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Dynamics and significance of cytokines in the three phases of major burned evolution

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# TABLE OF CONTENTS

## GENERAL DATA

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Evaluating and defining category of major burned.</td>
<td>3</td>
</tr>
<tr>
<td>II. Pathophysiology of shock from major burns in the three phases of evolution.</td>
<td>3</td>
</tr>
<tr>
<td>III. Pro-inflammatory and anti-inflammatory cytokines properties</td>
<td></td>
</tr>
<tr>
<td>IV. Adaptive Immunomodulation of major burned</td>
<td>4</td>
</tr>
<tr>
<td>V. Development. Prognosis</td>
<td>5</td>
</tr>
</tbody>
</table>

## PERSONAL STUDY

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. The purpose and objectives of the study</td>
<td>5</td>
</tr>
<tr>
<td>II. Materials and methods</td>
<td>7</td>
</tr>
<tr>
<td>III. Trial. Statistical analysis</td>
<td>7</td>
</tr>
<tr>
<td>Clinical trial results and statistical analysis</td>
<td>8</td>
</tr>
<tr>
<td>IV. Immunoassay study</td>
<td></td>
</tr>
<tr>
<td>Immunoassay results</td>
<td></td>
</tr>
<tr>
<td>V. Immunohistochemical study</td>
<td>9</td>
</tr>
<tr>
<td>The results of immunohistochemical and histopathological study</td>
<td></td>
</tr>
<tr>
<td>Final Conclusions</td>
<td>14</td>
</tr>
<tr>
<td>Selective References</td>
<td>16</td>
</tr>
</tbody>
</table>
GENERAL DATA
I. Evaluating and defining category of major burned.

Burns remain an important cause of morbidity and mortality worldwide. Infectious complications including sepsis, septic shock and multiple organ failure is a common pathology in patients with moderate and severe burns.

In 1992 was established consensus definitions for SIRS and MODS diagnosis. In 2001, The International Sepsis Definitions Conference held in Washington DC, the meeting of 29 participants from Europe and North America, revised and updated definitions for SIRS, sepsis, MODS.

The purpose of this conference was to identify methodologies for increasing accuracy, rehabilitation and clinical use of the definition of sepsis. In January 2007, the American Burn Association held a consensus conference for definition of sepsis and infection in burns.

Using this classification system, the Consensus Conference in 2001 implement a classification scheme called PIRO: Predisposing conditions, nature and extension of Injuries, nature and magnitude of the body's Response, and degree Organs dysfunction.

Postagressional inflammatory status was defined clinically as SIRS (Systemic Inflammatory Response Syndrome). SIRS can lead to multiple organic dysfunction syndrome (MODS) and death, particularly if infectious complications occur.

Assessment of burn injuries
Classification: depending on the vulnerable agent vulnerable against burns:
- thermal burns: produced by heat action
- chemical burns: result of the action of chemicals
- electrical burns: result of the action of electrical agents

Classification of burn injuries

Burning is a disease that begins when skin damage occur, developing a systemic evolution, affects all systems and organs of burned patient and continue long time after the local lesions were healed. All these elements requires monitoring burned patient for assessing injuries and determining a prognosis. For this reason it is proposed a classification considering based on two histological landmarks: dermo-epidermal junction defined by the epidermis (germinal epithelium) and skin annexes, represented by the hair follicle, sebaceous glands and sweat glands, very important elements because they contain germinative epithelium, essential for healing.

II. Pathophysiology of shock from major burns in the three phases of evolution.

Trigger's systemic response

Local lesion of burning is the result of the heat transfer in tissues and is a dynamic entity. Immune defense system fights infection by both defense mechanisms, innate and acquired or natural. Adaptive defense involves a rearrangement of genes, produce T and B cells highly specialized for antigen recognition. Defense by innate immunity, in contrast, is rapidly mobilized and is bactericidal.

Proinflammatory cytokines (TNF-α, IL-1β, IL-12, interferon γ) and chemokines (IL-8 and others) play a key role in local defense.

Systemic response to heat stress

Devitalized tissue acts as a self-antigen and is a powerful activator of the complement system. Meanwhile, limfokine stored in tissues or products of damaged cells, stimulates invasion of monocytes and enhances their maturation, cells which are responsible for clean burning through the process of phagocytosis, which aggravates the second insult.

Theory of "two-hit" refers to the fact that two activating sequential events may cause a maximum response that can cause systemic inflammation.
Physiological aspects of the response to thermal damage

Phase I or phase to initiate inflammation

Tissue damage causes activation of the five initiators of inflammation, initiators which are interactive and causes development of secondary mediator or effector signal.

Phase II or phagocyte response

Systemic response is a series of positive measures
- increase the serum concentration of cells (leukocytes, macrophages) to recognition of germs
- mobilizes white blood cells in circulation and
- increase the sanghin flow to the site of infection.

Systemic inflammation response prevents inflammation in other tissues by neutralizing the inflammation-inducing molecules such as cytokines, proteases and oxidants by reducing proinflamator response of circulating leukocytes.

Although local response of damaged tissue is predominantly proinflamator, many inflammatory cells or modulators are locally produced.

