Trauma Sub-Endotypes Identified Using Latent Class Analysis Have a Differential Response to Blood Product Transfusion Ratios. A secondary analysis of the PROPPR Randomized Trial

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Abstract

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Background: The American Red Cross declared the first ever national blood shortage crisis amid the COVID epidemic in 2022. In severe traumatic injury, large volumes of blood products are often required to resuscitate patients with hemorrhagic shock. A 1:1:1 transfusion ratio (plasma:platelets:red blood cells) compared to a 1:1:2 ratio improves time to bleeding cessation in severely injured patients with hemorrhagic shock, but has not been associated with reduced mortality in randomized trials. It remains unclear whether certain patients within the trauma population benefit from a 1:1:1 or 1:1:2 resuscitation strategy. My objective was to derive trauma sub-endotypes using latent class analysis (LCA) of molecular data and determine whether these subgroups were associated with mortality and differential treatment response to 1:1:1 vs. 1:1:2 resuscitation strategies.

Methods: A secondary analysis of the PROPPR trial, a randomized trial published in *JAMA* in 2015 that demonstrated no difference in 30-day mortality between the two blood transfusion resuscitation groups. LCA was performed on a panel of twelve plasma biomarkers in 478 severely injured patients (out of a total of 680 patients in the original PROPPR Trial) who had a complete panel of biomarkers drawn at the time of presentation and before blood product resuscitation. I tested for an association between trauma endotypes and 30-day mortality using multivariable relative risk (RR) regression adjusting for age, sex, trauma center, mechanism of injury, and injury severity score (ISS). I tested for a differential treatment response to transfusion strategy using a RR regression model for 30-day mortality by incorporating an interaction term for the product of endotype group and treatment group adjusting for age, sex, trauma center, mechanism of injury, and ISS.

Results: LCA identified two endotype groups as the optimal model within the population. Trauma endotype-1 (TE-1, n = 248) was associated with significantly higher risk for 30-day mortality compared with trauma endotype-2 (TE-2, n = 230). Mortality in TE-1 was 28.6% with 1:1:2 treatment vs. 32.6% with 1:1:1 treatment,

whereas mortality in TE-2 was 24.5% with 1:1:2 treatment vs. 7.3% with 1:1:1 treatment. There was a significant interaction between treatment arm and TE for 30-day mortality (*p*-value for interaction = 0.02).

Conclusions: Endotypes derived from plasma biomarkers in trauma patients at hospital arrival were associated with a differential response to 1:1:1 vs. 1:1:2 resuscitation strategies in severely injured trauma patients. These findings support the concept of molecular heterogeneity in critically ill trauma populations and have implications for tailoring therapy for patients at high risk for adverse outcomes.

Background and Significance:

Major trauma comprises 10% of all admissions to the intensive care unit (ICU), is the leading cause of death among people under the age of 45, and accounts for approximately $37 billion in U.S. healthcare costs annually[1,2]. An estimated 40% of trauma deaths involve massive hemorrhage, and rapid hemorrhage control coupled with early resuscitation are the two principles of initial management[3,4]. The Transfusion of Plasma, Platelets, and Red Blood Cells in a 1:1:1 vs a 1:1:2 Ratio and Mortality in Patients with Severe Trauma (PROPPR) trial assessed the safety and efficacy of a 1:1:1 ratio (plasma: platelets: RBCs) compared with a ratio of 1:1:2 in patients with hemorrhagic trauma[5,6]. Patients randomized to 1:1:1 were more likely to achieve hemostasis, but there were no differences in the primary outcomes of mortality at 24 hours or 30 days between resuscitation groups. In 2022, the American Red Cross declared a national blood crisis due to a shortage of blood products, noting that it was the worst shortage in over a decade[7]. Thus ensuring the appropriate ratio of blood product transfusion is not only important for the treatment of severe hemorrhagic shock, but also to ensure appropriate conservation of a precious resource due to critical shortage.

Heterogeneity within the trauma population may have been one of the contributing factors for why the PROPPR Trial failed to identify a significant signal for mortality benefit in 1:1:1 vs. 1:1:2 resuscitation. Sub-phenotypes of critically ill populations with sepsis[8], acute respiratory distress syndrome (ARDS)[9,10], and acute kidney injury (AKI)[11,12] have previously been identified by applying clustering algorithms to molecular measurements. Identification of these biologic endotypes have deepened our understanding of disease pathogenesis and helped identify sub-groups of patients with differing risk for poor clinical outcomes and response to therapies[10,13].

I hypothesized that unique molecular endotypes exist early in the presentation of hemorrhagic trauma, and these molecular endotypes are associated with differential treatment response to resuscitation strategies. In this analysis, I applied latent class analysis (LCA) to 36 pre-specified plasma biomarkers that were measured prior to treatment randomization and resuscitation in the PROPPR trial to identify sub-phenotypes within the trauma population. For my primary analysis, I tested whether identified endotypes were associated with a primary outcome of mortality at 30 days. Secondary outcomes we tested were mortality at 24 hours, death by traumatic brain injury, development of acute kidney injury (AKI), and development of acute respiratory distress syndrome (ARDS). I then performed a secondary analysis to determine if these endotypes responded

differently to 1:1:1 versus 1:1:2 resuscitation, or if no difference was detected between the two treatment arms, as previously identified in the original PROPPR trial.

