

A STUDY ON BIOMARKERS, CYTOKINES, AND GROWTH FACTORS IN CHILDREN WITH BURN INJURIES

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SUMMARY. *Background.* Burns are a unique injury which not only is devastating for the patients but also puts a great burden on society by consuming enormous health care resources. Despite improvements in burn wound care and treatment, understanding the role of pro-inflammatory and anti-inflammatory cytokines as well as the mechanisms responsible for the healing process remains to be clarified. Although leptin is regarded as a circulating hormone, it can exert a direct effect on T cells and monocytes, causing the release of cytokines. It may induce angiogenesis or influence angiogenic factors. The aim of the present work is to determine serum levels of leptin, tumour necrosis factor α (TNF α), interleukin-6 (IL-6), basic fibroblast growth factor (bFGF), procalcitonin (PCT), and C-reactive protein (CRP) in a group of children with thermal burns and to determine the changes in these parameters in relation to the duration of hospital stay, the presence of infection, and the total body surface area (TBSA) burned. *Patients and methods.* The study included 42 children with burns (22 males and 20 females; age range, 2 months to 7 years). The study also included 26 age-matched controls. Besides full clinical assessment, including assessment of TBSA burned and the presence or absence of sepsis, all the patients and controls had the following investigations performed: complete blood count, CRP, IL-6, TNF α , PCT, serum leptin, bFGF, and transforming growth factor α (TGF α). *Results.* The fatality rate in this study was 28.6%. Burn cases as a whole showed significantly higher values of white blood cells (WBC), CRP, PCT, TNF α , IL-6, leptin, bFGF, and TGF α than controls. Cases with sepsis showed significantly higher values of WBC, CRP, PCT, TNF α , and IL-6 than cases without sepsis. They showed significantly lower values of TGF α than cases without sepsis. Patients with larger TBSA (>30%) showed significantly higher levels of WBC, CRP, PCT, TNF α , IL-6, and leptin than cases with smaller TBSA. They showed significantly lower levels of bFGF and TGF α than patients with smaller TBSA. Non-survivors showed significantly higher levels of WBC, CRP, PCT, TNF α , and IL-6 than survivors. They showed significantly lower levels of leptin, bFGF, and TGF α than survivors. Correlation studies showed a significant positive correlation between TBSA and each of IL-6, TNF α , and leptin. *Conclusions.* Cytokines and leptin increased in severely burned patients, cases associated with sepsis, and in fatal cases, while bFGF and TGF α levels were lower in severe cases. This may point to impaired healing in such cases and to their poorer prognosis. *Recommendations.* It is highly recommended to monitor immunological parameters such as PCT and/or IL-6 for early detection of infectious complications following thermal injury. Leptin can be regarded as a novel treatment modality to diminish burn-induced inflammation, reduce post-burn immune dysfunction, and enhance burn healing.

Introduction

Burns are a unique injury which not only is devastating for the patients but also puts a great burden on society by consuming enormous health care resources.¹ Despite improvements in burn wound care and treatment, understanding the role of pro-inflammatory and anti-inflammatory cytokines and the mechanisms responsible for the healing process remains to be clarified. Cytokines have been thought to increase in the serum of burn patients.² Levels of inflammatory mediators such as tumour necrosis factor α (TNF α), interleukin-6 (IL-6), and IL-8 were found to be elevated post-burn. TNF- α is secreted mainly by activated macrophages and although it is primarily involved in inflammation and immunity, it has also been found to play a role in the process of angiogenesis. It pro-

motes endothelial cell proliferation and together with fibroblast growth factors stimulates endothelial cell tube formation, a necessary step in the healing process.³ Leptin is regarded as a circulating hormone produced primarily by adipose tissue and acts by regulating feeding and energy homeostasis through central nervous system afferent pathways.⁴ However, a growing number of reports depict a rather broad spectrum of physiological actions for leptin. It has been found to stimulate angiogenesis⁵ and to exert a direct effect on T cells and monocytes, causing the release of cytokines such as the granulocyte macrophage colony stimulating factor, TNF α , and IL-6.⁶ Recent studies have addressed the possibility that leptin may either induce angiogenesis or influence angiogenic factors.^{7,8} Despite the use of new treatment modalities, improvements in technology, and increased experience, mortality rates in

burns and sepsis remain high.⁹ Recently, the biomarkers procalcitonin (PCT) and C-reactive protein (CRP) were added as diagnostic criteria for sepsis.¹⁰

The aim of the present work is to determine serum levels of leptin, TNF α , IL-6, transforming growth factor α (TGF α), and basic fibroblast growth factor (bFGF), PCT, and CRP in a group of children with thermal burns and to determine the changes in these parameters in relation to the duration of hospital stay, the presence of infection, and total body surface (TBSA) burned.

Subjects and methods

Patients and control subjects

This study was performed with the consent of the children's parents or guardians and under the guidelines of the ethics committee of Assiut University, Egypt.

The study was prospective and included 42 children admitted to the burn unit of Assiut University Hospital within 24 h of the burn in the period March-September 2006. There were 22 males and 20 females and their ages ranged from 2 months to 7 years. Twenty-six apparently healthy children of matchable age were recruited for the study as controls. The TBSA burned ranged from 15 to 55%. The burns were mainly scalds affecting the abdomen, chest, and lower limbs and were associated with sepsis during the clinical course in 47.62% of cases. Patients were diagnosed as having sepsis when the following criteria were met simultaneously, as defined by Yamada et al.:¹¹ 1. evidence of obvious wound infection or positive blood culture; 2. hypothermia or hyperthermia (35.5 °C or >38.5 °C); 3. leucocytes <3000 or >15,000/mm³. The average stay in the burn unit was 33.17 \pm 1.31 days.

Exclusion criteria

Patients with known protein energy malnutrition, cardiac, pulmonary or chronic liver diseases, or other debilitating diseases were excluded from the study. Patients with pre-existing infections or on antibiotic therapy, with coagulation abnormalities, on steroid therapy, or with a malignancy were also excluded.

The patients were managed according to the conventional lines of burn treatment in the unit. All patients received resuscitation measures and nutrition according to body requirements. Additionally, they received their usual regimen of drugs and appropriate antibiotic therapy, initially on an empirical basis and then according to culture and sensitivity tests. On the second day of admission, blood was collected from the patients in a heparinized endotoxin-free sampling tube and immediately centrifuged at 3000 rpm and 4 °C for 40 sec to obtain platelet-rich plasma which was stored frozen at -80 °C until used. A second sample was collected at day 8 of hospital stay. All patients and controls had the following investigations performed initially and after 8 days of admission: complete blood

count (CBC), CRP, PCT, TNF α , IL-6, leptin, bFGF, and TGF α . The patients were classified according to the presence or absence of sepsis, TBSA, and outcome.

