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Identification of injury and shock driven effects on ex vivo platelet aggregometry: A cautionary tale of phenotyping

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Abstract

BACKGROUND: Platelet behavior in trauma-induced coagulopathy is poorly understood. Injured patients have impaired platelet aggregation (*dysfunction*) in ex vivo agonist-stimulated platelet aggregometry (PA). However, PA assumes that platelets are inactivated before ex vivo stimulated aggregation, which may be altered by injury. We hypothesized that following trauma, platelet aggregation (area under the curve) is decreased regardless of injury burden, but that (1) minor injury is associated with an increased baseline electrical impedance, characteristic of a *functional* platelet phenotype (platelets that activate in response to injury), and that (2) severe injury is not associated with an increased baseline electrical impedance, characteristic of a *dysfunctional* phenotype (platelets that do not activate well in response to injury) compared with healthy controls.

METHODS: Blood from 458 trauma patients and 30 healthy donors was collected for PA. Baseline electrical impedance (Ω); platelet aggregation stimulated by adenosine diphosphate, collagen, thrombin, and arachidonic acid; and rotational thromboelastometry were measured. Multivariate regression was performed to identify associations of PA measures with blood transfusion.

RESULTS: Compared with healthy controls, injured patients had impaired platelet aggregation in response to ex vivo stimulation, regardless of injury burden. However, minorly injured patients had increased endogenous platelet activation (baseline electrical impedance, Ω: with shock, $p = 0.012$;

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AUTHORSHIP

N.E.S. and L.Z.K. performed the study design, literature search, data analysis, data interpretation, and writing. Z.A.M. and L.Z.K. performed the data interpretation, literature search, writing, and critical revision. Z.A. M., A.T.F., and B.N.-G. performed the data collection. R.A.C. and M.J.C. performed the data interpretation and critical revision.

N.E.S. and Z.A.M. are co-primary authors.

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DISCLOSURE

For all authors, no conflicts are declared.

without shock, $p = 0.084$), but severely injured patients did not have significant increases in endogenous platelet activation (baseline electrical impedance, Ω: with shock, $p = 0.86$; without shock, $p = 0.37$). For every 10 Ω increase in baseline electrical impedance, there was an 8% decrease in units of blood transfused in the first 24 h (−0.08; confidence interval, −0.14 to −0.02; $p = 0.015$).

CONCLUSION: Injury and shock confer differential patterns of platelet aggregation in PA. Minor injury overestimates the presence of platelet dysfunction, while severe injury induces a truly dysfunctional phenotype—platelets that do not activate nor aggregate appropriately after injury. This is consequential in improving accurate phenotyping of postinjury platelet behavior for platelet-based therapeutics.

LEVEL OF EVIDENCE: Prognostic, level IV.

Keywords

Platelets; platelet activation; platelet aggregation; platelet function tests; trauma

Trauma-induced coagulopathy (TIC) remains a prevalent driver of hemorrhage-related morbidity and mortality after injury.[1–3] Given the central role of platelets in hemostasis,[4–7] research efforts have increasingly focused on elucidating the contribution of platelets to failures of clot formation and breakdown that are characteristic of TIC.[2,3,8–11] Importantly, the majority of these studies have identified that, even with normal platelet counts, impaired platelet aggregation, measured by ex vivo assays, is present in nearly half of injured patients.[8,9,11–13] However, to what extent these identified impairments in platelet aggregation are pathologic remains uncertain. This is the result of both an incomplete understanding of the molecular mechanisms driving these impairments and limitations of the ex vivo assays used to assess platelet function.

Specifically, it has been demonstrated that, even in patients with minor burdens of injury, impaired ex vivo platelet aggregation is prevalent.[14] Furthermore, despite impairments in platelet aggregation after injury, data support concomitant increased platelet activation (increased surface receptor expression and circulating platelet microparticles).[9,15] This contradictory biologic pattern of increased activation but impaired aggregation of platelets in the setting of injury is challenging to interpret because the majority of studies identifying impairments in platelet aggregation have relied on ex vivo aggregometry assays.[8,11,14,16] Importantly, these aggregometry assays are based on the principle that the platelets are in a nonthrombogenic inactivated state prior to ex vivo stimulated aggregation. However, the assumption that circulating platelets in whole blood samples drawn from injured patients truly exist in a nonthrombogenic and inactivated state is called into question.

Methodologically, ex vivo platelet aggregometry (PA) assays such as multiple electrode PA (Multiplate) detect changes in electrical impedance in ohms (Ω) across metal electrodes as platelets aggregate in a sample of whole blood following ex vivo addition of a platelet-stimulating agonist. The aggregation response to the ex vivo stimulation is measured as an area under the curve (AUC), providing a measure of platelet aggregation[10,17] (Fig. 1A). Importantly, to calculate an AUC, the baseline electrical impedance of the whole blood

before ex vivo platelet stimulation must be measured (Fig. 1A). However, because the AUC is then normalized to the baseline electrical impedance, variations in baseline electrical impedance are not accounted for. While this baseline electrical impedance is unlikely to have significant variability nor be elevated in a healthy individual, in the setting of a disease state such as trauma, circulating platelets are activated as part of the endogenous process of coagulation.[9,16] For patients with normal coagulation responses to injury and bleeding, this may translate into increased baseline electrical impedance before ex vivo stimulation used to induce aggregation (Fig. 1B). If so, the AUC measure of platelet aggregation will overestimate the presence of *dysfunction* due to falsely low measurements of platelet aggregation in response to ex vivo platelet stimulation (Fig. 1B).

