Predictive Value of IL-8 for Sepsis and Severe Infections after Burn Injury - A Clinical Study

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Abstract

The inflammatory response induced by burn injury contributes to increased incidence of infections, sepsis, organ failure, and mortality. Thus, monitoring post-burn inflammation is of paramount importance but so far there are no reliable biomarkers available to monitor and/or predict infectious complications after burn. As IL-8 is a major mediator for inflammatory responses, the aim of our study was to determine whether IL-8 expression can be used to predict post-burn sepsis, infections, and mortality other outcomes post-burn. Plasma cytokines, acute phase proteins, constitutive proteins, and hormones were analyzed during the first 60 days post injury from 468 pediatric burn patients. Demographics and clinical outcome variables (length of stay, infection, sepsis, multiorgan failure (MOF), and mortality were recorded. A cut-off level for IL-8 was determined using receiver operating characteristic (ROC) analysis. Statistical significance is set at (p<0.05). ROC analysis identified a cut-off level of 234 pg/ml for IL-8 for survival. Patients were grouped according to their average IL-8 levels relative to this cut off and stratified into high (H) (n=133) and low (L) (n=335) groups. In the L group, regression analysis revealed a significant predictive value of IL-8 to percent of total body surface area (TBSA) burned and incidence of MOF (p<0.001). In the H group IL-8 levels were able to predict sepsis (p<0.002). In the H group, elevated IL-8 was associated with increased inflammatory and acute phase responses compared to the L group (p<0.05). High levels of IL-8 correlated with increased MOF, sepsis, and mortality. These data suggest that serum levels of IL-8 may be a valid biomarker for monitoring sepsis, infections, and mortality in burn patients.

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Keywords

IL-8; infection; burn; sepsis; MOF; biomarker; mortality; organ failure

INTRODUCTION

The incidence of severe infectious complications after burn injury increases mortality by 40% (1); thus, successful treatment of infections and septic episodes are paramount to decrease death due to severe burn injury (2). Early identification of infections and sepsis remain one of the major clinical challenges. The destruction of large areas of the dermal barrier leaves the severely burned patient susceptible to bacterial, fungal, and viral infections (3, 4). Treatment is often empiric, not specific, and is frequently initiated too late (4). Timely identification of invasive pathogens can allow treatments targeted to the infection. Unfortunately current methods for identifying pathologic micro-organisms such as routine wound culturing or PCR are not sensitive or accurate enough at this stage. Positive identification of infectious agents by the microbiological laboratory is delayed in most cases, increasing the potential for developing life-threatening infectious complications. The ability to detect and treat emerging infections as early as possible would enable timely initiation of specific therapies targeted against the invading microorganism, thereby reducing post-burn morbidity and mortality, and improving patient outcome. The identification of a biomarker which is sensitive and that can be quickly measured would greatly advance the care for these critically injured burn patients.

Burn injury itself leads to an acute and prolonged hyper-inflammatory state that has been well described by many (5-9). Although several studies have reported the utility of using pro-inflammatory markers such as tumor necrosis factor α (TNF-α), C-reactive protein (CRP) or interleukin 6 (IL-6) to monitor post-burn inflammation or immune dysfunction (5, 6, 10, 11), the successful establishment of these same inflammatory markers as biomarkers of infection have been less well explored. Our earlier work has shown that the ratio of IL-6 to TNF-α can be used to predict death from sepsis following burn injury (12). Furthermore, in studies of adult burn patients, we have shown that IL-4, IL-8, granulocyte macrophage colony-stimulating factor, and monocyte chemotactic protein 1 may serve as predictive biomarkers of death from sepsis and/or multiple organ failure (MOF) (13). The incidence of MOF has also been correlated with differential cytokine expression (Jeschke, Finnerty, Herndon, unpublished data). A major mediator of the acute inflammatory response to infection and injury, IL-8 acts quickly, suggesting that early monitoring of this chemokine may provide quick information regarding infection status (11, 14-19). Aside from the recruitment of neutrophils, IL-8 signaling is implicated in the mechanisms underlying angiogenesis, cell growth and tissue remodeling (20). IL-8 production is induced by cellular dysfunction, exogenous stimuli such as bacteria and viruses, and via TNF-α or nuclear transcription factor kappa B (NF-κB) (15, 21). Based on IL-8's role as a mediator inflammation and of proliferation (16, 22), we hypothesized that systemic levels of IL-8 could be used to predict infections and sepsis in severely burned patients.

PATIENTS AND METHODS

Four-hundred sixty-eight pediatric burn patients with severe burns over 30% of total body surface area (TBSA) were enrolled in this study. All patients received the standard of care described in previous publications (23, 24). Briefly, patients were resuscitated according to the Galveston formula (5000 cc/m^2 TBSA burned + 2000 cc/m^2 TBSA lactated Ringer's solution given in increments) over the first 24 hours. Within 48 hours of admission, all patients underwent total burn wound excision and their wounds were covered with autograft. Any remaining open areas were covered with homograft. After the first operative procedure, patients were taken back to the operation theater when donor sites were healed. This procedure was repeated until all open wound areas were covered with autologous skin.

