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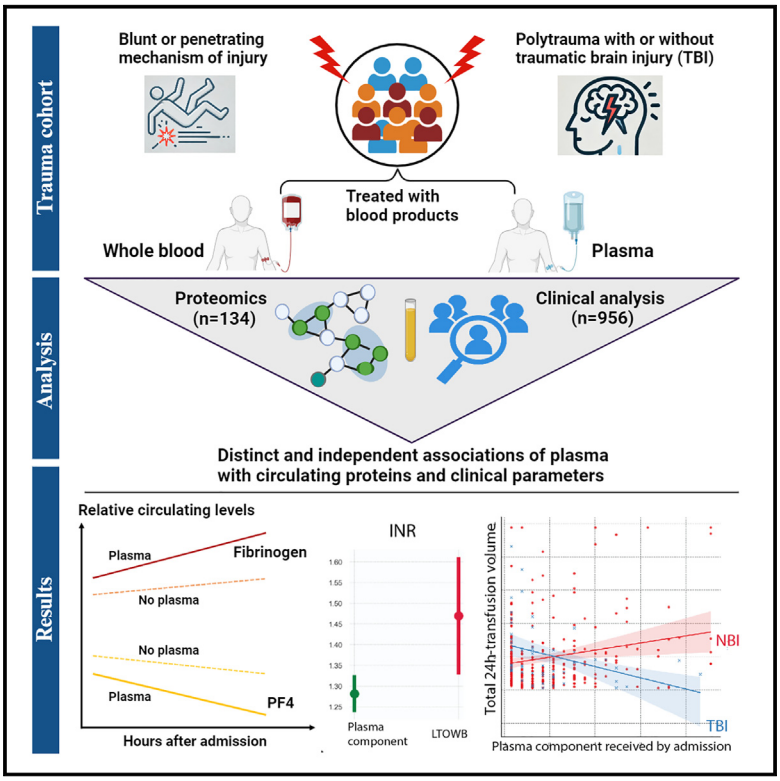
High-dimensional analysis of injured patients reveals distinct circulating proteomic profiles in plasma vs. whole blood resuscitation

Graphical abstract



Highlights

- LTOWB and plasma influence distinct immune and hemostatic pathways in patients with trauma
- Plasma transfusion is associated with higher blood fibrinogen and lower PF4 levels
- Early plasma correlates with improved INR and reduced first-day transfusion volume
- Patients with TBI, severe shock, and blunt injury selectively benefit from plasma

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In brief

Moheimani et al. perform proteomic profiling of patients with trauma, revealing how plasma component and LTOWB differentially modulate biological pathways. Plasma recipients show higher fibrinogen, lower PF4, and earlier INR normalization after severe shock. Plasma is distinctly associated with fewer transfusions in TBI and blunt injury, calling for patient-specific resuscitation strategies.

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High-dimensional analysis of injured patients reveals distinct circulating proteomic profiles in plasma vs. whole blood resuscitation

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SUMMARY

Early blood product resuscitation is often essential for optimal trauma care. However, the effects of different products on the underlying trauma-induced coagulopathy and immune dysfunction are not well described. Here, we use high-dimensional analysis and causal modeling in a longitudinal study to explore the circulating proteomic response to plasma as a distinct component versus low-titer O whole blood (LTOWB), which contains plasma. We highlight the differential impacts of plasma and LTOWB on immune mediator levels and the distinct capacity of plasma to modulate coagulation by elevating fibrinogen and factor XIII and reducing platelet factor 4. A higher proportion of plasma in prehospital resuscitation is associated with improved admission time coagulation parameters in patients with severe shock and elevated brain injury markers and reduced post-admission transfusion volumes in those suffering from traumatic brain injury (TBI) and blunt injury. While LTOWB offers broad hemostatic benefits, our findings demonstrate specific advantages of plasma and support individualized transfusion strategies.

INTRODUCTION

Trauma remains a leading cause of morbidity and mortality worldwide,[1] with hemorrhage and traumatic brain injury (TBI) being the critical drivers of early death among severely injured patients.[2] Despite advances in resuscitation strategies, the heterogeneity of patient responses to trauma and the variety of blood product administration protocols complicate our understanding of optimal treatment paradigms.[3,4] The interaction of early treatments with individualized immune and hemostatic responses creates diverse clinical trajectories that dictate patient outcomes.[5,6]

Increasingly, the benefits of early administration of blood products such as plasma in its various permutations (hereafter referred to as plasma) and low-titer O whole blood (LTOWB) have become apparent.[4,7–10] Previous studies have demonstrated the subgroup-specific survival benefit of prehospital plasma administration in patients with TBI and specific underlying molecular endotypes.[11–15] Others have shown that LTOWB resuscitation might not consistently confer survival benefits across all patients with trauma.[10,16–18] Emerging evidence suggests that treatment effects may vary based on patient demographics and injury characteristics such as the severity of shock and brain injury[19–21]; however, the differential impact of these

treatments in correcting coagulopathy and mitigating downstream immuno-inflammatory cascades is not well explained.[5,22–24] Furthermore, while plasma is a component of LTOWB, it is not understood whether administering plasma units, often as part of conventional component therapy, acts differently than LTOWB in impacting biological processes relevant to patient outcomes.[25] We posited that high-dimensional analysis of circulating proteins could characterize how plasma and LTOWB differentially modulate molecular pathways that drive recovery or deterioration and help bridge current knowledge gaps in planning patient-specific approaches to resuscitation.[14,26]

In this study, we aimed to integrate clinical data and longitudinal proteomic profiles from a cohort of severely injured patients with trauma (Shock, Whole Blood, and Assessment of TBI [SWAT] multi-center observational study)[16] to examine the interactions between resuscitation strategies and patient-specific factors. Building advanced causal machine learning models on temporal proteomic profiles allowed us to account for known confounders and leverage treatment variability to identify the differential associations of plasma versus LTOWB treatments with key immune and hemostatic pathway constituents. Our observational study connects molecular insights with clinical parameters to examine how the impact of early blood product administration can be moderated by characteristics like age, the presence of TBI, and severe shock. This high-dimensional analysis offers new potential avenues for improved personalized transfusion strategies in trauma care.

RESULTS

The overall analysis workflow can be viewed in Figures 1A–1D. As described previously,[16] 1,051 patients in hemorrhagic shock were enrolled in the SWAT prospective observational cohort. Notably, SWAT was designed to capture the characteristics of early transfusion protocols by only enrolling patients with a high probability of undergoing emergency surgery upon admission (Figure 1A). A subset of SWAT patients (hereafter referred to as "Omic" sub-cohort, n = 134) comprising polytrauma patients with blunt or penetrating injury, with or without TBI, were selected. All patients received at least one blood product unit (Packed Red Blood Cells [PRBC], plasma, platelets, or LTOWB) before admission and the first blood draw (time 0 h). The plasma derived from blood samples drawn at 0, 4, and 24 h post-admission time points was subjected to high-dimensional proteomic analysis. All individuals had plasma samples available for the three time points. Therefore, the patient sample size for the downstream omic analyses remained the same through all time points (total n = 134, TBI = 53, no brain injury [NBI] = 81). In addition, protein levels from 20 donor plasma samples obtained from a local Food and Drug Administration-licensed blood collector were simultaneously analyzed, providing a benchmark for downstream analyses (Figure 1B).[27]

Demographic and clinical patient information is shown in Table 1 and summarized in Figures 1E and 1F. Before any adjustments in the Omic sub-cohort, patients who received plasma (with or without LTOWB) were more likely to be female, had a lower initial Glasgow Coma Scale (GCS), and received more units of all blood products (including LTOWB) during their hospital stay. These patients spent more days in the intensive care unit (ICU) and a longer period on mechanical ventilation. As Figure 1G delineates, some patients were treated with plasma or LTOWB before the blood draws in the study. Therefore, for each analytical step, we have chosen an approach that facilitates the interpretation of specific treatment associations while accounting for other interventions as well as patient/injury variability.

Samples fromdonors and patients with trauma show clear and time-dependent differences in the overall circulating proteomic profiles

Volcano plots in Figure 2 demonstrate time-dependent relative differences between patient and donor samples for all assayed proteins (n = 7,596). The most significantly different (Z score difference >2 standard deviations [SDs]) plasma proteins between donor samples and 0 (2A), 4 (2B), and 24 h (2C) patient samples were highlighted. Integrin αIIbβ3 and a collection of mitochondrial matrix-located proteins were significantly higher in donor plasma at all three time points. It is unclear if the observed relative differences in the levels of such proteins are characteristic of plasma from healthy donors or suggestive of trauma-related changes. Integrin αIIbβ3 is a cell surface receptor associated with platelet aggregation through its attachment to fibrinogen and vWL factor, but the significance of its potential roles or associations when in circulation has not been adequately studied.[22,28] Similarly, unlike the well-investigated role of circulating mitochondrial products in injured patients, the consequences of such molecules in transfusion products are not known and may be an area for further investigation to identify novel functions.[29,30]

Several proteins were significantly higher in patients compared to donor plasma, including heart-type fatty acid-binding protein (a marker of myocardial injury)[31] at 0 h, matrix metalloproteinase-9, and heat shock protein 47 (wound repair and fibrosis)[32,33] at 4 h. Early increases in heart-type fatty acid-binding protein align with our previous findings that showed that this protein has a greater increase and a slower return to normal levels in patients with unfavorable outcomes.[34] Matrix metalloproteinases and their inhibitors (tissue inhibitors of metalloproteinases [TIMPs]) are directly involved in endothelial disruption and coagulation responses after trauma.[5] At 24 h, skeletal muscle-specific troponin I (TNNI) and β-enolase (associated with skeletal muscle injury)[35,36] were among the most differentially elevated proteins in the patient samples.

Among the transfused patients, Figure 2D depicts higher levels (>1 SD difference, adj. p log <−15) of tissue-specific (TNNI and TIMP1)[35,37] and immune-associated (SAA2, IL1RL1, and GCSF)[23] proteins at 4 h compared to 0 h. As seen in Figures 2E and 2F, both 0 and 4 h samples have higher levels of leukocyte cell-derived chemotaxin-2, CNS-associated protocadherin-8,[38] and cathepsin F.[39] In contrast, 24 h samples have higher levels of inflammatory and acute phase proteins (SAA1 and 2, CRP, and LBP),[40] chemokines (CCL7 and CCL23),[41,42] and immunoglobulin-like CD226 protein compared to the early time points. CD226 is known to associate with autoimmune, anti-viral, and anti-tumor immunity through its presence on natural killer and T cells.[43] Time-dependent pathway differences between patient

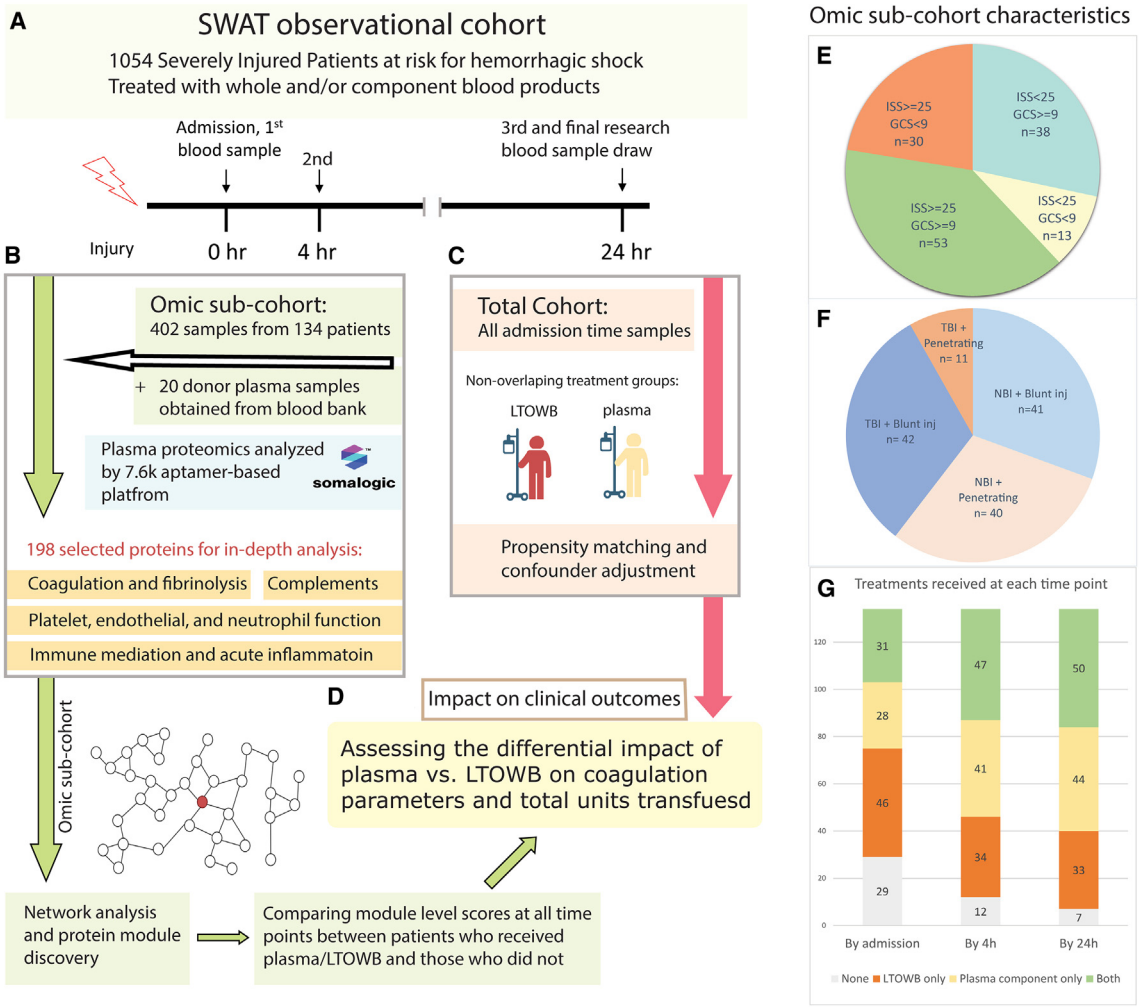

Figure 1. A summary of study flow and cohort characteristics

402 blood samples from 134 SWAT participants drawn at admission, 4 h, and 24 h, and 20 donor plasma samples were analyzed for circulating proteomics (A). Using network analysis, we identified homogeneous immune and coagulation-associated protein modules whose levels were compared between patients receiving different blood products (B). In parallel, we identified all SWAT patients who received either LTOWB or plasma component treatment by admission but not both (C). Propensity-matched subsets of these two treatment groups were compared for their clinical coagulation parameters. In addition, we assessed how receiving plasma could influence the total 24-h transfusion received in different patient subsets (D). (E) and (F) summarize the injury characteristics of patients in the omics sub-cohort. Patients could have received plasma from different sources (plasma component or LTOWB). (G) Summarizes the distribution of products as received before each blood draw.

and donor samples using gene set enrichment analysis (GSEA) are shown in Figures S1–S6. Taking collectively, this expanded view validates large differences in trauma and donated plasma samples and highlights marked biological consequences of trauma at different time points.

Patients receiving plasma show different hemostatic and immune proteomic profiles at all time points before and after accounting for injury and prehospital characteristics

198 well-known contributors to inflammatory and hemostatic processes following traumatic injury were selected for focused analysis. These included proteins associated with coagulation, fibrinolysis, platelets, endothelial function, neutrophils, acute phase inflammation, and immune mediation.[5,22,23,44–46] A complete protein list can be found in Table S7.

