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# Differential expression of the immunoinflammatory response in trauma patients: Burn vs. non-burn☆

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ARTICLE INFO

*Article history:*
Accepted 27 October 2011

*Keywords:*
Burn
Trauma
Cytokine
Inflammation
Interleukin-6
Interleukin-8
Multiple

ABSTRACT

*Rationale:* Cytokines are central mediators of the immune-inflammatory response to injury and subsequent multiple organ dysfunction syndrome (MODS). Although previous studies evaluated cytokine levels after trauma, differences between patients with burn and non-burn trauma have not been assessed systematically.

*Methods:* A prospective database of trauma patients admitted between May 2004 and September 2007 to the burn or surgical intensive care units within 24 h of injury with an anticipated stay of at least 72 h was analyzed. Sequential clinical and laboratory parameters were collected in the first week, including multiplex analysis data for plasma levels of inflammatory cytokines (IL-6, and IL-8). Patients with known pre-injury coagulopathy were excluded. A Marshall score of 10 or greater was defined as MODS.

*Results:* A total of 179 patients were enrolled (67 burn and 112 non-burn). Plasma IL-6 and IL-8 levels were markedly elevated in both burn and non-burn patients compared to healthy volunteers. Burn subjects had higher levels of IL-6 and IL-8 than the non-burn on days 1 through 7 after injury. Subjects with burns and at least 30% total body surface area were older and had a lower injury severity score, a higher prevalence of MODS, and correspondingly higher mortality. Multivariate analysis of injury type, MODS, and time did not demonstrate an influence of MODS.

*Conclusions:* Burns were associated with a greater and more sustained immune-inflammatory response than non-burn trauma as evidenced by elevated plasma IL-6 and IL-8 levels during the first week. There was no association between MODS and plasma cytokine levels.

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0305-4179/$36.00. Published by Elsevier Ltd and ISBI
doi:10.1016/j.burns.2011.10.013

# Report Documentation Page

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| **01 JUN 2012** | **N/A** | **-** |

| 4. TITLE AND SUBTITLE | 5a. CONTRACT NUMBER |
|---|---|
| **Differential expression of the immunoinflammatory response in trauma patients: burn vs. non-burn** | 5b. GRANT NUMBER |
| | 5c. PROGRAM ELEMENT NUMBER |
| 6. AUTHOR(S) | 5d. PROJECT NUMBER |
| **Mace J. E., Park M. S., Mora A. G., Chung K. K., Martini W., White C. E., Holcomb J. B., Merrill G. A., Dubick M. A., Wolf S. E., Wade C. E., Schwacha M. G.,** | 5e. TASK NUMBER |
| | 5f. WORK UNIT NUMBER |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) | 8. PERFORMING ORGANIZATION REPORT NUMBER |
| **United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX** | |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | 10. SPONSOR/MONITOR'S ACRONYM(S) |
| | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) |

12. DISTRIBUTION/AVAILABILITY STATEMENT
**Approved for public release, distribution unlimited**

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | |
| **unclassified** | **unclassified** | **unclassified** | **UU** | **8** | |

**Standard Form 298 (Rev. 8-98)**
Prescribed by ANSI Std Z39-18

## 1. Introduction

Critical injuries, including severe head trauma and polytrauma, result in an elaboration of a wide range of inflammatory mediators, such as cytokines [1,2]. Trauma can induce immune-inflammatory effects that are related to injury severity [3]. Severely injured patients have demonstrated early elevations in interleukin 6 (IL-6) and IL-8 [4–6], which have been associated with mortality [7,8]. Giannoudis et al. found elevated IL-6 levels to be associated with a systemic inflammatory response syndrome (SIRS) state in which early elevations were predictive of complications such as pneumonia, multiple-organ dysfunction syndrome (MODS), and death in blunt trauma patients [9]. Likewise, Maier et al. found elevated IL-6 and soluble tumor necrosis factor (TNF) receptors to be predictive of mortality but IL-8 levels predictive of MODS in trauma patients [10].

In patients with burns, immune-inflammatory response is associated with complications after injury, such as infection, MODS, and mortality [11]. Burns have been associated with increased plasma TNF-α, IL-6, and IL-8 levels in comparison to elective surgery controls. Elevated levels of IL-6 and IL-8 were identified locally in burned skin [12]. In effect, IL-1β, IL-6, IL-8, and TNF-α levels have been correlated with total body surface area (TBSA) in burn patients [13–19]. In addition to soluble cellular adhesion molecules, elevated levels of TNF-α, IL-6, and IL-8 were associated with severe burns complicated by septic shock or death [14,15,20–22]. Although Drost et al. demonstrated an association among infection, IL-6, and TNF-α, IL-6 alone was associated with mortality in patients with burns [13,23]. Unfortunately, not all studies are consistent. Gueugniaud et al., in a study of 10 patients with greater than 60% TBSA burned; found that survivors had higher IL-6 levels than non-survivors [24]. Recently, a study by Park et al. identified TNF-α as the only measured cytokine to predict mortality in burn and non-burn patient populations [25].

