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Acute kidney injury is associated with early cytokine changes after trauma

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Abstract

Background—Acute kidney injury (AKI) occurs in 26% of trauma patients and is associated with increased mortality and risk for nosocomial infections (NCI). We compared serial plasma cytokine levels in patients with posttraumatic AKI to determine whether the early cytokine changes are associated with the occurrence of AKI and NCI.

Methods—We performed a secondary analysis of the “Inflammation and the Host Response to Injury” database to include adult blunt trauma patients who had available plasma proteomic analyses. AKI was defined by the RIFLE (Risk, Injury, Failure, Loss, and End-stage Kidney) classification, which requires a 50% increase in serum creatinine. The association between AKI, NCI and plasma cytokines was analyzed using a mixed model analyses and logistic regression.

Results—Among 147 patients in the cohort, prevalence of NCI was 73% and 52% for patients with and without AKI, respectively. In mixed model analyses adjusted for clinical factors, AKI patients developed significant early increase in IL1ra, IL8, MCP1 and IL6, early decrease in sTNFR2, and late decrease in IL1ra, IL4 and IL6 concentrations, compared to patients without

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Author Contributions

Study concept and design: Drs. Moldawer, Bihorac, Baslanti, Cuenca and Hobson.
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Analysis and Interpretation of data: Drs. Bihorac, Baslanti, Cuenca, Moldawer, Maier, Hobson and Efron.
Drafting of the manuscript: Drs. Bihorac, Baslanti, Cuenca, Hobson and Moldawer.
Critical revision of the manuscript for important intellectual content: Drs. Bihorac, Baslanti, Hobson, Moore, Efron, Cuenca, Moldawer and Maier.
Statistical analysis: Drs. Bihorac, and Baslanti.
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AKI and regardless of NCI. The change in cytokine pattern differed for sIL1R2, CXCL1 and MIP1β depending on the occurrence of NCI: Patients with AKI and NCI had lower early and late sIL1R2 and higher early and late CXCL1 and MIP1β levels. Within first 24 hours of injury, adding plasma levels of IL1ra, IL8, MCP1, IL6, and sTNFR2 to clinical parameters of injury severity provided a predictive model for AKI superior to clinical model only (P<0.001).

Conclusion—AKI trauma patients exhibit simultaneous changes in pro and anti-inflammatory serial plasma cytokine levels. The predictive model for AKI that combines plasma cytokine levels with clinical data within 24 hours of injury requires further prospective validation in larger studies.

Keywords

Acute Kidney Injury; Nosocomial Infections; Trauma; Cytokine

INTRODUCTION

Although acute kidney injury (AKI) is independently associated with adverse outcomes among critically ill patients, only recently has it become appreciated as a common and serious complication of trauma[1,2]. The introduction of the RIFLE-AKI (Risk, Injury, Failure, Loss, and End-stage Kidney), a consensus definition for AKI, has increased awareness of the adverse effects of small changes in serum creatinine (sCr) level among different patient populations, and has facilitated a more uniform reporting of the prevalence and consequences of AKI[3].

RIFLE-AKI occurs in up to 26% of trauma patients and is associated with increased morbidity, mortality and cost[1,2]. AKI is characterized by local and systemic inflammatory reaction where many locally produced cytokines mediate not only AKI itself, but also exert distant organ injury through systemic release from leukocytes in the kidneys and renal tubular cells[4-6]. It is recognized that critically ill patients with severe AKI have an increased prevalence of infection[4], and we have recently demonstrated a higher prevalence of nosocomial infections (NCI) among trauma patients with even less severe stages of RIFLE-AKI [7].

The Trauma-Related DataBase (TRDB), a large multicenter database, contains prospectively collected clinical and biological data as a part of the *Inflammation and the Host Response to Injury* (Glue Grant), a large-scale interdisciplinary research program funded by the National Institute of General Medical Sciences to uncover the biological reasons for different clinical outcomes after traumatic injury. Previous studies of system-wide inflammation after trauma using quantification of serum cytokine levels from the TRDB demonstrated elevated levels of interleukin 6 (IL-6) and IL-10 as predictors of poor outcomes following trauma [8]. Since animal models of ischemic AKI have implicated complex changes in both pro and anti-inflammatory cytokine profile in association with kidney injury[5,6,9] we hypothesized that trauma patients with AKI may exhibit a similar changes in cytokine profile over time and that an increased prevalence of NCI among AKI trauma patients may reflect this association. The goal of this study was to assess the longitudinal change in serum cytokine levels among patients with severe blunt trauma enrolled in the Glue Grant database and their association with RIFLE-AKI and nosocomial infections.

