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ORIGINAL ARTICLE

Immature platelet dynamics are associated with clinical outcomes after major trauma

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Abstract

Background: Major trauma results in dramatic changes in platelet behavior. Newly formed platelets are more reactive than older platelets, but their contributions to hemostasis and thrombosis after severe injury have not been previously evaluated.

Objectives: To determine how immature platelet metrics and plasma thrombopoietin relate to clinical outcomes after major injury.

Methods: A prospective observational cohort study was performed in adult trauma patients. Platelet counts and the immature platelet fraction (IPF) were measured at admission and 24 hours, 72 hours, and 7 days after injury. Thromboelastometry was performed at admission. Plasma thrombopoietin, c-Mpl, and GPIbα were quantified in a separate cohort. The primary outcome was in-hospital mortality; secondary outcomes were venous thromboembolic events and multiple organ dysfunction syndrome (MODS).

Results: On admission, immature platelet counts (IPCs) were significantly lower in nonsurvivors (n = 40) than in survivors (n = 236; 7.3 × 10^9/L vs 10.6 × 10^9/L; P = .009), but IPF did not differ. Similarly, impaired platelet function on thromboelastometry was associated with lower admission IPC (9.1 × 10^9/L vs 11.9 × 10^9/L; P < .001). However, at later time points, we observed significantly higher IPF and IPC in patients who developed venous thromboembolism (21.0 × 10^9/L vs 11.1 × 10^9/L; P = .02) and prolonged MODS (20.9 × 10^9/L vs 11 × 10^9/L; P = .003) than in those who did not develop complications. Plasma thrombopoietin levels at admission were significantly lower in nonsurvivors (P < .001), in patients with MODS (P < .001), and in those who developed venous thromboembolism (P = .04).

Conclusion: Lower levels of immature platelets in the acute phase after major injury are associated with increased mortality, whereas higher immature platelet levels at later time points may predispose to thrombosis and MODS.

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Henry Schofield and Andrea Rossetto contributed equally to this study.

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KEYWORDS
multiple organ failure, platelets, thrombopoietin, trauma, venous thromboembolism

1 | INTRODUCTION

Platelet dysfunction is a central component of trauma-induced coagulopathy (TIC), a state of hemostatic incompetence that occurs in more than 25% of severely injured patients and is associated with a 4-fold increase in mortality [1,2]. A range of alterations in platelet form and function after major trauma have been described, including impaired responsiveness to *ex vivo* stimulation [3–5], loss of surface collagen receptors [6], and expansion of the procoagulant platelet subpopulation [7]. In patients who survive the bleeding phase, this initial coagulopathy switches toward a procoagulant state that predisposes to thrombotic events, which occur in up to a third of critically injured patients despite aggressive thromboprophylaxis [8]. Although our understanding of postinjury platelet biology has advanced significantly over the past decade, the precise mechanisms by which platelets contribute to TIC and their role in the subsequent prothrombotic switch are not fully understood.

Immature platelets are a highly reactive subpopulation that possess greater procoagulant and prothrombotic potential than older platelets [9]. These newly formed platelets, also known as reticulated platelets, are released from the bone marrow primarily in response to thrombopoietin (TPO) and contain higher levels of messenger RNA, which can be detected by nucleic acid dyes, enabling them to be distinguished from their older counterparts [10,11]. Commercially available assays for quantifying this immature platelet fraction (IPF) are widely available and have been primarily used to characterize thrombocytopenia, with a low IPF indicating a condition of decreased platelet production, while a normal or increased IPF is suggestive of increased platelet destruction or consumption [12–14]. The IPF has clinical utility in predicting recovery from thrombocytopenia after chemotherapy [15] and, more recently, has been shown to provide prognostic information in patients with COVID-19 and sepsis, with higher IPF values being predictive of mortality [16,17]. However, immature platelets have not previously been studied in patients with major trauma.

The objective of this study was to determine how immature platelet dynamics relate to clinical outcomes after major trauma. Given their procoagulant tendencies, we hypothesized that failure to mobilize immature platelets in the acute phase after injury would be associated with increased mortality due to bleeding and that increased production of immature platelets at later time points would increase the risk of thromboembolic events and multiple organ dysfunction syndrome (MODS). Finally, we aimed to investigate the changes in TPO levels in the acute phase after major trauma.

2 | METHODS

2.1 | Study design

We included patients recruited into the Activation of Coagulation and Inflammation in Trauma (ACIT)-II study at a single major trauma center. This is a platform prospective observational cohort study (REC reference 07/Q0603/29, ISRCTN12962642). Consent procedures and inclusion criteria have been described in detail previously [18]. For this analysis, we included 2 separate cohorts. To investigate the relationship between immature platelet dynamics and clinical outcomes, we included patients enrolled in the ACIT-II study between June 2020 and May 2022 for whom IPF was measured prospectively. To investigate the changes in TPO levels, we included patients enrolled in the ACIT-II study between February 2008 and March 2019 for whom biomarkers were measured retrospectively on prospectively collected samples as part of a separate proteomic discovery project. Patients who presented to the hospital more than 2 hours after injury, were transferred from another hospital, had sustained burns over >5% of their body surface area, received more than 2 liters of fluid prior to recruitment, or had a known bleeding diathesis were excluded.

2.2 | Study procedures

Blood samples were drawn on admission (within 2 hours of injury) and at 24 ± 2 hours, 72 ± 12 hours, and 7 days ± 24 hours. Demographics, injury characteristics, and outcomes were recorded prospectively by a trained member of the research team, and all patients were followed up daily until 28 days after injury unless preceded by death or hospital discharge. The primary outcome of interest was in-hospital mortality. Secondary outcomes included 24-hour mortality, mortality due to exsanguination, venous thromboembolism (VTE), and MODS.