However, existing data still supports the notion that inflammatory cytokines play a role in the development of subsequent complications, including MODS. Cytokines are central mediators of the immunoinflammatory response to injury. Nonetheless, little is known about the specific roles that cytokines play in the response to injury and whether differences exist in the immunoinflammatory profiles between various patient populations. Specifically, we speculated that unique cytokine profiles exist for burn and non-burn trauma patients that are associated with outcome.

## 2. Materials and methods

This study was conducted under a protocol reviewed and approved by the local institutional review board and in accordance with the approved protocol.

### 2.1. Patients

Patients eligible for enrollment in the study were at least 18 years of age, admitted to the intensive care unit (ICU) within 24 h of injury, and per treating physician, were expected to stay at least 72 h in the ICU. Reasons for ineligibility were admission greater than 24 h from time of injury, admission to the hospital ward, prisoner status, pre-existing therapeutic anticoagulation (exception made for aspirin or ibuprofen), or coagulopathy prior to trauma. Patients from both the burn and the surgical ICU's were enrolled in the study over a 41-month period (May 2004–September 2007).

### 2.2. Healthy volunteers

A group of healthy volunteers free of acute illness for at least 2 weeks prior, weighed at least 110 lbs, and not pregnant was recruited. In accordance with regulatory policy, volunteers were enrolled to donate a one-time sample of their blood after signing a consent form. This comparison group was our uninjured control group.

### 2.3. Clinical data

Sequential clinical and laboratory parameters were collected during the subjects' first week of hospitalization. Clinical data, including injury severity score (ISS) [26]; percent TBSA burned; inhalation injury (II); total ventilator-free, ICU-free, and hospital-free days; and mortality were entered into an Oracle database. Since nonsurviving patients tend to have deceivingly shorter hospitalization periods, we reported the number of days off mechanical ventilation (ventilator-free) and out of the ICU (ICU-free) during a 30-day period after admission in addition to the average length of hospital stay as measures of outcome. Laboratory results and other basic demographic data were recorded in the database via direct interface with electronic medical record. Daily MODS, defined as a Marshall score of 10 or greater, was determined based on clinical and laboratory parameters [27].

### 2.4. Blood collection

Blood samples were collected into citrated tubes via central venous or arterial catheters within 24 h of admission (Admit) and on post-injury days 1–3, 5, and 7. Blood sampling was discontinued upon transfer out of the ICU or the removal of central venous and arterial catheters. The blood samples were centrifuged, and plasma aliquots were stored in cryoprecipitate tubes at −80 °C for subsequent analysis of cytokine levels. Blood samples collected from healthy volunteers were also processed in the same manner.

### 2.5. Cytokine analysis

Multiplex analysis for plasma, IL-1β, TNF-α, GM-CSF, IL-6, and IL-8 levels was performed. The human inflammatory five-plex antibody bead kit (Biosource International, Camarillo, CA) and Luminex 100 luminescent analyzer (Luminex, Austin, TX) were used to measure plasma cytokine levels. The Luminex system was used in accordance to manufacturer's instructions. Standard curves were established based on various dilution factors where the sensitivity of our method was 3.0 pg/ml for IL-6 and IL-8 and 10 pg/ml for IL-1β, TNF-α, GM-CSF. When plasma levels were below Luminex analysis threshold, a value equal to the lowest detectable limit was assigned for statistical comparison.

