Published in final edited form as:

Shock. 2015 August ; 44(2): 101–109. doi:10.1097/SHK.0000000000000395.

Impact of Injury Severity on Dynamic Inflammation Networks Following Blunt Trauma

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Abstract

Introduction—Clinical outcomes following trauma depend on the extent of injury and the host's response to injury, along with medical care. We hypothesized that dynamic networks of systemic inflammation manifest differently as a function of injury severity in human blunt trauma.

Study Design—From a cohort of 472 blunt trauma survivors studied following IRB approval, three Injury Severity Score (ISS) sub-cohorts were derived after matching for age and gender: Mild ISS (49 patients [33 males, 16 females; age 42±1.9; ISS 9.5±0.4]); Moderate ISS: (49 patients [33 males, 16 females; age 42±1.9; ISS 19.9±0.4]) and Severe ISS: (49 patients [33 males, 16 females; age 42±2.5; ISS 33±1.1]). Multiple inflammatory mediators were assessed in serial blood samples. Dynamic Bayesian Network (DyBN) inference was utilized to infer causal relationships based on probabilistic measures.

Results—ICU length of stay [LOS], total LOS, days on mechanical ventilation, Marshall Multiple Organ Dysfunction Score, prevalence of pre-hospital hypotension and nosocomial infection, as well admission lactate and base deficit were elevated as a function of ISS. Multiple circulating inflammatory mediators were significantly elevated in Severe ISS vs. Moderate or Mild ISS over both the first 24 h and out to 7 days post-injury. Moderate and Mild ISS. DyBN suggested that IL-6 production in Severe ISS was affected by MCP-1/CCL2, MIG/CXCL9, and IP-10/CXCL10; by MCP-1/CCL2 and MIG/CXCL9 in Moderate ISS; and by MIG/CXCL9 alone in Mild ISS over 7 d post-injury.

Conclusion—ISS correlates linearly with morbidity, prevalence of infection, and early systemic inflammatory connectivity of chemokines to IL-6.

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Keywords

Trauma; ISS; Outcomes; MODS; Inflammation; immunomodulation; Chemokines; Cytokines; Mathematical Modeling; Systems Biology

INTRODUCTION

Trauma is the leading cause of death in the United States for people under the age of 45 (1), and incurs substantial short- and long term-disability (1). Outcomes of injury following trauma depend on the extent of injury, the host's attendant inflammatory and pathophysiologic responses, as well as timely care (2). A core question that has not yet been addressed is a basic one: "Does systemic inflammation increase as a function of injury severity, or does the character of this dynamic inflammatory response differ significantly as injury becomes greater?" Answering this question in a clinical setting, since trauma patients differ based on type of injury, location, and additional factors such as the individual's sex, the length of the interval between injury and surgery, the presence of shock at time of presentation, and the presence of cranial injuries (3). Since these different presentations of trauma along with the individual-specific demographic factors can in turn impact the inflammatory response differentially (4), an important starting point is the choice of injury severity assessment, which must be able to compare disparate types of trauma meaningfully (5, 6).

The need to improve the quality of trauma care – rather than a focus on the question of how injury severity affects physiology at a basic level – has led researchers over the past few decades to develop tools that allow physicians to predict the outcomes in poly-trauma patients (6), including many scoring systems (7). Among these injury severity scoring systems, the Injury Severity Score (ISS) (8), Revised Trauma Score (RTS) (9), and Trauma Injury Severity Score (TRISS) (10) have gained popularity and are widely employed. Despite the known limitation in the ISS score, it is still the one most commonly used to assess the extent of anatomic trauma injuries despite their cause (11) in most institutions, likely because of the ease in calculation. Although the ISS was developed in order to predict mortality (8), this scoring system has also been used to predict other factors such as clinical outcomes (12) and nosocomial infections postinjury (13). Compared to the other injury severity scores, the ISS comes closest to quantifying the degree of externally-driven injury (not the host's own physiological responses to that injury).

Systemic inflammation ensues in response to injury. To date, the focus of studies on systemic inflammation as a function of increasing injury severity has been on defining individual mediators that are elevated, typically in an attempt to help to better stratify patient outcomes (14). However, it is now increasingly clear that in order to gain new insights into the mechanisms of post-injury inflammation, as well as to define new diagnostic and therapeutic modalities, the inflammatory response must be viewed as a system of dynamic, interconnected processes (15, 16). Herein, we hypothesized that differential dynamic trajectories and networks of systemic inflammation are set in motion by injuries of different severity, in a manner that corresponds to increasingly adverse outcomes as a function of

injury severity. Accordingly, we sought to examine the impact of injury severity on the systemic inflammatory response – both in magnitude and interconnectivity – and to define the associated downstream clinical outcomes.

