Published in final edited form as:

J Crit Care. 2016 December ; 36: 146–153. doi:10.1016/j.jcrc.2016.07.002.

Individual-Specific Principal Component Analysis of Circulating Inflammatory Mediators Predicts Early Organ Dysfunction in Trauma Patients

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Abstract

Purpose—We hypothesized that early inflammation can drive, or impact, later Multiple Organ Dysfunction Syndrome (MODS), that patient-specific Principal Component Analysis (PCA) of circulating inflammatory mediators could reveal conserved dynamic responses that would not be apparent from the unprocessed data, and that this computational approach could segregate trauma patients with regard to subsequent MODS.

Methods—From a cohort of 472 blunt trauma survivors, two separate sub-cohorts of moderately/severely injured patients were studied. Multiple inflammatory mediators were assessed in serial blood samples in the first 24 h post-injury. PCA of these time course data was used to derive patient-specific “inflammation barcodes”, followed by hierarchical clustering to define patient sub-groups. To define the generalizability of this approach, two different but overlapping LuminexTM kits were used.

Results—PCA/hierarchical clustering of 24-h LuminexTM data segregated the patients into two groups that differed significantly in their Marshall MODScore on subsequent days, independently of the specific set of inflammatory mediators analyzed. Multiple inflammatory mediators and their dynamic networks were significantly different in the two groups in both patient cohorts,

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demonstrating that the groups defined based on “core” early responses exhibit truly different dynamic inflammatory trajectories.

Conclusion—Identification of patient-specific “core responses” can lead to early segregation of diverse trauma patients with regard to later MODS. Hence, we suggest that a focus on dynamic inflammatory networks rather than individual biomarkers is warranted.

Keywords

Blunt trauma; Multiple Organ Dysfunction Syndrome; Principal Component Analysis; Dynamic network analysis; Systemic inflammatory response; Inflammation biomarkers

INTRODUCTION

Traumatic injury, often accompanied by hemorrhage, represents the most common cause of death for young people, as well as a significant source of morbidity and mortality for all ages [1, 2]. Initial survivors of acute trauma are particularly susceptible to Multiple Organ Dysfunction Syndrome (MODS), a poorly understood syndrome of sequential impairment of organ function [3]. The early emergence of trauma-induced MODS appears to correlate with a complicated clinical course, accounting for substantial morbidity and mortality [4-6] post-injury. In addition, MODS is thought to be due, in part, to excessive or sustained activation of specific maladaptive inflammatory pathways [7]. Importantly, the post-traumatic inflammatory response is not in and of itself detrimental: an adequately robust early inflammatory response appears to be crucial for the survival of both human trauma patients and experimental animal models subjected to experimental trauma/hemorrhage [8]. Thus, focusing on the circulating levels of individual inflammatory mediators may be insufficient for stratifying injured patients with regard to their propensity to develop MODS. Indeed, single inflammatory mediators have been associated with adverse outcomes in studies of large trauma patient cohorts due to the large variability observed in the inflammatory response observed in trauma patients [9-11] but not on a patient-specific level.

Further complicating attempts to stratify trauma patient outcomes is the fact that the outcomes landscape in trauma/hemorrhage has expanded beyond mortality (now ~5-10%) to include not only MODS but also other complications (e.g. nosocomial infection), extended hospital and intensive care unit (ICU) length of stay, and long-term morbidity following discharge [12, 13]. However, the clinical trajectory of most blunt trauma patients is difficult to predict upon admission. Complicating this analysis is the multi-dimensional, complex, and apparently patient-specific interplay between inflammation and organ (dys)function that appears to drive outcomes in trauma [9-11, 14, 15]. Numerous prior studies have documented dynamic changes in circulating inflammatory mediators in trauma patients, which have in some settings correlated with detrimental outcomes such as MODS [9-11, 15] or nosocomial infection [13].