METHODS

Study design and subjects

We performed a secondary analysis of the TRDB's sampling cohort of adult severe blunt trauma patients with no previous history of chronic kidney disease (CKD) enrolled between

November 2003 and March 2008 (www.gluegrant.org). Criteria for enrollment were age ≥ 16 years, blunt trauma, arrival to hospital within 6 hours of injury, either hypotension or base deficit ≥ 6, blood transfusion within 12 hours of injury, body region exclusive of brain with an Abbreviated Injury Scale score ≥ 2, and intact cervical spinal cord. According to GlueGrant protocol biological samples for cytokine analyses were obtained only from the subgroup of patients younger than 55 years. The Steering Committee of the Glue Grant and the Institutional Review Board of the University of Florida approved this secondary use of the database.

Outcomes and covariate definition

Nosocomial infections and clinical covariates were defined by the Glue Grant protocol [10] (Supplemental Digital Content (SDC), Methods). AKI was defined by the RIFLE classification using the change in sCr during the first 28 days of hospitalization compared to reference sCr (RsCr) [11]. For the reference sCr we used the lower of two values: the lowest measured sCr in the first 24 hours after trauma or the estimated sCr as previously described[1]. Patients with RIFLE-AKI were stratified by severity determined by comparing the highest sCr with the RsCr. RIFLE-R corresponds to a 50% increase in sCr, RIFLE-I to a two-fold increase in sCr, and RIFLE-F to a three-fold increase in sCr. Duration of renal replacement therapy (RRT) was obtained from the database. Cytokine concentrations from thawed plasma were assayed with the LINCOplex kit (Millipore, Billerica, MA) using the LUMINEX (Millipore) technology according to analytical protocols [12] (SDC, Table 1). Time of measurements after injury was categorized as less than 12 hours (Day 0), 24-96 hours (Day 1-4) and ≥96 hours (≥Day 4).

Statistical analyses

The analytical plan followed the STROBE recommendations for observational cohort studies [13] (SDC, Methods). We utilized mixed model analysis of longitudinal changes in cytokines in order to properly account for correlations among repeated measurements for each patient. For each cytokine we modeled change over time of log-transformed cytokine concentration while accounting for time, AKI, nosocomial infections, and all two-way interactions. For AKI patients only we tested the additional effect of survival status as no deaths were observed in no AKI group. Each patient was entered into the model as the subject random effect. The Kenward-Roger method was used to calculate the denominator degrees of freedom due to the unbalanced study design[20]. Bonferroni adjustments were used for multiple comparisons. We performed sensitivity analyses by exclusion of outliers (values three standard deviations away from the mean after log-transformation) and missing values. Since none of the sensitivity analyses demonstrated statistically significant changes, we decided not to exclude any observations.

Using individual univariable logistic regression models, we tested the association between early (first 12 hours) log-transformed plasma cytokine levelsand the occurrence of adverse hospital outcomes - mortality, AKI and nosocomial infections- to determine optimal discrimination limits (ODL) while maximizing sensitivity and specificity along the receiver operating characteristic (ROC) curve. For each cytokine, we created a binary variable with high and low risk categories using ODL as a cut-off point. For each cytokine we constructed three multivariable logistic regression models (MLRL) testing the association between each outcome and the high-risk (values equal, above or below ODL depending on the direction of association) versus low-risk cytokine category as the main independent variable while adjusting for APACHE II and ISS [21]. Area under the receiver operating characteristic curve (ROC AUC) and their 95% confidence intervals (CI) were calculated for each model. Due to small number of deaths in the sample, we used bootstrapping as a resampling tool to derive bias-corrected CI for the effect dichotomized cytokines have on hospital mortality [21]. We

drew 1000 bootstrap samples and estimated the odds ratio (OR), CI and AUC in each sample. The values corresponding to the 2.5 and 97.5 percentiles of the 1000 bootstrap estimates were used as the limits of the 95% CI. For each model we performed model diagnostics to test for collinearity, interactions and model fit. We performed a sensitivity analysis using cytokines as continuous variable instead of as a binary variable. The difference between AUCs was tested using DeLong test[22]. Statistical analyses were performed with SAS (v.9.3, Cary, N.C.).

RESULTS

Clinical characteristics of trauma patients with acute kidney injury and nosocomial infections

We analyzed data for 147 adult patients with severe blunt trauma and no previous history of CKD who lived longer than 24 hours following injury, and for whom serial cytokine measurements were available (Table 1, SDC Table 2). Close to one fourth of these patients developed RIFLE-AKI in the first 28 days after trauma (23%, 33/147) with early onset (median time of AKI onset one day, 75th percentile 2 days) and two-thirds of these subjects had mild or moderately severe AKI (RIFLE-R and RIFLE-I) only. One third of the patients with AKI did not have complete recovery of their kidney function in the first 28 days after trauma, and 9% of patients with NCI and AKI remained RRT dependent at 28 days.

