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Temporal Patterns of Circulating Inflammation Biomarker Networks Differentiate Susceptibility to Nosocomial Infection Following Blunt Trauma in Humans

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Abstract

Background—Severe traumatic injury can lead to immune dysfunction that renders trauma patients susceptible to nosocomial infections (NI) and prolonged intensive care unit (ICU) stays. We hypothesized that early circulating biomarker patterns following trauma would correlate with sustained immune dysregulation associated with NI and remote organ failure.

Methods—In a cohort of 472 blunt trauma survivors studied over an 8-year period, 127 patients (27%) were diagnosed with NI versus 345 trauma patients without NI. To perform a pairwise, case-control study with 1:1 matching, 44 of the NI patients were compared with 44 no-NI trauma patients selected by matching patient demographics and injury characteristics. Plasma obtained upon admission and over time were assayed for 26 inflammatory mediators and analyzed for the presence of dynamic networks.

Results—Significant differences in ICU length of stay (LOS), hospital LOS, and days on mechanical ventilation were observed in the NI patients versus no-NI patients. Although NI was not detected until day 7, multiple mediators were significantly elevated within the first 24 hours in patients who developed NI. Circulating inflammation biomarkers exhibited 4 distinct dynamic

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patterns, of which 2 clearly distinguish patients destined to develop NI from those who did not. Mediator network connectivity analysis revealed a higher, coordinated degree of activation of both innate and lymphoid pathways in the NI patients over the initial 24 hours.

Conclusions—These studies implicate unique dynamic immune responses, reflected in circulating biomarkers that differentiate patients prone to persistent critical illness and infections following injury, independent of mechanism of injury, injury severity, age, or sex.

Keywords

dynamic network analysis; high-mobility group protein B1; injury severity score; intensive care unit; interleukin; multiple organ failure; nosocomial infection

Traumatic injury, often accompanied by hemorrhage, is the leading cause of death in patients younger than 45 years, and it represents a significant source of morbidity and mortality for all ages.¹ In patients who survive beyond the initial few hours after injury, early and sustained multiple organ failure (MOF) and delayed nosocomial infection (NI) are leading causes of late death^{2–4} and contribute to prolonged and resource-intensive hospital stays.^{5,6} It is currently unknown why some patients develop NI while other patients—with apparently similar demographics and injury characteristics—do not. One key mechanism by which traumatic injury contributes to an increased susceptibility to NI is through impairment of host defense mechanisms,^{7,8} in particular, dysregulation of the trauma-induced inflammatory response. However, the precise mechanisms underlying this impairment remain unclear,^{9,10} nor is it clear how dysregulated inflammation predisposes some patients to NI. Though inflammation is generally well-regulated, the posttraumatic inflammatory response can be either greater or lesser than that required to respond to the initial insult, resulting in immune dysregulation.^{11–13} Rather than promoting resolution and healing, this dysregulated inflammatory response may result in secondary damage to various tissues and organs,^{11,14,15} coining the term “second hit,” which renders trauma patients vulnerable to organ dysfunction and NI.

Inflammatory chemokines, cytokines, free radicals, and damage-associated molecular pattern molecules are the key regulators of the host inflammatory response to tissue injury after trauma.^{11,16} Previous studies^{17–20} have suggested an exaggerated systemic inflammatory response in patients who develop organ dysfunction. A recent genome-wide study examining the transcriptomic response within circulating leukocytes in severely injured humans identified distinct patterns between patients that followed either uncomplicated or complicated 28-day clinical course. In both patient cohorts, there was a simultaneous upregulation of transcripts associated with innate immunity, and downregulation in the expression of genes linked to adaptive immunity. This pattern was exaggerated both in terms of magnitude and duration in patients with complications. Thus, the magnitude of the immune response is one factor associated with complications after trauma. However, biomarker patterns that yield mechanistic insights or that predict MOF or NI have not been studied rigorously.

To address the phenotypic complexity of the trauma-induced inflammation associated with higher susceptibility to NI, we analyzed data retrospectively from a large cohort of blunt

trauma survivors (472 patients) studied over an 8-year period. To reduce confounding variables, we derived stringently matched subcohorts of NI and no-NI patients that still reflected the primary demographic and injury characteristics of the original large cohort. Our analyses revealed that early, persistent changes in postinjury inflammation manifest in unique biomarker patterns postinjury in patients prone to develop infections. We propose that a combination of traditional parameters of inflammation such as white blood cell differential combined with a novel subset of circulating biomarkers measured upon presentation and over the initial 24 hours could be used to stratify trauma intensive care unit (ICU) patients for more or less intensive care and monitoring and hence optimize resource utilization and outcomes.

