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Innate immune function predicts the development of nosocomial infection in critically injured children

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Abstract

Background—Critical injury has been associated with reduction in innate immune function in adults, with infection risk being related to degree of immune suppression. This relationship has not been reported in critically injured children.

Hypothesis—Innate immune function will be reduced in critically injured children, and the degree of reduction will predict the subsequent development of nosocomial infection.

Materials and Methods—Children (≤ 18 years of age) were enrolled in this longitudinal, prospective, observational, single-center study after admission to the pediatric intensive care unit (PICU) following critical injury, along with a cohort of outpatient controls. Serial blood sampling was performed to evaluate plasma cytokine levels and innate immune function as measured by *ex vivo* LPS-induced TNFα production capacity.

Results—Seventy-six critically injured children (and 21 outpatient controls) were enrolled. Sixteen critically injured subjects developed nosocomial infection. Those subjects had higher plasma IL-6 and IL-10 levels on post-trauma day (PTD) 1–2 compared to those who recovered without infection and outpatient controls. *Ex vivo* LPS-induced TNFα production capacity was lower in children on PTD 1–2 (p=0.006) and over the first week following injury (p=0.04) in those

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who went on to develop infection. A TNFα response of < 520 pg/ml at any time in the first week after injury was highly associated with infection risk by univariate and multivariate analysis. Among transfused children, longer red blood cell storage age, not transfusion volume, was associated with lower innate immune function ($p < 0.0001$). Trauma-induced innate immune suppression was reversible *ex vivo* via co-culture of whole blood with GM-CSF.

Conclusions—Trauma-induced innate immune suppression is common in critically injured children and is associated with increased risks for the development of nosocomial infection. Potential exacerbating factors, including red blood cell transfusion, and potential therapies for pediatric trauma-induced innate immune suppression are deserving of further study.

Keywords

trauma; suppression; complications; TNFα; pediatric; PICU

Introduction

Traumatic injury represents the leading cause of death for children outside the neonatal period in the United States (http://www.cdc.gov/nchs). In addition to the risks critically injured children face from their primary injuries, they also face substantial morbidity due to nosocomial infection (1). These hospital-acquired infections contribute to longer intensive care unit (ICU) stays and increase health care costs by tens of thousands of dollars per infection (2).

Host immune function likely contributes importantly to infection risk in the critically injured patient. Impairment of the innate immune system is common and measurable in the setting of critical illness, and is characterized by a *reduced* ability of whole blood to produce the pro-inflammatory cytokine, tumor necrosis factor (TNF)-a, in response to *ex vivo* stimulation with lipopolysaccharide (LPS) (3). In critically injured adults, *ex vivo* LPS-induced TNFα production capacity is reduced following trauma with a greater degree of reduction beingassociated with increased risks of nosocomial infection and death (4, 5). In children, lower *ex vivo* LPS-induced TNFα production capacity has been associated with adverse outcomes in the settings of multiple organ dysfunction syndrome (MODS) (6), influenza infection (7), and cardiopulmonary bypass (8), but these relationships have not been previously evaluated following critical trauma. Identifying these relationships is particularly important given that emerging evidence suggests that critical illness-induced immune suppression may be reversible, with beneficial effects on outcomes (6, 9). We therefore designed a single-center, prospective, longitudinal, observational study to test the hypothesis that reduced innate immune function will be associated with increased nosocomial infection risk in severely injured pediatric patients.

Materials and Methods

Setting

Nationwide Children's Hospital is a free-standing, quaternary-care pediatric referral center with a level-one pediatric trauma designation. The pediatric intensive care unit (PICU) is a 30-bed multidisciplinary unit that sees > 2000 admissions annually. Trauma patients

admitted to the PICU are collaboratively managed by trauma and critical care physicians. This study was approved by the Institutional Review Board at Nationwide Children's Hospital. Written informed consent, and subject assent when appropriate, was obtained for all subjects prior to study participation.

Subjects

Patients aged ≤ 18 years admitted to the PICU at Nationwide Children's Hospital with an admitting primary diagnosis of trauma and/or burn injury were included. Patients with a pre-existing limitation of care order and those with a weight of less than 3.3 kilograms were excluded (for blood volume reasons). Healthy control subjects were recruited from the outpatient phlebotomy area. Healthy subjects were excluded if they had subjective or objective fever within the past 24 hours, current receipt of antibiotics, use of nonsteroidal anti-inflammatory drugs within the past 48 hours, history of systemic corticosteroid administration within the past month, or history of a chronic inflammatory disease, malignancy or transplantation.

Measurements

Subjects were enrolled on the earliest possible weekday following ICU admission and underwent blood sampling at the time of enrollment and every Monday, Wednesday, and Friday thereafter until ICU discharge. Blood was collected in heparinized tubes (*Becton Dickinson*, Franklin Lakes, NJ) and placed on ice.