Nutritional intake is standardized for our hospital and was calculated as 1500 kcal/m^2 body surface + 1500 kcal/m^2 area burns previously described (25-27). The nutritional route of choice in our patient population was enteral nutrition via a naso-duodenal (Dobhoff) or nasogastric tube.

Patient demographics (age, date of burn and admission, sex, burn size, and depth of burn) and clinical data (concomitant injuries, inhalation injury, sepsis, morbidity, and mortality) were recorded throughout hospital course. Sepsis was defined as the combination of 1) a positive blood culture (≥10^5CFU) or pathologic identification of infectious agent from biopsy or post-mortem examination, and 2) at least three of the following: leucocytosis or leucopenia (>12,000 or <4,000/μl), hyperthermia or hypothermia (>38.5 or <36.5°C), tachycardia (>20% above normal value), refractory hypotension (systolic BP <20% below normal value), thrombocytopenia (platelets <50,000/mm^3), hyperglycemia (serum glucose >240 mg/dl), and enteral feeding intolerance (residuals > 200 cc/hr or diarrhea >1 L/day) in accordance with the American Burn Association consensus definition on sepsis (28) MOF overall organ function (DENVER2) were assessed as previously published (29, 30).

Patient data was collected and recorded prospectively using the clinical information system Emtek by physicians, nurses, and supportive staff. Data was processed and analyzed with Microsoft Access® and Excel® (Microsoft Corporation Inc. Redmond, WA, USA).

Hormones, proteins, and cytokines

Blood and/or urine were collected from burn patients at admission and during the first 60 days post-burn for serum hormone, protein, cytokine, and urine hormone analysis. Blood was drawn in a serum-separator collection tube and centrifuged for 10 minutes at 1320 rpm; the serum was removed and stored at −70°C until assayed.

Serum hormones and acute phase proteins were determined using HPLC (31), nephelometry (BNII, Plasma Protein Analyzer Dade Behring, MD), and ELISA techniques as previously published (24). The Bio-Plex Human Cytokine 17-Plex panel was used with the Bio-Plex Suspension Array System (Bio-Rad, Hercules, CA) to profile expression of seventeen inflammatory cytokines and chemokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70,IL-13,IL-17, granulocyte colony stimulating factor, granulocytemacrophage colony stimulating factor, interferon-gamma (IFN-γ), monocyte chemo attractant protein-1,

macrophage inflammatory protein-1beta, and TNF-α) as previously published by Finnerty et al. (5).

Ethics

The study was reviewed and approved by the Institutional Review Board of the University Texas Medical Branch, Galveston, Texas. Informed consent was obtained from each subject ≥18 years of age, parent or child's legal guardian. Assent was obtained from research subjects older than 7 years of age.

Data Analysis

For biochemical measurements, a Confidence Interval 2σ (CI= 0.9544997) was applied to improve reliability. Forward stepwise logistic regression, liner regression analysis, paired and unpaired Student's t-test, and Chi-square analysis tests were used where appropriate. For the cut-off analysis, Receiver Operating Characteristic (ROC) analysis was used. Statistical analysis was performed using Microsoft Excel® and Sigmastat® version 3.5 and Sigmaplot®, (Systat Software Inc., San Jose, CA, USA). Data are expressed as means±SD or SEM, where appropriate. Significance was accepted at $p<0.05$.

For determining the cut-off value, each patient's levels of IL-8 during the stay in the ICU were averaged. These values were processed with ROC analysis and a calculated cutoff value of 234 pg/ml of IL-8 was determined for highest sensitivity and specificity for survival. This value was then used to stratify the patients in the study. The calculated value is validated with an area under the curve (AUC) value of A=0.88 and considered having a good prognostic significance (Figure 1).

RESULTS

Demographics and clinical outcome

Four-hundred sixty-eight pediatric burn patients were included in this study and were stratified according to the cut-off values described above in two groups (Table 1). The cohort with levels of IL-8 below 234 pg/ml IL-8 (low group: L) consisted of 335 patients. The second cohort of patients with IL-8 greater than 234 pg/ml consisted of 133 patients (high group: H). Both groups were similar for many parameters (gender, ethnic background, age, and burn mechanism). The H group had significantly higher incidence of inhalation injury and greater burn sizes (Table 1) ($p<0.001$). Patients (in the H group) with high IL-8 levels had a significantly longer length of stay, higher incidences of MOF, infections, sepsis, and mortality, and greater incidence of infections compared to patients in the L group (Table 2) ($p<0.001$).

