Clinical significance of soluble Fas plasma levels in patients with sepsis

Klinički značaj nivoa rastvorljivog Fas u plazmi kod bolesnika sa sepsom

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Abstract

Background/Aim. The goal of modern clinical and experimental researches in the field of sepsis is to find one or more sensitive parameters that could predict the severity of sepsis and its outcome. In this study we investigated and compared the relationship of initial soluble Fas (sFas) plasma levels as well as Acute Physiology, Age and Chronic Health Evaluation II (APACHE II) score in 58 septic patients with severity and outcome of sepsis. **Methods.** The diagnosis and assessment of disease severity was performed on the same day, based on clinical and laboratory parameters. The blood samples were used for monitoring of laboratory standard parameters necessary for the diagnosis of sepsis, organ dysfunction and assessment of disease severity, as well as for determination of levels of sFas. According to consensus criteria, patients were divided into those with sepsis (n = 16), severe sepsis (n = 30) or septic shock (n = 12), those with (n = 26) and without (n = 32) multiple organ dysfunction syndrome (MODS), and survivors (n = 45) and non-survivors (n = 13). **Results.** Plasma sFas level (9.7 ± 10.1; 0–44.2 U/mL) was elevated in 54.4% of patients. All the patients with septic shock, 76.9% of the patients with MODS and 84.6% patients who died had elevated sFas level. We observed a strong positive correlation between sFas and APACHE II score ($p < 0.001$). The level of sFas was significantly higher in patients with septic shock compared to normotensive patients ($p < 0.001$), patients with MODS compared to those without MODS ($p < 0.001$) and survivors compared to nonsurvivors ($p < 0.01$). **Conclusions.** Our results suggest that initial sFas plasma levels in patients with sepsis correlated with the values of APACHE II score and separated very well the patients with septic shock *versus* the normotensive patients, the patients with and without MODS, and survivors *versus* non-survivors.

Key words:
Sepsis; antigens, cd95; plasma; prognosis.

Apstrakt

Uvod/Cilj. Cilj savremenih kliničkih i eksperimentalnih istraživanja u oblasti sepse je da se pronađe jedan ili više osetljivih parametara koji bi mogli da predvide težinu sepse i njen ishod. Cilj ovog rada bio je ispitivanje i upoređivanje odnosa početnih nivoa rastvorljivog Fas (sFas) u plazmi, kao i *Acute Physiology, Age and Chronic Health Evaluation* (APACHE) II skora sa težinom i ishodom sepse kod 58 bolesnika sa sepsom. **Metode.** Na osnovu kliničkih i laboratorijskih parametara istog dana postavljana je dijagnoza i vršena je procena težine bolesti. Iz uzoraka krvi određivani su standardni laboratorijski parametari potrebni za postavljanje dijagnoze sepse, disfunkcije organa i procenu težine bolesti, a, takođe, izmeren je i nivo sFas u plazmi. Prema konsenzus kriterijumima, bolesnici su podeljeni u grupe sa sepsom (n = 16), teškom sepsom (n = 30) ili septičkim šokom (n = 12), grupe sa (n = 26) i bez (n = 32) sindroma multiorganske disfunkcije (*multiple organ dysfunction syndrome* – MODS), i na preživele (n = 45) i bolesnike sa smrtnim ishodom (n = 13). **Rezultati.** Povišene nivoe sFas u plazmi (9,7 ± 10,1; 0–44.2 U/mL) imalo je 54,4% bolesnika, i to svi bolesnici sa septičkim šokom, 76,9% bolesnika sa MODS i 84,6% bolesnika sa smrtnim ishodom. Utvrđena je značajna pozitivna korelacija nivoa sFas u plazmi i APACHE II skora ($p < 0,001$). Bolesnici sa septičkim šokom imali su značajno više prosečne nivoe sFas u odnosu na normotenzivne bolesnike ($p < 0.001$). Značajno viši nivoi sFas utvrđeni su kod bolesnika sa MODS nego kod bolesnika bez MODS ($p < 0.001$), a značajno niži kod preživelih nego kod bolesnika sa smrtnim ishodom ($p < 0.01$). **Zaključak.** Početni nivoi sFas u plazmi kod bolesnika sa sepsom pozitivno korelišu sa vrednostima APACHE II skora i međusobno se razlikuju između bolesnika sa septičkim šokom i normotenzivnih bolesnika, bolesnika sa i bez MODS, kao i između preživelih i bolesnika sa smrtnim ishodom.

Ključne reči:
sepsa; antigeni, cd95; plazma; prognoza.

