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Traumatic Injury, Early Gene Expression, and Gram-Negative Bacteremia

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Abstract

Rationale—Bacteremic trauma victims have a higher risk of death than their non-bacteremic counterparts. The role that altered immunity plays in the development of bacteremia is unknown.

Objective—Using an existing dataset, we sought to determine if differences in early post-injury immune-related gene expression are associated with subsequent gram-negative bacteremia (GNB).

Design—Retrospective cohort study, a secondary analysis of the Glue Grant database.

Subjects—Severely injured blunt trauma patients.

Setting—Seven level one trauma centers across the United States.

Measurements—Total leukocyte gene expression was compared between the subjects that developed GNB and those that did not.

Main Results—We observed that GNB was an independent risk factor for death (OR, 1.86; $P=0.015$). We then compared gene expression at 12 and 96 hours after injury in ten subjects who subsequently developed GNB matched to 26 that did not. At 12 hours, expression of 64 probes differed 1.5-fold; none represented genes related to innate or adaptive immunity. By 96 hrs, 102 probes were differentially expressed with 20 representing 15 innate or adaptive immunity genes; including downregulation of *IL1B* and upregulation of *IL1R2*, reflecting suppression of innate immunity in GNB subjects. We also observed downregulation of adaptive immune genes in the GNB subjects.

Conclusions—By 96 hours after injury, there are differences in leukocyte gene expression associated with the development of GNB, reflecting suppression of *both* innate and adaptive immunity. GNB after trauma is, in part, consequence of host immunity failure and may not be completely preventable by standard infection-control techniques.

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Keywords

Trauma; immunity; gram-negative bacteremia

INTRODUCTION

Traumatic injury is the leading cause of death among young people and the 5th leading cause of death overall in the United States.[1] The majority of these deaths occur immediately after the injury. Those who survive this initial time period are at risk for developing nosocomial infections. Bacteremia, one of many possible nosocomial infections, is associated with poor outcomes and its incidence, particularly due to gram-negative organisms, is increasing.[2–5] We observed that 14% of trauma victims with ventilator associated pneumonia (VAP) had concomitant bacteremia which was associated with markedly increased length of intensive care unit (ICU) stays and a 2.5-fold increased risk of death.[6]

Immune competence requires the detection, recognition and destruction of invading microorganisms. The ability of bacteria to access and survive in the bloodstream likely represents a profound failure of the immune response.[7] Impaired immune competence after trauma is common. However, it has not been possible to correlate post-injury immune and inflammatory alterations with subsequent infectious complications.

The “*Inflammation and the Host Response to Injury*” program was a multicenter collaborative research project designed to increase our understanding of the host's response to injury and the severe systemic inflammatory response that accompanies it. The data and samples collected as part of this program have improved our understanding of the overall response to traumatic injury and the development of subsequent complications. The data have also allowed us to address two objectives regarding bacteremia. First, we sought to confirm whether bacteremia was associated with a poor prognosis, as we previously observed in trauma patients with VAP. Second, we tested whether early gene expression patterns in patients with bacteremia differed from other severely injured patients.

MATERIALS AND METHODS

Patient Recruitment and Enrollment

We analyzed data gathered from the “*Inflammation and the Host Response to Injury*” research program. The organization details, overall scope, and patient recruitment procedures of the program have been previously described.[8] Briefly, 1,819 subjects were enrolled from seven US level I trauma centers from 2003 to 2009. Subjects were enrolled if they had a blunt injury mechanism, had pre-hospital or emergency department (ED) hypotension [systolic blood pressure (SBP) <90mmHg] or an elevated base deficit (>6mEq/L), required blood transfusion within the first 12 hours after injury, and had an Abbreviated Injury Scale (AIS) score of >2 for any body region other than the brain.

