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It's About Time: Transfusion Effects on Post-Injury Platelet Aggregation Over Time

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Abstract

Background—Impaired post-injury platelet aggregation is common, but the effect of transfusion on this remains unclear. Data suggests that following injury platelet transfusion may not correct impaired platelet aggregation and impaired platelet aggregation may not predict the need for platelet transfusion. We sought to further investigate platelet aggregation responses to transfusions, using regression statistics to isolate the independent effects of transfusions given in discrete time intervals from injury on both *immediate* and *longitudinal* platelet aggregation. We hypothesized that platelet aggregation response to platelet transfusion increases over time from injury.

Methods—Serial (0–96h) blood samples were collected from 248 trauma patients. Platelet aggregation was assessed *in vitro* with impedance aggregometry stimulated by adenosine diphosphate (ADP), collagen, and thrombin receptor-activating peptide-6 (TRAP). Using regression, transfusion exposure was modeled against platelet aggregation at each subsequent timepoint and adjusted for confounders (injury severity [ISS], INR, base deficit, platelet count, and interval transfusions). The expected change in platelet aggregation at each timepoint under the intervention of transfusion exposure was calculated and compared to the observed platelet aggregation.

Results—The 248 patients analyzed were severely injured (ISS 21 +/− 19), with normal platelet counts (mean 268×10^9/L +/−90), and 62% were transfused in 24 hours. The independent effect of transfusions on subsequent platelet aggregation over time was modeled with observed platelet

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LZK, AD, ASC, RAC, and MJC contributed to study design, data collection, data analysis, data interpretation, writing, and clinical revision.

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aggregation under hypothetical treatment of one unit transfusion of blood, plasma, or platelets. Platelet transfusions had increasing expected effects on subsequent platelet aggregation over time, with the maximal expected effect occurring late (4–5 days from injury).

Conclusions—Controversy exists on whether transfusions improve impaired post-injury platelet aggregation. Using regression modeling, we identified that expected transfusion effects on subsequent platelet aggregation are maximal with platelet transfusion given late after injury. This is critical for tailored resuscitation, identifying a potential early period of resistance to platelet transfusion that resolves by 96 hours.

Level of Evidence—IV

Study Type—Prognostic

Keywords

wounds and injury; platelet transfusion; hemorrhage

BACKGROUND

Despite significant advances in trauma resuscitation and delivery of care, uncontrolled hemorrhage remains the primary cause of early preventable deaths after injury(1). Contributing to this is that approximately one-third of hemorrhaging trauma patients suffer from trauma-induced coagulopathy (TIC), a multi-factorial disorder of clot formation and lysis that is driven by injury and shock, and is associated with hemorrhage, organ failure, and mortality(2,3). Identifying distinct pathways implicated in TIC is critical to tailoring targeted resuscitation practices for improved outcomes after injury, and the practice of transfusing platelets in equal ratios to blood and plasma has become standard of care regardless of platelet count because platelets are known to play a pivotal role in normal coagulation and maintenance of endothelial integrity(4–8).

Specifically, platelets are anucleate fragments of megakaryocytes central to the ‘cell-based model of hemostasis’(9). Clot formation is reliant on functioning platelets that activate in response to signals of injury, and aggregate in response to activation. Platelets provide structure and functions for assembly of procoagulant proteins that generate large amounts of thrombin, leading to fibrin polymerization and clot formation. Despite this, the behavior of platelets in TIC remains poorly understood.

Post-injury thrombocytopenia is associated with bleeding, progression of brain injury, and mortality(6), but accumulating evidence has identified that up to half of injured patients demonstrate impaired platelet aggregation *invitro* despite having normal platelet counts(4,10,11). It remains unknown if this is a maladaptive response to injury. This uncertainty is supported by identified contradictory patterns of post-injury platelet behavior (activated but resistant to aggregation *invitro*(11)), coupled with known complexities of measuring platelet function *invitro*(12), and the finding that impaired post-injury platelet aggregation is not reversed with platelet transfusion(13–15).

These knowledge gaps in platelet behavior after injury and how platelet transfusions affect their behavior raise uncertainty regarding the best clinical application of platelet-based treatments for hemorrhaging trauma patients(16). Although the current standard-of-care in post-injury hemorrhage is transfusion of platelets in a balanced ratio with red blood cells and plasma (regardless of platelet count)(17), it remains unclear if this approach favorably affects vascular homeostasis and consequent clinical outcomes in trauma(16). In fact, recent data has uncovered that impaired platelet aggregation *invitro* in injured patients does not predict the need for platelet transfusion(13), platelet transfusion does not correct impaired platelet aggregation *invitro*(14), and platelet transfusion does not even improve the outcomes in patients with brain injury on antiplatelet therapy(15). We sought to further investigate platelet aggregation responses to transfusions, using regression statistics to isolate the independent effects of transfusions given in discrete time intervals from injury on both *immediate* and *longitudinal* platelet aggregation. Given previous investigations demonstrated that platelets are activated but do not aggregate well early after injury, we hypothesized that platelet aggregation responses to platelet transfusion increase over time from injury.