Figures 2G–2L provide the results of the principal component analyses (PCAs) on the 198 selected proteins in all 422 patients and the 20 donor plasma samples. The first two principal dimensions separate donor plasma samples from patient samples drawn at the three time points (Figure 3G). The patient samples were then segregated based on receiving plasma (here defined as receiving plasma with or without LTOWB in the first 24 h, see Figure 1G) or receiving LTOWB (with or without plasma in the first 24 h). No clear difference was observed between LTOWB recipients and non-recipients (Figure 2I). However, plasma recipients showed a distinct proteomic profile from plasma non-recipients (Figure 2H). Although not yet adjusted

Table 1. Characteristics of the patients in the Omic sub-cohort

Variables % or median (IQR)[a]	Total (*n* = 134)	Treatment (received before 0 h)[b]				Univariate differences (*p* value)[c]
		Only LTOWB (*n* = 46)	Only plasma (*n* = 28)	Both (*n* = 31)	None (*n* = 29)	
Demographics						
Age (years)	34 (23.5)	35 (23)	29 (27.5)	35 (26)	28 (15)	0.03 (0.1)
Gender (%female)	35.6%	21.8%	57.2%	22.6%	41.4%	0.3 (0.01)*
Injury characteristics						
Penetrating injury (%)	38.1%	28.3%	39.3%	45.1%	44.8%	0.15 (0.37)
ISS	29 (14)	24 (8.75)	34 (21.25)	34 (15.5)	27 (14)	0.02 (0.13)
Head_AIS >2 (%)	39.6%	43.5%	35.7%	45.1%	31.0%	0.11 (0.62)
Head_AIS	0 (3)	1 (3)	0 (3)	2 (3)	0 (3)	0 (0.68)
Face_AIS	0 (1)	0 (1.75)	0 (0.25)	0 (0)	0 (0)	0 (0.62)
Chest_AIS	3 (2)	2 (1.75)	3 (3.25)	3 (2)	3 (2)	0 (0.58)
Abdomen_AIS	3 (2)	3 (2)	3 (2)	4 (2)	2 (4)	0 (0.46)
Extremities_AIS	2 (3)	2 (3)	2.5 (3)	3 (3.35)	2 (3)	0 (0.38)
External_AIS	1 (1)	1 (0)	1 (0.25)	1 (1)	1 (1)	0 (0.9)
Prehospital vitals						
GCS	13.5 (12)	15 (5.75)	15 (12)	9 (11)	13 (12)	0.05 (0.02)*
Systolic blood pressure	95 (30.75)	100 (24)	89 (41)	90 (32)	97 (28)	0 (0.33)
Heart rate	118 (40.75)	114 (23)	116 (37.5)	126 (50)	119 (37.5)	0 (0.68)
Shock index	1.18 (0.46)	1.09 (0.38)	1.25 (0.43)	1.17 (0.61)	1.31 (0.5)	0.02 (0.13)
Respiratory rate	19 (6)	18 (4)	18.5 (6.5)	19 (10)	20 (7)	0 (0.6)
Treatments						
LTOWB (units)	1 (2)	2 (2)	0	2 (2)	0	0.8 (<0.01)*
Plasma	0 (3)	0	4 (3.25)	4 (3)	0	0.9 (<0.01)*
PRBC	2 (4)	0 (1.75)	5 (4)	5 (5.5)	1 (1)	0.54 (<0.01)*
Platelets	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0.23 (<0.01)*
Cryoprecipitate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.02 (0.16)
Total blood products	4 (7)	3 (2)	9 (8)	13 (8)	1 (1)	0.44 (<0.01)*
Total fluids (mL)	1,400 (1,975)	1,625 (2,000)	950 (1,000)	1,200 (2,350)	1,000 (2,000)	0.01 (0.29)
Coagulation parameters						
PT (s)	15.35 (2.7)	15.75 (2.9)	15.2 (2.5)	15.3 (6.6)	14.7 (4.4)	0 (0.35)
INR	1.29 (0.23)	1.3 (0.31)	1.3 (0.19)	1.32 (0.61)	1.2 (0.30)	0.01 (0.39)
Platelets (count/mm^3)	179 (75)	176 (59)	156 (118)	140 (123)	240 (127)	0.17 (<0.01)*
Outcomes						
Hospital_los (days)	17 (21.25)	16 (24)	17 (19)	26 (21.5)	14 (14)	0.02 (0.16)
ICU_los	8.5 (13)	6.5 (11.5)	6.5 (10)	14 (14)	8 (9)	0.04 (0.03)*
Vent_los	4 (9)	3.5 (6)	4 (9.5)	8 (14)	3 (5)	0.04 (0.04)*
30d_mortality	9.0%	4.3%	14.3%	16.1%	3.4%	0.19 (0.27)

[a]Proportions and quantitative variables are respectively presented in percentages and median (interquartile range, IQR).
[b]Only LTOWB and only plasma groups might have received blood products other than plasma and LTOWB (RBC, etc.).
[c]Between-group differences of quantitative variables are compared using the non-parametric Kruskal-Wallis test, and the associated effect size is reported as eta squared (negative numbers are rounded to 0). For proportions, standardized differences are calculated using Cramer's V on a 2*4 contingency table, and the chi-squared test assesses associated significance.
*$p < 0.05$.

for confounders, these results suggest that plasma administration was associated with the altered circulating hemostatic and immune proteomic profiles while LTOWB was not. When samples were segregated based on injury characteristics, no separation was observed in high vs. low ISS (Figure 2J), while blunt vs. penetrating (Figure 2K) and TBI vs. NBI showed some separation (Figure 2L).

We generated heatmaps for the six selected protein families (Figures S7–S12 in the supplementary material) to visually compare protein levels in patients receiving different blood products. Confounder adjustment was performed using inverse probability of treatment weighting as detailed in the Methods (Figures S13A–S13F). The results demonstrated that plasma recipients (with or without LTOWB) show differences in

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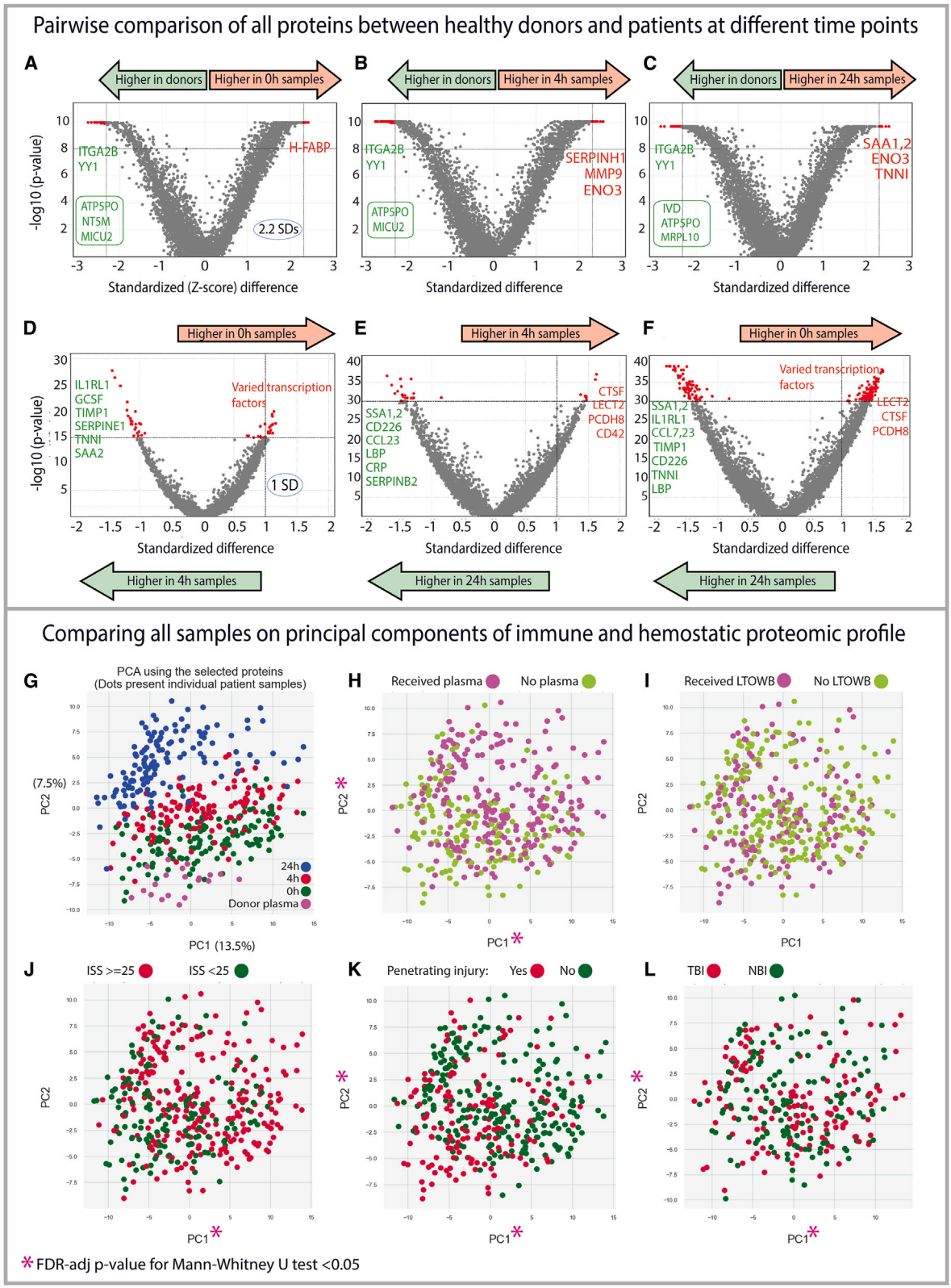

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constituents of all selected protein families, most of which were sustained up to 24 h. In particular, plasma recipients had higher levels of proteins associated with later stages of clot formation (fibrinogen) and degradation (plasmin)[5] and lower levels of several markers of platelet activation (CD63 and platelet factor 4 [PF4])[44] and innate immune pathways (protein s100-A9, CD44, Toll-like receptors, and C5-6 complex).[23,45] Most observed patterns persisted in heatmaps regenerated in the confounder-adjusted analysis. Minimal changes were observed between LTOWB recipients and non-recipients.

Prehospital plasma, but not LTOWB, is associated with immune pathway alterations in severely injured patients

As Figure 3A shows, in admission-time plasma samples, patients who had received plasma with or without LTOWB (n = 59 recipients vs. 75 non-recipients) had higher levels of vitamin K-dependent protein C (hemostasis associated),[5,22] GRFA1 (associated with neuron survival),[47] SMOC1 (involved in endothelial and platelet function as well as thrombin activation),[48,49] VSIG4 (potential inflammation mediator),[50] FIII (associated with wound repair),[51] and MMP8 (important mediator of neutrophil chemotaxis).[52] To help account for confounding differences between plasma recipients and non-recipients, a model was built to predict resolution (defined as surviving 1 month and ICU stay $\leq$ 10 days, which represents the 75th percentile of ICU length of stay in SWAT) utilizing relevant injury, patient, and other clinical variables (Figures S13G–S13I). In the regenerated plots on a subsample of patients who had ISS >24 and the chance of resolution <90% (n = 41 plasma vs. 42 non-recipients, see Figure S13J), the previously identified differences mainly persisted (Figure 3B). Conversely, severely injured plasma recipients had lower levels of PF4, DPYSL4 (intracellular protein with diverse interactions),[53] and PLSCR3 (associated with mitochondrial sensitivity to apoptosis),[54] as well as immune mediators INFA5 and CCL5. CCL5 has been associated with blood barrier integrity.[55] Interestingly, receiving LTOWB (with or without receiving plasma) did not lead to significant proteomic differences in the overall cohort or only severely injured patients (Figure 3C).

To isolate the pathway-level plasma treatment associations, we performed GSEA on the patients with ISS >24 and survival probability <90% after excluding patients who received both plasma and LTOWB before the admission (Figure S13J). GSEA showed the interleukin (IL)-2 production pathway to be significantly (adj. $p < 0.1$) up-regulated in severely injured plasma recipients (n = 22) compared to LTOWB recipients (n = 17) (Figure 3D).

Plasma recipients show a distinct pattern in coagulation and platelet-associated proteins that are moderated by shock severity and head trauma

We performed a multi-step process to find modules of highly correlated proteins and calculated the signature score of these modules (see Methods for step-by-step description). In short, we utilized the correlation between all 198 selected proteins to generate a network. Performing Leiden community detection[56] on the drawn network yielded 13 distinct protein modules. We performed two additional clustering methods for sensitivity analysis (see Figure S14 for the results of Louvain community detection and affinity propagation; see Table S8 for module details). Module scores were derived using a weighted average scheme[57] on constituent values. Next, we utilized causal forest machine learning to estimate treatment associations with each module.[58,59] All longitudinal analyses were performed once to compare plasma recipients and non-recipients (with or without LTOWB) and a second time for LTOWB treatment. This framework facilitated analyzing protein module trajectories by mitigating technical noise in measuring individual proteins. This approach adjusted for confounding due to patient differences while providing insights on the moderating roles of each covariate. We included relevant injury and patient characteristics, and other clinical variables of relevance as both confounders and effect modifiers for all analyses.

Table S1 shows the size and multiple-testing adjusted p values of LTOWB and plasma associations with all modules. Distinct and significant associations of plasma were observed in two hemostasis-associated modules (referred to as CLOT and ALPHA) that retained all their constituents (Figures S14A and S14B) and the significance of treatment associations (Figure S14C) in the sensitivity analyses. We further flagged three more heterogeneous immune-associated modules as potentially affected by plasma or LTOWB (SURF, FLAME1, and FLAME2, see Figures S15A and S16). Tables S9 and S10 show module constituents and their associated loadings for hemostasis and immune-associated modules, respectively.

A fibrin clot-associated module (CLOT, comprising fibrinogen, fibrinogen gamma-dimer, fibronectin, coagulation factor XIII, and von Willebrand factor)[5] was significantly (adj. $p < 0.05$) higher in the plasma-treated group (Figure 4A) at both the

Figure 2. Samples from donors and patients with trauma show clear and time-dependent differences in the overall circulating proteomic profiles

Volcano plots (A)–(F) demonstrate time-dependent differences between patient and donor samples, highlighting the most significantly different (size >2.2 SDs, false discovery rate (FDR)-adjusted Mann-Whitney U p value log <-8) proteins at 0 (A), 4 (B), and 24 h (C). Integrin αIIbβ3 protein and a collection of mitochondrial matrix-associated proteins (green box) were higher in donors at all time points. Heart-specific fatty acid-binding protein at 0 h, extracellular matrix-associated proteins MMP9 and HSP47 at 4 h, skeletal muscle-specific troponin I, and beta enolase at 24 h had a relatively higher level in patients. (D) Identifies higher levels (size >1 SD, FDR-adjusted Mann-Whitney U p log <-15) of tissue-specific (TNNI, TIMP1) and immune-associated (SAA2, IL1RL1, and GCSF) proteins in 4 h samples compared to 0 h samples. As seen in (E) and (F), both 0 and 4 h samples have higher levels of leukocyte cell-derived chemotaxin-2, cathepsin F, and CNS-associated protocadherin-8. 24 h samples have higher levels of acute inflammatory proteins (SAA1 and 2, CRP, and LBP), chemokines (CCL 7 and 23), and immunoglobulin-like CD226 protein compared to the early time points. (G–L) Show principal component analysis using 198 immune and hemostasis-associated proteins. The red asterisks represent significant component-level differences at FDR-adj. $p < 0.05$ as estimated by the Mann-Whitney U test. Patient and donor samples are easily distinguishable in the first two dimensions (G). Plasma recipients show a distinct proteomic profile (H), but no clear separation is visible between LTOWB and no-LTOWB groups (I). A minor separation can be seen when patients are dichotomized using severity (J), penetrating trauma (K), or brain injury (L).