MATERIALS AND METHODS

Patient Enrollment and Data Collection

All human sampling was carried out after approval by the University of Pittsburgh institutional review board, and informed consent was obtained from each patient or next of kin as per institutional review board regulations. Patients eligible for enrollment in the study were at least 18 years of age, admitted to the ICU after being resuscitated, and, per treating physician, were expected to live more than 24 hours. Reasons for ineligibility were isolated head injury or brain death criteria, pregnant women, and penetrating trauma. Laboratory results and other basic demographic data were recorded in the database via direct interface with electronic medical record. Three plasma samples, starting with the initial blood draw upon arrival, were assayed within the first 24 hours after injury and then from days 1 to 7 after injury. Clinical data, including Injury Severity Score (ISS), Abbreviated Injury Scale (AIS) score, Marshall Multiple Organ Dysfunction (MOD) score,²¹ ICU LOS, hospital LOS, and days on mechanical ventilation were collected from hospital inpatient electronic trauma registry database. AIS-05 (according to the updated 2005 injury code)²² and ISS²³ scores were calculated for each patient by a single trauma surgeon after attending radiology evaluations were finalized.

Study Design and Case Identification

The study period ran from January 1, 2004, to May 1, 2012, during which time 493 blunt trauma patients were admitted to the emergency department of the Presbyterian University hospital, a level I trauma center which receives trauma patients directly from the accident scene or transfers from regional hospitals. This relatively small number of patients enrolled during the study period is attributed to the willingness of patients to consent and the aforementioned stringent selection criteria (see earlier). The overall demographics, mechanism of injury, comorbidities, and outcomes of the 493 patients are shown in Table S1, Supplemental Digital Content, <http://links.lww.com/SLA/A677>. After excluding 21 trauma in-hospital nonsurvivors, clinical data from 472 blunt trauma survivors were analyzed for the presence of NI. Using the United States Centers for Disease Control clinical criteria for diagnosis of NI,²⁴ we identified 127 blunt trauma patients (prevalence = 27%) with NI.

To perform a pairwise, retrospective case-control study with 1:1 matching, we initially sought to avoid confounding factors related to type of mechanism of injury by selecting the predominant mechanism of injury in both subcohorts. Accordingly, we selected patients in the motor vehicle accident (MVA) category, as MVA was the predominant mechanism of injury in NI and no-NI trauma patients (65.4% and 53.9%, respectively). Next, we further excluded patients who received blood transfusions or underwent emergency surgical procedures within the first 24 hours after trauma to avoid the confounding impact of these interventions on the overall inflammatory response. As a result of this stringent selection criteria, 44 NI trauma patients were identified and subsequently analyzed for their inflammatory biomarker profile.

We next sought to select a control subcohort (no-NI) according to the following matching criteria: age (± 5 years), sex, ISS calculated on hospital discharge (± 5 points), similar mechanism of injury (MVA), and no history of blood transfusions or major surgical intervention within 24-hours after trauma. A control was required to be without evidence of NI at any time during hospitalization in the ICU. Accordingly, a computer-generated list of potential controls was obtained from a database including 345 trauma patients (see earlier). According to these patient-matching criteria, 44 trauma patients without NI were selected and subsequently compared their inflammatory biomarker profile to the 44 NI trauma patients (Table S3, Supplemental Digital Content, <http://links.lww.com/SLA/A679>).

Analysis of Inflammation Biomarkers

Blood samples were collected into citrated tubes via central venous or arterial catheters within 24 hours of admission and daily up to 7 days after injury. The blood samples were centrifuged, and plasma aliquots were stored in cryoprecipitate tubes at -80°C for subsequent analysis of inflammatory mediators. The human inflammatory MILLIPLEX MAP Human Cytokine/Chemokine Panel-Pre-mixed 24-Plex (Millipore Corporation, Billerica, MA) and Luminex 100 IS (Luminex, Austin, TX) were used to measure plasma levels of interleukin (IL)-1 β , IL-1 receptor antagonist (IL-1RA), IL-2, soluble IL-2 receptor- α (sIL-2R α), IL-4, IL-5, IL-6, IL-7, IL-8 (CCL8), IL-10, IL-13, IL-15, IL-17A, interferon (IFN)- γ , IFN- γ inducible protein (IP)-10 (CXCL10), monokine induced by gamma interferon (MIG; CXCL9), macrophage inflammatory protein (MIP)-1 α (CCL3), MIP-1 β (CCL4), monocyte chemotactic protein (MCP)-1 (CCL2), granulocyte-macrophage colony stimulating factor (GM-CSF), Eotaxin (CCL11), and tumor necrosis factor alpha (TNF- α). The Luminex system was used in accordance to manufacturer's instructions. High-mobility group protein B1 (HMGB1) measurement was performed using ELISA (Shino-Test Corp, Kanagawa, Japan, distributed by IBL international, Toronto, Ontario, Canada) according to the manufacturer's instructions.