Typical statistical analyses are geared toward identifying the average behavior of a population. However, computational techniques such as Principal Components Analysis (PCA) are aimed at determining key variables within a dynamic, complex response by examining the variance in a given time-varying dataset [16]. We have previously shown the

utility of PCA to distinguish circulating inflammatory mediator profiles in mice subjected to either minor or severe injury [17], for highlighting inflammatory re-compartmentalization and reprogramming in experimental Gram-negative sepsis [18], and for suggesting patient sub-groups in the setting of pediatric acute liver failure [19]. However, the use of PCA on a patient-specific level as a predictive tool in trauma has not been tested yet. We therefore hypothesized that though the inflammatory responses of individual patients might be variable, these individual responses are characterized by a core set of mediators that could be discerned in these individuals via PCA. Our findings suggest that PCA based on circulating inflammatory mediators assessed within the first 24 h post-injury is capable of segregating moderately/severely injured patients into distinct sub-groups, which are associated with differential degree of MODS that persist up to 5 days post-injury. Importantly, the unprocessed inflammatory mediator data were incapable of similar outcome segregation. These results suggest that it is not any one individual inflammatory mediator that distinguishes patients; rather, it is the PCA-based “inflammation barcode”, which denotes core, dynamic inflammatory responses that actually distinguishes patients.

MATERIALS AND METHODS

Human trauma patients and analyses

Patient recruitment, sampling, and data elements—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 ineligibility were isolated head injury, pregnancy, and penetrating trauma.

From a cohort of 472 blunt trauma survivors detailed recently [13], we identified 132 patients with injury severity score (ISS) > 16 and admission base deficit (BD) > 4 mEq/L (Fig. 1). This large cohort reflected moderately/severely injured patients from which we derived two separate sub-cohorts with at least 3 samples within the first 24 h of injury and complete Marshall MODScores from time of injury up to day 5 (Fig. 1): Derivation Cohort 1 (33 patients [19 males, 14 females; age: 44 ± 3 (mean $\pm$ SEM); ISS: 24 ± 3]); Validation Cohort 2: (33 patients [19 males, 14 females; age: 46 ± 1 ; ISS: 22 ± 1]). The overall demographics, mechanism of injury, and clinical outcomes for both sub-cohorts is shown in Table 1. Clinical data, including ISS, ICU length of stay (LOS), hospital LOS, days on mechanical ventilation, admission BD, and shock index (which identifies the degree of shock in trauma patients, calculated based upon the ratio of heart rate to the systolic blood pressure, where an index > 1 signifies hypovolemic shock) were collected from the hospital inpatient electronic database. 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 5 post-injury. The blood samples were centrifuged, and plasma aliquots were stored in cryopreservation tubes at -80°C for subsequent analysis of inflammatory mediators.

Marshall MODScore (Multiple Organ Dysfunction Score)—Marshall MODScore [20] was calculated daily as described previously [13, 21, 22].

Analysis of inflammatory mediators—To define the generalizability of the patient-specific PCA approach, plasma inflammatory mediators from Cohorts 1 and 2 were assayed using two different but overlapping human-specific Luminex™ beadsets (Invitrogen and Millipore, respectively) using a Luminex™ 100 IS apparatus (MiraiBio, Austin, TX). For Cohort 1, the Invitrogen assay kit (BioSource-Invitrogen, Austin, TX) included twenty-five plasma cytokines/chemokines (interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-2 receptor (IL-2R), IL-4, IL-5, IL-6, IL-7, IL-8 (CCL8), IL-10, IL-12p70/p40, IL-13, IL-15, IL-17A, interferon (IFN)-α, IFN-γ, IFN-γ inducible protein (IP)-10 (CXCL10), RANTES, monokine induced by gamma interferon (MIG; CXCL9), macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), monocyte chemoattractant protein (MCP)-1 (CCL2), granulocyte-macrophage colony stimulating factor (GM-CSF), Eotaxin (CCL11), and tumor necrosis factor alpha (TNF-α). For Cohort 2, the Millipore assay kit (Millipore Corporation, Billerica, MA) was used to measure twenty-five plasma cytokines/chemokines (IL-1β, IL-1Ra, IL-2, sIL-2Ra, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, TNF-α, IFN-α2, IFN-γ, GM-CSF, MIP-1α, MIP-1β, IP-10, MIG, Eotaxin, and MCP-1). Plasma NO₂⁻/NO₃⁻ in samples from both cohorts was measured by the nitrate reductase method using a commercially available kit (Cayman Chemical, Ann Arbor, MI).

Statistical analysis and data-driven modeling

Statistical analyses—All data were analyzed using SigmaPlot™ software (Systat Software, Inc., San Jose, CA). Statistical difference between sub-groups was determined by either Student's *t*-Test or Chi-square as appropriate. Group-time interaction of plasma inflammatory mediators' levels and injury was determined by Two-Way Analysis of Variance (ANOVA). To quantify the overall production of the statistically significant mediators, we calculated the area under the curve (AUC) using the mean values for each time point in a given time frame, then calculating the fold change difference between the two sub-groups inferred from the post-PCA hierarchical clustering. *P* < 0.05 was considered statistically significant for all analyses. *P* < 0.05 was considered statistically significant for all analyses.