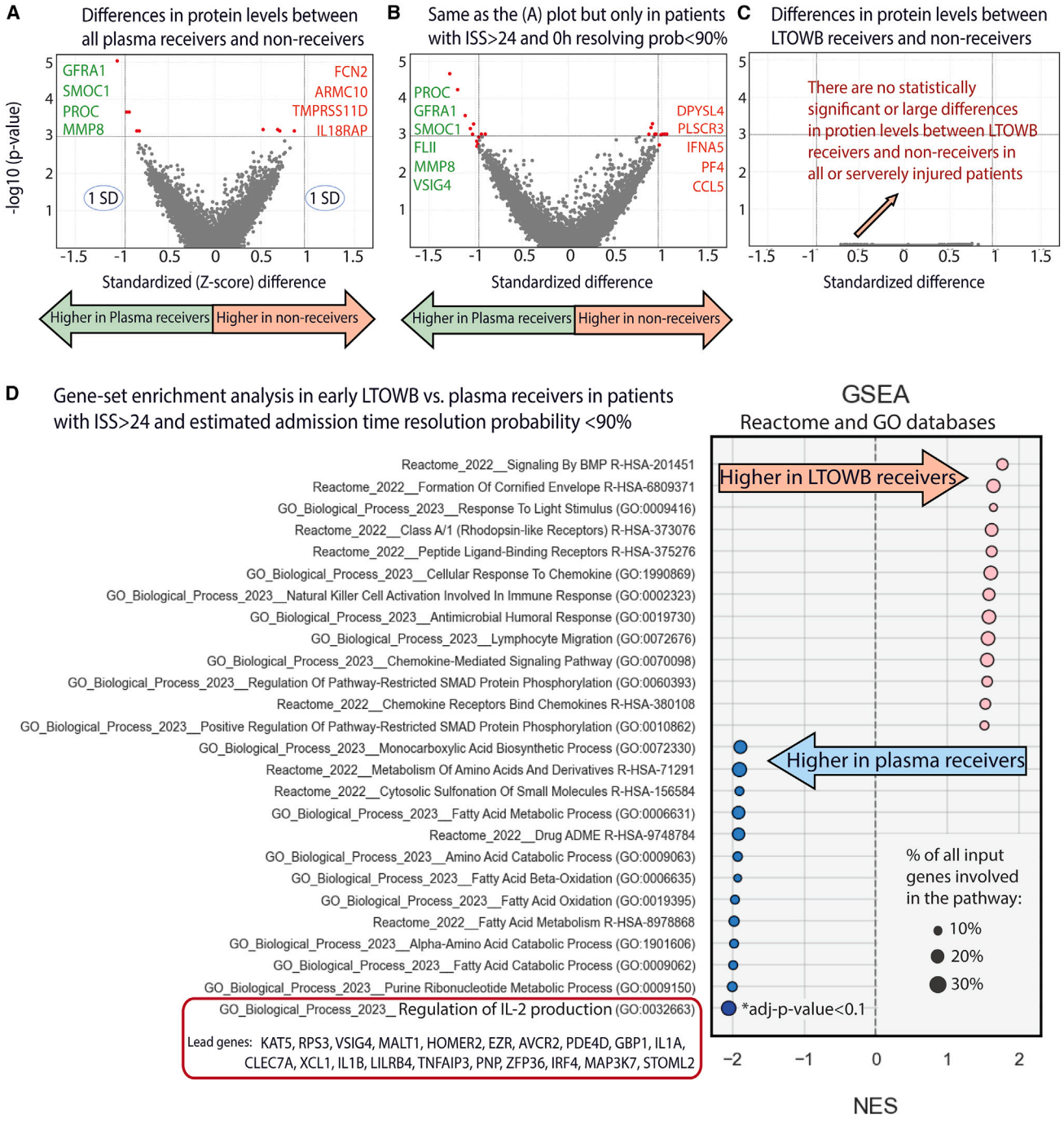
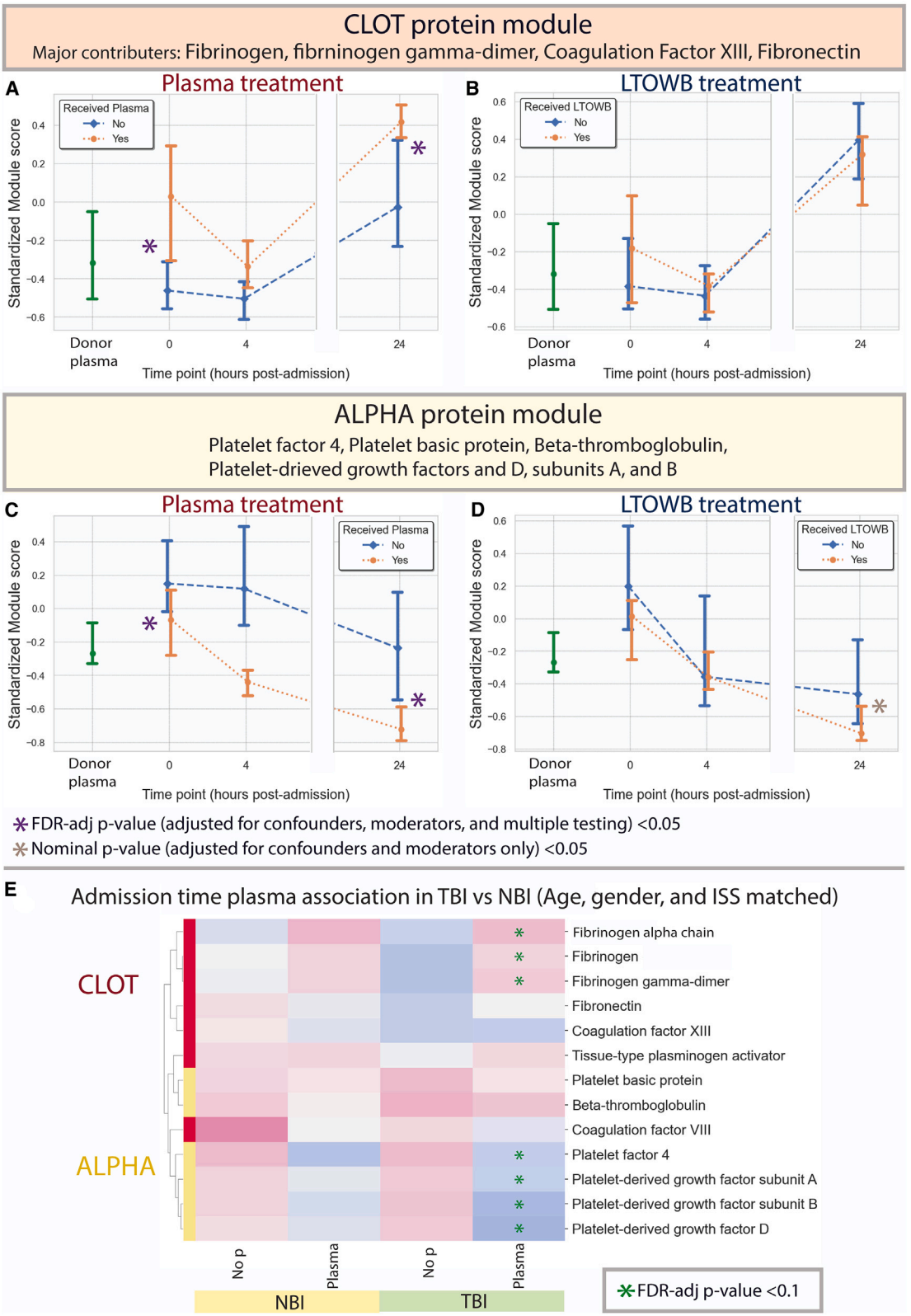


Figure 3. Prehospital plasma, but not LTOWB, is associated with immune pathway alterations in severely injured patients

Volcano plot (A) highlights the most different (size >1 SD, FDR-adjusted Mann-Whitney U *p* value <0.001) circulating proteins between 59 plasma recipients and 75 non-recipients (with or without LTOWB). Patients treated with plasma had higher levels of protein C, GRFA1, SMOC1, FIII, and MMP8. As (B) shows, the differences mainly persisted in severely injured patients who had a <90% chance of resolution ($n = 41$ plasma recipients vs. 42 non-receivers). Severely injured plasma recipients had lower levels of PF4, cellular proteins DPYSL4 and PLSCR3, as well as immune mediators INFA5 and CCL5. As seen in (C), receiving LTOWB (with or without plasma) did not lead to significant differences, whether in all or only in severely injured patients. (D) Shows relative enrichments in Gene Ontology and Reactome databases between severely injured plasma ($n = 17$) and LTOWB recipients ($n = 22$) at 0 h (none had received both treatments). We found higher levels of IL-2 (Family-wise error rate [FWER] *p* value <0.1, absolute normalized enrichment score >2) in plasma recipients.



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0 and 24 h time points. Yet, no similar association was seen for LTOWB (Figure 4B). At admission, the plasma treatment magnitude was significantly larger in patients with advanced age (>65 years) or with head AIS >2 and blunt injury mechanisms (Figure 5A). At 24 h, severe shock moderated the positive plasma association (Figure 5B). Notably, injury severity (as measured by ISS) did not affect the magnitude of these relationships.

A platelet alpha-granule-associated module (ALPHA, including PF4, platelet basic protein, beta-thromboglobulin [βTG], and platelet-derived growth factors)[44] had a significantly lower level in the plasma group at 0 and 24 h (Figure 4C). At both the 0 and 24 h time points, the plasma treatment association was significantly larger in younger patients with the highest prehospital shock index. However, no effect modification was observed by head AIS, the mechanism of injury, or ISS (Figures 5C and 5D). No such association was seen for LTOWB treatment (Figure 4D) except for a nominally significant LTOWB association in patients who had not received plasma in addition to LTOWB by 24 h (Figure S18F).

Furthermore, plasma-based associations for both hemostasis-associated modules were independent of treatment with red blood cells (RBCs), platelets, and cryoprecipitate and were not moderated by LTOWB treatment. Figure 4E shows the admission time associations of plasma treatment (with or without LTOWB) with each CLOT and ALPHA constituent in age, gender, and ISS-matched TBI ($n = 50$) vs. NBI ($n = 50$). Several individual proteins are significantly different only in the TBI plasma recipients.

Figure S15A assesses the potential dose-response relationships between treatments and the admission time level of protein modules using the Spearman correlation coefficient. CLOT was positively and significantly correlated with plasma treatment share (defined as the proportion of plasma units to all blood product units received before hospital admission) but not with LTOWB share (the proportion of LTOWB to all blood units). Similarly, plasma share (but not LTOWB share) was significantly and negatively correlated with ALPHA.

LTOWB and plasma treatment affect inflammation and immunity-associated proteins differently in patient subgroups

Compared to its associations with CLOT and ALPHA, a smaller but significant association of plasma with a third, more heterogeneous module called SURF was identified. SURF consisted of cell surface proteins, including TLR4, TLR10 (cell surface activation of immune and platelet cells), integrin alpha-m, CD59 (cell surface complement inhibitor), CD63, and other immune regulators such as IL-22. Unlike LTOWB recipients, plasma recipients had significantly lower SURF modules at 0 and 24 h time points (Figures S16E and S16F). Figures S15B and S15C detail the moderators of plasma association with SURF. The donor plasma level of the SURF module showed a large variance, which partially obscures a simple interpretation of this observed association.

Two highly correlated immune-enriched protein modules (Figure S16) showed mixed and weakly positive associations with both plasma and LTOWB at different time points. These are FLAME 1 (including platelet endothelial cell adhesion molecule, CD47, CD40L, ILs 11, 24, 25, 17a, and 17f, CXCL 9 and 10, histone H2a.z, and histone H3) and FLAME 2 (consisting of GCSF, ILs 6, 8, and 10, CCL2, P-selectin, and neutrophil elastase). Figures S17 and S18 show treatment associations and their moderators for plasma and LTOWB, respectively. Potential treatment associations with a tier of less consistently identified modules are presented in Figure S19.

Plasma component recipients showed shorter PT/INR compared to patients receiving LTOWB but no plasma component therapy

Both CLOT and ALPHA include proteins that contribute to post-trauma coagulation pathways.[5,22] Based on the association of plasma with distinct alteration of these two modules in specific patient subgroups (TBI and severe shock), we hypothesized that such subgroups of plasma recipients could achieve earlier normalization of coagulation parameters compared with patients whose resuscitation did not include plasma as a separate component.

To assess the clinical relevance of our biological findings, we first analyzed the dose-response association of plasma share (plasma component units to all blood units received) by admission with Prothrombin time (PT) in the Omic sub-cohort. A partial correlation framework that took the effect of known confounders into account showed a significant negative correlation between admission time PT and plasma share ($r_s = -0.22$, 95% confidence interval [CI] [−0.38, −0.04], $p = 0.015$).

To validate the specificity of brain injury in moderating this plasma treatment association, we curated two sets of proteins that are known to be markers of brain injury (GFAP, UCHL1, NSE, NEFL, NEFH, and MAPT,[60] see Table S7) and heart muscle injury (cTnT, cTnI, ANF, BNP, MYBPC, and H-FABP).[34] As seen in Table S2 and detailed in the Methods, we used PCA on the mentioned proteins to derive Brain Injury and Heart Injury Scores and define high and low injury sub-categories for each organ. As Figure 6A shows, a significant partial correlation of plasma with lower PT was only observed in the high Brain Injury Score group ($r_S = -0.26$, 95% CI [−0.49, −0.01] vs. −0.11, 95% CI [−0.36, 0.15]). The analysis was repeated with International Normalized Ratio (INR) as the outcome with four additional adjustments (platelet share, cryoprecipitate share, clinically determined TBI,

Figure 4. Plasma recipients show a distinct pattern in coagulation and platelet-associated proteins

Using a causal forest model to account for confounders and heterogeneity of treatment effects, CLOT (A) and ALPHA (C) modules were found to be significantly associated with plasma in admission and 24 h samples, but not with LTOWB (B, D). In (A)–(D), the error bars show mean ± SEM (standard error of the mean) at each time point. Gold and purple asterisks indicate $p < 0.05$ for, respectively, nominal and adjusted p values from the Mann-Whitney U test of between-subgroup effect differences. (E) Compares the relative level of admission time ALPHA and CLOT constituents in plasma recipients (with or without LTOWB) to that of plasma non-recipients in ISS, gender, and age-matched TBI vs. NBI groups of patients. The green asterisks present FDR-adjusted p values as estimated by the Mann-Whitney U test between treatment recipients and non-recipients. In the TBI group, several individual proteins remained significantly different after multiple testing adjustments.

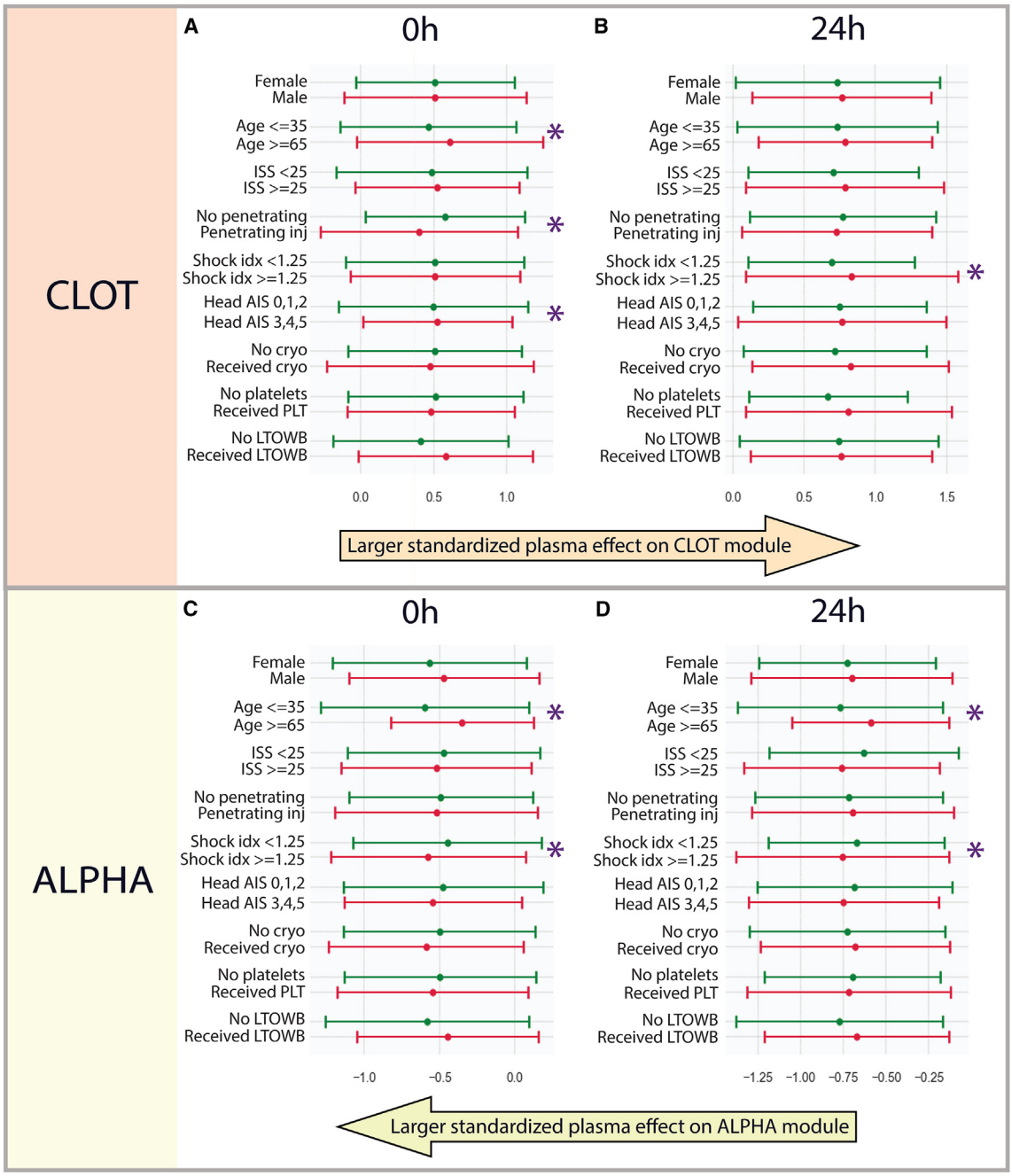

Figure 5. Severe shock and head trauma moderate the association of plasma with coagulation and platelet-associated protein modules

The error bars show mean ± sub-sample SD (standard deviation) at each time point, and purple asterisks indicate $p < 0.01$ after multiple testing adjustments on the Mann-Whitney U test and between-subgroup effect size difference >0.5. Based on this conditional effect analysis, the positive association of plasma component treatment with CLOT was significantly stronger in patients with head injury, old age, and blunt mechanism at 0 h (A) and severe shock at 24 h (B). The plasma association with lower ALPHA levels was moderated by age and severe shock at both time points (C and D).

and Heart Injury Score) to assess the results' sensitivity to our choice of variables. These results confirmed that the association of plasma with improved coagulation was exclusive to patients with a high biomarker-estimated Brain Injury Score and was independent of treatment with other products ($r_S = -0.29$, 95% CI [−0.52, −0.03]). Conversely, the lack of effect modification by cardiac muscle injury (95% CI [−0.47, 0.04] for plasma/PT association in high Heart Injury Score group) further validates the

