Early dynamic orchestration of immunologic mediators identifies multiply injured patients who are tolerant or sensitive to hemorrhage

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BACKGROUND: Multiply injured patients (MIPs) are at risk of complications including infections, and acute and prolonged organ dysfunction. The immunologic response to injury has been shown to affect outcomes. Recent advances in computational capabilities have shown that early dynamic coordination of the immunologic response is associated with improved outcomes after trauma. We hypothesized that patients who were sensitive or tolerant of hemorrhage would demonstrate differences in dynamic immunologic orchestration within hours of injury.

METHODS: We identified two groups of MIPs who demonstrated distinct clinical tolerance to hemorrhage (n = 10) or distinct clinical sensitivity to hemorrhage (n = 9) from a consecutive cohort of 100 MIPs. Hemorrhage was quantified by integrating elevated shock index values for 24 hours after injury (shock volume). Clinical outcomes were quantified by average Marshall Organ Dysfunction Scores from days 2 to 5 after injury. Shock-sensitive patients had high cumulative organ dysfunction after lower magnitude hemorrhage. Shock-tolerant (ST) patients had low cumulative organ dysfunction after higher magnitude hemorrhage. Computational methods were used to analyze a panel of 20 immunologic mediators collected serially over the initial 72 hours after injury.

RESULTS: Dynamic network analysis demonstrated the ST patients had increased orchestration of cytokines that are reparative and protective including interleukins 9, 17E/25, 21, 22, 23, and 33 during the initial 0- to 8-hour and 8- to 24-hour intervals after injury. Shock-sensitive patients had delayed immunologic orchestration of a network of largely proinflammatory and anti-inflammatory mediators. Elastic net linear regression demonstrated that a group of five mediators could discriminate between shock-sensitive and ST patients.

CONCLUSIONS: Preliminary evidence from this study suggests that early immunologic orchestration discriminates between patients who are notably tolerant or sensitive to hemorrhage. Early orchestration of a group of reparative/protective mediators was amplified in shock-tolerant patients. (*J Trauma Acute Care Surg.* 2021;90: 441–450. Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.)

LEVEL OF EVIDENCE: Prospective clinical outcomes study, level III.

KEY WORDS: Hemorrhagic shock; immunologic response; dynamic network analysis; trauma tolerance; trauma sensitivity.

Multiply injured patients (MIPs) are at risk of developing complications. Typically, complications are related to the magnitude of injury and hemorrhage. For example, in a recent work, the magnitude of cumulative hypoperfusion corresponded to organ dysfunction, duration of mechanical ventilation, and nosocomial infections (NIs).[1] However, there are anecdotal examples of patients who recover uneventfully after major injury or, conversely, are plagued with complications after less severe injury.

Outcomes after trauma and hemorrhagic shock are clearly affected by the immunologic response to injury.[2–6] The immunologic response is complex, with multidimensional temporal and spatial relationships among immune cells and the biochemical communication orchestrated by the cells primarily through various inflammatory mediators. The complexities of the response are highlighted by uniform failure in the clinical trauma arena to improve outcomes by mitigating individual immunologic mediators expressed after injury.[7] Recently, multiple studies have yielded novel insights into the trauma immunologic response by using computational methods that can account for temporal and spatial networks of mediators.[2–6,8–10] Rather than focusing on isolated mediators, typically cytokines and chemokines, several studies have shown that immunologic orchestration among mediators better corresponds to favorable versus unfavorable outcomes.[8,11,12] Patients who demonstrate early immunologic coordination followed by subsequent dissipation of dynamic networks of mediators have more favorable outcomes. In contrast, patients with poor early network orchestration followed by networks that grow in complexity have poor outcomes and higher mortality.[2,10] Delayed immunologic network formation is affected by the magnitude of injury severity,[4,5,8] but it is distinctly possible that tolerance to trauma is affected by individual capability to orchestrate a favorable coordinated immune response at the time of injury.

We hypothesized that differences in the composite immunologic response would stratify tolerance to trauma and hemorrhagic shock. We explored this concept in a prospective cohort of MIPs. Specifically, we identified two demographically similar groups of patients from a prospective cohort of 100 MIPs, who exhibited significant clinical tolerance or sensitivity to hemorrhage. Shock-tolerant (ST) patients had uncomplicated outcomes despite having increased cumulative hypoperfusion in the first 24 hours after injury. In contrast, shock-sensitive (SS) patients had poor outcomes despite having significantly less cumulative hypoperfusion during the same period. We hypothesized that there would be differences in individual circulating concentrations of immunologic mediators and distinct feature differences in dynamic networks of mediators between ST and SS cohorts. Our results identified two consistent clusters of immunologic mediators that occurred in trauma patients. Furthermore, our results showed that SS patients had delayed overall mediator orchestration, delayed formation of a distinct mediator cluster associated with tissue repair and protection, and time-dependent increases in mediator network connectivity in a second cluster of inflammatory mediators. In contrast, ST patients had robust early mediator orchestration, specific orchestration primarily within the tissue protective/reparative cluster, and dissolution of mediator connectivity over time.

