

**IL-6, MMP 3 AND PROGNOSIS IN PREVIOUSLY HEALTHY SEPSIS PATIENTS***IL-6, MMP-3 Y PRONÓSTICO EN PACIENTES CON SEPSIS PREVIAMENTE SANOS**Juan P. Ricarte-Bratti, Nilda Y. Brizuela, Nicolás Jaime-Albarran, Hilda L. Montrull***Abstract**

Sepsis and septic shock are clinical conditions with high mortality despite advances in technology and are the leading cause of death in intensive care. Clinical manifestations and morbidity may be attributable to a disproportionate increase in proinflammatory cytokines. The aim of this study was to evaluate the ability to predict mortality from interleukin 6 [IL-6] and matrix metalloproteinase 3 [MMP-3]. This single-center, observational, prospective study included 48 adult patients admitted to the Hospital Nacional de Clinicas in Cordoba, Argentina, with sepsis or septic shock. Serum levels of IL-6 and MMP-3 were measured at the time of diagnosis and 72 hours later. At time of admission, MMP-3 was 13.77 mg/ml in patients who died and 10.55 mg/ml in patients who survived up to 28 days after hospitalization [p = 0.012], while IL-6 did not differ between the groups. The change in IL-6 over 72 hours was increased in nonsurvivors by  $21.11 \pm 11.81$  pg/ml and decreased in survivors by  $40.87 \pm 14.94$  pg/ml [p = 0.007]. No difference in the change of MMP-3 over 72 hours was observed between survivors and nonsurvivors. This study shows that MMP-3 at admission and the change in IL-6 over the first 72 hours of hospitalization could provide prognostic information in septic patients. Further studies are needed to define the utility of these cytokines as a measure of sepsis severity and as predictors of mortality.

**Keywords:** Sepsis, Cytokines, Matrix Metalloproteases, Interleukin 6, Prognosis, Mortality

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## Introduction

Sepsis and septic shock refer to systemic responses of the body to an infection, wherein organ damage occurs at sites remote from the initial insult. The terms sepsis, severe sepsis, and septic shock were defined in 1992, [1] reviewed in 2002, [2] and are now widely used in intensive care and internal medicine. Sepsis has a very high occurrence rate and is characterized by hemodynamic disturbances and imbalances in tissue oxygenation along with organ dysfunction.

Septic shock is a major factor associated with morbidity and mortality among critically ill patients and remains to be a condition with high mortality, [3, 4] despite recent advances in understanding its pathophysiology and the development of novel treatment strategies.

In 1995, 751,000 new cases of sepsis were estimated to have occurred in the United States, causing 215,000 deaths, and this incidence was projected to grow by 1.5% per year. [5] In Argentina, no reliable large-scale population data about sepsis incidence is available.

Sepsis is the culmination of complex interactions between the infecting organism and the immune response and inflammatory and coagulation systems. The rational use of therapeutic targets in sepsis comes from a good understanding of its underlying pathophysiology. A massive inflammatory reaction occurs in the initial phase of sepsis, which is followed by an anti-inflammatory response. [6] These phenomena are regulated by chemical mediators including cytokines, chemokines, and inflammatory cells such as polymorphonuclear neutrophils and macrophages. Therefore, inflammatory as well as anti-inflammatory biomarkers could potentially be used to diagnose and predict outcome in sepsis.

Cytokines, including tumor necrosis factor-alpha, interleukin [IL]-1, IL-6, and nitric oxide are involved in the clinical progression of sepsis, leading to inflammation, vasodilation, increase in capillary permeability with vascular leakage, hypotension, multiorgan failure, shock, and finally death. [6] Some cytokines have shown potential for diagnostic and/or prognostic inference in sepsis. [7] A panel of cytokines including metalloprotease [MMP]-3 and -10, IL-1, and eotaxin have been

tested in sepsis and proposed for validation in clinical trials. [8] IL-6 is primarily produced by monocytes/macrophages, fibroblasts, endothelial cells, T cells, and stromal cells of the bone marrow. Together with IL-1, IL-6 is a primary inducer of the synthesis of acute phase proteins, especially fibrinogen, which connects the immune system with the hemostatic system.