**Table 1 – Demographic and outcome data.[a]**

| | Healthy volunteer ($n = 20$) | Burn ($n = 67$) | Non-burn; ($n = 112$) | $p$ Value |
|---|---|---|---|---|
| Data | Mean ± SD Median [95% CI] | Mean ± SD Median [95% CI] | Mean ± SD Median [95% CI] | |
| Initial demographics | | | | |
| Sex (% male) | 55 | 73 | 79 | NS |
| Age | 36 ± 10.5; 37 [27.0–43.5] | 49 ± 18.9; 46 [43.9–53.1] | 41 ± 17.7; 39 [37.7–44.3] | $p < 0.008$ |
| Injury severity score | – | 22 ± 12.6; 25 [18.7–24.9] | 27 ± 11.7; 26 [23.5–27.8] | $p < 0.04$ |
| Total body surface area | – | 34 ± 21.4; 30 [28.5–39.0] | – | – |
| Inhalation injury (% yes) | – | 36 | – | – |
| Outcomes | | | | |
| Hospital days | – | 40 ± 38.9; 25 [30.2–49.1] | 25 ± 24.8; 17 [19.9–29.2] | $p < 0.005$ |
| Ventilator-free days | – | 12 ± 12.6; 11 [9.3–15.5] | 19 ± 10.3; 22 [17.0–20.8] | $p < 0.001$ |
| ICU-free days | – | 9 ± 11.0; 0 [6.3–11.7] | 16 ± 9.7; 18 [14.0–17.7] | $p < 0.001$ |
| Multiple organ dysfunction syndrome (%) | – | 42 | 14 | $p < 0.001$ |
| Mortality (%) | – | 37 | 10 | $p < 0.001$ |

[a] Continuous variables were compared using $t$-test or analysis of variance where appropriate. Dichotomous variables are expressed as percentages with the number in parentheses and were compared using the chi-square test.

### 2.6. *Statistical analysis*

Data was analyzed with SPSS 16.0 (SPSS Inc., Chicago, IL) and SAS 9.1 (SAS Institute Inc., Cary, NC). Categorical variables were compared by chi-square. Continuous variables were assessed for normality. Variables that failed to pass the test of normality were logarithmically transformed to fit a normal distribution. Group–time interaction of plasma cytokine levels and injury was determined by two-way analysis of variance (ANOVA) with repeated measures, and multivariate ANOVA was used for determining the effect of MODS on plasma cytokine levels. A Dunnett's adjustment was used to evaluate differences in plasma cytokine levels compared to the initial plasma level. A $p$-value $< 0.05$ was considered significant for all analyses.

## 3. Results

During the study period, 179 hospitalized subjects and 20 healthy volunteers were enrolled and included in the analysis. Of the 179 subjects, 67 were admitted to the burn ICU and 112 to the surgical ICU with either blunt or penetrating traumatic injuries. Though the percentage of men and women between the groups was similar, subjects with burns were older with a lower ISS as an average for this population (Table 1). As a reflection of injury severity, subjects with burns had a high prevalence of MODS and a higher mortality rate. Burn patients had significantly fewer ventilator- and ICU-free days than non-burn trauma patients. Hence, as a result of traumatic injuries, these patients underwent critical care for a longer period.

### 3.1. *Cytokine analysis*

The first blood samples obtained from both burn and non-burn subjects were at similar ($p < 0.05$) times after admission, 13 ± 6 h and 12 ± 6 h, respectively. The majority of plasma samples from volunteers, trauma, and burn patients had values below the assay's limit of detection for IL-1β, TNF-α, and GM-CSF (75%, 73%, and 52%, respectively). A significant portion of the IL-1β, TNF-α, and GM-CSF values were below traceable levels; thus, we excluded these cytokines from further analysis since we would not be able to fully evaluate whether there was an association between cytokine measurements and injury type or patient trajectory. In contrast, 98% and 90% of samples had detectable concentrations of IL-6 and IL-8, respectively. As an initial assessment of the inflammatory response to type of injury and injury severity, we analyzed the association among ISS, TBSA, IL-6, and IL-8. In burns, there were significant but weak positive relationships among TBSA, IL-6, and IL-8 (Fig. 1, $r = 0.16$, $p = 0.005$; Fig. 2, $r = 0.12$, $p = 0.04$; respectively). There was also a significant but weak relationship between ISS and IL-8 (Fig. 3, $r = 0.20$, $p < 0.001$). No significant relationship between ISS and IL-6 was observed. In contrast, no relationships between ISS and IL-6 or between ISS and IL-8 were identified for non-burn trauma subjects.

In comparison to the plasma IL-6 levels of the healthy volunteers, both burn and non-burn subjects had markedly greater levels at every time point assessed ($p < 0.01$). The burn

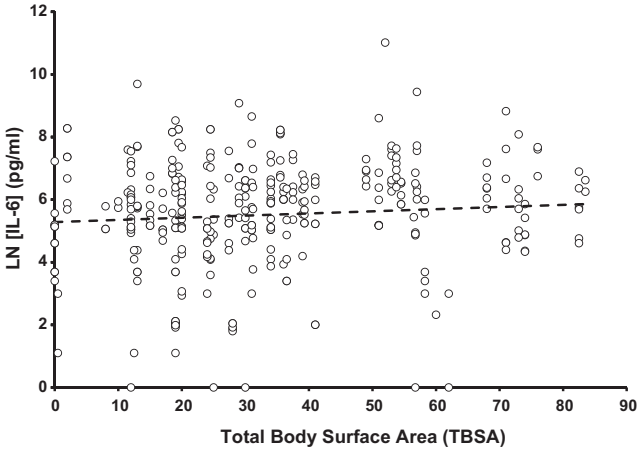


**Fig. 1 – Relationship between burn size and plasma concentrations of IL-6 in subjects with burns. Open circle represents data coming from subjects with burns; $r = 0.16$, $r^2 = 0.02$; $p = 0.005$.**