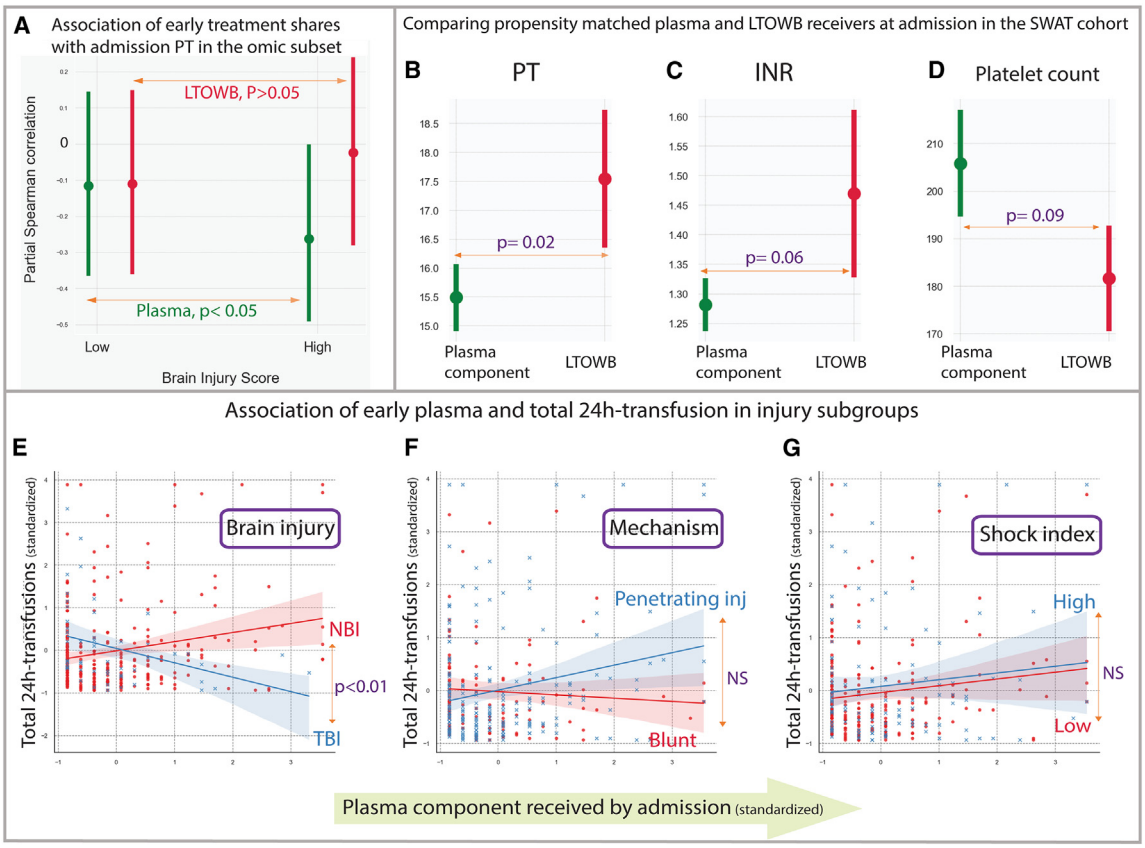

Figure 6. Clinical impacts of prehospital treatment with plasma

We divided patients into high- and low-brain injury groups based on a Brain Injury Score created with PCA on admission levels of six brain injury-specific proteins (see Table S7).

(A) Shows partial correlation analysis between treatment share (product units to total units received) and PT at admission. Error bars indicate mean+SEM for the correlation measure in each subgroup. A significant plasma association with lower PT was only observed in the high Brain Injury Score plasma recipients.

(B–D) Compare coagulation parameters in propensity-matched plasma ($n = 36$) and LTOWB recipients ($n = 35$) selected from patients who had not received both treatments. PT and INR are significantly lower in the plasma component group, while platelet count is similar. For (A)–(D), p values were estimated by the Mann-Whitney U test.

(E–G) Show the standardized effect measures regarding the association of pre-admission plasma with the sum of total units of blood products received on the first admission day as estimated by the multi-variable mixed-effects regression model. Plasma is significantly associated with lower transfusion volume only in the TBI subgroup (E). A trend toward a higher response in patients with blunt mechanisms of injury can be observed (F). The shock index does not moderate this association (G) (see Tables S5 and S6; Figure S20).

specificity of brain injury in moderating the plasma association. LTOWB share did not show a significant association in any of the corresponding analyses.

Next, we aimed to validate the same relationship exclusively in a subset of patients whose treatment with plasma and LTOWB did not overlap. Using the total SWAT cohort data, we started with 447 patients whose treatment by admission included plasma (n = 258) or LTOWB (n = 189) but not both (Table S3). After removing patients with missing PT/INR data at 0 h, propensity matching was performed on the remaining 301 patients, which resulted in 35 LTOWB and 36 plasma recipients. Figure S20A and Table S3 show the quality of propensity score matching and resulting patient characteristics, respectively. The results of this analysis (Figures 6B–6D) show that receiving only plasma was associated with lower admission time PT vs. only LTOWB (p = 0.02, Cohen's D = 0.37). As expected, the INR difference replicated the PT results. Both results were robust to random common cause refutation ($p = 0.8$). There was no statistically significant difference between platelet count at 0 h, 30-day mortality, or ICU length of stay.

A second analysis with PT/INR values from all 447 patients (including imputed values replacing the 146 missing) was performed to assess the sensitivity of this finding to treatment definition and adjustment specifics. Using a causal forest model similar in covariates to the one previously described in the omics analysis, we found that in line with the matched analysis, receiving plasma was associated with shorter PT times (standardized coefficient [std_coef.]: −0.21, p = 0.04). The subsequent conditional treatment assessment (Figure S20B) identified a stronger plasma association in patients with head injury ($p < 0.1$) and severe shock ($p < 0.05$). Of note, the presence of penetrating injury did not modify the significance of the plasma association.

Patients with TBI with a blunt mechanism of injury show an association of pre-admission plasma with reduced total transfusions in the first 24 h of hospital stay

We aimed to find whether the identified plasma treatment associations with coagulation further correlated with clinically important outcomes. A model was built to assess the relationship of total fluids, plasma, LTOWB, and other components administered before hospital admission with patient illness resolution (surviving a month with ICU stay $\leq$10 days, see the Methods for details on all covariates). The multivariable analysis on all 956 patients showed that patients with lower ISS, higher GCS, NBI, and a penetrating mechanism, receiving lower quantities of total prehospital PRBC, platelet, and cryoprecipitate and higher quantities of plasma (but not LTOWB), had a significantly higher chance of resolution (Table S4). We performed a secondary analysis to assess the relationship of the same set of variables with the total quantity of all blood products transfused between admission and the 24 h time point. This model was built on a sub-sample of 318 patients who had received at least one unit of plasma and LTOWB 4 h after admission to ensure similarity in logistics of product availability and a comparable degree of early patient care. As Table S5 shows, advanced age, lower GCS, higher quantities of the sum of prehospital products other than LTOWB or plasma, and less fluids received were significantly ($p < 0.05$) associated with higher total transfusion volumes on the first post-admission day. Smaller associations of total transfusion volume with ISS (std_coef. = 0.11, $p = 0.06$) and with LTOWB received by admission (std_coef. = -0.11, $p < 0.08$) were observed. Most importantly, the beneficial plasma association interacted with the presence of brain injury (std_coef. = 0.55, $p < 0.001$), revealing that higher amounts of plasma transfused before admission were significantly associated with fewer total transfusions on the first hospital day only in the TBI subgroup (Figure 6E). No significant interactions between plasma and injury mechanisms or shock severity were identified (Figures 6F and 6G; Figure S20C). Sensitivity analysis using product proportions instead of units and taking trauma care center into account validated the association of first-day transfusion volume with age, GCS, and plasma/TBI interaction (see Table S5 and Methods for details). Table S6 provides a better picture of this interaction using partial correlations for the plasma component interaction with reduced first-day transfusion in different injury subgroups. A significant association can only be seen in the TBI with blunt injury subgroup ($n = 52$, $r_s = -0.39$, 95% CI [-0.62, -0.1], $p = 0.011$).

DISCUSSION

We assessed how trauma resuscitation strategies interact with demographic and injury characteristics in influencing biological responses and shaping patient outcomes. By leveraging high-dimensional proteomics, this work provides an inference of possible mechanisms for the hemostatic and immunomodulatory consequences of plasma transfusion. The identified treatment associations are more potent in patients with severe shock or TBI and are distinct from those of LTOWB in similar patient subgroups. In addition, plasma-associated proteomic changes correlate with earlier normalization of PT/INR and a reduced number of blood products administered on the first admission day. The beneficial effects of plasma transfusion in correcting coagulation parameters and reducing the need for blood products have previously been documented.[5,61] However, the identified proteomic signatures and their observed associations with important clinical measures suggest that the plasma that is part of LTOWB may not act the same way as the separately administered plasma component. Thus, plasma could be considered a therapeutic intervention for certain patients even if LTOWB is used early in the resuscitation.

The multi-step network and causal analysis implemented in this study provide an expanded picture of patient/treatment interactions. Trauma-associated tissue injury and shock lead to immune activation, endothelial and platelet dysfunction, and rapid fibrinogen depletion, resulting in trauma-induced coagulopathy (TIC).[5,22] Low fibrinogen levels and activity after trauma are associated with the severity of injury and shock[62,63] and predict more extensive transfusion needs and early post-admission mortality.[64] Factor XIII circulates in the same complex as fibrinogen and exerts antifibrinolytic effects.[65] In this study, treatment with plasma but not LTOWB showed sustained associations with increased levels of fibrinogen, factor XIII, and fibronectin, all of which are principal constituents of cryoprecipitate. However, the observed associations in this work were independent of receiving adjunct cryoprecipitate.[66] Higher head AIS, but not aggregate ISS, moderated the association of plasma with fibrinogen levels at the time of admission. This can suggest nuanced differences in the pathophysiology of fibrinogen depletion and its response to treatment in polytrauma patients with severe head injury compared to those without.[67,68]

Plasma transfusion was associated with reduced circulating levels of several platelet alpha-granule constituents. Alpha granules contain adhesion molecules, coagulation/fibrinolysis factors, and immune molecules such as PF4 (CXCL4) and βTG (CXCL7).[44] Therefore, platelets can participate in maladaptive hemostatic and immunoregulatory post-trauma responses.[69] Studies show that severely injured patients have higher circulating βTGs but lower PF4 levels.[70] Conversely, PF4 serves as the target for auto-antibodies responsible for immune thrombotic thrombocytopenia, and an association of higher circulating PF4 with arterial and venous thrombosis is observed in patients suffering from related syndromes.[71,72] The post-trauma rise in platelet-leukocyte aggregates, increased platelet ballooning, extracellular vesicles, and PF4 release is an investigation target for early and late TIC.[69,73,74] In this study, lower ALPHA and SURF levels in plasma recipients, especially those in severe shock, might indicate reduced dysfunctional platelet activation. This is consistent with studies showing admission base deficit as an independent predictor of platelet hypofunction after trauma.[75] Platelet dysfunction is also associated with lower factor XIII-dependent crosslinking.[76] Therefore, the observed plasma association with higher fibrinogen and factor XIII and lower PF4 and βTG may point to complementary roles in restoring a balanced coagulation process. Moreover, these findings connect previously identified fibrinolysis modulation by prehospital plasma[14] to its effect on PF4, a known regulator of activated protein C functionality.[77]

Several plasma recipients in SWAT had received platelet components, which can directly affect platelet-related measurements.[78] However, conditioning the plasma treatment associations on this variable did not alter their significance. Although our collective observations add to the evidence supporting the benefits of plasma in alleviating trauma-associated "platelet exhaustion,"[5,44] we cannot entirely eliminate the possibility that relatively higher ALPHA levels in LTOWB recipients are a consequence of extant platelets in the product.

Traumatic injury leads to a rapid immune system alteration characterized by a broad release of immune mediators.[79] While targeted cytokine release can mediate protective effects, a combination of severe injury, hemorrhagic shock, and surgical interventions can create dysregulated immune cascades.[23,45,80] Although limited transfusion of blood products can blunt early trauma-related elevations in IL-6, larger amounts add to IL-6 and MCP-1 rise.[6] Prehospital plasma decreases both IL-6 and MCP-1 at admission while increasing the levels of IL-1B, IL-17A, and IL-23[11] and is potentially more beneficial in patients with a "systemic storm" pattern of immune activation.[13] Here, we observed diverse associations of plasma and LTOWB with markers of immune activation (such as IL-6, CXCL8, and CCL2) at 4 and 24 h time points. Such effects could result from the immunogenicity of donated blood products and are likely to be altered by using components from younger donors[81] or immune-depleted products like solvent detergent plasma, which correlates with lower rates of adverse immune responses and associated lung injury.[82] We emphasize, however, that the variety of injuries and interventions in SWAT means the influence of residual confounding on immune mediator levels cannot be eliminated with certainty.

IL-2 is involved in T cell response, and its post-injury suppression facilitates the development of sepsis.[83,84] Previous works show that patients suffering from polytrauma with higher IL-2 levels at admission have shorter ICU stays and lower mortality.[85] We showed that receiving plasma vs. LTOWB is associated with higher IL-2 levels when a similar set of severely injured patients (ISS >24 with predicted resolution <90%) are compared. Adequately powered trials directly comparing blood products can investigate if a subgroup of patients can benefit from lower complication rates when treated with plasma regardless of LTOWB administration.

There is compelling evidence suggesting a disparity of post-trauma biological response in males vs. females.[6,86] While, overall, the observed associations in this study were not moderated by gender, we cannot claim that there was enough statistical power to identify nuanced differences beyond the background biological responses after severe injury. Conversely, we found that patient age significantly moderates the association of LTOWB and plasma on hemostasis-associated and immune-enriched modules. Previous work shows that the influence of advanced age on trauma outcomes extends beyond frailty-associated conditions.[87] Older patients have a propensity for imbalanced immuno-inflammatory responses after trauma[86] and major elective surgery.[88] Moreover, advanced age is associated with greater clotting factor consumption and fibrinolysis independent of injury and shock severity.[89] It is suggested that aged patients have higher fibrinogen levels early after injury, followed by a steeper fall.[90] Therefore, the greater CLOT module associations in aged plasma recipients could have resulted from plasma correcting a more defective clotting process. In addition, aged patients have higher circulating βTG and platelet membrane phospholipid levels.[91] This may explain the role of age in moderating the plasma association with ALPHA proteins. Taken collectively, these observations are consistent with the benefits of higher plasma ratios in massively transfused aged adults.[92,93]

Several reports have shown that plasma component benefits could be partly explained by transfusion logistics and timing[94–96] and/or patient/injury characteristics[12,14,15,97] besides molecular endotypes.[11,13] Our causal analysis provides further evidence that such associations may be rooted in distinct biological impacts marked by a difference in the levels of circulating proteins.[11,20,98] Although we did not consistently observe survival differences in plasma vs. LTOWB recipients, our analysis showed that receiving prehospital plasma correlates with improved illness resolution (defined as lower mortality or shorter ICU stay) and reduced amounts of transfusion products on the first day of admission. Propensity-matched and heterogeneity-adjusted analyses identified an association of plasma components with lower PT/INR. The magnitudes of beneficial plasma associations were larger in patients with TBI and those with higher circulating markers of brain injury. Notably, the association with reduced transfusion volumes was significantly stronger in patients suffering from TBI and blunt injury. These align well with earlier works on the influence of injury mechanisms on endothelial dysfunction and patient recovery[99] and others demonstrating the survival benefits of prehospital plasma in patients with TBI.[12,14,67] Furthermore, plasma showed a stronger PT-normalizing association in patients with severe shock. The source of beneficial plasma effects in hemorrhagic shock has been discussed, with some highlighting the largest impact in patients requiring moderate RBC transfusions.[82,100] Here, the relationship of plasma with PT at admission was measured after adjusting for several conditions, including the total volume of products and fluids received, suggesting a volume-independent plasma-specific procoagulant association.[82] Correcting endothelial dysfunction and glycocalyx stabilization could be one route through which plasma exerts its salutary effects.[11,101,102] While our results did not show a distinct plasma association with endothelial-associated proteins, a comprehensive assessment of plasma vs. LTOWB effect on endothelial permeability needs detailed measurements of shock duration and severity.[103]

Given the similarity of the plasma volume in the plasma component and LTOWB, the source of their differential associations is not immediately clear.[104] While clotting factor differences are unlikely to fully account for the observed proteomic patterns, it is intriguing to speculate on the contributions of product age, storage conditions, or donor-related factors.[105] The maximum age of plasma as a component is 5 days, but LTOWB can be stored for 21–35 days (depending on the collection solution). While some clotting factors decrease when LTOWB is stored at 4°C for up to 14 days,[106] the concentration of most factors is well maintained over the 5-day plasma component storage. Furthermore, the plasma in LTOWB is stored with RBCs, platelets, and white blood cells (depending on leukoreduction levels), making its composition distinct from plasma separated from

blood components within 24 h of collection and not exposed to extended incubation. The proximity of plasma to platelets in LTOWB during storage leads to alterations, such as increased phosphatidylserine and $CD41^+$ microparticles, compared to liquid never-frozen plasma stored for more than 15 days.[107] In addition, sex differences between plasma and LTOWB donors might influence transfusion-associated proteomics. While mortality differences between recipients of male and female-donated RBC units have not been demonstrated, these units contain small quantities of plasma.[108,109] Most blood centers collect LTOWB exclusively from male donors to mitigate the risk of transfusion-related acute lung injury that might occur with whole blood from multiparous human leukocyte antigen-sensitized females.