2.3 | Experimental methods

Platelet counts and the IPF were measured in our central hospital laboratory using a Sysmex XN-series analyzer according to standard operating procedures. From these variables, we calculated the immature platelet count (IPC) (total platelet count multiplied by IPF) and mature platelet count (MPC) (platelet count minus IPC) for each sample, as previously described [19]. Rotational thromboelastometry was performed using a Delta instrument (Tem Innovations GmbH) within 1 hour of blood collection in accordance with the manufacturers' instructions. Lactate and base deficit were measured with point-of-care

blood gas analysis. For proteomic studies, blood was drawn into P100 vacutainers (Becton Dickinson) and centrifuged at 2500 × *g* for 10 minutes; the plasma fraction was isolated and stored at −80 °C prior to analysis. Plasma proteins were measured using the SomaScan platform (SomaLogic, Inc) as previously described [20] and reported as relative fluorescence units (RFU). Among the proteomic data available, we investigated biomarkers related to thrombopoiesis and platelet consumption: TPO, the TPO receptor c-Mpl, and GPIbα [21].

2.4 | Definitions

Severe injury and critical injury were defined as an injury severity score (ISS) of >15 and >25, respectively. Previously established rotational thromboelastometry cutoffs were used to define TIC (tissue-factor activated rotational thromboelastometry [EXTEM] clot amplitude at 5 minutes, <40 mm) and platelet dysfunction (EXTEM minus FIBTEM [tissue-factor activated rotational thromboelastometry with cytochalasin D] clot amplitude at 5 minutes <30 mm) [22]. MODS was defined as a sequential organ failure assessment score of ≥6 on any day after the first day of admission and subdivided into early-resolving MODS (ERMODS) where the sequential organ failure assessment score fell to <6 within 7 days of onset and prolonged MODS (PRMODS) when MODS persisted beyond 7 days [23]. VTE was defined as either deep vein thrombosis or pulmonary embolism confirmed on imaging and was subdivided into early (<7 days) and late (≥7 days) events. In some analyses, we divided patients into groups according to the median platelet count ($Count_{LOW}$ and $Count_{HIGH}$) and median IPF (IPF_{LOW} and IPF_{HIGH}).

2.5 | Data analysis

Analysis was performed using Prism v8.0 (GraphPad Software Inc) and RStudio (R version 4.1.3 [2022-03-10], R Core Team). Nonparametric data are reported as median with IQR and were compared using Mann–Whitney U-test or Kruskal–Wallis test with Dunn's posttest correction. Categorical data are reported as number and percentage and were compared with chi-squared or Fisher's exact test, with correction for multiple comparisons using the Bonferroni method. Multivariable logistic regression models were constructed for main clinical outcomes (in-hospital mortality, all and late VTE events, MODS, and PRMODS) and hemostatic parameters of coagulopathy and platelet dysfunction. As confounding factors, we considered demographic variables (age and assigned sex), key descriptors of trauma severity (ISS and base deficit), and the number of platelet pools transfused. The latter was not included in the models for coagulopathy and platelet dysfunction at admission as no platelet transfusions were administered prior to admission samples. No variables were considered as effect modifiers. All variables were included in the multivariable regressions independent of their statistical significance in the univariable analysis [24]. Due to the low frequency of VTE events and PRMODS and to avoid the risk of model overfitting, we performed multiple multivariable regressions for these outcomes, investigating the presence or absence of a consistent independent effect of IPC/IPF by adjusting for other confounding factors separately. Correlation between continuous variables was quantified with Spearman's ρ. Survival times between groups were compared using log-rank test and presented as Kaplan–Meier curves. A 2-tailed *P* value of <.05 was considered significant throughout, and a complete case analysis was performed.

3 | RESULTS

This study included 276 trauma patients recruited into the ACIT-II study between June 2020 and May 2022. The cohort comprised patients with a broad spectrum of injury severity (median ISS, 13 [2–26]), of whom 86% (241/276) were male and 60% (166/276) had sustained a blunt mechanism of injury; detailed characteristics are presented in Supplementary Table S1. The median admission platelet count was 246 × 10^9/L (199-299), and significant thrombocytopenia was rare (count, <100 × 10^9/L; 14/276, 5%), in keeping with previous reports [3,25]. No patients received platelet transfusion prior to baseline blood sampling.

3.1 | Low IPCs at admission are associated with increased mortality

We first examined the relationship between immature platelet metrics measured at admission and survival. Compared to survivors (*n* = 236), nonsurvivors (*n* = 40) had significantly lower IPCs (7.3 × 10^9/L [4.8-13.2 × 10^9/L] vs 10.6 × 10^9/L [7.9-16.2 × 10^9/L]; *P* = .009), but the IPF did not differ (4.0% [3.1%-6.3%] vs 4.7% [2.9%-6.5%]; *P* = .50). This pattern of lower levels of immature platelets in nonsurvivors persisted when we grouped patients according to injury severity, with markedly lower IPCs among nonsurvivors in patients with severe and critical injuries (Figure 1A), whereas there were no significant differences in IPF (Figure 1B). Similarly, we observed a lower IPC in patients who died within 24 hours of admission (*n* = 12; 5.0 × 10^9/L [1.8-11.6 × 10^9/L] vs 10.4 × 10^9/L [7.7-15.4 × 10^9/L]; *P* = .002; Supplementary Figure S1A); but again, there were no statistically significant differences in IPF (3.9% [2.5%-4.4%] vs 4.6% [2.9%-6.5%]; *P* = .14; Supplementary Figure S1B).