Statistical Analysis

All data were analyzed using SigmaPlot 11 software (Systat Software, Inc, San Jose, CA). Statistical difference between NI and no-NI groups was determined by either Student *t* test or χ^2 test as appropriate. Group-time interaction of plasma inflammatory mediators' levels between NI and no-NI groups was determined by 2-way analysis of variance (ANOVA). To quantify the differences between the statistically significant mediators, we calculated the

area under the curve (AUC) using the mean values for each time point, then calculating NI/no-NI AUC fold change. $P < 0.05$ was considered statistically significant for all analyses.

Data-driven Modeling: Dynamic Network Analysis (DyNA)

The goal of this analysis was to gain insights into dynamic changes in network connectivity of the posttraumatic inflammatory response to NI and no-NI over time. The mathematical formulation of this method is essentially to calculate the correlation among variables by which we can examine their dependence. To do so, inflammatory mediator networks were created in adjacent 8-hour time periods (0–8 hours, 8–16 hours, and 16–24 hours) using MAT-LAB (The MathWorks, Inc, Natick, MA). Connections in the network were created if the correlation coefficient between 2 nodes (inflammatory mediators) was greater or equal to a threshold of 0.7.

RESULTS

Characteristics of NI and No-NI Subcohorts: Demographics and Outcomes

Hypothesizing that distinct inflammation biomarker patterns could characterize patients who develop NI as a consequence of a unique immune phenotype, we identified 127 patients with NI and 345 patients without NI (Table S2, Supplemental Digital Content, <http://links.lww.com/SLA/A678>). Overall, males were predominant in both NI and no-NI subcohorts (66.1% and 71.3%, respectively) with no statistical difference in mean age ($P = 0.7$) between the 2 subcohorts. However, ISS was statistically significantly higher in the NI cohort ($P < 0.001$) than in the no-NI cohort. Interestingly, we observed a statistically significantly longer ICU LOS ($P < 0.001$), hospital LOS ($P < 0.001$), and days on mechanical ventilator ($P < 0.001$) in the NI cohort when compared with the no-NI cohort.

Description of Nosocomial Infections in NI Subcohort

Overall, 127 of the ICU patients studied had at least 1 nosocomial infection, 15 cases (12%) had multiple infections: 2 nosocomial infections developed in 14 of these cases and 3 or more nosocomial infections developed in 1 case. During their ICU stay, the 127 identified case patients developed 141 episodes of nosocomial infections (1.1 episodes per patient) with an overall infection rate (number of infections per 100 admissions) of 30%. The sites of infection were as follows: 79 episodes of pneumonia (56%), 39 urinary tract infections (UTIs, 28%), 15 bloodstream infections (11%), 5 wound infections (3%), 2 empyema (1.6%), and 1 *Clostridium difficile* infection (0.4%). Of the 79 episodes of pneumonia, 66 were primary; the other 13 were complicated by the following nosocomial infections: 7 UTIs, 4 bloodstream infections, and 2 wound infections.

To establish the diagnosis of suspected hospital-acquired pneumonia (HAP), we used clinical criteria that included new or progressive pulmonary infiltrates on radiograph after 48 hours of hospital admission, and 1 or more of the following: fever, leukocytosis, or leukopenia, an increase in purulent endotracheal secretions. Clinically suspected pneumonia was diagnosed when a bacterial cell culture of 10,000 or more colony forming units per milliliter of bronchoalveolar lavage fluid was grown.

Differences in Circulating Inflammatory Mediators in NI Versus No-NI Patients

We first sought to determine if there was a statistically significant difference in the levels of inflammatory mediators in either subcohort relative to baseline values. To establish baseline, inflammation biomarker data were obtained from 12 healthy volunteers matched to both subcohorts based on age and sex distribution (healthy volunteers; age: 46 ± 2.1 ; 8 men and 4 women) with no history of recent infections or existing comorbidities. This analysis showed that even upon admission, multiple inflammation biomarkers were statistically significantly different in both NI and no-NI sub-cohorts over the baseline levels (Figure S1, Supplemental Digital Content, <http://links.lww.com/SLA/A673>).

We next hypothesized that dynamics of circulating inflammatory mediators would differ in patients with NI from patients without NI, especially with regard to early (<24 hours) inflammatory responses that might predispose to NI. We tested this hypothesis by analyzing an extensive time course of plasma inflammation biomarkers from onset of injury and up to 7 days after injury in both subcohorts. The overall analysis of the inflammation biomarkers over the 7 days' course after injury between NI and no-NI subcohorts showed that multiple circulating levels of cytokines and chemokines were significantly higher in the NI cohort when compared with the no-NI cohort over the 7 day course (Figure S2, Supplemental Digital Content, <http://links.lww.com/SLA/A673>).