Data-driven modeling: Principal Component Analysis (PCA)—The goal of this analysis was to identify the subsets of mediators (in the form of orthogonal normalized linear combinations of the original mediator variables, called principal components) that are most strongly correlated with the inflammatory response in individual trauma patients, and that thereby might be considered principal characteristics of each response. We adapted an approach used previously in the setting of mouse trauma/hemorrhage [17] and human pediatric acute liver failure [19] to define patient-specific “inflammation barcodes” using time course data and subsequent PCA (see Supplementary Material, Materials and Methods for details).

Data-driven modeling: Hierarchical clustering analysis (HCA) of inflammatory mediator data—The goal of this analysis was to group trauma patients into similar groups based on time courses of circulating inflammatory mediators, either pre- or post-PCA, as we have shown previously for mouse trauma/hemorrhage [17], rat sepsis [18], and human pediatric acute liver failure [19] (see Supplementary Material, Materials and Methods for details).

Data-driven modeling: Dynamic Network Analysis (DyNA)—The goal of this analysis was to gain insights into the network complexity of the post-traumatic inflammatory response between sub-groups, as we have done recently in related settings [13, 17, 23, 24] (see Supplementary Material, Materials and Methods for details).

RESULTS

PCA of circulating inflammatory biomarkers, but not unprocessed biomarker data, segregates trauma patients into sub-groups within the first 24 h post-injury

We hypothesized that *early* inflammation can drive, or impact, later MODS. Thus, the central goal of the present study was to determine if early (<24 h) circulating inflammatory biomarkers would stratify trauma patients that present with moderate/severe injury and initial metabolic derangements with regard to their likelihood to later develop MODS. To test this hypothesis, a minimum of three serial plasma samples were obtained in the first 24 h post-injury from patients with moderate/severe injury (based on ISS) and metabolic derangement (as assessed by having admission BD > 4 mEq/L) (Fig.1). These blood samples were assessed for 26 inflammatory biomarkers (see *Materials and Methods*). To determine if the inflammatory biomarker dynamics over the first 24 h post-injury could segregate patients without further processing, the raw biomarker data were subjected to Hierarchical Clustering Analysis (HCA) as described previously [17]. This analysis suggested that patients could be clustered into two sub-groups (to which we refer as Group 1 and Group 2) based on the dynamic trajectories of their systemic inflammatory responses (Fig. 2A). Group 1 (n = 28; 17 males and 11 females) had longer average days in the ICU ($P = 0.02$) when compared to Group 2 (n = 5; 2 males and 3 females). However, there was no statistical difference between Group 1 and Group 2 with regard to age ($P = 0.44$), ISS ($P = 0.09$), hospital total LOS ($P = 0.22$), days on mechanical ventilation ($P = 0.1$), or their degree of MODS (as indicated by the Marshall MODScore over the 5 days post-injury) (Fig. 2B).

We next hypothesized that it would be more informative to focus on the “core” characteristics of the dynamic inflammatory process in individual patients, rather than focusing on the raw values of inflammatory mediators. PCA is a variance-based, statistical data reduction method in which data are transformed so as to yield such primary characteristics [16, 25, 26]. As seen in Figure 3A, patient-specific PCA of circulating inflammatory biomarkers, followed by HCA of the post-processed data, segregated Cohort 1 blunt trauma patients into two major sub-groups (Group A and B). Group A consisted of 11 patients (4 males and 7 females) and Group B consisted of 22 patients (15 males and 7 females).

Different trajectories of systemic inflammation in Group A versus B

Next, we hypothesized that the differences in early inflammatory dynamics between Group A and Group B would have concomitantly different trajectories over the next 5 d post-injury. The overall analysis of the inflammatory mediators from time of injury and up to day 5 showed that circulating levels of GM-CSF, TNF- α , IFN- γ , IL-1 β , IL-2, IL-10, IL-13, IL-15, IL-1RA, sIL-2R α , IL-4, IL-5, IL-7, IL-8, IL-17A, IFN- α , IP-10, MIG, MIP-1 α , MIP-1 β , and MCP-1 were significantly higher in Group A when compared to Group B (*Supplementary Material*, Fig. S1). In contrast, circulating levels of RANTES and IL-12p70/p40 were significantly lower in Group A vs. Group B. In addition, there was no statistically significant difference in circulating levels of IL-6, Eotaxin, and NO₂⁻/NO₃⁻ between Group A and Group B (data not shown).