To quantify innate immune function, LPS-induced TNFα production capacity was measured as follows: Within 30 minutes of collection, 50μl of whole blood was added to 500μl of highly standardized stimulation solution containing 500 pg/ml of LPS (phenol-extracted from *Salmonella abortus equii* [*Sigma*, St. Louis, MO]) and incubated for four hours at 37°C. After four hours, the supernatant was collected and stored at −80°C for batch analysis of TNFα. Stimulation assays were performed in duplicate for each blood sample and values reported are the average values from each set of duplicates. Stimulation solution was manufactured monthly and quality controlled such that the intra-batch coefficient of variation for TNFα production from healthy donor replicates was < 10%. Plasma from un-stimulated whole blood was collected at each sampling point and stored at −80°C for batch analysis of the pro-inflammatory cytokine interleukin (IL)-6 and the anti-inflammatory cytokine IL-10. TNFα, IL-6, and IL-10 were quantified by chemiluminescence using the *Immulite* automated chemiluminometer (*Siemens Healthcare Diagnostics*, Deerfield, IL). Complete blood cell counts were obtained at the discretion of the treatment team. Absolute cell counts were calculated when present.

In order to evaluate reversibility of trauma-induced innate immune suppression, samples from three children were co-incubated at 37°C for four hours with LPS +/− granulocyte macrophage colony-stimulating factor (GM-CSF, 1000 pg/ml) with TNFα production capacity measured as described above.

Clinical Data and Definitions

Clinical data including age, gender, type of injury(ies), transfusion history, medications, treatments, and outcomes were gathered from the electronic medical record. Nosocomial infection was defined as a new bacterial or fungal infection diagnosed > 48hours from hospital admission according to CDC criteria (10), though the decision to obtain cultures was left to the discretion of the treatment team. Of note, nosocomial lung infections in this report could represent “lower respiratory tract infection”, “ventilator associated pneumonia”, or “ventilator associated tracheitis” per CDC definitions. Severity of injury within 24 hours of admission was measured by Injury Severity Score (ISS) (11, 12). Severe traumatic brain injury (TBI) was defined as intracranial pathology with an admission Glascow Coma Scale (GCS) score of ≤ 8. Non-severe TBI was defined as any intracranial pathology with an admission GCS score of greater than 8. For multiply-transfused subjects, storage age of transfused RBC was defined as the storage age of the oldest unit transfused. Hospital and ICU lengths of stay were calculated based on physical admission to and discharge from the hospital and ICU, respectively. Patients transferred to inpatient rehabilitation were considered to be discharged from the hospital. While there were no specific extubation or ICU discharge criteria in place during the study period, there were no systematic changes in those practices over that time period. Mortality was defined as death during the hospitalization.

Statistical Analyses

Our primary outcome variable was the development of nosocomial infection within 14 days post-injury. We also evaluated time to development of nosocomial infection. Due to the intermittent nature of our weekday sampling regimen, immune function and cytokine data were analyzed using 48-hour time windows (*e.g.* post-trauma day [PTD] 1–2, 3–4, 5–6, 7–8). PTD 1 was defined as the first calendar day after the day of injury. Since many subjects were discharged from the PICU within 48 hours of injury, we undertook separate analyses for PTD 1–2 data and data from subjects who had more longitudinal sampling. Given the reduced number of subjects at later time points, we concluded our immunologic analyses by PTD 7–8. Comparisons between groups were made with Mann-Whitney U test for continuous variables or Fisher’s exact test for categorical variables. Two-way analyses of variance with Bonferroni posttest analyses were performed to determine differences between groups over time. A receiver operating characteristic curve was constructed and area under the curve (AUC) analysis was performed to evaluate the relationships between the lowest *ex vivo* LPS-induced TNFα production capacity at any point in time and development of secondary infection within 14 days post injury. In addition, recursive partitioning algorithms were used to assess an “optimal” cutpoint of TNFα production in relation to development of infection. Kaplan-Meier plotting and Log Rank test were used to evaluate time to infection between groups determined by immune function cutpoint. Logistic regression analyses were used to assess the impact of various factors (*e.g.* gender, age, ISS, injury type, red blood cell transfusion, innate immune function) on incidence of nosocomial infection. All-subsets logistic regression models were also explored to assess optimal modeling in the multivariable setting; however, given the inherent limitations with these analyses with the relatively limited number of infection events, these were used to guide generation and

evaluation of two-covariate multivariable models in relation to incidence of nosocomial infection. Data were log transformed for analyses as appropriate. Analyses were performed using Prism6 (*GraphPad Inc.*, La Jolla, CA) and the Mac version of the statistical program R version 3.0.1 GUI 1.61 (*The R Foundation for Statistical Computing*, Vienna, Austria). A p value of 0.05 was considered significant throughout. Data are presented as median and interquartile range (IQR).

Results

Seventy-six critically injured patients were enrolled between November, 2007 and October, 2010. All trauma subjects were admitted directly to the PICU from the Emergency Department (ED) or Operating Room (OR). The median post-trauma day for the first sampling event was PTD 2 [IQR: 1 – 3], with 67% of patients (n=51) having their first samples collected within 48 hours of injury. Demographic information for enrolled subjects can be found in Table 1. Sixty-two subjects (81%) had blunt trauma, while two subjects (3%) had penetrating trauma, 10 subjects (13%) had burns (median total body surface area [TBSA]: 30%), and two subjects (3%) had asphyxial injury. Thirty-five subjects (46%) had TBI (either isolated or in the setting of polytrauma), of whom 10 had severe TBI. The most common mechanisms of injury were motor vehicle crash (49%), fall (13%), thermal injury (6% scald, 6% flame), and assault (11%). ISS data suggested moderate to severe injury (17 [10 – 26]), and RBC transfusion was given in 45% of subjects. No subjects were managed with therapeutic hypothermia, and three patients were treated with methylprednisolone on PTD 0 – 1 for treatment of spinal cord injury. No subjects were known to be immunocompromised at baseline, including the use of immunosuppressive medications prior to injury.

