0.874; collagen AUC, 0.904) and in-hospital mortality (AA AUC, 0.769; collagen AUC, 0.717).

CONCLUSION: In this prognostic study, we identify clinically significant platelet dysfunction after trauma in the presence of an otherwise reassuring platelet count and standard clotting studies, with profound implications for mortality. Multiple electrode impedance aggregometry reliably identifies this dysfunction in injured patients, and admission AA and collagen responsiveness are sensitive and specific independent predictors of both early and late mortality. (*J Trauma Acute Care Surg*. 2012;73: 13–19.

LEVEL OF EVIDENCE: Prognostic study, level II.
KEY WORDS: Platelets; impedance aggregometry; multiple electrode aggregometry.

Platelets play a pivotal role in hemostasis after injury.[1] Recent evidence identifies that admission platelet counts are inversely correlated with early mortality and transfusion for patients with critical injury and trauma, even for platelet counts well into the normal reference range.[2] Quantitative platelet deficits also predict progression of intracranial hemorrhage and mortality after traumatic brain injury.[3] Although the increased morbidity and mortality associated with enzymatic coagulopathy after trauma is well described, near-total impairment of clot formation can also occur as a result of platelet dysfunction despite the presence of reference range coagulation studies and platelet count.[4] Thorough study of platelet dysfunction has been hindered by the technical complexity of existing platelet function assays; however, recent advances in impedance-based platelet aggregometry allow for rapid, point-of-care assessment of platelet function.[5]

Impedance aggregometry assays platelet aggregation via electrical resistance across sets of silver-coated copper electrodes immersed in whole blood; nonthrombogenic resting platelets are activated using specific platelet agonists, causing platelets to aggregate on the charged surface and increasing impedance in proportion to the degree of platelet activation.[6] This principle underlies the recently developed Multiplate multiple electrode aggregometer, which uses disposable test cells containing duplicate pairs of sensor wires to measure platelet aggregation in response to agonists of interest in citrated, heparinized, or hirudin-anticoagulated whole blood.[5] Impedance aggregometry has been cross-validated with single platelet counting, turbidimetric platelet aggregation, vasodilator-stimulated phosphoprotein phosphorylation, and light aggregometry[5,7,8] in normal controls and in monitoring clopidogrel and aspirin effects; however, only preliminary investigations exist using impedance aggregometry to characterize platelet dysfunction related to trauma.[9]

Therefore, the purpose of this study was to prospectively quantify platelet function using multiple electrode aggregometry to identify previously undetected platelet dysfunction in patients with trauma. We further sought to relate any observed dysfunction to outcomes after severe injury.

PATIENTS AND METHODS

Blood samples were prospectively collected from 101 patients with critical injury and trauma on arrival and at 6, 12, 24, 48, 72, 96, and 120 hours after admission to a Level I urban trauma intensive care unit (ICU) from November 2010 to October 2011. Admission samples were collected via initial placement of a 16G or larger peripheral intravenous line; subsequent samples were collected via indwelling arterial catheters. Standard laboratory vacuum-sealed tubes containing 3.2% (0.109 mol/L) sodium citrate were used for all draws. A total of 376 samples were analyzed, with a median of three samples per patient (interquartile range, 2–4). Demographics, resuscitation data, clinical laboratory results, and outcomes were collected in parallel. Informed consent was obtained from all patients, as approved by the University of California Committee on Human Research.

Platelet function was assessed at point of care using the Multiplate multiple electrode aggregometer (Verum Diagnostica GmbH, Munich, Germany) immediately after sample collection. Briefly, 0.3 mL of whole blood was diluted in warmed isotonic sodium chloride solution containing 3-mmol/L $CaCl_2$ and incubated for 3 minutes at 37°C with continuous stirring in a Multiplate test cell. Each test cell contains two sets of 3-mm silver-coated copper wires, across which electrical resistance is measured at 0.57-second intervals. Platelet activation was induced by adenosine diphosphate (ADP; final concentration, 6.5 μmol/L; via P2 receptors), thrombin receptor-activating

peptide-6 (TRAP; final concentration, 32 μmol/L; via PAR receptors), arachidonic acid (AA; final concentration, 0.5 mmol/L; via the cyclo-oxygenase pathway), or collagen (final concentration, 3.2 μg/mL; via GpIa/IIa and GpVI receptors). Platelet adhesion to the electrodes was detected as increasing electrical impedance, measured by duplicate sets of sensor wires in each test cell. Agonist responses are reported as area under the aggregation curve in units (U) during a 6-minute measurement period. Reference ranges for citrated whole blood were provided by the manufacturer based on studies of healthy controls.

