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SERUM PROFILE OF IL-6, TNF-ALPHA, IL-12 AND IFN-GAMMA IN EARLY SEPSIS

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Abstract. Aim. Priming and modulation of the immune response in severe infections are driven by bacterial products and endogenous mediators, such as cytokines. We analyzed several proinflammatory cytokines in the early sepsis and studied their correlation with aetiology and severity.
Methods. In 24 septic patients we analyzed serum levels of IL-6, TNF-alpha, IL-12 and IFN-gamma in the early sepsis. 14 healthy volunteers served as controls.
Results. IL-12 and IFN-gamma were undetectable both in controls and patients. Septic patients had a median TNF-alpha concentration of 6 pg/ml at admission that decreased at the following time-points. In controls, TNF-alpha was undetectable. In patients, IL-6 reached a median of 78 pg/ml at admission and diminished subsequently. Patients with severe sepsis had the highest IL-6 levels. TNF-alpha showed similar levels and kinetics for urinary and respiratory sepsis, yet in respiratory sepsis IL-6 increased more prominently. Gram positive sepsis led to higher amounts of TNF-alpha and IL-6 than Gram negative sepsis. Two out of five non-survivors had very high IL-6 at admission that did not follow the later descendent trend.
Conclusions. Since we did not detect circulating levels of IL-12 and IFN-gamma, it appears that sepsis does not to elicit an adaptive immune response in its early phases. The low levels of TNF-alpha may be due to the short half-life of the molecule. IL-6 release might be influenced by the site of infection and aetiology, and it could serve as a potential parameter in monitoring septic patients.
Keywords: sepsis, cytokines, immune response, prognosis

Introduction

The clinical diagnosis of overt sepsis is relatively easy. Nonetheless, an early diagnosis and the classification of patients according to severity and prognosis, both essential elements in guiding the therapy and anticipating patients' future evolution are considerably harder to perform [1]. The current trend is to identify novel criteria for a faster, more efficient patient assessment based on the mechanisms involved in the systemic response [2]. In this context, the in-depth analysis of the inflammatory response to infection represents a major track in the current research on sepsis and seems to have a key role in establishing new diagnostic approaches.

We analyzed several proinflammatory cytokines in the early sepsis and studied their correlation with aetiology and severity.

Material and Methods

This was a prospective study designed to measure serial serum levels of TNF-alpha, IL-6, IL-12 and IFN-gamma in adult septic patients admitted to Clinic of Infectious Diseases from Cluj-Napoca over

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a time span of 1 year. The study closely followed the regulations outlined by NIH and the Declaration of Helsinki and was reviewed by the Ethical Committee of the University. The study did not entail any therapeutic intervention (observational study).

Inclusion criteria were based on the American College of Chest Physicians and the Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference Committee definitions [3]. Severity of illness was assessed by calculating the Sepsis-related Organ Failure Assessment (SOFA) score during the 24 h from admission [4]. Along with the data required for the severe sepsis criteria and calculation of SOFA score, we also recorded demographic and clinical data including age, gender, underlying illness, the primary site of infection, microbial culture results, and procalcitonin (PCT) levels at admission.

After the informed consent, venous blood from adult septic patients was collected the first 3 days and at day 7 upon admission. Serum was obtained, aliquoted and stored at -70° C until analyzed. Sera from 14 healthy volunteers were used as controls.

Serum levels of TNF-alpha, IL-6, IL-12 and IFN-gamma were determined by a sandwich ELISA (R&D Systems Europe) with a sensitivity of 1.6pg/ml for TNF-alpha, 0.7pg/ml for IL-6.5pg/ml for IL-12 and 8pg/ml for IFN-gamma. The minimum detectable concentrations of the assays were 15.6pg/ml for TNF-alpha, 3.12pg/ml for IL-6, 7.8pg/ml for IL-12 and 15.6pg/ml for IFN-gamma.

Assays were performed in duplicate and if cytokines were not detected, the result was imputed below the level of assay sensitivity, and recorded as 0 pg/ml.

Results

The patients (19 males/5 females) had the median age of 65. The primary infection had urinary [11], respiratory [11], dental [1] and cutaneous [1] origin.

19 patients survived and 5 patients (17%) died during the first week. Eleven (45.8%) out of 24 patients had positive cultures, 4 with Gram positive rods and 7 with Gram negative bacteria. At admission, median PCT was 10ng/ml (note that the results were obtained by a semi-quantitative method and they were imputed as above 0.5 for levels between 0.5 and 2ng/ml, and as above 2 for levels between 2 and 10ng/ml) and median SOFA score was 6 (range 1-11). IL-12 was undetectable in all of the samples. IFN-gamma concentrations varied between 0 and 29pg/ml, but the median concentration of IFN-gamma was below the level of assay sensitivity both in controls and septic patients at all time-points. Septic patients had median TNF-alpha concentration of 6pg/ml at admission and decreasing at the following time-points, while in controls TNF-alpha was undetectable. In septic patients, IL-6 had a median level of 78pg/ml at day 1 and decreased at 38 pg/ml, 9.15pg/ml, 2.97pg/ml at day 2, 3 and 7 respectively (figure 1).

