

# THE PROFILE OF CIRCULATING IL-8 AND GM-CSF IN PATIENTS WITH SEVERE SEPSIS

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## REZUMAT

**Objective:** Proliferarea și activarea neutrofilelor și monocitelor sunt elemente cheie în patogeneză sepsisului. GM-CSF și IL-8 sunt citokine implicate în aceste evenimente. Prezentul studiu și-a propus să investigheze dacă nivelurile serice ale GM-CSF și IL-8 sunt sugestive pentru prognostic și dacă există o relație între acestea și alte date clinice. **Material și metode:** Am analizat nivelurile serice ale GM-CSF și IL-8 în ziua 1, 2 și 7 de la internare la 11 pacienți întrunind criteriile ACCP/SCCM pentru sepsis sever. Șapte voluntari sănătoși au servit drept control. **Rezultate:** Atât la pacienții cu sepsis cât și la voluntari, nivelurile circulante de GM-CSF au fost sub valorile minime detectabile. IL-8 a fost sub valorile minime detectabile la 7 pacienți la toate momentele de determinare. Valoarea mediană pentru IL-8 pentru întreg grupul de pacienți a fost de 0 pg/ml, dar, în timp ce la pacienții cu sepsis de origine respiratorie IL-8 a fost absentă, aceasta a avut o valoare mediană de 32 pg/ml în grupul pacienților cu sepsis urinar. În mod surprinzător, IL-8 a fost detectabilă la 5 dintre cei 7 voluntari sănătoși, cu o mediană de 68,7 pg/ml. **Concluzii:** Nu am identificat niveluri circulante de GM-CSF nici la pacienții cu sepsis, nici la lotul de control. Nivelurile circulante de IL-8 au fost mai mici la pacienți decât la lotul control. După cum s-a sugerat și în studii anterioare, rolul GM-CSF în sepsis ar putea fi la nivel local. Nivelurile scăzute de IL-8 ar putea avea ca explicație imunodepresia asociată sepsisului sever. De asemenea, e posibil ca nivelurile de IL-8 să se coreleze cu situsul primar de infecție și cu imunodepresia legată de acesta.

**Cuvinte cheie:** sepsis, citokine, inflamație

## ABSTRACT

**Objective:** Neutrophils and monocytes proliferation and activation are key events in the pathogenesis of sepsis. GM-CSF and IL-8 are cytokines regulating these processes. The present study investigates if the serum levels of GM-CSF and IL-8 are indicative of prognosis in sepsis and whether there is an association between these and clinical data. **Material and methods:** In 11 patients fulfilling ACCP/SCCM criteria for severe sepsis we analyzed serum levels of GM-CSF and IL-8 at day 1, 2 and 7 after admission. Seven healthy volunteers served as controls. **Results:** GM-CSF was always below detection limit in septic patients and controls. In seven patients, serum IL-8 was below detection limit at all time points. The median value for IL-8 for the group of patients was 0 pg/ml, but while IL-8 was absent in the sepsis of respiratory origin, it had a median value of 32 pg/ml in urinary sepsis. Surprisingly, IL-8 was detectable in 5 out of 7 controls, with a median of 68,7 pg/ml. **Conclusions:** We failed to identify detectable circulating levels of GM-CSF in controls or in severe sepsis. The circulating levels of IL-8 were lower in septic patients than in healthy volunteers. As previously suggested, the locally produced GM-CSF might have a role in this condition. Initial low levels of IL-8 might be a component of the immunodepression seen in severe sepsis. Also, it is possible that the IL-8 levels correlate with the primary site of infection and the severity of the immunodepression related to that.

**Key Words:** sepsis, cytokines, inflammation

## INTRODUCTION

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a key regulatory cytokine that governs the proliferation, differentiation and maturation of polymorphonuclear (PMN) and mononuclear phagocytic progenitors.

This colony-stimulating factor also enhances the effector functions of mature neutrophils, such as adhesion to vascular endothelium, chemotaxis, phagocytosis, superoxide production, and microbial killing.