We also examined the total inflammatory mediator production across all time points in the first 24 h and from days 1 through 5 by calculating the AUC between Group A and Group B. The AUC was calculated for each biomarker and expressed as fold change difference between sub-groups (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 inflammatory mediators expressed an increased fold change over the first 24 h post-injury and over the 5 days in Group A when compared to Group B (*Supplementary Material*, Table 1).

PCA-defined trauma patient sub-groups differ in degree of MODS within the first 24 h, and these differences persist for 5 days post-injury

We next hypothesized that these patient sub-groups would differ based on their degree of MODS within the first 24 h post-injury, which may correlate with their initial differences in inflammatory trajectories. To test this hypothesis, we calculated the MODScore, a well-validated index of dysfunction in multiple organ systems [27-29] at each time point in which inflammation biomarkers were assessed. Figure 3B shows that the post-PCA/clustering-defined patient Groups A and B differed significantly with regard to their MODScore within the first 24 h post-injury. Indeed, Group A had a statistically significantly higher degree of organ dysfunction ($P = 0.02$) when compared to Group B.

Next, we sought to determine if this early segregation of trauma patients, based on PCA of circulating inflammatory biomarkers obtained within the first 24 h post-injury, would persist further in time. As seen in Figure 4A, statistically significant differences ($P < 0.05$) in MODScore were observed in Group A vs. Group B out to five days post-injury, in a manner consistent with the significant differences in dynamics of circulating inflammatory mediators.

The overall demographics, mechanism of injury, prevalence of infection, and clinical outcomes for both sub-groups is shown in Table 2. Despite the aforementioned difference in MODS over time, there was no statistical difference between Group A and Group B with regards to age ($P = 0.95$), ISS ($P = 0.4$), ICU LOS ($P = 0.24$), hospital total LOS ($P = 0.36$), and days on mechanical ventilation ($P = 0.13$). Moreover, there was no statistically

significant difference in prevalence of infection ($P = 0.45$), admission BD ($P = 0.63$), and shock index ($P = 0.48$) between Group A and B.

More complex network of systemic inflammation in patients with higher degree of MODS inferred from DyNA

Finally, we hypothesized that the patient sub-groups defined based on “core” early responses exhibit truly different dynamic inflammatory networks. To test this hypothesis, we first sought to quantify the degree of dynamic inflammatory network connectivity across five days from the time of injury in both sub-groups. This analysis suggested that Group A exhibited a higher network density within the initial 24 h post-injury when compared to Group B, remaining elevated up to day 5 (Fig. 4B). Interestingly, the inflammatory density/complexity tracked in parallel with the degree of MODS (*see above*) over the 5 days post-injury (Fig. 3A). Taken together with the AUC analyses, these analyses suggest a higher, coordinated degree of activation of both innate and lymphoid pathways in patients that developed higher degree of organ dysfunction that happens early even upon admission.

Generalizability of patient-specific PCA

We next sought to validate the generalizability of the patient-specific PCA method using data from Cohort 2 trauma patients and with a different but overlapping human-specific Luminex™ beadset (see *Materials and Methods*). Accordingly, three plasma samples were obtained in the first 24 h post-injury and were assessed serially for the 26 inflammatory biomarkers (see *Materials and Methods*), and the data were subjected to HCA as described previously. As in Cohort 1, HCA suggested the presence of two patient sub-groups (which we again termed Group 1 and Group 2) based on early dynamics of systemic inflammation post-injury (Fig. 5A). Analysis of these patients’ demographics and clinical characteristics showed no statistically significant difference between Group 1 ($n = 24$; 14 males and 10 females) and Group 2 ($n = 9$; 6 males and 3 females) with regard to age ($P = 0.98$), ISS ($P = 0.7$), ICU LOS ($P = 0.98$), hospital total LOS ($P = 0.68$), and days on mechanical ventilation ($P = 0.38$). Moreover, both groups had no difference in their degree of organ dysfunction (Fig. 5B).

