Pro- and Anti-Inflammatory Balance of Septic Patients is Associated with Severity and Outcome

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Abstract

***Purpose*: To study inflammatory profile in patients with sepsis, severe sepsis and septic shock with regards to organ dysfunction and outcome, and to identify a pattern associated with more catastrophic course of illness, organ failure and risk of death.**

***Material and methods*: Twenty-nine consecutive patients with sepsis admitted to a medical Intensive Care Unit of a tertiary university hospital (November 2002-December 2003). Plasmatic levels of interleukin-6 (IL-6) and interleukin-10 (IL-10) as pro-inflammatory and anti-inflammatory markers were measured at baseline, 12, 24 and 48 hours of evolution.**

***Results*: There is a positive association between higher levels of IL-6 and severity of the septic process, organ dysfunctions and risk of death, statistically significant at anytime (at baseline, 12, 24 and 48 hours, $p < 0.05$). Higher IL-6/IL-10 ratios associate significantly with risk of death at 24 hours (RR=1.45 if higher or equal to the median).**

***Conclusions*: Plasmatic biomarkers measurement during the initial phase of sepsis may help to individualize therapy. An evaluation at 24 h based on IL-6/IL-10 ratio may anticipate a more aggressive inflammatory profile. These patients would specially benefit from immunomodulating therapies to improve survival.**

Key words: Inflammation, sepsis, interleukin, severity, outcome.

Introduction

Over the past years our understanding of the pathophysiology of inflammation and sepsis has significantly progressed but efforts to lower mortality have been elusive and it still remains a highly lethal disease [1,2].

Evidence from experimental and clinical studies supports the concept that sepsis is initiated by an uncontrolled and unbalanced hyper-inflammatory response to infection that is elicited by mediators (cytokines) released by a wide range of cells (monocytes, macrophages, neutrophils and endothelial cells) once activated by bacterial constituents [1,3,4]. This state is denominated a systemic inflammatory response syndrome (SIRS). After that, early in the course of sepsis patients develop a so-called compensatory anti-inflammatory response syndrome (CARS), a hypo-inflammatory status [5] that is probably not compensatory at all. Furthermore, in any SIRS, and in the early phase of sepsis, changes in plasmatic concentrations of pro-inflammatory and anti-inflammatory cytokines directly correlates to sepsis mortality and the increase of IL-6/IL-10 ratio are predictive for outcome [6,7] . Despite certain shortcomings, plasma remains one of best sources for measurement of sepsis-related mediators enabling a

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rapid characterization of the patient's inflammatory status and prognosis [6,8,9].

Considering this, we decided to clinically study the pro- and anti-inflammatory balance of patients with sepsis, severe sepsis and septic shock with regards to organ dysfunction and outcome. The hypothesis was that patients who do not survive have or at least associate with a more pro-inflammatory profile determines more severe metabolic, microcirculatory and ultimately organic derangements that finally lead to multiple organ failure (MOF) and death. Also, we wanted to try to disclose if there is a pattern on the cytokine profile that could indicate a more aggressive course of the illness.

Materials and Methods

Twenty-nine patients diagnosed with sepsis were prospectively enrolled and admitted to the Medical Intensive Care Unit (ICU) of a tertiary university hospital from November 2002 to December 2003. It is a close ICU ruled by intensivists with consultation to other specialties as required. The study was approved by the Ethical Committee of the Catholic University of Chile and all patients or their relatives signed an informed consent. Plasmatic levels of interleukin-6 (IL-6) as a pro-inflammatory marker and interleukin-10 (IL-10) as an anti-inflammatory marker were measured at different times from baseline, 12, 24 and 48 hours of evolution.

Patient selection and management

The study population included all new consecutive adult patients meeting the criteria of sepsis, severe sepsis and septic shock according to international definitions and bacteriological evidence of infection was requested. Patients were included before 24 hours after ICU-admission. Exclusion criteria were: patients with suspected immunosuppression (recent immunosuppressive therapy, malignancy or neoplastic hematological disease, non-septic neutropenia, HIV-infected) and pregnant women.