It was found that although we find proinflamator local mediators, inflammatory cell concentration is higher at level of local inflammation than their blood concentration.

The normal systemic response can be immunosuppressive!

In ICU is known that trauma may induce immunosuppression. Intense activation of the systemic response normally installed posttrauma, can cause a paralysis status of immunity, the so-called "endogenous immunosuppression". Immunosuppression induced by trauma can disrupt lymphocytic function and inhibit delayed hypersensitivity reaction.

Pathophysiology of systemic manifestations of the severe burned

The clinical course in severe burned is characterized sequentially by SIRS, sepsis, septic shock, MODS, events that depend on stage of treatment occurs. Injury caused by systemic manifestations occur when burning covers more than 25% of body surface area for a healthy adult, but are common in smaller areas (10-15%) in children, the elderly or inhalation injuries.

In terms of pathophysiological, manifestations of systemic injury caused by burning undergoing two basic steps, partly overlapping clinical stages:

- hypovolemic shock phase;
- acute phase systemic inflammation.

Subgroups of T cells are altered in sepsis: Th cells can be divided into Th1 or Th2 cells. Th1 cells secret especially proinflamatorcyt cytokines and Th2 cells secret anti-inflamatory cytokines.

III. Pro-inflammatory and anti-inflammatory cytokines properties

Cytokines or imunocite term was originally used to separate a group of immunomodulatory proteins, also called immune transmitters, by another group of growth factors, or peptides regulators factors that modulate proliferation and bioactivity of non-immune cells. Original concept "production cell – cytokine - target cell” was invalidated by careful study of each cytokine separately.

Perhaps the most important characteristic that distinguishes them from hormones, is that they are not produced by specialized cells arranged in glandular tissues. Cytokines act on a very broad spectrum of cells, larger than hormones. Type, duration, and also scope of activities induced a specific cellular cytokine may be influenced considerably by microclimate cell. Cytokines are positive or negative regulators of cell cycle, of differentiation, of migration of cell survival, of apoptosis and transformation.

Cytokines are some strong double-edged weapon which can trigger a cascade of reactions and can have side effects beyond the expected therapeutic effect.
Cytokine receptors

Corresponding receptors and cytokines have been subdivided into several families based on structure and activity, cytokines acting on target cells by binding their specific membrane receptors.

- **Hematopoietine receptor family** are dimers or trimers that retain Trp-Ser-X-Trp-Ser sequence.
- **Interferon receptor family** preserve cysteine and includes receptors IFNα, IFNβ, and IFNγ.
- **Tumours Necrosis Factor receptor family** includes several extracellular domains: receptors for TNFα and TNFβ, membrane CD40 ligand (important for activation of B cells and macrophages), Fas (which indicates cell apoptosis).
- **Chemokine family** interacts with G protein. This family includes receptors for IL-8, MIP-1.

IV. Adaptive Immunomodulation of major burned

Skin immune system

Associated skin lymphoid tissue including keratinocytes, epidermal Langerhans cells, T cells, endothelial cells of skin vessels and regional lymph nodes (14). This complex is called the skin immune system (SIS).

After burning, reaction that occurs is characterized by hipermetabolism and catabolism, which compromise the immune system and lead to MODS. Acute phase mediators are proinflammatory cytokines like IL-1, IL-6, IL-8, TNF, or anti-inflammatory cytokines like IL-10, increased synthesis of pro-inflammatory cytokines contributing to the installation of hipermetabolism and catabolism (75).

T cells are numerous at the skinlevel, and about 90% of these cells are found only in the dermal perivascular unit. Most are represented by memory phenotype T cells (CD45RO), other isoforms (CD45RA) is represented by phenotype of naive T cells.

Sepsis and multiorgan failure are the most important cause of death in intensive care of burned patient. Patients with sepsis have massive apoptosis in lymphoid organs. T cells rapidly disappear after thermal insult, their growth was observed only after 48 hours. The severe damage of lymphocytes is considered to be closely related to severe immunosuppression that occurs after thermal injury. T cells from the skin surface due to location and permanent antigenic stimulation, determines a characteristic immune response (184). Extreme cases heat is commonly associated with suppression of immunity and a loss of lymphocyte subpopulations in blood and lymphoid organs.

Inadequate increase apoptosis of T cells during or following heat insult or sepsis, may contribute to loss of T cells potential responder.

After SIRS installation, the immune response decreases drastically. In the first stage of SIRS, proinflammatory cytokines (IL-1, TNF-α) are produced in response to injury. If the original insult is severe proinflammatori mediators in the systemic circulation occurs, and so inflammatory cytokines may occur quickly to adjust the initial inflammation. If the mechanisms regulating the inflammatory response are not functional, a massive inflammatory reaction can lead to multiple organ failure.

Pathogenesis of immune response in major burns

**Cytokines in immune response after burn**

The presence of cytokines produced by monocytes, macrophages, neutrophils, involves a substantial magnification immune response to infection and determine a direct involvement in the pathogenesis of sepsis post burn. TNF-α and IL-1 is produced early in relation to cell activation and is a potent stimulus for activation of other cells locally or systemic.
Cytokine cascade.