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PATIENTS AND METHODS

Patient Population

This study was approved by our institutional review board. We prospectively enrolled 100 blunt trauma MIPs (Fig. 1). Patients were 18 to 55 years old and met the following criteria: (1) presented as a full trauma activation defined by the general surgical trauma team with the attending surgeon present at the initial resuscitation and (2) were admitted to surgical intensive care unit (ICU) or proceeded directly to surgery and were then admitted to surgical ICU. We excluded patients with nonsurvivable or severe traumatic brain injuries (TBIs; Glasgow Coma Score of ≤7 at presentation with no improvement after 48 hours after injury).

Fifty-one of the original 100 patients had TBI of which 15 were excluded when their Glasgow Coma Score remained ≤7 after 48 hours. Three patients with spinal cord injuries were also excluded. One additional patient sustained an iatrogenic air embolus with cardiac arrest and was excluded. This yielded the final study cohort of 81 patients (Fig. 1).

Shock Tolerance and Shock Sensitivity

The purpose of this study was to compare immunologic profiles in patients who demonstrated wide discrepancy in tolerating hemorrhage. We compared the magnitude of hemorrhage to the magnitude of organ dysfunction to stratify individual tolerance/sensitivity to hemorrhage. Cumulative hypoperfusion during the first 24 hours after injury was used to define the magnitude of hemorrhage.[1,13] Previously, we demonstrated that cumulative hypoperfusion, measured by temporal integration of abnormally elevated shock index values over the first 24 hours after injury, corresponded closely with transfusion requirements and organ dysfunction (Fig. 2).[1,13] The cumulative hypoperfusion index, 24-hour shock volume (24-hour SHVL), demonstrated significantly greater correspondence with outcomes including organ dysfunction compared with Injury Severity Score (ISS) and base deficit (BD). Notably, organ dysfunction, NIs, and transfusions increased abruptly in patients with 24-hour SHVL ≥ 100 units. The magnitude of organ dysfunction was calculated by using serial Marshall Organ Dysfunction Scores (MODS). Previously, we demonstrated that MODS averaged from days 2 to 5 after injury ($aMODS_{D2\text{-}D5}$) identified patients at risk for prolonged ICU length of stay (ICU_{Days}), prolonged mechanical ventilation (MV_{Days}), and NI.[1,11,12] In this work, there was a stark threshold of $aMODS_{D2\text{-}D5}$ of >4 that predicted poor outcomes.

From the cohort of 81 patients, we defined ST patients as those with $aMODS_{D2\text{-}D5}$ of ≤4 and 24-hour SHVL of ≥100 (Fig. 1, n = 10; Fig. 2, red dashed box). In contrast, SS patients were defined by an $aMODS_{D2\text{-}D5}$ >4 and 24-hour SHVL of <100 (Fig. 1, n = 9; Fig. 2, black dashed box). Initially, injury severity and demographics of the ST and SS groups were determined including ISS, age, sex, and Glasgow Coma Score. Subsequently, both groups were closely studied to identify more granular discrepancies in injury characteristics that may have accounted for differences in outcomes. Specifically, medical records were scrutinized to evaluate all preexisting comorbidities; trauma-related diagnoses; the magnitude, type, and resolution of TBI; the

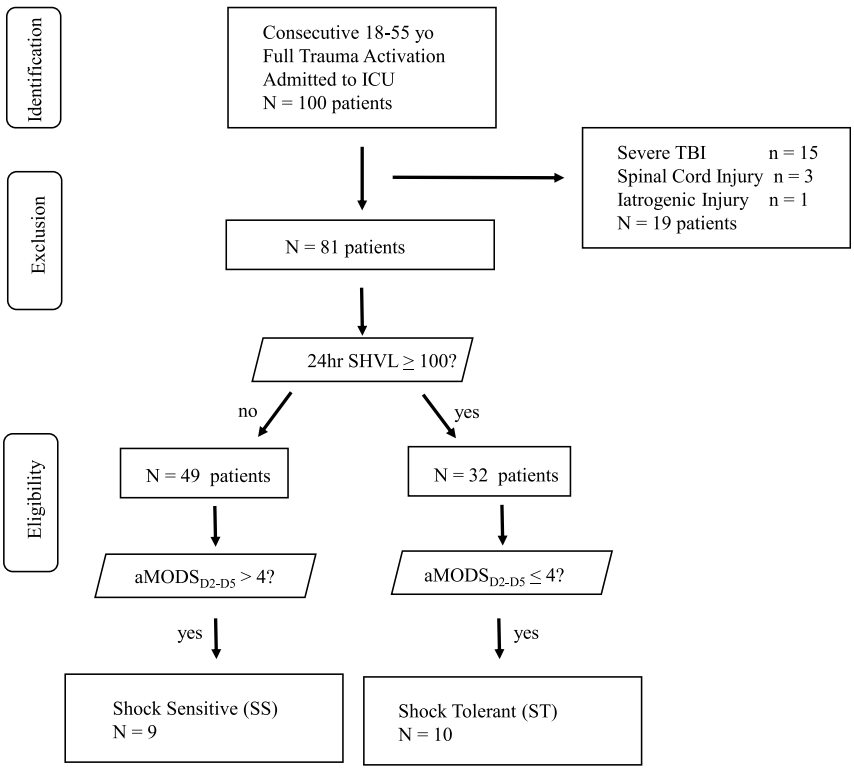

Figure 1. Experimental group mapping leading to the SS and ST groups. Nineteen patients were excluded from the original 100 enrollees. Note that 9 (18.8%) of 48 patients with 24-hour SHVL of <100 were SS, and 10 (30.3%) of 33 patients with 24-hour SHVL of ≥100 were ST by the screening criteria.