All patients were managed according to a standard hemodynamic and respiratory algorithm. In brief, it starts with fluid resuscitation to reach a median arterial pressure (MAP) >70 mmHg with a central venous pressure (CVP) >12 mmHg or a pulmonary artery occlusion pressure (PAOP) between 14-16 mmHg if available. If MAP <70 mmHg after that a norepinephrine (NE) infusion was started until 0.3 μg/kg/min. If hypotension persisted despite NE, epinephrine was started. In patients with low cardiac output or signs of persistent hypoperfusion (lactic acidosis, S_vO_2 <65%, oliguria or clinical signs of hypoperfusion) dobutamine was started. No patient received steroids or any immunosuppressive drug during the study period. Initial empiric antibiotic therapy was started early after recognition of the sepsis state under our local antimicrobial policy and was adjusted later (if appropriate) according to cultures and antimicrobial sensitivity.

Protocol and samples processing

All enrolled patients were classified as presenting sepsis, severe sepsis or septic shock. At admission (0 hours) and 12, 24 and 48 hours after; 10 ml venous blood samples were extracted from all patients using a heparinized tube, stored at -70 ºC and then processed to measure IL-6 and IL-10 levels.

Samples were centrifuged at 2500 rpm for 15 minutes using a centrifuge refrigerated at -4 °C (24.8 °F), and plasma was frozen at -70 °C (-94 °F). Cytoscreen TM, Biosource International Inc. (California, USA) was used to determine plasma levels of IL-10 and IL-6, according to the procedure recommended by the manufacturer. In short, the method consisted in quantitative determination by ELISA (Enzyme Linked-Immuno-Sorbent Assay) in a solid phase "sandwich" technique, in which antibodies directed against specific sequences of human IL-10 and IL-6 were bound. Antibodies allow the binding of their respective antigens (IL-6 and IL-10). This procedure is repeated once again. After removing the biotinized antibody surplus, streptavidine-peroxidase complex is added which, in its turn, is bound to the biotin. After the binding, the excess is washed off and tetramethylbenzydine is added, producing a colored compound, detectable at 450 nm in direct proportion to the antigen's concentration. This colorimetric change was determined in an ELISA reader. Previous studies

in our laboratory showed that plasma from patients with severe SIRS presented high concentrations of both interleukins (IL-6 and IL-10) in relation to the kit's detection range. Therefore, samples were diluted 1:5 for determination of IL-6 and IL-10 in order to maintain the linearity of concentration vs. 450 nm given by the standard curves of the respective kits.

Data collection

At admission the following data were registered: general demographic data, diagnosis, relevant medical history and APACHE II score. Additional daily clinical and laboratory information was registered in order to determine MODS (multiple organ dysfunction score), fluid balance, vasopressor doses and any other clinically relevant information. Follow-up of patients was carried out until discharge or death.

Statistical analysis

Interleukin levels are presented as medians ±25% of quartiles. Discrete data were analyzed by the chi-square test. Continuous variables were considered non parametric and analyzed with one-way analysis of variance using the Kruskal-Wallis test with the Dunn correction for multiple comparisons to test for differences among groups. Wilcoxon's test and the Mann-Whitney test were used for comparison of two sample cases. A p value of less than 0.05 by a two-tailed test was considered to indicate statistical significance.

Results

Twenty-nine consecutive patients fulfilled sepsis criteria and were eligible for entry into the study. Nine patients fulfilled criteria for sepsis, 6 for severe sepsis and 14 for septic shock (See **Table 1** for general characteristics).

As expected, APACHE II score, MODS score and lactate levels increase progressive and significantly as the severity of the sepsis increases (p <0.003). Mortality rate was zero for patients with sepsis and severe sepsis, and 50% patients with septic shock. Most frequent diagnoses in all groups were acute pyelonephritis and pneumonia. All patients included in the study had a known infectious agent.

Cytokine levels and kinetic analysis

All patients were followed up until the moment of discharge or death; 92% of all samples were obtained. When comparing IL-6 levels between septic, severe septic and septic shock patients we observe there is a positive association between higher levels of IL-6 and the severity of the septic process, a fact that is present from admission and persists at 48 hours and it is statistically significant (p <0.009 at admission; p <0.05 at 12 h; p <0.02 at 24 h and p <0.003 at 48 h) (**Table 2, Fig 1a**).

IL-6 levels were also significantly higher in patients who had 3 or more organs in dysfunctions at admission, at 12 and 48 h as compared to those who had 2 or fewer organs in dysfunctions (p <0.05 for all) and at 24 there was a trend towards significance (p <0.09) (**Table 3a**). Also, patients who died had significantly higher levels of IL-6 from admission to 48 h in comparison with those who survived (**Table 3b**).