Comparison of Stringently Matched NI and No-NI Subgroups

Injury severity,²⁵ the additional trauma of early surgical procedures,²⁶ and blood component transfusions²⁷ are all variables known to modulate the inflammatory response in blunt trauma patients. To determine if the susceptibility to NI was indeed associated with a unique temporal pattern of circulating inflammatory biomarkers independent of these aforementioned confounders, we matched 88 patients (44 from each sub-cohort) stringently according to age, sex distribution, and ISS and with no transfusions or major surgical interventions within the first 24-hours after injury (Table S3, Supplemental Digital Content, <http://links.lww.com/SLA/A679>). Among 44 NI patients, 27 were male and 17 were female (age: 48 ± 3 , ISS: 26.3 ± 1.7), whereas among 44 no-NI patients, 27 were male and 17 were female (age: 47.3 ± 2.3 , ISS: 26 ± 0.9). The extent of initial hypoperfusion was estimated by comparing blood lactate levels upon admission and over time in the NI and no-NI subgroups. Lactate levels were elevated in both groups upon admission being slightly but statistically significantly higher at 4 and 8 hours in the no-NI group and then gradually normalized over the initial 24-hours after injury (Fig. 1A). Importantly, these subgroups retained the key demographic and injury characteristics of the original, large sub-cohorts. Further supporting the validity of examining these stringently matched subgroups were the clinical outcomes: the NI group exhibited a prolonged ICU LOS ($P < 0.001$), hospital LOS ($P < 0.001$), and days on mechanical ventilation ($P < 0.001$) when compared with the no-NI group (Table S3, Supplemental Digital Content, <http://links.lww.com/SLA/A679>), independent of injury severity and major interventions within the first 24-hours after injury.

Site of Infection and Mean Day of Diagnosis in NI Patients

During their ICU stay, the 44-patient NI group developed 47 episodes of NI (1.1 infections per patient, as in the overall cohort): 22 episodes of pneumonia (47%), 14 UTIs (30%), 9

bloodstream infections (19%), and 2 wound infections (4%). Of the 22 episodes of pneumonia, 20 were primary; the other 2 were complicated with wound infections. The average time to the development of NI was 7 days after injury, and the mean day of diagnosis for each type of infection was as follows: pneumonia 6 ± 1 days, UTI 9 ± 2 days, bloodstream infections 7 ± 2 days, and wound infections 11 ± 4 days.

Comparison of Injuries by Body Region in NI Versus No-NI Subgroups

We next sought to calculate the AIS in NI and no-NI subgroups to identify whether specific body region injuries could be associated with an enhanced susceptibility to develop NI after trauma. The analysis of the injury patterns factored into the AIS revealed statistically significant differences in the head ($P = 0.04$) and chest ($P = 0.01$) regions in the NI group when compared with no-NI group (Fig. 1B). The clinical course, functional outcome, and total hospital LOS of trauma patients can be influenced substantially by the severity of brain injury after trauma.²⁸ The Glasgow Coma Scale is one of the most common tools used for gradation of central nervous system injury severity using clinical observations.²⁹ This analysis showed no statistical difference in the mean Glasgow Coma Scale score upon presentation ($P = 0.08$) between NI and no-NI subgroups (11.8 ± 0.7 and 13.6 ± 0.5 , respectively).

Multiple Organ Dysfunction and Circulating Leukocyte Patterns in NI and No-NI Patients

The NI and no-NI subgroups differed in their degree of MOD, as indicated by the Marshall MOD score, a well-validated index of dysfunction in multiple organ systems,^{30,31} which was calculated at each time point in which inflammation biomarkers were assessed. This analysis suggested that a similar level of organ dysfunction was present in NI and no-NI groups on the first day. However, by day 2, the NI group exhibited a statistically significantly higher degree of organ dysfunction ($P < 0.001$) and then up to day 7 after injury when compared with the no-NI group (lesser degree of organ dysfunction) (Fig. 1C). This difference seems to be due to not only an early increase in organ dysfunction in the NI group but also a persistence in organ dysfunction that rapidly resolves in the no-NI group.