To examine this further, we stratified the cohort into 4 groups according to the median total platelet count ($Count_{LOW}$ or $Count_{HIGH}$) and median IPF (IPF_{LOW} or IPF_{HIGH}); clinical characteristics of these groups are detailed in Supplementary Table S1. Patients in the $Count_{LOW}IPF_{LOW}$ group (*n* = 53) had a similar ISS and degree of shock compared to the remainder of the cohort but had significantly higher mortality (Figure 1C). These patients were also characterized by the highest proportion of mortality due to exsanguination (8% [4/53]; *P* = .002). Conversely, patients with lower platelet counts but a high IPF ($Count_{LOW}IPF_{HIGH}$, *n* = 87) had low mortality both at 24

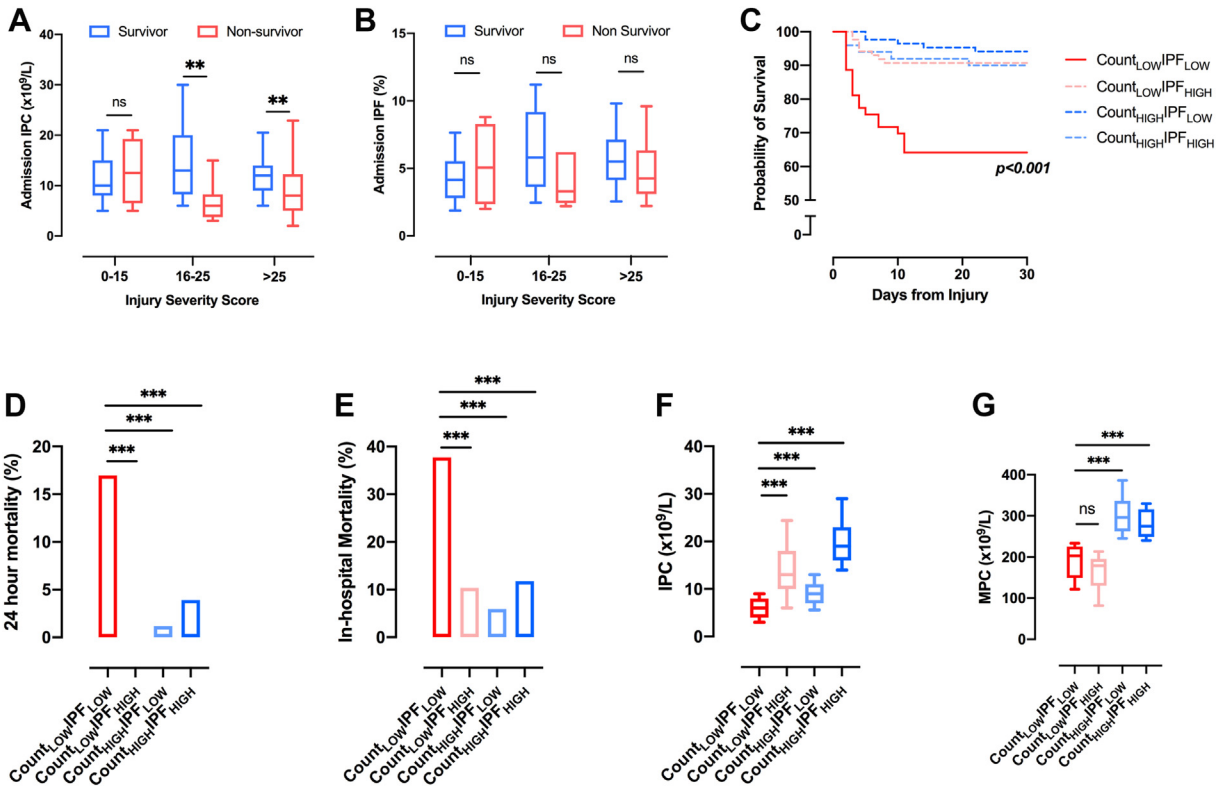

FIGURE 1 Admission immature platelet counts (IPC) and mortality after major trauma. (A) IPC and (B) immature platelet fraction (IPF) in survivors (n = 236) and nonsurvivors (n = 40) with mild-to-moderate (injury severity score [ISS], 0-15; n = 150), severe (ISS, 16-25; n = 50), and critical (ISS, >25; n = 74) injuries. Mann–Whitney U-test was used for comparisons. (C) Kaplan–Meier curves for survival in patients divided into 4 groups according to platelet count and IPF: $Count_{LOW}IPF_{LOW}$ (n = 53), $Count_{LOW}IPF_{HIGH}$ (n = 87), $Count_{HIGH}IPF_{LOW}$ (n = 87), and $Count_{HIGH}IPF_{HIGH}$ (n = 51). The P value was derived using log-rank test. (D) Twenty-four–hour mortality in patients grouped according to platelet count and IPF. (E) In-hospital mortality. Fisher's exact test was used for comparisons. (F) IPC. (G) Mature platelet count (MPC). Kruskal–Wallis test with Dunn's posttest correction was used for comparisons. Box and whisker plots depict 10th to 90th percentiles. $^{**}P <$ .01; $^{***}P <$ .001, ns, not significant.

hours (Figure 1D) and in-hospital (Figure 1E). Patients in the $Count_{LOW}IPF_{LOW}$ group had significantly lower IPCs compared to those in the remainder of the cohort (Figure 1F), but MPCs were not statistically different between $Count_{LOW}IPF_{LOW}$ and $Count_{LOW}IPF_{HIGH}$ (Figure 1G). On multivariate regression analysis adjusting for key confounders (age, sex, ISS, base deficit, and platelet transfusions), patients in the $Count_{LOW}IPF_{LOW}$ group had an 8.9-fold increase in odds of in-hospital mortality (95% CI, 2.8-28.3; $P <$ .001) compared to the remainder of the cohort (Table 1). Collectively, these data demonstrate that reduction of circulating immature platelets is a key signature associated with increased mortality after major trauma, whereas maintained IPCs are associated with reduced mortality.