C-reactive protein

CRP was measured by the semi-quantitative latex agglutination assay with a normal cut-off of <6.0 mg/l, and negative results were randomly assigned 0.0, 2.5, or 5 mg/l (cat. no. 40, 043, humatex CRP human Gesellschaft für Diagnostics mbH, D-65205 Wiesbaden, Germany).¹²

Procalcitonin

PCT was measured using an RIA kit that utilizes specific polyclonal antibodies and human ¹²⁵I-N-procalcitonin with a detection limit of 10 pg/tube (RIN 6025, Peninsula Lab., Inc., San Carlos, CA 94070, USA).¹³

Serum TNF α , IL-6, and bFGF

Commercially available ELISA methods used according to the manufacturer's instructions (BioSource Europe S.A., Nivelles, Belgium) were utilized to measure total TNF α (KAC1751, with a detection limit of 3.0 pg/ml). These assays utilized two monoclonal antibodies, of which one was conjugated with horseradish peroxidase along with positive and negative controls. A commercially available competitive EIA assay was utilized to measure IL-6 (KAC1301), with a detection limit of 0.7 pg/ml, and bFGF (product #410410), with a detection limit of 0.488 ng/ml, utilizing two specific antibodies with alkaline phosphatase as the conjugated enzyme (Cytimmune Sciences Inc., Lexington, KY, USA).¹⁴

Serum leptin level

Serum leptin level was quantitatively measured by the ELISA assay (cat. no. EZHL-80SK, LINCO Research, Missouri, USA), which utilized one polyclonal antibody, a biotinylated monoclonal antibody and streptavidin-peroxidase conjugate for colour development and human leptin standards (0.0-20 ng/ml). Briefly, 50 μ L assay buffer + 50 μ L of each standard or sample, incubated 2 h, solutions decanted and plate washed, 100 μ L detection antibody was dispensed, incubated 30 min, solutions decanted, 100 μ L enzyme solution was dispensed, incubated in dark for 30 min, solutions decanted and plate washed, 100 μ L substrate solution was dispensed and incubated with shaking for 10 min, reaction was stopped by dispensing 100 μ L stop solution, and colour was recorded at 450 nm against 590 nm. Sample content was calculated from the standard curve constructed (minimum detection limit, 0.125 ng/ml).¹⁵

Serum TGF α level

Serum TGF α level was quantitatively measured by the ELISA assay (Quantikine[®], cat. no. DTGA00, R&D Systems Inc., MN, USA), which utilized two polyclonal antibodies, of which one was peroxidase conjugated for colour development and human recombinant TGF α standards (0.0-1000 pg/ml). Briefly, 100 μ L assay diluent + 50 μ L of each standard or sample, incubated 2 h, solutions decanted and plate washed, 200 μ L antibody conjugate was dis-

Table I - Demographic data, burned surface area, length of hospital stay, frequency of sepsis, and types of antibiotics in the patients

Parameter	Total number = 42
Mean age (yr)	4.53 ± 0.7 (range, 2 months-7 yr)
Mean weight (kg)	19.12 ± 1.3
Sex	
Males	22
Females	20
Mean TBSA (percentage)	31.62 ± 12.01
Mean stay in burn unit (days)	33.17 ± 1.31 (range, 17-41)
Sepsis (number, percentage)	20 (47.62%)
Micro-organisms isolated in patients with sepsis	
<i>Staphylococcus aureus</i>	14
<i>Klebsiella pneumoniae</i>	17
<i>Haemophilus influenzae</i>	5

pensed, incubated 2 h, solutions decanted and plate washed, 200 µL substrate solution was dispensed, incubated in dark for 30 min, reaction was stopped by dispensing 50 µL stop solution, and colour was recorded at 450 nm against 570 nm. Sample content was calculated from the standard curve constructed (minimum detection limit, 2.27 pg/ml).¹⁶

Statistical analysis

The data were analysed by unpaired t-test, ANOVA with Newman-Keuls post-multiple comparison test, and multiple linear regression analysis by using the Prism statistical package version 3.0 (GraphPad, San Diego, CA, USA). A level of $p < 0.05$ was considered statistically significant. The data were expressed as mean ± SD. The chi-square test was also used when appropriate.

Results

The results are shown in *Tables I-V* and *Figs. 1-9*. The fatality rate was 28.6% (12 out of 42 patients).

Table II - Initial WBC, haemoglobin, platelets, CRP, PCT, TNFα, IL-6, leptin, bFGF, and TGFα in patients with burns compared with controls

	WBC x 1000 cells/ml	Hb g/dL	Platelets x 10 ³ /ml	CRP µg/ml	PCT ng/ml	TNFα ng/l	IL-6 pg/ml	Leptin ng/ml	bFGF ng/ml	TGFα pg/ml
Patients No. = 42	10.3 ± 4.2	10.2 ± 2.3	195.3 ± 110.4	32.12 ± 19.08	69.1 ± 11.4	98.3 ± 15.14	145.3 ± 36.4	15.7 ± 1.28	0.98 ± 0.22	170.81 ± 16.65
Controls No. = 26	5.1 ± 2.1	12.3 ± 1.4	355.6 ± 120.8	2.40 ± 0.40	0.17 ± 0.02	7.74 ± 3.03	12.4 ± 5.7	1.3 ± 0.4	0.56 ± 0.13	8.08 ± 1.66
Significance	<0.05	NS	NS	<0.005	<0.01	<0.001	<0.001	<0.001	<0.01	<0.001

Table III - Laboratory parameters studied in the patients with and without sepsis on follow-up

	WBC x 1000 cells/ml	Haemoglobin g/dl	Platelets x 10 ³ /ml	CRP nmol/l	PCT ng/ml	TNFα ng/l	IL-6 pg/ml	Leptin ng/mL	bFGF ng/ml	TGFα pg/ml
Patients with sepsis No. = 20 (I)	22.3 ± 4.2	10.2 ± 2.3	122.3 ± 110.4	112.12 ± 19.08	369.1 ± 11.4	116.3 ± 14.3	320.3 ± 36.4	14.7 ± 0.4	0.77 ± 0.22	131.81 ± 16.65
Patients without sepsis No. = 22 (II)	9.1 ± 2.1	11.3 ± 1.4	211.6 ± 120.8	28.70 ± 8.59	47.4 ± 10.7	34.02 ± 6.3	125.4 ± 5.7	11.3 ± 1.4	0.96 ± 0.13	169.08 ± 14.66
Controls No. = 26 (III)	5.1 ± 2.1	12.3 ± 1.4	355.6 ± 120.8	2.40 ± 0.40	0.17 ± 0.02	7.74 ± 3.03	12.4 ± 5.7	1.3 ± 0.4	0.56 ± 0.13	8.08 ± 1.66
I versus II	<0.001	NS	<0.01	<0.001	<0.001	<0.001	<0.001	NS	NS	<0.001
I versus III	<0.001	NS	<0.01	<0.001	<0.001	<0.001	<0.001	<0.01	<0.01	<0.001
II versus III	<0.001	NS	<0.01	<0.001	<0.001	<0.001	<0.001	<0.01	<0.01	<0.001

