IMMUNOLOGICAL PROFILE OVERVIEW IN BURNS

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Abstract

Introduction: The injury caused by burns induces an inflammatory response with activation of all inflammatory pathways. Sepsis and multiple organ failure are often death causes beyond the immunitary system dysregulation.
Methods: For this prospective study, we selected about 20 patients, from the age of 25 to 50, second and third degree burnt, ranging from 20% to 60% TBSA (total burned surface area). All these patients are submitted to balneotherapy and surgical treatment (grafting). None of the patients had physical disorders before the accident.
The analysed parameters are the cytokines IL-6, IL-8, IL-10, IL-12 , CD4/CD8 ratio, NK counting and C3c fraction. The monitoring is initiated very precociously and repeated three days after, with one week intervals or when clinical report justifies it. We tried to minimize the influence of procedures like plasma transfusion and surgical treatment by ensuring similarities among patients and collecting time adjusting.
Results and Conclusion: A dysregulation of cell-mediated immunity and alterations of cytokines occurs. We registered less NK cells and T helper and a higher value of CD4/CD8 ratio among non-survivors.
The rapid increase of C3, in all patients, is the consequence of an immune response, probably to prevent infection. The increase was continuous and persistent in non survivors potentiating inflammation wich is impeditive of tissue reconstruction.
Thus, the two major processes that are responsible by immune response failure and fatality are an extended inflammatory response mediated by the excessive production of IL-6, immediately at admission (partially related with burn area), and the depression of cell-mediated immunity. Even when we studied the ratio *concentration/burn area* comparing survivors/non survivors, the significant difference persists about the time of admission. There is no evidence of Th1 response commitment.

Introduction

The study of mortality in severe burns shows that most patients die because of septic problems and multiple organ failure. After the first barrier loss, the cutaneous barrier, immune system has a hard work to do. The increased susceptibility to infection and morbidity can be related with perturbations of natural and adaptive immunity. Many studies show immunological compromise but controversial results. There are no standard perturbations for similar situations.
At the time of injury, there are symptoms of chemotactic and phagocytic defects, increase in T-suppressor and a decrease of Th and NK cells (Dehne, 2002). Studies had registered an increased IL-4 and diminished production of interleukin-2, which is produced by Th cells (O'Sullivan, 1995). The exposure of naive Th cells may cause conversion to either Th1 or Th2 phenotype (Fig.1). A suppression of Th1 cells by conversion in Th2, rather than Th suppression seems to occur (Zhou, 1999; Dehne, 2002). IL-6 is produced by T cells, macrophages and fibroblasts and exerts important functions in haematopoiesis and antibody production. Same studies correlated this cytokine to mortality (Dehne, 2002). IL-8, produced by many cell types, is a neutrophil chemotactic and activating factor and has angiogenic activity. It seems to be strongly related to fatality in burn victims (Dehne, 2002, Dugan, 2004). Changes in IL-10 concentrations (elevation) were demonstrated in some studies (Dehne, 2002), but its specific role in the situations remains unclear. This cytokine, produced by activated macrophages, is an eosinophils activator, promotes antibody production and can suppress macrophages by TNF blocking. It has been demonstrated that IL-10 can down-regulate class II MHC expression on macrophages and, indirectly, inhibit the T cells and Natural Killer activation (Howard, 1992). IL-12 is produced by macrophages, dendritic cells and B lymphocytes. It acts on T naive cells to differentiate to Th1 cells. Also induces IFNδ production and NK cells and enhances NK activity. Some studies results, about this cytokine in burns, were inconclusive. On other hand, in a Kang (2003) study, changes of complement system seemed to correlate to the outcome of patients. They showed low C3 levels initially but increased on the following days. This increase was prominent in survivors.
Serum immunoglobulin levels are severely depressed in large burn injuries especially during the first week. The depletion may be due to increased catabolism and loss by wound exsudates (Oliva, 1993; Lehnhardt, 2004). There is a depletion of immunoglobulin G from periods ranging from days to weeks following thermal injury (Shirani, 1984). This suppression may enhance the risk of infection.
Intravenous immunoglobulins (IVIG), have been used for substitutive therapy in burnt patients and others. In several studies, IVIG therapeutic seems to prevent infections. However, there are contradictory results. Apart from few exceptions no clear benefit of IVIG therapy has been reported for the curative management of several infections. During autoimmune and systemic inflammatory disease IVIG has immune modulatory effects like Fc receptor-mediated effects, modulation of complement, modulation of cytokine production and superantigens neutralization (Emmi, 2002). Nevertheless, it is important know that IVIG is a heterogeneous product and so, it's difficult to determine the exact actuation mechanism in each disease.
A study demonstrated that IVIG treatment did not act on T cells but caused a significant decrease in the number of B lymphocytes and an increase in the number of Natural Killer cells (Dibirdik, 1995), but many other studies did not encounter this effect or change in the infection or mortality rates (Waymack, 1989). Most of these studies were done in 80s -90s and only looked for therapy replacement effects in symptoms and Igs concentrations themselves. Some studied phagocytosis by opsonic capacity of burn serum (Hansbrough, 1988). Recently the interest revived because IVIG treatment application continues among the burn therapeutics. Studies demonstrated inhibition of toxic necrolysis (Chang, 2004). The specific effect of IVIG therapeutic on immune response is still unclear.

