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The time-course of the inflammatory response to major burn injury and its relation to organ failure and outcome

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ABSTRACT

Burn injury causes major inflammatory activation and cytokine release, however, the temporal resolution of the acute and sub-acute inflammatory response has not yet been fully delineated. To this end, we have quantified 20 inflammatory mediators in plasma from 44 adult patients 0–21 days after burn injury and related the time course of these mediators to % total body surface area (TBSA) burned, clinical parameters, organ failure and outcome. Of the cytokines analyzed in these patients, interleukin 6 (IL-6), IL-8, IL-10 and monocyte chemoattractant protein 1 (MCP-1) correlated to the size of the injury at 24–48 h after burn injury. In our study, the concentration of IL-10 had prognostic value in patients with burn injury both measured at admission and at 24–48 h after injury. However, simple demographic data such as age, % burned TBSA, inhalation injury and their combination, the Baux score and modified Baux score, outperform most of the cytokines, with the exception of IL-8 and MCP-1 levels on admission, in predicting death.

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CRP, C-reactive protein; G-CSF, granulocyte colony-stimulating factor; HIV, human immunodeficiency virus; ICAM-1, intracellular adhesion molecule 1; ICU, intensive care unit; IFN-α, interferon alpha; IFN-γ, interferon gamma; IL-1α, interleukin 1α; IL-1β, interleukin 1β; IL-4, interleukin 4; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; IL-12p70, interleukin 12p70; IL-13, interleukin 13; IL-17A, interleukin 17A; IP-10, interferon gamma-induced protein 10; LAP, latency-associated peptide; LOS, length of stay; MAP, mean arterial pressure; MCP-1, monocyte chemotactic protein 1; MIP-1α, macrophage inflammatory protein 1 alpha; MIP-1β, macrophage inflammatory protein 1 beta; PaO_2/FiO_2, ratio of arterial oxygen partial pressure to fractional inspired oxygen; PEEP, positive end-expiratory pressure; PLT, blood platelets; ROC, receiver operating characteristics; SOFA, sequential organ failure assessment score; TBSA, total body surface area; TNF, tumor necrosis factor; VDI, vasopressor dependency index.

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1. Introduction

Major burn injury induces a profound inflammatory response that in severe cases may lead to multiple organ failure and in some cases death [1]. Common clinical predictors of mortality following burn injury are burn extent, age, presence of inhalation injury and sepsis [2], but many other factors may have a large impact on outcome. The injury itself causes immediate and subsequent cell damage or death, inevitably giving rise to inflammatory cascade activation, as well as altered levels of glucagon, catecholamines and endogenous steroids [3–5]. It has also been demonstrated that the early phase of injury is characterized by an overexpression of both pro- and anti-inflammatory mediators, simultaneously [6], further complicating the situation. Several roles of inflammatory pathways have been described for burn injury [7–11], but we know little about how these systems are activated in relation to the other and their impact on morbidity and mortality. Unlike other conditions with systemic inflammatory activation, such as sepsis or pancreatitis, burn injury has a precisely defined onset. Consequently, the time course of the inflammatory response to burn injury could presumably be more uniform initially than for other conditions with more insidious onset, as is the case for sepsis. A clinically predictable natural course of the inflammatory response after burn injury could facilitate timely therapeutic interventions to modulate the inflammatory response. Although studies report simultaneous measurement of pro and anti-inflammatory mediators [12], data on the temporal resolution of these mediators are scarce. Moreover, despite some reports on these biomarkers in predicting outcome [8,13], their performance compared to prediction models based on demographic data has not been assessed previously.

Our primary aim was to characterize the acute and subacute inflammatory response in adult patients with high temporal resolution from 0-21 days after severe and moderate burn injury analyzing 20 inflammatory mediators. A secondary aim was to relate the time course of these mediators to % total body surface area (TBSA) burned, clinical parameters, organ failure, and outcome.

2. Materials and methods

2.1. Patients and healthy subjects

The study protocol was approved by the local Institutional Review Boards (The Operative Ethics Committee of Helsinki University Central Hospital, 320/13/03/02/2011, and The Regional Ethical Review Board in Uppsala, 2011/484). Patients were recruited from a larger study cohort which has been described in a previous publication [14]. In short; patients admitted to the burn centers of the Uppsala University Hospital, Sweden, between March 1st, 2012 and February 28th, 2013, and Helsinki University Hospital, Helsinki, Finland, between April 1st 2012 and October 31st 2013, were screened for eligibility. These units admit annually about 280 and 150 burn injured patients, respectively, of whom 45 and 50 patients, respectively, have major burn injuries (>20% TBSA). We included adults (18 years or older) admitted to the burn centers regardless of type of burn injury. Exclusion criteria were malignancy, immune deficiency (HIV infection or medication with corticosteroids, cytostatic drugs, tetracyclines and certain bisphosphonates), known or suspected blood-transmitted infection and participation in another clinical study within 4 weeks. For analysis of the impact of inflammation, the patients were stratified according to the extent of the burned surface as mildly injured (<20% TBSA), moderately injured (20–40% TBSA) and severely injured (>40% TBSA). Written informed consent was obtained initially via next of kin and later directly from patients. Consenting healthy subjects (n=10) were recruited among hospital employees to determine normal values of circulating cytokines.