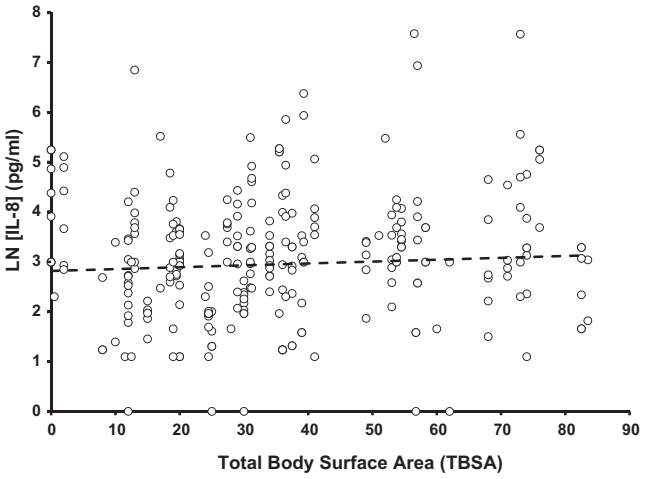


**Fig. 2 – Relationship between burn size and plasma concentrations of IL-8 in subjects with burns. Open circle represents data coming from subjects with burns. $r = 0.12$, $r^2 = 0.01$; $p = 0.04$.**

group had higher IL-6 levels on days 1–3, 5, and 7 compared to the levels of the non-burn group ($p < 0.05$) (Fig. 4). While plasma concentrations of IL-6 remained consistently elevated in burns throughout the study period, non-burn subjects exhibited a decreasing trend of plasma IL-6 levels ($p < 0.01$).

Plasma IL-8 levels of burn and non-burn subjects were higher than those of the healthy volunteers ($p < 0.01$). Similar to the findings of IL-6, there was no significant difference in admission plasma IL-8 levels between burn and non-burn subjects. In addition, burn subjects had higher levels of IL-8 on every subsequent day after injury measured (Fig. 5; $p < 0.01$). In comparison to admission values, plasma IL-8 of burn subjects was greater on days 1, 5, and 7. This group exhibited an increasing trend in plasma concentration of IL-8 throughout the study period. In contrast, non-burn subjects exhibited the opposite trend: IL-8 concentrations were lower on every day compared to admission ($p < 0.01$).

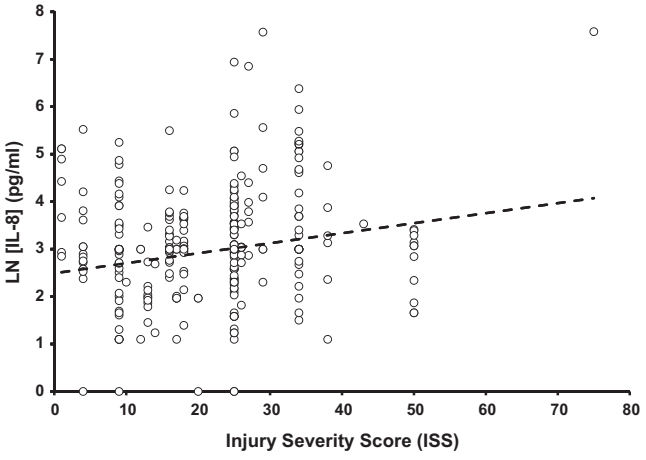


**Fig. 3 – Relationship between injury severity and plasma concentrations of IL-8 in subjects with burns. Open circle represents data coming from subjects with burns. $r = 0.20$, $r^2 = 0.04$; $p < 0.001$.**