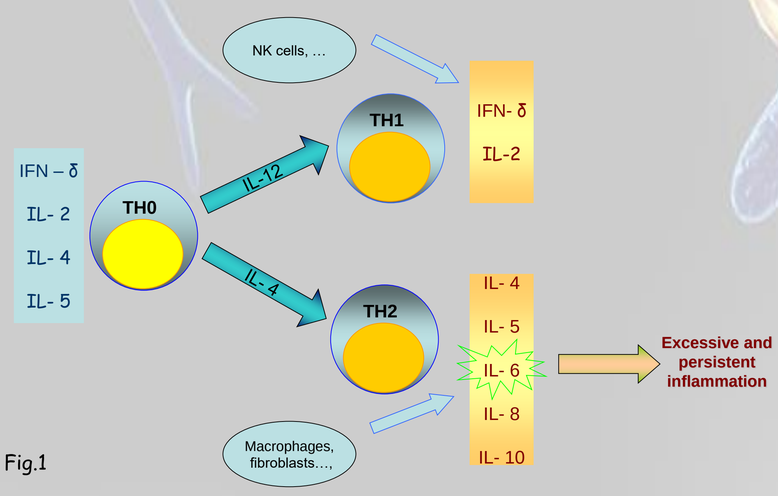

Fig.1

Material and Methods

Patients - This is a work in progress. For this prospective cohort study, we expect to study a 30 patients group (minimum). The study includes, for now, 18 patients.
We examined the burn victims, admitted to the Burns Unit of Coimbra University Hospitals, aged 25 to 50, second and third degree burnt, ranging from 20% to 60% TBSA (total burned surface area). All patients are submitted to balneotherapy and surgical treatment (grafting). We tried to minimize the influence of procedures like plasma transfusion and surgical treatment by ensuring similarities among patients and collecting time adjusting. None of the patients had physical disorders before the accident.
The group was monitored for the immunological profile in order to give an overview about immunological alterations in severely burned patients, correlated to burned area, gender, post-burn evolution and fatality looking for prognostic indicators. Thus, the present study and a more comprehensive understanding of the phenomena will hopefully provide a precocious detection of the more serious situations.
However one of the aims of the study is to select a small group of patients to submit to IVIG treatment and so investigate the utility of this therapeutic strategy, until now only three of the patients received this threatment. One of them is still in hospital.
The monitoring is initiated very precociously (immediately after admission), repeated three days after and with one week intervals. Other samples can however be collected when clinical report justifies it.
Lymphocytes subpopulations/NK counting – The procedure used to calculate percentage and the absolute values of lymphocytes (total), CD19+, NK, T cells, T suppressor and CD4/CD8 ratio was flow cytometry. The aim was the overview of the general immune response.
C3c complement fraction – We used nephelometry (Nephelometer Analyzer II - Behring) to

