High cytokine levels at admission are associated with fatal outcome in patients with necrotizing fasciitis

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ABSTRACT. We evaluated in a blinded fashion the cytokine profiles of patients with suspected necrotizing fasciitis. In 15 out of 20 patients, the diagnosis of necrotizing fasciitis was established; five patients had cellulitis. Eighteen of the 20 patients were i.v. drug users. Five of the 15 patients with necrotizing fasciitis died (33%). On admission, serum levels for interleukin-1β (IL-1β), IL-1-receptor antagonist (IL-1Ra), IL-18 and interferon-γ (IFNγ) as well as white blood cells (WBC) were significantly elevated in patients with fatal outcome compared to survivors with necrotizing fasciitis. IL-1Ra and WBC levels were also higher than in patients with cellulitis. No differences were observed between patients groups for IL-6 and IL-8. In summary, significantly elevated levels of proinflammatory cytokines and particularly IL-1Ra are associated with fatal outcome in patients with necrotizing fasciitis. The measurement of proinflammatory cytokines and IL-1Ra may help to establish early diagnosis of life-threatening necrotizing fasciitis and thus to initiate aggressive treatment.

Keywords: necrotizing fasciitis, cellulitis, fatal outcome, proinflammatory cytokines, aggressive treatment

INTRODUCTION

Necrotizing fasciitis (NF) is an uncommon, severe infection involving the subcutaneous soft tissues and particularly the superficial fascia. Predisposing factors include diabetes, peripheral vascular disease, immunological compromise and intravenous drug abuse [1]. The overall mortality rate of NF is high (about 30% percent) [2, 3]. The majority of necrotizing infections of soft tissues are polymicrobial and no bacterium is associated with a specific clinical entity [4, 5].

Initial diagnosis of NF is often missed because of the paucity of clinical signs and because the differentiation from cellulitis is difficult. In a recent report, the diagnosis of NF within a pediatric population was only made in 11/39 patients (28%) at admission [6]. The use of computerized tomography or magnetic resonance imaging have been shown to be helpful to assist in the diagnosis [7, 8]. However, no specific physiological parameter or serum markers were shown to be specifically associated with NF. The definitive diagnosis is usually established during surgical exploration.

Patients with NF may rapidly progress within few hours from a stable clinical condition to severe deterioration, sometimes leading to death. Beside broad spectrum antibiotic therapy, surgery is the primary treatment for NF and early treatment is mandatory. Early recognition by means of tissue biopsy, and aggressive surgical treatment has been shown to improve the survival rate [9]. Negative outcomes result from delay in diagnosis, inadequate surgical debridement, and complications of sepsis [2, 3, 5, 10].

We evaluated in a blinded fashion, the cytokine profiles of patients with suspected necrotizing fasciitis. High levels of proinflammatory cytokines or natural inhibitors were hypothesized to help to establish early diagnosis and to initiate aggressive treatment in order to reduce morbidity and mortality of this otherwise debilitating disease.

PATIENTS AND METHODS

Patients and samples

The study protocol was approved by the Investigational Review Board of the Highland General Hospital, and all patients gave written, informed consent. From July 2000 to October 2001, patients admitted to the emergency depart-

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ABBREVIATIONS: NF, necrotizing fasciitis; WBC, white blood cells; IL, interleukin

ment of the Highland General Hospital with suspected NF ($n = 15$) or suspected cellulitis ($n = 5$), along with a group of control patients (suspected acute myocardial infarction (MI), $n = 5$), were enrolled in this study. The diagnosis was confirmed retrospectively for each group of patients. NF was confirmed by histological evaluation of surgical specimens. Two groups of patients with NF were established according to the clinical outcome (survivors (NF/S) versus non-survivors (NF/D)). Thirteen of 14 patients with positive bacterial cultures were infected with a mixed flora of predominantly Gram-positive bacilli. Four of 5 patients with NF/D were infected with *Staphylococcus aureus* and either co-colonized with β-hemolytic group A *Streptococci*, *Clostridium sordelli*, *Enterobacter cloacae* or *Enterococcus faecalis*. From one NF/D patient, a mixed culture of *Clostridium baratii* and *Corynebacterium species* was isolated. Monomicrobial infections with either β-hemolytic group A *Streptococci* or multidrug-resistant *Staphylococcus aureus* were isolated from two patients with NF/S. All other NF/S patients were infected with more than one bacterium including *Staphylococcus aureus* (2), different species of *Streptococci* (5), *Pseudomonas stutzeri* (1) or mixed skin or respiratory type flora (2). *Clostridium perfringens* was co-isolated from one NF/S patient infected with *Streptococci*. One patient with NF/S was tissue culture negative.