Table IV - Laboratory parameters studied in the patients with TBSA $\leq 30\%$ and with TBSA $> 30\%$

	WBC x 1000 cells/ml	Haemoglobin g/dl	Platelets x 10 ³ /ml	CRP nmol/l	PCT ng/ml	TNF α ng/l	IL-6 pg/ml	Leptin ng/mL	bFGF ng/ml	TGF α pg/ml
Patients with TBSA $\leq 30\%$ No. = 22	10.3 \pm 4.2	10.2 \pm 2.3	195.3 \pm 110.4	29.12 \pm 19.08	58.1 \pm 11.4	89.3 \pm 15.14	112.3 \pm 36.4	12.7 \pm 1.28	0.98 \pm 0.22	170.81 \pm 16.65
Patients with TBSA $> 30\%$ No. = 20	15.1 \pm 2.1	8.3 \pm 1.4	110.6 \pm 120.8	44.40 \pm 0.40	88.17 \pm 0.02	112.74 \pm 3.03	155.4 \pm 5.7	18.3 \pm 0.4	0.58 \pm 0.13	89.08 \pm 1.66
Significance	<0.05	NS	NS	<0.005	<0.01	<0.001	<0.001	<0.001	<0.01	<0.001

Table V - Laboratory parameters studied in the patients who survived and in patients who did not survive

	WBC x 1000 cells/ml	Haemoglobin g/dl	Platelets x 10 ³ /ml	CRP nmol/l	PCT ng/ml	TNF α ng/l	IL-6 pg/ml	Leptin ng/mL	bFGF ng/ml	TGF α pg/ml
Survivors No. = 30	11.3 \pm 4.2	11.2 \pm 2.3	195.3 \pm 110.4	30.12 \pm 19.08	70.01 \pm 11.4	95.3 \pm 15.14	135.3 \pm 36.4	18.7 \pm 1.28	0.98 \pm 0.22	160.81 \pm 16.65
Non-survivors No. = 12	15.1 \pm 2.1	8.1 \pm 1.4	355.6 \pm 120.8	45.40 \pm 0.40	95.17 \pm 0.02	127.74 \pm 3.03	162.4 \pm 5.7	11.3 \pm 0.4	0.53 \pm 0.13	78.08 \pm 1.66
Significance	<0.05	NS	NS	<0.005	<0.01	<0.001	<0.001	<0.001	<0.01	<0.001

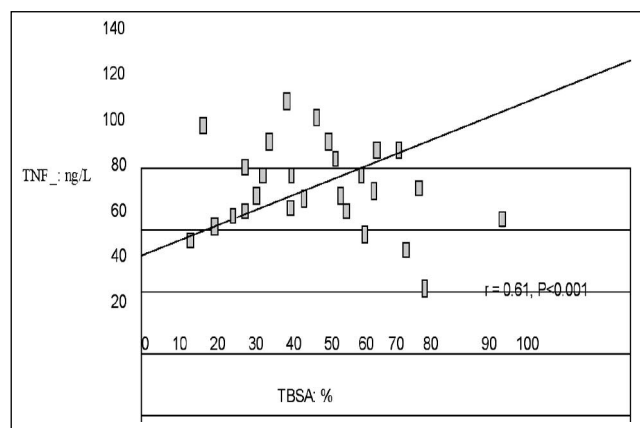


Fig. 1 - Correlation between TNF α and TBSA.

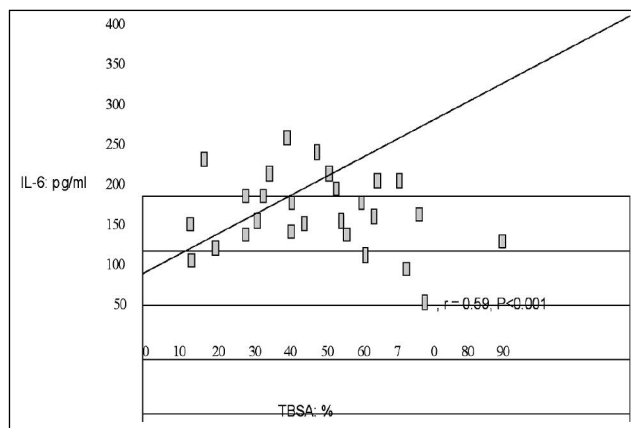


Fig. 3 - Correlation between IL-6 TBSA.

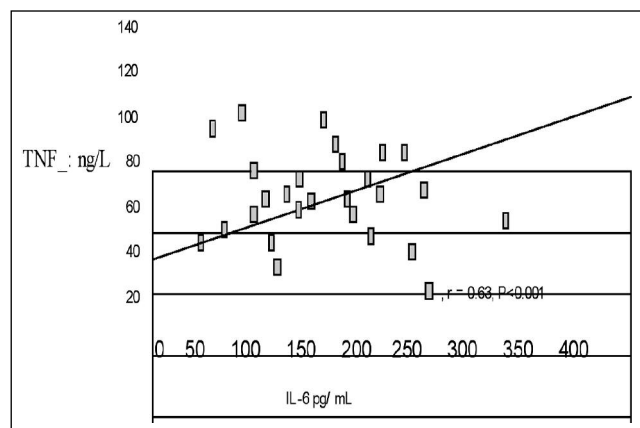


Fig. 2 - Correlation between TNF α and IL-6.

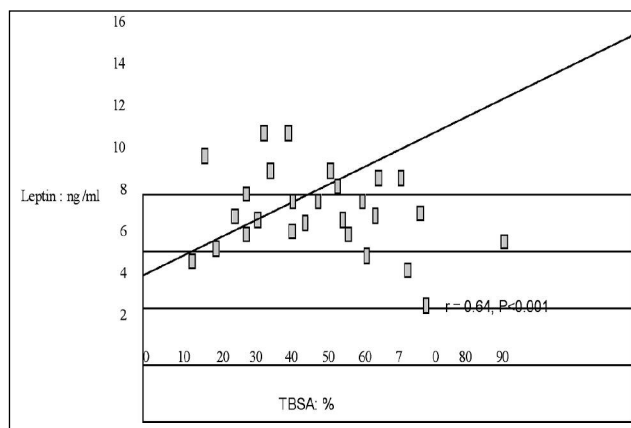


Fig. 4 - Correlation between leptin and TBSA.