Methods:

Study Population and Design

The PROPPR trial was a randomized, multicenter trial designed to assess the safety and efficacy of a 1:1:1 transfusion ratio compared with a 1:1:2 transfusion ratio in patients with trauma who were predicted to receive a massive transfusion greater than or equal to 10 units packed red blood cells. The original study included 680 level I trauma patients enrolled from Aug 2012 to Dec 2013 at 12 trauma centers in the US. Severely injured adults requiring the highest trauma level activation and massive transfusion (≥ 10 units of RBCs within 24 hours) were recruited within one hour of emergency department (ED) arrival.[5] Patients were randomized to receive either 1:1:1 or 1:1:2 transfusion ratios. The primary outcomes were 24-hour and 30-day mortality. All clinical data for our analyses were obtained from the original PROPPR datasets.

Biomarker Measurements

I evaluated plasma biomarkers in 478 patients enrolled in the original PROPPR study collected at the time of the first blood draw upon ED arrival. All patients arrived to the hospital directly from the injury site (those transferred from other hospitals were excluded from the original analysis). Patients were enrolled and received blood product resuscitation within an hour of arrival, as specified by the inclusion and exclusion criteria of the original trial[5]. Median time to enrollment was 26 minutes. Plasma biomarkers were measured using a multiplex analyzer (Luminex 100 platform, Luminex, Austin TX). The multiplex kits utilized were standardized panels (Milliplex MAP human cytokine and chemokine kit and Milliplex MAP high sensitivity cytokine kit Millipore, Billerica, MA) and analyzed using Biomanager computer software (Bio-Rad, Hercules, CA)[14,15]. I excluded patients who were missing > 10% of the biomarker measurements because LCA is not robust to missing data.[16]

Derivation of Trauma Endotypes

LCA was utilized for the derivation of trauma endotypes using an approach specified in prior reports[17]. LCA is a commonly used method of finite mixture modeling used to determine if unmeasured or unobserved groups exist within a population[17]. Unobserved or “latent” classes are inferred from patterns of observed variables used within the model. Once the model is fitted, the probability of class membership is estimated for each observation within the cohort, which is used to assign class. Whereas a k-means cluster methodology

utilizes an arbitrary distance measure to identify clusters, LCA provides model fit statistics, which allows for statistical inference when determining the number of appropriate clusters within a population[17].

For this analysis, 36 biomarkers obtained from the multiplex kit were initially considered as class-defining variables for the LCA models (Table S2). Highly correlated biomarkers were removed from the model prior to performing the analysis to minimize the influence of collinearity on cluster assignment. Biomarkers were removed in a stepwise process based on a Pearson's correlation coefficient $r > 0.5$ with any other biomarker (Figure S1), as described in previously reported protocols[11,18]. When two biomarkers had a correlation coefficient > 0.5, one biomarker was removed. When choosing between biomarkers that were highly correlated, specific biomarkers of inflammation, coagulopathy or endothelial injury that have been previously associated with mortality and organ injury in trauma and critical illness in the literature were preferentially selected. One exception was the inclusion of both tumor necrosis factor-alpha and interferon-gamma, which had an r equal to 0.73. These biomarkers were both included because both are easily attainable in the clinical setting and have previously been noted to be associated with illness severity and mortality in other critically ill populations such as sepsis and ARDS[9,19–21]. Twelve biomarkers were ultimately selected (Figure S1). All biomarkers were $\log_2$ transformed due to right-skewed distribution and rescaled to a common z-scale where the mean was set to zero and the standard deviation to 1 because the scales of each biomarker are distinct. Class assignment was conducted without inclusion of any clinical variables or outcome measures.

The Vuong-Lo-Mendell-Rubin (VLMR) likelihood ratio test, which tests whether a model with *n* classes provides a better fit to the data than a model with *n-1* classes, was utilized as the primary test for model fit. Additional criteria were considered, including Bayesian information criteria (BIC, in which lower values indicate model parsimony); the entropy statistic (a measure between 0 and 1, with optimal numbers greater than 0.8 indicating good class separation); and class generalizability, opting to select models with a class prevalence of at least 15% of the population to improve replicability.[17,22] LCA analysis was performed using Mplus v7.11.

Power Calculation

To determine power in LCA, I used bootstrapped likelihood ratio estimates[23]. I determined that I would need a sample size of at least 150 patients to differentiate a three-class model from a two-class model. Based on the

478 patients in the PROPPR trial, sample size was sufficient to compare the two models. Once groups were identified, we anticipated 80% power to detect a relative risk of 1.28 for mortality at 30 days assuming a Type 1 error rate of 5%, two sub-phenotype groups with prevalences of 40% and 60%, and a total population mortality of 25%.

Statistical Analysis

I determined the relative risk (RR) for the association of endotype class assignment with the primary outcome (30-day mortality) using a generalized linear model with a log link and robust Huber-White standard errors. The model was adjusted for age, sex, trauma center, injury mechanism (blunt vs. penetrating injury), and injury severity score (ISS)[24]. Trauma center was a categorical variable (labeled 1-12) for the twelve trauma centers that enrolled patients in the PROPPR trial. ISS, a validated score ranging from 0 to 75, with higher scores associated with increased risk for mortality[24], was modeled as a binary categorical variable, above or below the median score of 26. Secondary outcomes assessed included development of acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), death due to traumatic brain injury (TBI) and 24-hour mortality.

To examine whether the effect of transfusion strategy on mortality varied in subgroups defined by endotype and by other clinical characteristics, I fit four models, each with an interaction term for the product of treatment group with one of the following characteristics: endotype assignment, injury mechanism, ISS, and serious head trauma (using Abbreviated Injury Scale, or AIS[25], in which scores greater than or equal to 3 are considered “serious” or life threatening). All analyses were adjusted for age, sex, and trauma center location. The analysis with an interaction term for the product of treatment group and endotype assignment was also adjusted for injury mechanism and Injury Severity Score to determine whether treatment effect based on endotype was independent of clinical factors such as injury mechanism or severity of illness. ISS and injury mechanism were not considered confounders in the other three models because they were instead used as the primary independent variables. All analyses were conducted using R (version 4.1), and Stata (version 16).