Mortality rates in patients with low IL-8 levels (L group) were significantly reduced when compared to the H group during the first 60 days after injury (Figure 2). The absolute mortality levels were lower in the L group as well (Table 2, $p<0.001$).

Predictive value of IL-8 levels

Validation of the patient stratification can be shown with measurements of IL-8 over time showing persistently elevated levels of IL-8 in the H group compared to the L group (Figure 3A, $p < 0.05$).

Analysis of the prediction value of IL-8 levels for the L group (Figure 4A) revealed that levels of this cytokine increase with burn size ($p < 0.001$, highest F-to-Enter = 35.923). IL-8 is also specific ($p < 0.001$) for prediction of MOF and the maximum DENVER2 score. There was no correlation with between the incidence of sepsis and IL-8 levels.

In the H group (Figure 4B) IL-8 levels correlated strongly with the incidence of sepsis ($p = 0.002$; highest F-to-Enter = 9.874). IL-8 also correlated with the incidence of MOF and the maximum DENVER2 scores in the H group ($p < 0.005$). Interestingly, burn size showed no correlation with IL-levels in the H-group. A nearly linear increase in the probability of sepsis with increasing IL-8 levels occurs, indicating that IL-8 levels are correlated with burn size, but to a greater extent with infection and sepsis, indicating that these patients are experiencing even more inflammation.

To identify possible confounders for the incidence of sepsis, we conducted a linear regression analysis for both groups. In patients below the cut off, a significant relationship ($p < 0.001$) with third-degree burn size was found. In patients above the cut off, no relationship was found (Table 3). For all other variables such as age or time from burn to admit, no significant correlation was found in either group.

Inflammation is greater in patients with elevated IL-8

As previously reported, measured cytokine levels were elevated in both patient groups during the observed first sixty days post-burn. Significantly higher levels of IL-1β, IL-6, IL-10, IL12 (p70), and IL-13 can be found over time in the high IL-8 group compared to the low group, ($p < 0.05$) (Figures 3, 5, 6A-B).

The established pro-inflammatory marker CRP (Figure 7C) showed tremendous increases after day 1 post-burn with prolonged elevation in the H group over the first 60 days compared to the L group ($p < 0.05$).

Organ Function

Liver dysfunction, represented by serum bilirubin, increased in both groups directly after burn injury with levels in the L group decreasing to normal after the first week, whereas the H group remained significantly elevated from day 2 until day 60 post-burn ($p < 0.05$) (Figure 8A). Kidney dysfunction represented by creatinine (CRE) levels showed a significantly greater elevation in the high IL-8 group for the first 22 days ($p < 0.05$). Measurements of blood urea nitrogen revealed a remarkable elevation in both groups with significantly increased levels in the high IL-8 group till day 60 ($p < 0.05$) (Figure 8B, C).

DISCUSSION

Sepsis and severe infections are major complications after severe burn injury that are associated with a high mortality (32). Therefore, it is important to detect infectious complications at an early stage in order to initiate appropriate treatments, decreasing morbidity and mortality. As the severely burned patient is in a hyper-inflammatory state due to physiological reactions to the trauma (33-35), established pro-inflammatory markers such as CRP or IL-6 may have limited utility as biomarkers for specific outcomes (36, 37). Currently, there are no established biomarkers that can detect or monitor infections or sepsis (9, 19).We therefore explored the possibility of the chemokine IL-8 as a predictor because IL-8 is known for being a major mediator of the inflammatory response. IL-8 plays a major role in the activation of neutrophils, general inflammatory processes, and tissue repair mechanisms such as angiogenesis and cell proliferation (16, 17, 20). Based on these findings, we suggest that IL-8 has an important role in two major processes that may determine survival after burn injury: tissue remodeling (15, 20) and inflammatory response (16, 21). We hypothesized that IL-8 serves as an indicator for the extent of burn due to tissue repair as well as the ability to quantify the inflammatory response induced by infection. We further hypothesized that following burn injury there is a standard inflammatory response which is exacerbated by the presence of infectious stimuli.

The first analysis in this study identified a cut-off concentration of 234 pg/ml IL-8 as the most specific and sensitive indicator for survival. Patients with high levels of IL-8 had a ten-fold higher in-hospital mortality rate compared to patients with lower IL-8 levels. We further identified inhalation injury, burn size, and the incidence of MOF, sepsis, and infections as factors that contributes significantly to these outcomes. We also found a relationship between the clinical trajectory stratified by IL-8 levels and the expression of other pro-inflammatory regulatory cytokines such as IFN-γ and TNF-α. Interestingly both, inflammatory and anti-inflammatory cytokines were elevated. This observation is in accordance with the results of other studies conducted in burn patients (5-7, 9). Markers of organ function showed that liver and kidney function were also significantly worse in patients with average values of IL-8 greater than 234 pg/ml. These findings indicate that IL-8 levels are influenced by the injury discriminating factors for survival post-burn which are: inhalation injury, burn size and infectious complications. Studies in burned patients have shown that the incidence of infectious complications increases the mortality rate by 40%.