We analyzed data retrospectively from a large cohort of blunt trauma survivors (472 patients) studied over an 8-year period (17). Frequent sampling (3 samples within the first 24 h post-injury, and daily up to day 7 post-injury) allowed substantial precision to characterize the early and late inflammatory response post-trauma. To reduce the effects of confounding variables, we derived stringently-matched sub-cohorts of mild, moderate, and severe injured patients that still reflected the primary demographic and injury characteristics of the original large cohort. Utilizing emerging computational methods, we defined dynamic inflammation networks in these sub-cohorts. Persistent elevation of IL-6 post-injury is associated with worse outcomes in the severely-injured cohort. Our analyses suggest that different injury severity lead to injury-specific dynamic patterns of inflammation centered around differential impact of chemokines (MCP-1/ CCL2, MIG/ CXCL9, and IP-10/ CXCL10) on the key cytokine IL-6.

MATERIALS AND METHODS

Patient Enrollment and Sampling

All human sampling was done following 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 h. Reasons for ICU admission included: 1) hemodynamic instability requiring vasoactive drips; 2) fluid resuscitation or blood replacement needing invasive monitoring; 3) severe hypoxia necessitating complex ventilator management; 4) elderly with pre-existing coronary, pulmonary or peripheral vascular disease; and 5) patients status post damage control surgery with open abdomen or chest.

Reasons for ineligibility were isolated head injury, pregnancy, 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 h following trauma and then from days 1 to 7 post-injury. The blood samples were centrifuged, and plasma aliquots were stored in cryoprecipitate tubes at -80°C for subsequent analysis of inflammatory mediators (see below).

Study Design and Selection Criteria

This was a retrospective case control study, the salient characteristics of which were described recently (17). In brief, clinical data from 472 blunt trauma survivors (330 males and 142 females, age 48.4 ± 0.9 , ISS 19.6 ± 0.5) admitted to the Emergency Department of the UPMC-Presbyterian (a Level 1 trauma center) were analyzed as a function of injury severity. In an attempt to reduce the impact of any confounding factors present in the

general cohort, and similar to recent studies from our group (17–19), we used more stringent filtering criteria for the 472 patients by excluding those patients who had fewer than 3 blood samples in the first 24h post-injury, patients with documented alcohol intoxication and patients for whom data were incomplete. Furthermore, we matched patients according to age, sex, and mechanism of injury (MVC, falls, and motorcycles). Our final study cohort consisted of 49 mildly-injured patients, 49 moderately-injured patients, and 49 severely-injured patients who were as well-matched as possible. The overall demographics, mechanisms of injury, and clinical data of the 147 trauma patients are shown in Table 1. Importantly, this sub-cohort represents the age, the gender ratio, and mechanisms of injury ratios of the general cohort.

Clinical Data Collection

Clinical data, including Injury Severity Score (ISS; see below for calculation), Abbreviated Injury Scale (AIS), ICU length of stay (LOS), hospital LOS, days on mechanical ventilator, Marshall Multiple Organ Dysfunction (MOD) score, prevalence of pre-hospital hypotension, prevalence of nosocomial infections, surgical interventions, biochemical parameters (hemoglobin (Hgb), hematocrit (Hct), platelets count, white blood cell counts (WBC), lactate, base deficit (BD), heart rate (HR), respiratory rate (RR), temperature (T), and patient dispositions were collected from hospital inpatient electronic trauma registry database. Laboratory results and other basic demographic data were obtained from the electronic medical record.

Injury Severity Score Calculation

ISS (8, 20) and AIS-05 scores (according to the updated 2005 injury code) (21) were calculated for each patient by a single trauma surgeon after attending radiology evaluations were finalized. The ISS is based on an anatomical scoring system that provides an overall score for patients with multiple injuries (8). Each injury is assigned an AIS score, allocated to one of six body regions: head and neck, face, chest, abdomen, extremities (including pelvis), and external. Only the highest AIS-05 score in each body region is used. To calculate the ISS score, the score of the three most severely injured body regions is squared and the results are added to produce the ISS score. The ISS was categorized as mild (1–15), moderate (16–24), or severe (>24).

Marshall Multiple Organ Dysfunction (MOD) score

The Marshall MODScore (22) was calculated as an index of organ dysfunction. This score has six variables, including a) the respiratory system (PO_2/FIO_2 ratio); b) the renal system (serum creatinine concentration); c) the hepatic system (serum bilirubin concentration); d) the hematologic system (platelet count); e) the central nervous system (Glasgow Coma Scale) and f) the cardiovascular system- the pressure-adjusted heart rate (PAR). Marshall Score was calculated according to Marshall JC *et al* (22).

Analysis of Inflammation Biomarkers

Blood samples were collected into citrated tubes via central venous catheters within 24 h of admission and daily up to 7 days post-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-Premixed 23 Plex (Millipore Corporation, Billerica, MA) and Luminex™ 100 IS (Luminex, Austin, TX) was 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-17, 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. NO $_2^-$ /NO $_3^-$ was measured using the nitrate reductase/Griess assay (Cayman Chemical Co., Ann Arbor, MI).