Nosocomial infections developed in 73% and 52% of patients with and without RIFLE-AKI, respectively. Ventilator-associated pneumonia and bloodstream infections were more frequent in AKI patients compared to patients with no AKI. Injury severity score (ISS) was associated with the occurrence of NCI in both AKI and no AKI patients, with the highest prevalence of NCI among patients with severe and massive injury. Overall patients with NCI had higher APACHE II scores but that association was only observed among AKI patients when nosocomial infections were stratified by AKI status (Table 1). Overall, AKI patients received more blood transfusion and less crystalloids in the first 24 hours compared to no AKI patients, but the statistical significance was not present after stratifying for NCI status.

Although patients with AKI were more severely ill as reflected in the higher multiple organ dysfunction (MOD) scores, no difference in the score existed between AKI patients stratified by NCI (Table 1). However, the prevalence of organ dysfunction (MOD score ≥ 3) was very high in this cohort. Non-infectious complications and surgical site infections were more likely to occur in patients with AKI regardless of NCI. Patients with NCI required a longer ICU and hospital stay regardless of the presence of AKI. In-hospital mortality was observed for patients with NCI only if they had concomitant AKI (0% mortality for patients without AKI and 21% for patients with AKI, p-value=0.002) but the mortality rate in the AKI group did not differ depending on the development of NCI (22% mortality rate for AKI and no NCI and 21% for AKI and NCI).

Longitudinal change in plasma cytokine concentration

In multiple individual mixed model analyses adjusted for age, gender, race, ISS scores, NCI, AKI, time, AKI and time interaction, and AKI and NCI, AKI was independently associated with a change over time for several cytokines (Table 2, SDC Table 3). No change in association was observed when sensitivity analyses were performed with mixed models omitting age, gender, race and ISS scores (*data not shown*). In comparison with patients without AKI, AKI patients had significantly higher plasma levels of IL1ra, IL8, MCP1 and IL6 in the early time period (Day 0), regardless of the occurrence of NCI. Similarly, AKI was associated with the significant decrease in plasma levels for IL1ra, IL4, IL6 and

sTNFR2 in the late time period (after day 4) for both patients with and without NCI and in comparison to patients without AKI (Table 3). On the other hand, we observed statistically significant interaction between AKI and the occurrence of NCI for sIL1R2, CXCL1 and MIP1β (Table 2). As a result, among AKI patients with NCI sIL1R2 exhibited an early decrease as opposed to increase among AKI patients without NCI while CXC1 and MIP1β had an early increase in contrast to early decrease among AKI patients without NCI (Table 3). We observed that AKI non-survivors had significantly lower early and late MIP1α and MIP1β levels regardless of the occurrence of NCI (SDC Table 4).

Association between early cytokine levels and adverse outcomes

Using multiple individual univariable logistic regression models, ODL for each cytokine in relationship to hospital mortality, AKI and NCI were obtained while maximizing sensitivity and specificity along the ROC curve (Table 4). Interestingly, ODLs for each outcome were different; for MCP1 and MIP1β the direction of change associated with the mortality and AKI was opposite (lower levels associated with mortality and higher levels associated with AKI occurrence). We constructed three separate MLRM testing the association between hospital mortality, AKI and NCI occurrence as an outcome and high-risk versus low-risk category for each cytokine using ODL as cut-off while adjusting for injury severity using APACHE II and ISS scores. For hospital mortality, we demonstrated a significant association only between IL10 (high-risk defined as < 28 pg/ml) with AUC of the model of 0.72 (95% CI 0.49, 0.96). Bootstrap method yielded similar results with no statistically significant difference (SDC Table 5). Early cytokine levels for IL1ra > 4763 pg/ml, sIL1R2 > 5022 pg/ml, IL8 > 32 pg/ml, MCP1 > 205 pg/ml, IL6 > 178 pg/ml and sTNFR2 < 8307 pg/ml were associated with increased risk for AKI with AUC ranging from 0.61-0.74. For NCI, we demonstrated a significant association only for IL4 > 725 pg/ml (AUC 0.71 95% CI 0.62-0.80). The MLRM for AKI as an outcome using all cytokines demonstrated that there was significant confounding effect among cytokines causing > 10% changes in adjusted ORs but overall model performed superior to clinical model with APACHE II and ISS scores or any individual cytokine model alone. The sensitivity analysis using cytokines as continuous variables did not demonstrated any significant change in results (*data not shown*).

DISCUSSION

In a retrospective analysis of multi-center prospective cohort study of trauma patients with no previous kidney disease, patients with AKI developed early and late simultaneous changes in pro-and anti-inflammatory cytokines: early increase in IL1ra, IL8, MCP1 and IL6 concentrations, early decrease in sTNFR2, and late decrease in IL1ra, IL4 and IL6 concentrations, compared to patients without AKI and regardless of the occurrence of NCI. This association was confirmed in multivariable regression model where addition of early levels for these cytokines to clinical parameters provided a robust model with AUC superior to clinical model only. However, the change in cytokine pattern differed for sIL1R2, CXCL1 and MIP1β depending on whether AKI patients were to develop NCI. Patients with AKI who developed nosocomial infections demonstrated lower early and late anti-inflammatory sIL1R2 and higher early and late chemokines CXCL1 and MIP1β levels. Interestingly, for MIP1β this change was less prominent among patients with AKI and NCI who died as compared to those who survived. More importantly, only those patients with NCI who had concomitant AKI experienced a mortality rate of 20%. In a mixed model analysis adjusted for age, gender, race, APACHE II and ISS scores, AKI was independently associated with a change in cytokine levels over time.