Results:

Trauma Endotype Classification Reveals Two Distinct Subgroups

I analyzed data from 478 patients after excluding 86 patients that did not have biomarkers drawn in the original trial and an additional 116 patients who were missing > 10% of biomarker measurements (Figure S1). Mortality at 1 hour was higher in the patients who were excluded from our analysis; however, no differences were identified in baseline characteristics, abbreviated injury scores, or 24-hour and 30-day mortality among the 478 patients included and the 202 patients excluded from the analysis (Table S1).

I applied LCA to a panel of twelve plasma biomarkers of inflammation, coagulation and endothelial vessel function measured at the time of hospital presentation to identify molecular endotypes of severely injured patients (Table 1). A two-class model was identified as having the best fit within the sample population, with a VLMR p-value increasing as class number increased from 2 to 4 (VMLR p-value for a two-class model <0.001, Table 2. The two-class model demonstrated good model parsimony (BIC 15817 for the two-class model) and class separation (entropy 0.77) when compared to three and four-class models (Table 2). Fifty-two percent (n = 248) of patients were assigned to trauma-endotype 1 (TE-1) and forty-eight percent (n = 230) were assigned to trauma-endotype 2 (TE-2) (Figure 1). TE-1 was characterized by higher standardized concentrations of all 12 measured biomarkers when compared to TE-2 (Figure 2).

Trauma Endotypes Have Distinct Clinical Characteristics and Outcomes

Baseline demographics were similar among trauma endotypes, however the clinical characteristics of TE-1 and TE-2 were highly distinct (Table 3). Patients assigned to TE-1 were significantly more likely to present with blunt vs. penetrating injury (70% vs. 33% or 174/248 vs. 77/230 patients, $p < 0.01$), require massive transfusion (53% vs. 42% or 145/270 vs. 85/208 patients, $p < 0.01$), and had a higher ISS (median ISS 34 vs. 23, $p < 0.01$) compared to patients assigned to TE-2. Patients assigned to TE-1 were also significantly more likely to present with severe injury to the head, chest, or extremities according to the abbreviated injury score (AIS).[25] Compared with patients with TE-2, patients with TE-1 had higher risks of ARDS, death at 24 hours, and death at 30 days, after adjustment for age, sex, trauma center, injury mechanism and ISS (Table 4).

Trauma Endotypes, But Not Illness Severity or Injury Mechanism, Were Associated with Differential Response to 1:1:1 vs. 1:1:2 Resuscitation

I assessed whether the two endotypes responded differently, or demonstrated effect modification, to randomized transfusion strategies. Treatment with 1:1:1 vs. 1:1:2 was not significantly associated with 30-day mortality when analyzing all patients included in our cohort (Table 5). There was a significant treatment interaction between trauma endotype and resuscitation strategy for 30-day mortality when adjusted for age, sex, trauma center, injury mechanism and injury severity score (p = 0.02, Table 5). Patients assigned to TE-1 had a 30-day mortality of 32% (41 of 128 died) when randomized to a 1:1:2 strategy and a 33% mortality (40 of 120 patients died) when randomized to a 1:1:1 transfusion approach. In contrast, patients assigned to TE-2 had a 21% mortality (23 of 111 died) at 30-days when randomized to a 1:1:2 transfusion strategy, but only a 8% mortality 10 of 119 died) when assigned to 1:1:1.

I next assessed whether other clinically-derived groupings of patients similarly demonstrated effect modification to randomized transfusion strategies, after adjusting for age, sex, and trauma center, There was no significant treatment interaction between mechanism of injury (blunt or penetrating) and treatment resuscitation arm (1:1:1 vs. 1:1:2) for 30-day mortality (p-value for interaction = 0.76). Although there was no statistically significant interaction between ISS and treatment arm for 30-day mortality (p-value for interaction = 0.08), patients with a lower ISS experienced a statistically significant mortality benefit from 1:1:1 vs. 1:1:2 transfusion strategy (Table 5). Finally, although severe head injury was strongly associated with 30-day mortality, there was no differential response to 1:1:1 vs. 1:1:2 treatment between those with and without severe head injury (Table 5).

Discussion:

This study identified two distinct endotypes of severely injured patients with hemorrhagic shock associated with significant differences in 30-day mortality. Importantly, these two endotypes were associated with a differential response to 1:1:1 vs. 1:1:2 transfusion strategies, which was not previously identified in the original PROPPR trial. Differential response was not seen for other clinically-derived subgroups based on injury mechanism or severity. Our findings suggest substantial molecular heterogeneity exists in severely injured trauma patients, which may be identified within a few hours from the time of injury and leveraged to identify the most treatment-responsive trauma endotypes. Since the original PROPPR trial, most trauma centers have developed protocolized resuscitation strategies including a 1:1:1 transfusion ratio for severely injured patients at high risk for hemorrhagic shock.[26,27] However, our findings suggest that 1:1:1 may not benefit all patients equally.