2.2. Data collection

Demographics, co-morbidities and medications were registered, as well as clinical variables relevant for the degree of inflammation and organ dysfunction (plasma creatinine, plasma bilirubin, maximal daily vasoactive medication dose, vasopressor dependency index (VDI, (dopamine dose × 1) + (dobutamine dose × 1) + (epinephrine dose × 100) + (norepinephrine dose × 100) + (phenylephrine dose × 100)/MAP) and daily lowest ratio of arterial oxygen partial pressure to fractional inspired oxygen, PaO_2/FiO_2) were registered for all timepoints where applicable. Blood gas values and clinical laboratory data (C-reactive protein (CRP), leukocyte and platelet counts (PLT), and microbiological cultures) were obtained from laboratory records. Sequential organ failure assessment (SOFA) score was calculated on each sampling day for the severely injured patients. Patients' weight, cumulative fluid dose and cumulative urine output for the preceding 24 h were recorded on the days of sampling. Intensive care interventions, such as ventilator therapy, renal replacement therapy, and surgical interventions, were recorded as well as intensive care (ICU) length of stay (LOS). Baux-score was calculated from age and % TBSA burned [15] and adjusted for inhalation injury for modified Baux-score. Mortality was registered at 90 days after injury.

2.3. Sample collection

Blood samples were collected from patients as soon as possible after admission to the burn center and at 3-h intervals thereafter in association with routine intensive care laboratory samples during the first 24 h after injury. Thereafter, samples were collected on days 1, 2, 3, 7, 14, and 21 after injury. In patients with mild injury and no arterial line, sampling in three-hour intervals on day 1 was omitted and a single sample was taken by venipuncture. Samples were centrifuged at 1500 × *g* for 15 min and plasma was stored in −70 °C until analysis.

2.4. Cytokine analysis

Out of the originally recruited cohort, 44 patients where samples were available were included in the cytokine analysis (24 patients recruited in Finland and 20 patients in Uppsala). Interleukins (IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A), granulocyte colony-stimulating factor (G-CSF),

intracellular adhesion molecule 1 (ICAM-1), interferon alpha (IFN-α), interferon gamma (IFN-γ), interferon gamma-induced protein 10 (IP-10), latency-associated peptide (LAP), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1 alpha and beta (MIP-1α, MIP-1β), tumor necrosis factor (TNF), and E-selectin, were analyzed in plasma using the Human Inflammation 20-plex RTU FlowCytomix Kit (eBioscience) according to manufacturer's recommendations. In short, 25 mL of plasma was incubated with the bead mixture and biotin-conjugate at room temperature for 2 h, shaking at 500 rpm. After wash, the samples were incubated with 50 μL Streptavidin-PE solution at room temperature for 1 h, shaking at 500 rpm. After final washes, the samples were acquired in 280 μL assay buffer using a FACS CantoII (BD Biosciences) and analysed using Flow Cytomix Pro 3.0 (eBioscience). IL-10/TNF ratio was calculated as a measure of pro vs anti-inflammatory status [16].

2.5. *Statistical analysis*

Differences between groups were tested with the Kruskall–Wallis and Chi square test for continuous and discrete variables, respectively. The evolution of cytokines over time was assessed with Friedman ANOVA. Associations between clinical parameters and cytokines were evaluated using the Spearman rank correlation test. C-indexes were calculated using logistic regression. To include all patients in outcome analysis, admission levels of cytokines were used. Since

Table 1 – Patient demographics. Categorical variables are expressed as numbers and percentages. Continuous variables are expressed as medians and ranges. Comparisons are made between patients with burn injury >20% of TBSA, 20-40% of TBSA and <20% of TBSA using Kruskal-Wallis test for continuous variables and Chi Square test for categorical variables. P<0.05 is considered statistically significant.