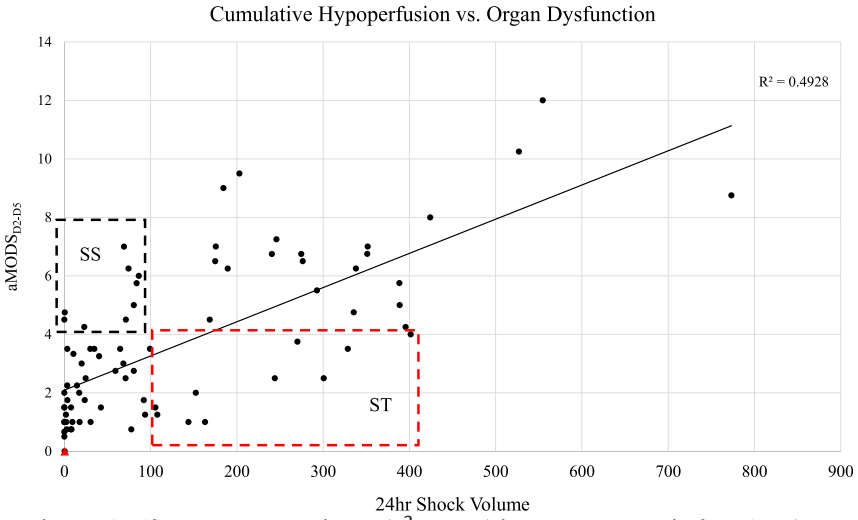

Figure 2. Linear regression shows significant correspondence (R^2 = 0.49) between organ dysfunction (aMODS$_{D2-D5}$) and cumulative hypoperfusion (24-hour SHVL) in 81 MIPs. Two outlier subcohorts were identified that had higher magnitude organ dysfunction with lower 24-hour SHVL (SS, black dashed box; aMODS$_{D2-D5}$, >4; 24-hour SHVL, <100) or lower magnitude organ dysfunction and higher 24-hour SHVL (ST, red dashed box; aMODS$_{D2-D5}$, ≤4; 24-hour SHVL, ≥100).

magnitude of initial hemorrhage; metabolic response to hemorrhage; transfusions; surgical interventions; and mechanism of injury.

Cytokine and Chemokine Measurements

Serial panels of 20 cytokines, chemokines, and high mobility group box 1 (HMGB1) were measured using a multiplex platform (Luminex; Luminex Corporation, Austin, TX). The following inflammatory mediators were measured: interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), soluble IL-2 receptor α, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-17A, IL-17E/IL-25, IL-21, IL-22, IL-23, IL-33, interferon γ–induced protein 10 (IP-10), monokine induced by interferon γ (MIG), monocyte chemoattractant protein 1 (MCP-1), and HMGB1. Mean values of individual mediators were calculated. Plasma was collected at the time of admission (0 hours) and subsequently at 8, 24, 48, and 72 hours after admission. Blood samples were all processed within 2 hours of collection. Samples were centrifuged at room temperature at 1,500 rpm for 10 minutes. About 1.0 mL of plasma was aliquoted into separate cryovial tubes and immediately frozen at −80°C.

Computational Modeling and Analyses

Two distinct analyses were conducted to evaluate immunologic orchestration and patterns of mediators. Time-dependent changes in mediator connectivity were measured using dynamic network analysis (DyNA).[4,14] Discriminant analysis[15–18] paired with elastic net linear regression (ENLR)[19] was used to identify cytokine networks that discriminated the ST and SS groups and the time windows in which the distinguishing networks were most evident.

Dynamic Network Analysis

These analyses explored temporal changes in network connectivity and complexity of the posttraumatic inflammatory response between the ST and SS groups. We have used DyNA in multiple studies, which specifically detail the methods.[2,9,14] Inflammatory mediator networks were created in the sampling intervals (0–8 hours, 8–24 hours, 24–48 hours, and 48–72 hours) using MATLAB (The MathWorks, Inc., Natick, MA) as we have done previously.[2,9,14,20] Connections in the network were created if the correlation coefficient between two nodes (inflammatory mediators) was greater or equal to a threshold of 0.85. For the network density calculation, to account for network sizes (number of significantly altered nodes) in the adjacent periods detailed previously, we used the following formula:

$$\frac{\text{Total number of edges} \times \text{Number of total nodes}}{\text{Maximum possible edges among total nodes}}$$