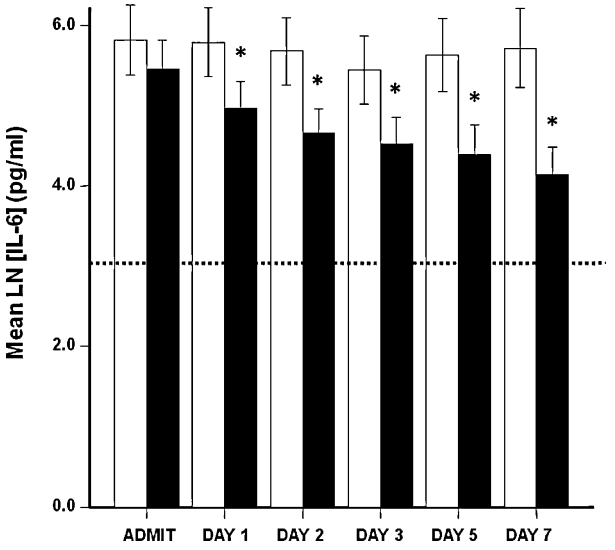


**Fig. 4 – Plasma IL-6 levels in subjects with trauma: burn vs. non-burn subjects. Data are expressed as an average for the group (with standard error represented by error bars). The burn group ($n = 67$) is represented by the white bars and the non-burn group ($n = 112$) by the black bars. The dashed line across the graph represents average plasma IL-6 concentrations of the healthy volunteers ($n = 20$). At each time point, the asterisk (*) indicates a difference ($p < 0.05$) between the burn and the non-burn group. All samples from the burn and the non-burn group had higher IL-6 levels than those of the healthy volunteers ($p < 0.01$).**

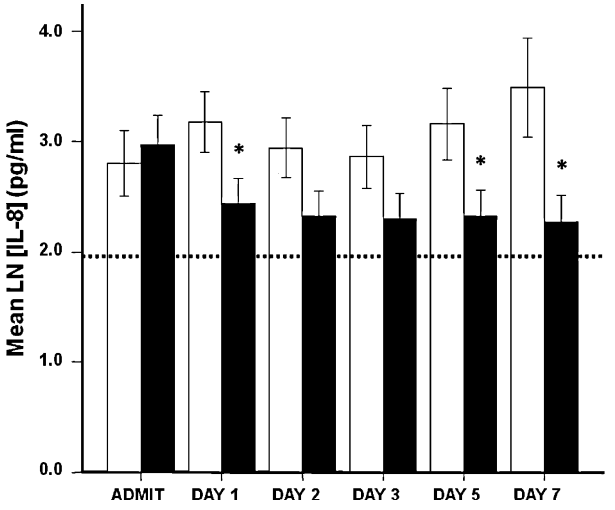


**Fig. 5 – Plasma IL-8 levels in subjects with trauma: burn vs. non-burn subjects. Data are expressed as an average for the group (with standard error represented by error bars). The burn group ($n = 67$) is represented by the white bars and the non-burn group ($n = 112$) by the black bars. The dashed line across the graph represents average plasma IL-8 concentrations of the healthy volunteers ($n = 20$). At each time point, an asterisk indicates a difference ($p < 0.05$) between the burn and the non-burn group. All samples from the burn and the non-burn group had higher IL-6 levels than those of the healthy volunteers ($p < 0.01$).**