MMPs are a family of zinc-containing endoproteases implicated in degradation and remodeling of the extracellular matrix; they play a role in sepsis by facilitating the recruitment and migration of leukocytes, modulating the inflammatory response, [9] and participating in the coagulation and fibrinolytic response. [10-12]

MMP-3 [stromelysin-1] is involved in proteolysis of extracellular matrix proteins such as collagen II, IV, and IX; proteoglycans; laminin; fibronectin; gelatins; elastin; [13] and fibrin. [14] In addition, it can activate several other MMPs. [13] This enzyme plays important roles in cardiovascular matrix remodeling. It has not yet been well studied in sepsis, but elevated plasma levels of MMP-3 have been reported in patients with sepsis. [15]

The aims of this study were to know the difference between survivors and non-survivors to sepsis or septic shock in serum levels of IL-6 and MMP-3 at admission, and the change in the first 72 hours of these cytokines with the treatment given.

## Materials and methods

### Population

This single-center observational study was conducted in the Emergency Department and Intensive Care Unit of Hospital Nacional de Clinicas, a university tertiary hospital in Cordoba, Argentina. Between July 2009 and July 2010, 48 consecutive previously healthy patients over 18 years of age who fulfilled the systemic inflammatory response syndrome [SIRS] criteria defined by the American College of Chest Physicians/Society of Critical Care Medicine [ACCP/SCCM], [1] were enrolled. Patients were followed for 28 days or until death, and they were treated with antibiotics and fluid resuscitation according to the "Surviving Sepsis Campaign" Guidelines. Vasoactive agents, mechanical ventilation, ultrafiltration, and any other therapies deemed necessary were administered.

The following exclusion criteria were applied: patients aged <18 years; patients [or their relatives] who declined to participate; and patients with comorbid conditions including diabetes, active cancer, chronic heart, kidney or liver failure, chronic obstructive pulmonary disease, human immunodeficiency virus infection, or connective tissue disease, in order to mitigate false positives in cytokine quantification.

This study was approved by the Hospital Ethics Committee. Written informed consent was obtained from all participants or relatives. Patients with sepsis who survived their stay in the intensive care unit were followed for 4 weeks through outpatient clinical assessment and phone follow-up. Those who died during that period from any cause were considered non-survivors. Patients who were alive at the end of the 4-week period were defined as survivors.

### Sepsis criteria

Sepsis was diagnosed according to the International Sepsis Definitions Conference criteria and it was defined as a documented or suspected infection with or without isolation or not of microorganisms plus general, inflammatory, or hemodynamic parameters and the presence of organ dysfunction and tissue perfusion. <sup>[2]</sup>

### Clinical and other variables

The following data were recorded for each patient: sex, age, urea, creatinine, leukocyte formulae, hemoglobin, lactic acid, platelets, activated partial thromboplastin time, arterial blood gas, sodium, potassium, and Acute Physiology and Chronic Health Evaluation II [APACHE II] score. Sepsis-related complications and mortality over the follow-up period were recorded. Cultures were obtained from any potential source of infection using standardized methods. Complementary imaging or other methods were performed according to the clinical scenario of the patient. Any necessary imaging performed was included in the patient records.

### Measurement methods

Subject data, medical history, and findings on physical examination were recorded at the time of

admission to the Emergency Department. In addition, blood samples were drawn by venipuncture for routine laboratory examinations and cytokine quantification. An additional blood sample was obtained 72 hours after admission. These samples were collected in heparinized tubes, separated by centrifugation at 2500 rpm for 10 minutes, serum samples were obtained and stored at -70°C until assayed. Samples that showed alterations, such as hemolysis or fibrin, were discarded, and a new sample was obtained. If the collection of a new sample was not possible for any reason, the patient was excluded from the protocol. Levels of MMP-3 and IL-6 were quantified by enzyme-linked immunosorbent assay using commercial kits [Invitrogen Corporation, Camarillo, CA, USA], following the manufacturer's instructions.