Discussion

Thermal burns and related injuries are a major cause of death and disability, especially in young persons. Most burns are caused by carelessness and appear to be preventable.¹⁷ Severe thermal injury induces an immunosuppressed state that predisposes patients to subsequent sepsis and multiple organ failure, which are the major causes of morbidity and mortality in burn patients.¹⁸ The immunological response to thermal injury is a depression in both the first and the second lines of defence. The epidermis of the skin becomes damaged, allowing microbial invasion as the coagulated skin and exudate of the patient create an ideal environment for microbial growth.¹⁹

Severe burn injury induces a distinct inflammatory response which is characterized by activation of all inflammatory pathways, dysregulation of cell-mediated immunity, and alterations of mediators of the immune system involving cytokines, growth factors, vascular endothelium,

and various immunocompetent cell populations.²⁰ The initiation of immune mechanisms takes place at the time of injury: chemotactic and phagocytic defects of PNLs, an increase in T-suppressor and a decrease in T-helper and NK cells, and a decrease in lymphokine production.²¹ Clinically, this general immune dysfunction may lead to organ failure and increased morbidity. The post-traumatic immune abnormalities generally consist of two pathways: an extended inflammatory response and a depression of cell-mediated immunity. The larger the burned surface area, the more intense the response usually becomes. Thermal injury is also stressful and the response of the body tends to be more intense when burns are complicated by infection. This is particularly marked in burn cases complicated by sepsis, septic shock, and septic multiple organ failure.¹⁹

Immune dysfunctions following burns may contribute to the increased susceptibility to infections observed in these patients.²⁰ Also, thermal injury increases macrophage

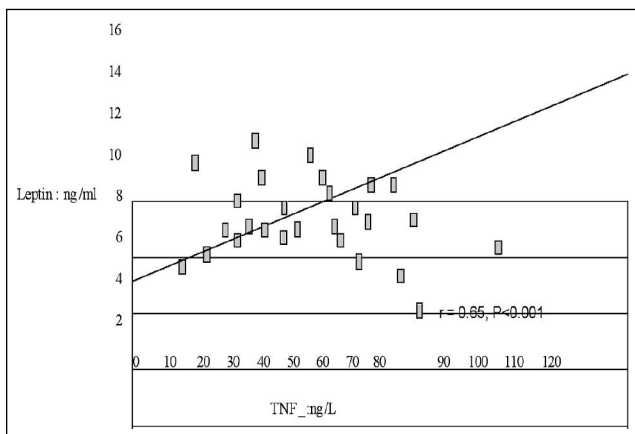


Fig. 5 - Correlation between leptin and TNF α .

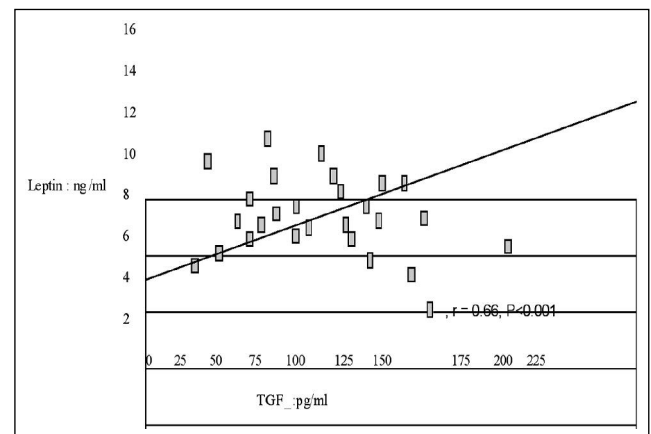


Fig. 7 - Correlation between leptin and TGF α .

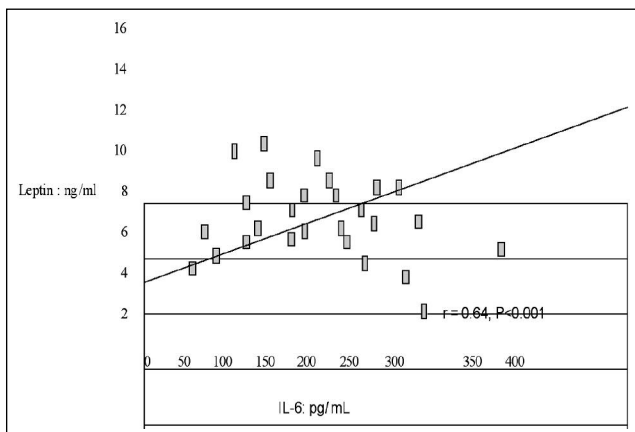


Fig. 6 - Correlation between leptin and IL-6.

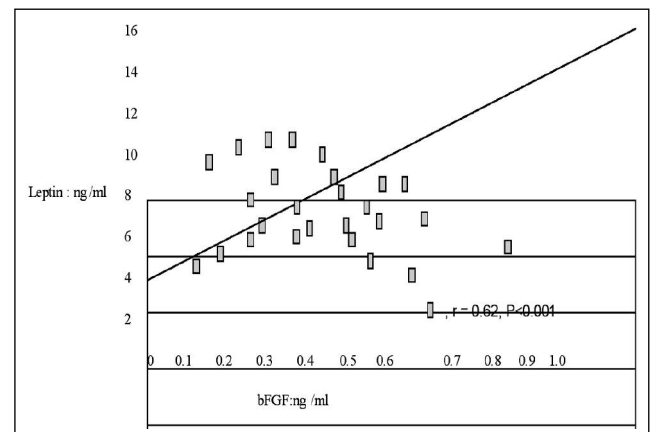


Fig. 8 - Correlation between leptin and bFGF.