The alteration in the host immune response after severe trauma involves simultaneous induction of innate and suppression of adaptive immunity genes with changes in both pro-

and anti-inflammatory cytokines[8,14,15]. Several studies have reported increased levels of MIP1β, IL10, IL6 and IL1ra as predictive of development of organ dysfunction after trauma[8,14,16]. Furthermore, studies have implicated a relationship between immune dysregulation after trauma and the subsequent development of sepsis and nosocomial pneumonia [17,18]. Suppressed macrophage regulatory and effector functions observed after trauma lead to alterations in cytokine production and decrease the ability to clear bacterial pathogens from the pulmonary airspace [19,20].

Using the TRDB epidemiological cohort we have recently demonstrated that RIFLE-AKI is not only common but is also associated with a 2.5-fold increase in the risk of dying in a multivariable regression model adjusted for baseline clinical characteristics, injury severity and resuscitation strategy [1]. In addition, AKI was significantly associated with a risk for nosocomial and surgical site infections[1,7]. This relationship between AKI and risk for infections is bidirectional as not only are patients with AKI more likely to develop sepsis and NCI, but AKI commonly complicates NCI and sepsis and worsen their outcomes[21-23].

It is becoming clear that AKI should no longer be viewed as just an indicator of overall severity of illness in critically ill patients. The injured kidney can exhibit independent effects on other distant organs, especially the lungs[24], through local and systemic inflammatory responses where many locally produced cytokines mediate not only AKI itself, but also exert distant organ injury through systemic release from leukocytes in kidneys and renal tubular cells [4-6]. In a mouse model, AKI sensitizes mice to endotoxin lipopolysaccharide (LPS) as evidenced by exaggerated renal production of TNFα, MCP1, and IL10 causing renal “hyperresponsive” state with either normal or reduced renal TLR4 expression[9]. In an ischemia/reperfusion (IR) AKI mouse model, expression profile of kidney tissue showed a biphasic expression of all up-regulated CXC chemokines (MCP-1, TARC, KC and MIP-2α, chemokine receptors CCR1 and CX3CR1) coinciding with the early inflammatory and late repair AKI phases, and was associated with the systemic leakage of pro-inflammatory chemokines released by injured kidney tissues [5,25]. In AKI trauma patients, we observed increase in both early and late chemokine plasma levels of IL8, CXCL1, MCP1 and MIP1β. Through infiltration of activated macrophages via CCR2 signaling[26], MCP1 plays a key role in the pathogenesis of both toxic and IR AKI as demonstrated by elevated levels of urinary MCP1 mRNA in mice and humans [27]. Both early and late plasma levels of MCP1 were uniformly increased among AKI patients in our cohort. A rise in renal keratinocyte-derived chemokine (KC) (analog of human CXCL1) was the earliest and most consistent change in mouse IR AKI, and urine and plasma levels were elevated well before sCr [28]. KC is regulated by the chemokine MCP1 after trauma-hemorrhage [29] and plays a pivotal role in neutrophil infiltration and organ damage after trauma-hemorrhage and resuscitation [29]. In our cohort, elevated serial levels of CXCL1 and MIP1β were observed only among trauma AKI patients who developed nosocomial infections. In both animal models and human studies, distant lung injury after AKI was associated with an increase in plasma levels of KC, IL6 and IL8 within 2 hours after AKI onset [30,31]. Genes related to innate immunity were significantly upregulated in both kidney and lung tissues of mouse IR AKI, including *Cd14* gene for the LPS receptor and *Il1r2* gene for IL-1 receptor type II[5]. Interestingly, significant and persistent increase in sIL1R2 was observed only among AKI patients who did not develop NCI while patients with NCI had a persistent decrease in sIL1R2 plasma levels.

Our study has several limitations. This is a retrospective analysis of prospectively collected data from which causal inference cannot be fully derived, and which could be subject to bias from unmeasured factors although we attempted to control for selection bias with appropriate statistical methods and risk adjustment. Although TRDB dataset represents a multicenter effort with potentially heterogeneous population, the strict standard operating

procedures for clinical management of the patients were maintained to minimize variation across centers involved in the data collection. The generalizability of results is limited to younger patients (< 55 years old) according to the Glue Grant protocol. Due to small number of deaths in the cohort, we used bootstrapping as a resampling tool to validate the model-based inferences and to derive bias-corrected confidence intervals. We improved internal validity by utilizing mixed model analysis for repeated measures to account for interactions, confounders and missing values and by performing multiple sensitivity analyses.