Discriminant Analysis With Elastic Net Linear Regression

We performed a set of discriminant analyses with several widely used statistical and machine learning methods to examine the predictive power of the biomarkers in differentiating the ST and SS groups. Initially, the biomarkers were analyzed based on (1) individual cytokines concentrations at each discreet time point (0, 8, 24, 48, and 72 hours) and (2) differences in each cytokine between adjacent time points (0–8 hours, 8–24 hours, 24–48 hours, and 48–72 hours). Multivariate discriminant analyses of the aforementioned biomarker measures were examined between the SS and ST cohorts. Pattern classification was performed with four widely used machine learning methods including (1) support vector machine (SVM) learning using either linear (linear SVM) or radial basis function (radial basis function SVM) as its kernel,[15,16] (2) decision tree analyses,[17] and (3) random forest modeling.[18] Classification performance was evaluated by leave-one-out cross-validation accuracy. Briefly, one observation was omitted, and the classifier was learned from the remaining n − 1 observations. The classifier was then applied to the hold out observation, and the accuracy was recorded. This was repeated for all observations, and the average of all the recorded accuracies was used to evaluate the classification performance. Based on these analyses, we determined that the 0- to 8-hour difference values between cytokines yielded the best cross-validation

accuracy. Accordingly, we applied ENLR analyses[19] to 0- to 8-hour cytokine difference values to identify networks of biomarkers that best discriminated between ST and SS cohorts.

Elastic net linear regression is a sparse learning model including both the least absolute shrinkage and selection operator and ridge regularizations. By adjusting a model parameter balancing the least absolute shrinkage and selection operator and ridge effects, we identified the 11 most predictive cytokines with varying sparsity levels ranging from 1 relevant cytokine through all 11 cytokines, respectively. To evaluate the power of the selected cytokines, we applied the aforementioned four classification methods (i.e., linear SVM, radial basis function SVM, decision tree, and random forest) using only the 11 identified cytokines as predictors and estimated the leave-one-out cross-validation accuracy. These focused analyses were conducted only on the 11 identified cytokines and we compared differences between SS and ST patients.

All the aforementioned machine learning analyses were implemented using Python (Python Software Corporation, Wilmington, DE) with its libraries including NumPy, Pandas, and Scikit-learn and were performed on a desktop running Ubuntu 18.04 (Canonical, London, United Kingdom) with Python 3.6 installed (Python Software Corporation, Wilmington, DE).

Statistical Analyses for Clinical Data

Continuous clinical and demographical data were compared with paired Student's *t* tests or analysis of variance when appropriate. Categorical data were compared by χ^2 analyses.

RESULTS

Organ dysfunction correlated with cumulative hypoperfusion. Shock-tolerant and SS groups were identified as outliers from regression analyses. Significant correspondence ($R^2 = 0.49$) was demonstrated between 24-hour SHVL and $aMODS_{D2\text{-}D5}$ from the entire cohort of 81 patients (Fig. 2). Two outlier groups were observed including a group of 10 ST patients with 24-hour SHVL of ≥100 and $aMODS_{D2\text{-}D5}$ of ≤4 (Fig. 2, red dashed box), and 9 SS patients with 24-hour SHVL of <100 and $aMODS_{D2\text{-}D5}$ of >4 (Fig. 2, black dashed box).

Demographics, injury severity, injury distribution, and surgical interventions were homogenous between SS and ST patients. There were no differences in age or sex between SS patients and ST patients (Table 1). The mean ISS for SS and ST patients was 32.0 (range, 9–50; SD, 13.2) and 29.6 (range, 21–48; SD, 8.6; $p = 0.65$), and the majority of patients in both groups were injured in motor vehicle collisions (Table 1). Demographics, injury severity, and mechanism of injury from both SS and ST patients reflected the overall cohort of 81 patients.

Injury distribution and surgical interventions were similar between the two experimental groups (Table 1). Shock-sensitive patients had more spine injuries in contrast to ST patients who had more abdominal and extremity injuries; however, none of the differences were significant. Surgical interventions were similar between groups and reflected differences in spine and abdominal injuries. Granular details demonstrated no substantial differences in the initial magnitude and resolution of TBI between ST and SS patients (Table 1).

TABLE 1. Demographic Variables, Comorbidities, Injury Profiles, and Surgical Interventions

	Entire Cohort (n = 81)	SS (n = 9)	ST (n = 10)	*p* Value (SS vs. ST)
Demographics, ISS, MVC				
Age, mean (SD),* y	36.6 (11.4)	33.7 (12.5)	36.6 (11.8)	0.61
Sex, male/female**	60/21	6/3	7/3	0.88
ISS, mean (SD)*	31.2 (14.1)	32.0 (13.2)	29.6 (8.5)	0.65
MVC, yes/no**	49/32	6/3	7/3	0.88
Comorbidities†				0.17
Smoking		3	4	
Alcohol abuse		1	1	
Diabetes		0	1	
COPD		1	1	
Cardiac disease		0	0	
Liver disease		0	0	
Kidney disease		0	0	
Injury profiles†				0.92
H/N		15	16	
Chest		33	20	
Abdomen		4	18	
Pelvis retro		6	7	
Spine		14	2	
Extremity		10	22	
TBI:		4	4	
TBI initial GCS, mean (SD)*		12.5 (3.5)	12.4 (3.7)	0.95
GCS 15, yes/no**		5/4	6/4	0.84
GCS ≤ 8, yes/no**		2/7	2/8	0.91
TBI final GCS, mean (SD)*		15.0 (0)	15.0 (0)	>0.99
Surgical interventions†				0.70
H/N		1	0	
Chest		3	1	
Abdomen		0	3	
Pelvis/retro		2	0	
Spine		4	0	
Lower extremity		5	10	
Upper extremity		2	7	

*Student's *t* test.
**χ^2 Test.
†Analysis of variance test.
GCS, Glasgow Coma Scale; COPD, chronic obstructive pulmonary disease; H/N, head and neck; MVC, motor vehicle crash.