The study subjects were treated in accordance with the Standard Operating Procedures (SOPs) adopted by all seven of the participating centers in order to minimize variations

across centers. The SOPs address resuscitation strategies, mechanical ventilation, sedation and analgesia use, diagnosis and treatment of VAP, nutritional support, and other specific aspects of care. The SOPs have been previously described.[9–16]

Subjects were excluded from our analysis if they had an ICU stay of less than three days (either due to death or discharge) using the rationale that bacterial infections in those subjects are less likely due to sequelae of nosocomial pathogen exposure in the ICU. The demographic information that was collected included age, gender, BMI, and measurements of severity of injury and illness [Injury Severity Score (ISS), Acute Physiology and Chronic Health Evaluation II (APACHE II), AIS scores], as well as details of each subject's hospital course including the development of gram-negative bacteremia.

This is a secondary analysis of de-identified data and is therefore classified as exempt by the University of Washington Human Subjects Division.

Statistical Analysis

Clinical data were evaluated using Stata 12 statistical software (StataCorp LP, College Station, TX). Continuous data, with the exception of the gene expression values, are presented as medians and range and were compared using Mann-Whitney U tests. Categorical data are presented as values and percentages and were compared using χ^2 or Fisher's exact tests. Actual *P*-values for all comparisons are presented. Both univariate and step-wise logistic regressions were performed to determine the risk of death associated with bacteremia. Variables were included in the final regression model if their individual *P*-value for association with death was ≤ 0.1 . Graphs were made using Prism (GraphPad Software, Inc. La Jolla, CA).

Propensity Score Matching

To eliminate some of the inherent heterogeneity in the gene expression values of our cohort we matched each subject who developed gram-negative bacteremia to 2–3 subjects who did not develop gram-negative bacteremia (non-gram-negative bacteremia) using propensity scores for the development of gram-negative bacteremia.[17] The propensity score for the development of gram-negative bacteremia was predicted from a multivariate logistic regression model that included the following variables: age, gender, body mass index (BMI), ISS, APACHE II scores, AIS scores for the head, face, neck, chest, abdomen, spine, and extremities, lowest SBP and GCS (Glasgow Coma Scale) from their ED stay, the ED base deficit, maximum blood glucose level in the first 24 hours, and the number of units of packed red blood cells (PRBCs) and volume of crystalloid received in the first 12 hours after injury. Using nearest neighbor matching, each subject with gram-negative bacteremia was matched to 2–3 subjects without gram-negative bacteremia based on their proximity in the database which also represented sequential enrollment in the study. Matching was done using ± 0.05 of the propensity score.

Gene Expression Data

The method for the collection of samples and generation of transcriptional profiles has been previously reported.[8, 18] Patient samples were applied to Affymetrix U133 plus 2.0 Gene

Chip platforms and the intensity signal was uploaded to the TRDB as CEL files. Only the CEL files of subjects with multiple time points and RNA quality ≥ 2 were included in the genomic analysis. The CEL files were perfect-matched normalized in dChip™ prior to analysis.[19] The signals were then converted and exported as expression values. Settings for the dChip™ analysis can be found in the supplemental material.

Gene Expression Analysis

The normalized expression values were adjusted for batch effect using the open access R program, ComBat.R for samples from time points of 12 hours and 96 hours post-injury in order to minimize non-biological variation.[20] ComBat.R processes and settings are detailed in supplemental material. The normalized and batch-corrected expression values for the subjects with gram-negative bacteremia and matched subjects without gram-negative bacteremia were then imported into GenePattern™ (Broad Institute, MIT, Cambridge, MA). [21] Using ComparativeMarkerSelection™[22–25] the values were analyzed to determine the probe sets that were differentially expressed between the subjects with gram-negative bacteremia and those without at each time point (12 hours, 96 hours after injury) using a two-sided t-test. The output of the analysis includes a ranking of the probe sets based on the value of the t-test and adjusts for multiple hypothesis testing using false discovery rate. Details of the settings used in GenePattern™ can be found in the supplemental material. Then, using the ExtractComparativeMarkerSelection™ feature in GenePattern™, we exported the top 200 ranked probe sets. Using a cut-off of ≥ 1.5 -fold differential expression, the probe sets representing genes that are known to be involved in either the innate or adaptive immune system were identified and compared to the expression of the same probe sets at the alternate time point from the normalized and batch-corrected data. Gene expression values are presented as mean $\pm$ standard deviation.