To test the hypothesis that high levels of IL-8 are triggered by infectious stimuli, we conducted a stepwise regression analysis for the patient populations in each groups resulting from stratifying by the lower and higher cut-off values. We included the parameters TBSA burn size, MOF and sepsis in the statistical model. The findings for the low-level group correlate best with the TBSA burn size area and consecutively with the third-degree burn and the incidence of MOF. These results correlate with linear regression modeling considering possible confounding factors for sepsis. These findings are similar to other studies that revealed a correlation of IL-8 to endothelial cell survival, cell proliferation, and the expression of matrix metalloproteases (MMP) (38, 39). These are essential mechanisms for the cleavage and remodeling of tissue after burn injury. Stepwise logistic regression

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analysis of the high IL-8 group showed a significant correlation with the incidence of sepsis, significant greater maximum DENVER2 scores, and incidence of MOF. Burn size did not contribute to IL-8 levels in the high-level group. Logistic analysis shows a nearly linear correlation above the cut-off level of 234 pg/ml for IL-8 with the incidence of sepsis in the high IL-8 patient population.

Summarizing our results, we found that IL-8 is a sensitive and specific biomarker for burn size below a threshold of 234 pg/ml and that at higher levels, the degree of plasma IL-8 correlates strongly with the incidence of septic episodes. We conclude that IL-8 may serve as a useful biomarker to predict infections and septic events in burn victims.