These findings have several implications for the interpretation of the original PROPPR data. First, TE-2 represents a large proportion of patients (48% of the cohort) that likely received a mortality benefit from a 1:1:1 resuscitation strategy. These findings address criticisms that 1:1:1 resuscitation is not associated with a mortality benefit in severe trauma,[28,29] particularly since 1:1:1 treatment has previously shown to improve early hemostasis-related mortality in prior observational studies.[26,30–33] Additional studies are needed to understand why 1:1:1 treatment was not associated with reduced mortality in patients with TE-1. The TE-1 endotype represented patients that were more critically ill, with higher levels of proinflammatory biomarkers, biomarkers of endothelial dysfunction, higher injury severity, and a higher proportion with blunt injury (Table 2). Studies from combat environments have demonstrated that transfusion within minutes of injury in the pre-hospital setting is associated with improved survival compared with delayed transfusion,[34] highlighting the importance of the timing of blood product administration in the most severely injured patients. Finally, this study demonstrates that molecular diagnostics can enrich trauma cohorts with patients most likely to experience clinical benefit from an intervention.[35] Although trauma endotype classification has significant overlap with injury severity, approaches to risk stratify trauma patients by using the ISS[24] alone are limited by the time, resources, and complexity required to ascertain multiple clinical variables and calculate scores.[36] For instance, ISS can only be calculated after a full description of a patient's injuries has been investigated at 24-hours post admission, potentially leading to critical delays in the early identification of patients most in need of additional resuscitation.[36] Future work will require the derivation of a predictive model using these biomarkers to

determine if endotype classification can be determined early in the hospital course for a patient with severe trauma, as well as to see if these endotypes have similar associations in other trauma cohorts beyond massive hemorrhagic shock.

Notably, the twelve biomarkers utilized in the LCA represented a broad range of biologic processes including immune function (IFN-gamma), endothelial function (Ang-2), tissue injury (TIMP1, TFPI), and coagulation (PAP, TFPI). Selected biomarkers of inflammation, including Tumor Necrosis Factor-alpha (TNF-alpha), Interferon gamma (IFN-gamma), Interleukin-6 (IL-6) are recognized for their associations with illness severity and mortality in sepsis[37–39] as well as severe organ injury such as AKI[40,41] and ARDS[9,19–21]. Other selected biomarkers of endothelial injury, including angiopoietin-2 (Ang-2) and Syndecan-1 (SDC-1), have been previously associated with injury severity, AKI[42,43] and mortality in sepsis[44,45] and trauma patients[46–48]. Taken together, we speculate that very early after severe injury, tissue damage, inflammation, and coagulopathy are all highly interconnected and different aspects of the host response as measured through plasma biomarkers may provide an early indication to future complications or treatment responsiveness.

This study has multiple strengths. On review, this analysis of 478 patients represents the largest molecular analysis of patients enrolled into a purely trauma cohort. Additionally, all biomarker measurements were collected from patients within 30 minutes of ED arrival and prior to randomization, meaning treatment assignment could not have confounded our molecular measurements and our endotype classifications effectively represent *de novo* trauma patients. Finally, these findings may help predictively enrich enrollment into future interventional trials testing trauma resuscitation strategies as well as be used as a clinical tool. Leveraging a set of plasma biomarkers to identify those at highest risk for poor outcomes is likely much more efficient and practical in a trauma setting than using clinical risk prediction tools such as the ISS that require a full description of a patient's injuries 24-hours post-hospital admission.[36]

Our study also has several important limitations. First, our study represents a secondary analysis of clinical trial data, and prospective studies testing whether trauma endotypes respond differently to 1:1:1 vs. 1:1:2 are required before any definitive conclusions can be made. Second, patients included in our analyses had lower 1-hour mortality compared with those from the broader PROPPR study who were excluded from our analyses. It is thus possible that selection bias led to our study population being slightly less sick at hospital arrival compared with the overall PROPPR cohort. Third, our biomarker panel – though broad – focused on

molecular signatures we *a priori* hypothesized were associated with severe trauma and clinical outcomes. It is likely other high-dimensional omics approaches could identify trauma endotypes our biomarker panel did not capture. Fourth, only a handful of the twelve biomarkers, including TNF-alpha, IFN-gamma, IL-6 and Ang-2 have the ability to be rapidly processed in the clinical setting. Future study is needed to determine if fewer biomarkers can be used to identify endotype classification, or if an assay consisting of all the biomarkers has practical utility as a classification tool. Additionally, the differences in outcomes between the trauma endotypes are likely driven in part by illness severity. Inclusion of ISS attenuated the associations between trauma endotype and all measures of organ injury, although TE was still significantly associated with 30-day mortality even after adjustment for ISS (Table 4). Finally, we only studied patients at highest risk for trauma-associated hemorrhagic shock, and thus our findings are not generalizable to a broader set of less severely injured patients requiring blood transfusions.

In conclusion, this study identified two novel endotypes of trauma patients based on molecular biomarkers obtained at the time of hospital arrival. Future investigation into these endotypes is warranted to better understand the biologic mechanisms that drive human host response to trauma, as well as to determine whether assignment of trauma endotypes during the early resuscitation phase of hemorrhagic trauma may lead more specific deployment of therapies that ultimately improve outcomes in patients at high risk.

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Table 1. Biomarker Panel Used to Derive Trauma Endotypes

Biomarkers Measured in the PROPPR Trial	
Tumor Necrosis Factor	TNF-α
Interferon-gamma	IFN-γ
Interleukin-1 Receptor Antagonist	IL-1Ra
Interleukin-1 beta	IL-1b
Interleukin-6	IL-6
Advanced glycosylation end-product specific receptor	RAGE
Mannose-Binding Lectin	MBL
Plasmin-α2-antiplasmin complex	PAP
Tissue factor pathway inhibitor	TFPI
TIMP metallopeptidase inhibitor 1	TIMP1
Angiopoietin-2	Ang-2
Syndecan-1	SDC-1

Table 2. Model fit statistics identify a two-class model as having the best fit in a Latent Class Analysis of 478 PROPPR patients

LCA Model	BIC	Entropy	Number of Individuals per Class				VLMR p-value
			N1	N2	N3	N4	
2 Group	15817	0.77	230	248			< 0.001
3 Group	15676	0.82	160	262	56		0.18
4 Group	15530	0.8	87	158	177	56	0.16

The Vuong-Lo-Mendell-Rubin (VLMR) likelihood ratio test, which tests whether class *n* model better fits the data than class *n-1*, was the primary test for model fit. VLMR identified a two-class model as having the best fit. Additional parameters included the Bayesian information criteria (BIC),in which lower values indicate model parsimony, and the entropy statistic, a measure between 0 and 1, with optimal numbers greater than 0.8 indicating good class separation. Additionally, we opt to select models with a class prevalence of at least 15% of the population to improve replicability.