Cytokine cascade consists of four steps:

- **Stage I (macrophage-dependent)**
  Cells responsible for carrying out step I are macrophages and cytokines which are involved at this level, is IL-1 and TNF-α.

- **Stage II (Th 1-dependent)**
  CD4 Th1 lymphocytes cells are responsible, and the main cytokines involved are represented by IL-2 and IFN γ, and less IL-12.

- **Stage III (Th 2-dependent)**
  Stage III is made by CD4 Th2 lymphocytes through cytokines related: IL-4, IL-10, IL-13.

- **Stage IV (inhibitory)**
  This step is achieved through the complex inhibit cytokine TGF-β, which is a inhibiting component of lymphoid cells Th1 CD4, CD8 and B, and on NK cells. B cells are activated by Th2 cells at lymph nodes.

Cytokines are a component of the immune system. Proinflammatory cytokines are produced mainly by monocytes and macrophages, TNF-α and IL-1β are early regulators of immune response and both causes the secondary release of other cytokines, IL-6, IL-8. IL-10 is an anti-inflammatory cytokine that reduces the synthesis of pro inflammatory mediators.

Early assessment of patients with major trauma and their early prognosis is difficult to perform, because of the many existing variables.

Severe burns induce an adaptive immune response by producing cytokines released under the influence of Th 2 cells.

In most cases, increased Th2 type of response does not appear immediately after thermal injury, but noted that the Th2 response is preceded by a Th 1 type response. TH1 cells play a major role in initiating cellular immune response, and Th2 cells causes production of antibodies and play a role in cytokine production. Moreover, Th2 cell response was linked to suppression of cellular immunity. Immunosuppression and subsequent development of sepsis is recognized as a major complication of thermal injury (21).

V. Development. Prognosis

**Vital prognosis**

Both types of cytokines, pro and anti inflammatory, appear in the systemic circulation in case of septic shock, MODS and immunosuppression, contributing to increased mortality. Early altered levels of serum IL-6 and IL-10 may be a predictive marker for identifying patients at increased risk for mortality after burn trauma. These cytokines may be used as predictors of mortality, but only within 24 hours, these parameters are not considered predictive, if death occurs later, after 6-15 days. These data demonstrate that the initial inflammatory response directly correlates with early mortality and did not correlate with late mortality.

**Quality of life**

Wound healing is a dynamic, interactive process involving soluble mediators, sanghine cells, extracellular matrix and parenchymal cells. The wound healing process has three phases: inflammation, tissue formation and tissue remodeling. Healing process reaches only 20% wound closure time in the first three weeks, then begin the process of wound healing and lteness.

Scar will never have the same flexibility as intact skin, the maximum that can be achieved is 70% of intact skin suppleness (14). The epidermis is capable of healing through cell strains. Strains skin cells is about 10% of keratinocytes of basal epidermal layer. These keratinocytes which are found around damaged tissue, migrate and proliferate in the epidermal and dermal edges they cover completely with a unicellular layer. Results of
research on wound healing of burns remains limited, due to an incomplete understanding of basic cellular mechanisms of wound healing.

PERSONAL STUDY
I. The purpose and objectives of the study

From desire and need to identify a possible problem solving complex issues raised by the systemic changes that occur in patients with severe burns, achieve the idea of this paper, titled “Dynamics and significance of cytokines in the three phases of major burned evolution”.

Due to the complexity and importance of issues considered outstanding I intend to carry out a comprehensive study in this direction, because prevent of complications caused by burning is a major goal.

The aim was to specify the pathophysiological implications and practical importance of determining and monitoring serum levels of cytokines in the three major phases of burned evolution, correlated with morphological and immunohistochemical local changes. Thus, I proposed to study the dynamics of serum levels of proinflammatory (IL-1, IL-6, TNF-α) and inflammatory cytokines (IL-10) depending on a number of clinical parameters (burned body surface area, degree of burn, age, gender, comorbidity, prognostic index, etc.).

Cytokines as intercellular signaling polypeptides produced by cells activated during inflammation, is the most important stimulator of systemic reaction. By evaluating their dynamic I proposed to monitor the evolution curve of cytokines in cases with severe burns, for the moments of decompensation detection. For this I proposed setting a specific cut-off for each cytokine studied.

Objectives are being achieved:

- Pathophysiological implications and practical implications of the determination and monitoring serum levels of cytokines in the main phases of evolution of patients with severe burns.
- Cytokine levels correlate with the appearance of local IHC.
- Correlation of severity and the progression of severe burns cases, as evidenced by the use of severity scores (prognostic indicators), with the dynamic evolution of cytokines.
- Obtaining a correct prognostic score as the major burned, both in terms of prognosis and clinical behavior, to study their reliability and applicability of prognostic mortality, by setting a cut-off value, from which to determine whether or not there is likelihood of death.
- Obtain new data regarding the “quality of life “, because the severe burns are followed by the formation of disabling and unsightly scars.

II. Material and methods

The prospective study included a group of 92 patients with severe burns in the period January 2005 - March 2009. The study was conducted in the ICU services and Plastic Surgery Clinic of the Emergency County Hospital Craiova. We studied 92 patients with severe burns (35-67% body surface area) admitted to these services, which accounted for 14.30% of the total number of patients who had burned and hospitalized. Patients were selected from a total of 643 adult burned patients admitted in the Department of Burns of Intensive Care Units and Plastic Surgery Clinic of Emergency County Hospital Craiova, in this period.