Therefore, we sought to identify the relationship of injury with baseline electrical impedance (injury-driven endogenous platelet activation) and AUC (measure of platelet aggregation) in multiple electrode PA. We hypothesized that following trauma, platelet aggregation (AUC) is decreased regardless of injury burden, but that (1) minor injury is associated with an increased baseline electrical impedance, characteristic of a *functional* platelet phenotype (platelets that activate in response to injury), and that (2) severe injury is not associated with an increased baseline electrical impedance, characteristic of a *dysfunctional* phenotype (platelets that do not activate well in response to injury) compared with healthy controls. To our knowledge, incorporating baseline electrical impedance measures into interpretation of ex vivo aggregometry assays has not previously been used to comprehensively phenotype PA results in the setting of trauma.

PATIENTS AND METHODS

Patient Enrollment

Citrated (3.2% [0.109 M] sodium citrate) whole blood samples were prospectively collected on 1,671 injured patients on arrival to the emergency department at Zuckerberg San Francisco General Hospital from 2011 to 2018 as part of Activation of Coagulation and Inflammation in Trauma, a longitudinal study examining coagulation and inflammation after trauma.[2,4,8,10,12,18–20] Highest-level trauma activations were enrolled under an initial waiver of consent that was approved by the University of California Institutional Review Board. Patients who were pediatric, pregnant, in-custody, had burns greater than 20% body surface area, were transferred from another facility, did not require ICU level care, or were found to be atraumatic were excluded. A subset of 458 patients was selected to include in this study because they were confirmed to not be on any anticoagulant/antiplatelet therapies and had whole blood assays performed. Multiple electrode PA and rotational thromboelastometry (ROTEM) were performed immediately on collection. Comprehensive patient demographics, injury characteristics, and clinical data were collected in parallel with laboratory results. An initial waiver of consent was followed by informed consent obtained from all patients as approved by the University of California Committee on Human Research. Citrated whole blood samples were additionally prospectively collected from 30 control healthy donors for comparison. Donors were healthy and not taking antiplatelet/anticoagulant medications. Healthy donor's informed consent was obtained with approval by the University of California Institutional Review Board.

Multiple Electrode PA

Platelet aggregation was measured with multiple electrode PA (Multiplate; Verum Diagnostica GmbH, Munich, Germany) immediately after sample collection.[8] Whole blood (0.3 mL) was diluted with 3 mM $CaCl_2$ in warmed normal saline, then incubated at 37°C, and continuously stirred for 3 minutes in a Multiplate test cell. Test cells measured electrical impedance across two sets of 3-mm silver-coated copper wires at 0.57-second intervals.

The baseline electrical impedance (Ω) was measured before addition of the platelet-stimulating agonists, providing a measure of the injury-driven endogenous platelet activation (Fig. 1). Standard platelet-stimulating agonists at concentrations provided by the manufacturer and validated in other studies[8,11,20] were used to stimulate platelet aggregation: adenosine diphosphate (final concentration, 6.5 μM; via P2 receptors), thrombin receptor activating peptide-6 (thrombin; final concentration, 32 μM; via PAR receptors), collagen (final concentration, 3.2 μg/mL; via GpIa/IIa and GpVI receptors), and arachidonic acid (final concentration, 0.5 mM; via the cyclooxygenase pathway). After platelet-stimulating agonists were added, platelet aggregation responses were recorded as increasing electrical impedance, which occurs as platelets adhere to the electrodes and to each other. After 6 minutes, the AUC was measured as aggregation units, representing the platelet aggregation in response to stimulation by each agonist. Reference ranges for platelet aggregation in response to stimulation by each agonist provided by the manufacturer are as follows: adenosine diphosphate, 36 to 101 U; thrombin, 75 to 137 U; collagen, 24 to 79 U; and arachidonic acid, 42 to 100 U.

Rotational Thromboelastometry

Viscoelastic clot strength was measured using the ROTEM delta machine (Pentapharm GmbH, Munich, Germany) with EXTEM reagents (star-TEM recalcifier; ex-Thromboelastometry (TEM) tissue factor extrinsic pathway activator). Citrated whole blood was warmed to 37°C in the TEM cup, prepared according to manufacturer specifications, and the test was initiated within 20 seconds of mixing 300 μL of whole blood with 20 μL of 0.2 M $CaCl_2$ for recalcification and respective activator. Maximum clot firmness (MCF) (mm) was recorded at completion of assay.

Statistical Analyses

Data are presented as mean (SD), median (interquartile range [IQR]), or percentage; univariate comparisons were made using Student's *t* test for normally distributed data, Wilcoxon rank-sum or Kruskal-Wallis testing for nonparametric data, and Fisher's exact test for proportions. The cohort was stratified by injury burden (Injury Severity Score [ISS] ≤ or >25) and shock severity (base excess [BE] ≤ or >−6). Univariate and multivariate linear regression analyses were performed to identify the association of baseline electrical impedance, AUC in response to stimulation by each agonist, and ROTEM maximum clot firmness by ROTEM extrinsic channel (EXTEM MCF) with units of blood transfusion in the initial 6 and 24 hours (as a surrogate for bleeding). Abbreviated Injury Scale for the head, chest, and abdomen were included in these models to control for injury burden to isolate the contribution of brain injury. The coefficients for linear regression reflect the estimated effect for each 10-U increase in baseline impedance (Ω) or AUC to support clinically meaningful

interpretations of the results. This is because the difference in baseline impedance (Ω) and AUC between groups are on the order of 10 to 40 U. The coefficients for ROTEM EXTEM MCF (mm) are not manipulated because of the smaller between group differences. In addition, we performed Pearson correlations to identify associations between PA results and viscoelastic clot strength. An α value of less than 0.05 was considered significant. Bonferroni corrections were applied for multiple comparisons. All analysis was performed using Stata version 15 (StataCorp, College Station, TX).