Data are presented as mean (SD), median (interquartile range), or percentage; univariate comparisons were made using Student's *t* test for normally distributed data, Wilcoxon rank sum testing for skewed data, and Fisher's exact test for proportions. Logistic regression was performed to identify predictors of platelet hypofunction. Kaplan-Meier time-to-event analysis was used to assess differences in mortality; Cox proportional hazards regression was used to identify adjusted predictors of mortality. Nonparametric receiver operating characteristic (ROC) analysis was performed to characterize the ability of continuous agonist responses to classify binary outcomes. An $\alpha = 0.05$ was considered significant. All analysis was performed by the authors using Stata version 12 (StataCorp, College Station, TX).

RESULTS

Our study population composed of 101 patients had a mean (SD) age of 41.3 (19.3) years and a mean (SD) Injury Severity Score (ISS) 23.2 (5.4); there was 31.0% penetrating injury and 61.2% brain injury. Mean platelet responsiveness to ADP, TRAP, AA, and collagen at admission were in the low reference range according to manufacturer-provided reference values (Table 1). Notably, the mean (SD) admission platelet count was 274.4 (85.4) $\times$ 10^3/μL, with no admission platelet count below 140 $\times$ 10^3/μL (Table 1). Significant correlations between agonist response and platelet count were observed for all agonists, with linear correlation between platelet response extending well into the clinically "normal" platelet range (Fig. 1). Platelet responsiveness was then longitudinally evaluated from ICU admission to ICU discharge or 120 hours. For all agonists, mean platelet responsiveness fell sharply to below the reference range by 6 hours (Fig. 2). TRAP and collagen responsiveness returned to the low reference range by 24 hours, whereas ADP and AA responsiveness (Fig. 2*A* and *B*) remained significantly impaired until 96 hours (ADP; Fig. 2*C*) and 120 hours (AA; Fig. 2*D*), respectively. Mean platelet count remained more than 100 $\times$ 10^3/μL for the entirety of the ICU stay (Fig. 2*E*).

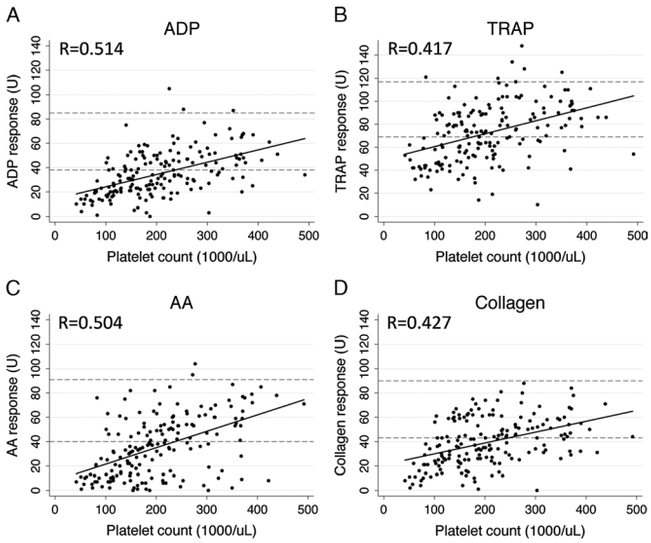

Figure 1. Scatter plots showing correlation between platelet count and ADP (*A*), TRAP (*B*), AA (*C*), and collagen (*D*) responsiveness using matched data from all time points collected. Manufacturer-provided reference ranges indicated by reference lines at the lower (5th percentile) and upper (95th percentile) boundaries. ρ values for each pairwise correlation given at upper left of each graph, with all associated $p < 0.001$.

Using the lower bound (fifth percentile) of manufacturer-provided reference ranges, 46 patients (45.5%) had below-normal platelet response to at least one agonist at admission; 92 patients (91.1%) had a below-normal response some time during their ICU stay. Of the 42 patients with confirmed prehospital medication data, 4 patients were taking aspirin and 1 patient was taking clopidogrel (Plavix) at the time of injury. Patients taking aspirin had significantly lower admission AA responsiveness (mean [SD], 5.8 [3.3] U vs. 48.0 [26.1] U; $p < 0.001$) and a trend toward lower collagen responsiveness (mean [SD], 24.5 [18.6] U vs. 46.7 [18.1] U; $p = 0.092$) but did not differ significantly in responsiveness to other agonists or by admission platelet count (all $p > 0.400$). Similarly, a single patient known to be taking Plavix had an admission ADP responsiveness of 27 U (below the 25th percentile in the study population) and a below-normal AA responsiveness of 38 U (reference range, 40–91 U). For all subsequent analysis, patients known to be taking aspirin or Plavix were excluded unless otherwise noted.