In conclusion, in a prospective cohort of severe trauma patients younger than 55 years, the observed risk for NCI and death among those with AKI was associated with an altered cytokine response characterized by simultaneous changes in both pro and anti-inflammatory serial plasma cytokine levels. Adding plasma cytokine concentrations within 12 hours of injury to initial clinical parameters of injury severity provided a predictive model with good internal validity that will require prospective validation in larger studies. Further prospective studies should focus on better characterization of systemic and local immune-modulatory effect of AKI and its resulting effect on clinical outcomes among trauma patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The corresponding author has full access to all the data in the study and has final responsibility for the decision to submit the manuscript for publication.

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Table I

Baseline host characteristics, anatomic and physiologic injury severity indicators and clinical outcomes for patients stratified by nosocomial infections (NCI) and acute kidney injury (AKI).

	NO NCI		NCI	
	No AKI (N=55)	**AKI (N=9)**	**No AKI (N=59)**	**AKI (N=24)**
Baseline host characteristics				
Age (years) (mean, 95% CI)	32 (29,35)	36 (27,45)	36 (33,39)	33 (29,38)
Male, N (%)	30 (55)	7 (78)	38 (64)	19 (79)
African-American ethnicity, N (%)	4 (7)	0 (0)	3 (5)	1 (4)
BMI (kg/m^2) (mean, 95% CI)	28 (26,29)	28 (23,33)	29 (27,30)	31 (28,34)
(median, 25th-75th)	27 (24,31)	29 (21,31)	28 (25,32)	29 (25,35)
Anatomic injury indicators (0 to 24 hours) [1]				
Injury Severity Score, N (%)				
Mild injury (<16)	1 (2)	0 (0)	3 (5)[b]	0 (0)[b]
Moderate injury (16-24)	13 (23)	1 (11)	3 (5)	1 (4)
Severe injury (25-40)	22 (40)	7 (78)	23 (39)	7 (29)
Massive injury (>40)	19 (35)	1 (11)	30 (51)	16 (67)
Physiologic injury indicators (0 to 24 hours) [1]				
Apache II (mean, 95% CI)	26 (24,28)	27 (25,28)	29 (28,30)[b]	29 (26,32)
Lactate ≥ 5 mmol/l, N (%)	19 (35)	5 (56)	26 (44)	14 (58)
Base deficit ≤ −10, N (%)	26 (47)	4 (44)	24 (41)	15 (63)
Blood glucose > 200 mg/dl, N (%)	18 (33)	5 (56)	27 (46)	7 (29)
RBC transfusion (U) (median, 25th-75th)	4 (2,8)	7 (6,9)	6 (3,9)	8 (4,16)
Crystalloids (L) (median, 25th-75th)	1.4 (1, 3.1)	0.8 (0.3, 1.7)	2.6 (1.1, 3.5)	1.3 (0.4, 3.1)
Multiple Organ Dysfunction (MOD) (first 28 days) [1]				
MOD_{max} score (mean, 95% CI)	4 (4,5)	7 (4,10)	6 (5,6)[b]	9 (8,10)[a]
Organ dysfunction/failure[2], N (%)	38 (69)	8 (89)	54 (92)[b]	24 (100)
Day of MOD_{max} score (median, 25th-75th)	2 (0,3)	4 (0,6)	2 (2,4)[b]	6 (3,11)[a]
Mechanical Ventilation > 24 hours, N (%)	52 (95)	9(100)	59 (100)	24 (100)
MV duration (days) (median, 25th-75th)	3 (2,5)	8 (7,10)[a]	10 (5,15)[b]	20 (12,27)[a, b]
AKI characteristics and renal outcomes (first 28 days) [1]				
AKI onset (days since injury) (median, 25th-75th)		1 (1, 2)		1 (1, 3)
RIFLE Risk (RIFLE-R), N (%)		7 (78)		9 (38)
RIFLE Injury (RIFLE-I), N (%)		0 (0)		8 (33)
Rifle Failure (RIFLE-F), N (%)		2 (22)		7 (29)
Renal replacement therapy, N (%)		0 (0)		4 (17)
Renal recovery, N (%)				