Regarding IL-10, the levels were also elevated in all patients as compared to normal (literature) levels, but we were unable to find any difference in IL-10 levels between septic, severe septic and septic shock patients at any time during the study period (**Table 2, Fig 1b**). In the same way, there were no statistical differences in IL-10 levels between patients with 3 or more organs in dysfunction as compared to those with 2 or fewer (**Table 3a, Fig 1b**) nor in patients who died as compared to those who survived (**Table 3b**).

IL-6/IL-10 ratio

When comparing IL-6/IL-10 ratio among patients with sepsis, severe sepsis and septic shock it is possible to observe significant higher values of this index in association with the severity of the sepsis. This association is significantly present from admission to 48 h (except at 12 h) (**Fig 1c, Table 2**). Higher values of the ratio are observed in patients with 3 or more organs in dysfunction from admission to 24 hours and in patients who died at 24 hours (**Fig 2b, Table 3**).

Consequently, the relative risk of death if IL-6/IL-10 ratio is higher or equal to the median is 1.45 than if it is lower than the median and the Odds Ratio (OR) is 4,24 (**Table 3**).

Discussion

The septic syndrome is not a single-shot disease but rather a repeated series of insults resulting in an overproduction of pro-inflammatory mediators followed by a prompt secretion of anti-inflammatory mediators, leading to alternate peaks of pro- and anti-inflammatory molecules in the circulation in a redundant, synergistic, and self-sustaining process [10, 11] . Here we describe the kinetics of pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines during the first 48 hours in patients with severe and non-severe sepsis. As expected, patients with severe sepsis are characterized by a significant higher mortality, number and intensity of organ dysfunction, higher APACHE II score, longer ICU and hospital stay and higher incidence of shock. Mortality in sepsis and severe sepsis is similar to that reported in the literature [2].

IL-6 and IL-10 as markers of inflammation

The validity of IL-6 as a marker of inflammation could be questioned. IL-6 is a pleiotropic cytokine with paramount roles in regulation of immune response, inflammation, and hematopoiesis [12]. It is a mediator as well as a biomarker and is rapidly induced in the course of acute inflammatory reactions associated with injury, infection and stress [13]. Some research has shown that IL-6 associates with a negative control of inflammation in certain contexts by inducing the release of IL-1ra (interleukin-1 receptor antagonist) and soluble TNF-a (tumor necrosis factor alpha) receptors and more important, inducing the release of acute phase proteins able to exert some anti-inflammatory effects that may limit the inflammatory process [14, 15] and these findings may explain why IL-6 has been shown to be protective in some models of septic shock. Overall, pro-inflammatory effects of IL-6 predominate: neutrophil cytotoxic potential enhancement [16], priming of neutrophils to produce platelet activating factor (PAF) and superoxide anion [17] and IL-6 has been described as an important mediator of coagulation pathway in sepsis [6]. Moreover, IL-6 may induce adhesion molecules (ICAM-1) expression [18,19], and unlike many early cytokines such as TNF-a and IL-1, IL-6 is readily secreted and circulates in large quantities and for longer time in sepsis [8,13]. For that reason, we and others consider IL-6 is a reliable marker of inflammation in sepsis.

IL-10 is expressed and secreted by a variety of cell types including T cells, monocytes-macrophages and epithelial cells, usually after activation. It exhibits potent anti-inflammatory effects and it is supposed to play a crucial role in both the resolution and pathogenesis of acute illnesses and sepsis [20]. Its concentrations associates with the magnitude of the inflammatory response and exerts its actions decreasing levels of pro-inflammatory cytokines or by inhibition of translocation of nuclear factor kappa-B in monocytes stimulated by lipopolysaccharide or TNF-a and promotes degradation of TNF, IL-1 alpha and beta mRNA [21,22]. Some pro-inflammatory effects of IL-10 have been found in patients treated pharmacologically with this cytokine and in volunteers with experimental endotoxemia [23,24].

At present, it is acknowledged that both IL-6 and IL-10 has not unique and exclusive biological effects, which are strongly modulated by the tissue, pathological setting and genetic background of the patient. Anyway, we may agree IL-6 has predominantly pro-inflammatory effects and IL-10 anti-inflammatory ones.