RESULTS

Baseline Characteristics

The 458 patients were young (median age, 37 years), 87% were male, and 54% of them suffered blunt mechanisms of injury. They were moderately injured (median ISS, 10; IQR, 2–26) but had normal initial platelet counts of 263 × 10^9/L (IQR, 218–310). Forty percent of patients required blood or plasma transfusions in the first 24 hours, while 12% received platelet transfusions. Twenty-four-hour mortality was 6%, and mortality at discharge was 12% (Table 1).

Injury and Shock Stratification

When stratified by injury burden (ISS ≤ or >25) and shock severity (BE ≤ or >−6), patients with minor injury (ISS ≤25) were less likely to have a blunt mechanism of injury (42% and 49% with and without shock, respectively) as compared with patients with severe injury (ISS >25; 64% and 80% with and without shock, respectively). Furthermore, patients with severe injury were more likely to have a traumatic brain injury (64% and 70% vs. 15% and 14% with and without shock, respectively) and coagulopathy (international normalized ratio (INR) ≥1.3; 55% and 33% vs. 17% and 4% with and without shock, respectively). They were also more likely to receive transfusions of blood (73% and 69% vs. 58% and 19% with and without shock), plasma (67% and 53% vs. 35% and 6% with and without shock), and platelets (36% and 28% vs. 14% and 2% with and without shock, respectively) in the first 24 hours. Furthermore, they had longer hospital and ICU stays, higher rates of acute lung injury and multiple organ failure, and higher rates of mortality at 24 hours and discharge. However, despite these differences, the initial platelet counts were normal across all injury and shock groups (median, 274 × 10^9/L and 266 × 10^9/L vs. 268 × 10^9/L and 261 × 10^9/L with and without shock, respectively; Table 1).

Multiple Electrode PA

Despite the lack of differences in platelet counts across injury and shock groups, there were notable differences in endogenous platelet activation (baseline electrical impedance, Ω) and platelet aggregation (AUC) in multiple electrode PA results. Compared with healthy controls, patients with minor injury had increased levels of endogenous platelet activation (baseline electrical impedances, Ω: with shock, 1,397 vs. 1,369, $p = 0.012$; without shock, 1,388 vs. 1,369, $p = 0.084$). However, patients with severe injury had no significant increases in endogenous platelet activation compared with healthy controls (baseline electrical impedances, Ω: with shock, 1,367 vs. 1,369, $p = 0.86$; without shock, 1,379 vs. 1,369, $p = 0.37$; Fig. 2 and Table 2).

Despite differences between the levels of endogenous platelet activation in injury groups, all injury groups had lower platelet aggregation in response to ex vivo stimulation with stimulating agonists thrombin, collagen, and arachidonic acid compared with healthy controls (Fig. 2 and Table 2). This was particularly notable for platelet aggregation in response to collagen stimulation, in which there was a significant and stepwise reduction in platelet aggregation, most significant in those with combined severe injury and shock (Fig. 2 and Table 2). Platelet aggregation in response to arachidonic acid stimulation followed a similar pattern in which the cohorts with severe injury had the lowest levels (Fig. 2 and Table 2). Platelet aggregation in response to thrombin stimulation was decreased for all levels of injury and shock (Fig. 2 and Table 2).

However, platelet aggregation in response to adenosine diphosphate stimulation followed a different pattern. Patients with minor injury trended toward higher but nonstatistically significant levels of platelet aggregation in response to adenosine diphosphate stimulation compared with healthy controls. Only patients with combined severe injury and shock showed a trend toward lower platelet aggregation in response to adenosine diphosphate stimulation (Fig. 2 and Table 2).

PA Predictors of Blood Transfusion

On multivariate linear regression controlling for baseline characteristics (age, sex, initial temperature), injury burden (Abbreviated Injury Scale for the head, chest, and abdomen), shock (BE), and platelet count, injury-driven endogenous platelet activation (baseline electrical impedance, Ω) was independently and negatively associated with number of units of blood transfusion in the first 6 hours. For every 10 Ω increase in baseline electrical impedance, there was an 8% independent decrease in the number of units of blood transfused in the first 6 hours (coefficient, −0.08; confidence interval, −0.13 to −0.03; $p = 0.004$; Table 3). When the multivariate linear regression was extended to number of units of blood transfused in the first 24 hours, the same independent association of baseline electrical impedance (Ω) with units of blood transfused was present (coefficient, −0.08; confidence interval, −0.14 to −0.02;$p = 0.015$; Table 4).

ROTEM Clot Strength

In aggregate, there was no linear correlation between baseline electrical impedance (Ω) and ROTEM EXTEM MCF (mm) (Pearson correlation, 0.09; Supplemental Digital Content, Supplementary Table 1, http://links.lww.com/TA/B637). However, there were moderate correlations between platelet aggregation measures and ROTEM EXTEM MCF (mm) (Pearson correlations, 0.45, adenosine diphosphate-stimulated AUC; 0.36, thrombin-stimulated AUC; 0.30, collagen-stimulated AUC; and 0.45, arachidonic acid-stimulated AUC, all $p < 0.05$; Supplemental Digital Content, Supplementary Table 1, http://links.lww.com/TA/B637). When stratified by injury burden and shock severity, the strongest correlations for each of the platelet aggregation measures with ROTEM EXTEM MCF were in the severe injury and shock group (all $p < 0.05$; Supplemental Digital Content, Supplementary Table 1, http://links.lww.com/TA/B637). However, importantly, baseline electrical impedance (Ω) and ROTEM EXTEM MCF (mm) had no significant correlations even when stratified by injury burden and shock severity.