3.2 | Patients with coagulopathy and platelet dysfunction have lower numbers of circulating immature platelets

Given that immature platelets have greater proaggregatory potential compared to mature platelets, we postulated that the reduction of the absolute number of immature platelets could contribute to TIC and platelet dysfunction. We compared patients with TIC (n = 98) and patients without TIC (n = 162) and found that coagulopathic patients had lower IPC levels (9.8×10^9/L [5.6-12.7×10^9/L] vs 11.6×10^9/L [8.4-18.5×10^9/L]; $P <$ .001), a pattern that was also evident when we grouped patients according to injury severity (Figure 2A) and when we stratified patients into $Count_{LOW}$ and $Count_{HIGH}$ groups as before (Figure 2B). A similar picture was evident when we compared patients with (n = 90) and without platelet dysfunction (n = 166), with significantly lower IPC counts in those with platelet dysfunction (9.1×10^9/L [5.5-12.6 $\times$ 10^9/L] vs 11.9×10^9/L [8.7-18.5 $\times$ 10^9/L]; $P <$ .001), which was again evident across injury subgroups (Figure 2C) and platelet count subgroups (Figure 2D). Conversely, IPF values were not significantly different in patients with TIC (4.8% [3.0%-6.4%] vs 4.6% [3.0%-6.9%]; P = .95) or platelet dysfunction (4.8% [2.8%-6.6%] vs 4.7% [3.2%-6.8%]; P = .91) in these analyses (Supplementary Figure S2). On multivariate regression, admission IPC, but not IPF, was independently associated with TIC ($P <$ .001) and platelet dysfunction ($P <$ .001) after adjusting for relevant confounders (Table 2).

TABLE 1 Multivariable regression analyses for in-hospital mortality.

Variable	OR	(95% CI)	*P* value	Adj. OR	(95% CI)	*P* value
Age (y)	1.028	1.009-1.047	.004	1.046	1.017-1.076	.002
Sex, male	0.910	0.288-2.874	.873	0.886	0.198-3.975	.875
ISS	1.085	1.054-1.117	<.001	1.075	1.034-1.119	<.001
BD (mEq/L)	1.128	1.062-1,198	<.001	1.047	0.950-1.155	.354
PLT transfusion, pools	2.292	1.459-3.599	<.001	1.377	0.728-2.606	.326
$Count_{LOW}IPF_{LOW}$	5.728	(2.457-13.35)	<.001	8.945	(2.823-28.34)	<.001

R^2 = 0.51.

Adj., adjusted; BD, base deficit; $Count_{LOW}IPF_{LOW}$, platelet count lower than the median and immature platelet fraction lower than the median; IPF, immature platelet fraction; ISS, injury severity score; OR, odds ratio; PLT, platelet.

3.3 | Increased immature platelets at later time points postinjury are associated with thrombosis and MODS

We next examined the relationship between immature platelet metrics and the risk of VTE and MODS. Patients who developed VTE (*n* = 12) had consistently higher IPF values over the first 7 days after injury (Supplementary Figure S3A) and a significantly higher peak IPF (10.3% [8.1%-13.6%] vs 5.0% [3.5%-7.2%]; *P* < .001), which was more pronounced in patients who developed VTE beyond the first week from injury (*n* = 5; Figure 3A). Peak IPCs were also significantly higher in patients who developed VTE (21.0 × 10^9/L [10.3-32.6 × 10^9/L] vs 11.1 × 10^9/L [8.0-18.0 × 10^9/L]; *P* = .02), particularly in those who developed late VTEs (Figure 3B); this difference was primarily attributable to higher IPCs on day 7 rather than at earlier time points (Supplementary Figure S3B). The association between peak IPC, but not peak IPF, with all and late VTE remained significant on regression analysis after adjusting for injury severity, shock, MPC, and platelet transfusions separately (Supplementary Table S2).