Due to its safety and effectiveness, LTOWB is increasingly favored in early trauma care, particularly in scenarios where quick and balanced resuscitation is critical.[7,10,16,97,110,111] However, this study adds to the evidence supporting the unique effects of plasma when given as a separate component. Our findings imply that plasma treatment could complement hemostatic stabilization provided by LTOWB, particularly in complex polytrauma cases with high immuno-inflammatory burdens, or where a nuanced hemostatic response is desired.[5,13,98,112] Further incorporation of insights from high-dimensional -omics studies with clinical research[113] could help shape personalized decision-making in trauma care.[21]

Limitations of the study

Several limitations qualify the conclusions from this study. First, because the data come from an observational cohort study rather than a randomized controlled trial (RCT), our ability to infer causality from the associations is inherently limited. Despite advanced sensitivity analyses and causal modeling to adjust for confounders and interpret effect modification, there may be persistent residual confounding, overmatching, or collider bias.[114] Definitive evidence synthesis should be built on RCTs that contrast well-defined blood product interventions. Second, this study was not powered to provide granular insights on the differential effects of varying ratios of RBC, platelet, and plasma components,[93] or their combinations with other products (cryoprecipitate, prothrombin complex, fibrinogen, etc.).[5] Similarly, although protein levels varied between donors, this study could not assess how donor characteristics influenced omics data or clinical outcomes. Third, standards of treatment in various SWAT participating centers were not identical, and these centers had different capabilities in prehospital product administration. Furthermore, transfusion practices evolved at some centers during the study period. Varied levels of leukoreduction and platelet-sparing filters in LTOWB products could have affected circulating immune and platelet-associated proteins although the evidence on the clinical consequences of such product alterations remains mixed.[16,115] Considering the limitations of available data on each intervention, it is not clear how much these disparities could have potentially influenced our results. Finally, previous studies have found instances of disagreement between aptamer-based proteomic assays (used in this work) and other assays.[116] Assessing the treatment associations with modules rather than individual proteins was a deliberate choice partly to mitigate the effect of potential sporadic inaccurate measurements. The presence of previously validated SomaLogic measurements of fibrinogen[117] and PDGFB,[118] which are the respective central nodes in CLOT and ALPHA modules, bolsters the case for the reliability of identified associations. However, validating highlighted biological pathways and ensuring the broader applicability of our findings across trauma populations necessitates replication in larger and more diverse cohorts through orthogonal quantitation methods.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Timothy R. Billiar (billiartr@upmc.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

A de-identified set of proteomics data has been deposited at Zenodo, a general-purpose repository, and is publicly available as of the date of publication at Zenodo: https://doi.org/10.5281/zenodo.14757173. All original code has been deposited on GitHub and is publicly available at Github: https://github.com/moheimanih/SWAT_proteomics_study as of the publication date. Any additional information required to reanalyze the data reported in this paper can be made available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.M., J.L.S., and T.R.B.; methodology, H.M. and J.D.; software, H.M., X.S., M.O., and J.L.D.; validation, J.D.; formal analysis, H.M., X.S., M.O., and J.L.D.; investigation, H.M.; resources, M.H.Y., F.X.G., S.R.W., B.A.C., J.W.C., M.A.S., E.E.M., N.N., J.P.M., and J.L.S.; data curation, H.M., U.K.K., and J.L.S.; writing – original draft, H.M.; writing – review and editing, H.M., E.P.O., T.O., M.H.Y., M.D.N., F.X.G., M.A.S., C.D.B., J.D., J.L.S., and T.R.B.; visualization, H.M. and T.O.; supervision, T.R.B.; project administration, H.M., U.K.K., J.L.S., and T.R.B.; funding acquisition, J.L.S. and T.R.B.

DECLARATION OF INTERESTS

M.D.N. serves as the Chief Medical Officer for Haima Therapeutics and has received personal fees from CSL Behring, Haemonetics, Cellphire, Octapharma, and Takeda and grants from Haemonetics, Alexion, National Institutes of Health, US Department of Defense, and DARPA outside the submitted work; in addition, M.D.N. has a patent for US 11,408,844 issued and a patent for US 9,072,760 issued. M.A.S. has received grant funding from Haemonetics and CSL Behring and is a consultant for Haemonetics, CSL Behring, Tricol, Velico Medical, and Octapharma. C.D.B. has patents issued or pending related to coagulation/fibrinolysis diagnostics, supplemental plasminogen in pleural space disease, and previously received grant support from Genentech and Werfen and consulting fees from Atheneum Partners.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Rossiter, N.D. (2022). Trauma—the forgotten pandemic? Int. Orthop. *46*, 3–11. https://doi.org/10.1007/s00264-021-05213-z.
2. Baker, C.C., Oppenheimer, L., Stephens, B., Lewis, F.R., and Trunkey, D.D. (1980). Epidemiology of trauma deaths. Am. J. Surg. *140*, 144–150. https://doi.org/10.1016/0002-9610(80)90431-6.
3. Saviano, A., Perotti, C., Zanza, C., Longhitano, Y., Ojetti, V., Franceschi, F., Bellou, A., Piccioni, A., Jannelli, E., Ceresa, I.F., and Savioli, G. (2024). Blood Transfusion for Major Trauma in Emergency Department. Diagnostics *14*, 708. https://doi.org/10.3390/diagnostics14070708.
4. Turnbull, C., Clegg, L., Santhakumar, A., and Micalos, P.S. (2024). Blood Product Administration in the Prehospital Setting: A Scoping Review. Prehosp. Emerg. Care *0*, 1–14. https://doi.org/10.1080/10903127.2024.2386007.
5. Moore, E.E., Moore, H.B., Kornblith, L.Z., Neal, M.D., Hoffman, M., Mutch, N.J., Schöchl, H., Hunt, B.J., and Sauaia, A. (2021). Trauma-induced coagulopathy. Nat. Rev. Dis. Primers *7*, 30. https://doi.org/10.1038/s41572-021-00264-3.
6. Jackman, R.P., Utter, G.H., Muench, M.O., Heitman, J.W., Munz, M.M., Jackman, R.W., Biswas, H.H., Rivers, R.M., Tobler, L.H., Busch, M.P., and Norris, P.J. (2012). Distinct roles of trauma and transfusion in induction of immune modulation after injury. Transfusion *52*, 2533–2550. https://doi.org/10.1111/j.1537-2995.2012.03618.x.
7. Dorken-Gallastegi, A., Spinella, P.C., Neal, M.D., Leeper, C., Sperry, J., Peitzman, A.B., and Brown, J.B. (2024). Whole Blood and Blood Component Resuscitation in Trauma: Interaction and Association with Mortality. Ann. Surg. *280*, 1014–1020. https://doi.org/10.1097/SLA.0000000000006316.
8. Sperry, J.L., Guyette, F.X., Brown, J.B., Yazer, M.H., Triulzi, D.J., Early-Young, B.J., Adams, P.W., Daley, B.J., Miller, R.S., Harbrecht, B.G., et al. (2018). Prehospital Plasma during Air Medical Transport in Trauma Patients at Risk for Hemorrhagic Shock. N. Engl. J. Med. *379*, 315–326. https://doi.org/10.1056/NEJMoa1802345.
9. Guyette, F.X., Zenati, M., Triulzi, D.J., Yazer, M.H., Skroczky, H., Early, B.J., Adams, P.W., Brown, J.B., Alarcon, L., Neal, M.D., et al. (2022). Prehospital low titer group O whole blood is feasible and safe: Results of a prospective randomized pilot trial. J. Trauma Acute Care Surg. *92*, 839–847. https://doi.org/10.1097/TA.0000000000003551.
10. Morgan, K.M., Abou Khalil, E., Feeney, E.V., Spinella, P.C., Lucisano, A.C., Gaines, B.A., and Leeper, C.M. (2024). The Efficacy of Low-Titer Group O Whole Blood Compared With Component Therapy in Civilian Trauma Patients: A Meta-Analysis. Crit. Care Med. *52*, e390–e404. https://doi.org/10.1097/CCM.0000000000006244.
11. Gruen, D.S., Brown, J.B., Guyette, F.X., Vodovotz, Y., Johansson, P.I., Stensballe, J., Barclay, D.A., Yin, J., Daley, B.J., Miller, R.S., et al. (2020). Prehospital plasma is associated with distinct biomarker expression following injury. JCI Insight *5*, 135350. https://doi.org/10.1172/jci.insight.135350.
12. Gruen, D.S., Guyette, F.X., Brown, J.B., Okonkwo, D.O., Puccio, A.M., Campwala, I.K., Tessmer, M.T., Daley, B.J., Miller, R.S., Harbrecht, B.G., et al. (2020). Association of Prehospital Plasma With Survival in Patients With Traumatic Brain Injury: A Secondary Analysis of the PAMPer Cluster Randomized Clinical Trial. JAMA Netw. Open *3*, e2016869. https://doi.org/10.1001/jamanetworkopen.2020.16869.
13. Wu, J., Vodovotz, Y., Abdelhamid, S., Guyette, F.X., Yaffe, M.B., Gruen, D.S., Cyr, A., Okonkwo, D.O., Kar, U.K., Krishnamoorthi, N., et al. (2021). Multi-omic analysis in injured humans: Patterns align with outcomes and treatment responses. Cell Rep. Med. *2*, 100478. https://doi.org/10.1016/j.xcrm.2021.100478.
14. Wu, J., Moheimani, H., Li, S., Kar, U.K., Bonaroti, J., Miller, R.S., Daley, B.J., Harbrecht, B.G., Claridge, J.A., Gruen, D.S., et al. (2022). High Dimensional Multiomics Reveals Unique Characteristics of Early Plasma Administration in Polytrauma Patients With TBI. Ann. Surg. *276*, 673–683. https://doi.org/10.1097/SLA.0000000000005610.
15. Reitz, K.M., Moore, H.B., Guyette, F.X., Sauaia, A., Pusateri, A.E., Moore, E.E., Hassoune, A., Chapman, M.P., Daley, B.J., Miller, R.S., et al. (2020). Prehospital plasma in injured patients is associated with survival principally in blunt injury: results from two randomized prehospital plasma trials. J. Trauma Acute Care Surg. *88*, 33–41. https://doi.org/10.1097/TA.0000000000002485.
16. Sperry, J.L., Cotton, B.A., Luther, J.F., Cannon, J.W., Schreiber, M.A., Moore, E.E., Namias, N., Minei, J.P., Wisniewski, S.R., and Guyette, F.X.; Shock, Whole Blood, and Assessment of Traumatic Brain Injury SWAT Study Group (2023). Whole Blood Resuscitation and Association with Survival in Injured Patients with an Elevated Probability of Mortality. J. Am. Coll. Surg. *237*, 206–219. https://doi.org/10.1097/XCS.0000000000000708.
17. Meizoso, J.P., Cotton, B.A., Lawless, R.A., Kodadek, L.M., Lynde, J.M., Russell, N., Gaspich, J., Maung, A., Anderson, C., Reynolds, J.M., et al. (2024). Whole blood resuscitation for injured patients requiring transfusion: A systematic review, meta-analysis, and practice management guideline from the Eastern Association for the Surgery of Trauma. J. Trauma Acute Care Surg. *97*, 460–470. https://doi.org/10.1097/TA.0000000000004327.
18. Malkin, M., Nevo, A., Brundage, S.I., and Schreiber, M. (2021). Effectiveness and safety of whole blood compared to balanced blood components in resuscitation of hemorrhaging trauma patients - A systematic review. Injury *52*, 182–188. https://doi.org/10.1016/j.injury.2020.10.095.
19. Dishong, D., Cap, A.P., Holcomb, J.B., Triulzi, D.J., and Yazer, M.H. (2021). The rebirth of the cool: a narrative review of the clinical outcomes of cold stored low titer group O whole blood recipients compared to conventional component recipients in trauma. Hematology *26*, 601–611. https://doi.org/10.1080/16078454.2021.1967257.
20. Jackson, B.P., Sperry, J.L., and Yazer, M.H. (2021). Prehospital Plasma Transfusion: What Does the Literature Show? Transfus. Med. Hemother. *48*, 358–365. https://doi.org/10.1159/000519627.
21. Davis, C.S., Wilkinson, K.H., Lin, E., Carpenter, N.J., Georgeades, C., Lomberk, G., and Urrutia, R. (2022). Precision medicine in trauma: a transformational frontier in patient care, education, and research. Eur. J. Trauma Emerg. Surg. *48*, 2607–2612. https://doi.org/10.1007/s00068-021-01817-7.
22. Savioli, G., Ceresa, I.F., Caneva, L., Gerosa, S., and Ricevuti, G. (2021). Trauma-Induced Coagulopathy: Overview of an Emerging Medical

Problem from Pathophysiology to Outcomes. Medicines (Basel) *8*, 16. https://doi.org/10.3390/medicines8040016.

