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RESEARCH ARTICLE

Establishing the Diagnostic and Prognostic Value of Serum Interleukin 6 Levels in Sepsis

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Objective: Establishing a serological profile of interleukin 6 (IL-6) in order to evaluate its usefulness as a biological marker for the diagnosis and early prognosis in sepsis. **Materials and Methods**: The study included 246 individuals, divided into 2 groups: 131 in the septic subgroup (S) and 115 in the septic shock (SS) subgroup. Inflammatory markers, bacteriological examinations and laboratory samples were determined within 24 hours of the first signs of infection. Severity scores were also calculated within the first day of the onset of sepsis. **Results**: The SS subgroup (median 121.2 pg/ml, 18.59-10235 pg/ml; SD = 1920) shows significantly higher values of IL-6, compared to the S subgroup (median 43.49 pg/ml, 13, 27-6566 pg/ml; SD = 1367) (p = 0.0026). The SS subgroup has a significantly higher death rate than S subgroup (p = 0.001). The cut-off values of the mortality prediction degree were 184.74 pg/ml. The area under the curve of the cytokine IL-6 for the differentiation of sepsis from septic shock was 0.693 (95% CI 0.582-0.790, p = 0.002). The optimal value of the cut-off that allows the differentiation of the septic subgroup from the one with organ dysfunction, was 52.72 pg / ml. **Conclusion**: Serum IL-6 values are significantly higher in the septic shock group. All deceased patients had higher IL-6 serum values.

Keywords: interleukin-6, sepsis, mortality

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Introduction

Sepsis, an organ dysfunction caused by an infection, is a major health problem [1]. It is a major cause of morbidity and mortality worldwide, with an estimated 18 million cases per year [2].

Targeted diagnosis and treatment, initiated early, can lead to a better prognosis in sepsis. Unfortunately, early diagnosis of sepsis remains an unresolved challenge. Sepsis and septic shock are important causes of admission in Intensive Care Unit (ICU) services and the most frequent cause of death in non-coronary ICUs [3].

Biomarkers improve the possibility of early diagnosis and treatment of sepsis. The literature has shown that they may be useful in the diagnosis, prognostic evaluation and therapeutic management of this syndrome. Numerous biomarkers were explored in order to validate their utility, while looking for the importance of associating different categories of biomarkers (marker panels) in the evolution of sepsis [4]. Biomarkers with high sensitivity and specificity are very important in sepsis diagnosis and this is the reason why nowadays studies analyzing the role of cytokine in early diagnosis of septic syndrome are showing an increasing trend.

Interleukin 6 (IL-6) is a proinflammatory cytokine and in the same time an anti-inflammatory myokine encoded in gene 6. Numerous studies demonstrated that IL-6 circulating levels are correlated to/ in correlation with sepsis severity and the mortality rates of septic patients [5, 6].

The objective of this study was to establish a serological profile of IL-6 biomarker in septic patients, correlated to the severity of clinical forms (sepsis /septic shock) in order to evaluate the utility of IL-6 as a biological marker of prognosis and mortality.

Materials and methods

The study was carried out after obtaining all ethical approvals required in research, the agreement of the Ethics Commission of the University of Medicine, Pharmacy, Sciences and Technology „G. E. Palade" from Târgu Mureș, no. 22462/13 October 2015, respectively the agreement of the Ethics Commission of the Târgu Mureș County Emergency Clinical Hospital, no. 123/2015. Sampling and processing of blood samples were performed based on the informed consent of the patient / legal caretaker.

This study makes part of an extended research regarding the significance of cytokines which have a role as biomarkers in sepsis. Patients were enrolled based on the same inclusion and exclusion criteria, as well the described methods earlier, are the same as the one published before. [7].

Patients were enrolled in the study based on inclusion criteria: Caucasian origin, age over 18 years, presence of sepsis (defined as the presence of a suspected / confirmed infectious outbreak, along with at least two SIRS criteria, among the following : fever> 38ºC or hypothermia <36ºC, tachycardia > 90/minute, tachypnea > 20/minute, leukocytosis >12000 mm3 or leukopenia <4000 mm3 or >10% immature cells [8].

Septic shock was defined as sepsis-induced hypotension (systolic arterial blood pressure <90 mmHg or mean arterial blood pressure <70 mmHg or decrease in systolic arterial blood pressure by> 40 mmHg), refractory to adequate volume resuscitation, serum lactate> 1 mmol / L or oliguria (diuresis <0.5 mL/kg for at least 2 hours) [9].

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Diagnosis criteria for sepsis are different from the actual SEPSIS 3 because the study design and data collection started before the appearance of new definitions.