Statistical Analysis

All data were analyzed using SigmaPlot™ 11 software (Systat Software, Inc., San Jose, CA). Statistical difference between sub-cohorts was determined by either Student's *t*-Test or Chi-square as appropriate. Group-time interaction of plasma inflammatory mediators' levels between mild, moderate, and severe cohorts was determined by Two-Way analysis of variance (ANOVA). To quantify the differences among the statistically significant mediators, we calculated the area under the curve (AUC) using the mean values for each time point, then calculating moderate injury patients / mild injury patients, and severe injury patients/ mild injury patients AUC fold change. $P < 0.05$ was considered statistically significant for all analyses.

Dynamic Bayesian Network (DyBN) Inference

Dynamic Bayesian Network (DyBN) inference was used to model the evolution of the probabilistic dependencies within a system over time. This analysis was carried out using MATLAB™ (The Math Works, Inc., Natick, MA), using an algorithm adapted from Grzegorzczuk & Husmeier (23) and revised recently by our group. In this analysis, inflammatory mediators were represented at multiple time points within the same network structure. In this approach, time was modeled discretely as in a discrete Markov chain. Each mediator was given a time index subscript indicating the time slice to which it belonged. Additional temporal dependencies were represented in a DyBN by edges between time slices. Each node in the network was associated with a conditional probability distribution of a variable that is conditioned upon its parents (upstream nodes). This particular network structure was used to assess the dominant inflammatory mediators and the probable interaction among various mediators, including possible feedback loops (18, 19, 23–25).

RESULTS

Demographics and clinical outcomes for the overall study cohort

The majority of 472 patients in the study population were males (70%), with a mean age of 48.4 ± 0.9 years and a mean ISS of 19.6 ± 0.5 . These patients sustained blunt trauma in the form of MVC (57%), falls (21.6%), motorcycle crashes (11.2%), all-terrain vehicle accidents (ATVs; 2.5%), pedestrian run-over (1.3%), crush injury (0.9%), machinery injury

(0.9%), cyclist injury (0.4%) and others (4.2%). The mean of ICU LOS was 6.9 ± 0.4 days, the mean hospital LOS was 12.7 ± 0.5 days, and the mean number of days on a mechanical ventilator was 2.9 ± 0.3 days.

Overall demographics and clinical outcomes of stringently-matched sub-cohorts

To test our core hypothesis regarding differential trajectories and networks of systemic inflammation as a function of injury severity, we sought to derive stringently-matched subcohorts that would be as similar as possible with regard to their basic demographics. As part of this process, we focused on the most common mechanisms of injury. Thus, from the above-described overall patient cohort, 147 patients were selected for this study: a sub-cohort of 49 patients with mild injury, a sub-cohort of 49 patients with moderate injury, and a sub-cohort of 49 patients with severe injury (See *Materials and Methods*). In this selection process, we focused on the MVC, falls, and motorcyclist injury groups, as those were the most common mechanisms of injury.

To determine whether the upper range value of one group is not statistically significantly different from the lower range value of the other group, we performed a statistical comparison on the mean ISS values of the mild vs. moderate groups and the moderate vs. severe groups (Table 1). As expected per our study design, ISS was significantly higher in the severe injury sub-cohort ($P < 0.001$) when compared to the moderate injury cohorts; similarly, ISS was significantly higher in the moderate injury group vs. the mild injury group ($P < 0.001$). Overall, males were predominant in the mild, moderate, and severe injury sub-cohorts (67.3%) with no statistical difference in mean age ($P = 0.8$) among the groups. Supporting the notion that the derived subcohorts reflected the known outcomes associated with increasing injury severity (8, 26), the ICU length of stay (LOS) ($P < 0.001$), hospital LOS ($P < 0.001$), and days on mechanical ventilation ($P < 0.001$) were all statistically significantly longer in the severely-injured cohort when compared to the mildly- and moderately-injured cohorts, respectively (Table 1). In addition, moderately-injured patients exhibited longer ICU LOS, hospital LOS, and requirement for mechanical ventilation as compared to the mildly-injured patients (Table 1).

Impact on distinct body regions as a function of injury severity

We next sought to compare the AIS in the stringently-matched sub-cohorts to identify whether injuries in particular body regions could be associated with the worse outcomes post-trauma as a function of increasing ISS. Accordingly, we used the 2005 AIS version to describe injuries in specific anatomic regions (see *Materials and Methods*). Though the sub-cohorts were matched for age, and were comprised of similar gender ratios of patients injured by similar mechanisms of injury, statistically significant differences were observed in the head and neck ($P < 0.001$), chest ($P < 0.001$), abdomen ($P = 0.001$), and extremities ($P = 0.002$) in the severely-injured sub-cohort when compared to mildly- and moderately-injured sub-cohorts (Fig. 1).

Prevalence of pre-hospital hypotension is higher as a function of injury severity

Several studies have reported a strong relationship between the severity of injury and the prevalence of pre-hospital hypotension, which in turn was associated with worse clinical

outcomes (19). The prevalence of pre-hospital hypotension in our cohorts was 31% in the mildly-injured sub-cohort, 41% in the moderately-injured sub-cohort, and 61% in the severely-injured sub-cohort ($P < 0.001$).