Although these effects normally increase the defensive response of the host against microbial invasion, activated neutrophils and their products are also capable of autoinjury.<sup>1,2</sup> Thus, excessive or inappropriate systemic granulocyte activation during severe infection has been postulated to contribute to the pathophysiology of the systemic inflammatory response syndrome.

Serum colony-stimulating activity has been noted in the acute stages of human infection for several decades, and elevated circulating levels of

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granulocyte colony-stimulating factor (G-CSF) have been described in a proportion of patients with bacterial infections and in neutropenic patients with fever.<sup>3-5</sup> In these types of patients, circulating GM-CSF usually is not detectable.<sup>6</sup>

However, little is known of endogenous G-CSF and GM-CSF levels in septic shock. Consequently, the potential of endogenously released G-CSF and GM-CSF to contribute to the phagocyte-mediated autoinjury in critical illness remains unknown.<sup>1</sup> Moreover, monocyte dysfunction has been shown to be associated with negative outcome in septic patients, and GM-CSF may be required for optimal monocyte function in these patients. It has been shown that GM-CSF induces monocyte activation and can protect monocytes from going into apoptosis.<sup>7,8</sup>

High levels of circulating proinflammatory cytokines including IL-8 have been described during sepsis, shock and MOF (multiple organ failure) and have been associated with poor outcome.<sup>9-12</sup> However, many of the existing results and correlations partially disagree.<sup>9-13</sup> Thus, while several reports had shown a diagnostic or prognostic value for different cytokines at various time-points other investigators indicate that persistently increased rather than peak values were found predictive for outcome, suggesting an iterative monitoring of cytokine levels.<sup>14-17</sup>

The controversy may be partly due to the fact that life-threatening localized infections such as ventilator-associated pneumonia cannot be diagnosed on the basis of blood-derived cytokine concentrations but generate increased cytokine levels reaching diagnostic levels locally, for instance in the tracheal aspirate.<sup>18</sup>

The present study was undertaken to investigate serum levels of GM-CSF and IL-8 in adult septic patients with the specific objective of determining whether serum levels of these cytokines are indicative of prognosis and whether there is an association between these cytokines levels and various clinical data.

## **MATERIAL AND METHODS**

### **Subjects and Study Design**

This was a prospective study designed to measure serial serum levels of IL-8 and GM-CSF in adult septic patients admitted at Clinic of Infectious Diseases from Cluj-Napoca over a 6 month period. The study followed closely the regulations outlined by NIH and the Declaration of Helsinki and was reviewed by the Ethical Committee of the University.

The study did not imply 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 as it follows.<sup>19</sup>

Sepsis was defined as a systemic inflammatory response syndrome associated with infection along with the criteria of ACCP/SCCM: body temperature  $<36^{\circ}\text{C}$  or  $>38^{\circ}\text{C}$ , tachycardia ( $>90$  beats/min), respiratory rate  $> 20$  breaths/min or  $\text{P}_a\text{CO}_2 < 32$  mmHg (unless the patient is ventilated), a white cell count  $>12\,000/\text{mm}^3$  or  $<4000/\text{mm}^3$  or  $> 10\%$  immature neutrophils (bands).

Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion or hypotension. Criteria for severe sepsis required the presence of at least one of the following: hypotension defined as a systolic blood pressure  $< 90$  mmHg or a reduction of  $> 40$  mmHg from baseline in the absence of other causes of hypotension.

Hypoperfusion was defined as acute alteration of the mental status (evaluated through the Glasgow Coma Score); elevated plasma lactate; unexplained metabolic acidosis with arterial pH  $< 7.3$ ; hypoxemia ( $\text{P}_a\text{O}_2 < 70$  mm Hg breathing room air, or an acute drop in  $\text{P}_a\text{O}_2$  of  $> 15$  mm Hg below baseline with breathing room air or hypoxemia requiring mechanical ventilation); prolonged prothrombin time or a decrease of platelet count of more than 50% or under  $100\,000/\text{mm}^3$ ; oliguria.