There were no statistical differences between the four groups of patients with respect to age, gender or vital signs *(Table 1)*.

WBC counts were obtained at the time of presentation in the emergency department. Serial serum samples were collected at admission and consecutively after six hrs and stored at – 50 °C until cytokine measurements.

Measurement of cytokines

All reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless indicated. The liquid-phase electrochemiluminescence (ECL) method was used to measure cytokines in serum samples [11]. The antibodies used for ECL were either labeled with biotin or ruthenium (TAG) as indicated and as previously described [12]. The following antibodies (in parenthesis) were used for cytokine measurements: IL-1β (biotinylated BAF201; TAG-labeled MAB201, R&D Systems, Minneapolis, MN, USA), IL-1Ra (biotinylated and TAG-labeled polyclonal rabbit IgG, own production), IL-18 (biotinylated BAF318; TAG-labeled MAB318, R&D Systems, Minneapolis, MN, USA), IL-6 (biotinylated BAF206; TAG-labeled MAB206, R&D Systems), IL-8 (biotinylated BAF208; TAG-labeled AF208NA, R&D Systems), IFNγ (biotinylated 10-I61, Fitzgerald Inc., Concord, MA, USA; TAG-labeled MAB285, R&D Systems). The amount of ECL was determined using an Origen Analyzer (Igen, Gaithersburg, MD, USA).

Statistical analysis

Statistical analyses were performed with the statistical package Statview™ 512 + (BrainPower, Inc. Calabasas, CA, USA). Comparisons between different groups of patients were calculated using the Mann-Whitney *U* rank test. Significant correlation between different parameters was determined with Spearmans's correlation test.

RESULTS

During a 15-month prospective clinical study we established in 15 of 20 patients the diagnosis of NF. Five patients were diagnosed as having cellulitis and five patients with MI served as controls. The diagnosis of NF was confirmed retrospectively by means of histological examination of a surgical specimen. Characteristics of patients are shown in *Table 1*. There was no difference with respect to age, gender or vital signs. The majority of the patients, 18 of 20, were intravenous drug abuser.

Serum concentrations of five different proinflammatory cytokines as IL-1β, IL-18, IL-6, IL-8 and IFNγ as well as IL-1-receptor antagonist (IL-1Ra) were obtained on admission to the emergency room, and consecutively after six, 12 and 18 hours. On admission, levels for IL-1β, IL-1Ra, IL-18 and IFNγ were significantly elevated in patients with fatal outcome (NF/D) compared to survivors with NF (NF/S). IL-1Ra was also higher in NF/D than in patients with cellulitis or MI. IL-8 was higher in NF/D than in MI. No differences amongst the groups were observed for IL-6 *(Figure 1)*.

WBC counts on admission were significantly higher in NF/D patients than in NF/S, cellulitis or MI patients *(Figure 2)*.

After six hours, significantly elevated levels for IL-1β and IL-1Ra were observed for NF/D as compared to NF/S patients. Higher levels of IL-8 were seen in NF/D patients as compared to those with cellulitis and MI. Higher levels of IFNγ were seen in NF/D compared to MI patients. At 12 hrs, IL-1Ra remained significantly elevated in NF/D compared to NF/S and MI patients; levels for IL-8 were still higher in NF/D compared to patients with MI. Within 18 hrs after admission, only levels of IL-1β and IFNγ rose significantly (data not shown).

The correlation between WBC counts, cytokines or IL-1Ra at admission was calculated for all patients with NF. As shown in *Figure 3*, WBC counts at admission

Table 1
Characteristics of study patients

Characteristic	NF/D *(n = 5)*	NF/S *(n = 10)*	Cellulitis *(n = 5)*	MI *(n = 5)*
Age/median* (range)	51 (26-59)	47 (32-57)	48 (45-60)	54 (45-63)
Gender* (male:female)	4:1	8:2	4:1	3:2
Temperature (°C)* (mean ± SD)	36.6 ± 1.3	37.5 ± 1.1	38.3 ± 1.3	36.7 ± 0.5
Blood pressure (MAP/mmHg)* (mean ± SD)	79 ± 14	88 ± 14	81 ± 0	92 ± 18

* No significant differences between all groups of patients using Mann-Whitney test for non-parametric variables