For MMP-3 coefficient of variation [CV] intra assay is 2.8 to 4.9%, while the inter-assay CV is 4.3 to 6.5%. For IL-6 intra assay CV is 5.7 to 7.7 % and inter assay CV is 6.5 to 9.3% [Invitrogen Corporation, Camarillo, CA, USA].

### Statistical methods

Serum levels of MMP-3 and IL-6 were compared between survivors and nonsurvivors, and at admission and 72 hours later. Results are expressed as mean  $\pm$  standard deviation. Variables with normal distribution were compared by Student's t test, and those without normal distribution were compared by the Mann-Whitney test. The relationships between various parameters were studied by regression analysis with Pearson's correlation matrix and calculated using Fischer's test. The statistical analysis was performed using SPSS statistical software [version 15.0, Chicago, USA]. P values less than 0.05 were considered statistically significant.

### Results

All patients were previously healthy and had no known comorbid conditions, excluding the cause of sepsis that led to their admission. All patients were followed for 28 days, and the finding of any disease mentioned in the exclusion criteria during that period of time led to the removal of the patient from the protocol. Patients without confirmed sepsis or any doubt of the diagnosis were also ex-

cluded.

Initially we included 88 patients, but 40 were excluded based on the exclusion criteria described above. The leading cause of exclusion was the diagnosis of cancer during hospitalization. The characteristics of the 48 patients ultimately included are shown in Table 1. Age, mean arterial pressure, pO<sub>2</sub>, creatinine, SOFA score, APACHE II score, septic shock, and multiorgan failure significantly differed between survivors and nonsurvivors. No other differences were found between groups.

IL-6 serum levels did not differ at time of admission: mean IL-6 concentration was 203 ± 26 pg/ml in nonsurvivors and 161 ± 24 pg/ml in survivors [p = 0.26] [Figure 1]. At 72 hours after treatment, mean IL-6 concentration was 121 ± 17 pg/ml in survivors and 205 ± 26 pg/ml in nonsurvivors [p =

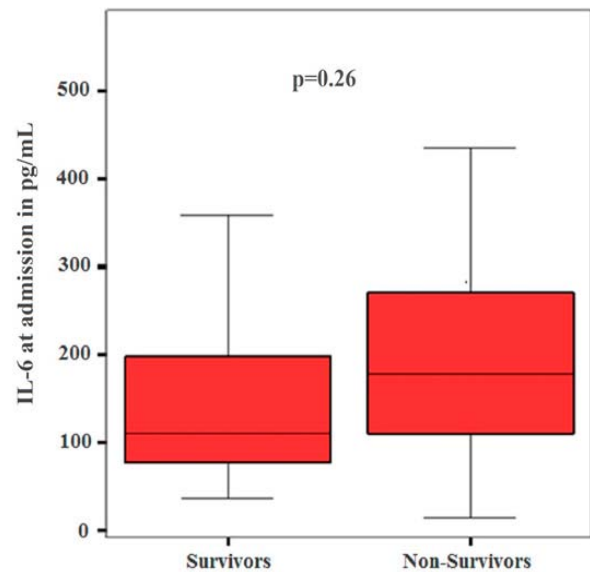
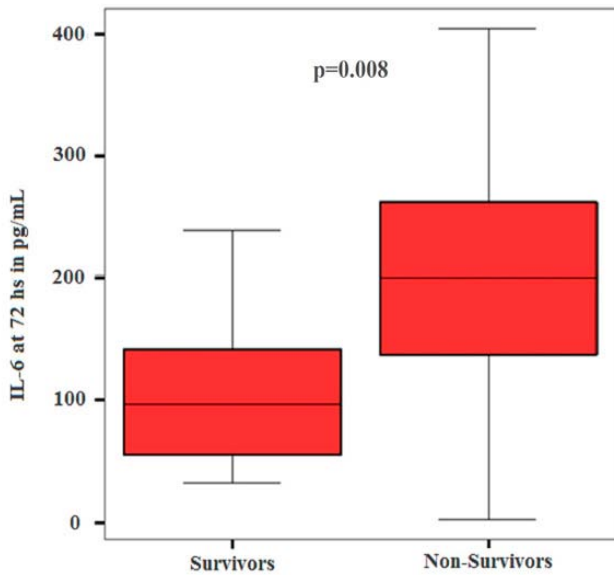