METHODS

Cohort

Blood samples and clinical data were prospectively collected on 1671 trauma patients from 2005–2016 who were enrolled in ‘Activation of Coagulation and Inflammation in Trauma’, a longitudinal study examining perturbations in coagulation and inflammation after trauma at Zuckerberg San Francisco General Hospital, a Level 1 Trauma Center(7,18,19). All highest-level trauma activations were included with initial waiver-of-consent approved by the University of California Institutional Review Board. Patients were excluded if: pediatric, pregnant, in-custody, burns >20% body surface area, transferred from another facility, did not require ICU level care, or were atraumatic. A subset of 248 patients were selected for this study because 1) they could be confirmed to not be on any anticoagulant/antiplatelet therapy, and 2) they had serial platelet aggregometry data needed to support regression modeling. Samples were collected on arrival via initial placement of a 16G or larger peripheral IV; and subsequently as part of clinical care. Standard laboratory vacuum-sealed tubes containing 3.2% (0.109M) sodium citrate were used for all draws. During the study period, there were no explicit changes to transfusion practices or protocols.

Assays

Platelet aggregation was assessed using Multiplate® multiple electrode impedance aggregometer (Verum Diagnostica GmbH; Munich, Germany) immediately after sample collection(4), which measures changes in electrical impedance across two electrodes in a sample of whole blood following *invitro* platelet activation with platelet surface receptor activating agonists. Following activation, platelets aggregate to platelets on the electrodes causing an increase in electrical impedance, and aggregation is measured by detecting the amount of increased impedance. Briefly, 0.3mL of whole blood was diluted in warmed normal saline containing 3mM $CaCl_2$ and incubated for 3 minutes at 37°C with continuous stirring in a Multiplate® test cell. Each test cell contains two sets of 3mm silver-coated copper wires, across which electrical resistance is measured at 0.57 second intervals. Platelet

activation was stimulated by platelet activating agonists targeted to receptor-ligand interactions of biologic relevance known to be altered in the setting of tissue injury: adenosine diphosphate (ADP, final concentration 6.5μM; P2 surface receptors; responsible for conformational changes in GpIIb/IIIa that induce binding to fibrinogen and formation of primary platelet plugs), thrombin receptor activating peptide-6 (TRAP, final concentration 32μM; PAR receptors; responsible for platelet aggregation, granular secretion, and stimulation of platelet procoagulant activity that accelerate the assembly of plasma coagulation factors on the platelet surface), and collagen (final concentration 3.2μg/mL; GpIa/IIa and GpVI receptors; responsible for platelet tethering, activation, adhesion, aggregation, degranulation, and procoagulant activity secondary to sub-endothelial vasculature exposure). Platelet aggregation stimulated by activating agonist was reported as area under the aggregation curve in units(U) over a 6-minute measurement period. Reference ranges were provided by the manufacturer based on studies of healthy controls.

Statistical Analyses

A retrospective analysis of prospectively collected data was performed. The independent effect of transfusions on subsequent platelet aggregation over time was modeled using regression. A parameter motivated by the causal inference literature(20,21) was estimated to assess the effect of transfusion on platelet aggregation. Under the assumptions of no unmeasured confounding and positivity, this parameter has causal interpretation. It measures the effect that a one-unit change in transfusion would have on platelet aggregation(20,21). The advantage of estimating this parameter is that it allows for the estimation of so-called counterfactual outcomes, that is, how the platelet aggregation would have changed if the transfusion had changed. It requires estimating the conditional distribution of platelet aggregation as a function of the exposure and covariates and then uses the model of this conditional distribution to make estimate counterfactual outcomes. The theory for causal effect estimation is established by Pearl *et al.* in 2009 and further explored in detail by van der Laan *et al.* in 2011(20,21).

The expected change in platelet aggregation under a hypothetical intervention of one-unit transfusion of blood, plasma, or platelets was calculated using the estimated regression equation and compared to the observed platelet aggregation. Transfusion exposure was modeled against platelet aggregation at each subsequent timepoint and adjusted for confounders: injury severity score(ISS) INR, base deficit, platelet count, and interval transfusions. Future platelet aggregation and platelet count variables were modeled as a function of transfusion variables and the adjustment set. Modeling assumed a linear functional form and was fit using regression, either linear or logistic. At each timepoint, the set of predictors grew in number since the patients' history of treatment grew over time. Once the conditional distribution of each outcome variable had been estimated, the impact of hypothetical interventions on platelet aggregation and count variables could be assessed. For example, the impact of a one-unit change in platelet transfusion given 0–6 hour(h) from injury was modeled by intervening on all the patients, increasing their platelet transfusion in that time interval, and using the regression equation to predict the change in platelet aggregation stimulated by each activating agonist (ADP, Collagen, and TRAP). Doing this for all the platelet aggregation outcomes and comparing the so-called counterfactual

outcomes under a hypothetical one-unit change in each treatment variable allowed for assessment of the impact of each treatment variable (transfusion). All analyses were performed using R version 3.5.0 (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The Cohort

The 248 patients were critically-injured (ISS 21±19) with 62% transfused (blood, plasma, or platelets) in the first 24h, 20% transfused platelets in the first 24h, and a hospital mortality rate of 13% (Table 1). Although they presented with normal platelet counts (mean 268×10^9/L +/−90; Table 1), 23% demonstrated impaired platelet aggregation response on presentation (Table 2) prior to any significant resuscitation interventions (mean pre-hospital crystalloid 0mL±250mL, no prehospital blood products; Table 1).

Natural History of Platelet Aggregation and Platelet Counts (Figure 1)

The post-injury unadjusted natural history of platelet aggregation and platelet counts are shown in Table 2 and Figure 1. The cohort demonstrated increasing impairment in platelet aggregation (when stimulated by ADP, Collagen, and TRAP) out to 12 hours after injury that steadily recovered toward baseline over 5 days (Figure 1, panel A; Table 2). Corresponding with this, there were declining platelet counts following presentation, nadiring at a mean of 156 +/− 59 at 48 hours (Figure 1, panel B; Table 2). Interestingly, the steady trend of recovery of platelet aggregation started earlier (12 hours from injury) than the trend of recovery of platelet counts (lag until 48–72 hours from injury; Table 2, Figure 1).