Table 3. Patient Characteristics Overall and in Endotype Groups Defined by Latent Class Analysis

Characteristics	*Total (n = 478)*	*TE-1 (n = 248)*	*TE-2 (n = 230)*	*P-value*
Demographics				
Age, years, median (IQR)	34.5 (25 – 51)	36 (25 – 53)	34 (25 – 50)	0.72
Female – n (%)	94 (20%)	55 (22%)	39 (17%)	0.15
Race/ Ethnicity – n (%)				
Black/African American	133 (27%)	51 (21%)	75 (32%)	< 0.01
Hispanic/Latinx	84(18%)	41 (17%)	43 (19%)	0.54
Asian	22 (4%)	8 (3%)	16 (7%)	0.06
White	313 (65%)	180 (73%)	132 (57%)	< 0.01
BMI kg/m^2, median (IQR)	26 (23 – 30)	26 (24 – 31)	26 (23 – 30)	0.2
Arrival time to Randomization, minutes, median (IQR)	26 (15 – 42)	25 (15 – 41)	28 (15 – 43)	0.5
Mechanism of Injury				
Blunt Injury – n (%)	251 (52%)	174 (70%)	77 (33%)	<0.01
Penetrating Injury – n (%)	222 (46%)	74 (30%)	153 (65%)	<0.01
Lab Values				
Pre-Randomization RBC units – median (IQR)	2 (1 – 3)	2 (1 – 2)	2 (1 – 2)	0.91
Hemoglobin ≤ 11 g/dL – n (%)	175 (38%)	87 (35%)	88 (38%)	0.47
INR > 1.5 – n (%)	78 (27%)	47 (19%)	31 (13%)	0.02
Platelets < 150,000/microliter – n (%)	77 (17%)	41 (19%)	36 (16%)	0.89
Severity of Illness				
Massive Transfusion – n (%)†	230 (48%)	138 (56%)	92 (40%)	<0.01
AIS Head >3 (severe) ††	113 (24%)	77 (31%)	36 (16%)	<0.01
AIS Chest >3 (severe)	293 (61%)	175 (70%)	118 (51%)	< 0.01
Total ISS – median (IQR)	26 (18 – 41)	34 (22 – 43)	24 (14 – 33.5)	<0.01

TE-1 – Trauma Endotype-1, TE-2 – Trauma Endotype-2, AIS – Abbreviated Injury Scale, AKI – acute kidney injury, ARDS – acute respiratory distress syndrome, BMI – Body Mass Index, INR – International Normalized Ratio, ISS – injury severity score, IQR – Interquartile range, RBC – red blood cells.

P-values calculated using Fisher's test for continuous variables or Wilcoxon Rank-Sum test for categorical variables as appropriate

† Defined as ≥ 10 units of RBCs within the first 24 hours
‡ Defined as ≥ 3 units of RBCs received at least once per 1-hour interval during the first 24-hour period
†† AIS greater than or equal to 3 is characterized as severe injury to that body part

Table 4. Patient Outcomes by Trauma Endotype

Outcome	*TE-1 (n = 248)*	*TE-2 (n = 230)*	*Unadjusted RR (95% CI) in TE-1 vs. TE-2*	*P-value*	*Adjusted RR (95% CI)† in TE-1 vs. TE-2*	*P-value*
AKI – n (%)	66 (27%)	46 (20%)	1.33 (0.96 – 1.85)	0.09	1.21 (0.85 - 1.74)	0.28
ARDS – n (%)	50 (20%)	18 (8%)	2.60 (1.55 – 4.28)	< 0.01	1.8 (1.08 – 3.06)	0.02
Death from TBI – n (%)	35 (14%)	9 (4%)	3.66 (1.77 – 7.34)	< 0.01	1.98 (0.98 - 4.01)	0.06
24-hour Mortality – n (%)	44 (18%)	19 (8%)	2.15 (1.29 – 3.57)	< 0.01	1.85 (1.05 - 3.26)	0.03
30-day Mortality – n (%)	82 (33%)	33 (14%)	2.29 (1.59 – 3.28)	< 0.01	1.66 (1.15 - 2.41)	< 0.01

ARDS – Acute Respiratory Distress Syndrome, AKI – Acute Kidney Injury, RR – Relative Risk, TBI – Traumatic Brain Injury, TE-1 – Trauma endotype 1, TE-2 – Trauma endotype 2, CI – confidence interval

† Adjusted for age, sex, trauma center, injury mechanism, and ISS

Table 5. Modification of Transfusion Strategy Effect on 30-Day Mortality by Trauma Endotype, Mechanism of Injury, Injury Severity Score and Severe Head Injury