Severity of illness was assessed by calculating the Sepsis-related Organ Failure Assessment (SOFA) score during the 24 h from admission.<sup>20</sup> (Table 1)

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.

### **Sample Collection**

After informed consent, venous blood from adult septic patients was collected at day 1, 2 and 7 upon admission. Serum was obtained, aliquoted and stored at  $-70^{\circ}\text{C}$  until analyzed. Sera from 7 healthy volunteers were used as controls.

### **Cytokines levels**

Plasma levels of IL-8 and GM-CSF were determined by a sandwich ELISA (R&D Systems Europe) with a sensitivity of 3.5 pg/ml for IL-8

and 3 pg/ml for GM-CSF. The minimum detectable concentrations of the assays were 31.2 pg/ml for IL-8 and 7.8 pg/ml for GM-CSF. 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.

### Statistical analysis

Median values were calculated in the groups of septic patients and healthy volunteers. Statistical evaluation was performed using the non-parametric Mann-Whitney U-test. Correlation between parameters was analysed by the Spearman rank correlation test.

## RESULTS

### Patient characteristics

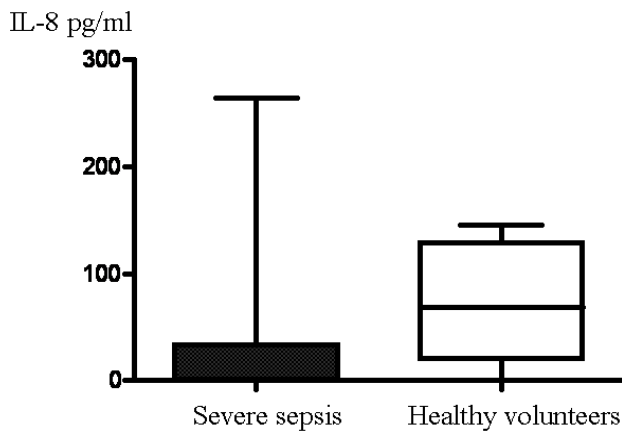
Demographic and clinical data are summarized in Table 2. Patients (10 males /one female) had the median age of 68 years (22-87). The primary infection had urinary (seven cases) and respiratory origin (four cases). The median number of SIRS criteria was three. All patients survived except one patient that died after five days upon admission following acute myocardial infarction.

**Table 2.** Patient characteristics

Patient No.	Age	Gender	Primary site of infection	No. of SIRS criteria	SOFA score Day 1
1	70	M	Urinary	2	9
2	81	F	Respiratory	3	11
3	87	M	Respiratory	3	7
4	77	M	Urinary	4	6
5	68	M	Urinary	2	6
6	54	M	Urinary	3	4
7	22	M	Respiratory	3	4
8	79	M	Urinary	3	5
9	65	M	Respiratory	2	5
10	24	M	Urinary	4	4
11	32	M	Urinary	3	2

**Table 1.** The SOFA score

SOFA score	1	2	3	4
			Respiration with respiratory support	
Respiration with respiratory support PaO <sub>2</sub> /FiO <sub>2</sub> , mmHg	< 400	< 300	< 200	< 100
Coagulation Platelets x10 <sup>3</sup> /mm <sup>3</sup>	< 150	< 100	< 50	< 20
Liver Bilirubin, mg/dl	1.2-1.9	2-5.9	6-11.9	> 12
Cardiovascular Hypotension >15 or (doses in ug/kg·min) catecholamines> 0,1	MAP < 70mmHg	Dopamine ≤ 5 or Dobutamine (any dose)	Dopamine > 5 or catecholamines ≤ 0.1	Dopamine
Neurologic Glasgow Coma Score	13-14	10-12	6-9	< 6
Renal Creatinine mg/dl or Urine output ml/zi	1.2-1.9	2-3.4	3.5-4.9 (200-500)	> 5 (< 200)