	All patients (n=44)	>40% TBSA (n=13)	20–40% TBSA (n=17)	<20% TBSA (n=14)	P
Hours to burn center	9 (2–37)	9 (2–37)	9 (2–20)	10 (7–25)	0.905
Male sex	31 (70%)	10 (77%)	12 (71%)	9 (64%)	0.772
Age, years	56.5 (18–88)	61 (18–88)	52 (24–84)	57.5 (22–83)	0.194
BMI	24.8 (19.6–41.5)	24.8 (20.4–41.5)	25.0 (19.6–29.7)	26.6 (20.3–33.7)	0.912
Total burn TBSA %	30 (3–80)	50 (44–80)	30 (22–39)	13 (3–17)	<0.0001
Inhalation injury	13 (30%)	6 (46%)	3 (18%)	4 (29%)	0.236
ICU days	9.5 (0–86)	13.5 (2–86)	14.5 (0–65)	2 (0–25)	0.078
Ventilator treatment	36 (82%)	13 (100%)	15 (88%)	6 (43%)	0.0007
Ventilator free days[a]	11.5 (0–21)	0 (0–15)	14 (0–21)	19 (0–21)	0.0004
Peak pressure	24 (15–33)	26 (16–33)	22 (16–27)	21 (15–31)	0.165
Highest PEEP	8 (5–14)	10 (5–14)	7 (5–10)	6.5 (5–11)	0.153
Driving pressure	16 (5–35)	16 (5–35)	16 (10–19)	14 (10–20)	0.925
Dynamic compliance	35.7 (13–141)	34 (13–140)	38 (31–53)	31 (24–52)	0.395
Highest PEEP/driving pressure	0.31 (0.11–1.08)	0.41 (0.22–1.08)	0.27 (0.11–0.70)	0.39 (0.15–0.55)	0.527
Vasopressor dependency index on admission	0.35 (0–29)	0.32 (0–10.6)	0.35 (0.1–29)	1.49 (0.2–2.8)	0.993
Clinical parameters on admission					
Plasma creatinine (μmol/L)	74 (43–481)	104 (56–205)	73 (49–481)	73.5 (43–98)	0.446
Plasma bilirubin (μmol/L)	13.5 (5–52)	15 (8–52)	16 (7–31)	9.5 (2–24)	0.128
PLT ($\times 10^9$)	250 (90–767)	275 (90–470)	246 (154–767)	224 (153–329)	0.458
CRP (Mg/L)	5 (0.22–121)	2.60 (0.71–121)	4.65 (0.22–21)	7.5 (1.7–21)	0.878
SOFA					
At 24–48 h	5 (0–13)[b]	7.5 (1–13)	4 (1–13)	2.5 (0–8)	0.125
At 48–72 h	6 (0–15)[c]	6 (2–15)	4 (1–11)	5 (0–7)	0.215
Mortality at 90 days	8 (18%)	6 (46%)	0 (0%)	2 (14%)	0.005

TBSA, total body surface area; SOFA, sequential organ failure assessment; BMI, body mass index; CRP, C reactive protein; PLT, platelet count; PEEP, positive end-expiratory pressure.

[a] During 21 days.

[b] n=28.

[c] n=27.