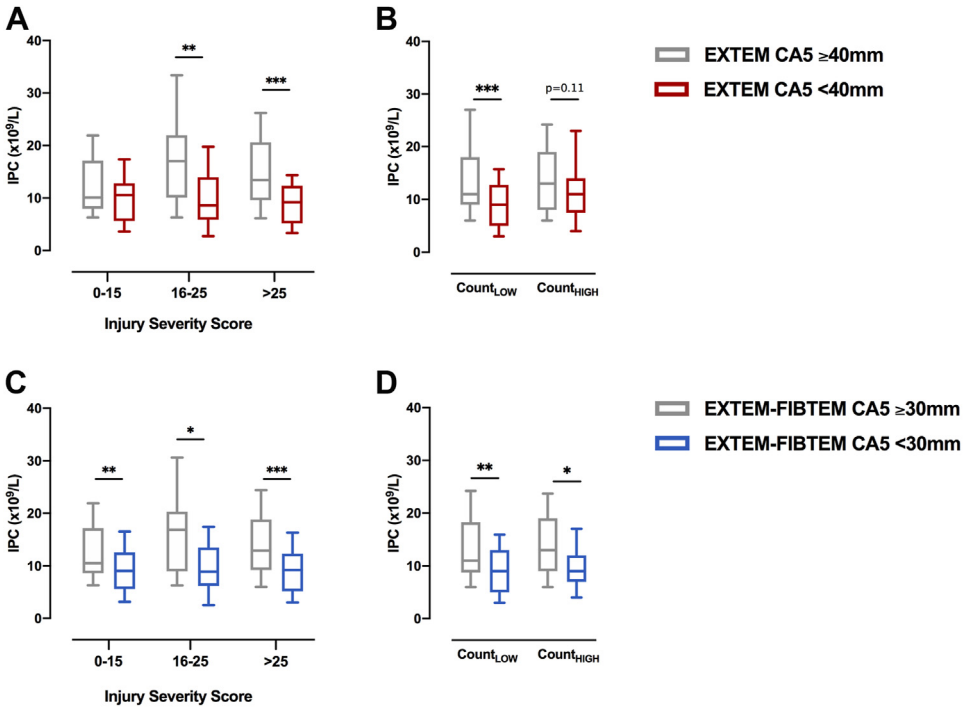

FIGURE 2 Immature platelet counts (IPCs) in patients with coagulopathy and platelet dysfunction. (A) IPC in patients with (*n* = 98) and without (*n* = 162) trauma-induced coagulopathy, defined as an tissue-factor activated rotational thromboelastometry (EXTEM) clot amplitude at 5 minutes (CA5) of <40 mm on rotational thromboelastometry in patients with mild-to-moderate (injury severity score [ISS], 0-15; *n* = 150), severe (ISS, 16-25;, *n* = 50), and critical (ISS, >25; *n* = 74) injuries. (B) IPC in patients stratified by the cohort median platelet count. (C) IPC in patients with (*n* = 90) and without (*n* = 166) platelet dysfunction on rotational thromboelastometry, defined as an EXTEM–tissue-factor activated rotational thromboelastometry with cytochalasin D (FIBTEM) CA5 of <30 mm, stratified by injury severity. (D) IPC in patients with and without platelet dysfunction stratified by the cohort median platelet count. Box and whisker plots depict 10th to 90th percentiles. **P* < .05; ***P* < .01; ****P* < .001; Mann–Whitney U-test.

TABLE 2 Multivariable logistic regression analysis of the association between immature platelet count and coagulopathy (top) and platelet dysfunction (bottom).

	EXTEM CA5 <40 mm						EXTEM-FIBTEM CA5 <30 mm					
Variable	OR	(95% CI)	*P* value	Adj. OR	(95% CI)	*P* value	OR	(95% CI)	*P* value	Adj. OR	(95% CI)	*P* value
IPC												
Age (y)	1.002	0.988-1.016	.786	0.999	0.982-1.016	.917	1.012	0.998-1.026	.106	1.011	0.995-1.027	.191
Sex, male	1.599	0.691-3.702	.184	1.956	0.727-5.264	.184	0.870	0.392-1.930	.732	0.946	0.388-2.304	.903
ISS	1.060	1.037-1.083	<.001	1.051	1.023-1.080	<.001	1.048	1.027-1.070	<.001	1.032	1.007-1.058	.012
BD (mEq/L)	1.163	1.093-1.238	<.001	1.083	1.010-1.161	.026	1.124	1.064-1.187	<.001	1.071	1.007-1.139	.030
IPC ($\times 10^9$/mL)	0.894	0.848-0.944	<.001	0.897	0.847-0.951	<.001	0.917	0.872-0.963	.001	0.926	0.880-0.974	.003
IPF												
Age (y)	1.002	0.988-1.016	.786	0.997	0.981-1.014	.758	1.012	0.998-1.026	.106	1.008	0.992-1.024	.345
Sex, male	1.599	0.691-3.702	.184	1.532	0.598-3.922	.374	0.870	0.392-1.930	.732	0.810	0.339-1.936	.636
ISS	1.060	1.037-1.083	<.001	1.044	1.018-1.072	.001	1.048	1.027-1.070	<.001	1.029	1.004-1.054	.021
BD (mEq/L)	1.163	1.093-1.238	<.001	1.104	1.033-1.180	.004	1.124	1.064-1.187	<.001	1.089	1.025-1.157	.006
IPF (%)	1.023	0.955-1.096	.509	0.997	0.919-1.081	.937	1.050	0.980-1.126	.167	1.033	0.955-1.117	.420

IPC: R^2 for EXTEM CA5 <40mm = 0.34. R^2 for EXTEM-FIBTEM CA5 <40mm = 0.24. IPF: R^2 for EXTEM CA5 <40mm = 0.25. R^2 for EXTEM-FIBTEM CA5 <40mm = 0.19.

Adj., adjusted; BD, base deficit; CA5, clot amplitude at 5 minutes; EXTEM, tissue-factor activated rotational thromboelastometry; FIBTEM, tissue-factor activated rotational thromboelastometry with cytochalasin D; IPC, immature platelet count; IPF, immature platelet fraction; ISS, injury severity score; OR, odds ratio.

Similarly, patients who developed ERMODS (*n* = 52) and PRMODS (*n* = 20) had significantly higher peak IPFs during the first 7 days after injury compared to those who did not develop MODS (Figure 3C and Supplementary Figure S4). Maximum IPCs were higher in patients who developed PRMODS but not in patients who developed ERMODS (Figure 3D), which again was primarily attributable to increases in IPC at day 7 after injury (Supplementary Figure S4). On regression analysis, peak IPC and IPF values were independently associated with PRMODS after adjusting for injury severity, shock, mature/total platelet count, and platelet transfusions separately (Supplementary Table S3).