Quantitative determination of IL-6; IL-8; IL-10; IL-12 – The concentrations were determined using plasma samples and sandwich enzyme immunoassay technique (ELISA) in a Behring ELISA processor II with Quantikine® of R & D Systems kits. The development of an effective immune response involves various cells. These molecules (IL), like all cytokines, are mediators of the interactions among the referred cells. So, we selected four cytokines, which could lead us to conclusions about immune response, searching for some associations, already referred to in literature although controversial.
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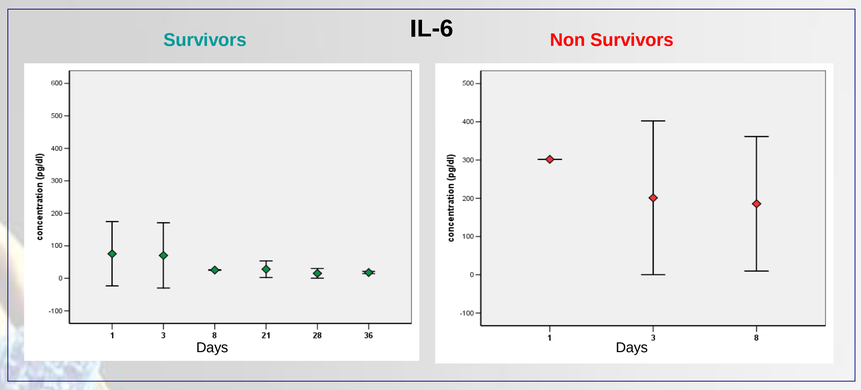
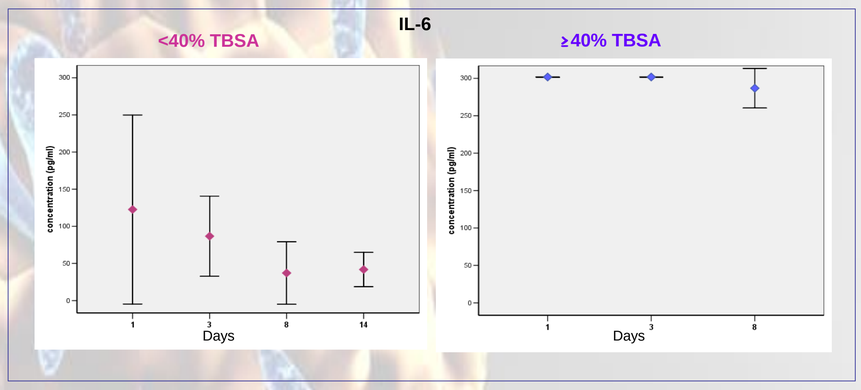
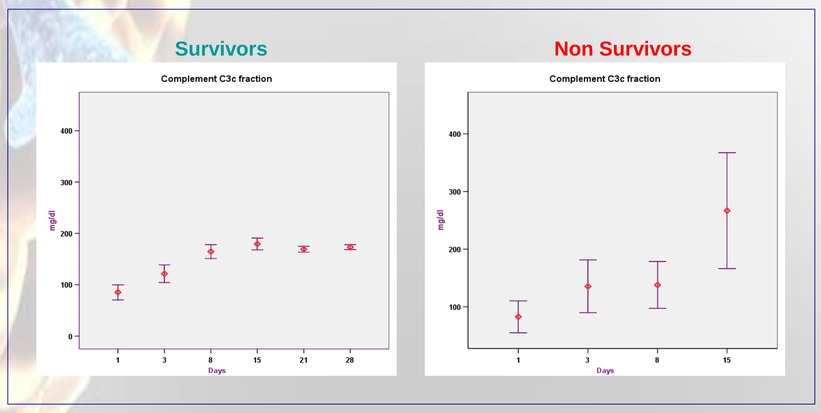
Statistical Analysis - Data was analysed using SPSS 14.0. The comparison of survival/non survival and › 40% TBSA/≤ 40%TBSA with the studied immune parameters was performed using Student t test. By extending the studied group, we will certainly be able to reach more reliable conclusions.

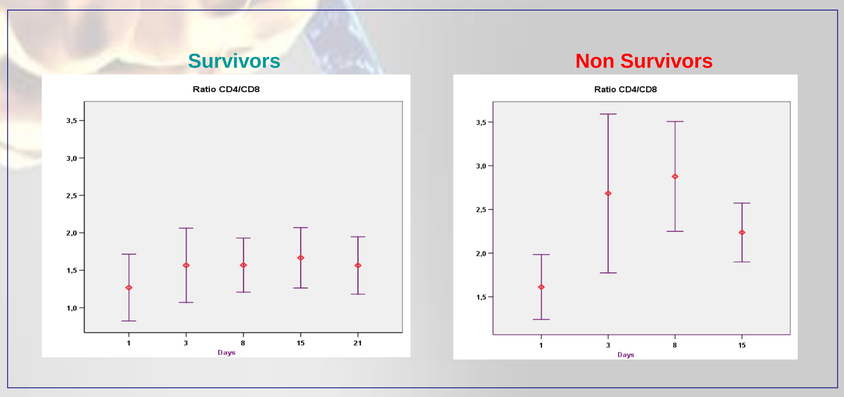
Results

The presented graphics show only the more reliable effects registered among study group:









Discussion and Conclusions

The injury caused by burns induces an inflammatory response with activation of all inflammatory pathways. A dysregulation of cell-mediated immunity and alterations of cytokines occurs. We registered less NK cells and T helper although with no statistical significance. At the same time, the CD4/CD8 ratio assumed higher value for the non survivors.
The rapid increase of C3, in all patients, is the consequence of an immune response, probably to prevent infection. The increase was continuous and persistent in non survivors potentiating inflammation.
There is evidence of an early increase in **IL-6**, possibly because this cytokine has a rapid response and it participates in acute phase. Non survivors had much higher levels of IL-6, especially at time of admission in hospital ($p<0,02$). These excessive levels cause a persistent and excessive inflammatory process which could lead to a multiple organ failure. The persistent inflammation is impeditive of tissue reconstruction. Thus, the two major processes that are responsible by immune response failure and fatality are an extended inflammatory response mediated by the excessive production of IL-6 (partially related with burn area), and the depression of cell-mediated immunity. Even when we studied the ratio *concentration/burn area* comparing survivors/non survivors, the significant difference persists about the time of admission. IL-6 seems to be a strong prognostic indicator in burned patients and an immunomodulation by exogenous agents, with this target, could be a therapeutic hope.
The concentration of **IL- 8** had an increase at the first-second week, between the non survivors ($p<0,04$ about third day). **IL-10**, a major immunoregulatory cytokine, showed some immunodepression, between the non survivors. Both phenomena are non significant.
IL-12 was similar in survivors and non survivors. Thus, there is no evidence of Th1 response commitment.
We can not conclude about the IVIG therapeutics effect. The patients to whom the Igs was applied had an immune response characterized by an increase of the number of lymphocytes, especially B