DISCUSSION

In this study, we aimed to improve phenotyping of postinjury platelet aggregation measured by multiple electrode PA via elucidating the effects of injury burden and shock severity on endogenous platelet activation, measured by baseline electrical impedance. We found that indeed, despite normal platelet counts, there were notable differences in endogenous platelet activation depending on the injury burden and severity of shock. As in previous ex vivo studies of platelet aggregation in injured patients,[8,11,14,16] across all injury burden and shock groups, we found lower platelet aggregation in response to ex vivo stimulation with platelet agonists thrombin, collagen, and arachidonic acid in comparison with healthy controls. However, distinct from prior studies, we found that patients with minor injury burdens had higher baseline electrical impedance, which we believe to be characteristic of a functionalplatelet phenotype in which circulating platelets activate in response to injury. The significance of these findings is that, in patients with minor injury, interpreting the AUC alone as a measure of platelet aggregation overestimates the presence of platelet dysfunction. In contrast, the patients with severe injury burdens had lower levels of baseline electrical impedance. This, in combination with the presence of impaired platelet aggregation responses, corresponds to a truly dysfunctional phenotype: platelets that do not activate nor aggregate appropriately after injury. Furthermore, when controlling for injury burden and shock in multivariate linear regression, we identified that, for every 10 Ω increase in baseline electrical impedance, there was an independent 8% decrease in the units of blood transfused in the first 6 and 24 hours. This supports that there is clinical relevance to increased levels of endogenous platelet activation and that patients with a functionalplatelet phenotype in which circulating platelets both activate in response to injury and aggregate may have reduced bleeding after injury.

Furthermore, in our analysis of correlations of PA and clot strength, we identified that injury-driven endogenous platelet activation did not correlate with viscoelastic clot strength, yet platelet aggregation responses correlated with viscoelastic clot strength for all injury and shock groups. This finding mirrors the divergent relationships of baseline electrical impedance with AUC in PA and suggests that viscoelastic measures of clot strength are also at risk of not adequately accounting for both the activation and aggregation hemostatic behaviors of platelets from injured patients.

To our knowledge, this is the first study incorporating baseline electrical impedance measures into the interpretation of ex vivo aggregometry assays in injured patients and demonstrates the need to better phenotype PA results in the setting of trauma. It is not a surprise to find that there are differential effects of injury and shock on platelet activation and aggregation in trauma, given that multiple mechanistic pathways known to be altered in TIC have been shown to be dependent on massive tissue injury in combination with systemic hypoperfusion.[1,2] In fact, our results for patients with severe injury (ISS >25) are consistent with prior studies demonstrating severe platelet aggregation impairments after major injury by aggregometry,[8,11,12 ,21] platelet mapping,[22,23] and platelet function analyzer measures.[9,16] The mechanisms responsible for these platelet aggregation impairments remain unclear. Some have proposed that platelet *exhaustion*, a term for a state in which platelets are activated by injury leading to degranulation of their contents, impairs the platelets from

further aggregation and clot formation.[16,24] However, a recent study of patients with TIC in which platelet function was measured by multiple assays including aggregometry, surface receptor expression, platelet mapping, and platelet function analyzer suggests that, in fact, platelets from patients with TIC retain their secretory function despite evidence of impaired aggregation.[16] Others have identified that inhibitory factors in the plasma of trauma patients may be driving platelet dysfunction[25] or may even lead to changes in platelet morphology and structure leading to subsequent destruction,[26] but the clinical implications of impaired platelet aggregation in the setting of injury remain controversial. While multiple studies have demonstrated strong associations between impaired platelet aggregation and up to 10-fold higher mortality rates,[8,9,21] others have shown that even minor injuries represented by low median ISS scores of 5 can result in platelet inhibition, questioning the clinical relevance of these findings.[14] In addition, studies highlighting that impaired platelet aggregation after injury may not depend on the degree of injury severity further support the need to improve our phenotyping of platelet dysfunction.[8]

Therefore, incorporating baseline electrical impedance as a marker of endogenous platelet activation in the interpretation of aggregometry data may provide clarity to previous reports of high rates of impaired platelet aggregation in patients with minor injuries.[8,14] Even with minor injury burdens, several biologic pathways including catecholamine responses and release of thrombogenic mediators secondary to endothelial disruption are expected to activate circulating platelets. However, in the setting of minor trauma without large tissue beds of injury for the platelets to locally incorporate into developing thrombi, activated platelets will circulate and whole blood aggregometry assays are thereby at risk of falsely identifying muted aggregation responses to ex vivo agonist stimulation because of the aforementioned limitations of AUC measurements in aggregometry assays.

This study has underscored the need for better phenotyping of platelet dysfunction and cautions against the use of platelet aggregation as a sole measure of platelet function in the setting of injury. Beyond incorporating baseline electrical impedance into the interpretation of aggregometry results, there remains a need for a better mechanistic understanding of the molecular drivers of altered platelet behavior after injury to identify new treatment targets. This is particularly important given that platelet transfusions do not reverse impairments in platelet aggregation that are identified ex vivo in injured patients, nor do they appear to significantly improve platelet counts.[20,27] It is possible that imprecise phenotyping of platelet dysfunction may contribute to this: transfused, appropriately functioning platelets may be activated endogenously after administration in injured patients appearing dysfunctional in aggregometry assays. Alternatively, transfused platelets may not correct platelet dysfunction as measured by aggregometry assays in studies of injured patients because the assay is actually capturing functional phenotypes of aggregation as well.