ACKNOWLEDGMENTS

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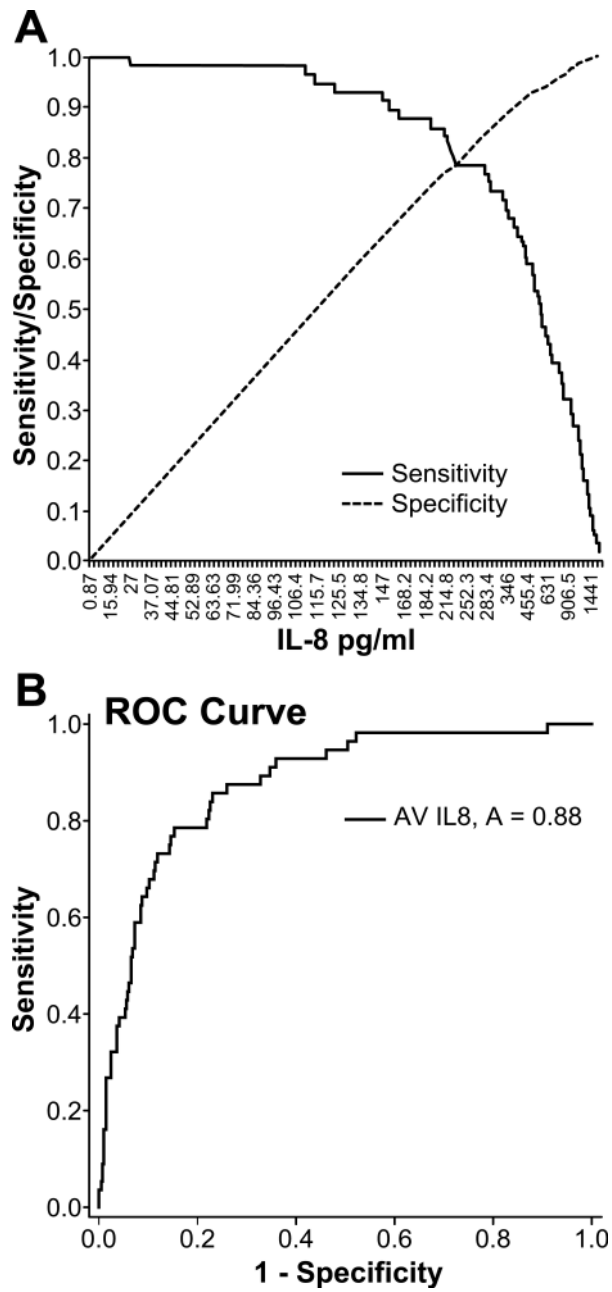
Figure 1. Cut-off determination using receiver operating characteristic (ROC) analysis
Cut-off value at the intersection of sensitivity and specificity (A). Validated by ROC curve with A=0.88 (B).
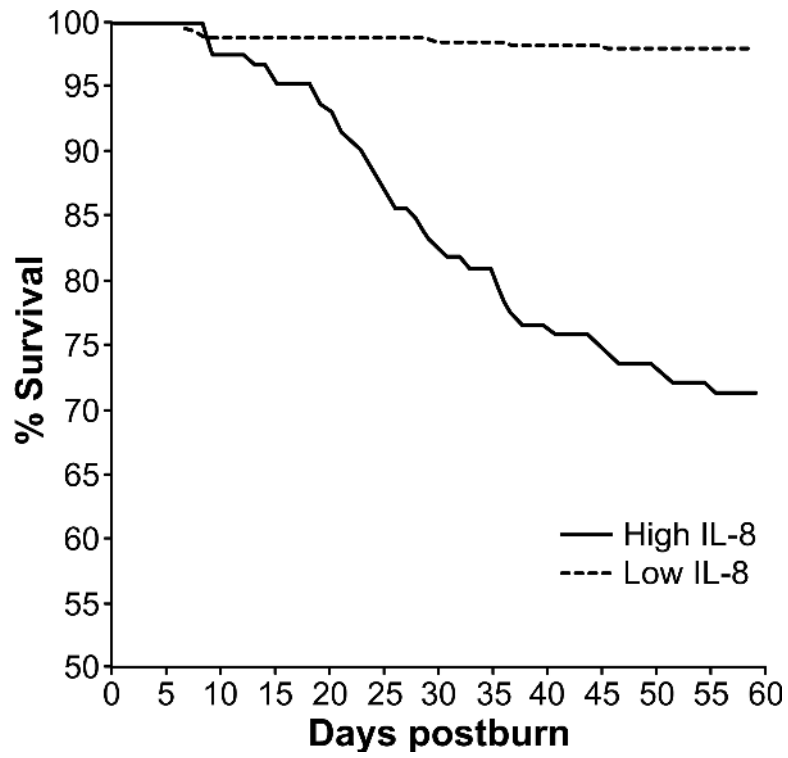
Figure 2. Kaplan Meier survival curve for the observed first 60 days after burn injury
There is a significant higher mortality rate in the high IL-8 level group.
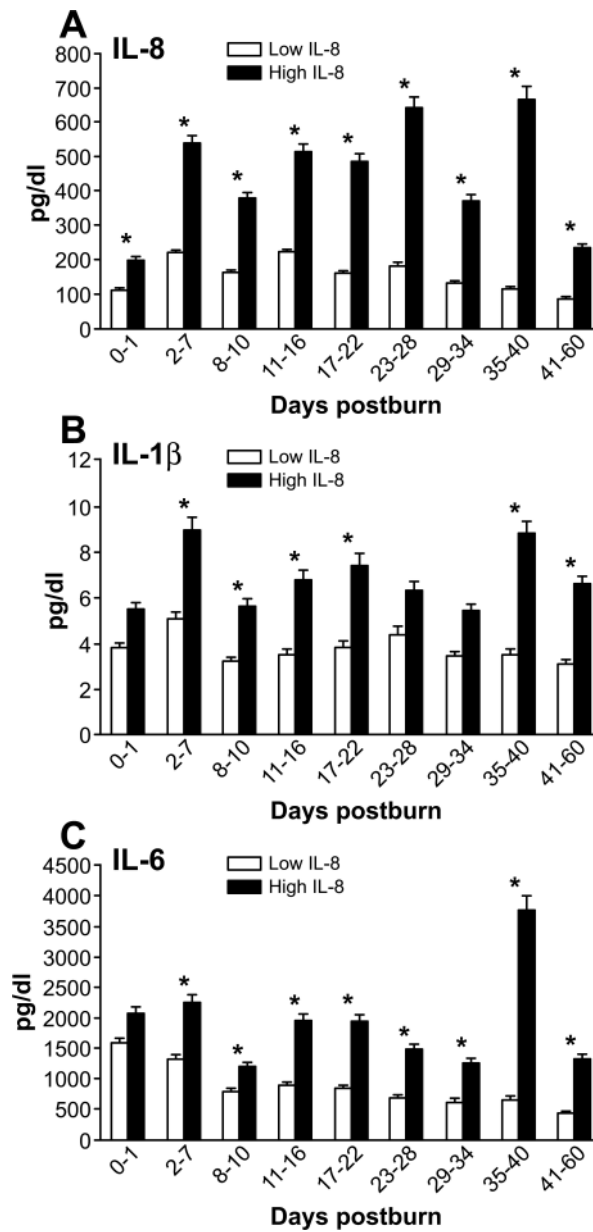
Figure 3. Pro-inflammatory Cytokines and Chemokines
IL-8 expression pattern shown over time (A). IL-1beta and IL-6 expression stratified based on IL-8 expression (B, C).