Were selected patients who had severe burns on 35-67% body surface area, cases below 35% body surface area and over 70% body surface area is excluded, because we considered a less critical risk burning in 35% and recovery is than satisfactory. At a burn rate of over 70%, the vital risk is very high and local developments is encumbered by multiple systemic complications.
Investigation protocol was established as measurable and specific set of parameters. It was intended clinical evaluation, laboratory evaluation and management of each case. It was intended to establish a clinical evaluation sheets burnt area assessment.

To determine the severity of burns and the development of outcome assessment, we used prognostic scores based on objective clinical and laboratory data as quickly and easily obtained. We used prognostic scores as prognostic index (PI), Baux score, ABSI score (Abbreviated Burn Severity Index)-Tobiasen, Blot score, score MODS (Multiple Organ Disfunction Scores), APACHE II score (Acute Physiology and Chronic Health Evaluation II), MPM (Mortality Probability Model).

Lots of patients included in the study were arranged according to body surface area burned, degree of burn, taking into account the associated pathology. Make clear that some of these scores are used in intensive care to calculate a prognostic score of mortality in all patients, not only in burned patients. We used these scores and prognosis, because the general trend in the early hours of burn patients in intensive care is similar to other trauma patients, thus trying to quantify burn-induced systemic pathology.

For the immune study peripheral blood was harvested, which was processed to be used for evaluation by ELISA test, samples were collected at admission in the Department of Burns, then harvesting was performed on days 3, 5, 7, 14 and in some cases, harvesting was carried out on the 21st day, the patients who survived to this day.

For immunohistochemical study, the material was the skin fragments (containing both healthy tissue area as well as lesion area), collected from a total of 92 patients who had burns of varying degrees IIa, IIb, IIb / III and grade III, on different days from the occurrence of burning. Tissue samples were collected on days 1, 5, 7 and 14 when the thermal injury.

For statistical processing of data were used software packages EPI2000, distributed by WHO, SPSS, specializing in scientific statistical calculations, SPSS product company and module Microsoft Excel Data Analysis Program, along with XLSTAT for MS Excel suite.

III. Trial. Statistical analysis

Clinical trial results and statistical analysis

To search for different characteristics of patients with burns, we divide the group of subjects in three subgroups:

- group 1: patients with burns surface <45% but ≥35%
- group 2: patients with burns surface ≥45%, but <55%
- group 3: patients with burns surface ≥55%, but < 65%

Also, we used a control group, called Lot 0 of patients with burns on the body surface area <10%.

We have established investigative protocol and set of measurable parameters and tracking some specific cases. In clinical evaluation we considered a broad range of clinical parameters: area burned, degree of burn, age, demographic data, type of causal agent, airway injury, evolution case. Calculating the distribution of deaths by burning agent, we note that if the heat deaths were most common (32.14%), this group being the one who presented the most cases.

Regarding the evolution of burned patients we correlated the rate of death by a series of clinical parameters, noting that if the difference between the two sexes death was minimal (2 cases), while the predominant group of male survivors.

I noticed a greater number of deaths in age groups 60-70 years and over 70 years, but we noticed, however, the difference between the two categories (death and survival) was not great as an absolute number, only 3 and 2 cases. In this situation we can not draw a conclusion about the deaths and the age of patients, but produced higher number of deaths from these types of patients who may be due to associated defects and insufficient immune response.
Burned patients were presented with several degrees of burn, at least two types of lesions in some cases two types of serious injuries, a high degree and only a few cases (8.69%), we had 1 and 2a burns degree. In 13.04% cases we found patients who had only 3 burns degree. The rest of burned patients had burns of varying degrees, combined, alternating with areas of severe burns a lower burns degree.

The results of statistical analysis of prognostic indices

Significant for statistical analysis of the cases were Student's test, comparing the averages for the two groups, Chi square test, Fisher's test, Odds Ratio and Relative Risk.

Please note that for a rigorous statistical analysis, we took into account the control group, statistical analysis performed on the entire group, 112 patients. Realizing the ROC curve when prognostic indices take different values, the following results stand out as useful limits in the use of prognostic indicators value for the probability of death.

Thus, for IP, we propose the cut-off value of 120 and we can say that the IP value > 120 indicates a significantly higher possibility of death.

For Baux index a cut-off value > 100 show a significantly higher risk of death.

Index ABSI analysis showed us that the cut-off value > 9 showed a significantly higher risk of death.

MODS score statistical analysis results showed that the MODS score value > 9 indicates a significantly higher possibility of death.

The results of statistical analysis of Blot mortality score showed that the amount of Blot mortality score > 3 showed a significantly higher risk of death.

Statistical analysis for APACHE II score showed a cut-off of 25 for APACHE II score.

The results of statistical analysis for MPM score (Mortality Prediction Models), showed a cut-off of 55 for MPM0 score, a cut-off of 40 for MPM24 score and a cut-off of 43 for MPM48 score. Because in burned Department of ICU, patients were hospitalized and more than 48 hours, we calculated the MPM score over time, to see which is the prediction of long-term patients. We found that the amount of MPMOT > 53 showed a significantly higher risk of death.