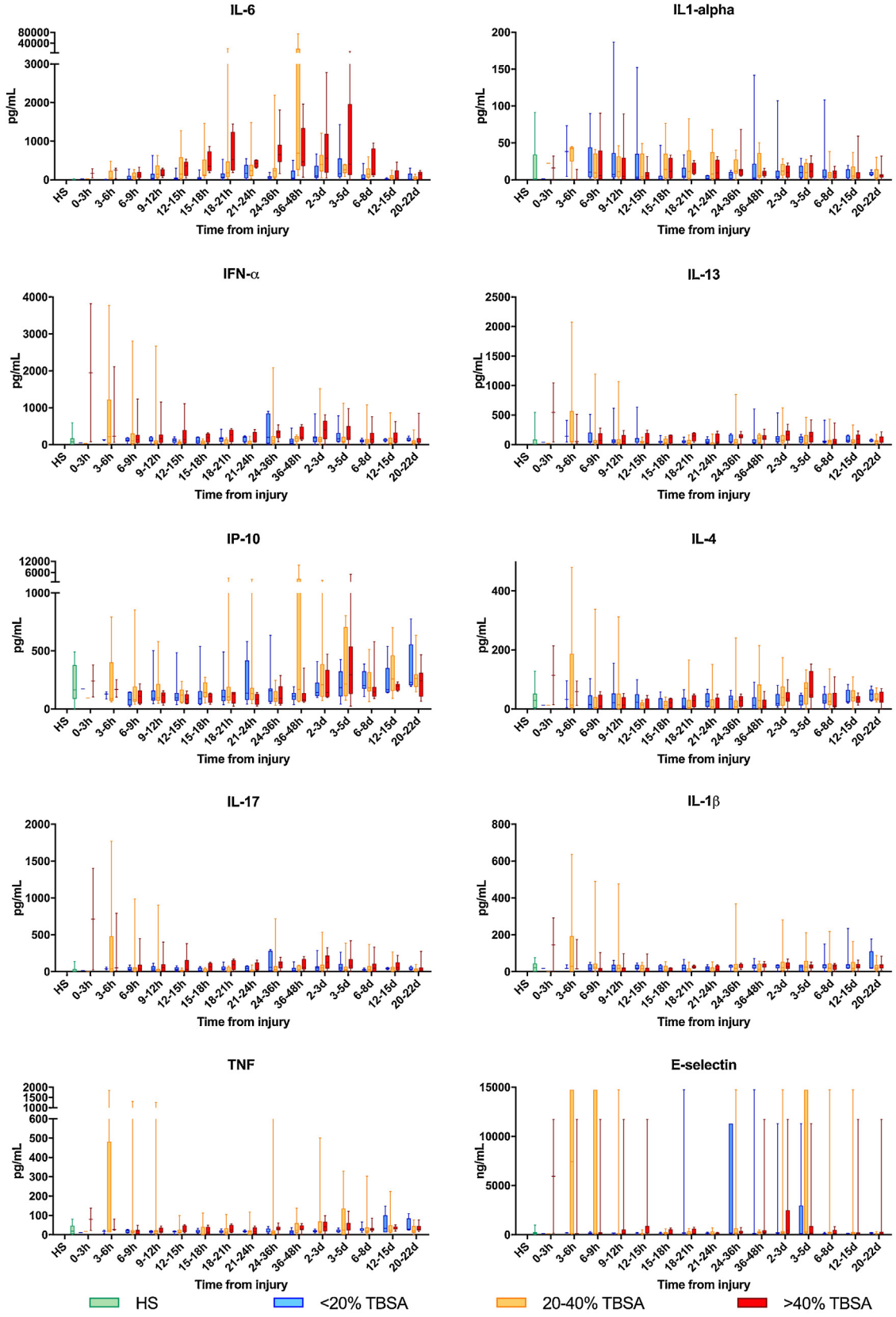

Fig. 1 – Blue bars represent cytokine concentrations in plasma from patients with burn injury of <20% TBSA (n=14), orange bars patients with 20-40% TBSA (n=17) and red bars patients with >20% TBSA burned (n=13). The green bar represents cytokine concentrations in healthy subjects (n=10). Results obtained during 21 days following burn injury are displayed in groups according to hours calculated from the time of injury. Box plots are representing interquartile ranges; horizontal lines represent

patients were admitted within the first day after the injury the peak value 24–48 h after the injury were used to standardize the early inflammatory response after the injury. Statistical analyses were performed using GraphPad Prism 7.0 for Windows (GraphPad Software Inc, La Jolla, CA, USA) and Statistica version 13.2 (Tulsa, OK, USA). P-values <0.05 were considered statistically significant. Given the explorative nature of the study, multiple comparisons were not corrected for.

3. Results

Demographics of the groups are presented in Table 1. Patients in the cohort had only thermal injuries. The patients in the mildly, moderately and severely injured strata were similar in their demographics and clinical presentation, but while less than half of the patients were mechanically ventilated in the mildly injured group, all patients were mechanically ventilated in the severely injured group.

3.1. *Temporal development of plasma cytokine concentrations in patients with burn injury*

The time courses of plasma cytokines are illustrated in Figs. 1 and 2. In the whole cohort IL-1α, IL-1β, IL-13, IL-17A, G-CSF, ICAM-1, IFN-α, IFN-γ, LAP and MIP-1α displayed peak median values in first few hours after burn injury, while IL-6 and IL-10 displayed highest concentrations after 24–48 h following burn injury. The levels of IL-6 ($p<0.0001$), IL-8 ($p<0.0001$), IL-10 ($p=0.001$), IL-13 ($p=0.01$), IL-17A ($p<0.001$), G-CSF ($p<0.01$), ICAM-1 ($p=0.04$), IFN-α ($p=0.03$), IFN-γ ($p=0.01$), MCP-1 ($p<0.001$), MIP-1α ($p=0.03$), MIP-1β ($p=0.01$) and E-selectin ($p=0.04$) were higher in burn injury patients than in healthy controls 24–48 h following burn injury. Time course of the ratio between IL-10/TNF is illustrated in Fig. 3.

The temporal development of cytokines in patients with severe, moderate and mild burn injury were similar, however average IL-1α, IL-6, IL-8, IL-10, LAP, MCP-1 and MIP-1β were all higher in patients with severe burn injuries. Mild injuries had relatively low concentrations of IL-6, IL-10, and the ratio IL-10/TNF. Patients with moderate injury displayed highest concentrations of IL-1β, IL-4, IL-12p70, IP-10 and TNF two to three weeks after the injury had occurred. In patients with severe burn injury, IL-1α, G-CSF, ICAM-1, IFN-α, MIP-1α and MIP-1β displayed peak concentrations within the first six hours following injury, while IL-1β, IL-4, IL-6, IL8, IL-10, IL-12p70, IL-13, IL-17A, IFN-γ, IP-10, TNF and E-selectin reached peak concentrations only at 48 h or later. For most of the patients, cytokine levels had retroceded to values similar to those of healthy subjects at the end of the study (21 days after injury). The ratio of IL-10/TNF peaked 36–48 h in patients with moderate burn injury, while 3–5 days after burn in patients with severe injuries.

3.2. *Correlations*

Correlations between cytokines and %TBSA burned on admission were generally weak, but were stronger 24–48 h after the injury. IL-6, IL-8, IL-10, MCP-1 and IL-10/TNF correlated to the size of the injury at 24–48 h after the injury occurred. IL-6 and IL-8 also correlated with SOFA score, CRP, PLT and ICU LOS (Table 2). At the same timepoint, PLT also displayed negative correlations to IL-13 ($r=-0.59$, $p=0.0004$), IL-17A ($r=-0.63$, $p=0.0001$), IFN-α ($r=-0.58$, $p=0.0005$), IFN-γ ($r=-0.58$, $p=0.0005$) and ICAM-1 ($r=-0.59$, $p=0.0003$). Age did not correlate with any of the analyzed cytokines, but displayed a negative correlation with ventilator free days ($r=-0.32$, $p=0.04$). %TBSA correlated with SOFA score 24–48 h after the injury (Fig. 4).