	NO NCI		NCI	
	No AKI (N=55)	**AKI (N=9)**	**No AKI (N=59)**	**AKI (N=24)**
Complete recovery		7 (78)		16 (67)
Partial recovery		2 (22)		5 (21)
No recovery		0 (0)		3 (12)
Type of NCI (first 28 days) [1]				
Ventilator-associated pneumonia (VAP), N (%)			34 (58)	19 (79)
Bloodstream infection (BSI), N (%)			14 (24)	10 (42)
Urinary tract infection, N (%)			17 (29)	7 (29)
Clostridium difficile colitis, N (%)			3 (5)	2 (8)
Central line associated blood stream infection, N (%)			3 (5)	2 (8)
NCI onset (days since injury) (median, 25th-75th)			5 (4, 10)	8 (4, 13)
VAP onset (days since AKI onset) (median, 25th-75th)				4 (0, 11)
BSI onset (days since AKI onset) (median, 25th-75th)				7.5 (7, 17)
Hospital Complications and Outcomes				
ICU LOS (days) (median, 25th-75th)	6 (5,9)	9 (8,10)	14 (8,18)[b]	23 (14,35)[a, b]
Hospital LOS (days) (median, 25th-75th)	14 (9, 19)	19 (12, 38)	26 (18, 33)[b]	34 (23, 49)
Non-infectious complications, N (%)	11 (20)	6 (67)[a]	38 (64)[b]	22 (92)[a]
Surgical site infections, N (%)	5 (9)	1 (11)	18 (31)[b]	11 (46)
Hospital mortality, N (%)	0 (0)	2 (22)[a]	0 (0)	5 (21)[a]
Discharge to Home, N (%)	28 (51)	3 (43)	19 (32)	5 (26)
Discharge to skilled nursing facility, N (%)	14 (25)	1 (14)	27 (46)	7 (36)
Discharge to inpatient rehabilitation, N (%)	9 (16)	3 (43)	10(17)	6 (32)

Abbreviations: AKI, acute kidney injury; NCI, nosocomial infection; CI, confidence interval; BMI, body mass index; RBC, red blood cells; U, units; MOD score, the Marshall multiple organ dysfunction score;ICU, Intensive care unit; MV, mechanical ventilation; LOS length of stay. All percentages were calculated as column percentages.

[1] All time points are determined from the time of injury.

[2] MOD score ≥ 3 was used as a cut-off point for an organ dysfunction/failure.

[a] Statistically significant differences between patients with and without AKI stratified by NCI (within NCI or no NCI group) as indicated at 0.05 significance level.

[b] Statistically significant differences between patients with and without NCI stratified by AKI(within AKI or no AKI group) as indicated at 0.05 significance level.

Table 2

Relationship between changes in cytokine levels over time and the occurrence of acute kidney injury (AKI) and nosocomial infections (NCI).

	NO NCI, No AKI (N=55)	No NCI and AKI (N=9)	NCI, No AKI (N=59)	NCI and AKI (N=24)	Significant effects
ILlra pg/mL					Time (p<0.0001), AKI*Time (p<0.0001),
Day 0[1]	913 (235, 2901)	9707 (8692,18387)	1171 (36, 3698)	2461 (36,22248)	
Day 1-4	175 (36, 1219)	36 (36,126)	172 (36, 1200)	62 (36,1382)	
After Day 4	384 (36, 1753)	36 (36,36)	127 (36, 1053)	36 (36,769)	
sIL1R2 pg/mL					AKI (p=0.003), AKI*NCI(p=0.022)
Day 0	2867 (1513, 4714)	7322 (5308,13897)	3311 (1712, 7402)	2303 (1036,9890)	
Day 1-4	2677 (1566, 4991)	11021 (3616,21111)	3035 (1526, 7532)	4008 (2110,11021)	
After Day 4	2674 (1265, 4811)	6567 (4931,10230)	3139 (1303, 6017	3296 (1239,8640)	
IL4 pg/mL					AKI (p=0.012), Time (p=0.015)
Day 0	227 (31, 457)	108 (23,256)	165 (49, 397)	177 (23,521)	
Day 1-4	125 (38, 385)	64 (29,125)	127 (39, 351)	132 (23,315)	
After Day 4	132 (23, 421)	24 (23,105)	123 (23, 354)	63 (23,243)	
sTNFR2 pg/mL					AKI (p=0.034)
Day 0	20156 (7097, 100403)	7938 (5445,15566)	25603 (9045, 88214)	8021 (6408,57499)	
Day 1-4	18454 (6066, 87469)	7916 (5025,20553)	11320 (4671, 60261)	13199 (5423,34165)	
After Day 4	12966 (5595, 92215)	11442 (3162,42229)	15091 (6504, 66558)	11354 (5271,42440)	
IL8 pg/mL					Time (p<.0001), AKI*Time (p=0.041)
Day 0	35 (15, 94)	43 (37,91)	58 (15, 121)	104 (49,405)	
Day 1-4	26 (7, 55)	17 (7,34)	24 (5, 49)	23 (4,158)	
After Day 4	19 (4, 49)	8 (3,35)	17 (6, 46)	30 (10,87)	
CXCL1 pg/mL					AKI (p=0.004), NCI (p=0.039), AKI*NCI (p=0.016)
Day 0	148 (44, 409)	40 (10,75)	95 (33, 271)	77 (27,240)	
Day 1-4	97 (19, 469)	27 (3,86)	81 (20, 343)	86 (7,191)	
After Day 4	126 (52, 820)	17 (6,76)	192 (40, 504)	111 (35,345)	
MCP1 pg/mL					Time (p<.0001), AKI*Time (p=0.021)
Day 0	243 (106, 433)	294 (226,857)	199 (77, 461)	332 (166,1442)	
Day 1-4	115 (39, 265)	179 (42,265)	113 (48, 284)	203 (129,558)	