The statistical study of prognostic indicators, were noted as markers that can be used for monitoring the development of burn patients, ABSI index and Blot mortality score, as most loyal death of prognostic markers in our study.

By setting the cut-off value of prognostic indicators studied, we can say that there is a statistically significant difference in the distribution of deaths according to the cut-off value, because both values exceeded the 95% confidence level (p < 0.05) as well as the confidence of 99% (p < 0.01).

IV. Immunoassay study

Immunoassay results

We considered the representative and we intend to study, as non-invasive method and easy to approach in medical practice, serum levels of proinflammatory cytokines (tumor necrosis factor TNF α, IL-6) and antiinflammatory cytokines (interleukin-10) by ELISA immunoassay technique using kits for human interleukins.

We considered these investigations since plasma levels of interleukins, pro-and anti-inflammatory cytokines are specific for severity of inflammatory response and may be correlated with progression or prognosis of patients. The evolution of cytokines was performed by linking each level and its dynamics on the time elapsed from the time of thermal injury, the vulnerable agent, sepsis, survival or death, the body surface area burned, installation of complications that can lead to death, offering me achieve a clear picture of the dynamics of the three cytokines in patients with severe burns.
**Dynamics of proinflammatory and antiinflammatory cytokines in burned patients**

Dynamic changes occurring in circulating levels of IL-6, IL-10 and TNFα, involved these cytokines in early response after thermal injury. A moderate increase of these cytokines is therefore beneficial, but when the balance between proinflammatory and antiinflammatory cytokines breaks, phenomenon associated with an exaggerated inflammatory response and hipermetabolism, patient outcome is unfavorable, as evidenced clinically after the first 7 days post thermal injury.

As the dynamics of TNFα, apparently, the shock is the essential element that binds TNFα production and release. TNFα is an cytokine alarm, she was among the first to be issued to activate phagocytes and that occurs at the beginning of cytokine cascade, then leading the release of other cytokines.

In this study, correlations made and follow the clinical course of burned patients, us to say that this cytokine is important not as diagnostic, but mainly because of the severity and prognosis evolution burned patients who were infected. Evolution must be correlated with IL 6 in management of patients, because it seems that TNFα can stimulate secondary synthesis of IL 6.

Correlating dynamics and TNFα levels with survival or death and also the role of TNFα in predicting sepsis, I found that this presents a significant increase in cytokine levels early hours of the application of thermal injury in patients subsequently died. In the subgroup of survivors, which are subsequently developed sepsis or not developed, I found that TNFα has been slow growth in the first 24 hours, then, plasma levels increase suddenly. This increase showed a peak on day 5, provides for patients who did not develop sepsis and a peak on day 7 of the patients who developed sepsis. After this peak, plasma levels started to decrease until the 14th day and then more slowly until the 21th day, but not back to normal until this day.

Although TNFα values remain high in patients who die, however, can not believe that we can use TNFα as a predictor of mortality, because in all patients, absolute values were high. Comparing groups of patients who died with those who survived, I noticed that an early and progressive increase in TNFα is associated with an increased risk of death in patients with severe burns.

Commenting on the cytokine and prognostic indicators, can be seen that the ABSI score had the best correlation with the cytokine in the 5th day, before the peak of the TNFα. This cytokine may be an indicator of burn severity and prognosis.

*TNFα appears to be an indicator of burn severity and prognosis of burned patients, and early and progressive increase in TNFα level is associated with a higher risk of death from major burn.*

The tests carried out, we have demonstrated that *IL 6 not only arises as a result of circulating endotoxin, but in particular because of the inflammatory response.*

In the study, increased IL 6 in the first three days was strictly due to post-inflammatory phenomenon of thermal injury. Clinical and laboratory data that we have on patients in the study group, we had no reason to believe that an infection had occurred during this period.

As the dynamics of IL 6 in light of evolution of the burned patients to death or survival, I found that the concentration levels of IL 6 in patients who developed sepsis and those who have died, but had no sepsis, were increased.

In patients who died and who presented sepsis noted a steady increase in the concentration of IL 6 by day 7, when he presented a peak of 610.5 pg / ml, after which values decreased until the 21th day, but no return to normal levels, 128.5 pg / ml.
IL 6 was increased in cases with sepsis, rather than in those without sepsis and control group but after day 14 these values showed no significant difference in surviving patients from patients who died, the dynamics of IL 6 have been the same, downward. The study found a significant early increase in IL 6, but only in patients who developed sepsis and those who survived. **IL 6 in this dynamic situation is biphasic thing that I have not found in the literature.** Probably the first increase in IL 6 is given by early release of IL 6 from the wound burned, especially in patients with severe burns, and the second is given by the systematic increase in the inflammatory response. This should be taken into account the fact that TNFα could play a key role, because this cytokine has the capacity to amplifies the release of other cytokines. Also burned patients released high levels of IL 6 immediately after injury. These data suggest that the production of IL 6 is more related to soft tissue damage than other cytokines.