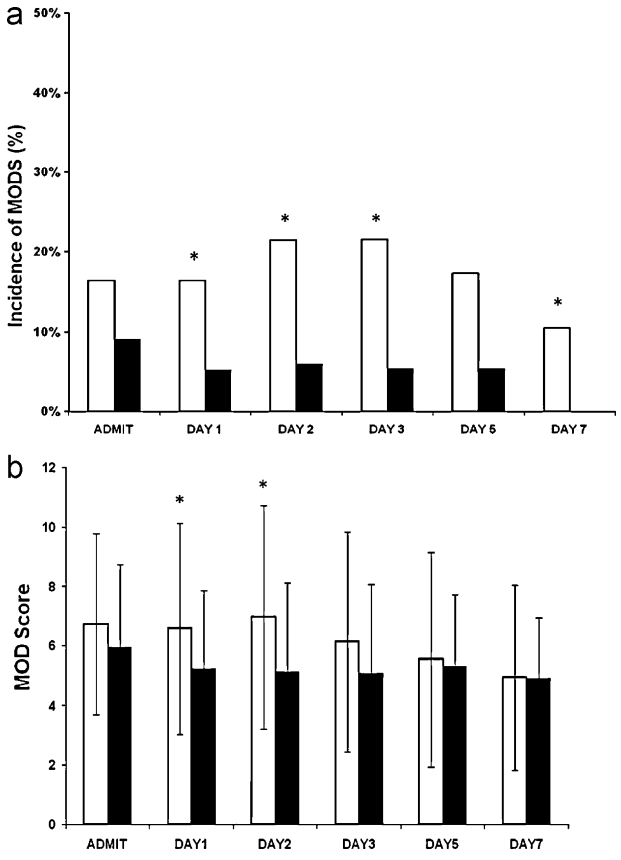


**Fig. 6 – (a) Daily rate of MODS in subjects with trauma: burn vs. non-burn subjects. While MODS was defined as a Marshall score of 10 or greater, the data are expressed as the percentage of population with MODS. The burn group is represented by white bars and the non-burn group by the black bars. At each time point, an asterisk indicates a difference ($p < 0.05$) between the burn and the non-burn group. Statistical analysis performed with chi-square test. (b) Daily average MOD score in subjects with trauma: burn vs. non-burn subjects. The burn group is represented by white bars and the non-burn group by the black bars. At each time point, an asterisk indicates a difference ($p < 0.05$) between the burn and the non-burn group.**

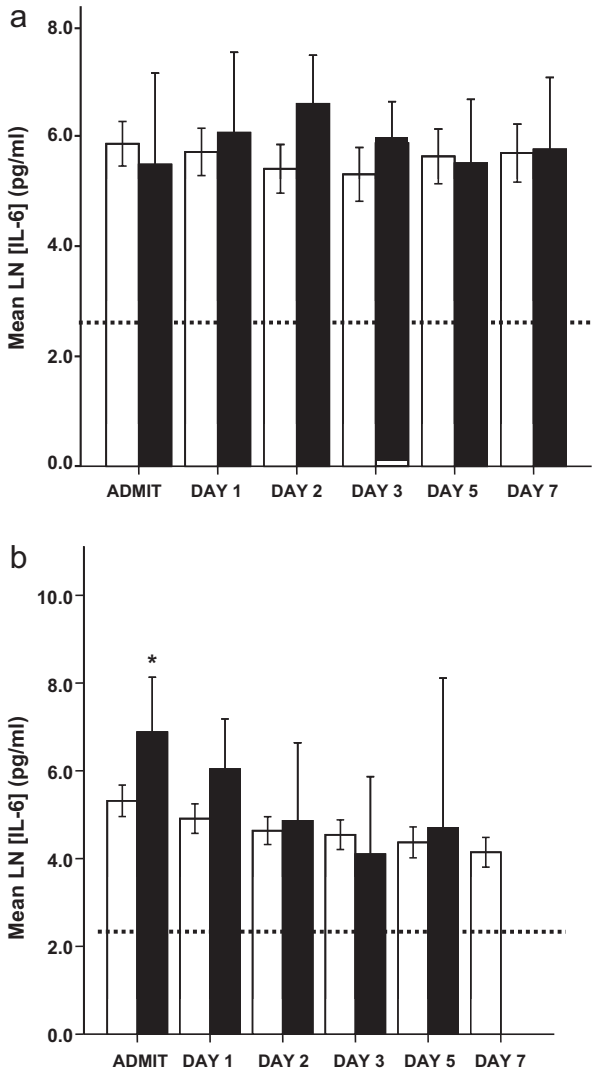


**Fig. 7 – (a) Mean concentration of plasma IL-6 levels in subjects with burns: no MODS vs. MODS. Data are expressed as an average for the group (with standard error represented by error bars). The no MODS group is represented by the white bars and the MODS group by the black bars. The dashed line across the graph represents average plasma IL-6 concentrations of the healthy volunteers ($n = 20$). At each time point, an asterisk indicates a difference ($p < 0.05$) between the no MODS and the MODS group. (b) Mean concentration of plasma IL-6 levels in subjects with non-burn trauma: no MODS vs. MODS. Data are expressed as an average for the group (with standard error represented by error bars). The no MODS group is represented by the white bars and the MODS group by the black bars. The dashed line across the graph represents average plasma IL-6 concentrations of the healthy volunteers ($n = 20$). At each time point, an asterisk indicates a difference ($p < 0.05$) between the no MODS and the MODS group. Of note, none of the subjects from the non-burn group met the Marshall criteria for MODS on day 7.**

### 3.2. *Multiple organ dysfunction syndrome (MODS)*

Overall burn subjects had a higher rate of MODS in comparison to non-burn subjects (Fig. 6a). Furthermore, subjects with burns had a higher MODS score on every study day, with the exception of admission ($p < 0.05$) (Fig. 6b). Multivariate ANOVA of injury type, MODS, and plasma IL-6 levels was performed with an overall significant group–time interaction ($p < 0.05$) (Fig. 7). The burn group without MODS had significantly higher plasma IL-6 than non-burn subjects without MODS on days 1 through 7 after injury($p < 0.05$). Although there were no differences between the burn and the non-burn groups with MODS, the subjects with burns and MODS had higher IL-6 levels than the non-burn group without MODS on days 2 and 3 ($p < 0.05$). However, non-burn subjects