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Introduction

Sepsis and its complications, septic shock and multiple organ dysfunction syndrome (MODS) despite the great advances in medical science, is still a very difficult clinical problem. It is unknown whether progress has been made in decreasing their mortality rate [1–4]. The goal of modern clinical and experimental research in the field of sepsis is to find one or more of sensitive parameters that could predict the severity of sepsis and its outcome. For the clinicians it is particularly important that these indicators are defined at the time of hospitalization, and the diagnosis of sepsis, in order to choose the most appropriate method of treatment [5–7]. Although some mediators of immune-inflammatory processes, especially those in the network of cytokines, may be important markers that reflect the degree of severity of sepsis, none of them is absolutely reliable indicator of the outcome of sepsis [8, 9].

Intensive studies of the pathophysiology of sepsis in recent twenty years have resulted in the knowledge that apoptosis is an important mechanism of cell death in animal models of sepsis and endotoxemia. Hypoperfusion and ischemia in experimental sepsis promote apoptosis in the gastrointestinal tract, liver, heart, and brain [10–14]. Accelerated apoptosis in the hematopoietic and lymphoid tissues due to the reduction in the number of mature T- and/or B-lymphocytes leads to the development of immunosuppression in ongoing, and after the sepsis [15–20]. Delaying apoptosis is associated with prolonged functional survival of neutrophils, which is reflected on their respiratory burst activity [21–23]. Apoptosis of endothelial cells in the course of sepsis has increased significantly and is an important mechanism for permeability disorders of microcirculation and the development of organ dysfunctions [24–28].

The findings that plasma of septic patients can significantly inhibit apoptosis of neutrophils in the blood of volunteers, indicates that there is a soluble circulating factors which can modify apoptotic processes [29]. Fas (CD95/APO-1) receptor is the main molecule involved in apoptosis during the sepsis. It is expressed on the surface of many cell types after their activation. Hotchkiss et al. [26] described increased apoptosis in different cells and organs in patients with fatal sepsis and MODS, while Fleck et al. [30] reported significantly higher levels of circulating (soluble) Fas molecule in patients with sepsis and septic shock [26, 30].

Several studies on a small number of patients demonstrated that sFas plasma levels might correlate with MODS and survival [31–33]. However, it remains still difficult to conclude whether circulating concentrations of this molecule are related to the severity of sepsis and outcome of septic patients and whether they would have prognostic value. This was the reason why we investigated and compared the relationship of initial sFas plasma levels in septic patients with severity and outcome of sepsis.

Methods

A total of 58 patients with sepsis were enrolled in this prospective study. The study was approved by the local Ethics Committee. For each patient, we recorded clinical data in a pre-established protocol that included demographic data, sepsis score, underlying diseases, microbiology results, final diagnosis and outcome. The diagnosis and assessment of disease severity was performed on the same day, based on clinical and laboratory parameters.

The blood samples were used for monitoring laboratory standard parameters necessary for the diagnosis of sepsis, organ dysfunction and assessment of disease severity, as well as for determination of levels of sFas. The microbiological results included the results of blood cultures and cultures of any other relevant sample (urine, cerebrospinal fluid, peritoneal fluid, and others).

The diagnosis of sepsis and classification of patients

The diagnosis of sepsis and its complications was made according to the consensus guidelines of the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) [34] and all patients were enrolled into the study within 24 hours since meeting those criteria. The diagnosis of organ dysfunction and MODS was made according to sepsis-related organ failure assessment (SOFA) score [35]. The severity of illness was scored by Acute Physiology, Age and Chronic Health Evaluation II (APACHE II) score [36]. Patients who died in the hospital were included in the group named as non-survivors. All the patients were followed until hospital discharge and classified into different groups on the basis of clinical and laboratory findings: patients with sepsis, severe sepsis or septic shock, patients with or without MODS, and survivors or non-survivors.

Quantification of sFas

Blood samples were obtained from each patient within 24 hours of meeting sepsis criteria, for determination of initial plasma sFas levels. After puncture of one forearm vein, blood was collected into pyrogen free tubes, centrifuged for 10 minutes at $1000 \times g$ aside plasma and immediately frozen and stored at -20ºC. Plasma concentrations of sFas were determined by commercially available ELISA kits (BIO-TRACK, Amerslam Pharmacia Biotech, Uppsala, Sweden). In healthy persons the normal range for human plasma sFas was up to 6 U/ml. The levels of sFas were compared between corresponding groups of patients and with APACHE II score.

Statistical analysis

The results are presented as the median values ± SD. To compare two independent samples we used Mann-Whitney *U*-test. Among the sFas and APACHE II score, Pearson's correlation coefficients were calculated to estimate the linear correlation between continuous variables. All *p*-values were two-sided, and a probability of less than 0.05 was considered statistically significant.

Results

The main demografic and clinical caracteristics of a total of 58 septic patients included in the study are given in Table 1.