RESULTS

Characteristics of the entire cohort

Data for 1,819 subjects were available and extracted from the database (data abstraction in June, 2011). Two hundred and twenty four of those subjects had an ICU stay of less than 3 days and were excluded from our analysis. The remaining 1,595 subjects were included in our initial analysis.

Patient and injury characteristics and outcomes are included in Table 1 and summarized here. A third of subjects had a severe traumatic brain injury, two-thirds had severe chest injury and nearly half had severe abdominal injury. Eight percent developed gram-negative bacteremia and eight percent developed gram-positive bacteremia. They had long ICU and hospital length of stays and in-hospital mortality of 11%.

Gram Negative, but not Gram Positive, Bacteremia is Associated with Increased Mortality

Gram-negative bacteremia developed in 127 subjects and gram-positive bacteremia occurred in 131. A number of factors were associated with mortality and these are shown in Table 2.

Gram-negative bacteremia was found to be an independent risk factor for death [odds ratio (OR), 1.86; 95% confidence interval (CI), 1.13–3.08; $P=0.015$]. However, gram-positive bacteremia was not. Other factors that were associated with death included age, APACHE II score and units of PRBCs in first 12 hours. The results of the logistic regression analysis are shown in Table 3.

We then focused on determining whether there were differences in early gene expression in these subjects that could explain a predisposition to subsequent gram-negative bacteremia.

Selection of Microarrays by Propensity Score Matching

A total of 121 subjects had total leukocyte microarray data that met sample quality specifications at within 12 hours and 96 hours after injury. Bacteremic subjects differed markedly from the non-bacteremic subjects as summarized in Table 4. Both amount of blood transfusion received and ISS have been associated with increased risk of bacteremia following trauma.[26] Additionally, transfusion of PRBCs alters gene expression, typically seen as an upregulation of inflammatory genes.[27] To minimize the impact of potential confounding factors; we used propensity score matching to select patients, and therefore microarrays, that were as closely matched as possible on factors that contribute to the risk of gram negative bacteremia and that might also directly affect gene expression. Propensity scores allowed us to match the 10 subjects with gram negative bacteremia to 26 subjects without (Table 5).

Genome-wide Expression Analysis

A heatmap of the top 100 differentially expressed at 12 hours probe sets is shown in Figure 1a. Of the top ranked probe sets with 1.5-fold differential expression (increased or decreased), none represented genes in the innate or adaptive immune system.

A heatmap illustrating the top 100 differentially expressed probe sets is shown in Figure 1b. Of the top ranked probes with 1.5 – fold differential expression, 20 represented 15 genes in the innate or adaptive immune system. The expression data for all 20 probe sets at both 96 hours and 12 hours after injury can be found in Supplemental Table 1.

Analysis of Individual Immune-related Genes

At the 96 hour time point, ten inflammatory, two counter-regulatory, and three adaptive immune system genes were differentially expressed. These include interleukin 1 beta (*IL1B*, Figure 2a) and interleukin 2 receptor beta (*IL2RB*, Figure 2b). *IL1B* expression was similar between groups at 12 hours but at 96 hours the subjects that developed gram-negative bacteremia had a progressive decrease in gene expression while the expression of *IL1B* in subjects that did not develop gram-negative bacteremia remained relatively constant. Expression of *IL2RB* followed a similar pattern. Granzyme A, chemokine (C-X3-C motif) receptor 1, chemokine (C-C motif) ligand 3-like 3 and 1, T-cell receptor alpha constant, killer cell lectin-like receptor subfamily B, member 1, T-cell activation RhoGTPase activating protein, and CD86 molecule expressions were also decreased at 96 hours after injury in the subjects that developed gram-negative bacteremia.

Counter-regulatory interleukin 1 receptor, type II (*IL1R2*, Figure 3a) and CD 163 molecule (*CD163*, Figure 3b) were increased at 96 hours after injury in the subjects that developed gram-negative bacteremia compared to those that did not. Specifically, *IL1R2* expression was similar between the two groups at 12 hours. At 96 hours expression remained relatively higher in those destined to develop gram-negative bacteremia. Conversely, *CD163* expression was similar between the two groups at 12 hours but decreased to a far greater degree by 96 hours in patients without bacteremia.