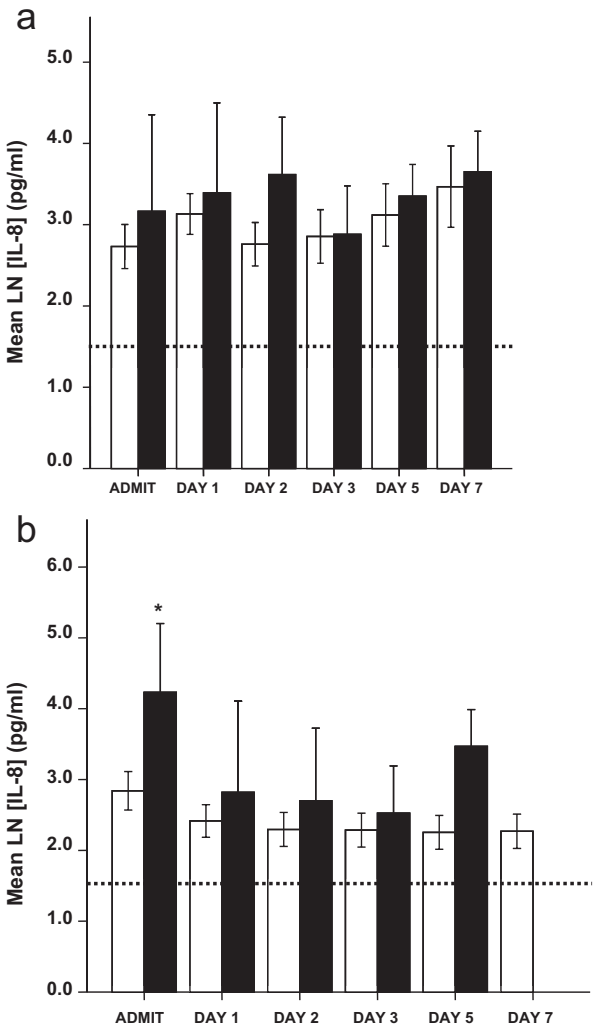


**Fig. 8 – (a) Mean concentration of plasma IL-8 levels in subjects with burns: no MODS vs. MODS. Data are expressed as an average for the group (with standard error represented by error bars). The no MODS group is represented by the white bars and the MODS group by the black bars. The dashed line across the graph represents average plasma IL-8 concentrations of the healthy volunteers ($n = 20$). At each time point, an asterisk indicates a difference ($p < 0.05$) between the no MODS and the MODS group. (b) Mean concentration of plasma IL-8 levels in subjects with non-burn trauma: no MODS vs. MODS. Data are expressed as an average for the group (with standard error represented by error bars). The no MODS group is represented by the white bars and the MODS group by the black bars. The dashed line across the graph represents average plasma IL-8 concentrations of the healthy volunteers ($n = 20$). At each time point, an asterisk indicates a difference ($p < 0.05$) between the no MODS and the MODS group. Of note, none of the subjects from the non-burn group met the Marshall criteria for MODS on day 7.**

with MODS had significantly higher plasma IL-6 at the time of admission than the non-burn group without MODS ($p < 0.05$).

The same multivariate ANOVA was performed with plasma IL-8 and an overall group–time interaction also ($p < 0.05$) (Fig. 8). Again, the burn group without MODS had higher IL-8 levels than the non-burn group without MODS on days 1 through 7 after injury ($p < 0.05$). The burn and non-burn groups with MODS did not differ and had similar IL-8 levels. As for days 2, 5, and 7, the burn group with MODS had higher IL-8 than the non-burn group without MODS ($p < 0.05$). On the day of admission, the non-burn group with MODS had a higher plasma IL-8 than the burn group without MODS ($p < 0.05$). In parallel to what was observed with IL-6, the non-burn group with MODS had higher plasma IL-8 level on admission than the non-burn group without MODS ($p < 0.05$).

## 4. Discussion

The period of recovery from a burn represents the extreme of hypermetabolism and catabolism possible in humans [1,28]. It was expected patients with burns would have higher plasma cytokine levels than non-burn trauma patients; however, this study quantitatively demonstrates the significant difference in terms of plasma IL-6 and IL-8 levels. Burn was associated with a greater and more sustained immunoinflammatory response in terms of elevated IL-6 and IL-8 levels during the first week post-injury compared to non-burn trauma patients. The exaggerated immunoinflammatory response with resultant imbalance in immune function places burn and trauma patients at increased risk for infection, sepsis, MODS, and consequently death. Our study did, however, demonstrate a distinct difference in plasma cytokine profiles between burn and non-burn trauma patients, the multivariate analysis of MODS was less conclusive.