Expected Platelet Counts Over Time *Without* Platelet Transfusion (Figure 2, panel A)

For the 186 patients who were not transfused platelets, their platelet counts over time after injury are shown in Figure 2, panel A (controlling for ISS, INR, base deficit, and interval blood and plasma transfusions with growing adjustments over time). When controlling for confounders, there was an expected similar pattern to the overall cohort, but with a more modest decline in platelet counts that nadired at 48h and recovered by 96h (Figure 2, panel A).

Expected Changes in Platelet Counts Over Time *With* Platelet Transfusion (Figure 2, panels B–F)

For the 62 patients who were transfused platelets, the independent effect of platelet transfusions given in discrete time ranges (0–24h, 25–48h, 49–72h) on subsequent longitudinal platelet counts at 24, 48, 72, 96, 120h from injury was modeled with observed platelet aggregation under hypothetical treatment of one unit transfusion of platelets (Figure 2, panel B–F) (controlling for ISS, INR, base deficit, platelet count, and interval transfusions with growing adjustments over time). Compared to the patients that did not receive platelet transfusions, those that received platelet transfusion in the first 24h (Figure 2, panel B) had a minimal expected initial increase in platelet counts at 48h (4×10^9/L), followed by a rapid decline to below their pre-transfusion platelet count at later timepoints (Figure 2, panel B). However, those that received platelet transfusion beyond 24h from injury, had greater

expected increases in their platelet counts post-transfusion (Figure 2, panels C–D). In fact, those that received platelet transfusion at 48h from injury (Figure 2, panels C), had expected increases in their post-transfusion platelet counts up to 40×10^9/L platelets at 72h, followed by a more gradual decline by 96h. Those that received platelet transfusion at 72h from injury also had greater expected increases in their post-transfusion platelet counts (up to 17.5×10^9/L platelets at 96h).

Expected Platelet Aggregation Over Time ***Without*** Platelet Transfusion (Figure 3, panel A)

For the 186 patients who did not receive platelet transfusions, the adjusted platelet aggregation over time after injury is shown in Figure 3, panel A. When controlling for confounders, there was an expected slight but steady increase in platelet aggregation over time (stimulated by ADP, collagen, TRAP; Figure 3, panel A).

Expected Changes in Platelet Aggregation Over Time ***With*** Platelet Transfusion (Figure 3, panels B–F)

For the 62 patients who were transfused platelets, the independent effect of platelet transfusion given at discrete time ranges (0–24h, 25–48h, and 49–72h) on subsequent longitudinal platelet aggregation at 6, 12, 24, 48, 72, 96, 120h from injury was modeled with observed platelet aggregation under hypothetical treatment of one unit transfusion of platelets (Figure 3, panel B–F) (controlling for ISS, INR, base deficit, platelet count, and interval transfusions with growing adjustments over time). Distinctly different expected patterns of platelet aggregation over time in response to platelet transfusion given in discrete time intervals were identified. Those that received platelet transfusion in the first 6h from injury had expected increases in their platelet aggregation (stimulated by ADP, Collagen, and TRAP) post-transfusion that persisted out to 12h, declined to a nadir at 72h, and recovered at 96h. However, those who received platelet transfusion between 7 and 12h from injury, had an expected longitudinal pattern of steep decline in platelet aggregation (stimulated by ADP, Collagen, and TRAP). This was most profound when stimulated by thrombin (TRAP), where the expected platelet aggregation response to platelet transfusion between 7 and 12h dropped below pre-transfusion baseline, with progressive decline down 15 units sub pre-transfusion platelet aggregation baseline by 96h (Figure 3, panel C). A similar pattern was also expected in those that received platelet transfusions between 13 and 24h, (Figure 3, panel D). Contrary, those that received platelet transfusion late after injury (49h and beyond; Figure 3, panel F) had recovery of this pattern with longitudinal increases in platelet aggregation (up to a max of 18U when stimulated by thrombin [TRAP] at 96h; Figure 3, panel F).

Expected Changes in Platelet Aggregation ***With*** Blood, Plasma, and Platelet Transfusions (Figure 4)

Following examining the expected longitudinal platelet aggregation responses after platelet transfusion given in each time interval, the *immediate* expected platelet aggregation response to a one unit transfusion of blood, plasma and platelets were examined (Figure 4, panel A–C) (controlling for ISS, INR, base deficit, platelet count, and interval transfusions with growing adjustments). Though transfusions of both blood and plasma resulted in patterns of expected increases in platelet aggregation out to 24–48h, beyond that there appeared to be a

nadir in response (Figure 4, panel A and B). Although platelet transfusion resulted in an expected pattern of modest response when given early after injury (0–6h), when platelet transfusion was given in the 7–24h range, the expected response in platelet aggregation nadired dropping below baseline when stimulated by ADP and thrombin [TRAP] (Figure 4, panel C). Most remarkable however was that beyond 24h, platelet aggregation in response to platelet transfusion rebounded (stimulated by ADP, Collagen, and TRAP). In fact, platelet transfusion given between 25–48h from injury had a maximal expected platelet aggregation stimulated by thrombin (TRAP) of 15 units, and platelet transfusion given between 49–72h from injury had a similarly high expected platelet aggregation stimulated by Collagen (Figure 4, panel C).