We next assessed whether leukocyte populations could differ between the NI and no-NI subgroups after injury. Figure S2, Supplemental Digital Content, <http://links.lww.com/SLA/A674>, shows that the NI group exhibited statistically significantly higher total leukocyte counts at time of presentation and at days 8, 9, and 10 after injury when compared with no-NI group (Figure S2A, Supplemental Digital Content, <http://links.lww.com/SLA/A674>). Polymorphonuclear neutrophils (PMN) percentages were statistically significantly higher in the NI group at time of presentation and days 8, 9, 11, and 12 after injury when compared with no-NI group (Figure S2B, Supplemental Digital Content, <http://links.lww.com/SLA/A674>). Moreover, total lymphocyte percentages were statistically significantly higher in no-NI group at multiple time points including the initial blood draw when compared with NI subgroup (Figure S2C, Supplemental Digital Content, <http://links.lww.com/SLA/A674>). In addition, monocyte percentages were statistically significantly higher in no-NI group at days 8, 9, 10, 11, 12, and 14 after injury when compared with NI group (Figure S2D, Supplemental Digital Content, <http://links.lww.com/SLA/A674>).

Unique Dynamic Patterns of Inflammation Biomarkers Emerge in NI Patients After Injury

We next sought to determine if levels of circulating inflammation biomarkers over 7 days after injury would differentiate trauma patients who would develop NI from stringently matched no-NI patients. Accordingly, at least 3 plasma samples were obtained in the first 24 hours after injury, including upon arrival as well as daily up to day 7 after injury, which corresponds to the mean day of diagnosis of NI. Significant differences were observed in multiple inflammation biomarkers upon admission over the baseline (healthy volunteers) in both the NI and no-NI subgroups (Figure S3, Supplemental Digital Content, <http://links.lww.com/SLA/A675>).

To determine difference in levels of inflammatory mediators between the NI and no-NI subgroups from time of admission and over the 7 days' course after injury, the biomarker data were analyzed using 2-way ANOVA (see Materials and Methods). This extensive analysis revealed statistically significantly higher circulating levels of multiple cytokines and chemokines as well as HMGB1 in the NI group when compared with the no-NI group (Figure S3, Supplemental Digital Content, <http://links.lww.com/SLA/A675>).

Importantly, this analysis revealed 4 distinct biomarker patterns; one that NI and no-NI patients share in common and 3 that clearly distinguish patients destined to develop NI from those who will not. These patterns are depicted qualitatively in Figure 2, and detailed data are shown in Figures S3A, S3B, S3C, and S3D, Supplemental Digital Content, <http://links.lww.com/SLA/A675>. In the first dynamic pattern, MIG/CXCL9, IL-10, IL-8/CCL8, and Eotaxin/CCL11 were elevated upon presentation in both NI and no-NI groups and then declined steadily within 24 hours and leveled off between days 1 and 7 after injury (Fig. 2A). In the second pattern, MCP-1/CCL2, IL-6, HMGB1 (Fig. 3), and IL-1RA were elevated upon presentation to a significantly greater degree in the NI group. Levels of these mediators declined sharply within 24 hours followed by moderate oscillations up to day 7 after injury (Fig. 2B). In the third pattern, mediators that were initially elevated minimally in both NI and no-NI patients began to increase early in the NI group in the second 12 hours after injury and then remained elevated to the end of the sampling period. This group included IL-7, IL-5, IL-17A, IL-4, IL-13, MIP-1 α /CCL3, MIP-1 β /CCL4, IFN- γ , IL-15, sIL-2R α , GM-CSF, and IP-10/CXCL10 (Fig. 2C). Finally, in the fourth pattern, IFN- α , IL-1 β , IL-2, and TNF- α were low on admission with minor but gradual elevation after 24 hours and up to day 7 after injury (Fig. 2D) only in the NI group.

The aforementioned biomarker patterns suggested that the posttraumatic inflammatory response in patients who developed NI diverges early after injury from similarly injured patients who experience an uncomplicated clinical course. Accordingly, we sought to examine the total inflammatory mediator production across all time points in the first 24 hours by calculating the AUC in NI and no-NI subgroups. The AUC was calculated for each biomarker and expressed as fold change difference between NI and no-NI subgroups (see Materials and Methods). Subsequently, the biomarkers were ranked according to their fold values (from highest to lowest fold change). This analysis suggested that multiple biomarkers expressed an increased fold change over the first 24 hours after injury in the NI group when compared with the no-NI group (Table S4, Supplemental Digital Content, <http://links.lww.com/SLA/A680>). Thus, both the levels and patterns of mediator accumulation in

the circulation, identified upon admission and over the first 24 hours, distinguish patients who will develop a sustained systemic inflammatory response, persistent organ dysfunction, and susceptibility to NI from similarly injured patients that will not. Furthermore, the dynamic changes identify a profile of mediators present within hours of injury, known to be derived from both immune and non-immune cells, which gives way to mediators associated predominantly with lymphoid cells.