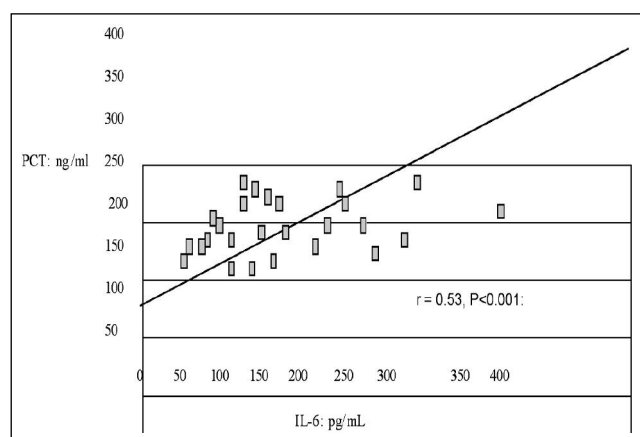


Fig. 9 - Correlation between PCT and IL-6.

activity, thereby increasing the productive capacity for the pro-inflammatory mediators, i.e. prostaglandin E2 (PGE2), reactive nitrogen intermediates, IL-6, and TNF α ²¹ and these factors may play a role as severity indicators.²² Dysregulation of macrophage activity leading to increased release of pro-inflammatory factors appears to be of fundamental importance in the development of post-burn immune dysfunction and increased susceptibility to sepsis following thermal injury.²³

In the present study, we analysed the plasma concentrations of certain biochemical markers as well as pro-inflammatory cytokines with respect to their potential use in differentiating between patients suffering from sepsis and those without sepsis but possibly with the systemic inflammatory response syndrome (SIRS). This assessment is of potential interest because systemic inflammation and sepsis are common problems in burn units, often leading to shock and death.

TNF α is considered the likely initiating factor in the activation of host response and subsequent cytokine release during trauma or infection. However, the diagnostic utility of TNF α is insufficient for distinguishing infectious from inflammatory conditions.²⁴ TNF α plays a central role in the inflammatory response towards burn injury. Levels were reported to be high early after burn, then decreasing after this initial phase of the immunological body response because of its short half-life of 17 min.²⁵ Levels were reported to increase once again with onset of infection.²⁶ Difficulties in using TNF α for sepsis diagnosis arise from its short-term concentration in response to bacterial challenge and from excessive concentrations of soluble receptors (sTNF-RI) and (sTNF-RII) during sepsis.²⁷ In agreement with these observations, in the present study, the serum level of TNF α was significantly higher in the patients as a whole than in controls (*Table II*) and was significantly higher in the group with sepsis than in that without sep-

sis (*Table III*).

IL-6 is expressed by a variety of normal and transformed cells including T and B cells, monocytes/macrophages, fibroblasts, vascular endothelial cells, and others.²⁰ IL-6 is implicated in the early host response to bacterial challenge. Increased concentrations correlating to infection have been demonstrated in several studies.²⁸ IL-6 exerts multiple functions on target cells and an important role in host defence, acute phase reaction, immune response, and haematopoiesis. It has effects on B-cell differentiation and antibody production, cytotoxic T-cell differentiation, T-cell activation, and IL-2 production in T cells. It can also induce the synthesis of hepatic acute phase proteins such as CRP. Furthermore, IL-6 is the cytokine whose levels best predict patient outcome in sepsis. Elevated levels of IL-6 following burn injury have been described,²⁰ coinciding with fever, tachycardia, leucocytosis with an associated left shift, and a decrease in albumin levels. This was also true in the present study, as serum IL-6 was significantly higher in the burn patients than in the control group and was significantly higher in the group with burns and sepsis than in patients with burns but without sepsis (*Tables II* and *III*, respectively).

In the present study, serum levels of TNF α and IL-6 were determined twice throughout the course, initially and on follow-up on the 8th day of admission. On the second day after admission, significantly higher values of these two cytokines were detected compared with controls (*Table II*). Interestingly, the second samples performed on day 8 revealed significantly increasing values in the group of patients with sepsis but decreasing values in those without sepsis (*Table III*), although these were still significantly higher than controls. Similar results were recorded by other researchers,²⁰ who stated that burns were perhaps the most intense stress experienced by the human body. It induces an inflammatory reaction to direct elements of body defence and the immune system to injury sites. The results of Yeh et al.²⁹ also showed that in the early course of burns, patients had increased levels of TNF α and IL-6 without any proven infection. They found also that IL-6 and TNF α correlated with the severity of skin burn injury. Similar results were found in the present study as significant positive correlations were observed between TNF α , IL-6, and TBSA (*Figs. 1,3*). This may indicate the importance of these cytokines not only as diagnostic parameters but also as measures of severity and progression. Furthermore, a significant positive correlation was found between TNF α and IL-6 (*Fig. 2*). This is consistent with previous researchers who reported that TNF α could potentially stimulate the production of second-wave cytokines such as IL-6, which act locally in tissues as well as systemically.²⁹

In the present study, the increasing levels of TNF α and IL-6 in the second week most probably coincided with

the detection of infection in these patients. Other investigators reported that burn plus infection could produce an additive effect on each cytokine and in addition IL-6 correlated better with mortality.²⁶ It has also been suggested that it is unlikely that complications due to infection occur immediately after burns. The rise in TNF α and IL-6 observed soon after a burn seems to represent a response of the body to the burn stimulus. Following complications of burns due to infection, cytokine levels become more raised.²⁰ Others¹⁹ reported that in infected burn patients IL-6 levels were higher in patients who were septic than in those who were not and that IL-6 levels in survivors decreased to normal values after an initial increase in the early post-burn period. In contrast, the non-survivors continuously exhibited increasing systemic concentrations of IL-6, which reached exceedingly high levels shortly before death.

Activated inflammatory mediators may themselves play a primary pathological role in the tissue injury and organ dysfunction associated with sepsis.³⁰ The release of these mediators can lead to several pathophysiological reactions, such as fever, leucocytosis, thrombocytopenia, haemodynamic changes, and disseminated intravascular coagulation, as well as leucocyte infiltration and inflammation in various organs, all of which may ultimately lead to death.³¹ In agreement with these observations, in the present study the total leucocyte count was significantly higher in the patients than in the controls, while the platelet count was significantly lower than in the controls on the second day of admission (*Table II*). In patients with sepsis, the leucocyte count increased significantly while the platelets decreased significantly in comparison with those without sepsis and with initial values (*Table III*).

In their study, Yamada et al.¹⁹ reported significantly higher values of TNF α in burn patients and a significant correlation between the overall TNF α level and TBSA. The levels were significantly higher in patients with TBSA >40% than in those with a lower TBSA. The same was also true for IL-6. In line with these findings, in the present study serum values of TNF α and IL-6 were significantly higher in burn patients with TBSA >30% than in those with smaller burn surface areas (*Table IV*), and significant positive correlations were observed between TNF α , IL-6, and TBSA (*Figs. 1,3*).