Clinical outcomes reflect higher organ dysfunction and resource utilization in SS patients compared with ST patients (Supplemental Digital Content, Supplementary Table 1, http://links.lww.com/TA/B832). Compared with ST patients, SS patients had a fivefold increase in the MV_{Days} ($p < 0.01$) and a threefold increase in ICU_{Days} ($p < 0.001$). Likewise, SS patients had a greater incidence of NI ($p = 0.011$) and a 2.3-fold increase in $aMODS_{D2\text{-}D5}$ ($p < 0.001$) compared with ST patients. Individual organ dysfunction trajectories confirmed that ST patients (Fig. 3, red lines) resolved organ dysfunction primarily between hospital days 2 and 3, compared with SS patients, which had little resolution of organ dysfunction from the time of injury to day 5 (Fig. 3, black lines).

Hemorrhage-based outcomes demonstrated trends toward more bleeding in ST patients but no differences in anaerobic

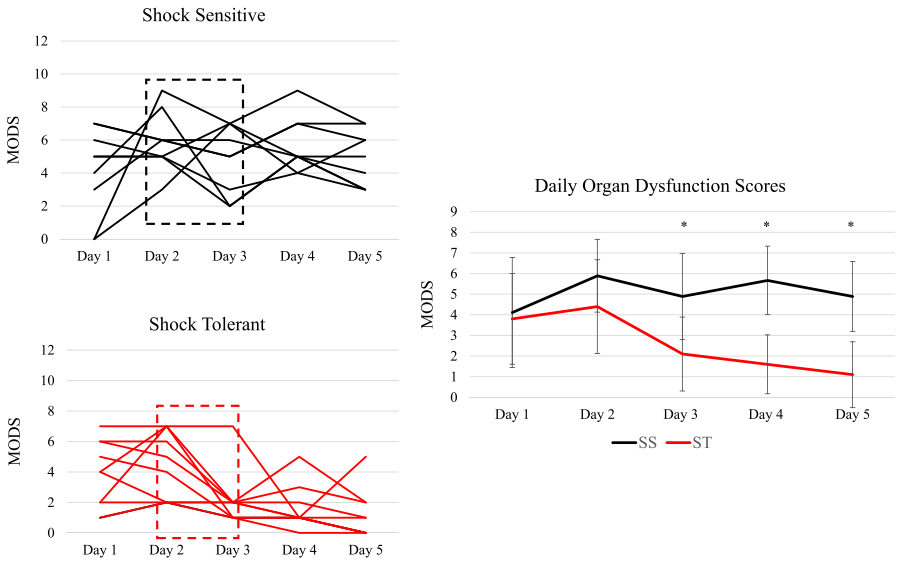

Figure 3. Individual daily MODS scores for SS (black lines) and ST (red lines) groups demonstrate divergence in organ dysfunction trajectories nearly uniformly occurred between days 2 and 3 after injury, with resolution of organ dysfunction in ST patients (* denotes significant difference with $p \leq 0.05$ between SS and ST groups).

metabolism (Supplemental Digital Content, Supplementary Table 2, http://links.lww.com/TA/B833). Bleeding indices, serum measurements of anaerobic metabolism, and transfusion requirements in the first 24 hours demonstrated that ST patients had greater 24-hour SHVL (by definition of the study design), which is reflected in significantly higher HR ($p < 0.01$) and a trend toward greater shock index ($p = 0.11$) at the time of injury compared with SS patients. However, there were no differences in anaerobic indices (BD and pH) during the initial 0 to 24 hours after injury between the groups. Initial hemoglobin level was 0.8 g/dL lower in ST patients compared with SS patients, but the difference was not significant. Four of 10 ST patients had a critical transfusion requirements[21] (3 or more units of packed red blood cells (PRBCs) transfused within a 60-minute period) compared with 2 of 9 patients in the SS group. Likewise, 3 of 10 of the ST patients had a massive transfusion (10 or more units of PRBCs in a 24-hour period) compared with 1 of 9 SS patients. With the small numbers of patients, differences in the incidence of massive transfusion and critical administration transfusions were not significant. Likewise, there were no statistical differences in mean units of PRBCs, platelet and fresh frozen plasma (FFP) transfusions between the groups. However, the mean 0- to 24-hour transfusion values in SS patients were largely influenced by a single patient who received a massive transfusion. There were minimal transfusions in either group after the first 24 hours (Supplemental Digital Content, Supplementary Table 2, http://links.lww.com/TA/B833). In summary, ST patients had greater cumulative hypoperfusion and tachycardia but no evidence of increased anaerobic metabolism compared with SS patients.