Twenty-one healthy control subjects were enrolled, 52% of whom were male. The healthy control cohort was not significantly different from the trauma cohort as a whole with respect to age (11.4 [8.2 – 13.3] vs 9.9 [4.5 – 14.5] years, p=0.7). Healthy control subjects demonstrated robust *ex vivo* TNFα production capacity (1297 [1088 – 1739] pg/ml), and all had undetectable plasma levels of IL-6 and IL-10.

As expected, critically injured children had higher plasma IL-6 levels on PTD 1–2 compared to healthy controls (44 [15 – 156] vs < 5 pg/ml). At the same time, critically injured children also demonstrated slightly higher levels of the anti-inflammatory cytokine IL-10 (5 [5 – 14] vs < 5 pg/ml). Lastly, critically injured subjects had reduced *ex vivo* TNFα production capacity compared to healthy controls (908 [506 – 1193] vs 1297 [1088 – 1739] pg/ml, p=0.0002).

Overall, 16 patients developed nosocomial infection within 14 days of injury (Table 2). The median time from injury to infection in these 16 patients was 4.5 days (range: 3 to 13 days). In the 51 children with PTD 1–2 samples available, those who developed nosocomial infection (n=7) had significantly lower *ex vivo* TNFα production capacity on PTD 1–2 compared to children who did not develop infection (342 vs 956 pg/ml, p=0.006; Figure 1A). Plasma levels of both IL-6 (p=0.02) and IL-10 (p=0.0005) were higher on PTD 1–2 in the subjects who went on to develop nosocomial infection (Figure 1 E,F). Among subjects

for whom CBC data were available (n=33), there were no significant differences in absolute cell counts between groups (Figure 1 B–D).

For those patients who remained in the PICU and underwent longitudinal sampling, *ex vivo* TNFα production capacity remained significantly lower over time in patients who developed infection (Figure 2A). Innate immune function returned to near normal levels in patients who remained infection free. Interestingly, absolute monocyte and neutrophil counts were also lower over time in patients who developed nosocomial infection (Figure 2B, D) though none were frankly leukopenic or neutropenic beyond PTD 3–4.

Receiver Operating Characteristic curve analysis revealed that the lowest value of LPS-induced TNFα production capacity in the first week after injury was strongly associated with the development of nosocomial infection (Figure 3A). Recursive partitioning algorithm analyses showed that having an *ex vivo* TNFα production capacity of < 520 pg/ml on PTD 1–2 was an optimum cutpoint for identifying those who would develop nosocomial infection within 14 days of trauma; infection rates in those with PTD 1–2 *ex vivo* TNFα levels < 520 were 43% vs. 3% in those ≥ 520 (p=0.004, ; sensitivity 82% [95% CI: 67–92%]; specificity 86% [95% CI: 42–100%]; PPV 97% [95% CI: 86–100%]; NPV 43% [95% CI: 18–71%]). This was still a highly significant cutpoint even when evaluating the minimum TNFα production capacity levels across all patients within the first week of trauma (48% vs. 8%, p=0.0002; sensitivity 78% [95% CI: 66–88%], 82%, specificity 75% [95% CI: 48–93%]; PPV 92% [95% CI: 81–98%]; NPV 48% [95% CI: 28–69%]). Overall, 25 patients in the cohort (33%) had at least one ex vivo LPS-induced TNF a production capacity value below this threshold. Time-to-event analysis shows that having a TNFα production capacity < 520 pg/ml in the first week is highly significantly associated with time to development of infection as well (Figure 3B). Multivariable modeling also supported using a cutpoint for *ex vivo* TNFα to predict development of nosocomial infection in children after trauma. By univariate logistic regression modeling, higher ISS, presence of severe TBI, burn injury, RBC transfusion during the first week, as well as an *ex vivo* LPS-induced TNFα production capacity < 520 pg/ml on day 1–2 *or* at any time during the post-trauma days 1–8 were all predictive of the development of nosocomial infection (Table 3). All-subsets logistic regression modeling showed that *ex vivo* TNFα production capacity < 520 pg/ml at any time in the first week after injury was the strongest predictor for infection and remained significant even when adjusting for other factors such as ISS, incidence and severity of traumatic brain injury, age, gender, and mechanism of injury. In all of the two-covariate logistic regression models, *ex vivo* TNFα levels <520 pg/ml was the best predictor of nosocomial infection (Table 3).

Thirty-four subjects received at least one RBC transfusion following injury, with 29/34 (85%) of those subjects receiving RBC within 72 hours of injury. As an exploratory analysis, we determined relationships between early red blood cell transfusion and innate immune function over time. Among those transfused within the first 72 hours post-injury, the median number of donor exposures was 2 (1 – 3), with a median volume of 15 (11 – 24) ml/kg transfused and a median RBC storage duration of 16 days (range: 5 to 42 days). In these patients, we found no association between total volume of red blood cells transfused and innate immune function over time (Figure 4A). However, children who received RBC

that had been stored for ≥ 14 days had lower *ex vivo* TNFα production capacity over time (Figure 4B) compared to those who received fresher red cells.