Similarly to Cohort 1, we utilized patient-specific PCA of circulating inflammatory biomarkers, followed by HCA of the post-processed data, which segregated Cohort 2 blunt trauma patients into two major sub-groups (Group A and B, Fig. 6A). The overall demographics, mechanism of injury, prevalence of infection, and clinical outcomes for both sub-groups is shown in Table 3. There was no statistical difference between Group A ($n = 9$; 5 males and 4 females) and Group B ($n = 24$; 14 males and 10 females) for age ($P = 0.69$), ISS ($P = 0.35$), ICU LOS ($P = 0.87$), hospital total LOS ($P = 1$), and days on mechanical ventilation ($P = 0.93$). Moreover, there was no statistically significant difference in prevalence of infection ($P = 0.08$), admission BD ($P = 0.61$), and shock index ($P = 0.27$) between Group A and B.

Overall analysis of the inflammatory mediators showed that circulating levels of IL-2, IL-1β, IL-13, IL-7, IL-17A, IL-5, IL-15, and IFN-γ (*Supplementary Material*, Fig.S2) were significantly higher in Group A when compared to Group B. Circulating levels of IL-10,

IFN- α , IP-10, TNF- α , and MCP-1 were significantly lower (*Supplementary Material*, Fig.S2) in Group A vs. Group B. There was no statistically significant difference in circulating levels of IL-4, IL-1RA, IL-6, MIG, GM-CSF, Eotaxin, sIL-2R α , IL-8, MIP-1 α , and NO $_2^-$ /NO $_3^-$ between Group A and Group B (data not shown). An AUC analysis revealed that multiple biomarkers expressed an increased fold change over the first 24 h post-injury and over the 5 days in Group A when compared to Group B (*Supplementary Material*, Table 2).

Figure 6B shows that the PCA/clustering-defined patient Groups A and B differed significantly ($P = 0.006$) with regard to their MODScore within the first 24 h post-injury, as in Cohort 1. Also in line with our findings in Cohort 1, this early segregation of trauma patients, based on PCA of circulating inflammatory biomarkers obtained within the first 24 h post-injury, persisted in Group A vs. Group B out to five days post-injury (Fig. 7A). In further concordance with our findings in Cohort 1, DyNA suggested that Group A exhibited a higher network density within the initial 24 h post-injury when compared to Group B which remained elevated up to day 5 (Fig. 7B).

DISCUSSION

The outcomes landscape in blunt trauma has shifted from mortality (now ~5% in patients not requiring operative management) to multiple organ dysfunction syndrome (MODS), nosocomial infection, leading to prolonged LOS [13]. The clinical challenge is now that of reducing morbidity and associated resource utilization, i.e. *late* events [30, 31], which we hypothesize are due in large part to *early* immune dysregulation that is driven by post-injury inflammation. We show here using relatively small numbers of blunt trauma patients that PCA of dynamic changes over 24 h in up to 26 different circulating inflammation biomarkers allows for the separation of patients based on MODS as the endpoint over the first 5 days post-injury.

A key observation regarding circulating inflammation biomarkers in trauma/hemorrhage is the large patient-to-patient variability, which generally requires relatively large numbers of patients in order to discern statistically significant associations between circulating inflammation biomarkers [9-11, 15]. This heterogeneity was indeed apparent in the present study. However, while the inflammatory response varies from patient to patient, we hypothesized that “core” dynamic responses are preserved across patients that share a limited set of clinical outcomes. This apparent paradox between highly variable inflammatory responses compared to a relatively homogenous set of outcomes led us to hypothesize that the core grouping of principal inflammatory characteristics would cluster patients into discrete sub-groups.

We also hypothesized that PCA, a well-known multivariate statistical method for data dimensional reduction [32], could be carried out on a patient-by-patient basis. Prior studies using PCA to study the inflammatory response and associated processes have had variable success. In animal models of inflammation, PCA was used to discern key differences between experimental hemorrhagic shock vs. the control sham cannulation [17], to suggest how multi-modal therapies such as hemoabsorption can impact experimental Gram-negative

sepsis [18], and as an adjunct to other data-driven or mechanistic modeling techniques [19, 33]. PCA was also used to gain insights into disturbances of the coagulation cascade in the context of acute traumatic coagulopathy [34]. In a recent study from our group on pediatric acute liver failure, we found that PCA/HCA-based segregation suggested some common dynamic inflammatory patterns between spontaneous survivors and those patients that received liver transplant. In addition, this methodology could, to some degree, segregate non-survivors from survivors and liver transplant recipients [19]. In sepsis patients, however, PCA could not discern differences among patient sub-groups [35].