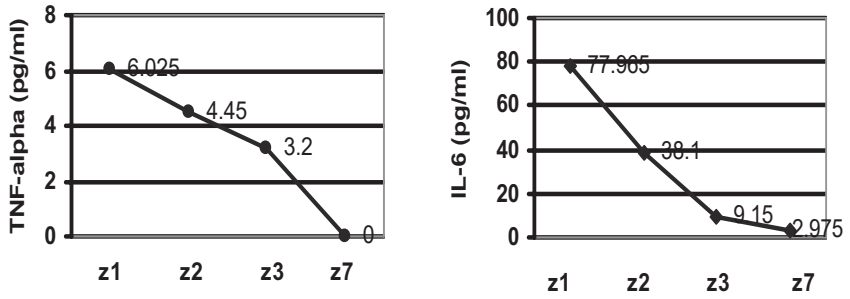

Figure 1 Dynamics of TNF-alpha and IL-6 concentrations in septic patients. Septic patients had median TNF-alpha and IL-6 concentration of 6pg/ml and 78 pg/ml respectively at admission, values that decreased at day 2, 3 and 7.

Patients with severe sepsis (n=18) had the highest IL-6 (reaching a median of 102.2pg/ml) and TNF-alpha levels (with a median of 6.775pg/ml). TNF-alpha showed similar levels and kinetics between urinary and respiratory sepsis, yet in respiratory sepsis IL-6 increased more prominently. Median concentration of IL-6 at admission was 123.3pg/ml in patients with respiratory sepsis versus 62.68pg/ml in patients with urinary sepsis (figure 2).

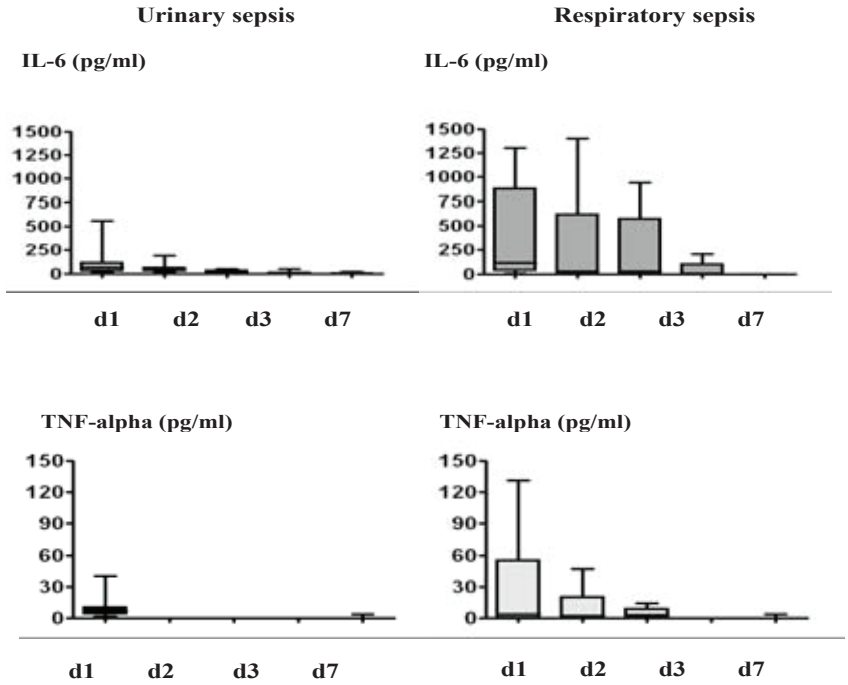

Figure 2 IL-6 and TNF-alpha levels in patients with sepsis of respiratory and urinary origin and in healthy volunteers. TNF-alpha showed similar levels and kinetics between urinary and respiratory sepsis, yet in respiratory sepsis IL-6 increased more prominently.

With respect to the aetiology, we compared the cytokine concentration and noticed that Gram positive sepsis (n=4) led to higher amounts of circulating TNF-a and IL-6, reaching 23,6pg/ml and 130pg/ml respectively, while in Gram negative sepsis (n=7), median levels of TNF-a and IL-6 were 6,6 pg/ml and 60pg/ml respectively. In 12 patients the aetiology was not established (figure 3).

We did not find a significant correlation between TNF-alpha or IL-6 levels and SOFA score, but two non-survivors had very high IL-6 at day 1 (over 1000 pg/ml) that did not follow the descendent trend of IL-6 levels at following time-points. The median values of serum IL-6 were higher in non-survivors than in survivors at all time-points. The median IL-6 concentration remained high in deceased patients while in patients who survived IL-6 showed declining values from day 1 to day 7 (table I).