The exclusion criteria from the study were: less than 18 years-old, HIV infection, immunosuppressive therapy, neoplasms.

Study design

The study was carried out in the Anesthesiology and Intensive Care Clinic of the Emergency Clinical County Hospital from Târgu Mures. All patients were enrolled no later than 24 hours after the onset of sepsis.

The study group consisted of 246 individuals, of which: 131 (53.25%) in the sepsis (S) subgroup and 115 (46.75%) patients in the septic shock (SS) subgroup.

Each patient included in the study was followed according to a follow-up file, which included: demographic and biometric data, main diagnosis and associated diseases, infection site, etiology of sepsis (according to bacteriological examinations), in-hospital death / survival, total number of days in ICU, vasopressors and mechanical ventilation, calculation of severity scores (APACHE II, SAPS II, SOFA) at ICU uptake, results obtained from collected biological samples (inflammation markers: IL-6, PCR, PCT, complete blood count, organ dysfunction markers: transaminases, serum bilirubin, albumin, creatinine, urea, INR, serum lactate, bacterial cultures, depending on the starting point of the infection, respectively the clinical suspicion).

Sampling and processing

Plasma samples for IL-6 levels were obtained from blood samples collected concurrently with those for blood culture. An amount of 3ml of blood was collected and centrifuged. Plasma obtained by centrifugation (2000 x 3 minutes) was then stored at -70 ° C until analysis. The determination of IL-6 values was performed using the hMagnetic LX Screening Assay 6 Plex Kit.

Establishing the serological profile of IL-6 in the septic patient and prognostic correlations

We looked for correlations between plasma levels and the severity of sepsis using the previously recorded parameters, we calculated the known severity and prognosis scores (APACHE II, SOFA, SAPS II), separately by subgroups (S vs SS). Subsequently, plasma levels of IL-6 were correlated with in-hospital mortality compared in subgroups.

Statistical Analysis

Normality tests were used to assess Gaussian distribution and were applied also parametric (mean, median, SD) or nonparametric (median, range) tests. Based on the normality tests, the quantitative variables were compared by the Kruskal-Wallis test, the t test and the Mann-Whitney U test.

Correlation with severity criteria and scores was analyzed by univariate correlations (Pearson and Spearman). To identify the prognostic power of IL-6, ROC curves were performed by graphical expression and interpretation of areas under the curve (AUC), as well as performance parameters - sensitivity and specificity - and cut-off values were identified, which have optimal predictability in differentiating survivors from non-survivors.

Results

In the subgroup of patients with septic shock (median 121.2 pg/ml, 18.59-10235pg /ml; SD = 1920), we obtained significant results compared to the septic subgroup (median 43.49 pg/ml, 13.27-6566 pg/ml; SD = 1367) (p = 0.0026). (Figure 1)

Spearman rho (nonparametric) correlations were performed to analyze a possible association between plasma levels of biomarkers and the severity of sepsis. The results show that there is no correlation between the number of days in ICU and artificial supportive therapies of vital functions and serum levels of IL-6 between the two septic subgroups, but if the whole group of septic patients is analyzed, the number of days of vasopressor drug therapy has a positive correlation with serum IL-6 values (p=0.021, correlation coefficient: 0.256).

The leukocyte count and hemoglobin level determined on first day after admission to ICU showed a positive correlation (p = 0.045; p = 0.031) in septic patients, which is not confirmed in the subgroup with organ dysfunction.

Also, the level of serum lactate shows a significant correlation only in the case of the group with sepsis without organ dysfunction (p = 0.030).

In terms of severity scores and circulating levels of IL-6, we did not obtain significant values in the two septic subgroups, but, by correlating the cytokine levels in the entire infectious group, in the case of APACHE II and SOFA scores, we found positive correlations (p=0.006/0.303 and p=0.037/0.231).

The area under the curve of the cytokine IL-6 for the differentiation of sepsis from septic shock was 0.693 (95% CI 0.582-0.790, p = 0.002). (Figure 2) The optimal value of the cut-off that allows the differentiation