Different Dynamic Networks of Systemic Inflammation Inferred in NI Versus No-NI Patients

On the basis of these findings, we hypothesized that the differences in the early dynamic, systemic inflammatory response between NI and no-NI could be explained, at least in part, by differential network connectivity among inflammatory mediators. This analysis was achieved by examining the time-dependent evolution of cytokine networks inferred from correlated changes in circulating inflammatory mediators. In this study, we wished not only to determine which networks were present at specific time intervals but also to assess the total degree of connectivity at each of these intervals. Figure 4 shows the detailed DyNA results for NI and no-NI over 3 different time periods following presentation (0–8 hours, 8–16 hours, and 16–24 hours). This analysis suggested that the connectivity among central nodes (> 6 connections) evolved rapidly in the NI group, whereas the no-NI group exhibited substantial reduction of network connectivity (<6 nodes) over the initial 24 hours after injury. Finally, we sought to go beyond an examination of inflammatory mediators and assess the global state of inflammatory networks, by quantifying the degree of network connectivity as a function of time in NI and no-NI subgroups. The NI group exhibited a higher network density at multiple time points (Figure S4A, Supplemental Digital Content, <http://links.lww.com/SLA/A676>), whereas the no-NI group had initially a high-network density within 8 to 16 hours after injury, which declined over the 16- to 24-hour time period (Figure S4B, Supplemental Digital Content, <http://links.lww.com/SLA/A676>). Taken together, these analyses suggest a higher, coordinated degree of activation of both innate and lymphoid pathways in NI patients as compared with no-NI, a difference that can be observed as early as the first 8 hours after injury.

DISCUSSION

Traumatic injury results in biochemical and physiological changes that are in part dependent on the nature and magnitude of the accompanying inflammatory response.³² Though properly regulated, self-resolving inflammation allows for timely recognition and effective responses to injury, yet, if in excess, inflammation can go awry resulting in immune dysregulation and subsequently impairment of host physiological functions.¹¹ This impairment is manifested by an early excessive and sustained systemic inflammatory response, which eventually becomes associated with persistent critical illness and a simultaneous persistent immune suppression with enhanced susceptibility to delayed infection.³³ We found that the magnitude and pattern of early circulating inflammatory biomarkers distinguished between similarly injured patients who would either improve or experience persistent organ dysfunction and delayed NI. The observed patterns suggest that patients prone to follow a complicated course could be predicted on the basis of biomarker analysis performed at early time points. The patterns also provide insights into the unique

nature of the immune-inflammatory response in patients that follow a more complicated course after injury.

Multiple prior studies have reported a strong association between injury severity and an increased susceptibility to NI after injury, which in turn contributes to higher in-hospital morbidity.^{7,34} In our analysis of the overall patient cohort, we noted that patients with NI had a higher injury severity, which was associated with elevations in multiple circulating inflammatory mediators over the 7 days' course after trauma when compared with the no-NI cohort. Although these results support the findings of these prior studies, they also pose a conundrum: Are changes in inflammation after injury related only to the severity of injury or to some other factors that predispose otherwise similarly injured patients to develop NI? We were also concerned about the potential confounding effect of invasive patient management on the inflammatory response. Our analyses using stringently matched cohorts recapitulate the clinical outcomes of the overall cohort, despite comparable degree of injury (ISS ~26). The notion that the magnitude of injury was comparable in these subcohorts is further supported by the observation that both groups had similar elevations in admission lactate levels and a subset of circulating inflammatory biomarkers (*Pattern A*, Figure 3A, Supplemental Digital Content, <http://links.lww.com/SLA/A675>).

On the basis of the observed changes in circulating biomarker levels, we identified 4 distinct temporal patterns from time of admission and through 7 days after injury in the NI group. Pattern A is virtually identical in the 2 cohorts and is remarkable for the early elevations in the chemokines MIG/CXCL9, IL-8/CXCL8, and Eotaxin. This observation suggest that these mediators represent a common component of the immune response to injury, which is aimed at modifying leukocyte trafficking. Moreover, AUC analysis showed that approximately half of the biomarkers that had a higher fold change in the NI group exhibit dynamics that suggest 2 distinct patterns, which are unique between the subgroups in this early time frame. In particular, the much higher levels of IL-6, MCP-1/CCL2, HMGB1, and IL-1RA (*Pattern B*) upon admission in the NI group suggest that a program is activated early in a subset of patients that is independent of mediator levels in Pattern A. What drives the differential expression of the Pattern B mediators is unclear; however, the early peak suggests that these mediators are produced in response to preexisting factors or variables associated with injury mechanism/pattern or prehospital management that are not revealed in our analysis. It is unclear, for example, if this pattern reflects the higher incidence of thoracic trauma in the NI group.