Inhalation injury correlated with IL-6 ($r=0.46$, $p=0.0108$) and MCP-1 ($r=0.42$, $p=0.00543$). No correlations at 24–48 h after the injury were found between cytokines and ventilator free days, dynamic compliance, vasopressor dependency index, highest positive end-expiratory pressure PEEP, highest PEEP/driving pressure or peak pressure.

3.3. *Association with outcome*

Mortality at 90 days was 18% in the whole cohort. Since two patients died within 24 h, the association between cytokine levels and outcome were analyzed both at admission and at 24–48 h. Patients who died were older ($p=0.0179$), had larger burn injuries ($p=0.01$) and almost all had inhalation injuries ($n=6$, $p=0.0013$). The deceased had higher IL-6 ($p=0.008$), IL-8 ($p=0.03$) and IL-10/TNF ($p<0.05$) at admission; they also had higher ventilator peak pressures ($p=0.002$), higher PEEP ($p=0.03$) during the ICU stay, fewer ventilator free days ($p=0.007$) and higher CRP ($p=0.01$). There were no differences between survivors and non-survivors in any of the analyzed cytokines 24–48 h from admission. Of the two patients in the group with burn injuries of <20% of TBSA, one died in a nursing home 89 days after injury and one patient died of a middle cerebral artery stroke, both unrelated to burn injury.

The discrimination of %TBSA burned Baux-score and modified Baux-score was high for 90 days mortality, while C-index values were generally lower for cytokines (Table 3). C-indexes for IL-6, IL-8, IL-10, MCP-1, IL-10/TNF were similar to Baux-score and modified Baux-score at admission, but 24–48 h after the injury only IL-10 was comparable to Baux-score and modified Baux-score, in discriminating survivors from non-survivors.

4. Discussion

4.1. *Key findings*

The inflammatory response after burn injury involves increased levels of a broad spectrum of cytokines, many of them

medians and whiskers represent ranges. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