Discussions

TNF-alpha and IL-6 are proinflammatory cytokines released by monocytes/macrophages and other cell types as a response of the innate immune system to different bacterial stimuli, while IL-12

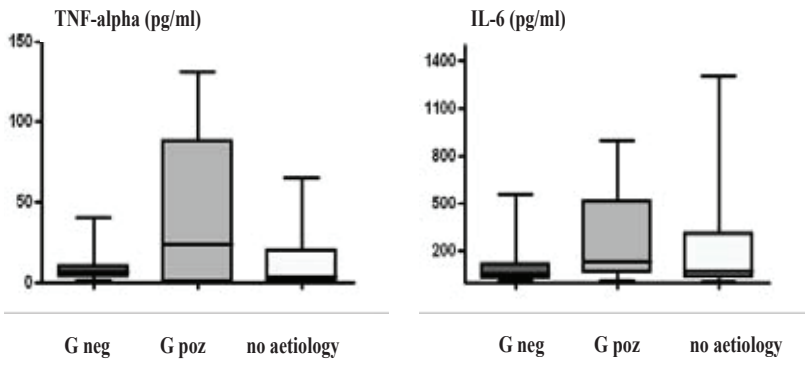

Figure 3 Median concentrations of TNF-alpha and IL-6 with respect to aetiology. TNF-alpha and IL-6 levels were higher in Gram positive sepsis (n=4) compared to Gram negative sepsis (n=7).

Day	Group	Median concentrations IL-6 (pg/ml)
1	Survivors	74,86
	Non-survivors	136,7
2	Survivors	38,1
	Non-survivors	512,5
3	Survivors	8,92
	Non-survivors	483
7	Survivors	2,97

Table I. IL-6 concentrations in survivors and non-survivors.

and IFN-gamma are cytokines having critical roles in regulating the Th1 response to antigens [5]. The present study analysed the dynamics of these four proinflammatory cytokines in the early stages of the septic syndrome and the results revealed that probably the proinflammatory component in the early phases of sepsis is promoted mainly by the innate immunity.

Numerous studies reported a positive correlation between the serum levels of different cytokines, the intensity of the inflammatory response and prognosis in sepsis. Because the serum levels of many cytokines vary largely and rapidly between subjects as well as in the same patients during the evolution of the syndrome, the majority of the authors are sceptic regarding the ability of a single cut-off value of a particular cytokine in orienting the diagnosis [6]. The variation could be explained both by the genetic polymorphism of the molecules, the pleiotropism of various inflammatory compounds or the presence of neutralising plasmatic receptors [7]. On the other hand, the impediments in measuring the levels of certain molecules could be due to some practical aspects associated with the short half life and the *ex-vivo* instability of specific molecules during collection and storage of the serum samples [8]. This hypothesis seems to be endorsed by the results of our clinical study showing very low values of serum TNF-alpha, in contrast with numerous experimental studies indicating moderate or high concentrations of the same molecule.

Increasing attention is nowadays given to the identification and analysis of specific dynamic trends (either increase or decrease) of various markers instead of isolated values. This approach could eliminate the intersubject variability of cut-off values seen with some biomolecules and may allow for a more accurate diagnosis and even earlier initiation of therapy. Several studies have indicated that the kinetics of various parameters may discriminate between favourable and unfavourable outcome. Thus, a gradual decrease in IL-6 levels has been associated with limitation and remission of the inflammatory syndrome and later on, with survival. In contrast, persistently increased IL-6 levels have been associated with subject death [6]. Our study revealed remarkably similar results. Thus, a decreasing trend of IL-6 levels has been present in 100% of survivors. Besides that, in 40% of non-survivors IL-6 revealed either increasing or persistently increased levels. Although obtained in a relatively small group of patients, these results further support the value of monitoring IL-6 time profile instead of isolated values.

During severe sepsis, the clinical evolution is greatly dependent on microbiological factors. Thus, the development of the systemic inflammatory response and the activation of cytokine network greatly differs between the sepsis with Gram-negative and Gram-postive bacteria [9]. With respect to that, the current results, indicating differences between IL-6 levels in Gram-negative and Gram-postive sepses are in line with the putative molecular mechanisms behind the synthesis and release of proinflammatory mediators.

The present study also revealed that the intensity of the inflammatory response depends on the site of infection. This is in line with a previous large multivariate analysis, in which the initial site of infection has been ranked second in importance among the variables predictive for severe sepsis. Moreover, several other authors reported that mortality has been significantly increased in patients with bacteraemia secondary to pulmonary or abdominal infections compared with urinary or catheter-related sepsis [10]. The current results are consistent with all these reports, which revealed higher IL-6 levels during sepsis with respiratory origin compared with urinary sepsis. However, we could not detect any correlations between the primary infection site and sepsis severity as determined using the ACCP/SCCM criteria or from the perspective of organ failure, as quantified by the SOFA score.

Conclusions

During early sepsis, a rapid inflammatory response is mounted and driven by the innate immunity, yet excessive response may prove detrimental. Since we did not detect circulating levels of IL-12 and IFN-g, it appears that sepsis does not elicit an adaptive immune response in its early phases. The low levels of TNF-alpha are maybe due to the short half-life of the molecule. IL-6 release might

be influenced by the primary site of infection and aetiology, and it could serve as a potential routine, reliable parameter in monitoring septic patients. It seems that persistence of IL-6 in the serum rather than peak levels of cytokines predicts a poor outcome in patients with sepsis.

Acknowledgements

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