Main demographic and clinical characteristics of 58 septic patients enrolled in the study

Characteristics	Values
Sex (men / women), n (%)	33 (56.9) / 25 (43.1)
Age (years), x̄ ± SD (range)	61.3 ± 16.3 (21–81)
APACHE II score, 24 h x̄ ± SD	19.5 ± 6.5 (8–36)
Underlying diseases, n (%)	21 (36.2)
Bacteremia, n (%)	24 (41.4)
Sepsis, n (%)	16 (27.6)
Severe sepsis, n (%)	30 (51.7)
Septic shock, n (%)	12 (20.7)
MODS, n (%)	26 (44.8)
Death, n (%)	13 (22.4)
Length of hospital stay (day), x̄ ± SD (range)	24.7 ± 8.3 (2–46)

APACHE II – Acute Physiology, Age and Chronic Health Evaluation II;
MODS – Multiple Organ Dysfunction Syndrome; n – number of patients.

A total of 40 (69,0%) patients were treated with antibiotics prior to admission. The etiology of sepsis was demonstrated in 50 (86.2%) of the patients, of whom 24 (41.4%) had positive blood cultures. Gram negative bacteria was the cause of sepsis in a total of 22 (37.8%) of the patients, gram-positive bacteria in 14 (24.1%), a mixed and anaerobic bacterial flora in 14 (24.1%).

Levels of sFas was elevated in 31 (54.4%) of 57 patients (9.7 ± 10.1; 0–44.2 U/mL) whereas the level of sFas was below the limit of detection in 9 (15.8%) of the patients.

All the patients with septic shock had sFas concentrations > 6.0 U/mL, and the highest levels (44.2 and 43.7 U/mL) were observed in two patients with septic shock. In patients with severe sepsis 15 (50.0%) had the level of sFas < 6.0 U/mL, and in 3 (10.0%) of them the concentrations were undetectable. At the same time, the concentrations of sFas < 6.0 U/mL were observed in 10 (62.5%) of the patients with sepsis, and in 5 (31.3%) of them they were undetectable.

Unmeasurable concentrations of sFas were found in one (4.0%) of the patients with MODS, and in 8 (25.0%) of patients without MODS. sFas concentrations < 6 U/mL were measured in 6 (24.0%) of the patients with MODS and in 20 (62.5%) without MODS.

Unmeasurable sFas levels were more frequently registered in the survivors (9 patients) compared to non-survivors (20.0% *vs* 0%). The values of sFas plasma levels and APACHE II score in different groups of septic patients are given in Table 2.

It was shown that initial plasma concentrations of sFas correlated positively with the APACHE II score ($r = 0.6046$, $p < 0.001$) (Figure 1).
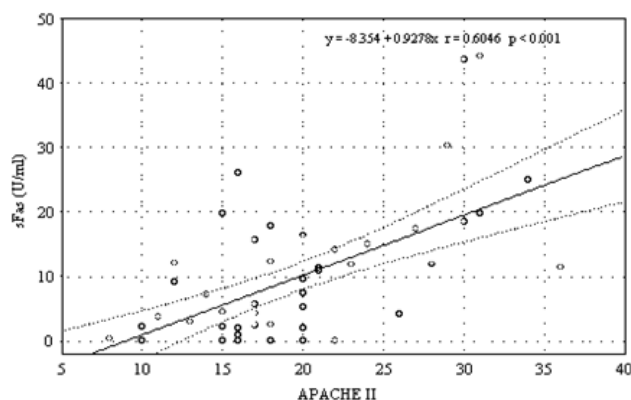

Fig. 1 – Correlation between sFas plasma levels and Acute Physiology, Age and Chronic Health Evaluation II (APACHE II) score in the patients with sepsis.

Discussion

In this study, we investigated the clinical significance of sFas in patients with sepsis and showed that, at the beginning of sepsis, the plasma levels of this biomolecule were increased in the majority of septic patients. These results are

**The relationship between sFas plasma levels and Acute Physiology,
Age and Chronic Health Evaluation (APACHE II) score value
in different groups of septic patients**

Characteristics of patients	sFas (U/mL), x̄ ± SD	APACHE II score, x̄ ± SD
Sepsis	4.8 ± 6.8*** [b]	13.8 ± 2.9*** [b]
Severe sepsis	7.5 ± 6.4	18.7 ± 3.3*** [c]
Septic shock	21.1 ± 12.5*** [a]	29.3 ± 4.4*** [a]
With MODS	14.8 ± 11.5***[d]	24.5 ± 6.0*** [d]
Without MODS	5.6 ± 6.4	15.4 ± 3.3
Survivors	8.0 ± 9.2	17.9 ± 5.6
Nonsurvivors	15.8 ± 11.2**[e]	24.9 ± 6.7** [e]

a – compared to the group with severe sepsis; b – compared to the group with septic shock;
c – compared to the group with sepsis; d – compared to the group without Multiple Organ Dysfunction Syndrome (MODS);
e – compared to survivors; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

generally in accordance with few published studies related to this topic [29–33, 37, 38]. Some observed differences may be due to different etiology of sepsis, different timing of sFas monitoring or different numbers of patients included in the studies.