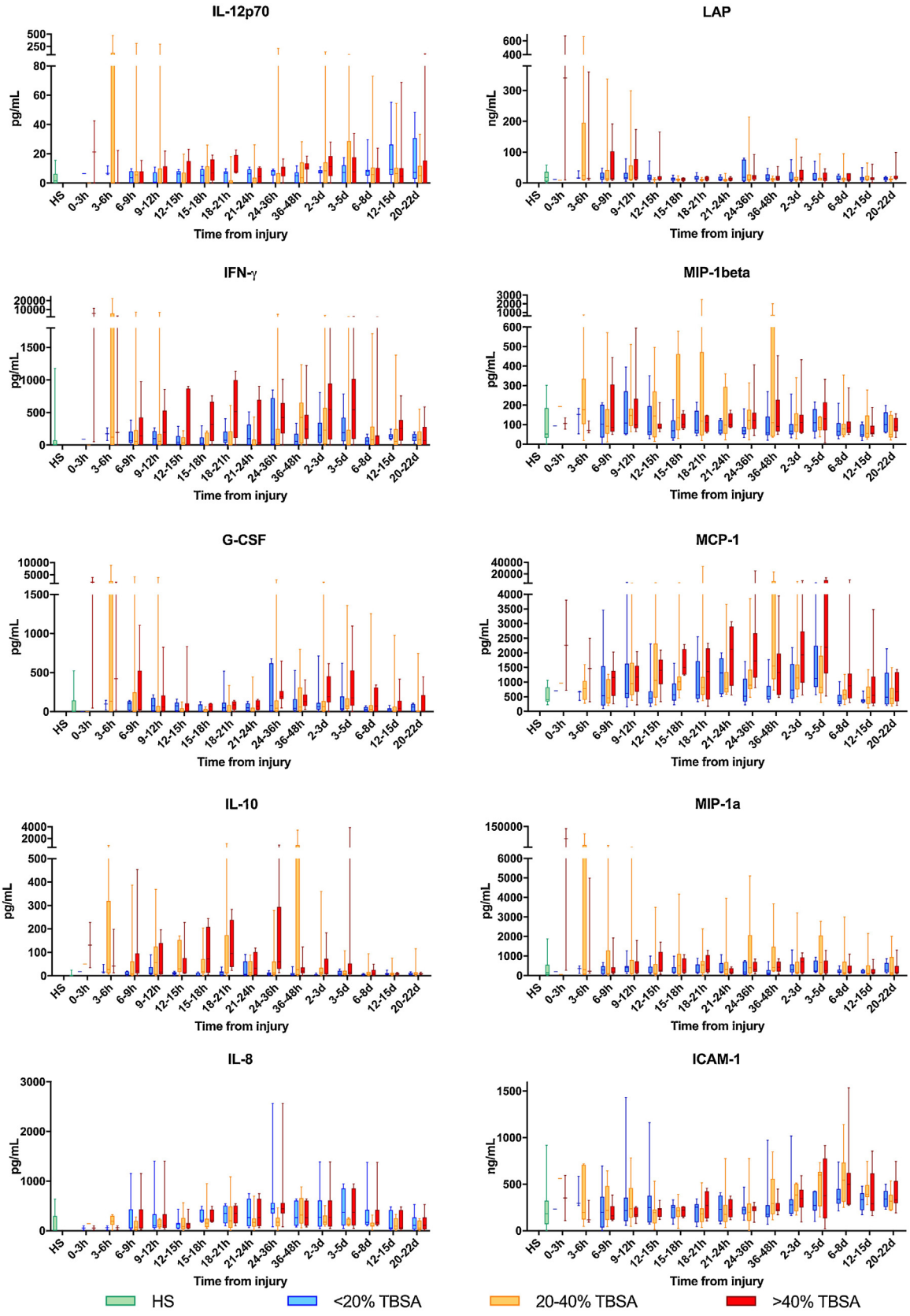

Fig. 2 – Blue bars represent cytokine concentrations in plasma from patients with burn injury of <20% TBSA (n=14), orange bars patients with 20-40% TBSA (n=17) and red bars patients with >20% TBSA (n=13). The green bar represents cytokine concentrations in healthy subjects (n=10). Results obtained during 21 days following burn injury are displayed in groups according to hours calculated from the time of injury. Box plots are representing interquartile ranges; horizontal lines represent

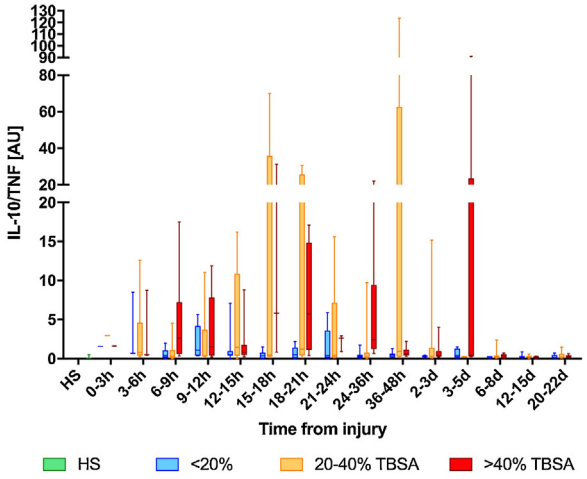

Fig. 3 – Blue bars represent the ratio between IL-10 and TNF in plasma from patients with burn injury of <20% TBSA (n=14), orange bars patients with 20–40% TBSA (n=17) and red bars patients with >20% TBSA (n=13). The green bar represents cytokine concentrations in healthy subjects (n=10). Results obtained during 21 days following burn injury are displayed in groups according to hours calculated from the time of injury. Box plots are representing interquartile ranges; horizontal lines represent medians and whiskers represent ranges. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

peaking a few days after the injury. IP-10, IL-4 and TNF levels were high even 20 days after the burn injury had occurred. A handful of cytokines were associated with the size of the burn injury, non-cytokine markers of systemic inflammatory response, organ failure and ICU length of stay. Simple demographic data such as burned TBSA and Baux-score and modified Baux-score discriminated survivors from non-survivors similarly or better than cytokines on admission or at 24–48 h.

As cytokines play a major part of the immunological response and the pathophysiological process following burn injury, contributing to increased risk of multi-organ failure and death [17], it is of high importance to further elucidate the immunological mechanisms driving the pathophysiology as well as seeking new trajectories to enable a more personalized approach to burn care and improve outcomes. In this prospective observational study of the acute and sub-acute phases of cytokine response after burn injury, we observed increased levels of a broad spectrum of cytokines, many of them peaking a few days after the injury occurred. Similar patterns of cytokine response have been described in some studies [4,12,17,18] while other studies do not report changes in cytokine levels during the first days [19]. This discrepancy between studies could be explained by the different timing of sampling after burn injury. Alterations in concentrations of mediators with highly dynamic levels during the early inflammatory response could potentially be missed if sampling is delayed after the burn injury or if sampling frequency is low. To the best of our knowledge, cytokine profiles have not previously been delineated in patients with burn injury with the high temporal resolution presented in this study.