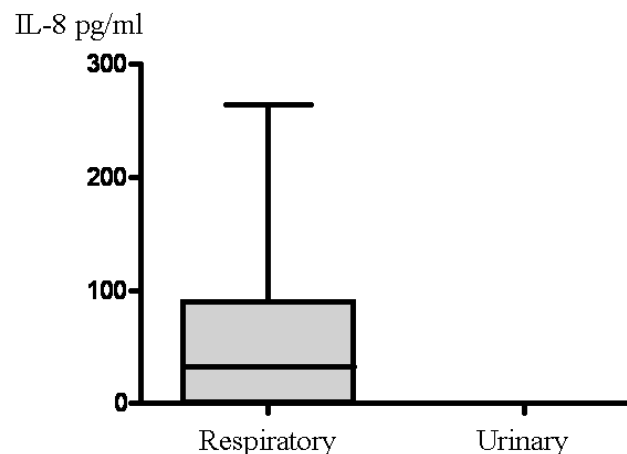
**Figure 1.** IL-8 levels in severe septic patients and in healthy volunteers. The median value of IL-8 for the group of patients (n = 11) was 0 pg/ml (0-264 pg/ml), while in controls (n = 7) it was 68.7 pg/ml (0-145.2 pg/ml), with detectable levels in five out of seven healthy volunteers.

### Cytokine concentrations, PCT and microbiological findings

Cytokines levels, PCT and microbiological findings are presented in Table 3. Four out of 11 patients had positive blood cultures (Gram-negative). The number of SIRS criteria positively correlated with positive blood cultures ( $r = 0.668$ ,  $P > 0.05$ ). At admission median PCT was 2ng/ml (0.5-10 ng/ml) and did not correlate with sepsis severity as assessed by the SOFA score ( $r = 0.184$ ,  $P > 0.05$ ).

GM-CSF was always below detection limit (7.8 pg/ml) in septic patients and controls. In seven patients, serum IL-8 was below detection limit (31.2 pg/ml) at all time points. We compared cytokines

concentrations among septic patients and in healthy volunteers and observed that IL-8 levels were higher in healthy subjects. The median value for IL-8 for the group of patients was 0 pg/ml (0-264 pg/ml) and, surprisingly, IL-8 was detectable in 5 out of 7 controls, with a median of 68,7 pg/ml (0-145.2 pg/ml). (Fig. 1) We noted an association between IL-8 levels and the primary site of infection. While IL-8 was absent in the sepsis of respiratory origin, it had a median value of 32 pg/ml in urinary sepsis. (Fig. 2)



**Figure 2.** The association between IL-8 levels and the primary site of infection IL-8 had a median value of 32 pg/ml (0-264 pg/ml) in urinary sepsis (n=7) and it was absent in sepsis of respiratory origin (n=4).

### Cytokine concentrations and organ failures

No significant correlation was found between the cytokine concentrations and the severity of acute organ dysfunction, as assessed using the SOFA score ( $r = -0.225$ ).

**Table 3.** Overview of the cytokine levels, PCT and microbiological findings

Patient No.	IL-8 (pg/ml)			GM-CSF (pg/ml) Day1	PCT (ng/ml)	Blood cultures	Other cultures
	Day 1	Day 2	Day 7				
1	<30	< 30	< 30	< 7.8	10	negative	
2	< 30	< 30		< 7.8	0.5	negative	negative
3	< 30	< 30	< 30	< 7.8	10	negative	negative
4	< 30	< 30	< 30	< 7.8	10	E. coli	negative
5			< 30	< 7.8	2	negative	negative
6			< 30	< 7.8	10	E. coli	negative
7	< 30	< 30	< 30	< 7.8	2	negative	negative
8		< 30	< 30	< 7.8	2	negative	negative
9	< 30	< 30		< 7.8	0.5	negative	Moraxella catharalis
10		< 30	< 30	< 7.8	2	Klebsiella	Klebsiella
11	< 30	< 30	< 30	< 7.8	0.5	E. coli	E. coli

## **DISCUSSIONS**

The current study found lower IL-8 levels in septic patients compared with healthy volunteers and no GM-CSF increase in the early course of severe sepsis.

Numerous reports previously indicated considerable increase of several proinflammatory including IL-8 in septic patients. Several studies have even suggested a relationship between pro-inflammatory cytokines and outcome, but their conclusions are sometimes inconsistent.