It has been reported that TNF α rises as the burn becomes more severe, irrespective of the presence or absence of accompanying infection. Following burn complications due to infection, TNF α levels become higher as the patient's condition grows more severe.¹⁹ Thus TNF α seems to be a useful indicator of the severity and prognosis of burns complicated by infection. In Yeh et al.'s study,²⁹ significantly higher values of serum IL-6 were found in patients with higher TBSA. When changes in serum IL-6 were compared with changes in serum TNF α and IL-8, a

good correlation was observed. Significantly higher levels of TNF α , IL-6, and IL-8 were all detected in septic and deceased burn patients. Furthermore, IL-6 was found to be the cytokine whose levels predicted patient outcome. In their study, Dehne et al.²⁰ reported that at the time of admission to the hospital, the plasma levels of TNF α and IL-6 were increased in all the burn patients but were significantly higher in patients with TBSA >30%. The levels remained on this level for one week and decreased continuously in patients with TBSA \leq 30%. In the other group with TBSA >30%, the concentration remained at an increased level.

Despite the use of new treatment modalities and improvements in diagnostics, mortality rates in sepsis remain high.⁹ A positive culture from blood, urine, cerebrospinal fluid, or bronchial fluid represents the most certain method of diagnosis of sepsis. Of particular interest was the inclusion of the biomarkers PCT and CRP in the diagnosis of sepsis.¹⁰ The PCT plasma level in healthy individuals is low, usually below 0.1 ng/ml.³² PCT concentration was found to be elevated in patients with organ dysfunction and sepsis.^{9,20} Its implication in the cytokine cascade stems from the demonstrated increases of TNF α before increases in PCT and the documented correlation between the concentrations of the two biomarkers.³³ In the present study, significantly higher values of PCT were detected in the patients than the controls initially (*Table II*). On day 8, patients with sepsis had significantly higher values than the non-sepsis group and their initial values (*Table III*). Luzzani et al.³⁴ reported that measurement of PCT as a biomarker for sepsis was highly favourable, given its half-life of 22-29 h and its prolonged increase during sepsis. In this respect our results are in keeping with those of Luzzani et al.³⁴

Wanner et al.³⁵ reported higher PCT levels in patients with clinically documented infection than in those fulfilling the criteria for SIRS. Furthermore, Muller et al.³⁶ investigated 101 patients admitted to a medical ICU and suggested that PCT was a more reliable marker of sepsis than CRP, IL-6, and lactate levels. PCT yielded the highest discriminative value for differentiating patients with SIRS from those with sepsis, followed by IL-6. In agreement with these observations, a significant positive correlation was detected in the present study between PCT and IL-6 (*Fig. 9*). Others also concluded that PCT, IL-6, and C3a concentrations were more reliable parameters for differentiating between septic and SIRS patients than CRP and elastase.²⁸ Mechanical and burn trauma causes elevated PCT levels, the degree of which depends on the severity of the injury. Levels peak on days 1-3 and fall thereafter. High concentrations of circulating PCT during the first three days after injury discriminate between patients at risk for SIRS, sepsis, and multiple organ dysfunctions in the early and late post-traumatic course.³³

CRP, an acute-phase protein, is an additional biomarker widely used in sepsis diagnosis. CRP increases late during the onset of sepsis. Opinions on the diagnostic usefulness of CRP vary, with reports claiming both high and low values. Reported concentrations in septic patients range from 12 to 159 mg/l.^{9,37} Regarding our results, significantly higher values of CRP were observed in patients than in controls (*Table II*). The level was not however markedly elevated. On the other hand, the level increased markedly and significantly in patients with sepsis and decreased in the group without sepsis at day 8 (*Table III*). The results of the present study also revealed an increase of PCT in all patients until day 8 after the burn trauma. In patients with TBSA $\leq 30\%$, CRP concentrations decreased while levels of patients with TBSA $>30\%$ increased significantly. The same was also true for PCT values (*Table IV*). Furthermore, the CRP level was significantly higher in non-survivors than in patients who survived (*Table V*). Thus patients with TBSA $>30\%$ showed different reactions to burn trauma from those with $<30\%$ and developed an extended inflammatory response. Similar results were reported by Dehne et al.,²⁰ who also reported that IL-6 could induce synthesis of hepatic acute phase proteins including CRP.

Leptin is a cytokine-like hormone that links nutritional status with the immune system. It regulates body weight through inhibition of food intake and stimulation of energy expenditure. Moreover, leptin enhances both innate and adaptive immunity.³⁸ TNF α induces the adipocytes to secrete the lipostatic hormone leptin. The increased production of leptin in response to TNF α may be involved in the negative energy balance that is common post-injury. The induced leptin may be an adaptive response helping the clearance of invading pathogenic micro-organisms and may have an anti-inflammatory effect on potentially toxic stimuli.³⁹

Leptin has been studied in a variety of disease states.⁴⁰ However, to the best of our knowledge, few studies have considered the leptin level in acute burn injury. A significantly higher value of serum leptin is reported in the present study in burn patients initially than in the control group (*Table II*) and leptin was significantly higher in the group with sepsis than in the non-sepsis group (*Table III*). Similar results were reported by Kino et al.,⁵ who reported that circulating levels of IL-6 and TNF α were increased in patients with burn injury. The mechanisms regulating the plasma leptin level in burn injury have not yet been established. Pro-inflammatory cytokines such as IL-1 α , IL-6, and TNF α have been suggested as stimulators of leptin production.⁴¹ Accordingly, the plasma concentration of leptin may increase along with the levels of pro-inflammatory cytokines after burn injury.¹⁹ This was also true in the present study, where significant positive correlations were noticed between leptin and each of IL-6 and TNF α

(*Figs. 5,6*). There were also significant positive correlations between levels of the cytokines IL-6 and TNF α (*Fig. 2*). Interestingly significant positive correlations were detected between each of TNF α , IL-6, leptin, and TBSA (*Figs. 1,3,4*).