Among cytokines which were increased in patients with severe burn injury as compared to moderate injury, IL-10, LAP and IL-1a displayed peak concentrations within the first 12 h after injury. Other cytokines (IL-6, IL-8 and MCP-1) displayed peak concentrations in these patients more than 24 h after injury. Despite IL-6 and IL-8 being broadly considered to be early markers of inflammation [17,20], our findings are in line with recent data demonstrating higher plasma concentrations of IL-6 and IL-8 in non-survivors 4–7 days after burn injury [13,21,22]. IL-6 is considered to have both pro- and anti-inflammatory properties, increasing activity of immune cells driving post-injury inflammation and tissue injury as well as limiting inflammation through stimulating the production and release of protective acute phase proteins [23]. IL-8 is primarily produced by macrophages, recruits leucocytes to primary sites of injury and has been correlated with mortality caused by multi-organ failure [24]. Although IL-6 and IL-8 concentrations peaked in plasma at 36–48 h after the injury occurred, these cytokines performed similar to Baux-score and modified Baux-score at predicting outcome at the time of admission. Moreover, IL-6 and IL-8 were higher in survivors compared to non-survivors on the second day after burn injury, suggesting that there is a relationship between the inflammatory response, organ failure and outcome. Similar findings for IL-6 levels have been described [25], but are challenged by two other studies that describe higher IL-6 or IL-8 in patients who do not survive burn injury a week or more after the injury occurred [13,26]. The contrasting cytokine response could possibly be explained by the timing of the highly dynamic cytokine response and systemic infections, underlining changing nature or inflammatory response and difficulties of interpreting the inflammatory response without clinical information. Although a very simplistic way of characterizing pro and anti-inflammatory response, we calculated IL-10/TNF ratio that has previously been described for sepsis patients [16]. Our data showed that a high ratio suggesting that early inflammatory response with anti-inflammatory predominance was a negative prognostic predictor that is line with the previous findings in sepsis patients.

The size of the tissue damage caused by the burn injury is thought to have major impact on the extent of the inflammatory response. In this study, IL-6, IL-8, IL-10 and MCP-1 correlated to the %TBSA burned on the second day after burn injury, corresponding to a previous report [27]. While IL-6, IL-8 and IL-10 are commonly discussed cytokines in burn injury, MCP-1 is getting increased attention in this context. MCP-1 is a key chemokine in recruiting and regulating migration and infiltration of effector leucocytes to primary

medians and whiskers represent ranges. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2 – Cytokine correlations to clinical parameters in patients 24–48 h after burn injury.

Cytokine [pg/mL]	TBSA [%]	SOFA	CRP [mg/L]	PLT	ICU LOS [days]
IL-6	r=0.59; p<0.0001	r=0.74; p<0.0001	r=0.63; p=0.0002	r=−0.35; p=0.05	r=0.52; p=0.0007
IL-8	r=0.60; p<0.0001	r=0.57; p=0.001	r=0.38; p=0.03	r=−0.45; p=0.01	r=0.36; p=0.02
IL-10	r=0.52; p=0.0003	NS	NS	NS	NS
MCP-1	r=0.55; p=0.0001	r=0.51; p=0.005	r=0.60; p=0.0003	NS	r=0.43; p=0.006
IL-10/TNF	r=0.39; p=0.01	r=0.43; p<0.05	NS	NS	NS

Cytokine correlations to clinical parameters were calculated using Spearman's correlation test in patients at 24–48 h after burn injury. $P<0.05$ was considered statistically significant. TBSA, total body surface area; SOFA, sequential organ failure assessment; CRP, C reactive protein; PLT, platelet count; ICU, intensive care unit; LOS, length of stay; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor.

sites of inflammation [28,29]. In this study, we observed that MCP-1 correlated to size of burn injury, inhalation injury and several clinical parameters and could discriminate survivors from non-survivors comparable to Baux-score and modified Baux-score. Previous studies have shown increased levels of circulating MCP-1 in non-survivors of severe burn injury and in patients with inhalation injury [30], but not a correlation to clinical parameters [26]. In the current study IL-6, IL-8, IL-10 and MCP-1, cytokines with high levels 24–48 h after the burn injury, exhibited a high degree of correlation to SOFA score during the same period and also to ICU-LOS.

Although organ failure is an important link between the inflammatory response and outcome, previous data on the association of these cytokines and organ failure after burn injury has been limited. In our study, the concentration of IL-10 had prognostic value in patients with burn injury both measured at admission and at 24–48 h after injury. Other studies have also reported differences in IL-10 between survivors and non-survivors [21,31]. IL-10 is an important mediator in inhibiting inflammation and increased IL-10 correlates with the incidence of sepsis in trauma patients [32,33]. Moreover, patients with severe injury are known to carry increased risk of developing life-threatening opportunistic infections [34]. The underlying mechanism behind the increased risk has been suggested to be explained by an imbalance in T cells and cytokine profiles [35] as well as elevated IL-10 [36]. An immunosuppression following a burn trauma manifesting in decreased numbers of circulating immune cells and key cytokines could potentially predispose patients to sepsis and multi-organ failure, potentially life-threatening conditions [21,37,38].

In this study, patients who survived a burn injury, and those who did not survive a burn injury of similar size and severity, seem to have similar immune responses as measured by most of these 20 cytokines one day after injury. In fact, simple demographic data such as age, %TBSA burned, inhalation injury, their combination, the Baux-score [15] and modified Baux-score that is adjusted for inhalation injury, outperform most of the cytokines, with the exception of IL-8 and MCP-1 levels on admission, in predicting death. When more than 24 h had passed after burn injury, only IL-10 could serve as a clinical biomarker for outcome. These findings confirm previous findings [8,13], that although inflammatory response is important, other factors such as the body's capacity to handle injury, have impact on outcome. The physiologic reserve decreases [39,40] and comorbidities as well as immune senescence become more common with increasing age [41,42], and since major burn injury is an extreme test of physiologic reserves, it is not surprising that the sum of these are strongly related to outcome.