There were minimal differences in individual mediator concentrations between the SS and ST patients at any time point. Differences in individual mediators between the groups were scattered (Supplemental Digital Content, Supplementary Fig. 1, http://links.lww.com/TA/B831), but the only individual mediator that was significantly different between the two groups was HMGB1, which was higher in SS patients. None of the cytokines or chemokines were different between the two groups.

Two distinct clusters of mediators were observed in both experimental groups. Dynamic network analysis quantifies coordination between individual mediators within a time interval. Two clusters of mediators were consistently observed in DyNA in both groups (Fig. 4; Supplemental Digital Content, Supplementary Fig. 2, http://links.lww.com/TA/B830). The first cluster included 11 cytokines, primarily associated with proinflammatory and anti-inflammatory functions,[22,23] including IL-1β, IL-17A, IL-1RA, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IP-10, and MCP-1 (Cluster One; Fig. 4, black dashed boxes). The second cluster had six cytokines including IL-9, IL-21, IL-22, IL-23, IL-33, and IL-17E/25 (Cluster Two; Fig. 4, red dashed boxes) that have been associated with reparative functions and boundary organ protection.[24–26]

Shock-sensitive patients had reduced overall early dynamic mediator orchestration and delayed coordination of Cluster Two compared with ST patients. In the first 0- to 8-hour interval, there were only 5 overall mediator connections in SS patients compared with 11 connections in ST patients (Fig. 4). Overall DyNA connections increased to 14 connections in the 8- to 24-hour interval (Fig. 4) in SS patients, which primarily reflected robust development of Cluster One (Fig. 4, black dashed box). Conversely, in the 0- to 8-hour and 8- to 24-hour intervals, SS patients had only three DyNA connections between mediators in Cluster Two (Fig. 4, red dashed boxes). In contrast, ST patients had early and robust dynamic orchestration of Cluster Two (Fig. 4, red dashed boxes) forming 7 connections in the 0- to 8-hour interval, which expanded to 12 connections in the 8- to 24-hour interval. In the 8- to 24-hour interval, IL-21 and IL-9 were both connected to five other mediators, and IL-17E/25 and IL-33 formed four connections. Interestingly, in the 48- to 72-hour interval, there was complete dissolution of all connectivity in ST patients.

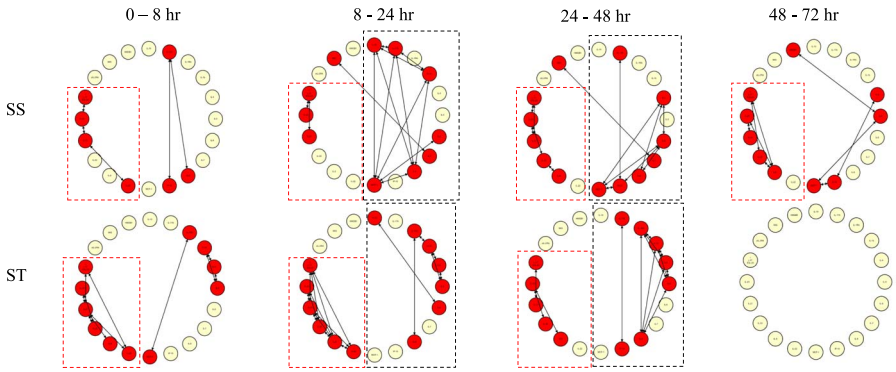

Figure 4. Dynamic network analysis plots at each time interval for SS and ST patients demonstrate distinguishing features in mediator orchestration and clustering. Individual cytokines are denoted by the red and yellow nodes on the periphery of each circle (an enlarged nodal map detailing each mediator is available in Supplemental Digital Content, Supplementary Fig. 2, http://links.lww.com/TA/B830). Two-way arrows between cytokine nodes denote that they are connected within that time interval. Two clusters of coordinated mediators were consistently identified including Cluster One (black dashed boxes) of 11 cytokines including IL-10, IL-17A, IL-1RA, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IP-10, and MCP-1 and six cytokines in Cluster Two (red dashed boxes) that included IL-9, IL-21, IL-22, IL-23, IL-33, and IL-17E/25. The most notable features include early robust coordination of Cluster Two in ST patients in contrast to delayed coordination of Cluster Two in SS patients, high-magnitude orchestration in Cluster One in the 8- to 24-hour interval in SS patients, and complete dissolution of immunologic coordination in ST patients by the 48- to 72-hour interval. Shock-tolerant patients maintained robust coordination of Cluster Two for the first 48 hours after injury. Overall connectivity graphs demonstrate delayed connectivity in SS patients in the initial 0- to 8-hour interval compared with ST patients.

Shock-sensitive patients had greater connectivity of Cluster One compared with ST patients. Shock-sensitive patients developed a highly orchestrated network involving Cluster One (Fig. 4, black dashed box) in the 8- to 24-hour interval forming 11 overall connections. Monocyte chemoattractant protein 1 was connected to five other mediators. Interleukin-1β, IL-6, and IL-10 were connected to four other mediators. In contrast, ST patients formed only six connections in Cluster One in the 8- to 24-hour interval and MCP-1 formed no connections (Fig. 4, black dashed box).