Elevated lactate and base deficit as a function of injury severity

Several biochemical parameters at admission in trauma patients have been shown to be associated with higher morbidity and mortality following injury. Accordingly, we evaluated heart rate (HR), respiratory rate (RR), temperature, lactate, base deficit (BD), hemoglobin (Hgb), hematocrit (Hct), platelet counts, and white blood cell counts (WBC) in our stringently-matched sub-cohorts. This analysis showed that lactate (4.1 ± 0.3 vs. 2.8 ± 0.2 vs. 2.9 ± 0.3 ; $P = 0.002$; Fig. 2A) and BD (7.2 ± 0.6 vs. 4.3 ± 0.5 vs. 5.2 ± 0.7 ; $P = 0.004$; Fig. 2B) assessed upon admission were significantly different, respectively, in severely-injured patients when compared to mildly- or moderately-injured patients. There were no significant differences among the three cohorts with regard to HR, RR, T, Hgb, Hct, platelet counts, or WBC counts upon admission (see Supplementary Material Table S1).

Greater requirement for surgical interventions as a function of injury severity

Trauma patients either required surgical interventions to achieve hemodynamic stability, or they presented with simple or compound fractures that required either reduction and/or fixation. In the stringently-matched sub-cohorts, we found that 14/49 (28.6%) of mild injury patients, 20/49 (40.8%) of moderate injury patients, and 28/49 (57%) of severe injury patients underwent laparotomy explore the source of bleeding and control it. Furthermore, 6/49 (12.2%) patients in the mild injury sub-cohort, 14/49 (28.6%) patients in the moderate injury sub-cohort, and 25/49 (51%) in the severe injury sub-cohort had either simple or compound fractures that required either fixation and/or reduction. Finally, 29/49 (59.2%) of the mild injury patients, 18/49 (36.7%) of the moderate injury patients, and 7/49 (14.3%) of the severe injury patients did not require any surgical intervention through their clinical course. ($P < 0.001$). These data are depicted in Table 1.

Greater multiple organ dysfunction a function of injury severity

The mild, moderate, and severe injury sub-cohorts differed in their degree of MODS, as indicated by the Marshall MODScore (a well-validated index of dysfunction in multiple organ systems (27), which was calculated at each time point in which inflammation biomarkers were assessed. This analysis suggested that severely injured patients had a statistically significantly higher degree of organ dysfunction ($P < 0.001$) at day 1 and up to day 7 post-injury when compared to the moderately- and mildly-injured patients, respectively (Fig. 3).

Prevalence of nosocomial infection is higher as a function of injury severity

Susceptibility to nosocomial infection (NI) depends on severity of injury, as well as the specific body region injured (17). Moreover, NI is associated with a longer hospital LOS and ICU LOS (17). In agreement with these prior studies, our data showed that the prevalence of NI increased significantly with severity of injury: 16% in the mildly-injured

sub-cohort, 29% in the moderately-injured sub-cohort, and 45% in the severely-injured sub-cohort ($P = 0.008$).

Different trajectories of systemic inflammation as a function of injury severity

Both blunt trauma itself as well as MODS help drive systemic inflammation (28). Inflammation post-injury is a complex, dynamic process that involves multiple body compartments, spills out into the systemic circulation, and defies simple characterization; we have utilized computational modeling to gain insights into this process (29, 30). Accordingly, we next sought to define the dynamic patterns and networks of systemic inflammation as a function of injury severity. We first hypothesized that dynamics of circulating inflammatory mediators would differ in the severe injury sub-cohort as compared to the mild and moderate injury subcohorts. We tested this hypothesis by obtaining an extensive time course of circulating inflammation biomarkers from close to the onset of injury and up to 7 days post-injury in each of the injury severity sub-cohorts. Over the full 7-days' time course, this analysis showed that circulating levels of IL-6 ($P < 0.001$), IL-7 ($P = 0.04$), IL-17 ($P < 0.001$), sIL-2R α ($P < 0.001$), GM-CSF ($P = 0.01$), IP-10/CXCL10 ($P = 0.03$), MIG/CXCL9 ($P = 0.03$), and MCP-1/CCL2 ($P = 0.02$) were significantly higher in severely-injured patients when compared to mildly- and moderately-injured patients (see Supplementary Material, Fig. S1). An analysis of area under the curve (AUC), in which circulating inflammatory mediators were ranked according to the fold change (moderate injury patients / mild injury patients, and severe injury patients/ mild injury patients) is shown in Table 2.