DISCUSSION

This study uses regression to investigate the independent effect of transfusions given in discrete time intervals from injury on both *immediate* and *longitudinal* platelet aggregation. We identified that 1)there are variable expected *immediate effects* on platelet aggregation following transfusion of blood, plasma, and platelets, particularly notable with platelet transfusion given early vs. late after injury; and 2)there are variable expected *longitudinal* patterns of platelet aggregation over time following platelet transfusion depending on when the platelet transfusion is given from injury (0–6, 7–12, 13–24, 25–48, 49–72h). The most marked finding we identified is that following injury the maximal expected platelet aggregation is following platelet transfusion given at late timepoints after injury (4–5d). This finding is pivotal as it builds on our understanding of several recent studies that have uncovered a lack of platelet aggregation response to platelet transfusions in the setting of injury(13–15). We have identified a more nuanced relationship between platelet transfusion and platelet aggregation that is temporal in nature.

Transfusion of platelets in the hemorrhaging trauma patient makes theoretical biologic and clinical sense given platelets have a central role in hemostasis(22), post-injury thrombocytopenia is associated with bleeding(6,23), and even in the absence of thrombocytopenia injured patients have impaired platelet aggregation(4,11,24,25). There is ample evidence that stored platelets demonstrate qualitative platelet dysfunction in hemostatic measures, mitochondrial respiration, and protection of endothelial integrity(14,26–31). Furthermore, recent studies have uncovered evidence that impaired platelet aggregation in injured patients may not be effectively treated with platelet transfusion(13–15). The implications of these findings are potentially two-fold: *1)*platelet transfusion may not actually improve impaired platelet aggregation in the setting of injury if circulating platelets are inhibited by overwhelming vascular perturbations of tissue injury and shock, or *2)*platelet transfusion may not improve platelet aggregation in the setting of injury because impaired platelet aggregation *invitro* may actually be physiologic evidence of well-functioning and activated platelets that do not need treatment.

*1)*First, the evidence supporting the implication that platelet transfusion may not actually improve impaired platelet aggregation in the setting of injury is highlighted by several recent studies. Stettler *et al*. used thromboelastographic platelet mapping in 303 injured patients identifying that impairment in platelet aggregation, as evidenced by ADP-inhibition, did not

significantly predict death, massive transfusion, or platelet transfusion in injured patients(13). In addition, Henriksen *et al.* identified that in 46 injured patients, reductions in platelet aggregation measured by multiple-electrode aggregometry were actually greatest after transfusion of platelets(14). Finally, Holzmacher *et al.* identified in 66 brain injured patients that platelet transfusion did not actually correct impaired platelet aggregation, as measured by thromboelastographic platelet mapping, for patients with clopidogrel inhibited platelets(15). Combining this with evidence highlighting platelet transfusion related mortality(14,32) and morbidity, including increased rates of lung injury(33,34), makes elucidating these relationships of utmost importance. However, these studies did not take into account severity of injury and illness, transfusions, and temporal relationship of transfusion from injury. By doing this, our results have identified a more nuanced relationship between platelet transfusion and platelet aggregation that is time dependent: earlier platelet transfusions have minimal to negative effects on platelet aggregation, whereas later platelet transfusions have appropriate expected effects on aggregation. Similarly, the lasting positive effects on platelet aggregation appears to be more robust when platelet transfusion is given at later timepoints from injury.

*2)*Second, the implication that platelet transfusion may not improve platelet aggregation in the setting of injury because impaired platelet aggregation may actually be physiologic evidence of well-functioning and activated platelets that do not need treatment, stems from studies that have identified phenotypic patterns of activated platelets that do not aggregate well in *invitro* aggregometry assays after injury. Jacoby *et al.* studied flow cytometric markers of platelet activation (platelet microparticles, P-selection, and activated glycoprotein IIb/IIIa) coupled with a platelet aggregation assay platelet function analyzer (PFA-100) to measure shear-induced occlusion of an aperture in an agonist-impregnated cartridge in injured patients(11). The authors identified a contradictory biologic pattern of increased platelet activation but decreased platelet aggregation in 100 injured patients, uncovering that the platelets are activated after injury. This may mean that the platelets are functioning and their impairments in aggregation may be a product of using aggregation assays *invitro* on already activated platelets. Specific to this, measuring platelet function is complex, dependent on multiple factors including platelet count, hematocrit, endothelium, and flow, and hard to interpret *invitro*(12). In addition, platelet aggregation assays rely on the activation of platelets with stimulating agonists (such as ADP, Collagen, and TRAP) and were originally intended for the measurement of platelet inhibition from anti-platelet agents. Therefore, these assays rely on a principle that platelets are in an inactivated state and will only aggregate when stimulated by an agonist. In fact, it may be that platelets are activated by injury itself and are functioning normally but do not respond to platelet stimulating agonists making them appear impaired in their ability to aggregate *invitro*. Beyond the identification of a more nuanced time-dependent relationship of platelet transfusion and platelet aggregation in the setting of injury, our results may actually support this theory as well. If the circulating platelet milieu immediately following injury is one of activated platelets that do not respond well to aggregometry, it may not be until later timepoints from injury that aggregometry assays can effectively measure platelet aggregation to stimulating agonists in an injured patient. As platelets are released from megakaryocytes in the bone marrow, they repopulate circulating platelets with non-thrombogenic, non-activated

populations that have not been exposed to the acutely perturbed milieu of vascular endothelial and tissue damage immediately following injury. These platelets that were not in circulation at the time of injury likely aggregate appropriately in aggregometry assays. This would correspond with our results of apparent improved platelet aggregation responses to platelet transfusion in the 4 to 5d timeframe following injury.