IL-6 is expressed in peripheral leukocytes, spleen, liver, kidney, and intestine and potentially from a multitude of other stimulated cell types. It modulates local and systemic inflammation with effects on other leukocytes and the acute phase response to injury [3]. IL-8 is a chemotactic cytokine (chemokine) predominantly produced from monocytes and macrophages that recruit neutrophils to the site of inflammation [29]. In burn patients, a significant source of IL-6 and IL-8 is the thermally injured skin itself [12]. Local sources of the cytokines after non-burn trauma have been demonstrated by elevated IL-6 levels in cerebrospinal fluid after traumatic brain injury [30] and in femoral intramedullary blood after fracture [31] as well as elevated bronchoalveolar fluid levels of IL-8 and IL-1β after chest trauma [32]. In spite of the various reported findings, the physiologic levels locally or systemically necessary to propagate an immune or inflammatory response has yet been clearly characterized.

In this study, unexpectedly, the presence of MODS did not have a significant effect on either plasma IL-6 or IL-8 levels. Nast-Kolb et al. demonstrated that blunt trauma patients with subsequent multiple-organ failure had early peaks of plasma IL-6 and IL-8 that were significantly greater than those of blunt trauma patients who recovered without multiple-organ failure [8]. One difference may be that our statistical analysis included time as a factor using ANOVA with repeated measurements as opposed to using the Kruskal–Wallis test for comparisons at isolated time points. Also, it may be that the heightened level of inflammatory response that ensued after a severe burn reaches an individually dependent

maximal threshold in which the added complication of MODS is not distinguishable. On the other hand, the same could be said about the response that MODS incites irrespective of injury type. The lack of significant effect of MODS may also be due to less power in small number of patients with MODS coupled with the large variance of plasma cytokine levels.

Interestingly, the burn patient population in this study had a slightly lower ISS than the trauma patient population, yet burn patients had a significantly higher prevalence of MODS and a higher mortality. Although ISS has been found to be an independent risk factor for mortality in patients with a combination of burn and trauma [33], the two populations in this study had a relatively similar ISS. This finding may represent a limitation of the ISS in terms of predicting outcomes specifically for burn patients even though it is well established in trauma patients. At a given ISS, burn patients also had worse survival and higher prevalence of MODS that may be related to a greater immunoinflammatory response in addition to other factors such as inhalation injury and loss of the innate immunity of skin.

A limitation of this study is the statistically significant baseline difference between the burn and the non-burn trauma populations. Burn patients were older than non-burn trauma patients, and inhalation injury was present in a third of the burn patients. This difference places the burn group at greater risk of MODS and death than the non-burn group. However, the 7-year age difference is small enough that it is unlikely to have resulted in the different cytokine pattern. Factors such as gender and the presence of inhalation injury may also impact cytokine levels; but, in this initial evaluation of the study they were not specifically addressed.

Additionally, we speculate that initial cytokine levels, especially in the burn patients, may be affected by the massive fluid resuscitation typically required in patients with larger burns. As such, mediators may have been potentially falsely diluted as a result and underestimated. Obtaining and comparing tissue levels or levels in other bodily fluids may be necessary.

## 5. Conclusions

In conclusion, burn patients had a greater and more sustained immune-inflammatory response, as reflected by plasma IL-6 and IL-8 levels, during the first week after injury than the non-burn trauma patients. The presence of MODS did not have a significant effect on plasma cytokine levels. Though we did not find associations between cytokine levels and outcome in this study, burn patients had a significantly worse outcome with higher mortality and more frequent multiple-organ dysfunction compared to non-burn patients. Our data suggests systemic measurements of IL-6 and IL-8 over the first week of hospitalization is reflective of injury severity rather than a predictor of patient outcomes.

## Conflict of interest statement

None of the authors have any conflicts of interest or disclosures to report.

## Acknowledgements

We acknowledge the laboratory support of the Department of Clinical Investigations by Kimberly Farrow, David Ho, Lisa Pérez, and Houston Kim in conducting the cytokine analyses and logistical support by Charlie Guymon. We also recognize the research nurses who supported this protocol, including Nancy Molter, Chaya Galin, Peggy Bielke, Kari Williams, Deb Deja, Elizabeth Frail, Juliette Castillo, and Michelle Morrow and the information technologists Nikolaos Kypreos and Jesús Morales, who assisted with database creation. Finally, we would like to thank Otilia Sánchez for editing the final manuscript.

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