We observed that septic patients without severity criteria displayed a balanced immune response between the pro-inflammatory and the anti-inflammatory compounds, reflected in a low IL-6/IL-10 ratio that is not associated with organ dysfunctions or mortality. On the other hand, we found that the more severe the septic process (addressed by SIRS criteria, organ dysfunctions and mortality) the higher the levels of IL-6 observed from admission to 48 h. IL-6 was elevated in all patients with sepsis, but was significantly higher in those with severe sepsis, patients with shock and in those who died. IL-10, although also elevated in all septic patients in comparison with healthy controls, remains steadily high along the study period in contrast with IL-6 and do not reach significantly higher levels

as severity of sepsis increase and we think this is a consequence of the small size of sample. Septic shock patients display significantly higher levels of IL-6 that may reflect a highly active pro-inflammatory state, with an unbalanced immune response more properly reflected in an elevated IL-6/IL-10 ratio that in turn is associated with a high incidence of organ dysfunction and mortality. This unbalanced response starts early in the course of the septic process and persists at least for 48 hours.

Taniguchi *et al* [7] in a study of 25 patients with SIRS secondary to multiple trauma (11/22 patients, 44%), infection (11/22 patients, 44%) and other reasons (3/25 patients, 12%), reported that in non-survivors IL-6 concentrations remain high and stable, IL-10 concentration decreased, and the ratio of IL-6/IL-10 increased. Also, IL-6/IL-10 ratio at 48 h was significantly higher in non-survivors than in survivors. Despite the small number of patients we found that IL-6/IL-10 ratio was significantly elevated in patients with severe sepsis, in patients with shock and in patients with three or more organs in dysfunctions at different times, reflecting a predominance of this pro-inflammatory response over the anti-inflammatory one as severity increases. Most important, in terms of outcome, at 24-h the IL-6/IL-10 ratio is significantly higher in patients who die compared with those that survive. Our findings confirm and extend the reported results to a clinical setting, a population of exclusive septic patients and are in concordance with a large body of evidence derived from studies in animals and humans that show that the synthesis of pro-inflammatory cytokines is a prerequisite for initiating the anti-infectious process whereas their exacerbated production during severe sepsis may contribute to the deleterious consequences observed in septic shock and that the balance of tissue pro- to anti-inflammatory cytokines directly correlates with severity of infection and mortality [6,25].

The linear transition where septic patients begin with SIRS, characterized by excessive production of pro-inflammatory mediators (hyper-inflammatory status) that is progressively suppressed by the development of the compensatory anti-inflammatory response (hypo-inflammatory status) syndrome (CARS) is not supported by the current data [5,26]. Pro-inflammatory cytokines are elevated in septic patients, and also the presence of anti-inflammatory mediators at the onset of sepsis have been confirmed [27]. It is still unclear whether septic shock patients die because the anti-inflammatory response is insufficient to control an overwhelming inflammatory response or because it induces a state of relative immunosuppression that impairs host defense mechanisms [28]. After the initial hyper-inflammatory state, the development of a sustained anti-inflammatory or immunosuppressive state ('immunoparalysis') has been demonstrated [5, 29] and could be responsible of late complications and/or death. The fact that many of the pathogens responsible for the secondary, hospital-acquired infections are not particularly virulent in patients with normal, competent immune systems highlights this concept. We were unable to determinate this in our small cohort but this clinical question demands further study.

Based on our results and in the literature, it is clear that patients with more severe sepsis have an unbalanced immune response, with a predominantly pro-inflammatory pattern during (at last) the first 48 hours of evolution of sepsis. This group of patients may be unable to raise an appropriate anti-inflammatory response to the infection in the early course of the disease, leading to a state that may be associated with more severity, shock, organ failure and death. It is still a challenge to try to identify this group of patients at the beginning of the septic process in order to anticipate a catastrophic course. We believe that a high IL-6/IL-10 ratio could anticipate it and that these patients would specially benefit from immunomodulating therapies (e.g. high-volume hemofiltration) [30]. As experimental data [6] suggest that the simple measurement of a pro-inflammatory cytokine is inappropriate for classifying the inflammatory status during sepsis and to address outcome, based on our results, we propose an evaluation at 24 h based on IL-6/IL-10 ratio to disclose a more aggressive inflammatory profile that allow making an intervention. This concept definitely must be more studied and a specific trial with the appropriate design and size of patients is needed.