We then dichotomized the study population into 39 patients (42.9%) with a below-normal response to any agonist at admission ("platelet hypofunction") compared with 52 patients (57.1%) with all reference range responses ("normal function"). Platelet hypofunction was associated with low admission Glasgow Coma Scale (GCS) score ($p = 0.007$), higher mechanical ventilation requirements ($p = 0.040$), and a nearly 10-fold higher early mortality ($p = 0.009$; Table 2). Logistic regression identified base deficit (odds ratio [OR], 0.872;

TABLE 1. Admission Platelet Agonist Responses and Platelet Counts

	Admission Values (n = 78), Mean (SD)	Observed Range	Reference Range
ADP, U	44.6 (20.4)	0–105	38–85
TRAP, U	86.6 (27.0)	10–170	69–117
AA, U	44.3 (28.3)	0–104	40–91
Collagen, U	44.7 (19.7)	0–88	43–90
Platelets, $\times 10^3$/μL	274.4 (85.4)	140–605	150–400

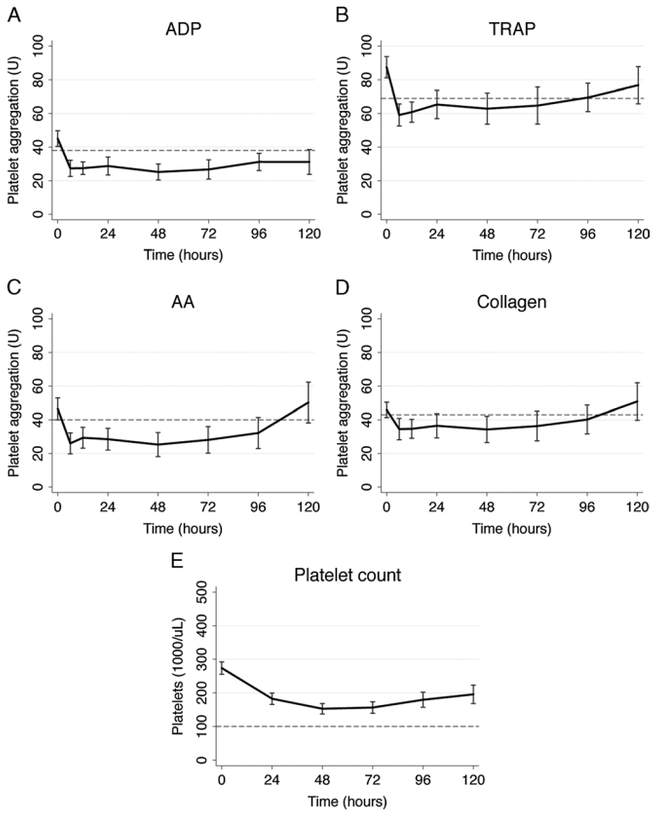

Figure 2. Platelet ADP (*A*), TRAP (*B*), AA (*C*), and collagen (*D*) responsiveness as area under the aggregation curve in units (U) over time. Platelet count measurements (*E*) are shown for comparison. Data points are mean values, with *capped bars* indicating 95% confidence intervals; *dotted lines* indicate the lower bound (fifth percentile) of normal values for each measurement.

$p = 0.033$) and GCS score (OR, 0.819, $p = 0.008$) as independent predictors of admission platelet hypofunction; platelet count was not a significant predictor of hypofunction ($p = 0.150$). Analysis was repeated in an intention-to-treat fashion including patients taking aspirin and Plavix: ORs and significance were similar for base deficit and GCS score, with older age identified as an additional significant predictor (OR, 1.041; $p = 0.032$).