Next, we sought to focus on the dynamics of systemic inflammation over the first 24 h post-injury in the stringently-matched sub-cohorts, since most of the inflammatory changes happened in the early phase, influenced by different factors including magnitude of injury, resuscitation by fluids, blood components, and surgical interventions. The analysis of inflammation biomarkers for the first 24 h samples among the mild, moderate, and severe injured sub-cohorts showed that circulating levels of IL-6 ($P = 0.005$), MCP-1/CCL2 ($P < 0.001$), IL-7 ($P = 0.01$), IL-17 ($P = 0.018$), IL-1RA ($P = 0.031$), and sIL-2R α ($P = 0.011$) were significantly higher in severely-injured patients when compared to mildly- and moderately-injured patients (see Supplementary Material, Fig. S1). An analysis of area under the curve (AUC), in which circulating inflammatory mediators were ranked according to the fold change (moderate injury patients / mild injury patients, and severe injury patients/ mild injury patients) is shown in Table 3.

Different dynamic networks of systemic inflammation as a function of injury severity

Based on these findings, we next hypothesized that the differences in the dynamic, systemic inflammatory response among the mild, moderate, and severe injury cohorts could be explained, at least in part, by differential expression of dynamic networks. We have recently demonstrated the utility of DyBN as a framework for biomarker discovery and to gain insight into acute inflammation in clinical settings (18, 19, 24). Based on DyBN, and similar to our prior studies in injured patients (18), we inferred that the chemokines MIG/CXCL9, IP-10/CXCL10, and MCP-1/CCL2 were central mediators of systemic inflammation in the mild injury subcohort, exhibiting self-feedback on their own production, with MIG/CXCL9 /

CXCL9 being a central node of inflammation in these patients. This chemokine-based network appeared to affect the levels of IL-6, IL-8, (MIP)-1 α (CCL3), and IL-1RA in mildly-injured patients, while, MCP-1/CCL2 affects the levels of IL-1RA, Eotaxin (CCL11), and sIL-2R α (Fig. 4A). In the moderate injury sub-cohort, DyBN inference suggested that IP-10/CXCL10, MIG/CXCL9, and MCP-1/CCL2 exhibited self-feedback on their own production, with MCP-1/CCL2 being a central node in the dynamic circulating inflammatory response of these patients. This MCP-1/CCL2 sub-network was inferred to affect IL-6, IL-1RA, sIL-2R α , IL-10, MIP-1 α (CCL3), and Eotaxin (CCL11). This analysis also suggested that MIG/CXCL9 affects the levels of IFN- α and IL-6, while IP-10/CXCL10 affects the levels of IL-10, MIP-1 α (CCL3), and Eotaxin (CCL11) (Fig. 4B). In the severe injury sub-cohort, DyBN suggested that, as in the other cohorts, IP-10/CXCL10, MIG/CXCL9, and MCP-1/CCL2 exhibited self-feedback on their own production. In these patients, MCP-1/CCL2 was inferred to affect IL-6, Eotaxin (CCL11), NO₂⁻/NO₃⁻, MIP-1 β (CCL3), and sIL-2R α ; MIG/CXCL9 was inferred to affect IL-6 and Eotaxin (CCL11); and IP-10/CXCL10 was inferred to affect IL-6 and sIL-2R α (Fig. 4C).

Taken together, this analysis of dynamic networks of systemic inflammation suggested that, among multiple mediators, the key injury-associated cytokine IL-6 (31) is affected by three chemokines (MCP-1/CCL2, MIG/CXCL9, and IP-10/CXCL10) in severely-injured patients. In contrast, IL-6 appeared to be affected only by two chemokines (MCP-1/CCL2 and MIG/CXCL9) in moderately-injured patients, while being regulated solely by MIG/CXCL9 in mildly-injured patients.

DISCUSSION

Multiple factors, including age (32), gender (33), and the presence of hypotension (19) can affect the outcomes of blunt trauma. However, the most common factor to consider when deciding how to best target care is the severity of the injury itself. This core consideration has driven the development and refinement of multiple trauma scoring systems over the last few decades, among which ISS remains the most commonly used (20) for anatomical injury assessment. Though imperfect in certain circumstances, and worse than other scoring systems such as TRISS with regard to outcome prediction, ISS correlates linearly with outcomes in the posttraumatic clinical course as well as with the late complications. Despite its limitations and compared to other injury severity scores, the ISS comes closest to quantifying the degree of externally-driven injury independently of the host's physiological responses to that injury. Furthermore, ISS affects the final destination for patients post-hospital, as there is a relation between the severity of the injury (calculated via the ISS) and the functional status of the patients following hospitalization (34).

Another key factor that drives outcomes post-injury is the systemic inflammatory and pathophysiologic response that ensues. Trauma and hemorrhage trigger a complex cascade of events associated with alterations in inflammatory/immune responses that are largely orchestrated by cytokines, chemokines, damage-associated molecular patterns molecules (DAMPs), and free radicals (35, 36). The dynamic interplay among these factors, which occurs at multiple scale s from the molecular to the whole organism and is associated with a massive reprogramming of gene expression (37), is a complex cascade of events. Thus,

focusing on single mediators or pathways as a “magic bullet” approach is likely to be non-productive; instead, a systems approach is needed to address this complexity (38).