	NO NCI, No AKI (N=55)	No NCI and AKI (N=9)	NCI, No AKI (N=59)	NCI and AKI (N=24)	Significant effects
After Day 4	83 (31, 182)	51 (34,144)	65 (29, 187)	82 (14,346)	
MIPIα pg/mL					Time (p=0.011)
Day 0	31 (10, 79)	66 (10,100)	17 (10, 92)	20 (10,71)	
Day 1-4	29 (10, 86)	31 (14,42)	22 (10, 110)	10 (10,80)	
After Day 4	16 (10, 78)	38 (10,54)	17(10, 55)	10 (10,44)	
MIP1β pg/mL					NCI (p=0.010), Time (p=0.003), AKI*NCI (p=0.017)
Day 0	188 (99, 585)	147 (86,248)	169 (99, 543)	234 (98,921)	
Day 1-4	146 (83, 299)	96 (52,115)	188 (95, 353)	226 (90,600)	
After Day 4	198 (103, 387)	84 (60,134)	131 (87, 272)	166 (97,474)	
IL6 pg/mL					Time (p<.0001)
Day 0	163 (87, 412)	477 (253,725)	141 (74, 549)	289 (140,858)	
Day 1-4	90 (25, 309)	80 (11,139)	95 (31, 379)	111 (24,581)	
After Day 4	52 (9, 293)	27 (5,74)	44 (8, 154)	25 (6,106)	
IL1β pg/mL					Time (p=0.021)
Day 0	46 (3, 95)	9 (4,53)	27 (5, 74)	14 (1,110)	
Day 1-4	24 (7, 72)	16 (7,35)	23 (5, 58)	14 (1,56)	
After Day 4	20 (4, 85)	7 (1,19)	12 (2, 52)	8 (1,36)	

Abbreviations: NCI, nosocomial infection; AKI, acute kidney injury.

[1]Time points were defined using time elapsed since injury as described in Methods. Data are presented as medians (25th percentile, 75th percentile). Significant effects were derived from multiple individual mixed effect models that included AKI, NCI, time, AKI and time interaction, and AKI and NCI interaction as independent covariates and log-transformation of cytokine levels as dependent values while adjusting for age, gender, race and injury severity score (ISS).

Table 3

Summary of early and late changes in cytokines in the presence of acute kidney injury (AKI) and nosocomial infections (NCI).

	AKI	
	No NCI	**NCI**
Anti-inflammatory cytokines		
IL1ra	Early ↑ Late ↓	Early ↑ Late ↓
sIL1R2	Early ↑ Late ↑	Early ↓ Late ↓
IL4	Late ↓	Late ↓
sTNFR2	Early ↓	Early ↓
Chemokines		
IL7	Early ↑	Early ↑
CXCL1	Early ↓ Late ↓	Early ↑ Late ↑
MCP1	Early ↑ Late ↑	Early ↑ Late ↑
MIP1β	Early ↓ Late ↓	Early ↑ Late ↑
Pro-inflammatory cytokines		
IL6	Early ↑ Late ↓	Early ↑ Late ↓

All statistically significant changes are derived from the mixed model analyses in Table 2.

↑ indicates higher values for AKI patients vs. no AKI patients for each nosocomial infection group at early (within 12 hours of the injury) and late (after day 4) time points.

↓ indicates lower values for AKI patients vs. no AKI patients for each nosocomial infection group at early(within 12 hours of the injury) and late (after day 4) time points.