To identify patient factors related to differential agonist responses, the study population was dichotomized by age (≥55 vs. <55 years), admission base deficit (≤−6 vs. >−6), traumatic brain injury (head Abbreviated Injury Scale [AIS] score, ≥3 vs. <3), and admission GCS score (≥8 vs. <8). Older patients had significantly lower responsiveness to TRAP (mean [SD], 74.5 [27.2] U vs. 91.9 [27.2] U; $p = 0.030$) and AA (mean [SD], 26.8 [23.5] U vs. 51.8 [26.2] U; $p = 0.001$); ADP ($p = 0.074$) and collagen ($p = 0.375$) responsiveness did not differ by age. Patients in shock as defined by admission base deficit had significantly lower responsiveness to collagen (mean [SD], 34.7 [20.5] U vs. 50.8 [16.7] U; $p = 0.011$), with no differences in ADP, TRAP, or AA responsiveness ($p > 0.200$). No significant differences were observed for patients with traumatic brain injury as identified by AIS score (all $p > 0.500$); however, patients with lower admission GCS score had lower responsiveness to ADP (mean [SD], 38.6 [20.7] U vs. 48.6 [20.3] U; $p = 0.016$) and collagen (mean [SD], 35.5 [20.1] U vs. 47.2 [18.3] U; $p = 0.008$), with no differences in TRAP or AA ($p > 0.300$). Admission platelet count did not statistically differ by age, base deficit, head AIS score, or GCS score (all $p > 0.100$).

Agonist responses were then examined for differences by mortality. For patients who died in-hospital at any time, admission AA and collagen responsiveness were significantly lower than those of survivors (AA: mean [SD], 22.4 [24.3] U vs. 48.8 [25.6] U; $p = 0.001$ (collagen: mean [SD], 29.6 [21.4] vs. 47.0 [17.6] U; $p = 0.008$), whereas admission platelet count did not differ significantly ($p = 0.278$). To account for the contribution of other patient and injury characteristics, Cox proportional hazards regression was used to adjust for age, GCS score, base deficit, and platelet count. In multivariate analysis, low TRAP (hazards ratio, 0.980; $p = 0.047$), AA (hazards ratio 0.968, $p = 0.003$), and collagen responsiveness (hazards ratio, 0.955; $p = 0.031$) were independent predictors of in-hospital mortality (Fig. 3). These results were unchanged when including patients taking aspirin or Plavix.

TABLE 2. Patient Characteristics by Platelet Hypofunction on Admission

	Platelet Hypofunction (n = 39)	Normal Function (n = 52)	*p*
Age, mean (SD), y	44.4 (20.7)	36.7 (16.5)	0.060
BMI, mean (SD), kg/m^2	26.3 (5.7)	25.6 (4.9)	0.543
Blunt injury, %	65.7	70.2	0.811
ISS, mean (SD)	22.8 (14.3)	25.4 (15.3)	0.513
GCS score,* median (IQR)	7 (3–10)	13 (6–15)	0.007
Temperature, mean (SD), °C	35.7 (0.7)	35.8 (0.8)	0.539
Prehospital IVF, median (IQR), mL	250 (50–1,000)	250 (50–750)	0.711
pH, mean (SD)	7.23 (0.20)	7.31 (0.14)	0.178
Base deficit, mean (SD)	−6.9 (6.4)	−3.9 (5.6)	0.072
INR, median (IQR),	1.2 (1.1–1.3)	1.2 (1.1–1.3)	0.875
PTT, median (IQR), s	28.6 (26.3–31.5)	26.4 (25.3–31.1)	0.322
Hematocrit, mean (SD), %	40.4 (5.5)	39.7 (4.8)	0.553
Platelet count, mean (SD), × 10^3/μL	257.7 (75.3)	285.7 (88.5)	0.125
RBC/24 h, median (IQR)	0 (0–1)	0 (0–4)	0.688
FFP/24 h, median (IQR)	0 (0–2)	0 (0–2)	0.795
Plts/24 h, median (IQR)	0 (0–0)	0 (0–0)	0.405
Hospital days, median (IQR),	6 (2–27)	10 (6.5–20)	0.090
ICU days, median (IQR)	3.5 (1–14)	3 (2–14)	0.436
Vent-free days/28 d,* median (IQR)	12 (0–26)	26 (7.5–27)	0.040
Multiorgan failure, %	31.7	27.3	0.656
24 h mortality,* %	20.0	2.1	0.009
In-hospital mortality, %	34.3	14.6	0.062

*$p < 0.05$ by Student's *t* test, Mann-Whitney *U* test, or Fisher's exact test.

BMI, body mass index; FFP, fresh frozen plasma units; INR, international normalized ratio; IQR, interquartile range; IVF, intravenous fluid; Plts\, platelet units; PTT, partial thromboplastin time; RBC, red blood cell units.