Figure 1. Boxplot: Serum IL-6 levels in survivors and non-survivors of sepsis at time of admission, p = 0.26

Table 1. Characteristics of previously healthy sepsis patients according to 1-year survival in Córdoba, Argentina.

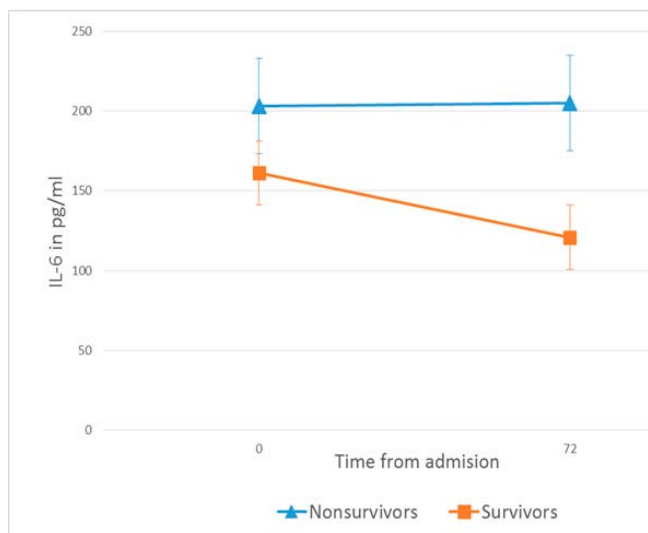
	Overall [N=48]	Survivors [N=27]	Nonsurvivors [N=21]	p
Age in years [SD]	61.9 [17]	53.8 [17.1]	73.2 [8.9]	<0.001
Male sex, number [%]	22 [45.8]	13 [48.1]	9 [42.8]	0.715
Body Mass Index [SD]	24.3 [3.5]	23.8[3.4]	25.5 [3.8]	0.137
Mean arterial pressure at admission [SD]	65.4 [28.7]	74.5 [26.3]	52.6 [27.6]	0.008
pO <sub>2</sub> at admission [SD]	66.7 [16.5]	72 [14.6]	59 [16.5]	0.008
Creatinine [mg/dL] at admission [SD]	1.44 [0.81]	1.18 [0.65]	1.79 [0.89]	0.009
pH at admission [SD]	7.4 [0.079]	7.42 [0.57]	7.36 [0.98]	0.056
HCO <sub>3</sub> [mEq/L] at admission [SD]	21.2 [4.6]	21.5 [3.3]	20.8 [6.1]	0.638
SOFA Score [SD]	8.2 [2.8]	5.5 [2]	10.8 [3.4]	0.006
APACHE II Score [SD]	17.6 [8.2]	13.9 [5.5]	22.9 [8.6]	<0.001
Length of stay in days [SD]	9.72 [7.3]	9.78 [6.9]	9.65 [8.0]	0.951
Septic shock, number [%]	18 [37.5]	6 [22.2]	12 [57.1]	0.013
Multiorgan failure, number [%]	21 [43.7]	6 [22.2]	15 [71.4]	<0.001
Inotropics/vasopressors	18 [37.5]	6 [22.2]	12 [57.1]	0.013
Mechanical Ventilation	9 [18.7]	0 [0]	9 [42.8]	<0.001
Source of Sepsis, number [%]				
Lung	22 [45.8]	14 [51.8]	8 [38.1]	NS
Genitourinary	11 [22.9]	9 [33.3]	2 [9.5]	NS
Abdominal	7 [14.6]	1 [3.7]	6 [28.6]	NS
Skin and Soft Tissue	5 [10.4]	2 [7.4]	3 [14.3]	NS
Meningitis	3 [6.3]	1 [3.7]	2 [9.5]	NS

0.008] [Figure 2].