In addition, we also suggest that the elevation in Pattern B mediators gives way to marked increase in Pattern C mediators after 12 hours of injury. These mediators reflect cytokines that, for the most part, are either produced by lymphoid cells or involved in the regulation of lymphocyte responses. In fact, both the AUC and time course analysis of inflammatory mediators show that the greatest fold changes occur in IL-7, IL-5, IL-17A, and IL-4, which correspond to Pattern C. These unique patterns (B and C) suggest that the phenotypic nature of the postinflammatory response that predisposes trauma patients to develop NI could be mediated by an early, robust proinflammatory signal through activation of the innate immune system. In addition, these patterns also suggest that the divergence of the response includes engagement of lymphocyte responses early in the clinical course, which correlate

with development of MOD and persist until onset of infection. This could reflect the activation of the innate lymphoid cell lineage with a concomitantly strong Th2 or type 2 immune signal, both of which persists over the 7 days' postinjury time course. We speculate that the gradual elevation of cytokines such as IL-1 β and TNF- α seen in Pattern D could reflect the response to microbes in the early phases of infection, though further studies are needed to test this hypothesis.

Several studies have reported on the pivotal role of the cellular component of the innate and T cell-mediated immune responses that precedes the development of NI and MOD following traumatic injury.^{35,36} In this study, both the total leukocyte counts and PMN percentage were significantly higher in patients who developed NI on admission and after 1 week of injury versus no-NI patients and elevated PMN corresponded with the mean day of NI diagnosis. Furthermore, our analysis revealed that the NI group exhibited sustained lymphopenia upon admission and up to 14 days after injury when compared with the no-NI group. Recent studies have demonstrated the association between prolonged lymphopenia and the development of NI and MOD,^{37,38} suggesting that lymphocyte depletion, at least in part, plays a detrimental role in the evolution of nosocomial sepsis-induced MOD after traumatic injury.

HMGB1 has emerged as a prototypical damage-associated molecular pattern in the response to sterile injury, including hemorrhagic shock and ischemia/reperfusion injury.³⁹ In addition, HMGB1 promotes processes required for host defense, tissue repair, and regeneration, including chemotaxis, angiogenesis, maturation of dendritic cells, and recruitment and proliferation of stem cells.⁴⁰ Other reports suggest that HMGB1 amplifies the inflammatory response by binding endogenous and exogenous inflammatory mediators, such as, cytokines or endotoxins.^{41,42} In agreement with prior findings,⁴³ our results show an early elevation of HMGB1 in patients who went on to develop infection, signifying a potential role of HMGB1 in earliest stages of the trauma-induced inflammatory response.

Data-driven computational modeling methods have emerged as adjuncts to in vitro and in vivo studies to help define the dynamic, multidimensional inflammatory response after injury.^{44,45} These methods are primarily based on associations among data variables and include logistic regression techniques that allow monitoring of either static or dynamic changes as well as more recent tools that enable graphical views of network interconnectivity.^{44,45} Utilizing one such tool, DyNA,⁴⁶ in parallel with 2-way ANOVA and AUC analysis, we suggest the presence of larger and more highly connected networks of systemic inflammation biomarkers in the NI group versus the no-NI group. These phenotypes were observed as early as we measured and persisted throughout the 24 hours' time course after injury. These findings suggest that, although mounting an adequately robust inflammatory response is essential for effective restoration of homeostasis after injury,⁴⁷ an overly exuberant and sustained immune response may be detrimental. Thus, to achieve complete resolution of trauma-induced inflammation, it is necessary to turn off both inflammatory mediator production and inflammatory cell accumulation, in particular activated PMNs, in a timely fashion.

Importantly, DyNA also suggested that the trauma-induced inflammation in the NI group was primarily driven by IL-7, IL-4, IL-2, IL-13, IL-15, and IL-1 β over the initial 24 hours after injury; these inflammatory mediators were persistently elevated in the NI group throughout 7 days after injury when compared with the no-NI group. In contrast, IL-13, IL-15, and IFN- α were the most connected nodes in the no-NI group over the initial 24 hours after injury. Collectively, these results imply a role for innate-like lymphoid cells and T-cells in both early and late inflammation in patients predisposed to develop NI. This hypothesis must be confirmed with additional studies.