Conclusion

Early outcome predictions based on plasma biomarkers during the initial phase of sepsis may help to individualize a therapy. Previous studies demonstrated association of high levels of IL-6, IL-10 and IL-6/IL-10 ratio with outcome in SIRS patients of different etiologies. Here we confirm these findings in a population of exclusive septic patients and propose an evaluation at 24 h based on IL-6/IL-10 ratio to disclose a more aggressive inflammatory profile. These patients would specially benefit from immunomodulating therapies to improve survival and more studies on this issue are needed.

Conflicts of Interest

None

Table 1. GENERAL CHARACTERISTICS OF PATIENTS

	Non severe sepsis (9)	**Severe sepsis (6)**	**Septic shock (14)**	***p* value**
Age (years)	60.8±22.4	67.8±14.1	70.1±12.62	ns
APACHE II score	5.63±2.5	12.2±7.73	19.1±6.46	<0.003*
MODS score	0	2.83±1.72	8.43±3.48	<0.0001*
Organs in dysfunction (n)	0	2.17±0.98	4.29±1.07	<0.0008*
Lactate (mmol/L)	1.2±0.5	1.45±0.35	4.6±2.7	<0.009*
Temperature (ºC)	38.4±0.5	38.3±0.5	38.2±1.4	ns
Heart rate (beats/min)	97.0±9.9	102.6±27	120±21.24	<0.009*
Respiratory rate (breath/min)	19.9±6.2	21±13.6	32.4±7.82	<0.006*
LOS (days)	2.5±1.5	5.75±2.5	9.08±8.15	<0.04*
Main diagnosis [n, (%)]				
Acute pyelonephritis	6 (66.6%)	2 (33.3%)	5 (35.7%)	
Pneumonia	1 (11.1%)	1 (16.6%)	2 (14.2%)	
Infectious endocarditis	0 (0%)	1 (16.6%)	1 (7.1%)	
Cellulitis	0 (0%)	0 (0%)	2 (14.2%)	
Others	2 (22.2%)	2 (16.6%)	4 (28.4%)	
Mortality [n, (%)]	0	0	7 (50%)	

ns = non statistical significance, *MODS*=multiple organ dysfunction score, *LOS*=length of ICU stay

* p <0.05 by analysis of variance using the Kruskal-Wallis test with the Dunn correction for multiple comparisons.

Table 2. IL-6, IL-10 AND IL-6/IL-10 RATIO LEVELS IN PATIENTS WITH SEPSIS, SEVERE SEPSIS AND SEPTIC SHOCK AT ADMISSION, 12, 24 AND 48 HOURS

	0 hours			12 hours			24 hours			48 hours		
	25%	**Median**	75%	25%	**Median**	75%	25%	**Median**	75%	25%	**Median**	75%
IL-6												
Sepsis	6,15	**9,30**	30,60	14,35	**26,85**	37,20	14,25	**21,60**	58,20	10,35	**15,85**	25,65
Severe Sepsis	4,75	**20,10**	148,95	24,85	**56,25**	129,25	11,70	**66,90**	198,00	27,50	**43,20**	142,80
Septic Shock	42,15	**104,45**	651,05	50,00	**74,55**	788,60	66,20	**84,05**	622,10	57,60	**88,80**	338,90
p value		*<0,009**			*0,05*			*<0,02**			*<0,003**	
IL-10												
Sepsis	34,20	**45,30**	109,50	18,90	**31,20**	68,50	15,90	**23,55**	38,55	5,40	**20,25**	61,55
Severe Sepsis	13,50	**34,65**	121,80	11,70	**66,90**	85,50	10,80	**28,80**	61,20	12,90	**30,60**	133,05
Septic Shock	12,00	**32,10**	155,85	19,20	**68,10**	111,30	16,90	**27,75**	76,05	16,05	**28,80**	65,10
p value		*ns*			*ns*			*ns*			*ns*	
IL-6/IL-10 ratio												
Sepsis	0,01	**0,44**	0,80	0,27	**0,64**	2,98	0,45	**0,63**	2,77	0,50	**0,79**	2,74
Severe Sepsis	0,15	**0,72**	7,88	0,33	**2,11**	5,15	0,97	**2,03**	6,88	0,52	**1,69**	4,81
Septic Shock	0,71	**4,00**	15,54	0,58	**2,55**	6,25	2,24	**4,99**	8,24	2,61	**4,68**	9,36
p value		*<0,03**			*ns*			*<0,02**			*<0,02**	