Human leukocyte antigen (*HLA*) genes and showed decreased expression at 96 hours after injury in the subjects that developed gram-negative bacteremia (Figure 4). Concentrating on the two genes highlighted in the figure, for both *HLA-DRA* and *HLA-DMB*, expression was similar between the two groups at the 12 hour time point and at 96 hours the expression in the subjects that developed gram-negative bacteremia persisted while the expression in the subjects that did not develop gram-negative bacteremia showed a progressive increase.

DISCUSSION

Our analysis of genome-wide gene expression demonstrates important differences between patients who subsequently developed gram-negative bacteremia and those who did not. Up until 12 hours after injury, differences were few and unremarkable. However, by 96 hours the differences were marked and suggest immune suppression. Genes that were downregulated at 96 hours in the subjects who developed gram-negative bacteremia represented genes of both the innate and adaptive immune system. In contrast, counter-regulatory genes of the innate immune system were upregulated in the subjects destined to develop gram-negative bacteremia consistent with an overall picture of suppression of *both* the innate and adaptive immune system genes at 96, but not before 12 hours. The “*Inflammation and the Host Response to Injury*” collaborative recently defined the overall response to traumatic injury and described the phenomenon as a “genomic storm”.[28] In the entire study cohort, the duration of altered gene expression correlated with a prolonged and “complicated” recovery, but there were no qualitative differences in the gene expression patterns.[28] We, however, do observe differences in inflammatory and immune gene expression in the subgroup of subjects who developed gram-negative bacteremia. By matching these bacteremic subjects to similarly severely injured control subjects, we have observed differences in both innate and adaptive immune gene expression that could lead to failure of the host to prevent gram-negative bacteria from accessing the bloodstream.

Four of the genes identified at 96 hours are important to innate immune responses. First is *IL1B* (2q14), which encodes IL-1 beta, which is central to the innate immune response.[29] We observed decreased expression of *IL1B* in the subjects that went on to develop gram-negative bacteremia. This is consistent with a study in which investigators observed lower perioperative *IL1B* expression in patients who subsequently developed postoperative sepsis.[30] Second, *IL2RB* (22q13.1) encodes the beta subunit of the interleukin-2 receptor. It is expressed by lymphocytes and by binding with interleukin-2 (IL-2) leads to T-cell proliferation.[31] We observed lower expression of this gene in subjects that developed gram-negative bacteremia, consistent with relative immune suppression. Third, is *IL1R2* (2q12), which encodes the interleukin-1 receptor type II (IL-1 type II receptor) and functions

as a decoy receptor for IL-1 beta. IL-1 type II receptor prevents processing of the propeptide and blocks the interaction of the mature form of IL-1 beta with its functional receptor.[32] We observed a relative increase in IL1R2 expression which likely contributes to suppressed innate immunity in the bacteremic patients. Finally, *CD163* (12p13.3) is expressed by monocytes and macrophages. Binding of such complexes to cells with CD163 binds to hemoglobin/haptoglobin complexes and increases secretion of interleukin-10, a known anti-inflammatory cytokine.[33] Lower *IL1B* and *IL2RB* gene expression and higher *IL1R2* and *CD163* suggests an overall suppression of innate immunity leading to gram-negative bacteremia.

We observed marked decreased expression of all five of the probe sets for HLA genes (chromosome 6) and the CD86 gene in the subjects who developed gram-negative bacteremia. Others have observed decreased expression of HLA antigens on monocytes after severe trauma.[34, 35] Decreased HLA expression has also been associated with higher infection rates in patients undergoing major operations and the degree of decreased expression has been associated with increase in mortality from sepsis.[36] [37] Our observations and these other reports are consistent with the notion that adaptive immune suppression follows severe traumatic injury and contributes to nosocomial infections and perhaps death.