Recently, leptin has been considered to be a candidate hormone for the regulation of stress⁴² and the results of the present study suggest that it may play a role in the pathophysiological response to burn injury. Serum leptin level was significantly higher in patients with a higher TBSA than in those with a lower TBSA, and in surviving patients than in non-survivors (*Tables IV,V*, respectively). Similar results were reported by Correia et al.,⁴³ who also stated that leptin might be important for survival in acute sepsis, and suggested that the high leptin level in survivors with sepsis or septic shock might represent a host defence mechanism against bacterial infection. Leptin was also reported to increase the expression of the corticotrophin-releasing factor and arterial blood pressure.⁴⁴ Studies have also shown that leptin may modulate functions of the cells of the non-specific immune response, such as phagocytosis by neutrophils, as well as secretion of cytokines by macrophages.⁴⁵ Moreover, leptin has been shown to have a direct effect on T lymphocytes, enhancing the T-helper proliferative response.⁴⁶ This immunomodulatory role of leptin during infection or inflammation makes it likely to act as an anti-inflammatory agent. Furthermore, it was shown that leptin administration inhibited susceptibility to endotoxic shock.⁴⁷

In the present study, patients with a larger TBSA had significantly higher TNF α and IL-6 values than patients with a lower TBSA (*Table IV*). Similarly, non-survivors showed significantly higher values of TNF α and IL-6 than survivors (*Table V*). These results are consistent with those of Yamada et al.,¹⁹ who found that the inflammatory reaction could be most marked when the TBSA was large. TNF α and IL-6 were also recorded as being good indicators of severity in the infectious phase in burn patients. Burn injury itself may become a stimulus for the production of TNF α throughout the entire course of burn injury. TNF α in the blood may stimulate the production of IL-6 and IL-8, which would be produced in massive amounts in local sites of infection and enter the blood. These cytokines become triggers to produce various mediators, and they may thus reflect the severity of the morbid condition of burn injury.⁴²

In their study, Schwacha et al.²³ reported that TNF α was more often detectable in burn patients with septicaemia than in those with negative blood cultures and in those who died than in survivors. They reported a relationship between high concentrations of IL-6 and poor clinical outcome. In this respect our results are in keeping with Schwacha's. Yamada et al.¹⁹ reported higher levels of TNF α in the group of patients that died than in those that survived, probably

because the TBSA was significantly higher in the non-survivors. Similarly, Yeh et al.²⁹ reported significant differences in serum TNF α and IL-6 values on admission between patients who survived or died from burn injury. They concluded that IL-6 was a prognostic factor of mortality at the time of admission. Dehne et al.²⁰ reported that IL-6 increased in proportion to the severity of the burn wound and was significantly higher in patients who died than in survivors. A greater increase in TNF α and IL-6 was observed in serum samples obtained shortly before the death of burned patients. Similar findings were reported by Yeh et al.,²⁹ i.e. that the kinetics of TNF α , and IL-6 plasma levels after burn injury were closely related to clinical outcome. In survivors, IL-6 plasma levels declined to normal values after an initial maximum increase in the early post-burn period. In contrast, non-survivors continuously exhibited increasing systemic concentrations of the cytokines, which reached exceedingly high levels shortly before death.

The process of wound healing is a highly regulated dynamic cascade of cellular and biochemical events that normally result in the successful repair of injured tissues. TNF- α has been thought to play a role in wound healing: it induces vessel growth, an important step in promoting wound healing. The mechanism seems to function through a direct effect on keratinocytes, which are relevant for epithelialization, and endothelial cells, which are important for angiogenesis.⁴⁸ Leptin has been thought to affect wound healing and stimulate angiogenesis. It can be produced in actively angiogenic tissues, suggesting that it may stimulate neovascularization in these tissues. Leptin is also known to be a proangiogenic factor and it is likely to be an active participant in the neovascularization process that accompanies wound healing.¹⁸

It was reported that leptin had a beneficial effect on wound healing, mostly due to direct mitogenic action on keratinocytes located at the wound margin. Growth factors stimulating re-epithelialization are central to the wound healing process, including keratinocyte growth factor, epidermal growth factor, and TGF α .⁴⁹ The expression of leptin receptors in keratinocytes was also reported by these researchers, who suggested a role of leptin in wound healing through activation of other growth factors. In line with these observations, the leptin level in the present study was significantly higher in the patients than in the controls throughout the course of hospital admission (initially and on day 8). This sustained elevation may in part be responsible for wound healing (*Tables II,III*). In addition, the results of the present study revealed that serum levels of the growth factors TGF α and bFGF were significantly higher in the patients than in the controls early and late in the hospital course (*Tables II,III*), and both correlated positively with leptin values (*Figs. 7,8*). Similar results were reported by other investigators,⁵⁰ who reported that leptin had induced acceleration of wound healing through in-

creased expression of transcripts for TGF α . The same results were reported by Cleary et al.⁵¹ These observations provide evidence for the beneficial effects of leptin in wound healing.

In their study, Murad et al.¹⁸ reported a local increase in leptin mRNA expression as early as 6 h post-wound. This increase was sustained throughout the healing process. On day 5, leptin mRNA levels remained elevated and were found to diminish only after 10 days, at which time repair was complete and scar remodelling was actively taking place. They also reported an increase in serum leptin levels and suggested that the increased production of leptin in the wounds could contribute to the sharp increase in the circulating level of leptin after the injury. The spillover of wound leptin into the circulation may likely occur owing to a combination of factors, such as increased local permeability of the blood vessels in the periphery of the wound, clearance of wound fluid, and direct upregulation of leptin synthesis in the wound.

The normal progression of healing in wounds involves the formation of granulation tissue, which requires neovascularization. In the present study, serum leptin levels carried significant positive correlations to each of TNF α , IL-6, TGF- α , and bFGF (*Figs. 5-8*, respectively). This is in keeping with the findings of other researchers,⁵² who observed that soluble factors such as FGFs, TGF- α and α , TNF α , and vascular endothelial growth factor (VEGF) were actively produced in wounds and stimulated angiogenesis either directly or through chemoattracted macrophages that actively secreted angiogenic molecules. The demonstrated role of leptin as a potent angiogenic factor, coupled with its presence in wounds, suggests that the healing augmentation effect of leptin may be the result of its ability to induce neovascularization during tissue repair.

The results of the present study revealed significantly higher levels of bFGF in the patients than in the controls initially and also on day 8. However, patients with sepsis had significantly lower values than the non-septic patients (*Tables II,III*). Similar results²¹ showed that basic fibroblast growth factor might set the stage for angiogenesis during the first four days of wound repair, whereas VEGF was critical for angiogenesis during the formation of granulation tissue. Hypoxia and tissue damage following tissue injury are stimulants for the production of bFGF and VEGF. Activated epidermal cells of the wound secrete large quantities of VEGF, while bFGF and VEGF are potent stimulators of endothelial cell proliferation and migration and contribute to endothelial cell activation in the process of angiogenesis. These factors stimulate endothelial cells to migrate and form new blood vessels at the injured site. Once the wound is filled by granulation tissue, angiogenesis ceases, owing to the activity of anti-angiogenic factors such as angiostatin and endostatin. This stage is followed by wound contraction and extracellular-matrix

reorganization, a complex process orchestrated by interacting cells, the extracellular matrix, and cytokines.⁵³