23. Huber-Lang, M., Lambris, J.D., and Ward, P.A. (2018). Innate immune responses to trauma. Nat. Immunol. *19*, 327–341. https://doi.org/10.1038/s41590-018-0064-8.
24. Yang, Y.-W., Wu, C.-H., Tsai, H.-T., Chen, Y.-R., Chang, Y.-P., Han, Y.-Y., Wu, T.E., and Hu, R.-H. (2023). Dynamics of immune responses are inconsistent when trauma patients are grouped by injury severity score and clinical outcomes. Sci. Rep. *13*, 1391. https://doi.org/10.1038/s41598-023-27969-7.
25. McCoy, C.C., Brenner, M., Duchesne, J., Roberts, D., Ferrada, P., Horer, T., Kauvar, D., Khan, M., Kirkpatrick, A., Ordonez, C., et al. (2021). Back to the Future: Whole Blood Resuscitation of the Severely Injured Trauma Patient. Shock *56*, 9–15. https://doi.org/10.1097/SHK.0000000000001685.
26. Xiao, W., Mindrinos, M.N., Seok, J., Cuschieri, J., Cuenca, A.G., Gao, H., Hayden, D.L., Hennessy, L., Moore, E.E., Minei, J.P., et al. (2011). A genomic storm in critically injured humans. J. Exp. Med. *208*, 2581–2590. https://doi.org/10.1084/jem.20111354.
27. Candia, J., Daya, G.N., Tanaka, T., Ferrucci, L., and Walker, K.A. (2022). Assessment of variability in the plasma 7k SomaScan proteomics assay. Sci. Rep. *12*, 17147. https://doi.org/10.1038/s41598-022-22116-0.
28. Huang, J., Li, X., Shi, X., Zhu, M., Wang, J., Huang, S., Huang, X., Wang, H., Li, L., Deng, H., et al. (2019). Platelet integrin αIIbβ3: signal transduction, regulation, and its therapeutic targeting. J. Hematol. Oncol. *12*, 26. https://doi.org/10.1186/s13045-019-0709-6.
29. Zong, Y., Li, H., Liao, P., Chen, L., Pan, Y., Zheng, Y., Zhang, C., Liu, D., Zheng, M., and Gao, J. (2024). Mitochondrial dysfunction: mechanisms and advances in therapy. Signal Transduct. Targeted Ther. *9*, 124–129. https://doi.org/10.1038/s41392-024-01839-8.
30. Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., Brohi, K., Itagaki, K., and Hauser, C.J. (2010). Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature *464*, 104–107. https://doi.org/10.1038/nature08780.
31. Goel, H., Melot, J., Krinock, M.D., Kumar, A., Nadar, S.K., and Lip, G.Y.H. (2020). Heart-type fatty acid-binding protein: an overlooked cardiac biomarker. Ann. Med. *52*, 444–461. https://doi.org/10.1080/07853890.2020.1800075.
32. Yabluchanskiy, A., Ma, Y., Iyer, R.P., Hall, M.E., and Lindsey, M.L. (2013). Matrix Metalloproteinase-9: Many Shades of Function in Cardiovascular Disease. Physiology *28*, 391–403. https://doi.org/10.1152/physiol.00029.2013.
33. Taguchi, T., Nazneen, A., Al-Shihri, A.A., Turkistani, K.A., and Razzaque, M.S. (2011). Heat Shock Protein 47: A Novel Biomarker of Phenotypically Altered Collagen-Producing Cells. Acta Histochem. Cytochem. *44*, 35–41. https://doi.org/10.1267/ahc.11001.
34. Li, S.R., Moheimani, H., Herzig, B., Kail, M., Krishnamoorthi, N., Wu, J., Abdelhamid, S., Scioscia, J., Sung, E., Rosengart, A., et al. (2023). High-dimensional proteomics identifies organ injury patterns associated with outcomes in human trauma. J. Trauma Acute Care Surg. *94*, 803–813. https://doi.org/10.1097/TA.0000000000003880.
35. Simpson, J.A., Labugger, R., Collier, C., Brison, R.J., Iscoe, S., and Van Eyk, J.E. (2005). Fast and Slow Skeletal Troponin I in Serum from Patients with Various Skeletal Muscle Disorders: A Pilot Study. Clin. Chem. *51*, 966–972. https://doi.org/10.1373/clinchem.2004.042671.
36. Chosa, E., Sekimoto, T., Sonoda, N., Yamamoto, K., Matsuda, H., Takahama, K., and Tajima, N. (2003). Evaluation of human beta-enolase as a serum marker for exercise-induced muscle damage. Clin. J. Sport Med. *13*, 209–212. https://doi.org/10.1097/00042752-200307000-00003.
37. Brew, K., Dinakarpandian, D., and Nagase, H. (2000). Tissue inhibitors of metalloproteinases: evolution, structure and function1. Biochim. Biophys. Acta *1477*, 267–283. https://doi.org/10.1016/S0167-4838(99)00279-4.
38. Wu, Q., and Jia, Z. (2021). Wiring the Brain by Clustered Protocadherin Neural Codes. Neurosci. Bull. *37*, 117–131. https://doi.org/10.1007/s12264-020-00578-4.
39. Patel, S., Homaei, A., El-Seedi, H.R., and Akhtar, N. (2018). Cathepsins: Proteases that are vital for survival but can also be fatal. Biomed. Pharmacother. *105*, 526–532. https://doi.org/10.1016/j.biopha.2018.05.148.
40. Jain, S., Gautam, V., and Naseem, S. (2011). Acute-phase proteins: As diagnostic tool. J. Pharm. BioAllied Sci. *3*, 118–127. https://doi.org/10.4103/0975-7406.76489.
41. Chang, T.-T., Chen, C., and Chen, J.-W. (2022). CCL7 as a novel inflammatory mediator in cardiovascular disease, diabetes mellitus, and kidney disease. Cardiovasc. Diabetol. *21*, 185. https://doi.org/10.1186/s12933-022-01626-1.
42. Arruda-Silva, F., Bianchetto-Aguilera, F., Gasperini, S., Polletti, S., Cosentino, E., Tamassia, N., and Cassatella, M.A. (2017). Human Neutrophils Produce CCL23 in Response to Various TLR-Agonists and TNFα. Front. Cell. Infect. Microbiol. *7*, 176. https://doi.org/10.3389/fcimb.2017.00176.
43. Huang, Z., Qi, G., Miller, J.S., and Zheng, S.G. (2020). CD226: An Emerging Role in Immunologic Diseases. Front. Cell Dev. Biol. *8*, 564. https://doi.org/10.3389/fcell.2020.00564.
44. Sloos, P.H., Vulliamy, P., van 't Veer, C., Gupta, A.S., Neal, M.D., Brohi, K., Juffermans, N.P., and Kleinveld, D.J.B. (2022). Platelet dysfunction after trauma: From mechanisms to targeted treatment. Transfusion *62*, S281–S300. https://doi.org/10.1111/trf.16971.
45. Chakraborty, S., Karasu, E., and Huber-Lang, M. (2018). Complement After Trauma: Suturing Innate and Adaptive Immunity. Front. Immunol. *9*, 2050. https://doi.org/10.3389/fimmu.2018.02050.
46. Cardenas, J.C., Dong, J.F., and Kozar, R.A. (2023). Injury-induced endotheliopathy: What you need to know. J. Trauma Acute Care Surg. *95*, 454–463. https://doi.org/10.1097/TA.0000000000004082.
47. Ibáñez, C.F. (2010). Beyond the cell surface: New mechanisms of receptor function. Biochem. Biophys. Res. Commun. *396*, 24–27. https://doi.org/10.1016/j.bbrc.2010.01.136.
48. Awwad, K., Hu, J., Shi, L., Mangels, N., Abdel Malik, R., Zippel, N., Fisslthaler, B., Eble, J.A., Pfeilschifter, J., Popp, R., and Fleming, I. (2015). Role of secreted modular calcium-binding protein 1 (SMOC1) in transforming growth factor β signalling and angiogenesis. Cardiovasc. Res. *106*, 284–294. https://doi.org/10.1093/cvr/cvv098.
49. Delgado Lagos, F., Elgheznawy, A., Kyselova, A., Meyer zu Heringdorf, D., Ratiu, C., Randriamboavonjy, V., Mann, A.W., Fisslthaler, B., Siragusa, M., and Fleming, I. (2021). Secreted modular calcium-binding protein 1 binds and activates thrombin to account for platelet hyperreactivity in diabetes. Blood *137*, 1641–1651. https://doi.org/10.1182/blood.2020009405.
50. Li, J., Diao, B., Guo, S., Huang, X., Yang, C., Feng, Z., Yan, W., Ning, Q., Zheng, L., Chen, Y., and Wu, Y. (2017). VSIG4 inhibits proinflammatory macrophage activation by reprogramming mitochondrial pyruvate metabolism. Nat. Commun. *8*, 1322. https://doi.org/10.1038/s41467-017-01327-4.
51. Kopecki, Z., and Cowin, A.J. (2008). Flightless I: an actin-remodelling protein and an important negative regulator of wound repair. Int. J. Biochem. Cell Biol. *40*, 1415–1419. https://doi.org/10.1016/j.biocel.2007.04.011.
52. Tester, A.M., Cox, J.H., Connor, A.R., Starr, A.E., Dean, R.A., Puente, X.S., López-Otín, C., and Overall, C.M. (2007). LPS Responsiveness and Neutrophil Chemotaxis In Vivo Require PMN MMP-8 Activity. PLoS One *2*, e312. https://doi.org/10.1371/journal.pone.0000312.
53. Desprez, F., Ung, D.C., Vourc'h, P., Jeanne, M., and Laumonnier, F. (2023). Contribution of the dihydropyrimidinase-like proteins family in synaptic physiology and in neurodevelopmental disorders. Front. Neurosci. *17*, 1154446. https://doi.org/10.3389/fnins.2023.1154446.

54. Liu, J., Dai, Q., Chen, J., Durrant, D., Freeman, A., Liu, T., Grossman, D., and Lee, R.M. (2003). Phospholipid scramblase 3 controls mitochondrial structure, function, and apoptotic response. Mol. Cancer Res. *1*, 892–902.

55. Lin, J., Xu, Y., Guo, P., Chen, Y.-J., Zhou, J., Xia, M., Tan, B., Liu, X., Feng, H., and Chen, Y. (2023). CCL5/CCR5-mediated peripheral inflammation exacerbates blood–brain barrier disruption after intracerebral hemorrhage in mice. J. Transl. Med. *21*, 196. https://doi.org/10.1186/s12967-023-04044-3.

56. Traag, V.A., Waltman, L., and van Eck, N.J. (2019). From Louvain to Leiden: guaranteeing well-connected communities. Sci. Rep. *9*, 5233. https://doi.org/10.1038/s41598-019-41695-z.

57. Bonacich, P. (2007). Some unique properties of eigenvector centrality. Soc. Netw. *29*, 555–564. https://doi.org/10.1016/j.socnet.2007.04.002.

58. Dudík, M., Erhan, D., Langford, J., and Li, L. (2014). Doubly Robust Policy Evaluation and Optimization. Stat. Sci. *29*, 485–511. https://doi.org/10.1214/14-STS500.

59. Wager, S., and Athey, S. (2018). Estimation and Inference of Heterogeneous Treatment Effects using Random Forests. J. Am. Stat. Assoc. *113*, 1228–1242. https://doi.org/10.1080/01621459.2017.1319839.

60. Hier, D.B., Obafemi-Ajayi, T., Thimgan, M.S., Olbricht, G.R., Azizi, S., Allen, B., Hadi, B.A., and Wunsch, D.C. (2021). Blood biomarkers for mild traumatic brain injury: a selective review of unresolved issues. Biomark. Res. *9*, 70. https://doi.org/10.1186/s40364-021-00325-5.

61. Lawless, R.A., and Holcomb, J.B. (2015). Plasma Transfusion. In Trauma Induced Coagulopathy (Springer), pp. 323–337. https://doi.org/10.1007/978-3-319-28308-1_20.

62. Rourke, C., Curry, N., Khan, S., Taylor, R., Raza, I., Davenport, R., Stanworth, S., and Brohi, K. (2012). Fibrinogen levels during trauma hemorrhage, response to replacement therapy, and association with patient outcomes. J. Thromb. Haemost. *10*, 1342–1351. https://doi.org/10.1111/j.1538-7836.2012.04752.x.

63. DeBot, M., Erickson, C., Schaid, T., LaCroix, I., Moore, E.E., Silliman, C., Cohen, M.J., D'Alessandro, A., and Hansen, K.C. (2024). Trauma-induced dysfibrinogenemia: the von Clauss assay does not accurately measure fibrinogen levels after injury. Blood Vessels Thrombosis Hemostasis *1*, 100017. https://doi.org/10.1016/j.bvth.2024.100017.

64. Parker, M.J., Crowder, E.W., Miles, M.V.P., Harrell, K.N., and Maxwell, R.A. (2023). Hypofibrinogenemic Massive Transfusion Trauma Patients Experience Worse Outcomes. Am. Surg. *89*, 3423–3428. https://doi.org/10.1177/00031348231162711.

65. Fraser, S.R., Booth, N.A., and Mutch, N.J. (2011). The antifibrinolytic function of factor XIII is exclusively expressed through α_2-antiplasmin cross-linking. Blood *117*, 6371–6374. https://doi.org/10.1182/blood-2011-02-333203.

66. Nair, P.M., Rendo, M.J., Reddoch-Cardenas, K.M., Burris, J.K., Meledeo, M.A., and Cap, A.P. (2020). Recent advances in use of fresh frozen plasma, cryoprecipitate, immunoglobulins, and clotting factors for transfusion support in patients with hematologic disease. Semin. Hematol. *57*, 73–82. https://doi.org/10.1053/j.seminhematol.2020.07.006.

67. Zhang, J., Zhang, F., and Dong, J.F. (2018). Coagulopathy induced by traumatic brain injury: systemic manifestation of a localized injury. Blood *131*, 2001–2006. https://doi.org/10.1182/blood-2017-11-784108.

68. Lv, K., Yuan, Q., Fu, P., Wu, G., Wu, X., Du, Z., Yu, J., Li, Z., and Hu, J. (2020). Impact of fibrinogen level on the prognosis of patients with traumatic brain injury: a single-center analysis of 2570 patients. World J. Emerg. Surg. *15*, 54. https://doi.org/10.1186/s13017-020-00332-1.

69. Vulliamy, P., and Armstrong, P.C. (2024). Platelets in Hemostasis, Thrombosis, and Inflammation After Major Trauma. Arterioscler. Thromb. Vasc. Biol. *44*, 545–557. https://doi.org/10.1161/ATVBAHA.123.318801.

70. Windeløv, N.A., Ostrowski, S.R., Johansson, P.I., Wanscher, M., Larsen, C.F., Sørensen, A.M., and Rasmussen, L.S. (2015). Circulating levels of platelet α-granule cytokines in trauma patients. Inflamm. Res. *64*, 235–241. https://doi.org/10.1007/s00011-015-0802-4.

71. Nevzorova, T.A., Mordakhanova, E.R., Daminova, A.G., Ponomareva, A.A., Andrianova, I.A., Le Minh, G., Rauova, L., Litvinov, R.I., and Weisel, J.W. (2019). Platelet factor 4-containing immune complexes induce platelet activation followed by calpain-dependent platelet death. Cell Death Discov. *5*, 106–112. https://doi.org/10.1038/s41420-019-0188-0.

72. Greinacher, A., and Warkentin, T.E. (2023). Platelet factor 4 triggers thrombo-inflammation by bridging innate and adaptive immunity. Int. J. Lab. Hematol. *45*, 11–22. https://doi.org/10.1111/ijlh.14075.

73. Zipperle, J., Altenburger, K., Ponschab, M., Schlimp, C.J., Spittler, A., Bahrami, S., Redl, H., and Schöchl, H. (2017). Potential role of platelet-leukocyte aggregation in trauma-induced coagulopathy: Ex vivo findings. J. Trauma Acute Care Surg. *82*, 921–926. https://doi.org/10.1097/TA.0000000000001410.

74. Vulliamy, P., Gillespie, S., Armstrong, P.C., Allan, H.E., Warner, T.D., and Brohi, K. (2019). Histone H4 induces platelet ballooning and microparticle release during trauma hemorrhage. Proc. Natl. Acad. Sci. USA *116*, 17444–17449. https://doi.org/10.1073/pnas.1904978116.

75. Kutcher, M.E., Redick, B.J., McCreery, R.C., Crane, I.M., Greenberg, M.D., Cachola, L.M., Nelson, M.F., and Cohen, M.J. (2012). Characterization of platelet dysfunction after trauma. J. Trauma Acute Care Surg. *73*, 13–19. https://doi.org/10.1097/TA.0b013e318256deab.

76. St. John, A.E., Wang, Y., Chen, J., Osborn, W., Wang, X., Lim, E., Chung, D.W., Stern, S., White, N., Fu, X., and Lopez, J.A. (2020). Plasma Proteomic Profile Associated with Platelet Dysfunction after Trauma. Blood *136*, 9–10. https://doi.org/10.1182/blood-2020-141361.

77. Preston, R.J.S., Tran, S., Johnson, J.A., Ní Áinle, F., Harmon, S., White, B., Smith, O.P., Jenkins, P.V., Dahlbäck, B., and O'Donnell, J.S. (2009). Platelet factor 4 impairs the anticoagulant activity of activated protein C. J. Biol. Chem. *284*, 5869–5875. https://doi.org/10.1074/jbc.M804703200.

78. Vulliamy, P., Kornblith, L.Z., Kutcher, M.E., Cohen, M.J., Brohi, K., and Neal, M.D. (2021). Alterations in platelet behavior after major trauma: adaptive or maladaptive? Platelets *32*, 295–304. https://doi.org/10.1080/09537104.2020.1718633.

79. Bonaroti, J., Billiar, I., Moheimani, H., Wu, J., Namas, R., Li, S., Kar, U.K., Vodovotz, Y., Neal, M.D., Sperry, J.L., and Billiar, T.R. (2022). Plasma proteomics reveals early, broad release of chemokine, cytokine, TNF, and interferon mediators following trauma with delayed increases in a subset of chemokines and cytokines in patients that remain critically ill. Front. Immunol. *13*, 1038086. https://doi.org/10.3389/fimmu.2022.1038086.

80. Cai, J., McKinley, T., Billiar, I., Zenati, M.S., Gaski, G., Vodovotz, Y., Gruen, D.S., Billiar, T.R., and Namas, R.A. (2021). Protective/reparative cytokines are suppressed at high injury severity in human trauma. Trauma Surg. Acute Care Open *6*, e000619. https://doi.org/10.1136/tsaco-2020-000619.

81. Amico, F., Briggs, G., and Balogh, Z.J. (2019). Transfused trauma patients have better outcomes when transfused with blood components from young donors. Med. Hypotheses *122*, 141–146. https://doi.org/10.1016/j.mehy.2018.11.016.

82. van den Brink, D.P., Kleinveld, D.J.B., Sloos, P.H., Thomas, K.A., Stensballe, J., Johansson, P.I., Pati, S., Sperry, J., Spinella, P.C., and Juffermans, N.P. (2021). Plasma as a resuscitation fluid for volume-depleted shock: Potential benefits and risks. Transfusion *61*, S301–S312. https://doi.org/10.1111/trf.16462.

83. Abraham, E., and Regan, R.F. (1985). The effects of hemorrhage and trauma on interleukin 2 production. Arch. Surg. *120*, 1341–1344. https://doi.org/10.1001/archsurg.1985.01390360007002.

84. Doganyigit, Z., Eroglu, E., and Akyuz, E. (2022). Inflammatory mediators of cytokines and chemokines in sepsis: From bench to bedside. Hum. Exp. Toxicol. *41*, 09603271221078871. https://doi.org/10.1177/09603271221078871.

85. Hinson, H.E., Rowell, S., Morris, C., Lin, A.L., and Schreiber, M.A. (2018). Early fever after trauma: Does it matter? J. Trauma Acute Care Surg. *84*, 19–24. https://doi.org/10.1097/TA.0000000000001627.

86. Frink, M., Pape, H.-C., van Griensven, M., Krettek, C., Chaudry, I.H., and Hildebrand, F. (2007). Influence of sex and age on mods and cytokines after multiple injuries. Shock *27*, 151–156. https://doi.org/10.1097/01.shk.0000239767.64786.de.

87. Atinga, A., Shekkeris, A., Fertleman, M., Batrick, N., Kashef, E., and Dick, E. (2018). Trauma in the elderly patient. Br. J. Radiol. *91*, 20170739. https://doi.org/10.1259/bjr.20170739.

88. Bonaroti, J.W., Ozel, M., Chen, T., Darby, J.L., Sun, X., Moheimani, H., Reitz, K.M., Kar, U.K., Zuckerbraun, B.S., Das, J., et al. (2024). Transcriptomic and Proteomic Characterization of the Immune Response to Elective Spinal Reconstructive Surgery: Impact of Aging and Comparison with Traumatic Injury Response. J. Am. Coll. Surg. *238*, 924–941. https://doi.org/10.1097/XCS.0000000000000922.

89. Curry, N.S., Davenport, R., Wong, H., Gaarder, C., Johansson, P., Juffermans, N.P., Maegele, M., Stensballe, J., Brohi, K., Laffan, M., and Stanworth, S.J. (2023). Traumatic coagulopathy in the older patient: analysis of coagulation profiles from the Activation of Coagulation and Inflammation in Trauma-2 (ACIT-2) observational, multicenter study. J. Thromb. Haemost. *21*, 215–226. https://doi.org/10.1016/j.jtha.2022.11.005.