3.4 | Rapid decrease of plasma TPO occurs in patients with severe injuries and adverse clinical outcomes

To investigate TPO levels after major trauma, we analyzed plasma proteomic data in blood samples drawn within 2 hours of injury from a separate cohort of patients recruited into the ACIT-II study (*n* = 421; Supplementary Table S4). This cohort contained a similar spectrum of injury to our main cohort, with a median ISS of 11 (IQR, 4-25) and in-hospital mortality of 5% (23/421). Plasma levels of TPO were negatively correlated with ISS ($\rho = -0.46$; $P < .001$), with the lowest levels in patients with critical injuries (Figure 4A). A similar pattern was evident with plasma base deficit, a marker of systemic hypoperfusion, which showed a significant negative correlation with TPO ($\rho = -0.29$; $P < .001$). Although TPO levels generally increase with a reduced platelet count due to lower availability of circulating TPO receptors [26], we found no correlation between TPO and platelet count ($\rho = 0.03$; $P = .54$; Figure 4B), and indeed, there was a nonsignificant trend toward lower TPO levels in patients with thrombocytopenia (*n* = 35; 640RFU [544-783] vs 685RFU [593-779]; $P = .12$). In contrast, TPO levels were inversely proportional to plasma GPIbα, a marker of platelet consumption ($\rho = -0.41$; $P < .001$; Figure 4C). Plasma concentrations of the TPO receptor c-Mpl showed no correlation with injury severity ($\rho = 0.10$; $P = .95$) and were not correlated with TPO levels ($\rho = 0.02$; $P = .67$, Supplementary Figure S5).

Finally, we examined the relationship between admission TPO levels and clinical outcomes. TPO concentrations were significantly lower in nonsurvivors (*n* = 23) than in survivors (*n* = 398; 559RFU [483-659] vs 688RFU [595-5779]; $P < .001$), in patients with MODS (*n* = 74) than in patients without MODS (*n* = 347; 616RFU [546-714] vs 737RFU [654-811]; $P < .001$), and in those who developed VTE (*n* = 11) than in those who did not develop VTE (*n* = 410; 628RFU [507-689] vs 683RFU [592-777]; $P = .04$). These differences were also evident when we stratified patients in quartiles according to the plasma TPO level, with the highest rates of in-hospital mortality, MODS, and VTE in the lowest quartile (Figure 4D–F). These data indicate that a mechanism of trauma-induced TPO reduction that occurs within hours of injury is not attributable to increased TPO receptor levels in the circulation and is associated with poor outcomes.

4 | DISCUSSION

In this cohort study of more than 250 trauma patients, we describe important associations between the number and proportion of

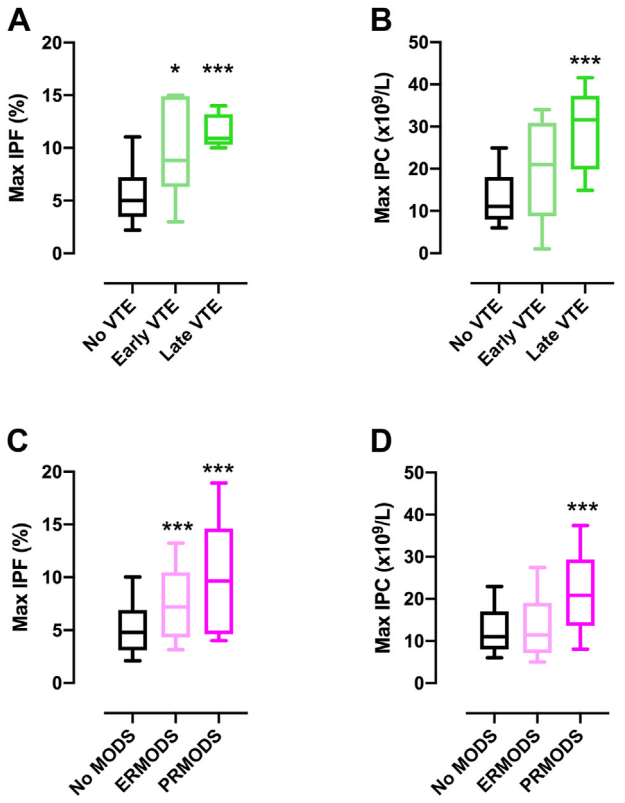

FIGURE 3 Immature platelet metrics in postinjury venous thromboembolism (VTE) and multiple organ dysfunction syndrome (MODS). Maximum (A) immature platelet fraction (IPF) and (B) immature platelet count (IPC) over the first 7 days after injury in patients who did not develop VTE (n = 264), developed VTE within the first week (early VTE, n = 7), and developed VTE beyond the first week (late VTE, n = 5). Maximum (C) IPF and (D) IPC over the first 7 days after injury in patients who did not develop MODS (n = 204), developed early-resolving MODS (ERMORDS, n = 52), and developed prolonged MODS (PRMODS, n = 20). Box and whisker plots depict 10th to 90th percentiles. $^{*}P < .05$, $^{***}P < .001$ vs no VTE/no MODS; Kruskal–Wallis test with Dunn's posttest correction.

immature platelets and clinical outcomes. Our results demonstrate that early reduction of platelets together with a low IPF is associated with a marked reduction in survival over the subsequent 10 days. Conversely, a larger increase in circulating immature platelets at later time points occurs in patients who develop MODS and thrombosis. Early reduction of plasma TPO levels occurs in proportion to injury severity and clinical outcome, which may indicate increased TPO uptake by platelet precursors to compensate for tissue damage and blood loss. This represents a potential mechanism for subsequent increases in circulating immature platelets among patients who develop thrombotic and inflammatory complications.