Lastly, to evaluate reversibility of trauma-induced innate immune suppression, whole blood samples from three critically injured subjects were incubated with LPS alone or LPS + GM-CSF (1,000 pg/ml). *Ex vivo* co-incubation of subjects' whole blood with LPS + GM-CSF resulted in higher TNFα production capacity than that seen with LPS alone in all three samples (Figure 4C).

Discussion

This is the first study to document innate immune responsiveness in critically injured children. In our cohort, innate immune suppression as measured by reduced *ex vivo* LPS-induced TNFα production capacity was common among critically injured children, and was significantly associated with the subsequent development of nosocomial infection. Using a highly standardized assay we found an *ex vivo* LPS-induced TNFα production capacity of < 520 pg/ml at any time in the first week following injury to be predictive of the development of nosocomial infection.

The immunosuppressive nature of critical illness and injury is increasingly recognized as an important feature of intensive care unit pathophysiology. In recent years, innate immune suppression has been described following the onset of sepsis, MODS, influenza, and cardiopulmonary bypass in adults and children (6–8, 13–15). It should be noted, however, that this phenomenon was described by Polk *et al* (16) more than 25 years ago, with impaired antigen presentation capacity demonstrated in innate immune cells from severely injured adults. Much of the literature in this field has continued to focus on antigen presenting capacity, as measured by reduction (or recovery) of expression of the cell surface marker HLA-DR (a class II major histocompatibility complex molecule) on circulating monocytes. Severe or persistent loss of monocyte HLA-DR expression has been consistently associated with increased sepsis risk following major trauma in adults (17, 18).

Another approach to innate immune monitoring is the quantitation of the ability of whole blood to make the pro-inflammatory cytokine TNFα upon *ex vivo* stimulation with LPS. While TNFα is typically absent from the circulating plasma, whole blood from immunocompetent individuals should readily produce TNFα when challenged *ex vivo*. Indeed, we observed robust TNFα production capacity in our samples from healthy children. Impairment of cytokine production capacity has been reported in adults following severe injury, with an associated increase in risk for infection-related mortality (4). We have previously demonstrated a relationship between reduced TNFα production capacity and adverse outcomes in critically ill children with MODS (6), influenza (7), and cardiopulmonary bypass (8) but this had not been previously studied in the setting of pediatric critical injury. This population is of particular interest due to our ability to clearly elucidate temporal relationships between injury and immune function, something that is more challenging with sepsis, for example, whose onset can be difficult to pinpoint.

Our findings suggest that innate immune suppression occurs as early as the first 48 hours following severe trauma, with the depth of suppression being predictive of subsequent nosocomial infection risk even at this early time point. Elevations in plasma biomarkers of both the pro-inflammatory (IL-6) and anti-inflammatory (IL-10) responses occurred along with innate immune suppression. This finding is consistent with our prior work which demonstrated high levels of plasma cytokines alongside impaired innate immune responsiveness in other forms of pediatric critical illness (6, 7). The association of elevations in early plasma IL-6 levels with adverse outcomes is also consistent with the adult trauma literature (18, 19). It is notable that impairment of TNFα production capacity was not associated with reduced innate immune cell numbers among subjects with absolute cell counts available on PTD 1–2. This is suggestive of impaired cellular function rather than leukopenia as an explanation for the reduced TNFα response.

The longitudinal time course of the innate immune response was also of interest in our study. Among subjects who underwent serial immune monitoring, recovery of TNFα production capacity was slower in children who went on to develop nosocomial infection, with infection typically occurring toward the end of the first week after injury. In these analyses, we did see a relationship between lower innate immune cell numbers and lower TNFα production capacity, though none of the subjects were frankly leukopenic or neutropenic.

One of the problems in the field of innate immune monitoring has been the lack of standardization of LPS stimulation procedures. Our highly standardized reagents and approach have now been used in single- and multi-center studies with consistent prediction of adverse outcomes (6–8) and are consistent with results obtained in adults using a similar approach (20). While we have repeatedly demonstrated a threshold of TNFα production capacity of around 250 pg/ml to be associated with mortality in critically ill children (6, 7), a higher threshold of 520 pg/ml was highly predictive of nosocomial infection risk in our trauma cohort. This still represents marked depression of innate immune responsiveness compared to healthy children, whose values were typically > 1,000 pg/ml. This also suggests that different immune function thresholds may be relevant for different outcomes or clinical contexts. We were unable to evaluate a mortality threshold due to the low incidence of death in our study population.

Mechanisms of innate immune suppression in the pediatric ICU remain poorly understood. It is likely that these mechanisms are multifactorial, and include patient-related factors as well as unintended immunomodulation by treatments provided in the ICU. Very few of our subjects (3/76) received overtly immunosuppressive medications, such as glucocorticoids. A growing body of evidence suggests that red blood cell transfusion may be immunosuppressive. Several retrospective analyses have shown red blood cell transfusion to be an independent risk factor for mortality and nosocomial infection in critically ill adult patient populations, including trauma (21–23). Receipt of red blood cells with longer storage duration has been shown to be associated with increased risks of adverse outcomes in critically ill adults and children (24–27). While exact mechanisms underlying these observations remain uncertain, studies from our laboratory indicate that stored red blood cells suppress monocytes *in vitro* as a function of red cell storage duration (28). These

findings suggest that red blood cell transfusion, particularly with red blood cells of longer storage duration, may be immunosuppressive. In our current study, the receipt of a red blood cell transfusion was associated with a strong trend toward increased infection risk even after adjusting for immune function. In addition, receipt of red blood cells with longer storage duration was associated with a failure to recover innate immune function, supporting the hypothesis that red blood cell storage-related factors may contribute to post-traumatic innate immune suppression.