**In conclusion, early growth, progressive and consistently high levels of IL 6 can make this cytokine a marker of sepsis,** because harvesting and determination of IL-6 were performed before the occurrence of sepsis.

Among patients who developed sepsis, 5th day we think may be regarded as discriminating between patients who survived and those who died, because in this day in surviving patients sepsis, plasma concentrations started to decrease. Conversely in patients with sepsis who died, plasma levels of IL 6 have continued to grow.

In this case, MODS score is the one who gave us the prognosis with the highest sensitivity, but in the days before the peak of IL-6, making it among the prognostic factors which announces increased cytokine IL-6. This is important, because maintaining a increased constant level of IL-6 from the early days after thermal injury, could be an aggravating factor for the progress of burn patient especially in patients who will die, particularly in patients with sepsis.

Inflammatory cytokine **IL-10** regulates the production of proinflammatory cytokines, prevents antigen-specific T cell activation and inhibits T cell proliferation.

Following the evolution of lots of burned patients and its correlation with the evolution of the IL 10 with / without sepsis I noticed a difference in the evolution of IL 10 in patients who survived to those who died. In all cases the levels of IL 10 produced a peak on 5th day, regardless of developments in cases, even in the control group. Absolute values were those that made differentiation, plasma values was 19.05 pg / ml in the control group, up to 64.3 in patients with sepsis who died.

A particular behavior of plasma IL 10 level was present in patients who died: after an initial value of 40.2 pg / ml in patients who died who had sepsis, and a plasma value of 45.6 pg / ml in patients without sepsis, followed a slight decrease in plasma levels on 3th day. 5th day peak of the deceased patients was much higher than in patients who survived (maximum value of 65.3 pg / ml in deaths compared to 50.4 pg / ml in survivors).

Thus, the of IL 10 curve took an **biphasic aspect,** thing I have not seen in other studies. Has been described a correlation between increased plasma levels of IL-10 with a poor prognosis, has been described even this growth which occurs in patients who die, but a preletal profile has not been achieved.

We believe that increased production of IL-10 in patients with severe sepsis as well as severe burns associated with precipitating factors, can be regarded as relevant to a deathly final, because an increase in IL-10 is associated with severe immunosuppression.

A **persistent response of inflammatory cytokines is characterized by a fatal severe sepsis development. It may be observed that any excess production of IL 10 is pathognomonic for the evolution of burned patient.**

Calculation of prognostic indicators correlated with IL-10, within 24 hours, showed us the best specificity and sensitivity of 0.97 and 0.89 respectively in APACHE II score. A
sensitivity of over 0.80 are also presented. IP (0.83), ABSI score, MPM intake and MODS (0.82).

To observe the variations that exist between the concentration of proinflammatory and antiinflammatory cytokines, networking to these changes with the emergence and development of MODS, I followed the correlation between plasma levels of TNFα, IL 6, IL 10 and MODS. Feature and the difference between TNFα and IL 6 was that while the increase and decrease TNFα occurred slowly, IL 6 increase and decrease in plasma levels followed a downward and upward sharp curve. Prolonged growth and increased concentration of IL 6 in burned patients may be considered when there is early and progressive, as a prognostic factor and evolution to install MODS.

A persistent inflammatory response by increasing the concentration of IL 10 causes immunosuppression, predispose patients to sepsis and / or MODS.

**Correlation of prognostic indicators with the dynamics of the three cytokines studied.** showed that IP is positively correlated with the dynamics of TNFα and IL-6, considering that IP may be a good indicator for early deaths. IP correlate with dynamic of IL-10 only in burned patients who died, in patients who survived, the IP values are inconsistent with the dynamic IL-10.

Regarding the Baux index, I noticed that its values are nearly constant in patients who died, without being able to make such a distinction between early and late deaths, but its evolution shows that Baux index has a positive correlation with the dynamics of TNFα, IL-6 and IL-10.

ABSI score may be a good indicator for early deaths, he is positively correlated with the dynamics of TNFα, IL-6 and IL-10, both in patients who survived and patients who died, an useful prognostic indicator of evolution of burned patients.

MODS score may be an early indicator for the development of MODS cases, having a positive correlation with the dynamics of TNFα, IL-6 and IL-10.

Blot mortality index is positively correlated with the dynamics of TNFα and IL-6, it is a good prognostic indicator of the progress of burn patients. We could say that the mortality index Blot can be a good indicator for death in general. I have not established a statistically significant correlation between the IL-10 dynamics and evaluation for mortality Blot index.

APACHE II score is positively correlated with the dynamics of TNFα, IL-6 and IL-10 it is a good prognostic indicator of the progress of burn patients, especially if performed every 24-48 hours.

MPM score with its variants, has not shown a positive correlation with the dynamics of cytokines than MPM admission and MPM-24 hours, remaining variables were low and not statistically significant.

V. Immunohistochemical study

**The results of immunohistochemical and histopathological study**

Taking into account the strong interaction and liaison between local and systemic factors, I wanted to do a immunohistochemical study of local burn injury, correlated with the cytokines dynamics and systemic reaction that ultimately based on the local level. Local release of pro-and anti-inflammatory cytokine following major trauma, indicates their potential to induce systemic immunological alterations, depending on the balance that exists between different types of cytokines. Cytokines are a component of the immune system, the local factor is involved in the evolution of the burned patient. I relied on these elements and we found it necessary a study of local lesion, to be able to match systemic dynamics of cytokines with local changes.