Finally, the expected platelet aggregation effects with blood transfusion that we identified are not surprising given the known importance of platelet and red blood cell interactions in clot formation driven by the rheologic influence of red cells as well as their coordinated release of ADP, ATP, nitric oxide, exposure of phosphatidylserine, and contribution to the generation of thrombin(35).

We have shown for the first time that although there is an early period of relative platelet aggregation resistance to platelet transfusion that is identified in *invitro* aggregometry assays, this appears to resolve by 72–96h after injury. This finding invites further study before paradigm changes in tailored resuscitation practices can be supported. Our study has some important limitations to be addressed going forward. In order to solely isolate injury effects on platelet aggregation, all patients on antiplatelet medication and anticoagulant medication were removed. In addition, in order to perform regression for causal inference, patients included in the analyses needed to have serial timepoints of platelet aggregometry data. This inclusion criteria limited the population we studied to severely injured patients that were critically ill long enough to get serial samples drawn out to 120h. Because of this, no inferences can be made on the natural history or expected changes in platelet aggregation to platelet transfusion in uninjured or moderately injured patients. However, understanding the platelet aggregation trajectories in those patients may be of lesser clinical significance. In addition, we did not examine bleeding or clotting outcomes associated with early or later transfusion of platelets, but this will be critical in the future to further explore these temporal relationships and elucidate whether there is actually a relative resistance to platelet transfusion early after injury or if this is due to the complexities inherent to measuring platelet aggregation in the setting of injury. In addition, we did not study trends of platelet counts or aggregation beyond 96h, so later patterns cannot be commented on and will be of particular interest in the setting of development of thromboembolic complications. We also do not have corresponding viscoelastic assay data to compare to the aggregometry data, but other investigators have identified that platelet transfusion is better correlated with viscoelastic clot strength parameters than measures of platelet aggregation or inhibition in injured patients(13).

Finally, there are statistical limitations to consider. We assumed a linear functional form for the relationship when modeling, and more flexible approaches that are data-adaptive might be considered in the future(36). The assumption of linearity is one that is always made whenever a regression model is used. Regression models are a useful way to summarize relationships despite this assumption and tend to be more interpretable than machine learning approaches. Additionally, like all statistical modeling, we assume no unmeasured confounding and that positivity must be met in order for the estimated parameters to have a causal interpretation. As in all prospective observational trauma cohort studies, these may be violated in the data. We do believe that we have measured and included as many of the

confounders that are possible and appropriate, and specifically that controlling for severity of tissue trauma (ISS), coagulopathy driven by other pathways of TIC (INR), shock states (base deficit), platelet count, and all interval transfusions at each timepoint is robust and will account for as much variability as possible in a prospectively collected observational dataset. We can assuredly not achieve complete control of all confounders in injured patients, particularly unmeasurable ones. However, the parameter estimated is still a useful statistical parameter that is interpretable and relevant and likely improves our understanding of these complex relationships between transfusion and platelet aggregation(21). In addition, there will likely not be clinical equipoise for randomizing injured patients to receive platelet transfusion vs. not, so we must make some assumptions in order to understand these nuanced relationships from a prospectively collected observational dataset.

In the future, we need to advance our ability to measure platelet biology in the setting of injury via improved *invitro* and potentially *in vivo* assays. This remains open science and is likely to include high-throughput microfluidics measurements (flow and endothelial environments), microscopy (light, fluorescence, and electron), mitochondrial respiration, platelet genomics, and broadened biomarkers of platelet activation and aggregation(8,30,37–39). In addition, further investigations of the temporally related variable responses of platelet biology to platelet transfusion after injury, and how this relates to clinical outcomes, will be critical. These avenues will open new research horizons for improving tailored resuscitation practices related to platelet transfusions with an ultimate goal of reduction of bleeding and clotting complications following injury.