Limitations

Our study has several notable limitations. First, this is an observational study, and therefore, our results may be subject to unaccounted biases. However, we did prospectively collect comprehensive clinical and demographic patient information and controlled for key known confounders. Second, whole blood PA is an ex vivo test that may not necessarily reflect the

true function of platelets in vivo in the absence of endothelium and flow. Furthermore, sampling of circulating blood may bias toward measuring less active or functional platelets that were not incorporated into clots in local injury environments. This could potentially exaggerate the identification of platelet dysfunction in trauma patients compared with healthy volunteers. It should also be noted that, while we found impaired platelet aggregation in response to collagen, thrombin, and arachidonic acid, we did not observe this relationship for adenosine diphosphate-stimulated responses, in contrast to prior studies.[8,11,16] This may be in part driven by the fact that, as a weak agonist, adenosine diphosphate may not induce sufficient aggregation responses required to reveal substantial differences in the AUC, particularly in the setting of platelets that are already endogenously activated by injury. Lastly, this is the first report of the use of baseline electrical impedance as a marker of endogenous injury-driven platelet activation, and without other validation, future work should focus on corroborating the relationship between baseline electrical impedance and other accepted measures of platelet activation, such as surface receptor analysis or microparticle release.

CONCLUSIONS

Elucidating the role of platelets and their function during the response to injury has important implications for future platelet-based therapies for TIC. This study suggests that the current interpretation of whole blood PA in injured patients may in fact overidentify platelet dysfunction in minorly injured patients while underestimating the impairment of platelets in severely injured patients, because of important differences in injury-driven endogenous platelet activation after injury. These findings provide further insights into the nuanced nature of altered platelet behaviors after injury in the context of TIC. Furthermore, this study highlights the potential pitfalls of ex vivo platelet assays and the need for better mechanistic investigations to decode these critical aspects of platelet biology and their associated clinical implications for trauma patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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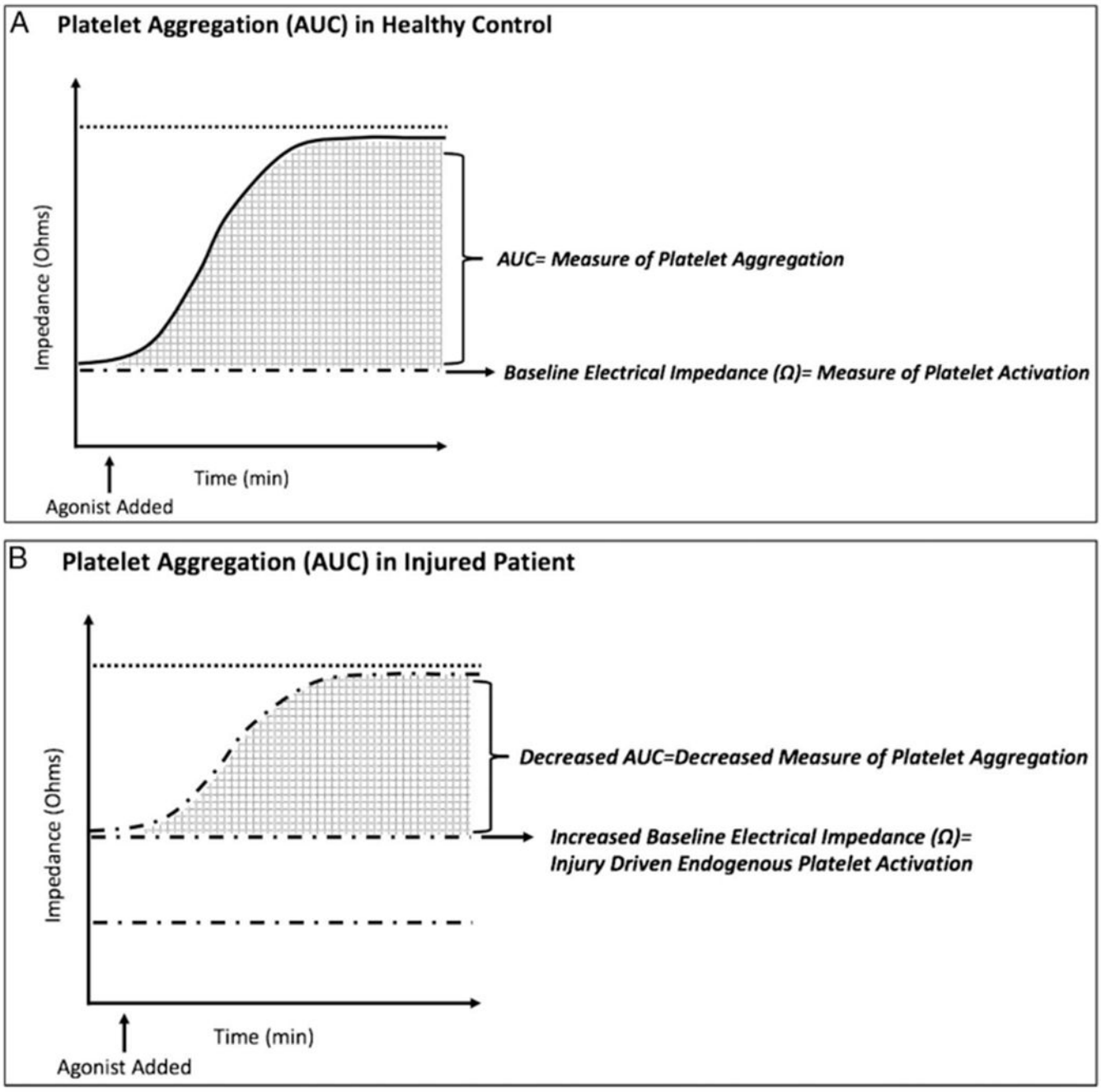

Figure 1.