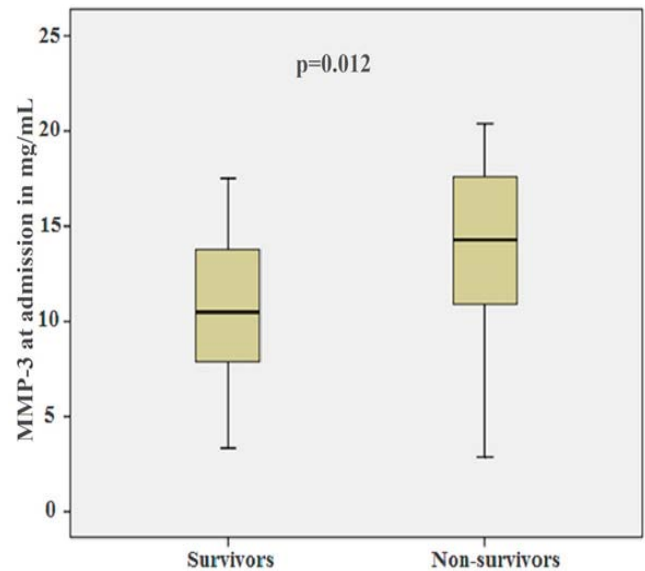
**Figure 2.** Boxplot: IL-6 at 72 hours after hospitalization in survivors and nonsurvivors of sepsis,  $p = 0.008$

From these data, we calculated the change in IL-6 plasma levels over the first 72 hours. Survivors had a mean value decrease in IL-6 of  $41 \pm 15$  pg/ml, and nonsurvivors had an mean value increase of  $21 \pm 12$  pg/ml [ $p = 0.007$ ] [Figure 3].



**Figure 3.** Change in serum IL-6 levels in survivors and non-survivors of sepsis over the first 72 hours after hospitalization,  $p = 0.007$

MMP-3 significantly differed between survivors, who had a mean plasma concentration of  $10.55 \pm 0.76$  mg/ml, and non-survivors, with a mean concentration of  $13.77 \pm 0.98$  mg/ml, at the time of admission [ $p = 0.012$ ], as shown in Figure 4.



**Figure 4.** Boxplot: MMP-3 in survivors and nonsurvivors of sepsis at time of admission,  $p = 0.012$

However, the change in MMP-3 over the first 72 hours after admission did not significantly differ: non-survivors and survivors demonstrated mean increases in MMP-3 of  $0.79 \pm 1.27$  mg/ml and  $0.169 \pm 0.86$  mg/ml, respectively [ $p = 0.677$ ].

## Discussion

At admission we found that IL-6 was not related with mortality, consistent with findings published by De Freitas,<sup>[16]</sup> and Calandra.<sup>[17]</sup> However consistent information exists for the role of IL-6 to predict mortality in sepsis.<sup>[18–21]</sup> The number of patients included in this study does not allow to draw prognostic value of these cytokines, so further studies with more patients are needed to validate these findings.