Immature platelets are generally considered to have greater procoagulant and prothrombotic potential compared to aged platelets, acting as seeds for the assembly of platelet aggregates at sites of vessel injury [9,27]. Patients with major injuries may have multiple bleeding sites and frequently have an established coagulopathy that involves multiple aspects of hemostasis, including impairment of several key aspects of platelet hemostatic function [28]. Our results suggest that maintenance of a circulating pool of immature platelets in these patients may protect against coagulopathy and ongoing bleeding. Conversely, depletion of immature platelets below a certain threshold may contribute to compromised platelet hemostatic capacity, with a consequent increase in mortality.

The IPF and the IPC are related variables that provide overlapping information, although there are important differences between them. The IPF is a more complex variable in that it can be reached by various combinations of IPCs and MCPs—eg, a high IPF can be due to either an increase in IPC or a decrease in MPC, whereas a low IPF can be due to a decrease in IPC or an increase in MPC [29]. Interpreting the IPC in the context of trauma can also be complex, as a low IPC may be due to a reduction in the release of new platelets and/or an increase in the rate of immature platelet consumption. Previous studies have demonstrated differences between the IPF and IPC in various clinical settings [17,30,31], and it has been suggested that IPC may provide a better real-time assessment of platelet production than the IPF [19]. However, the IPF has been shown to carry prognostic information in a range of diseases, including COVID-19 [17], sepsis [16], and bone marrow failure [15]. In trauma—particularly in the initial hours following injury—the balance between platelet consumption and production is likely to be highly dynamic given the potential for recruitment of platelets to sites of bleeding and tissue damage. This added complexity may explain why our results differ from studies in other diseases, such as sepsis and COVID-19, in which a high IPF was predictive of mortality [16,17], although it should also be noted that these patients often present once the disease process is established, and therefore, early levels of immature platelets are unknown. Our interpretation of the admission data presented here is that in the initial hours after injury, a reduced IPF in the context of a reduced total platelet count is strongly associated with increased mortality. We postulate that a low IPF within hours of injury may either reflect a major perturbation in platelet production and/or consumption, resulting in a low IPC, or instead reflect minimal changes in these processes such that the IPF is not markedly different from that of healthy individuals. This may explain why the IPF in isolation does not predict mortality; however, when combined with platelet count, it shows a strong association with outcome.

The mechanism underlying this early immature platelet reduction in trauma may either be inadequate production by the bone marrow, increased consumption of immature platelets in developing thrombi, preferential sequestration in the liver and spleen, or some combination of these. The relative contributions of each process in trauma patients require further study, but the fact that the mortality associated with low IPC is evident in patients who also have lower total platelet counts suggests that consumption is likely to be a contributor. However, our results show that reduced numbers of mature platelets in isolation do not appear to confer an increased risk of mortality or coagulopathy, suggesting that immature platelets may help compensate for overall reductions in platelet count and coagulopathy until a certain threshold is reached.