90. Nakae, R., Fujiki, Y., Takayama, Y., Kanaya, T., Igarashi, Y., Suzuki, G., Naoe, Y., and Yokobori, S. (2020). Age-Related Differences in the Time Course of Coagulation and Fibrinolytic Parameters in Patients with Traumatic Brain Injury. Int. J. Mol. Sci. *21*, 5613. https://doi.org/10.3390/ijms21165613.

91. Bastyr, E.J., Kadrofske, M.M., and Vinik, A.I. (1990). Platelet activity and phosphoinositide turnover increase with advancing age. Am. J. Med. *88*, 601–606. https://doi.org/10.1016/0002-9343(90)90525-I.

92. Hohle, R.D., Wothe, J.K., Hillmann, B.M., Tignanelli, C.J., Harmon, J.V., and Vakayil, V.R. (2022). Massive blood transfusion following older adult trauma: The effect of blood ratios on mortality. Acad. Emerg. Med. *29*, 1422–1430. https://doi.org/10.1111/acem.14580.

93. Fujiwara, G., Okada, Y., Ishii, W., Echigo, T., Shiomi, N., and Ohtsuru, S. (2024). High Fresh Frozen Plasma to Red Blood Cell Ratio and Survival Outcomes in Blunt Trauma. JAMA Surg. *159*, 1272–1280. https://doi.org/10.1001/jamasurg.2024.3097.

94. Lewis, R.E., Muluk, S.L., Reitz, K.M., Guyette, F.X., Brown, J.B., Miller, R.S., Harbrecht, B.G., Claridge, J.A., Phelan, H.A., Yazer, M.H., et al. (2022). Prehospital plasma is associated with survival principally in patients transferred from the scene of injury: A secondary analysis of the PAMPer trial. Surgery *172*, 1278–1284. https://doi.org/10.1016/j.surg.2022.04.039.

95. Zadorozny, E.V., Lin, H.-H.S., Luther, J., Wisniewski, S.R., Cotton, B.A., Fox, E.E., Harbrecht, B.G., Joseph, B.A., Moore, E.E., Ostenmayer, D.G., et al. (2024). Prehospital Time Following Traumatic Injury Is Independently Associated With the Need for In-Hospital Blood and Early Mortality for Specific Injury Types. Air Med. J. *43*, 47–54. https://doi.org/10.1016/j.amj.2023.09.013.

96. Pusateri, A.E., Moore, E.E., Moore, H.B., Le, T.D., Guyette, F.X., Chapman, M.P., Sauaia, A., Ghasabyan, A., Chandler, J., McVaney, K., et al. (2020). Association of Prehospital Plasma Transfusion With Survival in Trauma Patients With Hemorrhagic Shock When Transport Times Are Longer Than 20 Minutes. JAMA Surg. *155*, e195085. https://doi.org/10.1001/jamasurg.2019.5085.

97. Abuelazm, M., Rezq, H., Mahmoud, A., Tanashat, M., Salah, A., Saleh, O., Morsi, S., and Abdelazeem, B. (2024). The efficacy and safety of pre-hospital plasma in patients at risk for hemorrhagic shock: an updated systematic review and meta-analysis of randomized controlled trials. Eur. J. Trauma Emerg. Surg. *50*, 2697–2707. https://doi.org/10.1007/s00068-024-02461-7.

98. Cannon, J.W., Gruen, D.S., Zamora, R., Brostoff, N., Hurst, K., Harn, J.H., El-Dehaibi, F., Geng, Z., Namas, R., Sperry, J.L., et al. (2024). Digital twin mathematical models suggest individualized hemorrhagic shock resuscitation strategies. Commun. Med. *4*, 113–212. https://doi.org/10.1038/s43856-024-00535-6.

99. Donohue, J.K., Gruen, D.S., Iyanna, N., Lorence, J.M., Brown, J.B., Guyette, F.X., Daley, B.J., Eastridge, B.J., Miller, R.S., Nirula, R., et al. (2024). Mechanism matters: mortality and endothelial cell damage marker differences between blunt and penetrating traumatic injuries across three prehospital clinical trials. Sci. Rep. *14*, 2747. https://doi.org/10.1038/s41598-024-53398-1.

100. Anto, V.P., Guyette, F.X., Brown, J., Daley, B., Miller, R., Harbrecht, B., Claridge, J., Phelan, H., Neal, M., Forsythe, R., et al. (2020). Severity of hemorrhage and the survival benefit associated with plasma: Results from a randomized prehospital plasma trial. J. Trauma Acute Care Surg. *88*, 141–147. https://doi.org/10.1097/TA.0000000000002530.

101. Kravitz, M.S., Kattouf, N., Stewart, I.J., Ginde, A.A., Schmidt, E.P., and Shapiro, N.I. (2024). Plasma for prevention and treatment of glycocalyx degradation in trauma and sepsis. Crit. Care *28*, 254. https://doi.org/10.1186/s13054-024-05026-7.

102. Johansson, P.I., Stensballe, J., and Ostrowski, S.R. (2017). Shock induced endotheliopathy (SHINE) in acute critical illness - a unifying pathophysiologic mechanism. Crit. Care *21*, 25. https://doi.org/10.1186/s13054-017-1605-5.

103. Kregel, H.R., Hatton, G.E., Isbell, K.D., Henriksen, H.H., Stensballe, J., Johansson, P.I., Kao, L.S., and Wade, C.E. (2022). Shock-Induced Endothelial Dysfunction is Present in Patients With Occult Hypoperfusion After Trauma. Shock *57*, 106–112. https://doi.org/10.1097/SHK.0000000000001866.

104. Yazer, M.H., Cortese-Hassett, A., and Triulzi, D.J. (2008). Coagulation factor levels in plasma frozen within 24 hours of phlebotomy over 5 days of storage at 1 to 6 degrees C. Transfusion *48*, 2525–2530. https://doi.org/10.1111/j.1537-2995.2008.01913.x.

105. Chae, R.C., Sisak, S., Nguyen, C.Q., Wattley, L.J., Joseph, B., England, L., Schuster, R., Lentsch, A.B., Caldwell, C.C., Goodman, M.D., and Pritts, T.A. (2025). Age above all: The impact of storage duration in mice resuscitated with whole blood or packed red blood cells and plasma in a hemorrhagic shock model. Surgery *179*, 108792. https://doi.org/10.1016/j.surg.2024.07.054.

106. Pidcoke, H.F., McFaul, S.J., Ramasubramanian, A.K., Parida, B.K., Mora, A.G., Fedyk, C.G., Valdez-Delgado, K.K., Montgomery, R.K., Reddoch, K.M., Rodriguez, A.C., et al. (2013). Primary hemostatic capacity of whole blood: a comprehensive analysis of pathogen reduction and refrigeration effects over time. Transfusion *53*, 137S–149S. https://doi.org/10.1111/trf.12048.

107. Mihalko, E.P., Srinivasan, A.J., Rahn, K.C., Seheult, J.N., Spinella, P.C., Cap, A.P., Triulzi, D.J., Yazer, M.H., Neal, M.D., and Shea, S.M. (2023). Hemostatic In Vitro Properties of Novel Plasma Supernatants Produced from Late-storage Low-titer Type O Whole Blood. Anesthesiology *139*, 77–90. https://doi.org/10.1097/ALN.0000000000004574.

108. Edgren, G., Murphy, E.L., Brambilla, D.J., Westlake, M., Rostgaard, K., Lee, C., Cable, R.G., Triulzi, D., Bruhn, R., St Lezin, E.M., et al. (2019). Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. JAMA *321*, 2183–2192. https://doi.org/10.1001/jama.2019.7084.

109. Chassé, M., Fergusson, D.A., Tinmouth, A., Acker, J.P., Perelman, I., Tuttle, A., English, S.W., Hawken, S., Forster, A.J., Shehata, N., et al. (2023). Effect of Donor Sex on Recipient Mortality in Transfusion. N. Engl. J. Med. *388*, 1386–1395. https://doi.org/10.1056/NEJMoa2211523.

110. Sperry, J.L., and Brown, J.B. (2023). Whole-Blood Resuscitation Following Traumatic Injury and Hemorrhagic Shock—Should It Be Standard Care? JAMA Surg. *158*, 540. https://doi.org/10.1001/jamasurg.2022.6986.

111. Coulthard, S.L., Kaplan, L.J., and Cannon, J.W. (2024). What's new in whole blood resuscitation? In the trauma bay and beyond. Curr. Opin. Crit. Care *30*, 209–216. https://doi.org/10.1097/MCC.0000000000001140.

112. Hrebinko, K.A., Sperry, J.L., Guyette, F.X., Brown, J.B., Daley, B.J., Miller, R.S., Harbrecht, B.G., Claridge, J.A., Phelan, H.A., Neal, M.D., et al. (2021). Evaluating the Cost-effectiveness of Prehospital Plasma Transfusion in Unstable Trauma Patients: A Secondary Analysis of the PAMPer Trial. JAMA Surg. *156*, 1131–1139. https://doi.org/10.1001/jamasurg.2021.4529.

113. Marsden, M.E.R., Kellett, S., Bagga, R., Wohlgemut, J.M., Lyon, R.L., Perkins, Z.B., Gillies, K., and Tai, N.R. (2023). Understanding pre-hospital blood transfusion decision-making for injured patients: an interview study. Emerg. Med. J. *40*, 777–784. https://doi.org/10.1136/emermed-2023-213086.

114. del Junco, D.J., Bulger, E.M., Fox, E.E., Holcomb, J.B., Brasel, K.J., Hoyt, D.B., Grady, J.J., Duran, S., Klotz, P., Dubick, M.A., et al. (2015). Collider bias in trauma comparative effectiveness research: The stratification blues for systematic reviews. Injury *46*, 775–780. https://doi.org/10.1016/j.injury.2015.01.043.

115. Yazer, M.H., Seheult, J.N., Beckett, A., Triulzi, D.J., and Spinella, P.C. (2022). Rebirth of the cool: the modern renaissance of low titer group O whole blood for treating massively bleeding civilian patients. Ann. Blood *7*, 17. https://doi.org/10.21037/aob-21-34.

116. Eldjarn, G.H., Ferkingstad, E., Lund, S.H., Helgason, H., Magnusson, O.T., Gunnarsdottir, K., Olafsdottir, T.A., Halldorsson, B.V., Olason, P.I., Zink, F., et al. (2023). Large-scale plasma proteomics comparisons through genetics and disease associations. Nature *622*, 348–358. https://doi.org/10.1038/s41586-023-06563-x.

117. Hattori, K., Ota, M., Sasayama, D., Yoshida, S., Matsumura, R., Miyakawa, T., Yokota, Y., Yamaguchi, S., Noda, T., Teraishi, T., et al. (2015). Increased cerebrospinal fluid fibrinogen in major depressive disorder. Sci. Rep. *5*, 11412. https://doi.org/10.1038/srep11412.

118. Raffield, L.M., Dang, H., Pratte, K.A., Jacobson, S., Gillenwater, L.A., Ampleford, E., Barjaktarevic, I., Basta, P., Clish, C.B., Comellas, A.P., et al. (2020). Comparison of Proteomic Assessment Methods in Multiple Cohort Studies. Proteomics *20*, e1900278. https://doi.org/10.1002/pmic.201900278.

119. Gold, L., Ayers, D., Bertino, J., Bock, C., Bock, A., Brody, E.N., Carter, J., Dalby, A.B., Eaton, B.E., Fitzwater, T., et al. (2010). Aptamer-Based Multiplexed Proteomic Technology for Biomarker Discovery. PLoS One *5*, e15004. https://doi.org/10.1371/journal.pone.0015004.

120. Choung, R.S., Petralia, F., Torres, J., Ungaro, R.C., Porter, C., Sato, T., Telesco, S., Strauss, R.S., Plevy, S., Princen, F., et al. (2023). Preclinical Serological Signatures are Associated With Complicated Crohn's Disease Phenotype at Diagnosis. Clin. Gastroenterol. Hepatol. *21*, 2928–2937.e12. https://doi.org/10.1016/j.cgh.2023.01.033.

121. Faquih, T., Mook-Kanamori, D.O., Rosendaal, F.R., Baglin, T., Willems van Dijk, K., and van Hylckama Vlieg, A. (2021). Agreement of aptamer proteomics with standard methods for measuring venous thrombosis biomarkers. Res. Pract. Thromb. Haemost. *5*, e12526. https://doi.org/10.1002/rth2.12526.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Plasma samples from patients enrolled in the SWAT study	Sperry et al.[16]	N/A
Plasma samples from donors	FDA-Licensed blood collector	N/A
Critical commercial assays		
SomaScan® v4 assay	SomaLogic	SS-2216993
Deposited data		
SWAT cohort proteomics dataset	This paper	Zenodo: https://doi.org/10.5281/zenodo.14757173
Software and algorithms		
Python version 3.9	Python Software Foundation	https://www.python.org
Analytical programming codes	This paper	Github: https://github.com/moheimanih/SWAT_proteomics_study

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Shock, Whole Blood, and Assessment of TBI (SWAT) was a prospective cohort study with a planned enrollment period of 3.5 years. It was performed using seven busy level 1 trauma centers participating in the Linking Investigations in Trauma and Emergency Services (LITES, https://www.litesnetwork.org) clinical trials network with IRB approval from the University of Pittsburgh and the Human Research Protection Office of the Department of Defense. The single IRB approved both a waiver or alteration of the consent process and a waiver of Health Insurance Portability and Accountability Act authorization for a 36-h period. Treatment allocation was based on centers following their respective local resuscitation protocols, and specific indications for LTOWB transfusion (childbearing age status, etc.) were also at the discretion of each site's local resuscitation protocols.

Details of the cohort methodology have been reported previously and will be briefly described here.[16] The study included all eligible patients within the pre-planned enrollment period, determining the final sample size. The inclusion criteria were injured patients at risk of massive transfusion who met Assessment of Blood Consumption criteria that is two or more of the following: hypotension (systolic blood pressure $\leq$90 mmHg); penetrating mechanism of injury; positive Focused Assessment for the Sonography of Trauma (FAST) examination; heart rate $\geq$120; and who within 60 min of arrival required both transfusion and hemorrhage control procedures in the operating room or interventional radiology suite. The inclusion criteria also included patients with qualifying vital signs or receiving blood products during prehospital care. Exclusion criteria included age less than 15 years, penetrating brain injury, >5 min of consecutive CPR, death before initiation of hemorrhage control procedures, known prisoners, and known pregnancy.

Overall, out of 4440 patients assessed for eligibility, 1051 met all criteria and were included in the main SWAT cohort study. For the current work, a sub-cohort of 134 patients whose injury spanned TBI, NBI, blunt, and penetrating mechanisms and had all three research draw samples available were designated as the "Omic" sub-cohort and selected for proteomics analysis. In addition, 20 donor plasma samples not directly associated with the SWAT cohort were acquired from a local FDA-licensed blood collector and included in the omic analysis to aid reference setting and comparison.

METHOD DETAILS

Multiplexed proteomic assay

For multiplexed protein measurements, 100 μL of plasma from each of the 402 patients and 20 donor EDTA-treated samples were aliquoted and shipped on dry ice to SomaLogic Inc. (Boulder, CO, USA). SomaScan assay adopts an aptamer-based approach that can measure more than 7000 proteins in human plasma samples. The technical details have been explained elsewhere.[119] Briefly, this assay is based on protein-binding SOMAmer reagents constructed by modifying single-stranded DNA molecules. Sample protein levels are estimated by transforming protein concentrations into corresponding signatures of aptamer concentrations, which are then detected and quantified by DNA microarrays.

Quality control of proteomic data

Hybridization and calibration procedures were used to remove systematic biases from raw SomaScan assay data. The first normalization step involves using hybridization control that mitigates variation within the run that comes from the readout steps (transfer to Agilent slides, hybridization, wash, and scan) in individual microarrays. This is followed by median signal normalization across pooled calibrator replicates to mitigate within-run technical variation in the calibrator signal. The set of ratios of the calibrator reference value to the median of calibrator replicates for each SOMAmer reagent is calculated and decomposed into two terms: A plate scale adjusts for overall signal intensity differences, and a calibration scale adjusts SOMAmer reagent-specific assay differences between runs. Median signal normalization is performed using Adaptive Normalization by Maximum Likelihood. A total of 488 out of 7596 SOMAmers were identified as 'In Tails' during quality control, meaning their ratios on any plate were outside the accepted reference range of 0.8–1.2. Following closer evaluation, it was decided that "In Tail" values of this study could be explained by dramatic post-trauma deviation of concentrations from the standard reference. Therefore, no SOMAmer measurement was removed from the main analyses. Overall, in line with previous investigations,27 10, 50, and 90 percentiles of quality control CVs were estimated at 2.6%, 4.3%, and 8.8% respectively. Following previous orthogonal validation of SomaLogic measurements, the protein associated with SomaID SL000022 was annotated as "Fibrinogen", SL000424 as "Fibrinogen alpha chain", and SL003341 as "Fibrinogen gamma chain-dimer".[117,120,121] All other annotations are based on SomaLogic reference annotations.