There are doubtless many factors that contribute to alterations in early gene expression and may therefore ultimately lead to gram negative bacteremia. As shown in Table 4, there were many differences in measures of injury severity, shock severity and treatments received (crystalloid and blood transfusions) between patients with and without gram negative bacteremia. These risk factors for bacteremia and other infectious outcomes have been reported by others and each clearly could contribute to the differences in gene expression that we observed at 96 hours after injury. Differences that were not yet large or consistent enough to be manifest by the first sampling time point. Despite the effectiveness of propensity matching in reducing the differences between the bacteremic and non-bacteremic subjects, there were still some differences as could be seen in Table 5. Bacteremic patients were more likely male and received more blood in the first 12 hours. Both of these factors may have, in part contributed to the differences in gene expression and therefore to the development of gram negative bacteremia. Our data can only hint at the potential mechanisms linking the clinical risk factors with gene expression and eventual outcomes.

Our study has important limitations. First, the gene expression profiles were measured from total leukocyte samples, the majority of which are neutrophils; which potentially masks differences in gene expression from minority cell types, such as monocytes. However, the reciprocal increases in *CD163* and *IL1R2*, both primarily expressed by monocytes, indicates that we were not observing simply a relative reduction in monocyte RNA in subjects who subsequently developed gram-negative bacteremia. Second, our analysis was limited by having relatively few bacteremic subjects with available gene expression data. Having more cases may have given us more power to identify other differences in gene expression, which may have resulted in an overall different interpretation of global changes in gene expression. As it is, our limited number of subjects likely resulted in fewer genes meeting the genome-wide threshold for significance. Third, our data and conclusions rely solely on the results of

the microarray analyses without any confirmation individual gene rtPCR or other measurements of protein products. However, we have taken a number of steps to ensure that our gene expression results are robust and that they are derived from quality RNA samples that were managed and analyzed correctly. Nevertheless, as we progress further along this path we will look at individual gene expression with rtPCR. Although we have retrospectively reviewed the clinical and gene expression data, the datasets are robust, complete and include most of the data needed to address our research question. Importantly, having genome-wide expression data allowed us to assess the global inflammatory state in an unbiased manner.

We believe our observations have important implications for understanding and possibly preventing post-traumatic gram-negative bacteremia. For example, recent focus has been on reducing the incidence of bacteremia and other catheter-related infections by using “bundles” based on guidelines that were established to ensure consistency of technique.[38, 39] Doubtless, these interventions are important and have reduced infectious complications in hospitalized patients. However, despite these system-wide approaches, recent reports suggest an increasing incidence of bacteremia; particularly with gram-negative organisms. [3–5] These reports indicate that while bacteremia can be reduced by these interventions, many occur despite optimized care and likely reflect factors specific to the individual patient, such as alterations in their immune response, as demonstrated here. We believe that without interventions aimed at injury-induced alterations in both innate and adaptive immunity, system-wide interventions will not eliminate gram-negative bacteremia. Targeting the use of immunostimulants, such as interferon-gamma, in patients with decreased immune gene expression could potentially lead to improved outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Heatmap of top 100 ranked differentially expressed probe sets from within 12 hours after injury. B) Heatmap of top 100 ranked differentially expressed probe sets at 96 hours after injury. Blue color represents decreased expression and red color represents increased expression with the darker colors indicating a greater degree difference. GNB=subjects that developed gram-negative bacteremia, Non-GNB=subjects that did not develop gram-negative bacteremia

Figure 2.

Pro-inflammatory innate immune system genes interleukin 1 beta (*IL1B*) and interleukin 2 receptor beta (*IL2RB*) are similarly expressed in both subjects that developed gram-negative bacteremia (GNB) and those that did not (Non-GNB) within 12 hours after injury. At 96 hours after injury the gene expression in the Non-GNB subjects persisted while that in the GNB subjects progressively decreased so that both genes showed decreased expression in GNB subjects at 96 hours. Gene expression is presented as units $\pm$ standard deviation.

Figure 3.

Counter-regulatory innate immune system gene expression is increased in subjects that developed gram-negative bacteremia (GNB) compared to those that did not (non-GNB). Within 12 hours after injury, expression interleukin 1 receptor, type II (*IL1R2*) is similar in both groups but by 96 hours, expression in the GNB subjects has persisted while that in non-GNB subjects has decreased. Expression of CD163 molecule (*CD163*) is also similar between the two groups within 12 hours after injury and at 96 hours, expression has decreased in both groups but to a greater degree in the Non-GNB subjects. Gene expression is presented as units $\pm$ standard deviation.

Figure 4.