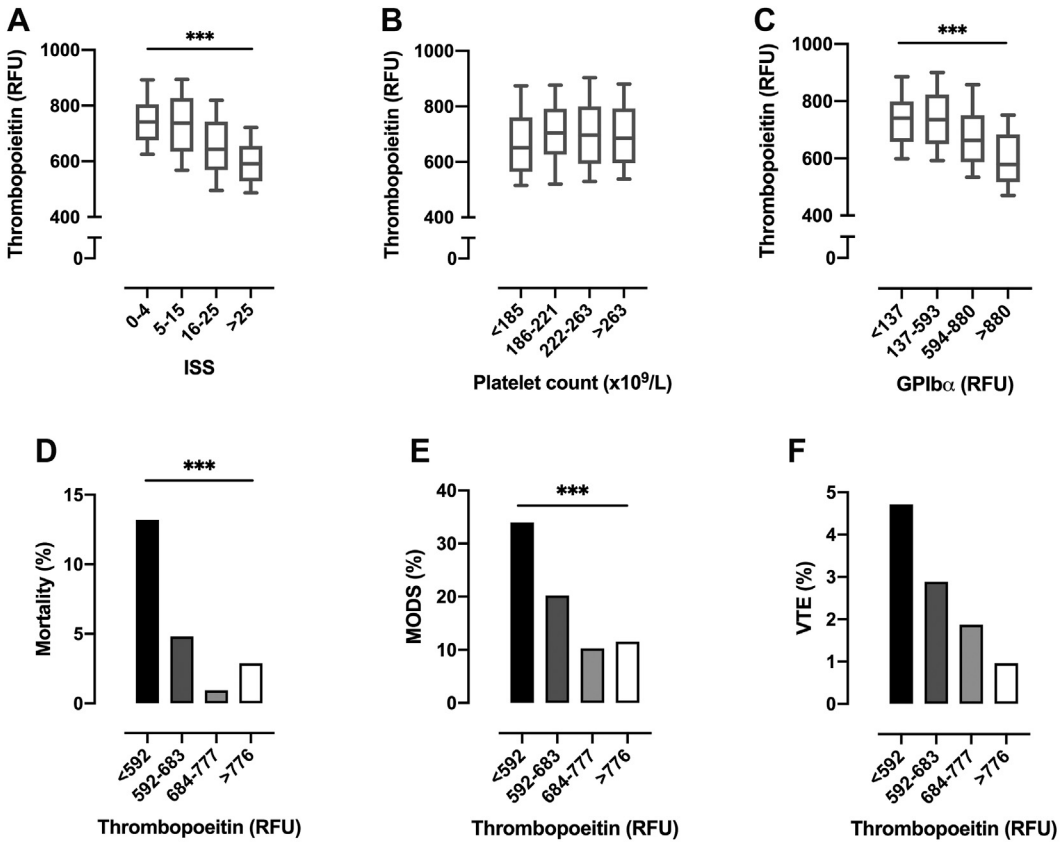

FIGURE 4 Admission plasma thrombopoietin (TPO) levels after major trauma (n = 421). Levels of TPO in patients stratified by (A) injury severity score (ISS) (mild: ISS, 0-4; n = 107; moderate: ISS, 5-15; n = 125; severe: ISS, 16-25; n = 94; critical: ISS, >25; n = 95), (B) admission platelet count quartiles, and (C) plasma GPIbα quartiles. (D) Mortality, (E) multiple organ dysfunction syndrome (MODS), and (F) venous thromboembolism (VTE) in patients stratified according to TPO quartiles. ***$P < .001$; Kruskal–Wallis test. Box and whisker plots depict 10th to 90th percentiles. RFU, relative fluorescence unit.

Beyond the initial bleeding phase, trauma patients who survive rapidly shift from an initial coagulopathy toward a highly prothrombotic and proinflammatory state that contributes to adverse outcomes [32–34]. Our results suggest that while immature platelets may be protective during the bleeding phase, they may also drive the development of subsequent complications in patients who survive to reach intensive care. Higher IPCs are associated with an increased risk of thrombotic events in atherosclerosis [35,36], and diseases with increased platelet turnover, such as diabetes mellitus, carry an increased rate of thrombosis [37]. Consistent with this, our results suggest that immature platelets may play a role in the development of postinjury thrombosis. Platelets are increasingly recognized as important players in the pathophysiology of VTE [38] but are not specifically targeted by current thromboprophylactic regimens. Our findings add to the body of evidence implicating platelets in the development of posttraumatic VTE [38,39] and suggest that targeting platelets may enhance the efficacy of existing preventative measures. We also found that immature platelet numbers are elevated in patients who develop MODS, adding weight to existing evidence for a role for platelets beyond hemostasis and thrombosis in major trauma [40,41]. These findings are consistent with previous studies showing that a higher IPF/IPC is associated with worse outcomes in sepsis and COVID-19 [16,17]. Further investigation into the characteristics of immature platelets released after injury and the mechanisms by which they may contribute to postinjury MODS and thrombosis could inform the development of new prophylactic and therapeutic strategies.

The primary mediator of platelet production is TPO, which is produced by the liver and is essential for the survival and differentiation of megakaryocytes (MKs) [42]. We found that TPO levels were reduced within 2 hours of major trauma, with lower levels in patients with critical injuries and shock. Given that the half-life of TPO is 20 to 40 hours [43], this is unlikely to reflect synthetic failure. On binding to its receptor c-Mpl, expressed on platelets and MKs, TPO is rapidly internalized and metabolized, providing a mechanism by which circulating levels of TPO fall [44]. We did not find evidence of increased c-Mpl in circulation, and TPO concentrations were not related to platelet count in trauma patients. Therefore, a potential explanation for the reduction of TPO levels in plasma is increased uptake by MKs and their precursors via increased expression of surface c-Mpl, although the mechanisms responsible for this are unclear. Given that TPO takes approximately 5 days to produce new platelets, we speculate that this could explain the delayed rise in IPCs that we

observed in patients who subsequently developed thrombotic events and MODS.

There are several limitations to this study. First, while we describe quantitative changes in immature platelets, we have not explored the characteristics of newly formed platelets released after injury. A pertinent question for further study is whether major injury and blood loss induce a change in the properties of newly formed platelets released after injury. Second, by virtue of the observational design of our study, we are only able to describe associations rather than causal relationships, and further mechanistic work is needed to build on our findings reported here. Third, the blood sampling schedule, which is inherent to the design of the ACIT-II study, does not include blood draws between 72 hours and 7 days and may miss biologically relevant processes occurring between these time points. Fourth, our definitions of coagulopathy and platelet dysfunction are based on *ex vivo* thromboelastometry, which has limitations in identifying patients with clinically significant hemostatic compromise and is not a test designed specifically to evaluate platelet function. Fifth, our study is limited by the small sample size of some subgroups included in our analysis, particularly in relation to clinical outcomes such as VTE and MODS. Larger studies are required to provide definitive evidence on the impact of IPC/IPF levels on these outcomes. Sixth, we did not measure levels of other immature cell types (such as red blood cells), which may have lent an insight into whether there is a global impairment in bone marrow function. Finally, we were not able to quantify TPO levels in the same cohort that immature platelet metrics were measured and, therefore, were not able to draw direct links between these variables. This limits our ability to draw conclusions about the relationship between immature platelets and the biomarkers measured, which requires investigation in future dedicated studies.

In conclusion, this study provides new insights into immature platelet kinetics after major trauma and demonstrates their relevance to both early and late outcomes after injury. Based on the data reported here, we propose a model of initial immature platelet reduction in a subgroup of critically injured patients that is associated with mortality, followed by an increased release of immature platelets at later time points that predisposes to thrombosis and MODS in those who survive the initial bleeding phase. These findings highlight the need for further work to define the mechanisms responsible for these changes and characterize the properties of newly formed platelets released after major injury.

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AUTHOR CONTRIBUTIONS

H.S., A.R., P.V., and K.B. designed the study. H.S., A.R., P.V., and P.C.A. analyzed the data and wrote the manuscript. H.E.A., P.C.A., T.D.W., and K.B. critically revised the intellectual content. All authors have read and approved the final version of the manuscript.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

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