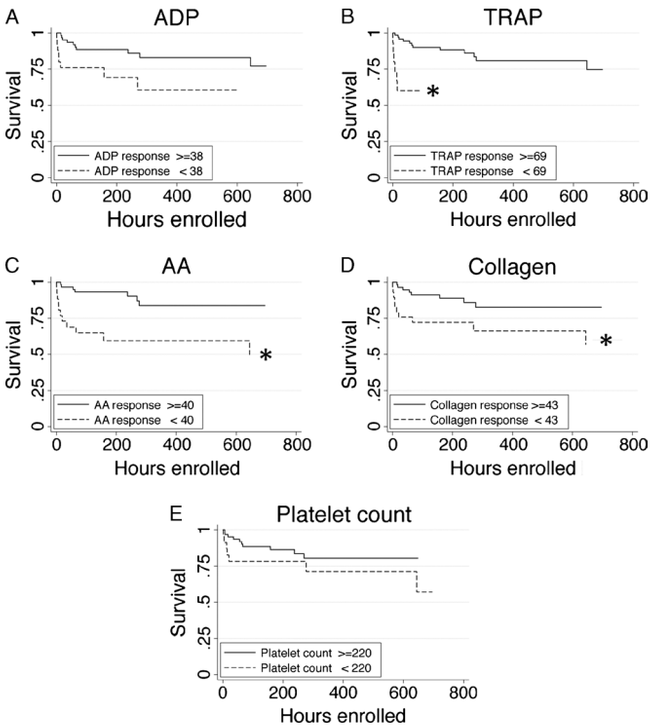

Figure 3. Kaplan-Meier 30-day survival curves showing survival differences between patients with below-normal admission platelet responsiveness to ADP (*A*), TRAP (*B*), AA (*C*), and collagen (*D*). Survival curves for patient admission platelet counts below the 25th percentile (*E*) are shown for comparison. *$p < 0.05$ by log-rank test.

To establish the utility of admission platelet function testing, nonparametric ROC analysis was used to identify clinically relevant cutoff values for admission agonist responsiveness as predictors of in-hospital mortality. Based on ROC curves (Fig. 4), AA (area under the curve, 0.769) and collagen (area under the curve, 0.717) responsiveness were robust predictors of mortality; in contrast, admission ADP responsiveness, TRAP responsiveness, and platelet count did not statistically differ from chance. Specifically, an admission AA responsiveness of 35 U or higher had 80.0% sensitivity and 68.8% specificity for in-hospital mortality (negative likelihood ratio, 0.291), correctly classifying mortality in 77.3% of patients; admission collagen responsiveness of 20 U or higher had 96.0% sensitivity and 37.5% specificity (negative likelihood ratio, 0.107), correctly classifying 81.8% of the patients.

DISCUSSION

Here, we report a prospective, impedance aggregometry-based analysis of platelet dysfunction after trauma. Using the Multiplate multiple electrode aggregometer, we serially assayed platelet activation to ADP, TRAP, AA, and collagen in 101 patients with critical injury on arrival and then serially for the remainder of their ICU stay. Despite uniformly normal admission platelet counts, platelet hypofunction was strikingly common, occurring in 45.5% of patients at admission and 91.1% some time during their ICU stay; mean responsiveness to some agonists remained abnormal for up to 120 hours after admission. We identified severe base deficit and low GCS score as multivariate predictors of admission platelet hypofunction. Using Cox proportional hazards regression, we demonstrated that admission platelet hyporesponsiveness to TRAP, AA, and collagen were independent predictors of mortality when adjusted for other patient and injury characteristics. Using ROC analysis to identify the most informative agonist responses, admission AA and collagen were found to be significant predictors of in-hospital mortality.

Platelet dysfunction after trauma has been systematically described in only three other studies. Jacoby et al.[10] used the platelet function analyzer-100 (PFA-100; which measures shear-induced occlusion of an aperture in an agonist-impregnated cartridge) and flow cytometric markers of platelet activation (platelet microparticles, P-selection, and activated glycoprotein IIb/IIIa) to prospectively assess platelet function in 100 patients with trauma. In this study, significantly impaired collagen/epinephrine closure times were observed in six nonsurvivors at later time points, although admission values were statistically similar to 94 survivors; similarly, closure times were impaired in 22 patients with significant head injury (head AIS score, ≥4) compared with 78 patients without head injury at 24 hours, with no difference at admission. This parallels our finding that