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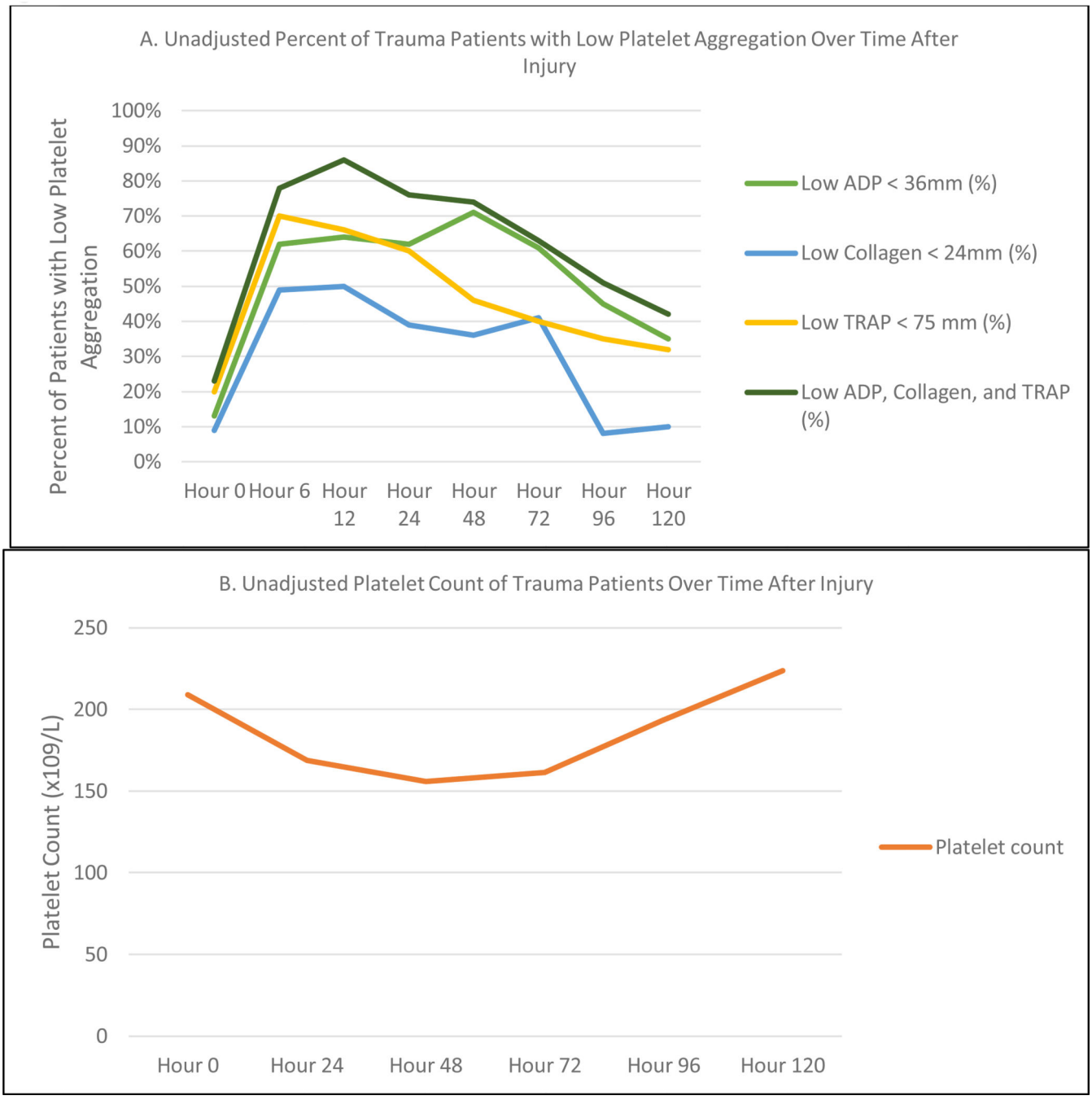

Figure 1. Natural History of Platelet Aggregation and Counts After Injury

Panel A: Percent of Trauma Patients with Impaired Platelet Aggregation Over Time After Injury. The cohort demonstrated increasing impairment in platelet aggregation (when stimulated by ADP, Collagen, and TRAP) out to 12h after injury that steadily recovered toward baseline over 5 d **(Figure 1, panel A**). Corresponding with this, there were declining platelet counts following presentation, nadiring at a mean of 156±59 at 48h (**Figure 1, panel B**). Interestingly, the steady trend of recovery of platelet aggregation started earlier (12h

from injury) than the trend of recovery of platelet counts (lag until 48–72h from injury; **Figure 1**).

Panel B: Mean Platelet Count of Trauma Patients Over Time After Injury

*Platelet activation was induced by adenosine diphosphate (ADP, final concentration 6.5μM; via P2 receptors), thrombin receptor activating peptide-6 (TRAP, final concentration 32μM; via PAR receptors), and collagen (final concentration 3.2μg/mL; via GpIa/IIa and GpVI receptors).