We suggest that PCA of circulating inflammation biomarkers may facilitate precision medicine. Precision medicine is an emerging concept for diagnosis and treatment based on quantitative segregation of patients rather than a reliance on historical diagnostic and staging criteria [36]. In this vein, we have suggested previously that data-driven patient segregation could differentiate sub-groups of patients in cohorts that could not be segregated based on clinical or standard laboratory parameters [37]. In the present study, we aimed to test this hypothesis. We applied patient-specific PCA followed by HCA to two separate cohorts of otherwise highly similar moderately/severely injured blunt trauma patients, all survivors. This methodology showed that trauma patients that were indistinguishable based on HCA of raw values of circulating inflammation biomarkers (or based on clinical/demographic variables) could indeed be segregated into distinct sub-groups based on data obtained within the first 24 h post-injury. Furthermore, these sub-groups remained distinct for 5 days post-injury. This segregation was independent of the exact set of mediators studied or the specific assay kit, though there was overlap in the mediators across kits. Given the uncertainty for how long ICU patients stay, we suggest that our post-PCA/HCA approach can early stratify patients with otherwise homogenous/uniform biomarker “barcodes” with the goal of characterizing the dynamics of trauma-induced inflammation and hence better target care.

A notable finding in our study was that IL-6, which has been shown repeatedly to be a biomarker of adverse outcomes in severely-injured trauma patients [38, 39], was not significantly different in the PCA-defined patient sub-groups. This supports our hypothesis that rather than focusing on individual mediators, it may be more fruitful to characterize the dynamic, multi-dimensional inflammatory response post-injury. To do so, we utilized DyNA, a method which is primarily based on associations among data variables. *In silico* inference of the dynamic network complexity suggested a bifurcation of well-regulated versus dysregulated inflammation in the high MODS group vs. the low MODS group, respectively, early post-injury. These findings suggest that the overall magnitude of the inflammatory response is greater in patients with higher degree of MODS which could drive adverse outcome post-injury. Importantly, these *in silico*-defined trajectories of inflammation mimic the clinical trajectories of MODS over the 5 days in both cohorts.

There are several limitations to our study. First, the size of each cohort is relatively small at 33 patients, though two cohorts were studied and extensive time courses of inflammation biomarkers were obtained for the present study. The key findings – namely, that PCA/HCA based on inflammatory mediators assessed at three time points within the first 24 h post-injury – could segregate patients with a higher degree of organ dysfunction from those with a lower degree – could be replicated in both patient cohorts. Second, the possibility exists

that adding more inflammatory mediators that the panel could further increase the predictive capability of this approach; further studies are needed to determine this point. Third, it may be argued to more frequent sampling within the first 24 h post-injury could improve the predictive capability of this approach. However, we note that given the exigencies of clinical care, obtaining three samples within the crucial, early post-injury time frame is likely to be the most that is feasible in a realistic clinical setting. However, we are examining the possibility that measurement of inflammatory mediators starting while en route from the scene of injury to the emergency room could improve the utility of this method. It is also well-known that the severity of MODS correlates with other clinical parameters such as incidence of nosocomial infection, days on mechanical ventilation, and ICU LOS [4, 6]. We did not see a difference in these parameters despite the differences in MODS severity which is probably based on the relatively small number of patients in our study sub-groups. We propose that these observations point to the greater specificity of inflammation networks in associating with MODS than even these major clinical endpoints.

In conclusion, we present a novel, practical approach to “precision medicine” as applied to blunt trauma patients, based on the concept of “inflammation barcodes” defined by patient-specific PCA. We note that the assays we used are commercially available, and that we applied a sampling frequency that is achievable within the first 24 h post-injury. These results raise the potential of using multiplexed inflammation biomarker analysis coupled with patient-specific PCA as framework for outcome prediction in the setting of blunt trauma. Moreover, this approach may be used to determine the principal drivers of inflammation in individual patients and patient sub-groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant P50-GM-53789.