A

Prediction for lower than Cut Off
Variables not in Model

Group	F-to-Enter	P
TBSA third	17.169	<0.001
MOF Code	10.572	0.001
Max DENVER2	22.801	<0.001
Sepsis code	0.675	0.412

Variables in Model

Group	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
Constant	28.401		11.875		
TBSA burn	1.208	0.315	0.202	35.923	<0.001

B

Prediction for higher than Cut Off
Variables not in Model

Group	F-to-Enter	P
TBSA burn	0.239	0.626
TBSA third	0.0964	0.757
MOF Code	6.034	0.015
Max DENVER2	9.429	0.003

Variables in Model

| Group	Coef.	Std. Coeff.	Std. Error	F-to-Remove	P
Constant	564.077		52.587		
Sepsis code	299.041	0.267	95.167	9.874	0.002

C
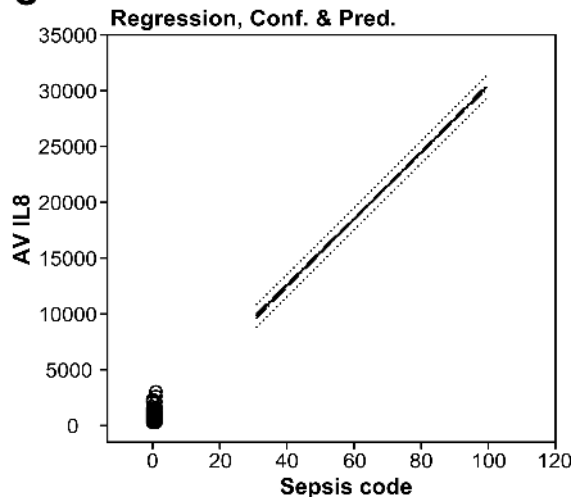
Figure 4. Forward stepwise logistic regression analysis model for the predictive value of IL-8