Table 4

Association between early changes in cytokine levels and adverse outcomes

Outcome	Hospital mortality ODL(Sensitivity, specificity)[1] Odds Ratio[2] (95% CI) AUC (95% CI)	Acute Kidney Injury ODL (Sensitivity, specificity) Odds Ratio (95% CI) AUC (95% CI)	Nosocomial Infections ODL (Sensitivity, specificity) Odds Ratio (95% CI) AUC (95% CI)
Clinical Model (CM)[3]			
APACHE II	1.14 (0.96, 1.34)	1.02 (0.94, 1.10)	**1.08 (1.00, 1.15)**
ISS	1.02 (0.95, 1.08)	1.00 (0.97, 1.04)	1.03 (0.99, 1.06)
	0.67 (0.46, 0.88)	0.49 (0.37, 0.61)	0.66 (0.57, 0.75)
CM and IL1ra (pg/ml)	(>) 14558 (0.43, 0.90)	(>) 4763 (0.59, 0.84)	(<) 36 (0.28, 0.86)
	3.54 (0.57, 22.14)	**7.87 (3.19, 19.41)**	2.48 (0.97, 6.31)
	0.70 (0.48, 0.92)	0.74 (0.63, 0.84) *	0.69 (0.60, 0.78)
CM and sIL1R2 (pg/ml)	(>) 5022 (0.71, 0.72)	(>) 5022 (0.5, 0.76)	(>) 8161 (0.24, 0.83)
	4.2(0.85, 21.58)	**2.84 (1.22, 6.59)**	1.58 (0.63, 3.92)
	0.77 (0.58, 0.96)	0.61 (0.49, 0.73)	0.67 (0.58, 0.77)
CM and IL4 (pg/ml)	(<) 60 (0.71, 0.72)	(<) 77 (0.47, 0.71)	(>) 725 (0.18, 0.98)
	4.1 (0.81,20.49)	2.15 (0.95, 4.90)	**10.38 (1.27, 84.60)**
	0.78 (0.61, 0.97)	0.59 (0.48, 0.71)	0.71 (0.62, 0.80)
CM and IL10 (pg/ml)	(<) 28 (0.29, 0.98)	(<) 482 (0.84, 0.35)	(<) 107 (0.61, 0.54)
	15.81 (1.93, 129.66)	2.39 (0.90, 6.37)	0.54 (0.26, 1.12)
	0.72 (0.49, 0.96)	0.56 (0.45, 0.66)	0.68 (0.59, 0.77)
CM and sTNFR2 (pg/ml)	(<) 69069 (1, 0.32)	(<) 8307 (0.56, 0.74)	(>) 6171 (0.87, 0.23)
	7.4 (0.44, 123.83)	**3.93 (1.45, 7.94)**	1.18 (0.45, 3.11)
	0.77 (0.63, 0.92) *	0.66 (0.55, 0.77) *	0.66 (0.57, 0.76)
CM and IL8 (pg/ml)	(>) 24 (1, 0.3)	(>) 32 (0.88, 0.44)	(>) 54 (0.61, 0.58)
	3.42 (0.38, 30.90)	**5.56 (1.81, 17.07)**	1.82 (0.88, 3.78)
	0.71 (0.56, 0.86)	0.63 (0.53, 0.73) *	0.68 (0.59, 0.78)
CM and MCP1 (pg/ml)	(<) 294 (0.86, 0.45)	(>) 205 (0.78, 0.51)	(<) 97 (0.31, 0.83)
	5.42 (0.62, 47.25)	**3.62 (1.43, 9.22)**	0.39 (0.15, 0.99)
	0.75 (0.55, 0.96)	0.62 (0.52, 0.72) *	0.68 (0.59, 0.77)
CM and IL6 (pg/ml)	(>) 241 (0.71, 0.59)	(>) 178 (0.78, 0.62)	(>) 1790 (0.15, 0.97)
	1.83 (0.38, 8.74)	**4.81 (1.96, 11.79)**	3.96 (0.81, 19.35)
	0.67 (0.46, 0.89)	0.68(0.58, 0.79) *	0.70 (0.61, 0.79)
CM and all significant cytokines			
APACHE II		1.02 (0.92, 1.13)	
ISS		1.00 (0.97, 1.05)	

Outcome	Hospital mortality ODL(Sensitivity, specificity)[1] Odds Ratio[2] (95% CI) AUC (95% CI)	Acute Kidney Injury ODL (Sensitivity, specificity) Odds Ratio (95% CI) AUC (95% CI)	Nosocomial Infections ODL (Sensitivity, specificity) Odds Ratio (95% CI) AUC (95% CI)
sTNFR2 <8307 pg/ml		**4.40 (1.56, 12.42)**	
sIL1RA >5022 pg/ml		**3.37 (1.18, 9.57)**	
IL6 > 178 pg/ml		**3.39 (1.11, 10.35)**	
IL8 > 32 pg/ml		2.53 (0.69, 9.26)	
MCP1 > 205 pg/ml		1.48 (0.48, 4.61)	
		0.83 (0.76, 0.91)**	

[1] Optimal discrimination limits (ODL) for early cytokine level {within 12 hours of injury) associated with each outcome are reported with sensitivity and specificity for each model.

[2] Odds ratios and area under receiver operator curve (AUC) with 95% confidence intervals (CI) are obtained for the effect of each cytokine as binary variable using ODL as cut-off in individual multivariate logistic regression models adjusted for clinical model.

[3] Clinical model included composite APACHE II and injury severity scores in first 24 hours of injury and odds ratios are reported per unit change.

* Implicates significant difference in AUC compared to clinical model at the significance level < 0.05.

** Implicates significant difference in AUC compared to clinical model and all individual cytokine models at the significance level < 0.05.