ABBREVIATIONS

PCA	Principal Component Analysis
HCA	Hierarchical Clustering Analysis
MODS	Multiple Organ Dysfunction Syndrome
ICU	Intensive Care Unit
IL	interleukin
IFN	interferon
MCP-1	monocyte chemotactic protein-1
MIG	monokine inducible by interferon- γ (CXCL-9)

MIP-1α	macrophage inflammatory protein-1α (CCL-3)
TNF-α	tumor necrosis factor-α

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Figure 1. Flow chart of recruitment and study participation

From a large cohort of 472 blunt trauma survivors and after exclusion of patients with injury severity score (ISS) < 16 and admission base deficit (BD) < 4 mEq/L, we identified 132 moderately/severely injured patients. From this cohort, we derived two separate sub-cohorts with at least three blood samples within the first 24 h of injury and with complete Marshall MODScores from time of injury up to day 5: Derivation Cohort 1 (n = 33) and Validation Cohort 2 (n = 33).

Figure 2. Hierarchical Clustering Analyses (HCA) of unprocessed <24 h circulating inflammation biomarkers in derivation trauma Cohort 1

Serial plasma samples were obtained from trauma patients (see Table 1) and assayed for 26 cytokines, chemokines, and reactive nitrogen species as described in the *Materials and Methods*. **Panel A:** The inflammation biomarker data obtained at three time points within the first 24 h post-injury were subjected to HCA as described in the *Materials and Methods*. Each row of graph represents the patients' average value of cytokines from three time points. **Panel B:** Marshall MODScore of the post-HCA Group 1 and Group 2 shows no statistical difference between Groups from day 1 and up to day 5 post-injury.

Figure 3. Individual-specific PCA clustering suggested two distinct sub-groups that differ in their degree of MODS

Panel A: The biomarker data were subjected to PCA and then followed by HCA (as described in the *Materials and Methods*), segregated Cohort 1 blunt trauma patients into two major sub-groups (Group A and B), along with several minor sub-groups (Groups A1, A2, B1, and B2). **Panel B:** The trauma-patient sub-groups inferred from the data depicted in Figure 2A were analyzed for their degree of MODS during the first 24 h post-injury. The main trauma patient sub-groups (Group A and B) differ significantly in their MODScore ($*P = 0.02$ by Mann–Whitney *U*Test).

Figure 4. Trauma patient sub-groups inferred from individual-specific PCA differ in their degree of MODS and differential network complexity

Panel A: MODScore in the trauma patient sub-groups shown in Figure 2A were assessed for up to 5 days post-injury, and showed statistically significant differences between Group A and B up to 5 days post-injury. * $P < 0.05$ by Two-Way ANOVA. **Panel B:** DyNA suggested a more complex inflammation network in Group A which persisted up to day 5 post-injury when compared to Group B.

Figure 5. Hierarchical Clustering Analyses (HCA) of unprocessed <24 h circulating inflammation biomarkers in validation trauma Cohort 2

Similar to Cohort 1, serial plasma samples were obtained from trauma patients (see Table 1) and assayed for 26 cytokines, chemokines, and reactive nitrogen species as described in the *Materials and Methods*. **Panel A:** The inflammation biomarker data obtained at three time points within the first 24 h post-injury were subjected to HCA as described in the *Materials and Methods*. Each row of graph represents the patients' average value of cytokines from three time points. **Panel B:** Marshall MODScore of the post-HCA Group 1 and Group 2 shows no statistical difference between groups from day 1 and up to day 5 post-injury.

Figure 6. Individual-specific PCA clustering suggested two distinct sub-groups that differ in their degree of MODS

Panel A: The biomarker data were subjected to PCA and then subsequently to HCA as described in the *Materials and Methods*. This analysis segregated Cohort 2 into two major sub-groups (Group A and B), along with several minor sub-groups (Groups A1, A2, B1, and B2). **Panel B:** The trauma-patient sub-groups inferred from the data depicted in Figure 5A were analyzed for their degree of MODS during the first 24 h post-injury. The main trauma patient sub-groups (Group A and B) differ significantly in their MODScore (* $P = 0.006$ by Mann–Whitney U Test).

Figure 7. Trauma patient sub-groups inferred from individual-specific PCA differ in their degree of MODS and differential network complexity

Panel A: MODScore in the trauma patient sub-groups shown in Figure 5A were assessed for up to 5 days post-injury, and showed statistically significant differences between Group A and Group B up to 5 days post-injury. * $P < 0.05$ by Two-Way ANOVA. **Panel B:** DyNA suggested a more complex inflammation network in Group A which persisted up to day 5 post-injury when compared to Group B.