Discriminant analyses and elastic net linear regression demonstrated that mediator differences between 0 hours and 8 hours were most discriminating between SS and ST patients. Discriminant analyses demonstrated that both random forest modeling and decision tree analysis were superior in identifying mediators that best distinguished ST from SS patients (Table 2). In addition, the highest discriminant values were consistently measured by modeling the 0- to 8-hour mediator difference values.

Elastic net linear regression (Table 2) identified a hierarchical order of mediators that best distinguished ST and SS patients. Monokine induced by interferon γ was the single most distinguishing mediator between SS and ST patients. Immune response differences between SS and ST patients were statistically distinguished by including 0- to 8-hour differences in five mediators (in descending order of influence) including MIG, soluble interleukin 2 receptor α, IL-23, MCP-1, and IL-1RA ($R^2 = 0.191$; $p = 0.021$). However, this initial model accounted for only 19% of the variance between the two groups. Adding 0- to 8-hour changes in IL-6, IP-10, and IL-22 to the model nearly doubled the discriminating power between ST and SS patients with an R^2 value of 0.368. Finally, incremental increases in regression correspondence were quantified with the addition of IL-10 and IL-17E/IL-25.

DISCUSSION

The data from this experiment demonstrated differences in the immunologic response in patients identified as ST compared with patients identified as SS. We used computational approaches to account for the complexity of the immunologic response after injury. The most distinguishing features of the immunologic response between the two groups demonstrated that SS patients had overall reduced dynamic immunologic orchestration in the initial 0- to 8-hour interval, ST patients had greater immunologic orchestration in the first 24 hours after injury involving a distinct cluster of protective/reparative cytokines, SS patients had greater orchestration of a second distinct cluster of pro/anti-inflammatory cytokines, and, in the latest 48- to 72-hour interval, ST patients had complete dissipation of all immunologic orchestration. In addition, the single most distinguishing mediator between SS and ST groups identified from ENLR analyses, MIG, did not fit into either cluster but formed a single connection with Cluster One in SS patients (Fig. 4). All observations need to be appropriately tempered by the small number of patients, and the pathomechanistic significance of any of the observations is unknown.

Cluster One contains cytokines that have been associated with proinflammatory (IL-1β, IL-5, IL-6, IL-8, IP-10, and MCP-1)

TABLE 2. Four Method Intervals Including SVM Learning Using Linear and Radial Based Functions, Random Forest Modeling, and Decision Tree Analysis Were Used for Discriminant Analyses

	Linear SVM, Mean (SD)	RBF SVM, Mean (SD)	Random Forest, Mean (SD)	Decision Tree, Mean (SD)	Regression, R^2	*t* Test, *p* Value
IL-10, IL-1RA, IL-6, IP-10, MCP-1, IL-22, IL-23, IL-17E/IL-25, sIL-2RA, MIG, HMGB1	0.16 (0.73)	0.16 (0.73)	0.21 (0.82)	0.68 (0.93)	0.487	0.001
Il-10, IL-1RA, Il-6, IP-10, MCP-1, IL-22, IL-23, IL-17E/IL-25, sIL-2RA, MIG	0.16 (0.73)	0.16 (0.73)	0.37 (0.96)	0.74 (0.88)	0.458	0.001
Il-10, IL-1RA, Il-6, IP-10, MCP-1, IL-22, IL-23, sIL-2RA, MIG	0.16 (0.73)	0.16 (0.73)	0.21 (0.82)	0.74 (0.88)	0.402	0.003
IL-1RA, Il-6, IP-10, MCP-1, IL-22, IL-23, sIL-2RA, MIG	0.26 (0.88)	0.21 (0.82)	0.47 (1.00)	0.47 (1.00)	0.368	0.006
IL-1RA, IP-10, MCP-1, IL-22, IL-23, sIL-2RA, MIG	0.21 (0.82)	0.21 (0.82)	0.47 (1.00)	0.53 (1.00)	0.301	0.009
IL-1RA, IP-10, MCP-1, IL-23, sIL-2RA, MIG	0.26 (0.88)	0.16 (0.73)	0.53 (1.00)	0.74 (0.88)	0.294	0.008
IL-1RA, MCP-1, IL-23, sIL-2RA, MIG	0.21 (0.82)	0.21 (0.82)	0.63 (0.96)	0.74 (0.88)	0.191	0.021
MCP-1, IL-23, sIL-2RA, MIG	0.21 (0.82)	0.11 (0.61)	0.21 (0.82)	0.21 (0.82)	0.046	0.372
MCP-1, IL-23, MIG	0.42 (0.99)	0.47 (1.00)	0.21 (0.82)	0.21 (0.82)	0.045	0.377
IL-23, MIG	0.53 (1.00)	0.53 (1.00)	0.21 (0.82)	0.21 (0.82)	0.035	0.436
MIG	0.53 (1.00)	0.53 (1.00)	0.11 (0.61)	0.11 (0.61)	0.029	0.458

Random forest modeling and decision tree analyses using mediator difference values in the 0 to 8 hours interval (SD in parentheses) numerically provided the greatest distinguishing differences between SS and ST patients. Subsequently, elastic net linear regression modeling was used to sequentially build discriminating rosters of mediators. Once five cytokines including IL-1RA, MCP-1, IL-23, sIL-2RA, and MIG were enrolled in the model, statistically significant discrimination was identified between SS and ST patients ($p = 0.021$), but predictive power was modest ($R^2 = 0.191$). Addition of three additional mediators including IL-6, IL-22, and IP-10 nearly doubled correspondence to an $R^2 = 0.368$.