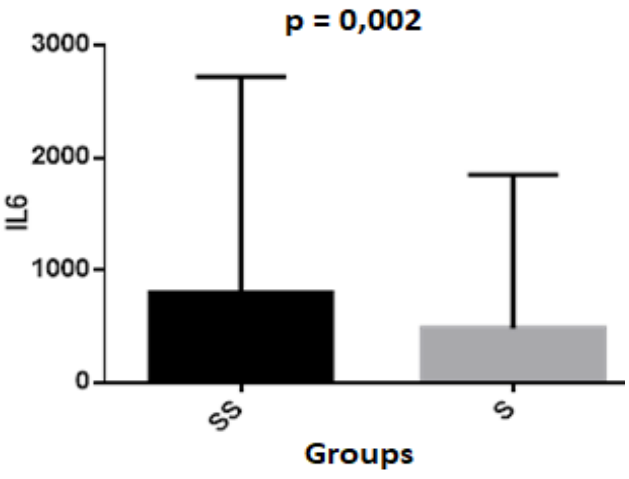

Fig. 1. IL-6 values in the two subgroups.
S = sepsis, SS = septic shock. Test Mann-Whitney U

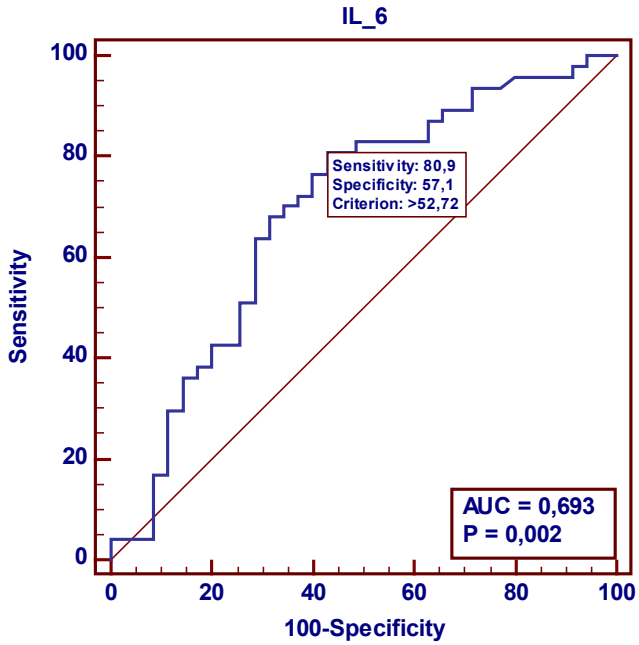

Fig. 2. ROC curve for differentiating sepsis from septic shock

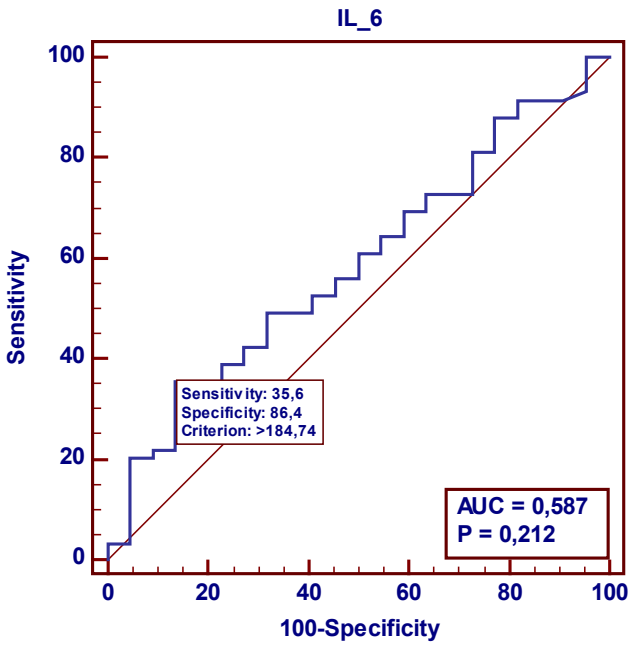

Fig. 3. ROC curve for mortality prediction capacity

of the septic subgroup from the one with organ dysfunction, was 52.72 pg / ml (sensitivity 80.9% and specificity 57.1%). (Table I).

Analyzing the in-hospital mortality rate, although the number of deaths is higher compared to the survivors (median 98.14 vs 75.91; SD = 1823 vs 1383), there is no significance in this respect (p = 0.2352). However, comparing the two septic groups, as expected, SS has a significantly higher death rate than infection without organ dysfunction (p = 0.001).

We also looked at whether the risk of mortality can be predicted depending on the circulating level of IL-6. Thus, through the ROC curve we established the cut-off values of the mortality prediction degree. The cut-off for it is 184.74 pg/ml. The sensitivity was 35.6% and the specificity was 86.4%. Above these cut-off values, the risk of mortality increases. (Figure 3)

Mortality in the group of septic patients included in the study was 75.6%. All 3 categories of admissions (medical, scheduled surgery, respectively surgical emergencies) in which patients were included, presented a higher number of deaths than survivors, the results being statistically significant (p = 0.0001).