Effect of increased baseline electrical impedance (Ω; injury-driven endogenous platelet activation) on AUC (measure of platelet aggregation) in ex vivo multiple electrode PA. (*A*) Healthy expected baseline electrical impedance (Ω) and subsequent expected platelet aggregation after addition of platelet-stimulating agonist (AUC, shaded). (*B*) Injury-driven endogenous platelet activation increases baseline electrical impedance (Ω) with decreased subsequent measures of platelet aggregation after addition of platelet-stimulating agonist (AUC, shaded).

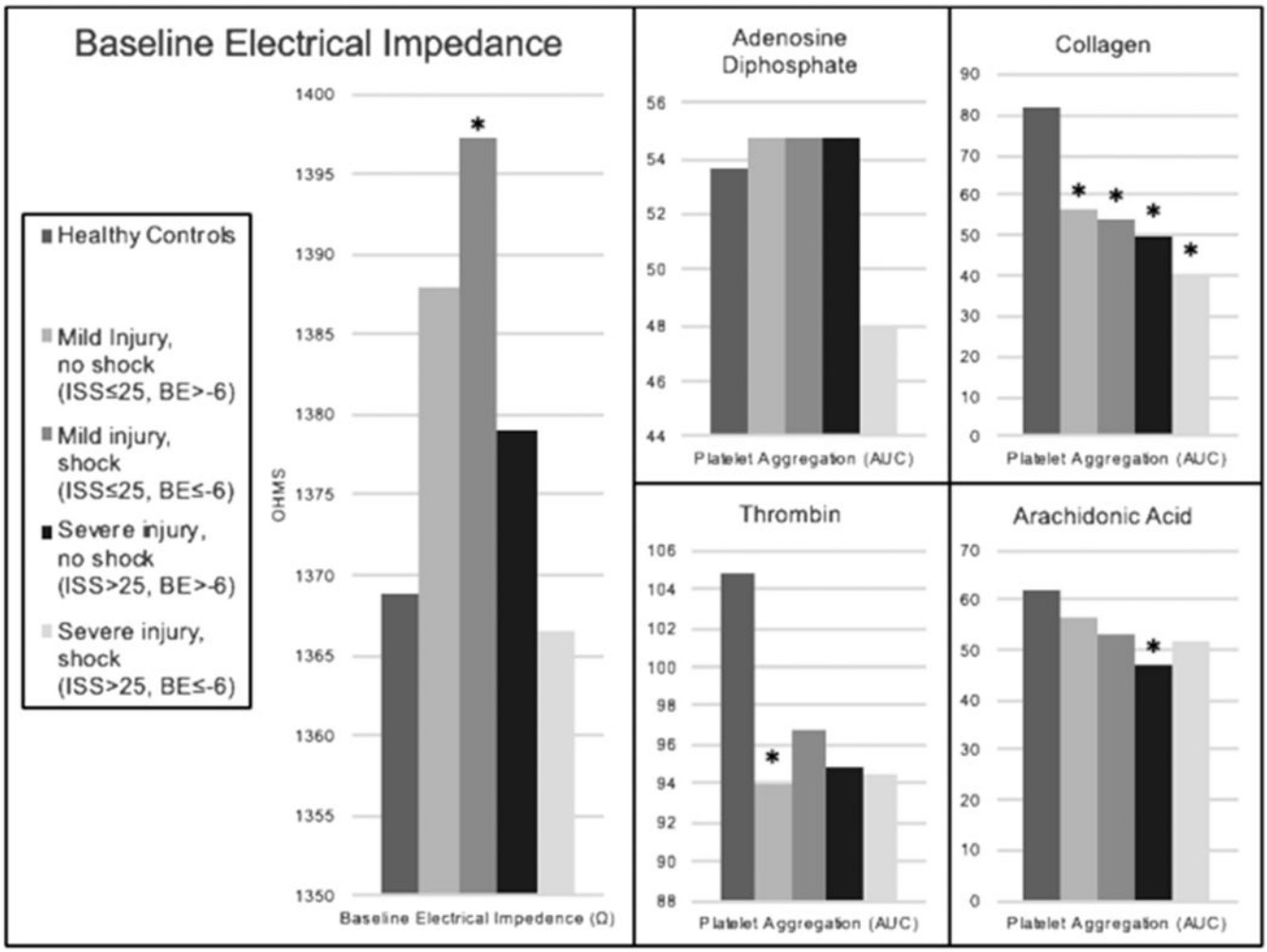

Figure 2.

Endogenous platelet activation (baseline electrical impedance, Ω) and platelet aggregation (AUC) in multiple electrode PA. *p Value of <0.05 for injury/shock group compared with healthy controls.

TABLE 1.