One to two days after injury, epidermal cells at the wound margin begin to proliferate. Local release of growth factors such as TGF α and keratinocyte growth factor, as well as increased expression of growth factor receptors, may stimulate these processes.⁵⁴ A series of experimental and clinical studies have demonstrated a positive effect of EGF and TGF- α on wound repair, suggesting that these endogenous growth factors are involved in the healing process.⁵⁵ TGF α is a major cytokine that may promote keratinocyte proliferation. On the basis of the presence of TGF α in wound fluid, its strong upregulation early after injury,⁵⁶ and the beneficial effect of exogenous TGF α on wound healing, TGF- α was expected to play an important role in the repair process.⁵⁵ A role was suggested for TGF α in the early phase of re-epithelialization and epidermal regeneration following mid-dermal injuries to the skin, a process that requires both proliferation and migration of keratinocytes.⁵⁷ In line with these observations, the results of the present study indicated significantly higher values of TGF α in the patients than in controls initially, with the level continuing to be significantly higher on follow-up (*Tables II,III*). However, patients with sepsis had significantly lower values than non-septic patients.

Yamamoto et al.⁵⁸ reported that the topical application of epidermal growth factor (EGF) accelerated epidermal regeneration of partial-thickness burns. Similarly, the topical application of TGF α in antibiotic cream on partial-thickness burns accelerated epidermal regeneration compared with untreated burns. They also reported that TGF α appeared to be more effective than EGF in stimulating epidermal regeneration. Furthermore, the regenerated epithelium from burns treated with TGF α appeared to be histologically normal. They also found that in addition to the proliferation of keratinocytes, TGF α accelerated the process of angiogenesis in the dermis and would appear to be very favourable for subsequent wound healing in the dermis.

Conclusions

The results of the present study strongly suggest that wound-associated leptin production plays an important role in epidermal re-epithelialization, neovascularization, and the development of granulation tissue, which are key events in wound repair.

The study of the relationship between leptin and other cytokines actively produced after burn injury may provide insights into the potential role of leptin as an important upstream regulator of the cytokine activation cascade that accompanies tissue repair.

Procalcitonin can be used as an early marker of sepsis in burn patients, and together with IL-6 it could have a significant prognostic importance.

IL-6 is a prognostic factor of mortality and can be closely related to clinical outcome.

Recommendations

1. Since septic shock and multiple organ failure are among the most frequent causes of death after thermal injury, modulation of the inflammatory response in severe sepsis is an important clinical problem. The control of the inflammatory reactions by anti-cytokines may be of great importance in the development of better outcome in burns. This point requires further study.

2. It is highly recommended that immunological parameters such as PCT and/or IL-6 should be monitored for early detection of infectious complications following thermal injury.

3. Leptin can be regarded as a novel treatment modality to diminish burn-induced inflammation and associated multiple organ failure and to reduce post-burn immune dysfunction.

4. The topical application of selected growth factors such as TGF α may be useful in accelerating the healing of partial-thickness injuries.

RÉSUMÉ. *Eléments de base.* Les brûlures constituent une lésion tout particulière qui non seulement est dévastatrice pour les patients mais en outre met un grand fardeau sur la société parce qu'elles dévorent de grandes quantités de ressources pour les soins sanitaires. Malgré les progrès dans les soins et le traitement des brûlures, il faut encore comprendre mieux le rôle des cytokines pro-inflammatoires et anti-inflammatoires comme aussi les mécanismes responsables du processus de la guérison. Bien que la leptine soit considérée une hormone circulante, elle peut exercer un effet direct sur les cellules T et les monocytes, en provoquant la libération des cytokines. La leptine peut induire l'angiogenèse ou influencer les facteurs angiogéniques. Les Auteurs de cette étude se sont proposés de déterminer les niveaux sériques de leptine, du facteur de nécrose tumorale α (TNF α), d'interleukine-6 (IL-6), du facteur de croissance de base des fibroblastes (bFGF), de la procalcitonine (PCT) et de la protéine C-réactive (CRP) dans un groupe d'enfants atteints de brûlures thermiques et de déterminer les modifications de ces paramètres relativement à la durée de l'hospitalisation, à la présence de l'infection et à la surface corporelle totale brûlée (TBSA). *Patients et méthodes.* L'étude comprenait 42 enfants brûlés (22 mâles et 20 femelles; variation d'âge, 2 mois jusqu'à 7 ans). L'étude comprenait aussi 26 enfants témoins d'âge équivalent. Outre l'évaluation clinique complète, y compris l'évaluation de la TBSA et la présence ou absence de la septicité, tous les patients et les témoins ont fait les investigations suivantes: analyse complète du sang, CRP, IL-6, TNF α , PCT, leptine sérique, facteur de croissance transformant α (TGF α). *Résultats.* Le taux de mortalité dans cette étude était de 28,6%. Dans l'ensemble les patients brûlés présentaient des valeurs des globules blancs (WBC), CRP, PCT, TNF α , IL-6, leptine, bFGF et TGF α

significativement plus élevées par rapport à celles des témoins. Les cas septiques présentaient des valeurs des WBC, CRP, PCT, TNF α et IL-6 plus élevées par rapport aux cas non septiques. Ils présentaient des valeurs de TGF α significativement inférieures aux cas non septiques. Les patients avec une TBSA plus étendue (>30%) présentaient des valeurs des WBC, CRP, PCT, TNF α , IL-6 et leptine significativement plus élevées par rapport aux patients avec une TBSA plus limitée. Les patients décédés présentaient des niveaux des WBC, CRP, PCT, TNF α et IL-6 significativement plus élevés par rapport aux patients survivants et des niveaux de leptine, bFGF et TGF α significativement inférieurs à ceux des survivants. Les études corrélatives démontraient une corrélation positive significative entre la TBSA et chacune des valeurs d'IL-6, TNF α et leptine. *Conclusions.* Les cytokines et la leptine augmentaient chez les patients sévèrement brûlés, qui étaient des cas associés au développement d'une condition septique et à la mortalité, tandis que les niveaux de bFGF et TGF α étaient inférieurs dans les cas sévères. Cela pourrait indiquer une guérison détériorée dans ces cas et un pronostic moins favorable. *Recommandations.* Les Auteurs recommandent vivement de monitorer les paramètres immunologiques comme PCT et/ou IL-6 pour l'identification précoce des complications infectieuses qui se produisent après les lésions thermiques. La leptine peut être considérée une nouvelle modalité thérapeutique pour diminuer l'inflammation provoquée par les brûlures, réduire la dysfonction immunitaire post-brûlure et améliorer la guérison.

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