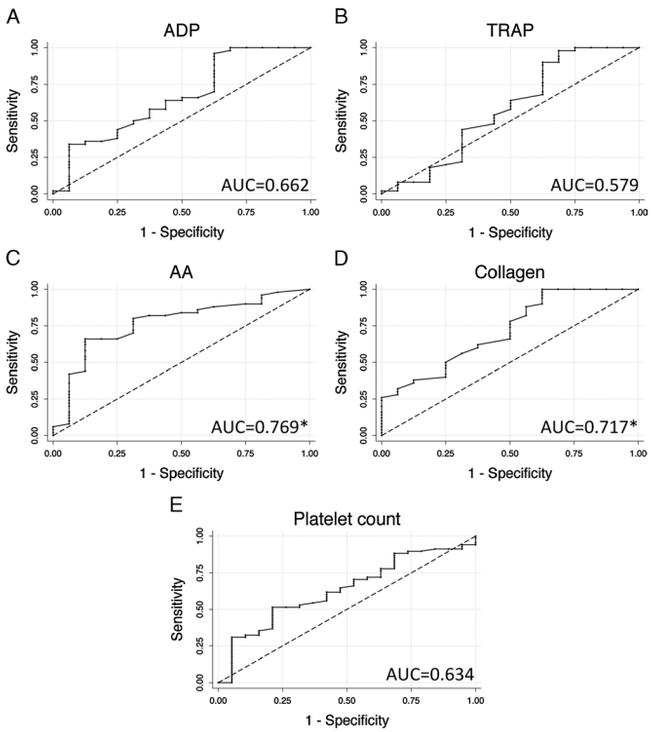

Figure 4. Receiver operating characteristic curves using admission platelet responsiveness to ADP (*A*), TRAP (*B*), AA (*C*), and collagen (*D*) as predictors of in-hospital mortality. Curve for admission platelet count (*E*) is shown for comparison. Area under the receiver operating characteristic curve (AUC) values given at the lower right in each graph. *Areas under the curve for which 95% confidence intervals differ significantly from chance.

platelet dysfunction is associated with mortality and that brain injury is a significant predictor of platelet dysfunction. This study found no differences in nonsurvivors or patients with brain injury at admission based on aperture closure time, although platelet microparticle levels were significantly higher at admission in these populations; this indicates that alterations in platelet function present on arrival are not reliably detected by PFA-100 aggregation. We here identify that impedance aggregometry is sensitive to these early differences and that poor admission AA-induced and collagen-induced responsiveness are associated with later mortality. Impedance aggregometry appears superior to PFA-100 aggregation in identifying platelet dysfunction on arrival, potentially allowing better triage and earlier targeted therapy.

Solomon et al.[9] recently reported a retrospective study of impedance aggregometry responses to ADP, TRAP, and collagen at admission in 163 patients with trauma. The incidence of platelet hypofunction in their study was notably lower than that reported here. They found platelet hyporesponsiveness to ADP in 13.9% of patients, TRAP in 13.7%, and collagen in 5.6%: comparatively, we identified hyporesponsiveness to ADP in 30.7%, TRAP in 18.7%, and collagen in 34.7%. Similarly, they identified only a weak correlation between platelet count and agonist responsiveness but did not report statistical significance. Many of these differences are likely attributable to differences in study population: their population had median ISS of 18 and overall mortality of 12.3%, compared with our median ISS of 25 and mortality rate of 22.7%. Because no adjusted analysis was performed, the absence of additional findings may be caused by the predominance of milder injury in their population. Their finding that ADP and TRAP responsiveness were significantly lower in seven patients with ISS of 50 or higher compared with 113 patients with ISS of 25 or lower supports this explanation. Despite these differences, the association of platelet dysfunction with mortality in their study parallels the unadjusted and multivariate analysis presented here.

Nekludov et al.[11] reported a smaller experience using thromboelastography (TEG)-based platelet mapping to evaluate platelet response to ADP and AA in 30 patients with trauma compared with controls. TEG-based platelet mapping measures the maximal amplitude of clot formation in heparin-treated and reptilase-treated whole blood in response to ADP and AA, comparing it to kaolin-activated maximal amplitude to generate an agonist-specific percentage of platelet inhibition.[12] The authors found significantly impaired AA responsiveness in patients with brain injury compared with patients with trauma without brain injury as well as controls but were unable to detect platelet dysfunction in 10 patients with trauma without brain injury. Although platelet mapping has been correlated with light aggregometry results,[13] to our knowledge, no direct comparison of TEG-based platelet mapping and impedance aggregometry exists to facilitate comparison of results. However, although we describe broader impaired AA responsiveness for patients with and without brain injury, our finding that low admission GCS score is a multivariate predictor of platelet hypofunction parallels the findings of Nekludov et al., pointing to important associations between platelet dysfunction and brain injury. These data have clear clinical implications for identifying patients at risk for intracranial hemorrhage progression.