In the low IL-8 group has only a significant relationship with burn size (A). In patients with higher levels than the determined cut-off IL-8 levels there is a significant correlation with the incidence of sepsis (B). IL-8: confidence, cutoff, and prediction of sepsis (C).
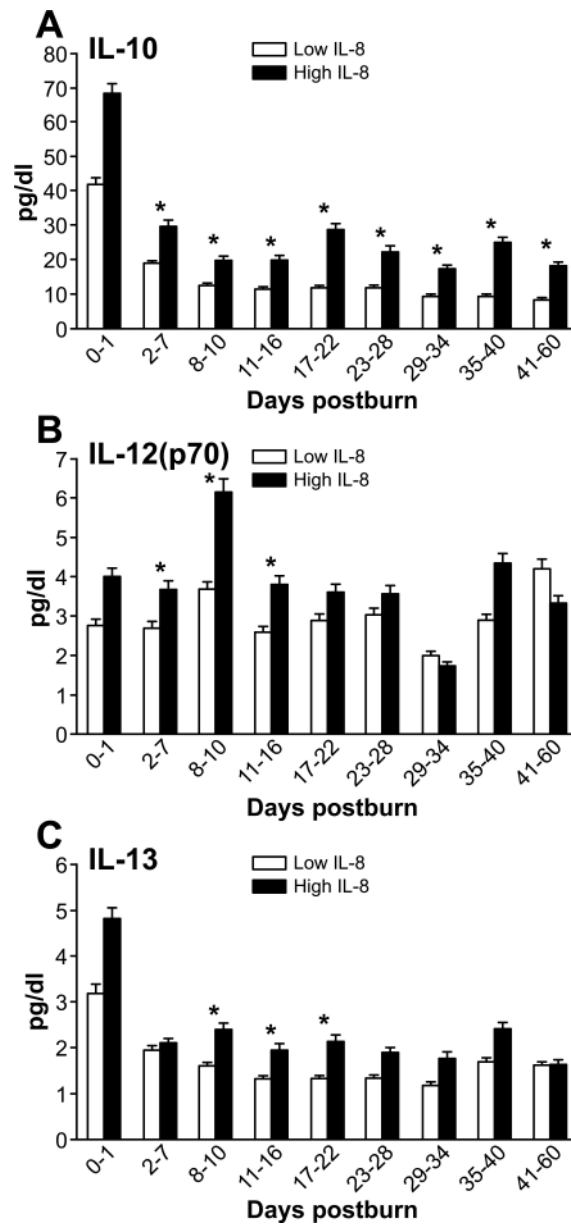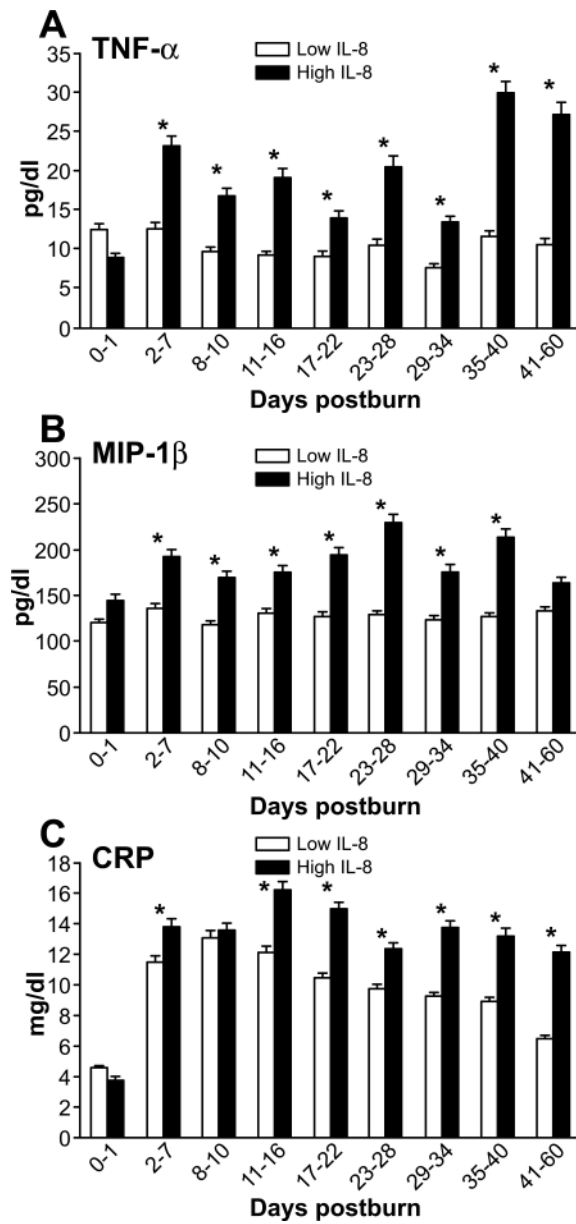
Figure 5. Profile of major regulatory cytokines
Expression of IL-10, IL-12(p70), and IL-13 is associated with IL-8 expression (A, B, C).
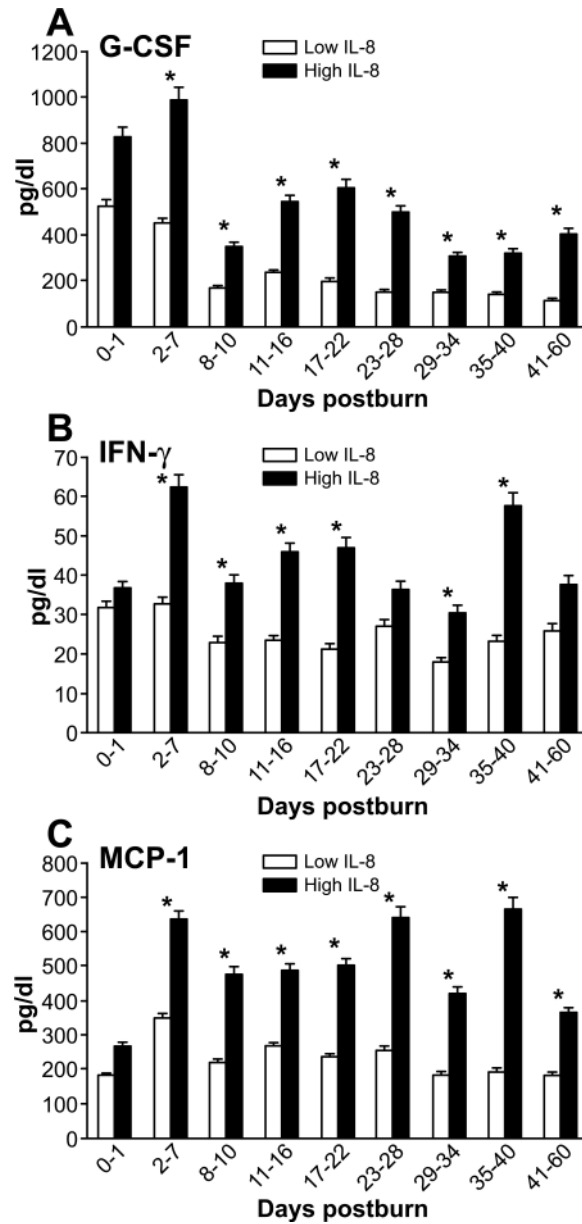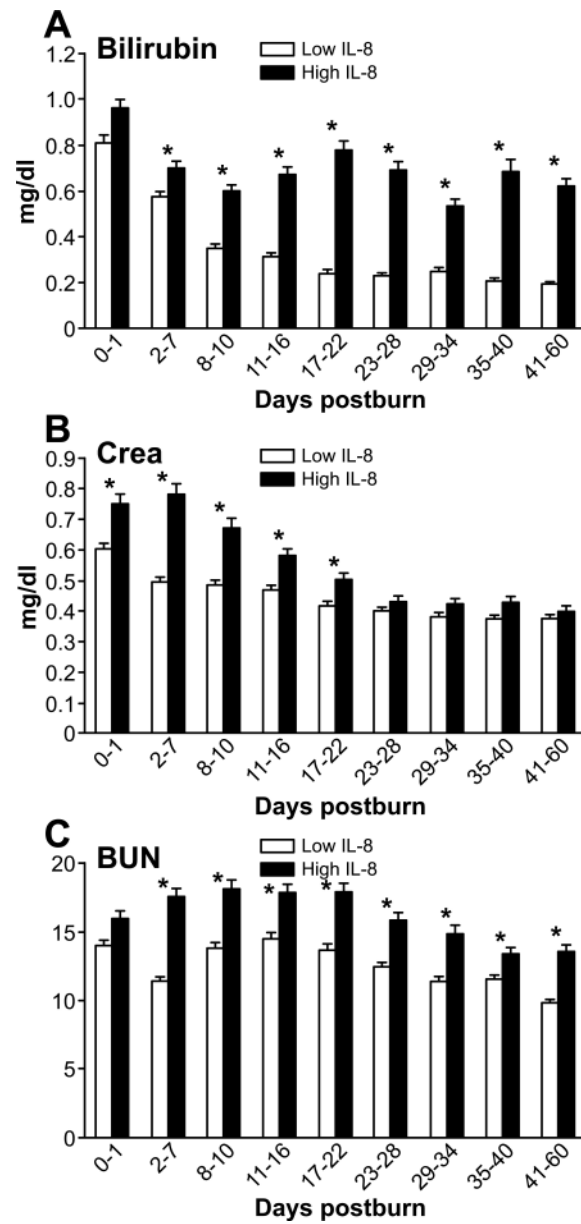
Figure 6. Major regulatory mediators
Expression of G-CSF, IFN-gamma, MCP-1 (markers for granulocyte activation (A), inflammation (B), and macrophage activation (C) is higher in the group with high IL-8 levels.