Our observed nosocomial infection rate of 21% is comparable to previously published studies of critically injured children (1). It should be noted that these infections included *all* nosocomial infections by CDC criteria, not just ventilator-associated pneumonia or catheter-associated bloodstream infections. The majority of patients who developed nosocomial infection in our cohort had either severe traumatic brain injury or burn injury. This is in keeping with higher rates of infectious complications reported in the literature for each of these two mechanisms of injury (29, 30). This increased susceptibility to infection may be due to severity of illness and increased need for invasive support (central venous catheters and mechanical ventilation), but evidence suggests that immune suppression following these injuries may be particularly problematic (31, 32). Indeed, burn injury and severe traumatic brain injury are likely to be immunologically distinct from other forms of trauma and warrant independent study.

Our data provide additional evidence that trauma-induced innate immune suppression may be reversible given that co-culture of whole blood from critically injured children with the stimulant GM-CSF was capable of restoring LPS-induced TNFα production capacity. These findings are in agreement with similar studies in injured adults which suggest that suppressed innate immune cells can be pharmacologically reactivated *ex vivo* (33, 34) and *in vivo* (35). GM-CSF has been FDA-approved since 1991 for the reconstitution of bone marrow following chemotherapy and bone marrow transplantation in adults and children. Limited evidence also suggests that it may reverse critical illness-induced innate immune suppression (6, 9). We are currently conducting an NIH-funded clinical trial of GM-CSF therapy for the reversal of trauma-induced innate immune suppression in critically injured children (NCT01495637, R01GM094203).

There are important limitations to our study. First, as a single-center study of critically injured children, we had a limited sample size including a relatively small number of children who went on to develop nosocomial infection. As such, we were limited in the number of covariables that could be included in multivariable regression models and it is possible that other confounding variables may have contributed to our results. Given the study logistics of weekday testing, not all patients had samples available on PTD 1–2, and many of the patients had left the ICU by day PTD 7–8. Despite this, we present the largest cohort of critically injured children to undergo immune monitoring to date, and were able to show robust associations between immune function and outcome. Second, our patient population was heterogeneous, representing the spectrum of critical injuries throughout the pediatric age range. For the purposes of this observational study, we intentionally cast a wide net in hopes of evaluating a diverse group of injured children. For subsequent multi-center and/or interventional trials, it will likely be important to stratify for the presence or

absence of important confounders such as severe traumatic brain injury and burns. Third, we employed a single method of quantitation of innate immune function. It is not clear if *ex vivo* LPS-induced TNFα production capacity is superior to monocyte HLA-DR expression in predicting risk of adverse outcomes following trauma. Our approach offers the advantage of standardization along with measurement of a functional output (cytokine production capacity). Multiple approaches for HLA-DR quantitation by flow cytometry exist (*e.g.* %-positive, molecules/cell) and it is not clear which approach is optimal. Further, flow cytometric approaches all have some potential to be affected by lot-to-lot variability in reagents and cytometer settings. Head-to-head comparison of all of these measures of innate immune function is badly needed in a large cohort of critically injured patients.

In summary, suppression of the innate immune response in the first week following trauma is common and is predictive of the development of nosocomial infection in critically injured children. Further multi-center work is warranted to determine mechanisms of innate immune suppression following trauma, including the effects of stored red blood cells, and to determine the efficacy of immunomodulatory treatments in preventing adverse outcomes in pediatric trauma patients.

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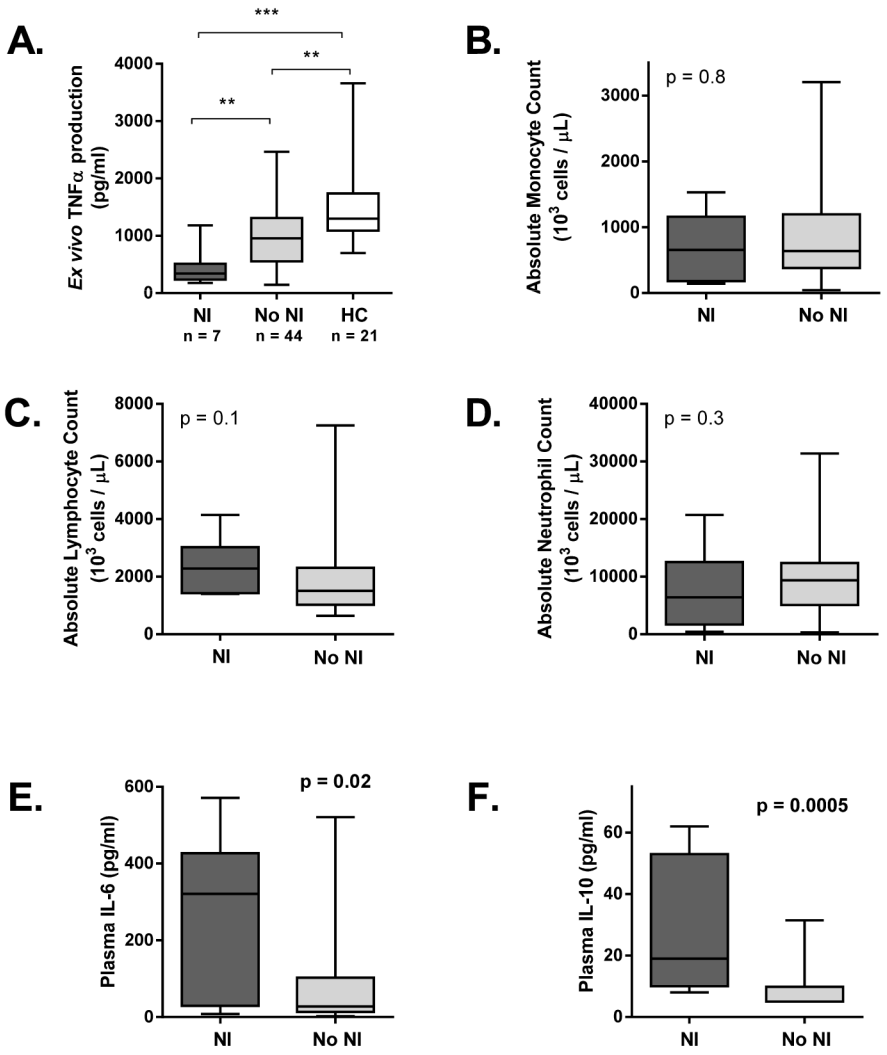