We and others have used both data-driven and mechanistic computational modeling approaches to address this complexity and to gain both basic and translational insights in trauma, hemorrhage, and related phenomena such as sepsis (30, 39). Our focus in this study was to address a basic question regarding whether the degree or character of systemic inflammation changes as a function of injury severity, since there is a lack of consensus in the literature with regard to the impact on inflammation of increasing injury severity. Our hypothesis in the present study was the differential immune/inflammatory mechanisms are set in motion by injury of varying severity, in a manner associated with differential clinical outcomes. The systemic circulation is both a locus for this dysregulated inflammation and a convenient compartment for assessing the inflammatory response (17–19). Accordingly, we sought to define the dynamic trajectories and networks of inflammation as a function of injury severity, and to correlate these differences with key in-hospital and discharge-related outcomes.

To carry out this study, we first obtained highly-granular clinical and inflammation biomarker data, with a focus on extensive early sampling (within 24 h and out to 7 days postinjury). Second, we generated stringently, *post hoc*-matched cohorts of mildly-, moderately-, and severely-injured patients, to reduce the impact of confounding factors while still retaining the core clinical characteristics. These sub-cohorts have face validity precisely because they reproduce known findings with regard to the generally worse clinical outcome as a function of injury severity (derived from multiple studies that did not filter out the confounding variables we removed, such as alcohol intoxication). These two goals were integral to the design of our clinical protocol, facilitating a data-driven, precision medicine approach to addressing our core questions “Does systemic inflammation increase as a function of injury severity, or does the character of this dynamic inflammatory response differ significantly as injury becomes greater?”

To address this question, we first confirmed key prior observations about the impact of different injury severity in blunt trauma patients, namely longer ICU and total LOS, greater requirement for mechanical ventilation, higher prevalence of prehospital hypotension, a higher degree of organ dysfunction, and a higher prevalence of nosocomial infection which were statistically different between the sub-cohorts. Furthermore, we examined several physiological parameters independent of ISS, and were unable to identify any significant differences among the three stringently-matched groups in: temperature, heart rate, respiratory rate, blood pressure, and Glasgow Coma Score. These outcomes, which in some cases varied linearly as a function of injury severity, were correlated with differential inflammatory trajectories and dynamic networks.

Our data suggest that the intensity of activation of multiple inflammatory pathways, including those pathways driven by macrophages/neutrophils as well as lymphoid cells, roughly increases as a function of injury severity. This result is in agreement with prior studies (35). In many prior studies of trauma/hemorrhage in both animals and patients, the cytokine IL-6 has persisted as a key inflammatory mediator and biomarker (40). Prior

studies from our groups have leveraged various computational modeling tools to discern the importance of chemokines in driving the inflammatory response – and IL-6 in particular – in both mice and blunt trauma patients(41). In this context, this study reinforces a key role for the cytokine IL-6 (as gleaned from both animal and clinical studies), but does so in a manner that puts this cytokine in the dynamic context of other inflammatory mediators, i.e. finding that differential network connectivity is a key feature of increasing injury severity. Most importantly, and by identifying key mediators in each group, our data-driven approach could increase our understanding of the acute, systemic inflammatory response to traumatic injury, and thereby aid in the implementation of innovative therapies that target specific components of the mediator cascade.

In the present study, we carried out DyBN, a network discovery method that allows for inference of feedback in network nodes. We have used this method previously as a novel biomarker framework in pediatric acute liver failure (24), and to suggest key dynamic networks of inflammation in blunt trauma and spinal cord injury (18, 19). Our analysis is perhaps best explained by stating that based on differential chemokine interactions over time, there is a resultant impact on the dynamic production of IL-6, which in turn might impact the peak systemic levels of IL-6. These results support our prior studies combining *in vitro*, *in silico*, and clinical studies, in which MCP-1/CCL2 was suggested to regulate IL-6 production (41). Further computational modeling studies from our group suggest that this injury-graded network can account for some aspects of the overall systemic inflammatory response as well as patient outcomes (Azhar *et al*, in preparation). Nonetheless, further mechanistic studies are needed in order to prove the hypothesis regarding chemokine-based regulation of systemic IL-6.

We recognize that there are several limitations in our 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 the current study. The use of the ISS as a surrogate for the extent of injury may itself be considered as a limitation, as many other scoring systems are available for quantifying the overall physiological burden associated with trauma/hemorrhage. Indeed, we have demonstrated that additional components beyond the injury itself (e.g. spinal cord injury (18), nosocomial infection (17), or hypotension (19)) drives complex changes in dynamic networks of systemic inflammation in the context of blunt trauma.