Table 1

Cohorts 1 and 2 trauma patients' demographic data, clinical characteristics and outcomes. ISS, Injury Severity Score; ICU LOS, intensive care unit length of stay; Hospital LOS, hospital length of stay; Ventilation days, number of days on mechanical ventilation. Values are mean $\pm$ SEM. Statistical significance set at $P < 0.05$ by either Mann–Whitney *U*Test or Chi-square as appropriate.

	Derivation Cohort 1 n = 33	Validation Cohort 2 n = 33	P value
<i>Demographics</i>			
Age, yr	44.3 $\pm$ 3.15	44.3 $\pm$ 1.3	1
Sex, male/female	M=19 F=14	M=19 F=14	1
Injury severity score (ISS)	24 $\pm$ 2.50	23.3 $\pm$ 1	0.65
<i>Mechanism of injury</i>			
Motor vehicle accidents (MVA), n (%)	28 (85%)	27 (82%)	1
Fall, n (%)	5 (15%)	5 (15%)	1
Others, n (%)	0	1 (3%)	N/A
<i>Co-morbid conditions</i>			
Psychiatric conditions, n (%)	4 (12%)	4 (12%)	1
Hypertension, n (%)	9 (27%)	8 (24%)	0.7
Diabetes mellitus, n (%)	5 (15%)	4 (12%)	0.7
Bronchial asthma, n (%)	3 (9%)	3 (9%)	1
Chronic anemia, n (%)	1 (3%)	0	N/A
Alcohol Use	4 (12%)	4 (12%)	1
Chronic liver diseases, n (%)	1 (3%)	1 (3%)	1
None, n (%)	10 (30%)	14 (42%)	1
<i>Outcomes</i>			
Intensive Care Unit length of stay, days	7.2 $\pm$ 1.36	8 $\pm$ 1.4	0.6
Mechanical ventilator, days	3.6 $\pm$ 0.9	4.3 $\pm$ 1.2	0.86
Hospital length of stay, days	14.3 $\pm$ 2	14.7 $\pm$ 2	0.77

Table 2

Demographic data, clinical characteristics and outcome of post-PCA/clustering of derivation Cohort 1 defined patient Groups A and B. Values are mean $\pm$ SEM. Statistical significance set at $P < 0.05$ by either Mann–Whitney U Test or Chi-square as appropriate.

	Group A n = 11	Group B n = 22	<i>P</i> value
<i>Demographics</i>			
Age, yr	44 $\pm$ 6	44.4 $\pm$ 3.7	0.95
Sex, male/female	M=4 F=7	M=15 F=7	0.13
Injury severity score (ISS)	27.1 $\pm$ 4.6	22.6 $\pm$ 3	0.4
<i>Mechanism of injury</i>			
Motor vehicle accidents (MVA), n (%)	9 (82%)	18 (78%)	0.75
Fall, n (%)	2 (18%)	3 (13%)	0.36
Others, n (%)	0	1 (9%)	N/A
<i>Outcomes</i>			
Intensive Care Unit length of stay, days	9.1 $\pm$ 2.4	6.2 $\pm$ 1.7	0.24
Mechanical ventilator, days	5.7 $\pm$ 2.1	2.5 $\pm$ 0.8	0.13
Hospital length of stay, days	16.4 $\pm$ 3.3	13.2 $\pm$ 2.5	0.36

Table 3

Demographic data, clinical characteristics and outcome of post-PCA/clustering of validation Cohort 2 defined patient Groups A and B. Values are mean $\pm$ SEM. Statistical significance set at $P < 0.05$ by either Mann–Whitney U Test or Chi-square as appropriate.

	Group A n = 9	Group B n = 24	<i>P</i> value
<i>Demographics</i>			
Age, yr	45 $\pm$ 3	44 $\pm$ 1.4	0.69
Sex, male/female	M=5 F=4	M=14 F=10	1
Injury severity score (ISS)	24.7 $\pm$ 1.6	22.8 $\pm$ 1	0.35
<i>Mechanism of injury</i>			
Motor vehicle accidents (MVA), n (%)	6 (67%)	16 (66%)	1
Fall, n (%)	1 (11%)	4 (17%)	0.33
Others, n (%)	2 (22%)	4 (17%)	0.33
<i>Outcomes</i>			
Intensive Care Unit length of stay, days	9 $\pm$ 3.5	7.6 $\pm$ 1.5	0.87
Mechanical ventilator, days	3.7 $\pm$ 1.8	4.5 $\pm$ 1.5	0.93
Hospital length of stay, days	16.3 $\pm$ 5	14 $\pm$ 2	1