The mechanisms underlying trauma-associated platelet dysfunction are poorly understood. One potential mechanism is suggested by Jacoby et al.,[10] who identified that flow cytometric markers of platelet activation were elevated in patients with trauma, despite impaired functional aperture closure times. In nontrauma studies, prolonged circulation of activated but hypofunctional platelets has been observed for up to 96 hours after activation.[14] These data mirror our finding that platelet function falls within 6 hours of admission and remains suppressed for up to 120 hours after injury. Taken together, this suggests that immediate platelet activation in response to tissue injury may induce a prolonged refractory state, in which a fraction of activated platelets remain in circulation but are dysfunctional. In light of the critical role played by platelets in the cell-based model of coagulation, this platelet hypofunction may correlate with functionally impaired thrombin generation even in the absence of classical explanations for coagulopathy (such as clotting factor depletion or hyperfibrinolysis) or may partially mediate the effects of hypothermia, hemodilution, and acidosis on clot formation. The ability to identify this state and assess the impact of targeted therapies would allow better guidance for the conduct of resuscitation and operative intervention.

Several limitations that are important for interpretation of this study exist. Similar to other platelet function studies, ours remains an initial, single-center experience; further work is needed to confirm and extend these findings. Although previous studies have cross-validated impedance aggregometry with several other assays of platelet function,[5,7,8] these studies were performed in healthy controls or were designed to detect antiplatelet medication effects. The reference ranges derived from these studies may not be ideal measures of platelet hypofunction in the setting of trauma. Point-of-care instrument use in a busy trauma center poses additional challenges in sample handling and evaluation of results that need to be addressed before these results can be clinically applied. Finally, although physiologic relevance is suggested by the prospective correlation of platelet function with later stent thrombosis in the cardiovascular literature,[15] further study is required to confirm that platelet aggregation in a laboratory test cell is a meaningful surrogate for hemostatic function in the bleeding trauma patient.

These results highlight two important clinical issues. First, although impairment of platelet response to ADP and AA have been characterized in response to clopidogrel and aspirin, respectively,[16] there is no a priori sense of which agonists are relevant in the setting of trauma. Given the wide availability of over-the-counter medications with antiplatelet effects and the known variability of platelet function in the population at large,[17] one could posit that the dysfunction of AA and collagen pathways seen here may be the result of an occult medication–related effect, as opposed to a trauma-related phenomenon. However, the observed hyporesponsiveness to TRAP argues that this platelet dysfunction is related to injury because neither thrombin generation nor platelet activation downstream of thrombin receptors is affected by cyclo-oxygenase-pathway blockade.[18] The data presented here

provide evidence for a specific platelet dysfunction induced by traumatic injury, but careful additional in vitro and clinical characterizations are required to elucidate the mechanism and to validate trauma-relevant agonists, reference ranges, and indications for clinical action.

Second, although we clearly identify the grave prognosis associated with platelet hypofunction, few therapeutic options exist to address it, calling into question the clinical utility of identifying an untreatable pathologic finding. Studies of platelet function-targeted therapy have been impeded by a lack of well-validated assays with which to demonstrate efficacy. However, the recent development of new functional analyzers has fostered a growing literature on several potential proplatelet therapies, including studies of desmopressin[19] and tranexamic acid[20] in reversing platelet dysfunction in cardiac surgical patients. Ongoing in vitro aggregometry studies and further prospective clinical studies will provide a platform for the evaluation of novel proplatelet agents, adding to the clinical armamentarium for treatment of trauma-associated platelet dysfunction.

Here, we demonstrate that clinically significant platelet dysfunction after trauma exists in the presence of an otherwise reassuring platelet count and clotting studies, with profound implications for mortality. Impedance aggregometry reliably identifies this dysfunction in injured patients, and admission AA and collagen responsiveness are significant predictors of both early and late mortality. The significance of low GCS score as an independent predictor of platelet hypofunction highlights the importance of further investigation into the link between traumatic brain injury and platelet dysfunction. The clinical availability of rapid, point-of-care platelet function testing will lead to improved triage, more appropriately targeted therapy, and better outcomes after trauma.