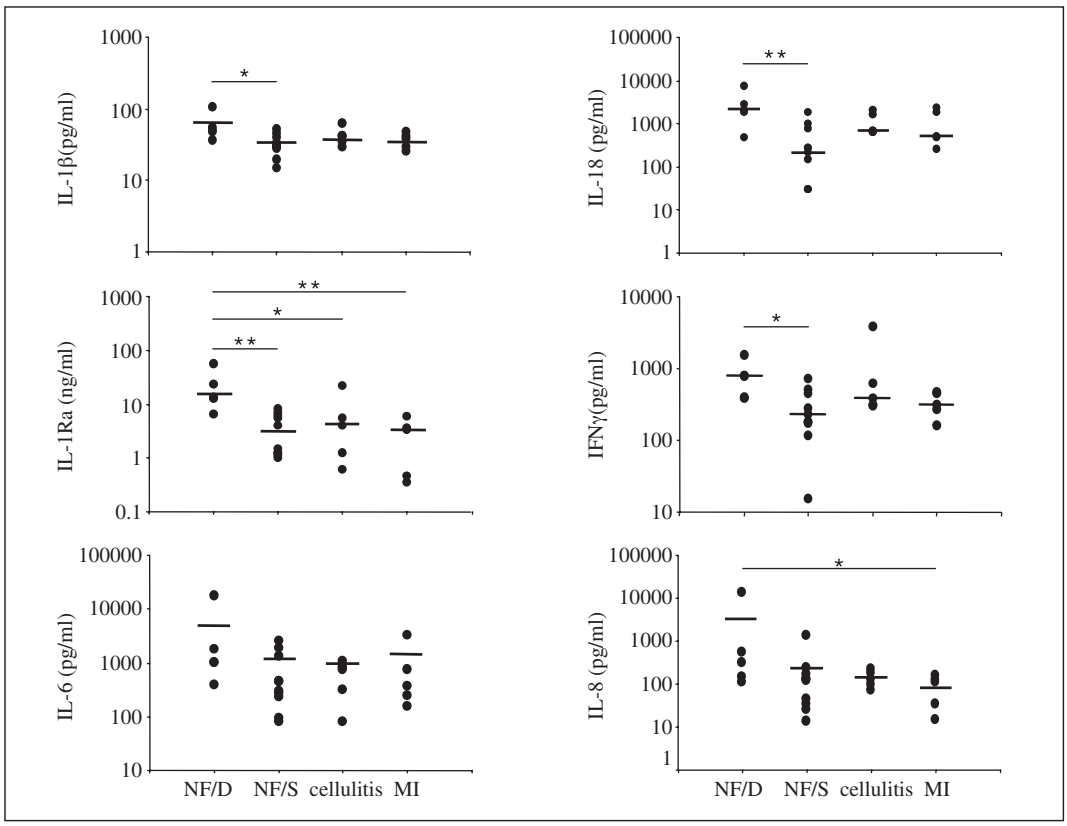

Figure 1
Blood samples were taken at admission to the emergency room from patients with suspected necrotizing fasciitis (NF) or control patients with cellulitis ($n = 5$) or myocardial infarction (MI, $n = 5$). According to the outcome, two groups of patients with necrotizing fasciitis were established as survivors (NF/S, $n = 10$) or non-survivors (NF/D, $n = 5$). Levels of IL-1β, IL-1Ra, IL-6, IL-18, IFNγ and IL-8.

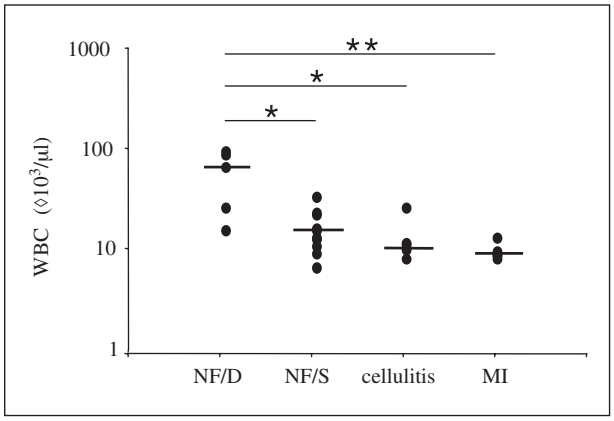

Figure 2
White blood cell counts at admission. Differences between the four groups of patients were calculated using the Mann-Whitney test for non-parametric variables. Horizontal lines indicate median values in each group.

correlated positively with levels of IL-1β, IL-1Ra and IL-18. No correlation was found for WBC with IFNγ, with IL-6 or with IL-8.

In all patients with NF, there was a positive correlation between IL-1β and IL-1Ra, IL-18 and IL-6 at admission. IL-18 correlated positively with IL-1Ra, IL-6 and IL-8. There was no correlation between IL-18 and IFNγ. IL-8 correlated positively with IL-6. Infection with *Staphylococcus aureus* in patients with NF was associated with higher levels of IL-1β and IL-18 at admission (data not shown).