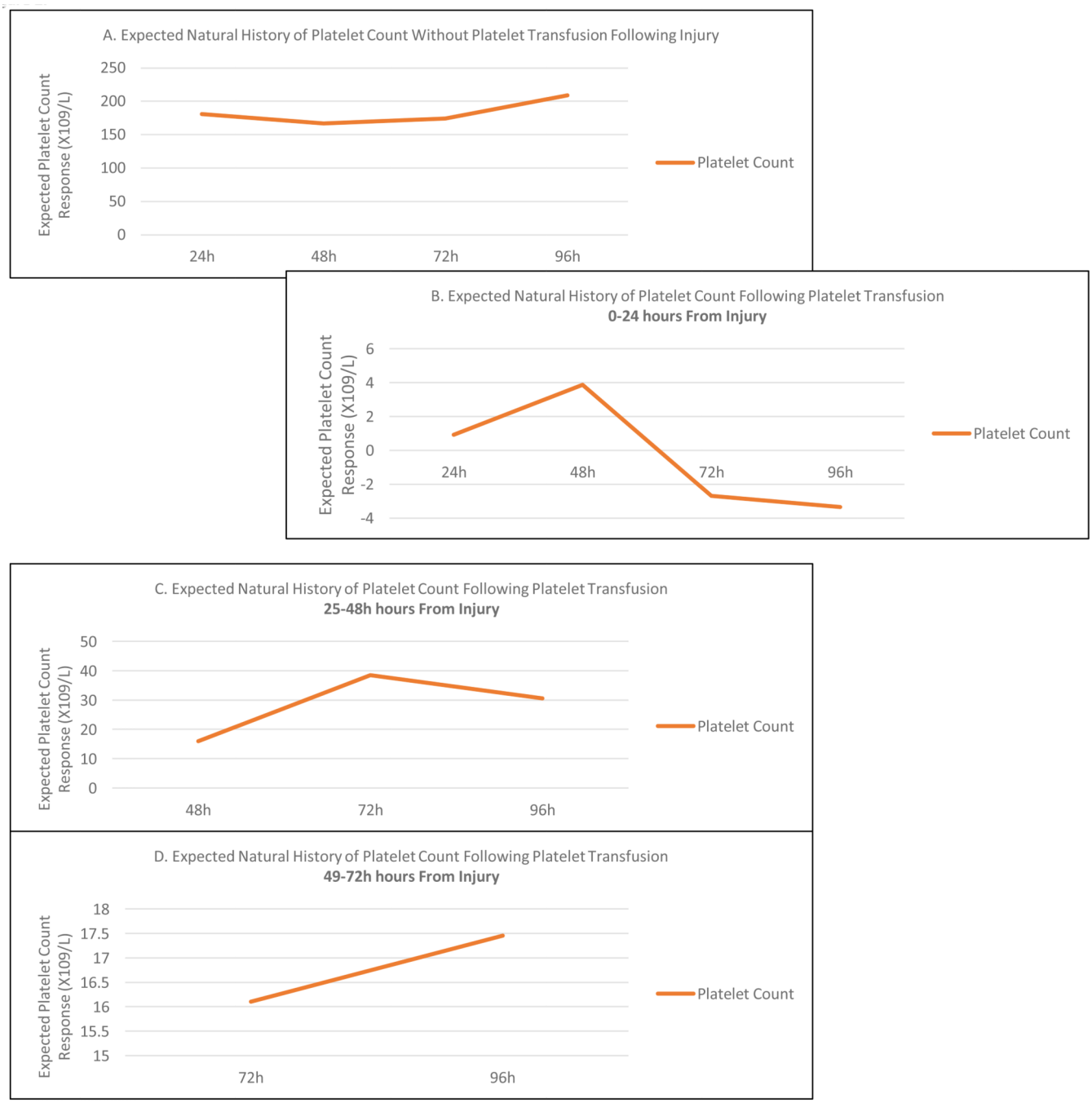

Figure 2. Adjusted Expected Changes in Platelet Counts Over Time After Injury.

Expected changes in platelet count (controlling for ISS/INR/base deficit/interval transfusions). **A single unit of transfused platelets would be expected to increase platelet count by 15×10^9/L based on previous observational studies*(16).

Panel A: Expected platelet count over time without transfusion of platelets.

Panel B: Expected changes in platelet count over time with a hypothetical transfusion of one unit of platelets 0–24h after injury.

Panel C: Expected changes in platelet count over time with a hypothetical transfusion of one unit of platelets 25–48h after injury.

Panel D: Expected changes in platelet count over time with a hypothetical transfusion of one unit of platelets 49–72h after injury.

Figure 3. Adjusted Expected Changes In Platelet Aggregation Over Time After Injury

Panel A: Expected platelet aggregation over time without transfusion of platelets.

Panel B-F: Expected changes in platelet aggregation over time with a hypothetical transfusion of one unit of platelets 0–6h of injury (**B**), 7–12h after injury (**C**), 13–24h after injury (**D**), 25–48h after injury (**E**), 49–72h after injury (**F**).

* Platelet activation was induced by adenosine diphosphate (ADP, final concentration 6.5μM; via P2 receptors), thrombin receptor activating peptide-6 (TRAP, final concentration

32μM; via PAR receptors), and collagen (final concentration 3.2μg/mL; via GpIa/IIa and GpVI receptors).