Serum levels of IL-6 associated to APACHE II score was a prognostic indicator of sepsis mortality,<sup>[22]</sup> although not independently for predict mortality. Our findings were not consistent with this re-

port, because we found that the non-fall in plasma IL-6 concentration over the first 72 hours under treatment was associated with death independently, and may predict mortality. We observed a statistically significant difference between survivors, in whom IL-6 decreased, and nonsurvivors, in whom IL-6 remains constant. These findings were reported previously in other publications, [17, 24] but only with gram negative infections or acute distress respiratory syndrome, with no previously healthy patients. It is possible that IL-6 generates these effects over time by its power to modulate cardiovascular function in inflammatory response. [23-25]

IL-6 may be "controlled" by several molecules. It is known the relation between TNF alpha and IL-1 beta in sepsis and other conditions, [26] and the chronovariation of IL-6. It is possible that IL-6 could be influenced by these and more molecules, but the predictive power that we have found in the kinetics of IL-6 over time could not be minimized. Moreover Tambuyzer et al found in pigs that IL-6 may have considerable individual variations and a static quantification of this cytokine could not be representative of the inflammatory state, so they concluded that IL-6 fluctuations should be used as an indicator of the infection state. [27]

We further found that MMP-3 at admission may predict mortality at 4 weeks, because non-survivors had higher serum concentrations at admission than survivors. Thus, measurement of MMP-3 at admission could guide physicians to opt for more intensive care and aggressive therapies. However this difference was small and need to be validated prospectively.

Some medications used in sepsis, like vasopressors, hydrocortisone, and activated protein C, can affect MMP expression. [28, 29] In the present study, hydrocortisone was used in more than 50% of patients, and norepinephrine in 37.5%. The use of hydrocortisone beyond septic shock due to bronchospasm with poor response to bronchodilators. All patients that received norepinephrine at medium-high doses received hydrocortisone too. In addition hydrocortisone were given to patients with severe airflow obstruction by respiratory secretions and in some patients with acute distress respiratory syndrome. We cannot exclude these

therapies from the arsenal of therapies to treat sepsis.

Time-dependent changes in other MMPs in sepsis have been well documented. For example, MMP-9 has a peak early in sepsis induced by lipopolysaccharide from *Escherichia coli*, returning to normal values in 24 hours. [30, 31] However, MMP-9 did not predict multiorgan failure, [32] and more some authors even reported that this metalloprotease was decreased in sepsis patients. [33] MMP-3, 8, 9, and 10 were shown to differ between patients with and without sepsis, [34] but demonstrated no significant difference when compared between survivors and nonsurvivors. It is crucial to mention that despite the conflicting reports of previous studies, MMPs play an important role in the physiopathology of sepsis, [34, 35] although their precise functions are not well understood.

MMPs may play a complex role in sepsis because this process connects the inflammatory and coagulation systems. MMPs participate in the recruitment of leukocytes from the bloodstream to the site of infection, [36, 37], in inflammation, [38, 39] and in coagulation/fibrinolysis response. [10-12, 41] MMPs further mediate proteolysis in the basement membrane and recruit immune cells to the site of disorder [36], and also modulate and are modulated by cytokines. [39, 40] Plasmin activates pro-MMPs, acting as a regulator over their function. [10] To elucidate if MMP-3 or another MMP is responsible for each function mentioned may be the subject for future research, with the objective to control this response and ultimately progress the treatment of sepsis toward improving its morbidity and mortality.

Metalloproteases and Cytokines may connect in many phases. Perhaps the union of IL-6 to its receptor may be for the time being the most important nexus of both systems. IL-6 Receptor alpha generates significantly changes in levels of ADAM 10 and 17 (metalloproteases). [42]

In conclusion, we found that in patients with sepsis, the increase of IL-6 in the first 72 hours after admission was associated with a higher risk in mortality, while the decrease of serum IL-6 levels was associated with a lower risk, although IL-6 at admission did not differ between survivors and non-survivors. MMP-3 at the time of admis-

sion was significantly higher in patients who did not survive, but its change over the first 72 hours after admission did not differ between groups. Thus, MMP-3 at admission and the change in IL-6 could provide prognostic information beyond the traditional risk scores. The main limitation of this study was the small number of patients included. Further studies are needed to define the utility of these molecules as a measure of sepsis severity and mortality prediction.

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