AUTHORSHIP

M.E.K. and M.J.C. prepared the article, performed all data analysis, and take full responsibility for the data as presented. B.J.R. and M.F.N. made significant contributions to the study design and implementation. R.C.M., I.M.C., M.D.G., and L.M.C. performed all clinical data collection.

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DISCLOSURE

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REFERENCES

1. Davenport RA, Brohi K. Coagulopathy in trauma patients: importance of thrombocyte function? *Curr Opin Anaesthesiol*. 2009;22:261–266.
2. Brown LM, Call MS, Margaret Knudson M, et al. A normal platelet count may not be enough: the impact of admission platelet count on mortality and transfusion in severely injured trauma patients. *J Trauma*. 2011;71: S337–S342.
3. Schnuriger B, Inaba K, Abdelsayed GA, et al. The impact of platelets on the progression of traumatic intracranial hemorrhage. *J Trauma*. 2010; 68:881–885.
4. Hess JR, Brohi K, Dutton RP, et al. The coagulopathy of trauma: a review of mechanisms. *J Trauma*. 2008;65:748–754.
5. Toth O, Calatzis A, Penz S, et al. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. *Thromb Haemost*. 2006;96:781–788.
6. Cardinal DC, Flower RJ. The electronic aggregometer: a novel device for assessing platelet behavior in blood. *J Pharmacol Methods*. 1980;3: 135–158.
7. Seyfert UT, Haubelt H, Vogt A, et al. Variables influencing Multiplate(TM) whole blood impedance platelet aggregometry and turbidimetric platelet aggregation in healthy individuals. *Platelets*. 2007;18:199–206.
8. Siller-Matula JM, Gouya G, Wolzt M, et al. Cross validation of the Multiple Electrode Aggregometry. A prospective trial in healthy volunteers. *Thromb Haemost*. 2009;102:397–403.
9. Solomon C, Traintinger S, Ziegler B, et al. Platelet function following trauma. A Multiple Electrode Aggregometry study. *Thromb Haemost*. 2011;106:322–330.
10. Jacoby RC, Owings JT, Holmes J, et al. Platelet activation and function after trauma. *J Trauma*. 2001;51:639–647.
11. Nekludov M, Bellander BM, Blomback M, et al. Platelet dysfunction in patients with severe traumatic brain injury. *J Neurotrauma*. 2007;24: 1699–1706.
12. Bochsen L, Wiinberg B, Kjelgaard-Hansen M, et al. Evaluation of the TEG platelet mapping assay in blood donors. *Thromb J*. 2007;5:3.
13. Craft RM, Chavez JJ, Bresee SJ, et al. A novel modification of the Thrombelastograph assay, isolating platelet function, correlates with optical platelet aggregation. *J Lab Clin Med*. 2004;143:301–309.
14. Wun T, Paglieroni T, Holland P. Prolonged circulation of activated platelets following plasmapheresis. *J Clin Apher*. 1994;9:10–16.
15. Sibbing D, Braun S, Morath T, et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. *J Am Coll Cardiol*. 2009;53:849–856.
16. Chen F, Maridakis V, O'Neill E A, et al. A randomized clinical trial comparing point-of-care platelet function assays and bleeding time in healthy subjects treated with aspirin or clopidogrel. *Platelets*. Sept 15, 2011. [ePub ahead of print].
17. Can MM, Tanboga IH, Turkyilmaz E, et al. The risk of false results in the assessment of platelet function in the absence of antiplatelet medication: comparison of the PFA-100, multiplate electrical impedance aggregometry and verify now assays. *Thromb Res*. 2010;125:e132–e137.
18. Armstrong PC, Truss NJ, Ali FY, et al. Aspirin and the in vitro linear relationship between thromboxane A2-mediated platelet aggregation and platelet production of thromboxane A2. *J Thromb Haemost*. 2008;6: 1933–1943.
19. Weber CF, Dietrich W, Spannagl M, et al. A point-of-care assessment of the effects of desmopressin on impaired platelet function using multiple electrode whole-blood aggregometry in patients after cardiac surgery. *Anesth Analg*. 2010;110:702–707.
20. Weber CF, Gorlinger K, Byhahn C, et al. Tranexamic acid partially improves platelet function in patients treated with dual antiplatelet therapy. *Eur J Anaesthesiol*. 2011;28:57–62.