Pro-inflammatory adaptive immune system gene expression is decreased in subjects that developed gram-negative bacteremia (GNB) compared to those that did not (non-GNB). Within 12 hours after injury, expression of major histocompatibility complex, class II, DR alpha (*HLA-DRA*) and major histocompatibility complex class II, DM beta (*HLA-DMB*) are similar between the two groups but at 96 hours, expression in the Non-GNB subjects had increased whereas the expression in the GNB subjects had persisted. Gene expression is presented as units $\pm$ standard deviation.

Table 1

Patient Demographics, Injury Characteristics, and Clinical Outcomes (N=1595)

Age (yrs.)	41 (26–55)
Male Gender	1043 (65)
Body Mass Index	29.9 (24–70)
Injury Severity Score	34 (24–41)
APACHE II Score	29 (25–33)
Initial GCS	6 (3–15)
Severe Head Injury	575 (36)
Severe Chest Injury	1055 (66)
Severe Abdominal Injury	705 (44)
Lowest ED SBP (mmHg)	83 (72–97)
Initial Base Deficit (mEq/L)	8 (6–11)
Total PRBCs Received in 1st 12hrs (units)	2 (1–3)
Total Crystalloid Received in 1st 12hrs (l)	10 (7–14)
Developed Gram Negative Bacteremia	127 (8)
Day of Onset	8 (5–8)
Development of Gram Positive bacteremia	131 (8.2)
Day of Onset	9 (5 – 9)
Mortality	181 (11.4)
Ventilator Days	8 (3–15)
ICU Length of Stay (days)	11 (6–19)
Hospital Length of Stay (days)	20 (12–32)

GNB=gram-negative bacteremia; APACHE II=Acute Physiology and Chronic Health Evaluation II; Severe Head/Chest/Abdominal Injury=body region abbreviated injury score 3; ED=Emergency Department; SBP=systolic blood pressure; PRBC=packed red blood cells; ICU=intensive care unit. Categorical data are presented as count (%); Continuous data are presented as median (interquartile range).

Table 2

Risk Factors for Mortality (N=1595)

	Died (n=181)	Survived (n=1414)	P-Value
Age >= 56 years	74 (41)	311 (22)	<0.0001
Male Gender	116 (64)	927 (66)	0.7
BMI	30 (25–70)	30 (25–70)	0.23
ISS	34 (29–49)	34 (22–41)	<0.0001
APACHE II >= 34	105 (58)	288 (20)	<0.0001
Severe Head Injury	87 (48)	488 (34)	<0.001
Severe Chest Injury	129 (71)	926 (66)	0.12
Severe Abdominal Injury	75 (41)	630 (45)	0.43
Initial Base Deficit (mEq/L)	9 (6–13)	8 (6–11)	0.005
Lowest ED SBP (mmHg)	77 (63–90)	84 (73–98)	<0.0001
> 3 units PRBC in 1st 12 hrs	87 (48)	307 (22)	<0.0001
Total Crystalloid in 1st 12 hrs. (l)	11 (7–15)	9.6 (7–13)	0.003
Gram-negative Bacteremia	27 (15)	100 (7)	<0.001
Gram-positive Bacteremia	13 (7)	118 (8)	0.59

GNB=subjects who developed gram-negative bacteremia; Non-GNB=subjects who did not develop gram-negative bacteremia; BMI=body mass index; ISS=injury severity score; APACHE II=Acute Physiology and Chronic Health Evaluation II; Severe Head/Chest/Abdominal Injury=body region abbreviated injury score 3; ED=Emergency Department; SBP=systolic blood pressure; PRBC=packed red blood cells; Categorical data are presented as count(%) and compared using χ^2 test; Continuous data are presented as median (interquartile range) and compared with Mann-Whitney U test.

Table 3

Logistic Regression Results, Adjusted Risk of Death

Variable	OR for Death	95% CI	P-value
Gram Negative Bacteremia	1.86	1.13–3.08	0.015
Age 56 yrs.	2.33	1.4–3.86	0.001
APACHE II 34	5.17	2.73–9.79	<0.001
PRB>3 units in 1st 12hrs	2.94	1.58–5.45	0.001

OR=Odds ratio for death; CI=Confidence Interval; APACHE II=Acute Physiology and Chronic Health Evaluation II; PRBC=packed red blood cells. Gram-positive bacteremia, gender, injury severity score, volume of crystalloid infused in first 12 hours, lowest systolic blood pressure in emergency department, maximum glucose in first 24 hours, initial base deficit and severe head injury were included in the regression and were not significant risk factors for mortality.