Table 3.IL-6, IL-10 AND IL-6/IL-10 RATIO AT ADMISSION, 12, 24 AND 48 HOURS IN PATIENTS WITH RESPECT TO ORGAN DYSFUNCTION (OD) (A) AND TO SURVIVAL (B)

(A)	0 hours			12 hours			24 hours			48 hours		
	25%	**m**	75%	25%	**m**	75%	25%	**m**	75%	25%	**m**	75%
IL-6												
≤2 OD	7,8	**13,5**	30,6	14,4	**27,3**	62,4	14,7	**31,5**	95,1	13,4	**25,7**	88,2
≥3 OD	42,2	**104,5**	415,3	45,6	**68,7**	788,6	57,0	**75**	622,1	32,7	**66**	287
p value		*<0.003**			*<0.03**			*ns*			*ns*	
IL-10												
≤2 OD	40,5	**48,3**	163,2	25,8	**51,5**	93,3	22,1	**27,9**	48,8	5,4	**20,3**	45,5
≥3 OD	13,2	**31,2**	111,3	12	**26,7**	90,2	15,3	**22,5**	76,1	14,1	**28,8**	66,6
p value		*ns*			*ns*			*ns*			*ns*	
IL-6/IL-10 ratio												
≤2 OD	0,04	**0,4**	0,9	0,3	**0,6**	2,4	0,6	**1,5**	3,2	0,7	**1,0**	4,5
≥3 OD	1,0	**3,7**	12,7	2,1	**3,7**	5,9	1,2	**4,6**	8,3	1,5	**4,5**	7,5
p value		*<0.004**			*<0.01**			*<0.03**			*ns*	

(B)	0 hours			12 hours			24 hours			48 hours		
	25%	**m**	75%	25%	**m**	75%	25%	**m**	75%	25%	**m**	75%
IL-6												
Survival	8,9	**27,3**	96,8	23	**42**	76,5	18,3	**56,9**	87,2	15,9	**33,2**	61,1
Non survival	87,9	**573,6**	2056	58	**431,5**	1809	66,2	**610,6**	1145	88,8	**262,5**	464,4
p value		*<0.01***			*<0.04***			*<0.01***			*<0.001***	
IL-10												
Survival	21	**40,5**	109,5	16,7	**30**	83,4	16,4	**24**	46,8	10,5	**21,9**	67,1
Non survival.	31,2	**75**	169,5	10,5	**69**	111,3	14,3	**56,6**	145,1	19,5	**60,3**	66,6
p value		*ns*			*ns*			*ns*			*ns*	
IL-6/IL-10 ratio												
Survival	0,3	**0,90**	2,4	0,3	**2,1**	3,2	0,6	**2,0**	3,9	0,7	**1,3**	5,3
Non survival.	0,2	**9,71**	18,4	0,6	**5,5**	27,4	3,1	**6,2**	38,9	3,7	**4,5**	7,0
p value		*ns*			*ns*			*<0.02***			*ns*	

m = median, *ns* = non statistically significant

* $p < 0.05$ by analysis of variance using the Kruskal-Wallis test with the Dunn correction for multiple comparisons

** $p < 0.05$ by analysis using the Wilcoxon's and Mann-Whitney test for comparison of two samples cases

Figure 1. A) IL-6 (PG/ML), B) IL-10 (PG/ML) AND C) IL-6/IL-10 RATIO IN PATIENTS WITH SEPSIS, SEVERE SEPSIS AND SEPTIC SHOCK, ACCORDING TO TIME (0, 12, 24 AND 48 HOURS)