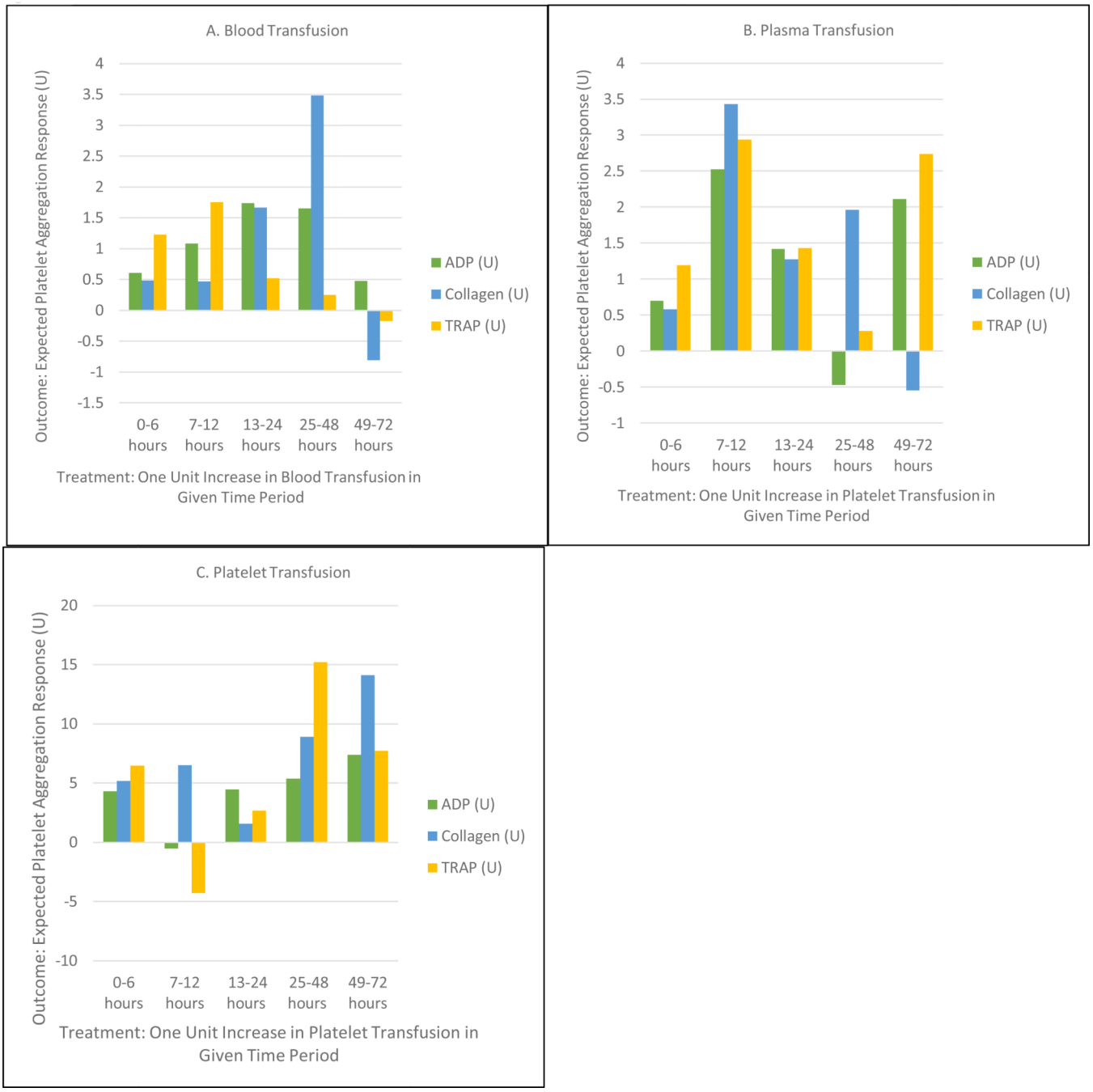

Figure 4. Adjusted Expected Immediate Platelet Aggregation Responses to Blood, Plasma, and Platelet Transfusions Given At Each Time Interval From Injury.

Panel A: Expected changes in platelet aggregation with a hypothetical transfusion of one unit of blood in each time interval.

Panel B: Expected changes in platelet aggregation with a hypothetical transfusion of one unit of plasma in each time interval.

Panel C: Expected changes in platelet aggregation with a hypothetical transfusion of one unit of platelets in each time interval.

* Platelet activation was induced by adenosine diphosphate (ADP, final concentration 6.5μM; via P2 receptors), thrombin receptor activating peptide-6 (TRAP, final concentration 32μM; via PAR receptors), and collagen (final concentration 3.2μg/mL; via GpIa/IIa and GpVI receptors).

TABLE 1.

Patient Demographics/Outcomes

	N= 248
Age (years)	37 (26)
Male (%)	85
BMI (kg/m2)	26 (6)
Blunt mechanism (%)	68
Injury severity score	21 (19)
Admit GCS	14 (8)
TBI (%)	44
Pre-hospital crystalloid volume (mL)	0 (250)
Admit temperature (°)	36.4 (0.8)
Admit pH	7.31 (0.13)
Admit base deficit	2.60 (6.35)
Admit INR >=1.3 (%)	13
Admit platelets (× 10^9/L)	268 (90)
Transfused product in 24 hours (%)	62
Transfused platelets in 24 hours (%)	20
Total hospital days	13 (16)
Total ICU days (to 28 days)	5 (10)
Ventilator free days (to 28 days)	25 (9.75)
Multi-organ failure (%)	15
Mortality at 24 hours (%)	2
Mortality at discharge (%)	13

* Patient demographics for the 248 patients. Data are mean +/− SD, or percentage. Clinical data was collected out to 28 days. The Glasgow coma score (GCS) used was the first recorded score in the ED. Multi-organ failure (MOF) was defined using the Denver Post-Injury Multiple Organ Failure Score (40). Ventilator free days were counted for the first 28 days of hospitalization and subjects who expired received zero ventilator free days.

TABLE 2.

Unadjusted Platelet Aggregometry and Counts Over Time After Injury

	Hour 0	Hour 6	Hour 12	Hour 24	Hour 48	Hour 72	Hour 96	Hour 120
ADP (U)	59 (22)	31 (18)	31 (16)	32 (17)	28 (16)	35 (19)	45 (23)	49 (23)
Collagen (U)	47 (18)	27 (16)	26 (15)	31 (18)	30 (18)	39 (19)	45 (18)	50 (21)
TRAP (U)	97 (27)	63 (28)	66 (25)	66 (26)	75 (28)	83 (30)	87 (29)	87 (30)
Low ADP < 36mm (%)	13%	62%	64%	62%	71%	61%	45%	35%
Low Collagen < 24mm (%)	9%	49%	50%	39%	36%	41%	8%	10%
Low TRAP < 75 mm (%)	20%	70%	66%	60%	46%	40%	35%	32%
Low ADP, Collagen, and TRAP (%)	23%	78%	86%	76%	74%	63%	51%	42%
Platelet count ($\times 10^9$/L)	209 (87)	NA	NA	169 (65)	156 (59)	162 (61)	194 (73)	224 (87)

*Mean platelet aggregometry, counts, and clot strength values over time for the 248 patients. Data are mean +/− SD. Platelet activation was induced by adenosine diphosphate (ADP, final concentration 6.5μM; via P2 receptors), thrombin receptor activating peptide-6 (TRAP, final concentration 32μM; via PAR receptors), and collagen (final concentration 3.2μg/mL; via GpIa/IIa and GpVI receptors). Manufacturer cutoffs were used for impaired platelet aggregometry: ADP<36mm, Collagen <24mm, and TRAP <75mm. Platelet counts obtained daily, no 6 or 12 hour platelet count data.