Figure 1.

Among subjects with PTD 1–2 data available, innate immune function **(A)** was lower in critically injured children compared to healthy controls, but was lowest in children who went on to develop nosocomial infection. Among those with CBC data (n=33), there were no differences in absolute cell counts between groups **(B-D)**. PTD 1–2 plasma IL-6 **(E)** and plasma IL-10 **(F)** levels were bother higher in critically injured children who went on to develop nosocomial infection. Lines and boxes represent median and IQR, with whiskers representing range throughout. NI: nosocomial infection, HC: healthy control . **p < 0.001, ***p < 0.0001.

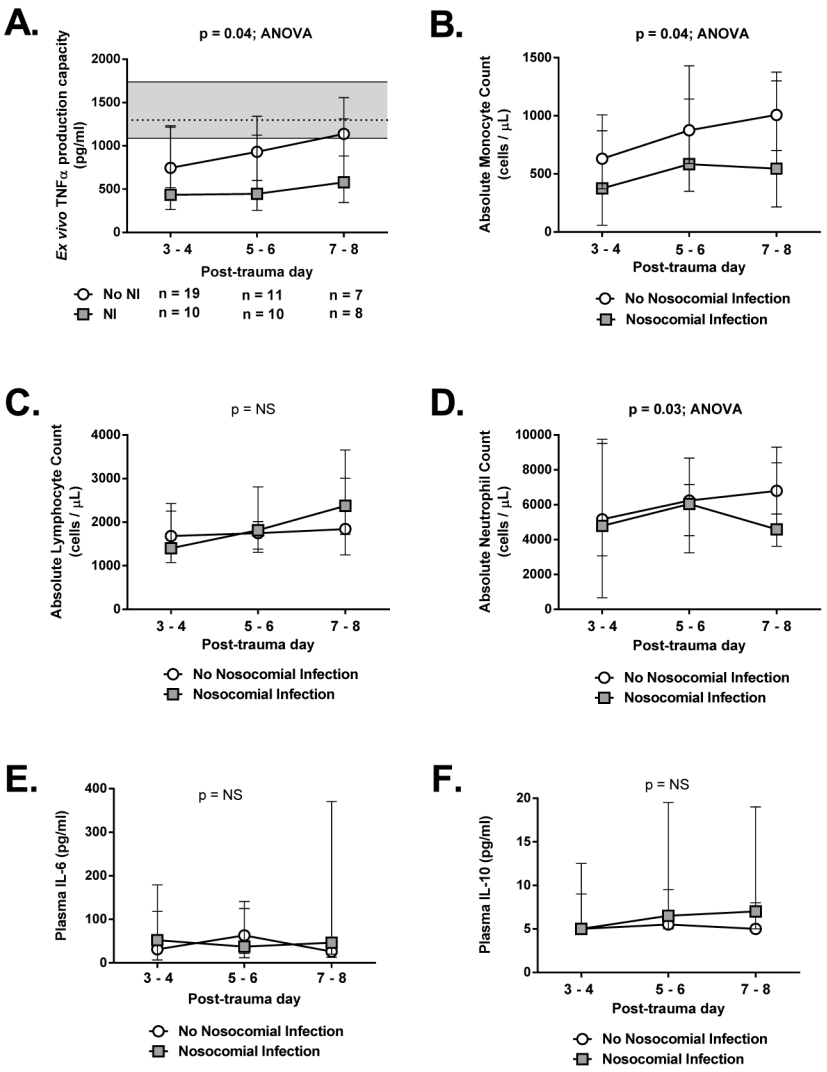

Figure 2.

In critically injured children who underwent longitudinal sampling, those who went on to develop nosocomial infection (gray squares) demonstrated lower innate immune function **(A)** over time compared to those who did not develop infection (open circles). The dotted line and shaded area represent median and IQR for healthy controls. Absolute monocyte counts **(B)** and absolute neutrophil counts **(D)** but not absolute lymphocyte counts **(C)** were lower over time in the group which developed nosocomial infection. There were no differences in plasma IL-6 **(E)** or IL-10 **(F)** levels over time. Data represent median and IQR. NI: nosocomial infection.