The objective of this study was to find a link between systemic response of cytokines and local characteristics of burn injury, because severe burns causes an adaptive immune response by producing cytokines released under the influence of Th1 and Th 2 cells.
In most cases, increased Th2-type response does not appear immediately after thermal injury, but noted that the Th2 response is preceded by a Th1 type response. Th1 cells play a major role in initiating cellular immune response, and Th2 cells determines the production of antibodies and plays a role in the production of cytokines. Moreover, Th2 cell response was linked to suppression of cellular immunity.

Skin tissue injury causes an inflammatory response initiation, with local release of cytokines by keratinocytes, fibroblasts, endothelial cells. Secretory role of the epidermis is particularly interested because keratinocytes by autocrine secretion of additional proteins most secret and cytokines in intercellular space, influencing local growth function, metabolic function, inflammatory and immunological function.

Analysis of the immune marker CD45 RO, in this study, revealed a marked inflammatory cell membrane isolated with this antibody on the first day. Analysis of the immune marker CD45 RO in the coming days, revealed a more marked inflammatory cell membrane by this antibody, suggesting that increased inflammatory process was achieved at the expense of increasing the number of T lymphocytes. The cells were predominantly located on the outskirts of structural nodular thus having a similar distribution of lymphoid follicles.

Correlating these data with the dynamics of cytokines, we find that an increase from first day is positively correlated with IL 6 whose values were slightly elevated, but especially with IL 10, which presented values 10 times higher than the maximum standard.

Correlation is negative for this marker with TNFα within 24 hours. For following days, the correlation with cytokines is negative for the expression of proinflammatory cytokines and positive for the anti-inflammatory cytokines.

A large number of T cells expressing a membrane glycoprotein, CLA (cutaneous lymphocyte-associated antigen). After 5 days of thermal injury, immune marker analysis to CLA showed a slight increase in inflammatory cells labeled with this antibody membrane, located mainly around vessels.

T lymphocytes seem to disappear quickly after thermal injury, their growth was observed only after 48 hours, which confirms immunosuppression almost immediately after the heat action.

Also in the study of the skin immune system we have pursued and mature B cells, those that produce immunoglobulins. In sepsis, the B cells (CD20), represents a dramatic reduction in the lymphoid follicles, compared to other major trauma.

Analysis of the immune marker CD20 was negative, suggesting the absence of B lymphocytes within 7 days after thermal injury. Only after two weeks analyzing the immune marker CD20 revealed the presence of a membrane marker in inflammatory cells arranged this time in the center of the nodular structures of superficial and deep dermis. It was thus proved that the immunosuppression in severe burns, stretching over a long period of time, compared to the control group in which immunosuppression was transient and without complications.

There are many cell populations which are involved in the immunosuppression that occurs after thermal injury, but CD8 cells remain the main factor involved in immune response after burn. In patients with severe burns, the predominant source of inflammatory interleukins is represented by CD8 cells that are capable of rising above their five-fold after the 5th day. Weak expression of CD8 on day 5 to show that burns induce a significant change in the direction of Th1 cytokine response and not to Th 2, towards producing inflammatory cytokines. CD 8 expression from the 7th day began to grow. The 7th day many more cytokines are secreted, achieving the peak of the proinflammatory cytokines, showing that CD8 is involved in the increased expression of proinflammatory cytokines IL 6 and TNFα.
CD8 massive reduction occurs at the 14th day, as we found in this study, viewed through a weak positive immuno marking this day. Late decrease of CD8 expression may be a consequence of apoptosis induced by burn injury.

Collagen is the basic substance necessary to wound healing and epithelization rate is partly dependent on the presence of MMP. Determination of MMP-2 expression on the first day showed a weak positive immuno mark in all layers of the epidermis, only more intense in keratinocytes of basal layer. In our study, MMP-2 immuno marking became intensely positive since the 5th day, which leads us to hypothesize that a marked and earlier expression of MMP-2 could be associated with a better quality scar.

Analysis of MMP 9 immuno marking the first day, showed an negative immune marker MMP-9 to studied cases. In the early stages of thermal trauma can not be found proMMP-9 activation in conditions in which TNF-α is an activator of proMMP-9. MMP9 correlation with TNF-α level was positive, as the 5th day of TNF-α level was high, leading to TNF-α transformation of proMMP-9 in MMP-9 mature, TNF-α values in this day being over 11 times higher than the maximum standard.

Analysis of MMP 9 in immuno marking the 14th day, showed a positive mark in the squamous epithelium, predominantly in the basal layer, the sebaceous and sweat glands, and inflammatory cells (lymphocytes, plasma cells, rare polymorphonuclear), showing involvement of the MMP system in the healing process.

The role that apoptosis plays in septic syndrome and the development of CARS and MODS has not yet been sufficiently explored, but it was suggested that apoptosis may play a role in complications of septic status. Increased apoptosis, especially in the lymphoid tissue and some parenchymal tissues may contribute to the association of MODS to sepsis and may be a target for therapeutic intervention.