Table 4

Demographics, Injury Characteristics and Outcomes by Development of Gram-Negative Bacteremia for Total Cohort (1,595)

	GNB (n=127)	Non-GNB (n=1468)	P-Value
ISS	36 (29–45)	34 (22–41)	0.0006
APACHE II	32 (27–35)	29 (25–33)	0.0002
Severe Abdominal Injury	71 (56)	634 (43)	0.006
Initial Base Deficit (mEq/L)	9.5 (6–13)	8 ()	0.001
Total PRBC transfusion in 1st 12 hrs. (units)	2.8 (1–6)	1.5 (1–3)	<0.0001
Total Crystalloid in 1st 12 hrs. (l)	12.3 (9–18)	9.6 (7–13)	<0.0001
Ventilator Days	19 (11–28)	7 (3–14)	<0.0001
ICU Length of Stay (d)	22 (15–32)	10 (5–18)	<0.0001
Length of Stay (d)	31 (21–53)	20 (12–31)	<0.0001
Mortality	27 (21)	154 (11)	<0.001

GNB=subjects who developed gram-negative bacteremia; Non-GNB=subjects who did not develop gram-negative bacteremia; ISS=injury severity score; APACHE II=Acute Physiology and Chronic Health Evaluation II; Severe Head/Chest/Abdominal Injury=body region abbreviated injury score 3; PRBC=packed red blood cells; ICU=intensive care unit; Categorical data are presented as count(%) and compared using χ^2 test; Continuous data are presented as median(range) and compared with Mann-Whitney U test. Age, male gender, body mass index, severe head and chest injury, and lowest emergency department systolic blood pressure; *p*=not significant.

Table 5

Demographics, Injury Characteristics and Outcomes by Development of Gram-Negative Bacteremia for Matched Cohort (N=36)

	GNB (n=10)	Non-GNB (n=26)	P-Value
Age (yrs.)	28 (23–37)	33 (24–47)	0.57
Male Gender	9 (90)	15 (59)	0.06
BMI	29 (24–33)	30 (24–33)	0.94
ISS	40 (33–43)	40 (27–46)	0.86
APACHE II	33 (25–37)	30 (27–33)	0.6
Severe Head Injury	4 (40)	7 (27)	0.45
Severe Chest Injury	7 (70)	16 (62)	0.71
Severe Abdominal Injury	8 (80)	17 (65)	0.68
Initial Base Deficit (mEq/L)	9 (4–15)	11 (8–14)	0.27
Lowest ED SBP (mmHg)	81 (75–87)	84 (77–96)	0.6
Total PRBC transfusion in 1st 12 hrs. (units)	5 (2–7)	3 (2–4)	0.07
Total Crystalloid in 1st 12 hrs. (l)	19 (12–28)	15 (9–19)	0.24
Maximum Glucose in 1st 24hrs (mg/dL)	209 (151–295)	180 (152–226)	0.43
Ventilator Days	16 (10–28)	12 (6–17)	0.11
ICU Length of Stay (d)	20 (13–32)	17 (10–21)	0.24
Length of Stay (d)	44 (22–57)	29 (21–41)	0.22
Mortality	1 (10)	2 (8)	0.82

GNB=subjects who developed gram-negative bacteremia; Non-GNB=subjects who did not develop gram-negative bacteremia; BMI=body mass index; ISS=injury severity score; APACHE II=Acute Physiology and Chronic Health Evaluation II; Severe Head/Chest/Abdominal Injury=body region abbreviated injury score 3; ED=Emergency Department; SBP=systolic blood pressure; PRBC=packed red blood cells; ICU=intensive care unit; Categorical data are presented as count (%) and compared using Fisher's exact test; Continuous data are presented as median(interquartile range) and compared with Mann-Whitney U test.