Class	*1:1:2 Treatment 30-day mortality/total (%)*	*1:1:1 Treatment 30-day mortality/total (%)*	*Relative Risk†(95% CI) for 30-day Mortality for 1:1:1 Resuscitation vs. 1:1:2 Resuscitation*	*P-Value for treatment Interaction*
All Patients				
All Patients (n = 478)	64/238 (27%)	50/240 (21%)	0.79 (0.58 – 1.08)	NA
Trauma Endotypes				
TE-1 (n = 248)	41/128 (32%)	40/120 (33%)	1.14 (0.82 – 1.68)	0.02
TE-2 (n = 230)	23/111 (21%)	10/119 (8%)	0.30 (0.14 – 0.63)	
Mechanism of Injury				
Blunt (n = 256)	46/128 (36%)	34/128 (27%)	0.77 (0.53 – 1.12)	0.76
Penetrating (n = 222)	20/110 (18%)	16/112 (14%)	0.77 (0.42 – 1.42)	
Overall Injury Severity				
ISS > 26 (n = 234)	45/113 (40%)	42/120 (35%)	0.91 (0.65 – 1.27)	0.08
ISS ≤ 26 (n = 244)	19/125 (15%)	8/119 (7%)	0.44 (0.20 – 0.94)	
Severe Head Injury				
AIS Head ≥ 3 (n = 113)	33/61 (54%)	24/52 (46%)	0.88 (0.59 – 1.30)	0.74
AIS Head < 3 (n = 365)	31/178 (17%)	26/187 (14%)	0.79 (0.50 – 1.26)	

AIS – Abbreviated Injury Scale, ISS – Injury Severity Score, TE-1 – Trauma endotype 1, TE-2 – Trauma endotype 2, 1-1-1 Treatment – Transfusion ratio of 1:1:1 (plasma:platelets:red blood cells), 1-1-2 Treatment – Transfusion ratio of 1:1:2 (plasma:platelets:red blood cells),

† All RR models adjusted for age, sex, trauma center. The model for trauma endotype assignment is also adjusted injury mechanism, and injury severity score

Figure 1.

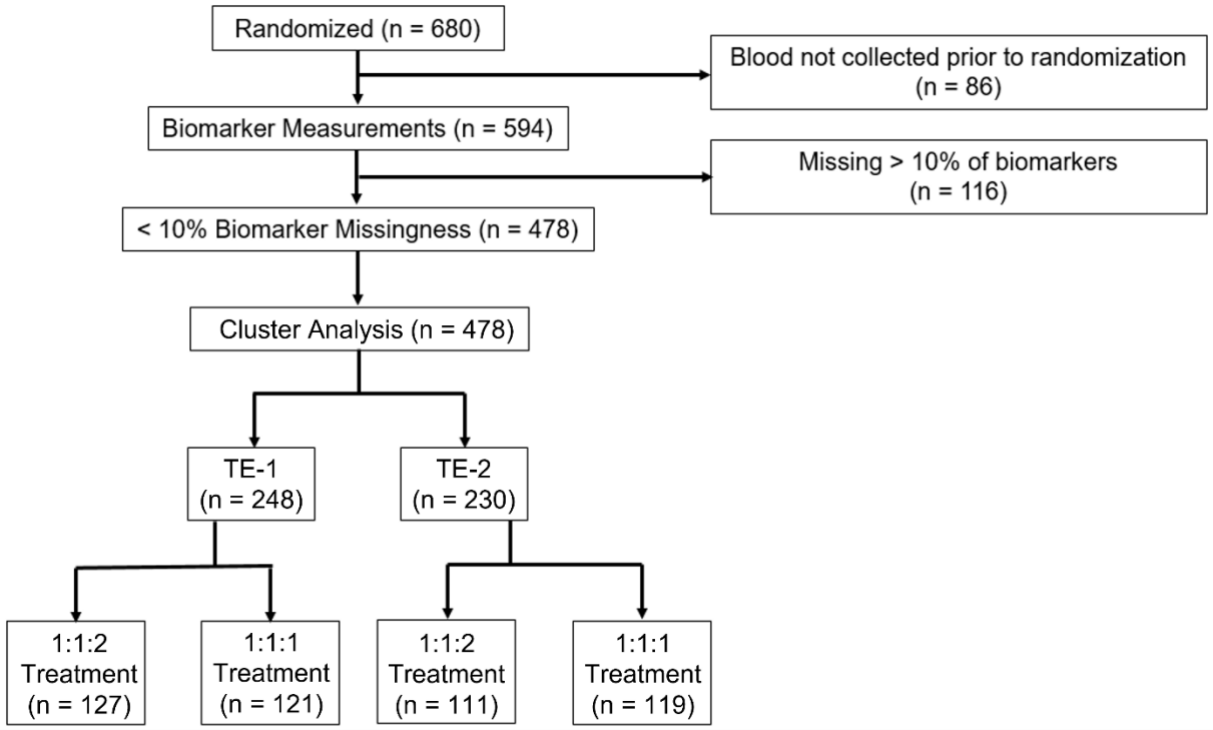

Figure 1. Consort Diagram of Patients Included in the Secondary Analysis of the PROPPR Trial for Classification of Trauma Endotypes.

Figure 2.