Cytokine profiling of patients with severe sepsis may represent a valuable tool for delineating different patterns of immunological response, thus allowing identification of groups of patients with homogeneous biological derangements.<sup>21</sup> However, the cytokine patterns associated with the evolution of organ dysfunction are not well established. IL-1 beta, IL-6, IL-8, IL-10, MCP-1 and G-CSF, were found to correlate positively with organ dysfunction, as assessed by the SOFA score on Day one.<sup>15</sup>

Surprisingly, we did not find increased circulating GM-CSF at any time point, despite robust leukocytosis in some patients. Previous studies reported significant increases in circulating G-CSF, in conjunction with several other cytokines.<sup>15</sup> Many of these studies were based on larger but more heterogeneous groups of patients, having diverse etiologies and different degrees of severity. A relationship between severity of sepsis and circulating GM-CSF has never been clearly established. However, an inversely proportional relationship between the two entities may be possible.<sup>7</sup>

The absent circulating GM-CSF in septic patients argues against a pathophysiologic role for GM-CSF in the systemic modulation of granulopoiesis during infection. However, a paracrine role, synergistically with other cytokines in the microenvironment of tissues such as the bone marrow and the lung remains more likely.<sup>1</sup>

In contrast with several previous studies showing increased IL-8 levels early during sepsis, we found lower circulating IL-8 in patients with severe sepsis as compared with the healthy controls.

This feature has not been previously reported, and may be an expression of the immunodepression observed during sepsis. In response to infectious stimuli, monocytes/macrophages release a number of mediators, including cytokines, involved in the proinflammatory response that underlies sepsis. The excessive release of these mediators triggers the development of systemic inflammation, and has essential roles in the pathogenesis of sepsis. Moreover,

septic patients also undergo an anti-inflammatory phase (the compensatory anti-inflammatory response syndrome-CARS) and sometimes, a mixed response with both pro-and anti-inflammatory components (the mixed antagonistic response syndrome-MARS). Also, it has been demonstrated that sepsis shows a biphasic immunological pattern during the initial and later phase: the early hyperinflammatory phase is counterbalanced by an anti-inflammatory response which may lead to a hypoinflammatory state.<sup>22</sup> This so-called immunoparalysis is characterised by monocytic deactivation and has been associated with poor prognosis in septic patients. Impaired monocyte function is reflected by decreased cytokine production, decreased HLA-DR surface expression and associated with decreased capacity to present antigens. Thus, fatal outcome seems to be more frequent in patients with lower HLA-DR expression.<sup>23</sup>

The present findings are consistent with an earlier report showing that neutrophils obtained from severely septic patients display reduced IL-1beta and IL-8 production upon LPS or streptococcal stimulation.<sup>24</sup> IL-8 may be produced by various types of cells in response to a wide variety of stimuli, including proinflammatory cytokines, microbes and their products, and environmental changes such as hypoxia or reperfusion. However, numerous observations have pointed out the role of IL-8, a main cytokine produced by the neutrophils to perpetuate the neutrophil-mediated acute inflammation within an autocrine loop.<sup>24</sup>

It may be argued that the main drawback of the study could be represented by the small number of patients. However, the patients formed a rather homogenous group in terms of severity, comparable with existing studies and revealed differences and patterns within subgroups. The subanalysis according to the infection site suggested that respiratory sepsis leads to a more profound reduction of IL-8 in sepsis than urosepsis. This concurs with earlier observations indicating that respiratory sepsis represents a more severe form, associated with higher mortality than those with urosepsis or catheter associated bacteremia.<sup>25</sup>

## **CONCLUSIONS**

In conclusion, we found lower circulating levels of IL-8 in septic patients than in healthy controls while GM-CSF levels remained undetectable in both healthy individuals and septic patients. The initial low levels of IL-8 might be a component of the immunodepression seen in severe sepsis. It is also possible that the IL-8

levels correlate with the primary site of infection and the severity of the immunodepression related to that.

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