RBF, radial basis function; sIL-2RA, soluble interleukin 2 receptor α.

and anti-inflammatory (IL-1RA, IL-4, IL-10) functions.[22,23] Cluster Two is comprised of cytokines that have substantial tissue protective/reparative effects.[24–26] Cluster Two cytokines are particularly protective of barrier organs including the skin, lung, and gut, all of which have been shown to be significantly compromised by injury.[25,26] Interestingly, concentrations of IL-17E/25, IL-21, IL-23, and IL-33 were higher in survivors of blunt trauma at the time of admission compared with nonsurvivors. Furthermore, prehospital administration of plasma led to early increases in IL-17E/25, IL-21, IL-23, and IL-33 and increased survival compared with untreated patients.[27] In another retrospective report, IL-33 was elevated in blunt trauma survivors compared with a propensity matched group of nonsurvivors.[28]

Researchers have established that the immunologic response to injury plays a major and potentially dominant role in acute outcomes.[2,4–6,9,11,22,23] Likewise, it is increasingly recognized that immunologic dysfunction affects longer-term outcomes after injury.[29,30] Numerous studies have quantified association between immunologic mediators and outcomes[31–35]; however, causation models linking individual mediator changes with postinjury phenotypes are notably absent. Accordingly, researchers are increasingly leveraging computational methods to understand how injury incites and propagates the immunologic response and how the response affects outcomes.[2,4,9,14,36,37] For example, Abboud et al.[2] demonstrated distinct immunologic feature differences, using DyNA, in blunt trauma survivors and nonsurvivors in closely matched cohorts. Survivors had early orchestration of predominantly lymphoid-based cytokines. Nonsurvivors had greater innate immunity-based cytokine networks that were initially delayed and then expanded in complexity over a 72-hour time frame.[2] In another study, patients with poor outcomes after subarachnoid hemorrhage had reduced initial cytokine orchestration with delayed progressive orchestration of cytokine networks that included MCP-1, IL-6, and IL-1RA (Cluster One cytokines). Conversely, in patients with good outcomes, network orchestration was early, and IL-9 (Cluster Two cytokine) played a central role in mediator networks in survivors.[36] In summary, computational capabilities in trauma-based immunologic studies have uncovered consistent themes that (1) increasing injury severity uncouples early immunologic coordination; (2) early immunologic orchestration is associated with improved outcomes; and (3) patients with reduced initial immunologic orchestration followed by delayed expansion of immunologic network connectivity are at risk for poor outcomes. Methodologic advancements will be necessary to develop immunologic assay platforms and computational methods that can quantify individual immunologic networks at the time of injury to inform clinical decisions and interventions.

Our data are preliminary, and the experimental groups are small, which could clearly affect the results. Accordingly, clinical extrapolation of these results is not possible. For example, transfusions in a single patient in the SS group more than doubled the mean values of transfused units of PRBCs, platelets and fresh frozen plasma within the SS group. Likewise, while there were more spine injuries in SS patients and more abdominal injuries in ST patients (Table 1), with the small group numbers, the differences were not significant. It is possible that these differences may have affected the results. However, three of the four SS patients who had spine surgery did so within 36 hours of injury, and there were no additional transfusions associated with these three surgeries. Our findings will need to be validated in an expanded prospective trial. Our definition of ST and SS is admittedly arbitrary. However, in our foundational studies,[1,13] cumulative hypoperfusion was more accurate than ISS and BD in predicting outcomes. Other components specific to the injury and specific to the patient may better account for the clinical differences between the ST and SS cohorts. Transfusions were reported only for the initial 24 hours in these groups. However, there were minimal transfusions in either group after the first 24 hours (Supplemental Digital Content, Supplementary Table 2, http://links.lww.com/TA/B833). We scrutinized injury-associated and demographic variables to identify alternative explanations for the clinical disparities between the two groups and found no meaningful differences

(Table 1). Collectively, it is unlikely that clinical differences were attributable to demographics, injury magnitude and distribution, or interventions. Our analyses are singularly focused on the immunologic response to injury. We used two different computational methods to provide an in-depth exploration of the immunologic response at distinct cross-sections in the injury time frame and dynamically during progression of injury. However, it is possible that other global response mechanisms to injury such as metabolic response were more critical determinants of clinical outcomes.

In summary, from a larger cohort of MIPs, two selected subcohorts that had ST and SS clinical trajectories demonstrated fundamentally different computational immunologic responses. Increased early orchestration in cytokine networks corresponded to improved outcomes. In particular, early dynamic orchestration of a reparative/protective cluster of cytokines was increased in ST compared with SS patients. Larger populations of patients need to be interrogated to explore this model.

AUTHORSHIP

T.O.M., G.E.G., T.R.B., and Y.V. participated in study design, data collection and analysis, statistical analysis, and article preparation. R.Z., L.S., Q.S., and R.A.N. participated in data collection and analysis, and article preparation.

DISCLOSURE

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