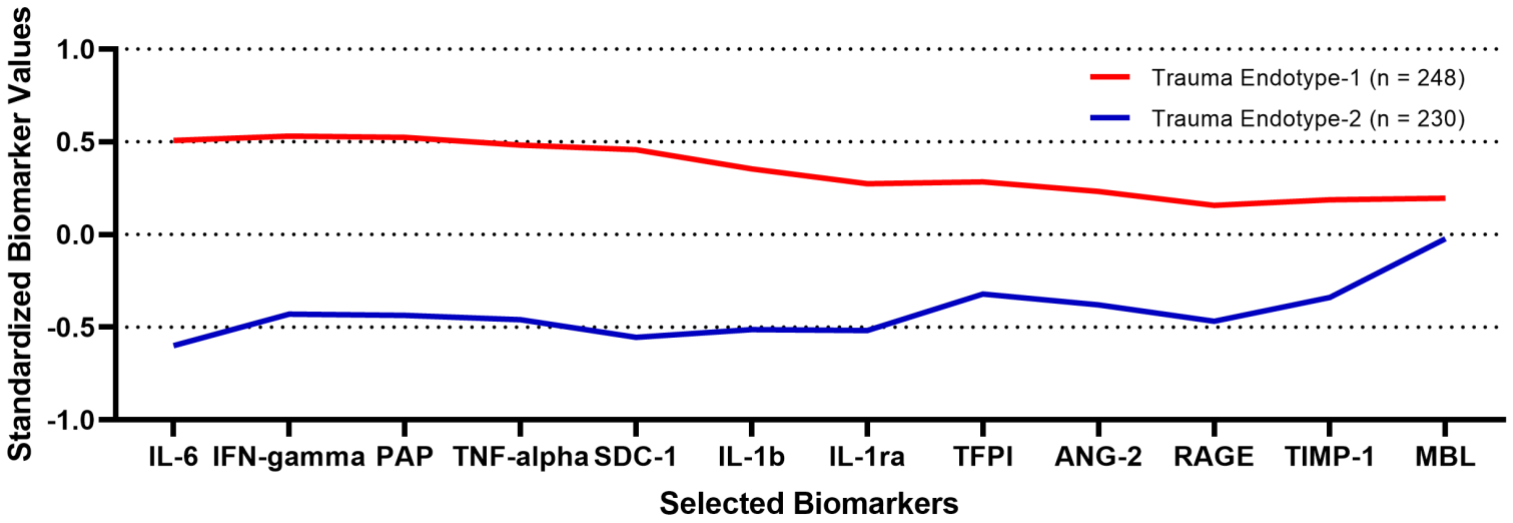

Figure 2. Standardized biomarker values by trauma endotype used in the K-means cluster analysis of patients enrolled in the PROPPR Trial. Biomarker variables were placed on a z-scale with a mean of zero and SD of one. Variables are presented from left to right in order of largest separation between trauma endotype-1 (TE-1) and 2 (TE-2). All standardized biomarkers are higher in TE-1. Biomarkers were selected *a priori* as part of the original PROPPR trial investigation (Table 1).

Supplemental Tables and Figures

Table S1. Subject Characteristics of PROPPR Trial Participants Included in the Secondary Analysis

Characteristics	*Total Included (n = 478)*	*Excluded from analysis (n = 202)*	*P-value*
Demographics			
Age - median (IQR)	34.5 (25 – 51)	33 (23 – 47)	0.28
Female – n (%)	94 (20%)	40 (20%)	0.96
Race/ Ethnicity – n (%)			
Black/African American	133 (27%)	61 (30%)	0.31
Hispanic/Latinx	84(18%)	46 (18%)	0.94
Asian	22 (4%)	6 (3%)	0.37
White	313 (65%)	122 (60%)	0.23
BMI (IQR)	26 (23 – 30)	26 (23 – 31)	0.82
Arrival time to Randomization	26 (15 – 42)	29 (18 – 48)	0.02
Mechanism of Injury			
Blunt Only – n (%)	256 (52%)	102 (50%)	0.46
Penetrating Only – n (%)	227 (48%)	100 (50%)	0.40
Lab Values			
Hemoglobin Level – median (IQR)	11.7 (10.1 – 13.4)	11.9 (9.9 – 13.2)	0.91
Hemoglobin ≤ 11 g/dL – n (%)	175 (38%)	72 (38.0%)	0.95
INR Level – median (IQR)	1.3 (1.12 – 1.54)	1.3 (1.20 – 1.62)	0.81
INR > 1.5 – n (%)	78 (26.5%)	38 (27%)	0.95
Platelet Count – median (IQR)	213 (164 – 261)	215.5 (165 – 263)	0.88
Platelets < 150,000 – n (%)	77 (17.2%)	37 (20%)	0.44
Base Excess – median (IQR)	-8 (-12.1 – -4)	-9 (-14 - -5)	0.03
Severity of Illness			
Massive Transfusion – n (%)†	230 (48%)	83 (41%)	0.08
AIS Head Severe	113 (24%)	40 (20%)	0.27
AIS Thorax Severe	293 (61%)	120 (59%)	0.64
AIS Abdomen Severe	248 (52%)	92 (46%)	0.13
AIS Extremity Severe	211 (44%)	88 (44%)	0.89
Total ISS – median (IQR)	26 (17 – 38)	26 (18 – 41)	0.58
Mortality			
Death within 1 hour	14 (3%)	17(8%)	< 0.001
Death within 12 hours	55 (11.5%)	34 (17%)	0.06
Death within 24 hours	64 (13%)	37 (18%)	0.08
Death within 30 days	114 (24%)	50 (25%)	0.8

AIS – Abbreviated Injury Scale, AKI – acute kidney injury, ARDS – acute respiratory distress syndrome, BMI – Body Mass Index, INR – International Normalized Ratio, IQR – Interquartile range, ISS – injury severity score, RBC – red blood cells.