Though our three study sub-cohorts were matched stringently with regard to age and mechanism of injury, as well as comprising a similar mix of males and females, statistically significant differences were observed in multiple body regions in the severely-injured sub-cohort when compared to mildly- and moderately-injured groups. Thus, we cannot fully rule out that it is these differences in injured body regions, rather than the ISS as a composite score thereof, that drove the differential inflammatory responses and clinical outcomes observed. Moreover, significant differences were observed in the prevalence of pre-hospital hypotension and nosocomial infection among cohorts, which may play a role in the different patterns of the inflammatory response, which cannot be ignored. Another important

limitation of this retrospective study is the potential impact of blood transfusion and surgical interventions on the temporal dynamics of the inflammatory response. We note that these interventions are by necessity an intrinsic element of clinical care for management of trauma patients with evidence of blood loss. We suggest that these patients who received this type of interventions reflect patients with persistent hemodynamic instability, and that by excluding these patients we will be missing the opportunity to capture the early dynamic changes that accompany persistent hypotension. Another limitation concerns the set of inflammatory mediators assessed in this study, which represent a broad cross-section of innate and lymphoid mediators and thus interrogate most pathways examined to date in blunt trauma. Furthermore, the mediators represented in this beadset interrogate most pathways examined to date in blunt trauma, though additional mediators need to be studied in order to examine their potential role in following injury. Finally, we note that data-driven modeling relies on available data, and as such depends on the quality of those data. These tools do not provide any direct mechanistic information about the biology beneath it, however, they are suggesting possible interactions among inflammatory mediators.

In conclusion, the current study demonstrates the presence of differential, injury-graded early systemic inflammatory responses, which may explain why IL-6 may be a persistently useful inflammation biomarker. These inflammatory responses are associated fairly tightly with significantly different clinical outcomes. We suggest that by using extensive early sampling coupled to stringent *post hoc* matching of sub-cohorts, we were able to identify key networks that characterize the trauma-induced inflammatory response, and that we identified differential dynamic network connectivity as a key output of increasing injury severity. Using this methodology, we identified key networks that characterize the trauma-induced inflammatory response in a manner that was as free from confounding co-morbidities and other clinical factors that could obscure these findings. By identifying key mediators in each group, our data-driven approach could facilitate the implementation of innovative therapies that target specific components of the mediator cascade in an attempt to improve patients' outcomes following trauma. In addition, and in the context of precision medicine, we suggest that analysis of the dynamic networks (by DyBN) during the initial 24 h post-injury could eventually allow for early stratification of patients who develop critical illness, and hence improve allocation of resource intensive care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Nabil Azhar (University of Pittsburgh, Department of Department of Computational and Systems Biology) for developing a MATLAB[®] integrated interface which was used to run the Dynamic Bayesian Network Analysis.

Funding:

- National Institutes of Health (NIH) grant P50-GM-53789
- National Institution of Health Trauma Training grant T32-GM008516.

ABBREVIATIONS

MODS	multiple organ dysfunction syndrome
ICU	intensive care unit
ISS	injury severity scale
AIS	Abbreviated Injury Scale
IL	interleukin
IFN	interferon
MCP-1	monocyte chemotactic protein-1 (CCL2)
MIG	monokine inducible by interferon- γ (CXCL9)
MIP-1α	macrophage inflammatory protein-1 α
TNF-α	tumor necrosis factor- α (CCL3)
DyBN	Dynamic Bayesian Network

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Figure 1. Differences in the Abbreviated Injury Scale (AIS)

AIS scores were statistically significantly higher in the head and neck, chest, abdomen, and extremities regions in the severe injury sub-cohort when compared to mild and moderate injury sub-cohorts (* $P < 0.05$ vs. mild injury analyzed by One-Way ANOVA).

Figure 2. Differences in biochemical parameters between mild, moderate, and severe injury patients at time of admission

(A) Plasma lactate levels assessed at the time of admission statistically significant higher in the severe injury sub-cohort when compared to mild and moderate injury sub-cohorts. (B) Base deficit (BD) assessed at the time of admission was statistically significantly higher in severe injury sub-cohort when compared to mild and moderate injury sub-cohorts. Values are mean $\pm$ SEM. (* $P < 0.05$ vs. mild injury, ** $P < 0.05$ vs. moderate injury, analyzed by One-Way ANOVA).

Figure 3. Multiple organ dysfunction (MOD)

The severe injury sub-cohort exhibited a higher degree of MOD from day 1 up to day 7 post-injury when compared to mild and moderate injury sub-cohorts. (* $P < 0.05$ vs. mild, ** $P < 0.05$ vs. moderate, analyzed by Two-Way ANOVA).

A

Figure 4. Dynamic Bayesian network (DyBN) inference suggests injury-graded impact of chemokines on IL-6 and other systemic inflammation biomarkers

(A) DyBN inference of the mild injury sub-cohort suggested that systemic IL-6 is affected by MIG/CXCL9 only. (B) DyBN inference of the moderate injury sub-cohort suggested that systemic IL-6 is affected jointly by MCP-1/CCL2 and MIG/CXCL9. (C) DyBN inference of the severe injury sub-cohort suggested that systemic IL-6 in the severe injury sub-cohort was affected jointly by MCP-1/CCL2, MIG/CXCL9, and IP-10/CXCL10.