However, comparing these three categories of patients admitted to ICU in terms of circulating levels of IL-6, we did not find any statistical difference (p = 0.6157).

Discussion

The diagnosis of sepsis and the assessment of its severity are difficult because sepsis is manifested by a series of nonspecific signs and symptoms and varies individually. Early diagnosis, as mentioned before, is the key element that plays an important role in the outcome of the infectious process. BMs might play an important role in sepsis because they can indicate its presence, absence or even severity and can differentiate bacterial infections from viral and fungal ones, respectively systemic infections from localized ones [7].

In 2011, the National Institutes of Health (USA) defined biomarkers as elements that can be measured and used as indicators of normal biological processes, pathological ones, respectively as markers of pharmacological responses arising from the use of various therapies [4].

The diagnosis of sepsis has become a sensitive process, which nowadays leads to the use of biomarkers that could reflect the dysfunctions that occur in an infectious process. Thus, the present study attempted to analyze these biomarkers, which are known to be involved in organ and immune dysfunctions.

As expected, the severity of the infectious process also influenced the circulating level of IL-6, which has much higher values in patients with severe infections. The same can be said about the gender of patients, since it seems that males are more prone to severe disease forms.

Table I. Diagnostic values for patients in the septic and septic shock subgroups and cut-off values for predicting mortality

Severity	AUC (95% CI)	Cut-off value pg/ml	Sensitivity %	Specificity %	P
S vs SS	0.693 (0.582-0.790)	52.72	80.9	57.1	0.002*
Mortality	0.587 (0.472-0.695)	184.74	35.59	86.36	0.212

AUC: area under the curve. S: Sepsis, SS: septic shock, *: statistically significant

Consistent with the present study, studies in the literature confirm that high levels of IL-6 in deceased patients are significant compared to survivors and those with mild infections [8], being an early marker of mortality in ICU [9]. Erik H et al. argue that circulating levels are significantly increased in groups of patients with organ dysfunction and that there is also a positive correlation with serum lactate levels and death [10]. Using routine severity scores - APACHE II, SOFA and SAPS II - our study showed a significant correlation between APACHE II and SOFA and the serum level of cytokine analyzed IL-6, as demonstrated by other specialized studies, thus influencing patients' prognosis [8].

The high rate of deaths in the infectious group could also be explained by the fact that many patients had a number of other comorbidities, in addition to pulmonary pathologies, which is also described by the study of Abe T et al. which shows that patients with diabetes have a higher risk to develop sepsis and a higher mortality compared to those who do not present this pathology [23].

Because sepsis is a serious health problem, it would be ideal to find diagnostic markers with sensitivity close to 100%. Therefore, we performed the ROC curves, where we found the cut-off values of IL-6 in case of mortality in our study of 184.74 pg/ml with specificity 86.4% and sensitivity 35.6%. Some studies show cut-off values between 10 and 500 pg / ml [21]; Onal et al. reported cut-off values for IL-6 of 20 pg/ml [16], and Ng et al. of 31 pg / ml in 45 patients with sepsis, with a sensitivity of 89% and a specificity of 95% [22]. At the same time, Barre M et al. argue that IL-6 influences patients' short- and medium-term prognosis and implicitly their mortality rate [17], and Naffa M et al. the fact that the IL-6 level at discharge has a better ability to predict mortality than at admission [19].

Conclusions

Serum IL-6 levels are significantly higher in the group with organ dysfunction and there are also significant relationships in the number of days of vasopressor drug therapy, results that highlights its possible role in establishing the prognosis of sepsis.

Among the severity scores, only the most common ones, respectively APACHE II and SOFA, presented significantly different values between the studied groups.

Regarding the survival rate all deceased patients had higher values of this cytokine, which again emphasizes that this biomarker could be useful in the prognosis of patients with severe infections complicated by organ dysfunction.

Abbreviations

IL-6 -Interleukin 6
BM - Biomarkers
APACHE II - Acute physiology and chronic health evaluation
AUC - Area under curve
BMI -Body mass index
SAPS - Simplified acute physiology score
SIRS - Systemic inflammatory response syndrome
SOFA -Sequential Organ Failure Assessment
PCR - Polymerase chain reaction
OR - Odds ratio

Authors' Contribution

IMB (Conceptualization; Methodology; Data Curation; Writing - rewiew & editing); AET (Conceptualization; Methodology; Formal Analysis); OC (Data Curation; Investigation); VN (Methodology; Writing- review & editing)

Conflict of interest

The authors declare that they have no conflict of interest.

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