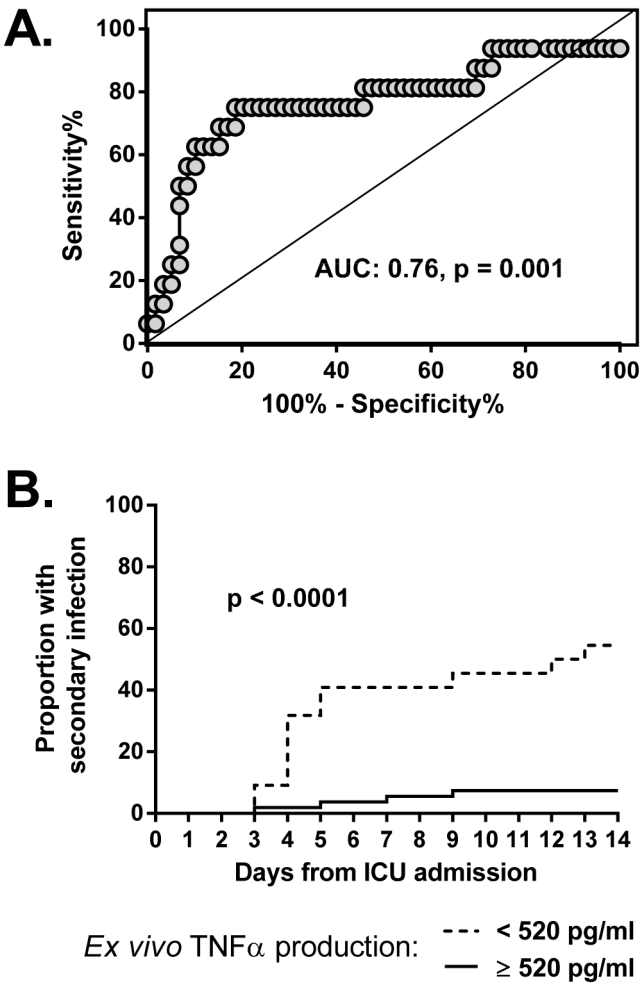

Figure 3.

(A) ROC curve analysis suggests that *ex vivo* TNFα production capacity is predictive of nosocomial infection with an optimal cutoff value of 520 pg/ml (sensitivity: 75% [95%CI 48– 93%], specificity: 81% [95%CI 69–90%]). **(B)** Time to event analysis suggests a strong association between an *ex vivo* TNFα production capacity < 520 pg/ml and development of nosocomial infection, particularly in the first week following injury ($p < 0.0001$, log rank test).

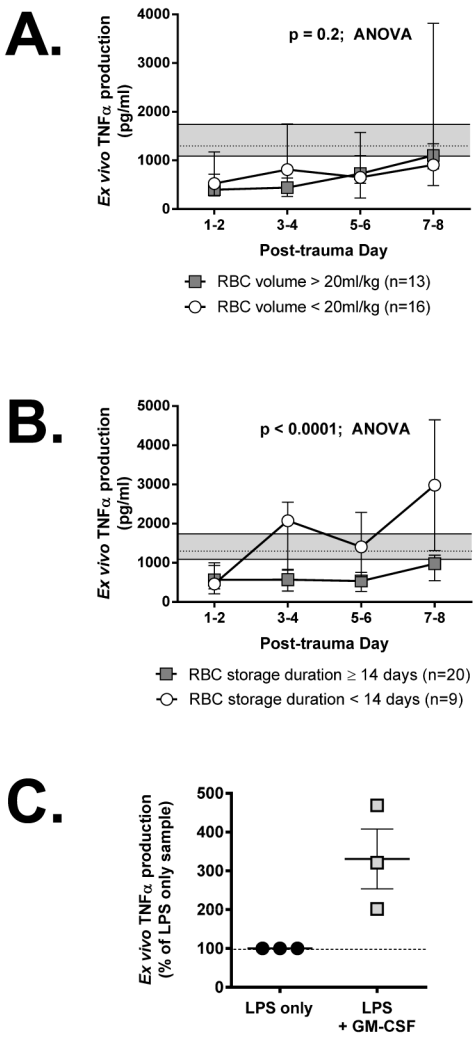

Figure 4.

(A) Among children transfused with RBC within the first 72 hours after injury (n = 29), there was no difference in innate immune function between those who received < 20 ml/kg of RBC (n=16, open circles) and those who received ≥ 20 ml/kg (n=13, gray squares). **(B)** Immune function was lower over in time, however, in transfused children whose RBC storage age was ≥ 14 days (n=20, gray squares) versus those receiving fresher RBC (n=9, open circles). Data represent median and IQR. The dotted line and shaded area represent median and IQR for healthy controls. **(C)** Whole blood samples from three critically injured children were concurrently incubated with LPS alone and with LPS + GM-CSF (1000 pg/ml). Co-incubation with GM-CSF resulted in increased *ex vivo* TNFα production capacity in these samples, suggesting reversibility of trauma-induced innate immune suppression.