Table 1

Demographic data, clinical characteristics and outcomes of stringently-matched sub-cohorts (mild injury cohort [n = 49], moderate injury cohort [n = 49], and severe injury cohort [n = 49]). Values are mean $\pm$ SEM. Statistical significance set at $P < 0.05$ by either Student's t -Test or Chi-square, or One-way ANOVA as appropriate.

	Mild Injury Cohort n=49	Moderate Injury Cohort n=49	Severe Injury Cohort n=49	P- Value
Demographics				
Age, year	42 $\pm$ 1.9	42 $\pm$ 1.9	42 $\pm$ 2.5	1
Sex, male/female	M=33 F=16	M=33 F=16	M=33 F=16	1
Injury severity score (ISS)	9.5 $\pm$ 0.4	19.9 $\pm$ 0.4	33 $\pm$ 1.1	< 0.001
Mechanism of injury				$P = 0.5$
Motor vehicle accidents (MVA), n (%)	34 (69.4%)	36 (73.5%)	35 (71.4%)	N/A
Fall, n (%)	8 (16.3%)	7 (14.3%)	9 (18.4%)	N/A
Motorcycle, n (%)	7 (14.3%)	6 (12.2%)	5 (10.2%)	N/A
Surgical Interventions				$P < 0.001$
Fracture Fixation, n (%)	6/49 (12.2%)	14/49 (28.6%)	25/49 (51%)	N/A
Laparotomy, n (%)	14/49 (28.6%)	20/49 (40.8%)	28/49 (57%)	N/A
None, n (%)	29/49 (59.2%)	18/49 (36.7%)	7/49 (14.3%)	N/A
Outcomes				
Intensive Care Unit length of stay, days	3.3 $\pm$ 0.5	6.2 $\pm$ 1	10.1 $\pm$ 1.2	< 0.001
Mechanical ventilator, days	1 $\pm$ 0.2	3.7 $\pm$ 1.1	6.5 $\pm$ 0.9	< 0.001
Hospital length of stay, days	9.2 $\pm$ 1.1	11.4 $\pm$ 1.2	17.6 $\pm$ 1.7	< 0.001
Disposition				$P = 0.2$
Inpatient rehabilitation facility, n (%)	2 (4.1%)	5 (10.2%)	3 (6.1%)	N/A
Home, n (%)	33 (67.4%)	23 (47%)	20 (40.8%)	N/A
Home with service, n (%)	3 (6.1%)	2 (4.1%)	3 (6.1%)	N/A
Rehabilitation, n (%)	4 (8.2%)	8 (16.3%)	12 (24.5%)	N/A
Skilled nursing facility, n (%)	7 (14.2%)	11 (22.5%)	11 (22.5%)	N/A

Table 2

Summarizes the area under the curve (AUC) analysis for the statistically significantly different inflammatory mediators (by Two-way ANOVA) between mild, moderate, and severe injury patients in the time course of 7 days.

Inflammatory Mediators (Time of injury – 7 days)	Mild Injury Cohort	Moderate injury Cohort	Fold change	P- value
GM-CSF	61	198	3.3	0.01
MCP-1	2499	3472	1.4	0.02
MIG	4132	4960	1.2	0.032
IL-6	505	586	1.2	< 0.001
IL-7	127	132	1.0	0.04
IP-10	3359	3257	1.0	0.03
Eotaxin	483	358	0.7	< 0.001
sIL-2Rα	1501	1042	0.7	< 0.001
IL-17	304	200	0.7	< 0.001

Inflammatory Mediators (Time of injury – 7days)	Mild Injury Cohort	Severe Injury Cohort	Fold change	P- value
IL-7	127	532	4.2	0.01
GM-CSF	61	198	3.3	0.02
MCP-1	2499	4792	1.9	0.032
IL-6	505	957	1.9	< 0.001
sIL-2Rα	1501	2641	1.8	0.04
IL-17	304	365	1.2	0.03
MIG	4132	4404	1.1	< 0.001
IP-10	3359	2882	0.9	< 0.001
Eotaxin	483	309	0.6	< 0.001

Table 3

Summarizes the area under the curve (AUC) analysis for the statistically significantly different inflammatory mediators (by Two-way ANOVA) among mild, moderate, and severe injury patients in the first 24 h post-injury.

Inflammatory Mediators (0 – 24 h Post-injury)	Mild Injury Cohort	Moderate Injury Cohort	Fold change	P- value
IL-6	700	1554	2.2	0.005
MCP-1	2299	3783	1.7	< 0.001
IL-7	110	95	0.9	0.01
IL-1RA	2319	1372	0.6	0.031
sIL-2Rα	1052	615	0.6	0.011
IL-17	250	88	0.4	0.018

Inflammatory Mediators (0 – 24 h Post-injury)	Mild Injury Cohort	Severe Injury Cohort	Fold change	Pvalue
IL-7	110	437	4.0	0.01
IL-6	700	2176	3.1	0.005
IL-1RA	2319	5493	2.4	0.031
MCP-1	2299	4984	2.2	< 0.001
sIL-2Rα	1052	1621	1.5	0.011
IL-17	250	214	0.9	0.018