DISCUSSION

This is a prospective clinical study to evaluate the cytokine profile of patients with suspected NF. Here we show that high levels of IL-1 β, IL-1Ra, IL-18 and IFN γ, as well as WBC at admission are associated with fatal outcome in NF. In particular, IL-1Ra and WBC were elevated as compared to patients with cellulites, and this might help to distinguish NF from cellulitis at an early stage of disease. In contrast to Gram-negative infections, the cytokines of the IL-1-family such as IL-1β and IL-18, as well as IL-1Ra, were positively correlated with WBC counts in this predominantly Gram-positive infection.

In accordance with previous studies [4, 5], the bacteriological findings for most subjects in our cohort of NF-patients demonstrated a polymicrobial infection consisting of at least one Gram-positive bacterium. Only two of 15 patients (13%) were infected with a single pathogen. As reported by Singh *et al.* [4], *Staphylococci* were isolated most frequently (seven of 15 patients) and Group A *Streptococci* were found in only 13% of patients (two of 15). Interestingly, the infection with *Staphylococcus aureus* was associated with elevated levels of IL-1β and IL-18.

Early diagnosis is mandatory to allow initiation of aggressive treatment by surgical debridement, which clearly improves outcome in NF [5]. Most difficult is the clinical discrimination between NF and routine cellulitis on presentation [5, 6]. Beside pain, high fever but stable blood pressure was described at early presentation with NF in a recent retrospective evaluation of 163 patients [2]. All of our patients were hemodynamically stable, but the major-

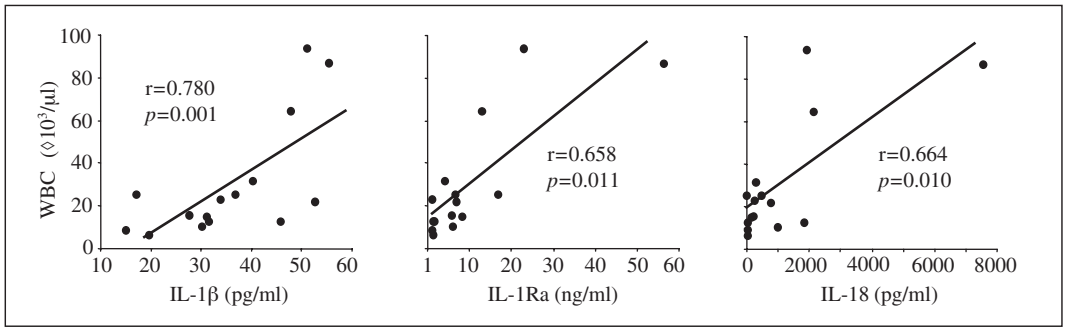

Figure 3
Cytokine levels in patients with necrotizing fasciitis (both NF/D and NF/S) were correlated with WBC counts at admission using the Spearman test for non-parametric variables. Corresponding r-values and *P*-values are shown for each graph. A *P*-value of < 0.05 was considered statistically significant; * $P < 0.05$, ** $P < 0.01$.

ity presented without fever or with only a moderately elevated temperature. There was no difference in body temperature between surviving or non-surviving patients. Poor outcome in NF has been reported to be associated with age (> 60 yr), gender (female), organ failure at admission and elevated blood lactate [10].

In order to establish a more specific marker for NF and the severity of disease, we evaluated the cytokine profile in a series of patients with suspected NF. High levels of proinflammatory cytokines were shown to correlate with the outcome in septic patients [13]. Norrby-Teglund and colleagues reported higher local and systemic cytokine levels in severe cases of invasive group A streptococcal disease including NF [14, 15]. We found that levels of IL-1β, IL-18, IFNγ and IL-1Ra, as well as WBC were significantly elevated in NF patients with fatal outcome. This is a significant finding since, at the time of initial presentation, all patients were in a similar clinical condition. In particular, IL-1Ra and WBC at admission were significantly higher in NF patients than in patients with cellulitis. Thus, high levels of IL-1Ra and WBC may help to distinguish NF from cellulitis and facilitate the decision to start aggressive surgical therapy. No rapid analysis of IL-1Ra is currently available. Therefore, at present, marked leukocytosis without fever should make one suspicious of NF and justifies aggressive treatment. IL-1 induces leukocytosis [16]. In our study, we also demonstrate that cytokines of the IL-1 family and IFNγ positively correlate with WBC in NF as an example of severe Gram-positive infection. In contrast, in severe Gram-negative infection IL-6 and IL-8, but not members of the IL-1 family of cytokines or IFNγ were shown to correlate with WBC [17].

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