QUANTIFICATION AND STATISTICAL ANALYSIS

Normalization, transformation, and scaling

Clinical data were collected from the research electronic data capture (REDCap) online data repository for all participating sites. Missingness was assessed at the individual patient level as well as independent and dependent variable levels. In the Omic sub-cohort, all clinical independent variables had less than 5% of missing data, and no individual had missing values for more than three independent variables. Only pre-hospital GCS (24%) had above 20% missingness in the total cohort. Individuals with missing values for at least half of (i.e., 13) independent variables of interest were removed from the total cohort in all downstream analyses (remaining $n = 956$). Multiple imputations by chained equation (30 iterations of a random forest model) were implemented to fill the remaining missing values to account for the possibility of random unrecording. Tables 1 and S3 show descriptive statistics after the imputation procedure was completed. In the case of clinical lab measures used as outcomes, however, there was considerably more missingness both in the Omic sub-cohort (admission time PT/INR missing at 21%) and total cohort (admission time PT/INR 37%, platelet count 50%). To eliminate the possibility of non-random missingness influencing clinical interpretations, the downstream propensity-matched comparing coagulation measures were only performed on patients with complete coagulation data records.

Before downstream analysis, the proteomic data were log2 transformed to approximate the normal distribution, and subsequently, all clinical and proteomic variables were standardized (Z score) across each variable before modeling. Unless otherwise specified throughout the paper, the multiple testing adjustments for p-values were performed using the Benjamini-Hochberg false discovery rate (BH-FDR), and effect size for continuous variables was measured by Cohen's D.

Statistical modeling and software

Visualization

Volcano plots simultaneously visualize the size and significance of between-group differences for several proteins on a scatterplot. The x axis shows the standardized difference between (Z score) log2 transformed values of proteins. The y axis shows the log10 transformation of Benjamini-Hochberg-adjusted p-values obtained from the non-parametric Mann-Whitney U test of mean group differences. Thresholds for proteins of interest are shown with vertical (standardized difference threshold) and horizontal (adj-p-value threshold) lines.

Heatmaps show relative levels of proteins across different patient groups and treatments. For each protein, patient/treatment groups that, on average, have higher standardized values of that protein will be shown with a warmer (red) color. The intensity of blue or cold color signifies low relative levels.

Pathway enrichment analysis

Pathway enrichment analysis is a statistical technique that adds to mechanistic insights by identifying biological pathways with differences in their gene expression (or associated protein levels) more than we would expect by chance alone. Here, we used Gene Ontology and Reactome Databases to search for differentially expressed pathways (protein sets) between time points or treatment groups of interest. In GSEA visualizations, we have utilized color intensity to signify multiple test adjusted $p < 0.05$ unless explicitly noted in the legends.

Dimensionality reduction

We used dimensionality reduction with the principal component analysis (PCA) to show how the proteomic profile of each sample places it close or far from patients having a specific characteristic. PCA was conducted using standardized proteomics data from 198 selected proteins (Table S7) of all 3-time points. The first two dimensions were used to visualize each sample on a scatterplot, and then, each sample was colored according to its clinical category. The significance of differences in the first two principal components for each dichotomous categorization was then estimated using the Mann-Whitney U test.

In this study, PCA was also used to derive biomarker-estimated Brain and Heart Injury Scores using two sets of proteins that are known to be markers of brain (GFAP, UCHL1, NSE, NEFL, NEFH, and MAPT,[60] see Table S2) and heart muscle injuries (cTnT, cTnI, ANF, BNP, MYBPC, H-FABP). The first three dimensions of each organ-specific PCA were assessed regarding their relationship with different clinical injury metrics (Table S2). It was decided that Brain PC2, and Heart PC1 were specific markers of associated tissue injuries, and their values were used as the Brain Injury and Heart Injury Scores. High and low injury categories were defined based on individual values placed higher or lower than the median value.

Confounder adjustment with predictive modeling and partial correlations

To emulate a randomized design, our choice of clinical covariates included major early injury characteristics and patient status (ISS, Head AIS, prehospital GCS, heart rate, systolic blood pressure, respiratory rate, age, and gender). Of note, while the presence of TBI for the main cohort study was designated by positive CT scan brain imaging, here, to approximate early clinical decision-making, we defined patients with head AIS values of 3, 4, and 5 as having suffered a traumatic brain injury. The inclusion of different treatments as confounders and nuances of adjustment schemes depended on the specific objective of each analysis and -as detailed below- differed between the sections.

For protein group heatmaps, we aimed to compare patients who had received Plasma (or LTOWB) to patients who had not, regardless of other product treatments. Therefore, we chose a propensity weighting scheme that would bring balance between receivers and non-receivers. First, we built a logistic regression model that predicted receiving plasma (or LTOWB) treatment by the 24-h time point. The model was built on the total cohort excluding the omic subset (n = 822), and its inputs were: Age, gender, head injury (AIS), ISS, pre-hospital shock index, respiratory rate, and GCS, total fluid volumes, platelet components, cryoprecipitate, and LTOWB (or plasma) received by 24h (Figure S1). Next, we assigned a probability of not receiving the respective treatment for each individual of the Omic sub-cohort (n = 134) and weighted each patient with this probability to reverse the effect of confounders in the subsequent analysis. In Figures S7–S12, sub-figures C and D have undergone this adjustment. Effective post-weighting sample sizes were estimated at 67 for comparing plasma to no plasma group, and 118 for comparing LTOWB and no LTOWB groups using the following estimation:

Effective n = $(\sum w_i)^2/\sum(w_i^2)$, where w_i is the weight of the i-th individual.

Due to major differences in patients who had received only one of the treatments before the 0h time point, for the volcano plot and GSEA analysis in Figures 2B and 2D we took another adjustment path that would be more robust to logistical and center-dependent confounders. We used logistic regression to create a model that predicts patient illness resolution defined as surviving at least a month with an ICU stay of 10 days or less. This composite definition of resolution is consistent with our previous work.[34] We chose 10 as the threshold, due to it being the 75th percentile of ICU length stay in the SWAT cohort. The model was built on the total cohort excluding the Omic subset and its inputs were: age, gender, ISS, head injury, prehospital GCS, earliest measured heart rate, respiratory rate, systolic blood pressure, and total prehospital fluids and blood products received (Figure S13). Resolution probabilities were then derived by testing the model on the Omic sub-cohort. Using the model outputs, we performed head-to-head plasma vs. LTOWB GSEA analysis only in the severely injured (ISS>24) patients that had a resolution probability <90%.

Assessing the relationship between the amount of plasma received by patients and coagulation parameters required more direct adjustments for the ratio of products received. Here, we defined "plasma share" (and similarly other product "share"s) as the proportion of the total amount of plasma received to total blood product units by admission. Then, we assessed the relationship of this well-defined treatment with admission time PT/INR using a partial Spearman correlation that took other relevant covariates (similar set to the resolution model above) into account.

Protein correlation network and module detection

We performed a multi-step process to find modules of highly correlated proteins and calculate the plasma signature score of each module. First, we generated a network where each of the selected proteins constituted a node, and edge weights were calculated according to the formula: $R=([r_{0h}+r_{24h}]/2)^2 + r^2_{0h\text{-}to\text{-}24h}$, $Edge\_weight = (R/2)^2$.

Where r_{0h} and r_{24h} are Spearman correlation values of the 198 selected proteins at 0h and 24h, respectively. The $r_{0h\text{-}to\text{-}24h}$ term is the Spearman correlation for 0 to 24h changes of protein values. After trialing powers of 1, 2, and 4 of R for edge weights, the power of 2 was observed to provide a good balance of within-cluster similarity and constituent number. Overall, this weighting structure allowed us to maximize the probability of homogeneous and consistent protein clusters across time points while keeping the edge weights connected to the interpretable framework of ranked R-squared.

Next, we performed the Leiden community detection algorithm[56] on the drawn network that initially yielded 9 clusters with at least two proteins. The influence of each protein in their cluster was quantified with eigenvector-centrality values.[57] To ease interpretation, arbitrary thresholds of 0.01, 0.25, and 0.5 were used as cut-off values for minimal, low, moderate, and high protein centrality and are presented as module contributions in Tables S9 and S10. Module constituents with minimal eigen-centrality were removed from downstream analysis. Due to the unsigned nature of the edge weights, four clusters included both positively and negatively correlated proteins, which were not amenable to providing a single summary score. These four clusters were each divided into two modules of positively correlated proteins, yielding a new total of 13 distinct modules (and separating SURF and FLAME1 modules in the process). All final modules and their constituents are shown in Table S8. Of note, 20% of the selected proteins were either clustered alone or were minimally influential and are therefore designated as uncategorized.

We assessed the sensitivity of modules to detection techniques and our weighting scheme by performing two additional clustering methods. The Louvain network community detection was implemented on a network generated with the same weights as the main

method described above (Figure S14A). As an additional non-network-based method, an Euclidean-distance-based affinity propagation procedure was performed with 0 and 24h values of all proteins. The resulting clusters were visualized on a 2-D densmap (nonlinear method for dimension reduction) generated on 198 protein values (Figure S14B). Two hemostasis-associated modules (called CLOT and ALPHA) were thoroughly consistent across all methods. Affinity propagation selected fibrinogen and platelet-derived growth factor B as respective module examplars. Three immune-associated models (called FLAME1, FLAME2, and SURF) experienced some levels of merging and/or more fine-clustering by Louvain and affinity propagation.

To calculate a composite module signature score, we first standardized the protein levels, then averaged the eigen-centrality-weighted values[57] of each of the Leiden-detected module constituents. The resulting module signature scores were used for downstream analysis.

Causal forests for treatment effect on proteomic modules

We utilized the doubly robust Causal Forest machine-learning framework to eliminate the influence of known confounders and generate insights into the moderating associations of each covariate (heterogeneity in response to treatments).[58,59] Random forest classifier and light gradient boosting regressor were respectively selected as treatment and outcome models and ran for 500 iterations, with other hyperparameters left as default). For all analyses, we included relevant covariates, namely age, gender, head injury (AIS), ISS, pre-hospital shock index, respiratory rate, GCS, fluid volumes, PRBC, platelets, cryoprecipitate, and LTOWB (or plasma) received to be assessed both as confounders and modifiers of plasma (or LTOWB) associations. All inputs were standardized across all participants. The treatment variable for plasma (or LTOWB) was filled as 1 if received, and 0 if not received. All treatment interventions were calculated as received by the research draw time point. In the Omic sub-cohort, we ran this model three times (one for each time point) to assess plasma treatment associations with standardized values of all 13 protein modules. The analysis was repeated for LTOWB treatment. For each treatment (plasma or LTOWB), the multiple test adjustments of p-values were performed using the Benjamini-Hochberg method.

Based on the consistency of modules and the significance of treatment associations, three tiers of modules were selected for downstream analysis at different levels of detail. Tier one included homogeneous ALPHA and CLOT modules that were identified consistently and showed significant associations with treatments at more than one time point. These two were thoroughly analyzed regarding differential treatment associations and moderators. Additionally, to assess the sensitivity of results to signature score calculation methods, a second score for ALPHA and CLOT modules was calculated in which their protein constituents were equally weighted. The results were visualized in error plots that were generated after performing the propensity-weighting scheme explained in the confounder adjustment section above. As Figure S14C shows, other than the CLOT module level at 24h, which showed very high variance in most groups, the observed treatment associations confirmed the primary findings.

FLAME1, FLAME2, and SURF were more heterogeneous, fairly consistent across algorithms, and showed significant (nominal or adjusted) associations with treatments at more than one time point and were therefore included in the second tier. Analysis of these second-tier treatment associations and their moderators are available briefly in the results and in detail in the supplements. A third tier of Leiden-detected modules were sensitive to the choice clustering algorithm but were selected for a brief discussion in Figure S19 based on showing some treatment associations. In all figures presenting temporal module levels, the error bars show mean ± SEM at each time point), and Gold and Purple asterisks indicate $p < 0.05$ for nominal and adjusted p-values, respectively.

In all modules of interest, effect modification was assessed across seven patient/injury categories: Male vs. Female; Age>65 vs. <35; ISS>24 vs. =<24; head AIS ≥3 vs. <3, shock index>=1.25 vs. <1.25; platelet received vs. not received; cryoprecipitate received vs. not; LTOWB (or plasma) received vs. not. Treatment associations conditioned on the variable of interest were calculated for each category, while the causal forest model adjusted for other confounders. False discovery rate adjusted p-value <0.01 on the Mann-Whitney U test and between-subgroup effect size difference >0.5 were used as the significant and substantial effect modification threshold, and its instances were marked by a purple asterisk in associated figures.

Causal forests for treatment effect on the coagulation parameters

In the total cohort, a similarly designed causal forest model was used to assess plasma treatment (input as 1 or 0) associations with standardized PT values. For this analysis, all patients (including those with treatment overlap and imputed PT value) were included (n = 956). This was followed by an assessment of significant effect modification across the following categories: patient/injury categories: Male vs. Female; Age>65 vs. <35; ISS>24 vs. =<24; head Ais ≥3 vs. <3, shock index>=1.25 vs. <1.25.

Propensity score matching for coagulation parameter comparison.

To robustly compare coagulation parameters between plasma and LTOWB receivers, we first identified 447 patients whose treatment by admission time did not include both plasma and LTOWB. As shown in Table S3, this subset received either plasma (n = 258) or LTOWB (n = 189) but not both. After removing patients with missing PT/INR data at 0h (remaining n = 301), we performed propensity matching using a logistic model taking age, gender, ISS, prehospital shock, RR, GCS, and platelet, cryoprecipitate, and fluid and total blood product volumes received as of admission time as confounders. Matching with a caliper of 0.15 resulted in 35 LTOWB-only and 36 plasma-only patients (Figure S20A). The Mann-Whitney U test was used to compare PT, INR, and platelet count between Plasma-only and LTOWB-only groups. The sensitivity of the results to confounders was assessed by common cause refutation implementation of the Python DoWhy library, with an estimated p-value comparing the associations before and after adding a random common cause for treatments and outcomes.

Multivariable modeling of resolution and total transfusion volumes

A linear multivariable regression model was built to assess the relationship of resolution (defined as surviving a month with ICU stay $\leq$10 days) with the following covariates: Age, gender, ISS, brain injury, penetrating mechanism, prehospital GCS, earliest systolic blood pressure, heart rate, respiratory rate, total fluids, plasma, LTOWB, and other components (sum total of packed RBC, platelet component, cryoprecipitate) received by admission. Extreme outliers of transfusion volumes were Winsorized at the 99th percentile. We repeated this model on a limited sample of 318 patients who had received at least one unit of plasma and LTOWB by 4 h after admission (Table S4). A secondary analysis in this subset was performed to assess the relationship of the same covariates with the total first-day (0h–24h) transfusion volume. Four Interaction terms between plasma and LTOWB with brain and penetrating injury were introduced to the preliminary model. The only significant interaction term that was kept in the final model was the (TBI) *(plasma treatment) term. The model was generated with robust (heteroskedasticity controlled) standard errors. A sensitivity analysis that utilized product shares (as a proportion of total prehospital transfusion volume) instead of units was performed with structural equations modeling that received treatment center as a random effects variable (see Table S5 for results and the complete list of covariates).

Additionally, A similar set of covariates was used for partial Spearman correlation analyses that assessed the relationship between plasma units received by admission and total first-day transfusion in each brain injury, shock, and injury mechanism subgroup (Table S6).

Software

All analyses were performed using Python programming language (v. 3.9.15), using the following libraries: pandas (v. 2.2) and numpy (v. 1.23) for data frame manipulation and descriptive statistics; miceforest (v. 5.6) for multiple imputations; scipy (v. 1.12) and Pinguin (v. 0.5) for statistical tests, including the Spearman rank (partial) correlation, Mann-Whitney U test of non-parametric between-group comparisons, and mediation analysis; statsmodels (v. 0.14) and semopy (v. 2.3) for multivariable linear regression and structural equations modeling; gseapy (v. 1.0) for gene set enrichment analysis; sklearn (v. 1.2) for affinity propagation, principal component analysis and all instances of logistic regression and random forest models; lightgbm (v. 5.5) for light gradient boosting; umap (v. 0.5) for Densmap generation; networkx (v. 3.2) and igraph (v. 0.10) for correlation networks; leidenalg (v. 0.10) community (v. 0.16) for Leiden and Louvain community detection algorithms; econml (v. 0.14) for causal forest models and heterogeneity assessment; psmpy (v. 0.3) for propensity matching; dowhay (v. 0.11) for common cause refutation.

All visualizations were created with Microsoft Excel (Microsoft Inc, Redmond, WA, USA) And seaborn (v. 0.13), and matplotlib (v. 3.8) libraries of Python. The visual abstract was created on a BioRender.com template. Figure panels were designed with Adobe Illustrator (Adobe Inc, San Jose, CA, USA). All software licensing was provided by the University of Pittsburgh.

ADDITIONAL RESOURCES

ClinicalTrials.gov, ID: NCT03402035.