P-values calculated using Fisher's Exact test or Wilcoxon rank-sum test as appropriate

† Defined as ≥ 10 units of RBCs within the first 24 hours

S2. Thirty-Six Biomarker Panel Used to Derive Trauma Endotypes

Biomarkers Measured in the PROPPR Trial	
Tumor Necrosis Factor (TNF-α)	Platelet- Derived Growth Factor (PDGF)
Interferon-gamma (IFN-γ)	TIMP metallopeptidase inhibitor 1 (TIMP1)
IL-1 Receptor Antagonist (IL-1Ra)	TIMP metallopeptidase inhibitor 2 (TIMP2)
IL-1 beta (IL-1b)	TIMP metallopeptidase inhibitor 3 (TIMP3)
IL-4	TIMP metallopeptidase inhibitor 4 (TIMP4)
IL-5	Advanced glycosylation end-product specific receptor (RAGE)
IL-6	Thrombin–antithrombin complex (TAT)
IL-7	Granulocyte-macrophage colony-stimulating factor (GM-CSF)
IL-8	High mobility group box 1 (HMGB1)
IL-9	Monocyte Chemoattractant Protein-1 (MCP-1)
IL-10	Chemokine Ligand 5 (RANTES)
IL-13	Plasmin-α2-antiplasmin complex (PAP)
Macrophage Inflammatory Protein 1-alpha (MIP-1α)	Eotaxin
Macrophage Inflammatory Protein 1-beta (MIP-1β)	Tissue plasminogen activator (tPA)
Angiopoietin-1 (Ang-1)	Plasminogen activator inhibitor-1 (PAI-1)
Angiopoietin-2 (Ang-2)	Tissue factor pathway inhibitor (TFPI)
Soluble Thrombomodulin (sTM)	Mannose-Binding Lectin (MBL)
Syndecan-1 (SDC-1)	

Due to high degree of collinearity, these biomarkers were ultimately pared down to 12 biomarkers with a correlation of $R < 0.5$

Figure S1.

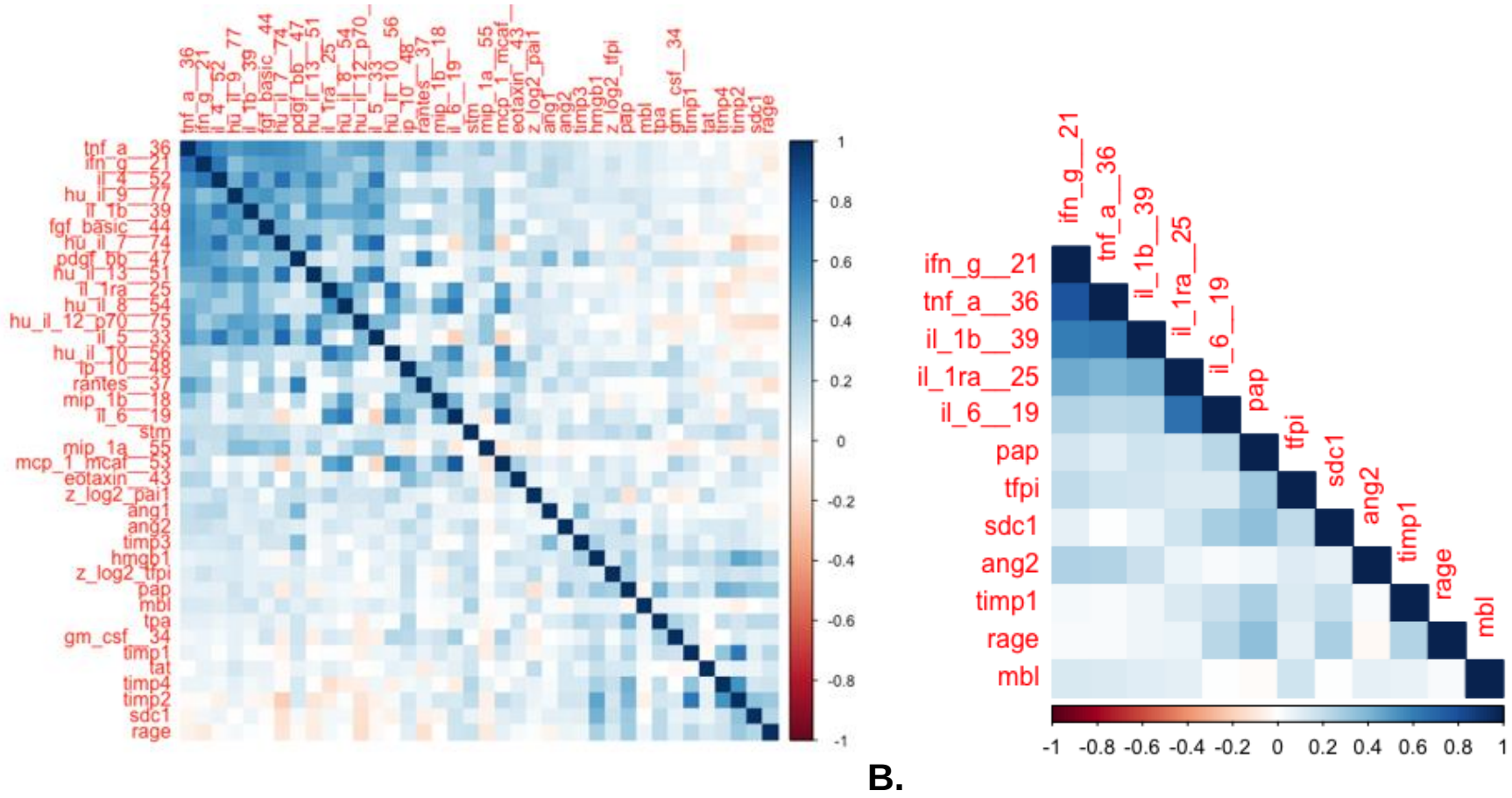

Figure S1. Derivation of Trauma Endotypes using Latent Class Analysis. Pearson's correlation matrix (**Panel A**) of all 36 plasma biomarkers measured in PROPPR. We removed biomarkers in a step-wise fashion based on a Pearson' correlation of > 0.5 with any other variable. If two variables had any correlation > 0.5, one of the biomarkers was removed. The 12 biomarkers that were not highly collinear are shown in **Panel B** and Table 1.