4.2. *Strengths and limitations*

This study reports the evolution of cytokine response to burn injury with high resolution and starting early after the injury, links these to the extent of organ failure and mortality in adult patients. No patients were lost on follow up. Since many

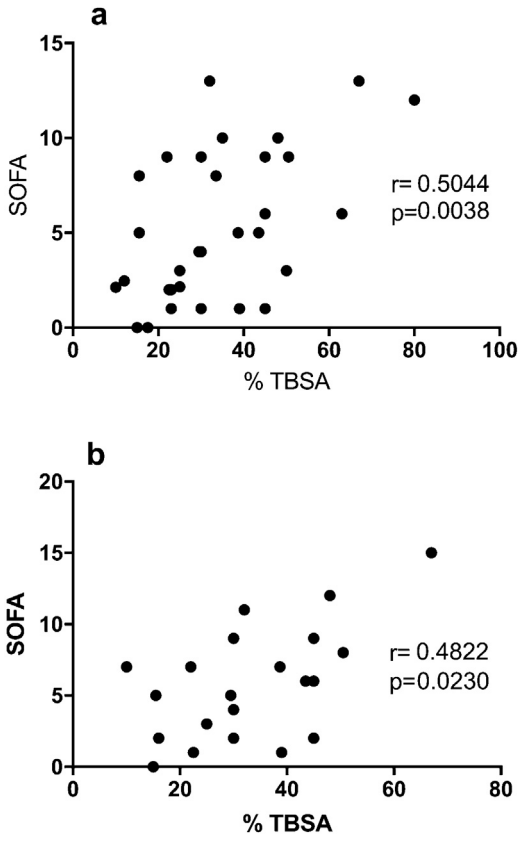

Fig. 4 – Correlation of sequential organ failure assessment (SOFA) to extent of burn injury (a) at 24–48 h and (b) at 48–72 h after injury. Scatter plots represent Spearman's correlation between SOFA score and percentage of total body surface area (%TBSA) in all patients (n=44).

Table 3 – C-index, i.e. area under the curves (AUC) from receiver operating characteristics (ROC) curves, showing discrimination for age, total burned surface area (TBSA), Baux score, modified Baux score and biomarkers between survivors and non-survivors at admission and 90 days.

Predictor variable	C-index admission	C-index for peak value 24–48 h
Age	0.66	
TBSA	0.82	
Baux score	0.82	
Modified Baux score	0.89	
IL-1α	0.71	0.64
IL-6	0.81	0.60
IL-8	0.86	0.63
IL-10	0.79	0.77
ICAM-1	0.47	0.60
MCP-1	0.87	0.70
MIP-1α	0.45	0.61
TNF	0.42	0.42
IL-10/TNF ratio	0.81	0.71

patients with major burn injuries develop infections within a few days after the injury obscuring the inflammatory response, our data on the early cytokine response are of particular value. However, after the initial phase it is not possible to distinguish the inflammatory response triggered by the burn injury and that triggered by infections and surgery. Thus, our and others' [19,27] findings of elevated cytokines weeks after the burn injury could represent both infection and burn induced inflammation. This is a limitation of virtually all studies in patients with burns. Different types of burn injuries (chemical, electrical and thermal burns) may give rise to different inflammatory responses. For this study, we have only included patients with thermal burn injuries. The results of this study will be limited by the considerable interdependence of inflammation, organ failure, requirement of organ support and death making inferences on causality difficult. Additionally, minor differences in the treatment traditions between the two participating centers, such as early administration of ascorbic acid in Uppsala, could have influenced the inflammatory response [43]. However, this would not have impact on the inflammatory response at admission. Finally, the relatively broad range of age groups and %TBSA burned could have increased the variability of cytokine response making conclusions more difficult. One the other hand, these diversities increase the validity of the findings of this study.

4.3. *Clinical implications*

Our results suggest most of the cytokine biomarkers after burn injury are of limited value in predicting outcome in terms of morbidity and mortality on their own.

4.4. *Future studies*

Although the number of patients with major burn injuries is low, larger studies would be of value since they would allow the development of more complex prediction models. These studies can be achieved using multiple sites to collect data. Also, trying to connect tissue cytokine concentrations with plasma concentrations could fill in important gaps.

5. Conclusions

The levels of cytokines have a variable course after burn injury. However, a handful of these are strongly associated with morbidity and mortality but do not necessarily perform better than simple prediction models based on demographical data.

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Conflict of interest statement

We have no conflicts of interest to disclose for the manuscript entitled "The time-course of the inflammatory response to major burn injury and its relation to organ failure and outcome".

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