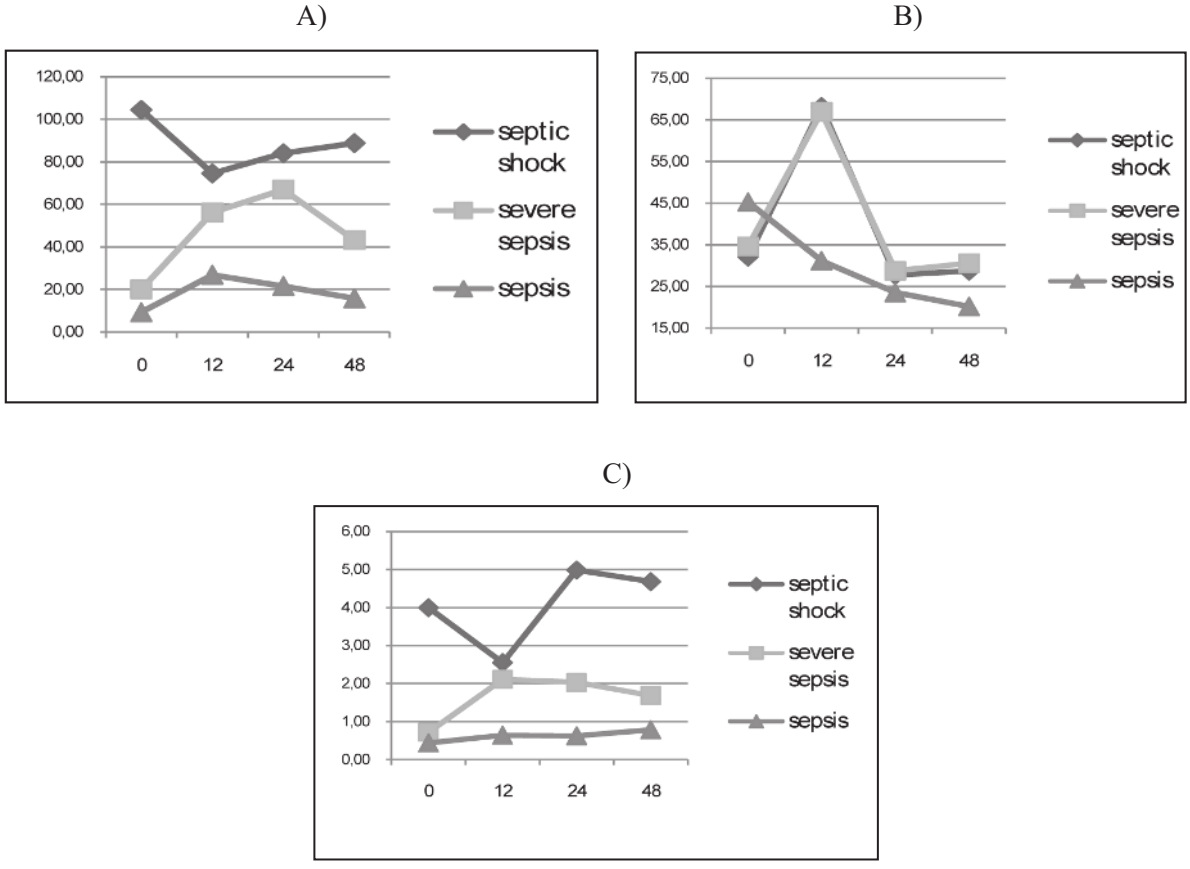
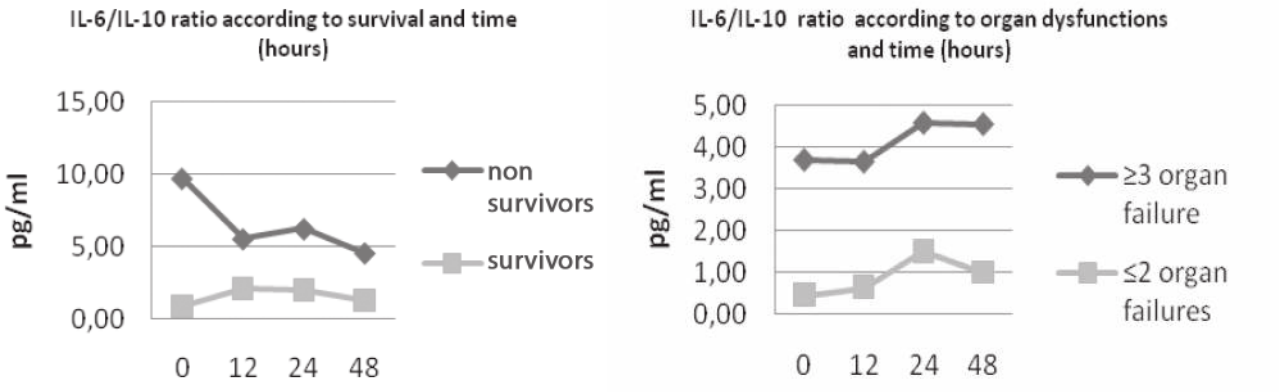


Figure 2. IL-6/IL-10 RATIO IN PATIENTS WITH REGARD TO SURVIVAL AND ORGAN DYSFUNCTION (OD)



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