Table 1

Clinical data

Characteristic	All Patients (n=76)	No NI (n=60)	NI (n=16)	p
Age, years	9.9 [4.5–14.5]	9.7 [5–14.7]	8.3 [1.9–11.2]	0.1
Male Gender, n (%)	52 (68%)	44 (73%)	8 (50%)	0.12
Injury Severity Score	17 [10–26]	16 [10–21]	26 [20–30]	**0.0001**
Injury Type, n (%)				
Isolated TBI, severe	4 (5)	3 (5)	1 (6)	< **0.0001**
Isolated TBI, non-severe	21 (28)	19 (32)	2 (13)	
Polytrauma without TBI	31 (41)	29 (48)	2 (13)	
Polytrauma with TBI, severe	6 (8)	1 (2)	5 (31)	
Polytrauma with TBI, non-severe	4 (5)	3 (5)	1 (6)	
Burn	10 (13)	5 (8)	5 (31)	
Support Devices, n (%)				
Mechanical ventilation	54 (71%)	38 (63%)	16 (100%)	**0.004**
Central venous catheter	31 (41%)	18 (30%)	13 (81%)	**0.0004**
Arterial catheter	38 (50%)	24 (40%)	14 (88%)	**0.001**
Foley catheter	65 (86%)	49 (82%)	16 (100%)	0.11
Transfusion, n (%)				
Red blood cell	34 (45%)	22 (37%)	12 (75%)	**0.01**
Fresh frozen plasma	11 (14%)	7 (12%)	4 (25%)	0.23
PICU LOS, days	4.1 [1–10.2]	1.8 [0.9–6]	13.5 [8.8–28.6]	<**0.0001**
Hospital LOS, days	10.2 [4.5–22]	7 [3.5–12.8]	21.9 [13.9–46.8]	<**0.0001**
Mortality, n (%)	3 (4%)	2 (3%)	1 (6%)	0.54

NI = nosocomial infection; TBI = traumatic brain injury; PICU = pediatric intensive care unit; LOS = length of stay. Data are median [interquartile range] unless otherwise specified.

Table 2

Characteristics of subjects with infection within 14 days of injury.

Subject	Age (yr)	Injury	Mechanism	ISS	Site	Organism	PTD
1	10.7	Polytrauma/TBI (mod)	MVC	30	Urine	*E. coli*	9
3	0.75	Burn	Scald	25	Blood	*C. albicans*	12
4	0.16	Isolated TBI (mod)	NAT	17	Lung	*S. aureus*	4
14	2.8	Burn	Fire	9	Lung	*S. pneumoniae*	4
20	3.2	Isolated TBI (severe)	NAT	18	Lung; CSF	*K. pneumoniae; S. epidermidis*	3; 8
23	10.4	Spinal cord injury	MVC	26	Lung	*S. aureus*	3
27	11.4	Polytrauma/ TBI (severe)	MVC	59	Lung	*A. baumanii*	7
28	13	Polytrauma/TBI (severe)	MVC	35	Lung	*E. coli*	3
33	6.5	Polytrauma	MVC	30	Urine	*K. oxytoca*	13
47	1.2	Burn	Scald	25	Urine; Blood	*P. aeruginosa; S. pneumoniae*	4; 4
60	13	Polytrauma/TBI (severe)	MVC	42	Lung	*C. albicans*	4
61	10	Isolated TBI (mod)	MVC	10	Lung	*H. influenzae*	5
62	10.2	Polytrauma/TBI (severe)	MVC	29	Lung	*S. aureus*	4
66	1.9	Burn	Fire	25	Wound	*E. fecalis, C. tropicalis*	9, 10
67	2	Burn	Scald	25	Urine; Lung	*P. aeruginosa; M. catarrhalis, H. influenzae, S. pneumoniae*	5; 5
78	15.7	Isolated TBI (severe)	Fall	26	Lung	*S. aureus, C. albicans*	5

PTD: post-trauma day, TBI: traumatic brain injury, MVC: motor vehicle crash, NAT: non-accidental trauma, CSF: cerebrospinal fluid,

Table 3

Univariate and two-covariate analyses of risk factors for nosocomial infection

Univariate			
Factor	**OR**	**95% CI**	**p-value**
Gender (male)	0.36	0.12 – 1.1	0.081
Age	0.90	0.80 – 1.0	0.054
ISS	**1.11**	**1.04 – 1.2**	**0.0018**
TBI (ref=none)			
Moderate	0.66	0.15 – 2.8	0.58
Severe	**7.29**	**1.6 – 32.8**	**0.0097**
Mechanism of injury*	1.26	0.67 – 2.4	0.47
Burn injury	**5.0**	**1.2 – 20.2**	**0.024**
TNFα < 520 pg/ml on Day 1–2	**27.0**	**2.8 – 257**	**0.004**
Any TNFα < 520 pg/ml#	**10.85**	**3.0 – 39.3**	**0.0003**
Two-covariate			
Any TNFα < 520 pg/ml#	**11.1**	**2.6 – 46.8**	**0.0011**
ISS	**1.1**	**1.03 – 1.2**	**0.0041**
Any TNFα < 520 pg/ml#	**9.6**	**3.1 – 29.5**	**0.0011**
Severe TBI	**6.7**	**1.3 – 34.4**	**0.024**
Any TNFα < 520 pg/ml#	**9.1**	**2.4 – 34.1**	**0.001**
Burn injury	2.5	0.52 – 12.2	0.25
Any TNFα < 520 pg/ml#	**8.2**	**2.2 – 30.9**	**0.002**
RBC transfusion#	3.15	0.8 – 12.3	0.098

ISS: injury severity score; TBI: traumatic brain injury;

*blunt, penetrating, burn, asphyxia;

#at any time days 1 through 8.