Studying the influence of apoptosis, we found that Bcl-2 immuno marking in the 5th day, showed a weak positive expression in the basal layer of epidermis cells and focal weak positive in the skin annexes. Immune marker analysis of day 7 of the Bcl-2, showed changes from the previous stages, which allowed us to conclude that while the rapidly proliferating cell population for tissue reconstruction, cell growth is balanced by apoptosis. Bcl-2 immune marker analysis performed on the 14th, showed the same characteristic expression of that on the 7th. Since this time we should have a low apoptosis and thus a marked expression of Bcl-2 for normal wound healing, the presence of only focal expression of Bcl-2, could indicate a defect in wound healing. But this issue has not been met in all cases with keloid scars, so we can not draw a definite conclusion.

What I noticed is that Bcl-2 expression in the fibroblasts could be the most significant element, because I met so intensely positive expression in the epidermis and the skin annexes. Apoptosis is a local process but that is not significantly affected by the overall condition of the patient, but especially by the dermal hypoxia.

**FINAL CONCLUSIONS**

1. Accurate assessment of the severity and prognosis of the burned can be linking by clinical severity scores with the dynamics of plasma cytokines and immunohistochemical study of local lesion.

2. By setting the cut-off scores of studied indices, we can say that there is a statistically significant difference in the distribution of deaths according to the cut-off value because the values have exceeded the 95% confidence (p <0.05) and the confidence of 99% (p <0.01).

3. **Immunological study.**

   Dynamics of serum cytokines studied showed the following data:

   3.A. **TNFα**
TNFα is a cytokine whose plasma levels increase as early as 24 hours early after the onset of thermal aggression. Growth has been explosive (15-20 times) primarily in patients who later died. Growth was much slower in patients who survived. It can be said to have a prognostic value in certain cases, especially when correlated with other prognostic factors.

TNFα is correlated in dynamic with Baux score, ABSI, APACHE II and mortality index Blot.

3.B. IL-6

For IL-6 the most significant element is that there was an increase mainly as a result of plasma levels of inflammatory response and as a result of septic complications and may thus be a prognostic indicator.

In patients who developed sepsis and survived, increased plasma IL-6 appeared early and had a biphasic dynamic.

In patients complicated with sepsis and MODS, increased plasma IL-6 has occurred early, was progressive and fatal cases, remained elevated until death.

Correlation of IL-6 with clinical prognostic indicators was positive for IP, Baux index, the score ABSI, Blot mortality index, APACHE II score (repeated every 21-48 hours), MPM admission and MPM 24 hours.

3.C. IL-10

IL-10 was associated with a strong immunosuppression.

Plasma levels of IL-10 were initially raised in the first 24-48 hours, regardless of body surface area burned. Subsequently, the dynamics of IL-10 was varied according to body surface area burned, septic complications and MODS, it can be considered a prognostic marker.

A persistent inflammatory response, accompanied by increased serum IL-10 induces immunosuppression that predisposes to sepsis and MODS.

Linking the dynamics of IL-10 with clinical studied prognostic index was positive for Baux, ABSI score, MODS and APACHE II score (repeated every 21-48 hours).

4. Immunohistochemical study of local lesion

This study argues serious about the relationship between local and systemic factors.

Dynamics of systemic cytokines was correlated with local changes.

T lymphocytes are the main immune system cells to the wound, had a different behavior from the burned skin.

The study included modifications occurred on days 1, 5, 7, 14 postburn, IHC antibody CLA CD45RO, CD20, CD8, MMP-2, MMP-9, Bcl 2, correlated with plasma dynamics studied cytokines.

CLA is involved in the skin's immune system. Positive immune marking there was existence of inflammatory lesions and was positively correlated with growth of proinflammatory cytokines (TNFα, IL-6) and negatively with anti-inflammatory cytokines (IL-10).

CD45 RO derived from endothelial cells was stimulated by proinflammatory cytokines studied (TNFα, IL-6), until the 14th day, when serum concentration of inflammatory cytokines has been declining. After that day, he got a positive marker linked immuno-inflammatory cytokines (IL-10).

CD20 is involved in regulating the expression of B lymphocytes. Immune marker analysis was negative, suggesting the absence of B lymphocytes, which correlated with immunosuppression given by immediate increase of IL-6, IL-10 and TNFα slow growth.
CD 8, which is an important factor in the immune system, represented an important source of stimulating the production of cytokines by day 5, 7th.

In this study, late decreased of CD8, seems to be a consequence of burn injury-induced apoptosis.

MMP-2 correlated with the dynamics of cytokines, was negative on the first day, but intensely positive in the coming days in all epidermal layers.

The presence of immunohistochemical detection of MMP-2 expression may be associated with a scar quality.

MMP-9 had a positive correlation with its expression in the dynamics of IL-6 and IL-10 and a negative correlation with TNFα, having a role in collagen remodeling.

Cytokines that may control cellular mechanisms of interaction have a role in wound healing.

Modulation of cytokine secretion is extremely important, such as cytokines monitoring can give an image of generalized inflammatory response assessment, which can support a clinical diagnosis and may be a prognostic method.

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