We recognize that there are several limitations in this study. First, this study was performed at a single level I trauma center and thus may not be generalizable or pertinent to other centers with differing admission demographics, injury characteristics, or management practices. This issue warrants additional, similar studies in other trauma centers to validate the results suggested from this study. Another important limitation is the number of inflammatory mediators analyzed, which was limited to the number of analytes we could measure using commercially available Luminex beadsets. Further studies examining a larger panel of inflammatory mediators are warranted. We also note that the diagnosis of HAP is difficult to establish, as there are no reliable tools to determine whether the patient has HAP, particularly in trauma patients with multiple other possible reasons such as lung contusion, acute respiratory distress syndrome, and transfusion-related acute lung injury with abnormal pulmonary gas exchange and abnormal chest radiograph on presentation. Furthermore, when interpreting data from registry-based and institutional series, the limitations of retrospective analyses must be considered. These limitations include patient selection bias and misclassification or information bias as a result of the retrospective aspect. In this study, we tried to overcome this limitation by selecting controls (ie, no-NI) through a computer-generated list with 1:1 matching based on stringent selection criteria (see Material and Methods). Finally, we note that data-driven modeling relies on available data and as such depends on the quality of those data. One of the key drawbacks of purely data-driven modeling techniques for monitoring of biological processes is their input-output nature, which does not provide any knowledge of the internal state of the process. In addition, an input-output system description cannot deal with physical system interconnections. Hence, these methods do not provide any direct mechanistic information about the system; rather they are based on association among data variables in some fashion or another. In multiple prior studies of trauma and sepsis, we have leveraged the counterpart to data-driven modeling, namely mechanistic modeling, to overcome some of these limitations.⁴⁵

CONCLUSIONS

This study demonstrates unique inflammatory biomarker patterns, particularly in the early events after injury, which emerge in patients prone to develop infections, suggesting that specific inflammatory biomarkers can potentially predict or drive processes that increase the susceptibility to infection. In addition, our study suggests the diagnostic value of these temporal patterns coupled with network analysis within the initial 24 hours after injury and based on admission characteristics that could allow early patient stratification and allocation of resource intensive care resource allocation. Finally, and in line with recent calls for data-

driven patient stratification within the context of Precision Medicine, our study highlights the power of computational analysis for gaining insights into complex patient cohorts.⁴⁸

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FIGURE 1.

A, Plasma lactate levels on admission and over 7 days after injury in NI and no-NI patients after trauma. Both subgroups had elevated lactate levels on admission, being statistically significantly higher in no-NI group at 4 and 8 hours after injury. $*P < 0.05$ by 2-way ANOVA. B, AIS of NI and no-NI subgroups. Head/neck and thoracic injuries were assessed as part of the AIS and were significantly higher in the NI group as than in the no-infection group. $*P < 0.05$ by Student *t* test. C, MOD score in NI vs no-NI subgroups over 7 days' time course after injury. Initially, both subgroups had higher degree of MOD at day 1, and by day 2 the NI group had persistently higher degree of MOD when compared to no-NI group. $*P < 0.05$ by 2-way ANOVA.

FIGURE 2.

Temporal dynamic patterns of inflammation biomarkers in NI and no-NI subgroups. A, *Pattern A* includes MIG, IL-10, IL-8, and Eotaxin; B, *Pattern B* includes MCP-1, IL-6, and IL-1RA; C, *Pattern C* includes IL-7, IL-5, IL-17A, IL-4, IL-13, MIP-1 α , IFN- γ , IL-15, sIL-2R α , IP-10, GM-CSF, and MIP-1 β ; D, *Pattern D* includes IFN- α , IL-1 β , IL-2, and TNF- α . Actual concentration levels for each biomarker are provided in Figure S3, Supplemental Digital Content, <http://links.lww.com/SLA/A680>.

FIGURE 3.

Plasma HMGB1 levels within 24 hours after trauma in NI and no-NI subgroups compared to healthy volunteers. HMGB1 levels were statistically significantly elevated within 4 hours of injury in NI group when compared with no-NI group. Both groups had elevated levels of HMGB1 at multiple time points when compared with healthy volunteers. * $P < 0.05$ by 2-way ANOVA between NI and no-NI subgroups; † $P < 0.05$ by 1-way ANOVA vs baseline (healthy volunteers).

FIGURE 4.

DyNA of inflammation biomarkers in NI and no-NI subgroups suggests differential network connectivity within 24 hours after injury. DyNA at 0 to 8 hours suggested that IL-7/IL-15/IL-13/IL-4/IL-1β/IL-2/IFN-γ/IL-17A were highly connected in the NI group (A), whereas the no-NI group (D) exhibited a lesser degree of connected nodes: IL-15/IFN-α/IL-13 with 2 isolated networks. DyNA at 8 to 16 hours suggested that the NI group (B) retained the connectivity among IL-7/IL-15/IL-5/IL-4/IL-1β/IL-2/IL-13, whereas the no-NI group (E) continued to exhibit a lesser degree of connections with 1 main network: IL-13/IL-15/IL-5/IL-4/IFN-γ/IL-17A/IFN-α and 2 isolated networks. DyNA at 16 to 24 hours revealed that the NI group (C) had an increasing connectivity: IL-7/IL-15/IL-5/IL-13/IL-17A/IL-4/IL-2/IL-1β/IFN-γ/GM-CSF. In contrast, the no-NI group (F) exhibited a substantial reduction of network connectivity with 4 isolated networks when compared with NI group.