Figure 7. Markers for Inflammation
Regulatory mediator (TNF-alpha), chemokine (MIP-1beta) and the established marker for inflammation CRP (C) in the high IL-8 group (A, B, C).

Figure 8. Organ specific markers

Elevation of organ specific markers for liver (A: bilirubin) and kidneys (B: Creatinine; C: BUN).

Table 1

Patients demographics.

	High IL-8	Low IL-8	P value
n	133	335	
Gender			
male	84	243	
female	49	92	
Ethnicity			
African American	6	15	
Caucasian	21	33	
Hispanic	105	283	
Other	1	4	
Age at admit (years)	8 ± 6	8 ± 5	NS
Inhalation injury (%)	73 (55)	129 (39)	<0.01
Type of burn			
Flame n (%)	104 (78)	259 (77)	
Scald n (%)	26 (20)	51 (15)	
Other n (%)	3 (2)	25 (8)	
TBSA burn (%)	67 ± 18	57± 15	< 0.001
TBSA second (%)	20 ± 20	19 ± 19	NS
TBSA third (%)	55 ± 26	45 ± 22	< 0.001
Burn to admit (days)	3 ± 3	4 ± 4	<0.05

Table 2

Patient outcome and critical events during stay in the ICU.

	High IL-8	Low IL-8	P value
n	133	335	
Number of OR	6 ± 4	4 ± 3	< 0.001
Time btw OR (days)	5 ± 2	5 ± 3	NS
LOS ICU (days)	39 ± 32	32 ± 24	<0.05
LOS/TBSA	0.6 ± 0.4	0.5 ± 0.3	NS
died n (%)	44 (33)	12 (4)	<0.001
Max DENVER2	5 ± 2	3 ± 1	< 0.001
MOF n (%)	67 (50)	49 (15)	< 0.001
Sepsis n (%)	41 (31)	39 (12)	< 0.001
Infections n	3.3 ± 2.7	2.1 ± 2.3	< 0.001

Patients with high IL-8 levels show a higher morbidity and mortality.

Table 3

Linear regression analysis.

	Coefficient	Std. Error	t	P	VIF
Linear Regression below cutoff for sepsis					
Constant	0.0794	0.0729	1.089	0.277	
AV IL8	−0.000623	0.00038	−1.641	0.102	1.094
Age admit	0.00171	0.00401	0.426	0.67	1.016
Burn to Admit	−0.00593	0.00549	−1.08	0.281	1.053
TBSA third	0.00412	0.000994	4.145	‹0.001	1.083
Linear Regression above cutoff for sepsis					
Constant	0.236	0.0951	2.486	0.014	
AV IL8	−0.0000126	0.0000624	−0.202	0.84	1.003
Age admit	−0.00728	0.00597	−1.219	0.225	1.084
Burn to Admit	−0.00956	0.0104	−0.919	0.36	1.01
TBSA third	0.0003	0.0013	0.23	0.818	1.088