Patient Demographics/Outcomes

Factor	Overall	Minor Injury, No Shock (ISS ≤25, BE >−6)	Minor Injury, Shock (ISS ≤25, BE ≤−6)	Severe Injury, No Shock (ISS >25, BE >−6)	Severe Injury Shock (ISS >25, BE ≤−6)	*p* (Injury Groups)
n	458	264	76	79	39	
Age, y	37 (26–50)	37 (28–48)	31 (22–45)	42 (28–67)	28 (23–42)	**<0.001**
Male	398 (87%)	233 (88%)	69 (91%)	62 (79%)	34 (87%)	0.095
BMI, kg/m^2	26 (23–30)	26 (23–29)	27 (23–32)	27 (24–30)	28 (25–29)	0.079
Blunt mechanism	247 (54%)	127 (49%)	32 (42%)	63 (80%)	25 (64%)	**<0.001**
ISS	10 (2–26)	5 (1–11)	5 (1.5–17)	30 (27–35)	34 (27–38)	**<0.001**
Initial systolic blood pressure (mmHg)	130 (115–151)	133 (122–150)	121 (104–149)	129 (112–164)	120 (107–137)	**0.002**
Admit GCS	14 (8–15)	15 (12–15)	14 (8–15)	13 (4–15)	5 (3–14)	**<0.001**
TBI	128 (28%)	37 (14%)	11 (15%)	55 (70%)	25 (64%)	**<0.001**
Prehospital crystalloid, mL	30 (0–200)	0 (0–150)	50 (0–250)	100 (0–250)	100 (0–350)	**0.011**
Admit temperature, °C	36.6 (36.2–36.9)	36.6 (36.3–36.9)	36.6 (36.0–36.9)	36.4 (36.0–36.6)	36.3 (35.2–36.4)	**0.001**
Admit pH	7.3 (7.3–7.4)	7.4 (7.3–7.4)	7.2 (7.2–7.3)	7.3 (7.3–7.4)	7.2 (7.1–7.3)	**<0.001**
Admit BE, mmol/L	−2.1 (−6.0 to 1.0)	0.0 (−2.6 to 2.3)	−9.8 (12.7–7.4)	−1.6 (3.5–0.8)	−8.6 (12.8–7.6)	**<0.001**
Admit INR ≥1.3	67 (16%)	10 (4%)	12 (17%)	24 (33%)	21 (55%)	**<0.001**
Admit INR	1.1 (1.0–1.2)	1.0 (1.0–1.1)	1.1 (1.0–1.2)	1.2 (1.0–1.3)	1.3 (1.2–1.4)	**<0.001**
Admit PTT, s	28 (25.4–30.3)	28 (25.1–29.4)	27 (25.3–29.7)	30 (25.1–34.2)	32 (27.1–37.5)	**<0.001**
Admit platelets, ×10^9/L	263 (218–310)	261 (222–308)	268 (227–318)	266 (200–321)	274 (216–307)	0.730
Admit fibrinogen, mg/dL	208 (162–275)	218 (182–277)	229 (159–294)	182 (130–268)	162 (119–231)	0.018
Transfused blood in 24 h	136 (40%)	35 (19%)	30 (58%)	47 (69%)	24 (73%)	**<0.001**
Transfused plasma in 24 h	88 (26%)	12 (6%)	18 (35%)	36 (53%)	22 (67%)	**<0.001**
Transfused platelets in 24 h	42 (12%)	4 (2%)	7 (14%)	19 (28%)	12 (36%)	**<0.001**
Blood in 24 h, U	0 (0–4)	0 (0–0)	1 (0–4)	2 (0–5)	4 (0–9)	**<0.001**
Plasma in 24 h, U	0 (0–2)	0 (0–0)	0 (0–2)	1 (0–4)	4 (0–6)	**<0.001**
Platelets in 24 h, U	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–1)	**<0.001**
Total hospital days	5 (2–12)	3 (2–7)	4.5 (2–10.5)	14 (5–22)	10 (3–28)	**<0.001**
Total ICU days (to 28 d)	2 (0–5)	0 (0–2.5)	2 (0–3.5)	7 (3–14)	4 (2–15)	**<0.001**

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Factor	Overall	Minor Injury, No Shock (ISS ≤25, BE >−6)	Minor Injury, Shock (ISS ≤25, BE ≤−6)	Severe Injury, No Shock (ISS >25, BE >−6)	Severe Injury Shock (ISS >25, BE ≤−6)	*p* (Injury Groups)
Ventilator-free days (to 28 d)	27 (24–28)	28 (27–28)	27 (25–28)	16 (0–25)	10 (0–24)	**<0.001**
Acute lung injury	34 (10%)	5 (3%)	2 (3%)	19 (28%)	8 (22%)	**<0.001**
Multiorgan failure	24 (8%)	3 (2%)	3 (6%)	11 (18%)	7 (19%)	**<0.001**
Mortality at 24 h	26 (6%)	2 (1%)	4 (5%)	13 (17%)	7 (18%)	**<0.001**
Mortality at discharge	55 (12%)	7 (3%)	6 (8%)	28 (35%)	14 (36%)	**<0.001**

Patient demographics for the 458 patients. Data are presented as mean (SD), median (IQR), or percentage. Data for skewed variables are reported as median with IQRs. Ventilator-free days are counted for the first 28 days of hospitalization. Patients who expired received 0 ventilator-free days.

Bolded, $p < 0.0125$ significance level corrected for multiple comparisons between injury/shock groups.

BMI, body mass index; GCS, Glasgow Coma Scale; TBI, traumatic brain injury; INR, international normalized ratio; PTT, partial thromboplastin time.

TABLE 2.

Direction and Magnitude of Changes in PA and ROTEM in Injured Patients Compared With Healthy Controls

	Healthy Control	Overall Injured	Minor Injury, No Shock (ISS ≤25, BE >−6)	Minor Injury, Shock (ISS ≤25, BE ≤−6)	Severe Injury, No Shock (ISS >25, BE >−6)	Severe Injury, Shock (ISS >25, BE ≤6)
Baseline electrical impedance, Ω	1,369 (39)	↑↑ 1,387 (54)	↑↑ 1,388 (56)	↑↑* 1,397 (51)	↑ 1,379 (52)	↓↓ 1,367 (48)
Adenosine diphosphate, AUC	54 (14)	↓ 54 (22)	↑ 55 (20)	↑ 55 (22)	↑ 55 (27)	↓ 48 (27)
Collagen, AUC	82 (27)	↓↓↓* 54 (27)	↓↓↓* 54 (28)	↓↓↓* 54 (29)	↓↓↓* 50 (26)	↓↓↓↓* 40 (21)
Thrombin, AUC	105 (19)	↓ 95 (28)	↓↓* 94 (25)	↓ 97 (28)	↓ 95 (32)	↓ 95 (36)
Arachidonic acid, AUC	62 (18)	↓* 54 (23)	↓ 56 (21)	↓ 53 (22)	↓↓* 47 (26)	↓ 52 (25)
ROTEM EXTEM MCF, mm	63 (6)	↓↓↓* 60	↑ 64 (5)	↓ 62 (8)	↓↓* 62 (7)	↓↓↓* 60 (7)

PA: ↑, 0–10 U > baseline; ↑↑, 11–20 U > baseline; ↑↑↑, 21–40 U > baseline; ↑↑↑↑, >40 U > baseline; ↓, 0–10 U < baseline; ↓↓, 11–20 U < baseline; ↓↓↓, 21–40 U < baseline; and ↓↓↓↓, >40 U < baseline.

ROTEM: ↑, 0–1 mm > baseline; ↓, 0–1 mm > baseline; ↓↓, 1–2 mm < baseline; and ↓↓↓, 2–3 mm < baseline.

* *p* Value ≤0.05.

Values reported are median (IQR). BE is presented as mmol/L.

TABLE 3.

Linear Regression of Units of Blood Transfusion at 6 Hours With PA and ROTEM Clot Strength

	Univariate Regression					Multivariate Regression				
	Coef	**CI**			***p***	**Coef**	**CI**			***p***
Age, y	0.00	−0.03	—	0.02	0.817					
Male sex	0.19	−1.03	—	1.41	0.761					
BE ≤−6, mmol/L	−0.18	−0.25	—	−0.11	<0.001					
AIS head	0.53	0.33	—	0.73	<0.001					
AIS chest	0.88	0.62	—	1.14	<0.001					
AIS abdomen	0.60	0.30	—	0.91	<0.001					
Admit temperature, °C	−0.37	−0.80	—	0.05	0.081					
Admit platelets, $\times 10^9$/L †	0.01	−0.04	—	0.06	0.676					
Baseline electrical impedance, Ω †	−0.11	−0.17	—	−0.05	<0.001	**−0.08**	**−0.13**	—	**−0.03**	**0.004**
Adenosine diphosphate, AUC †	−0.27	−0.46	—	−0.09	0.004	**−0.27**	**−0.41**	—	**−0.12**	**<0.001**
Collagen, AUC †	−0.24	−0.36	—	−0.12	<0.001	**−0.21**	**−0.33**	—	**−0.09**	**0.001**
Thrombin, AUC †	−0.20	−0.35	—	−0.06	0.007	**−0.15**	**−0.26**	—	**−0.04**	**0.01**
Arachidonic acid, AUC †	−0.12	−0.27	—	0.04	0.146	−0.01	−0.15	—	0.14	0.943
ROTEM EXTEM MCF, mm	−0.22	−0.29	—	−0.15	<0.001	**−0.10**	**−0.16**	—	**−0.04**	**0.001**

Bolded, $p < 0.05$ for independent associations of PA and viscoelastic clot strength with units of blood transfused in initial 6 hours.

† Ten times linear combinations of estimators.

Coef, coefficient; AIS, Abbreviated Injury Scale; CI, confidence interval.

TABLE 4.

Linear Regression of Units of Blood Transfusion at 24 Hours With PA and ROTEM Clot Strength

	Univariate Regression					Multivariate Regression				
	Coef	**CI**			***p***	**Coef**	**CI**			***p***
Age, y	−0.01	−0.03	—	0.02	0.626					
Male sex	0.19	−1.23	—	1.62	0.792					
BE ≤-6, mmol/L	−0.20	−0.28	—	−0.12	<0.001					
AIS head	0.50	0.27	—	0.73	<0.001					
AIS chest	0.73	0.44	—	1.02	<0.001					
AIS abdomen	0.65	0.34	—	0.97	<0.001					
Admit temperature, °C	−0.12	−0.66	—	0.42	0.658					
Admit platelets, $\times 10^9$/L †	0.02	−0.04	—	0.08	0.507					
Baseline electrical impedance, Ω †	−0.11	−0.19	—	−0.04	0.003	**−0.08**	**−0.14**	—	**−0.02**	**0.015**
Adenosine diphosphate, AUC †	−0.02	−0.04	—	0.00	0.08	**−0.29**	**−0.47**	—	**−0.11**	**0.001**
Collagen, AUC †	−0.28	−0.44	—	−0.13	<0.001	**−0.24**	**−0.42**	—	**−0.07**	**0.006**
Thrombin, AUC †	−0.06	−0.23	—	0.11	0.489	−0.10	−0.25	—	0.05	0.178
Arachidonic acid, AUC †	−0.13	−0.33	—	0.07	0.214	0.04	−0.15	—	0.22	0.701
ROTEM EXTEM MCF, mm	−0.21	−0.29	—	−0.13	<0.001	**−0.12**	**−0.20**	—	**−0.05**	**0.001**

Bolded, $p < 0.05$ for independent associations of PA and viscoelastic clot strength with units of blood transfused in initial 24 hours.

† Ten times linear combinations of estimators.

Coef, coefficient; CI, confidence interval; AIS, Abbreviated Injury Scale.