It is known that the Fas molecule could occur as a cell surface receptor as well as a soluble protein. sFas is derived by proteolytic cleavage of membranous Fas or by alternative splicing of membrane-bound Fas [29, 39]. sFas functions as an inhibitor of apoptosis due to the competitive binding to Fas-L, but it also down-regulates the expression of membranous Fas receptor. However, the levels of this biomolecule follow the extent of Fas expression and thus can serve as a marker of apoptosis intensity [40]. Apoptosis has been documented as an important mechanism involved in pathophysiology of septic shock and MODS and therefore determination of sFas levels might be an indirect parameter of cell death in sepsis [29–33, 37, 38].

Many factors, including pro-inflammatory mediators in sepsis increase the expression of Fas as well as Fas-L on different cells such as lymphocytes, cells of innate immunity, vascular endothelial cells and various parenchymatous cells [11–13]. It is interesting that both monocytes and monocyte-derived macrophages release TNF-α and IL-8 following Fas ligation, two important cytokines associated with many events in sepsis, suggesting that the Fas signaling pathway can also lead to proinflammatoty cytokine induction [41]. Apoptosis of endothelial cells, could be an important cause of the development of septic shock and our results showing significantly higher levels of sFas in septic shock patients compared to normotensive septic patients are in agreement with this hypothesis. Huttunen et al. [42] also demonstrated that high sFas concentrations and increased sFas/Fas-L were associated with hypotension and high SOFA score in patients with bacteremia. However, some authors found that the levels of sFas did not correlate with other apoptotic markers in sepsis implicating that these and other endothelial-damaging parameters are better indicators for the development and the severity of septic shock than sFas [31, 33, 38].

It has been documented that sFas may also influence the adaptive T-cell mediated immunity [43, 44] and thus contributing to T-cell anergy in sepsis. However, prolonged survival of neutrophils due to reduced neutrophil apoptosis was accompanied with hyperactivity of these cells and subsequent release of neutrophil elastase upon degranulation, the mechanisms which significantly contribute to the development of organ dysfunction. It has been shown that the values of neutrophil elastase and leukocyte counts correlated with serum sFas levels in patients with sepsis. Moreover, Fas-mediated neutrophil apoptosis was efficiently inhibited by serum sFas from septic patients as efficiently as recombinant sFas [23]. These results demonstrate the involvement of sFas in neutrophil-mediated pathology in sepsis and suggest that sFas may represent a target for new therapeutic approaches to prevent neutrophil hyperactivity and sepsis.

The levels of sFas in septic patients with MODS are of particular importance since MODS is a frequent complica-

tion of severe sepsis and septic shock [45]. Although massive inflammatory reaction is considered to be one of the main triggers for the development of MODS, outcome is not necessarily improved by blocking the action of these mediators [46]. Therefore, the downstream effects of these inflammatory mediators, such as the induction of apoptosis, might be pivotal in the pathogenesis of MODS [47–49].

Papathanassoglou et al. [31] examined 35 critically ill patients with MODS and analyzed association between sFas concentrations and severity of organ dysfunction, survival and levels of certain mediators. In these patients, they registered significantly elevated sFas levels during the first two weeks of hospitalization, compared with controls. Moreover, the expression of Fas and FasL on the peripheral blood mononuclear cells in the most severe patient with MODS correlated with the severity of the disease defined according to APACHE II score, and increased along with the increase in the severity of the clinical picture [33]. However, since they did not find a correlation between the concentrations of sFas and expression of Fas on the surface of mononuclear cells, they considered that in critically ill patients some other factors emerge as mediators of apoptosis in MODS [31, 33]. Similar observations about the relationship between sFas and MODS were noticed by other authors, who additionally demonstrated that recovery of patients with organ dysfunction was followed by rapid decrease of sFas concentration [29, 32]. Paunel-Görgülü et al. [23] demonstrated increased sFas levels in patients with sepsis after major trauma at day 5 and day 9 compared with patients with uneventful recovery. At the same time apoptosis of neutrophils was significantly decreased. They also showed a high correlation between sFas and SOFA or MOD scores and thus provided evidence for the clinical significance of this biomolecule as a predictor for the development of sepsis and MODS in traumatized patients. In our study we observed initially elevated concentrations of sFas in almost all the patients with MODS and its levels were significantly higher than in the patients without MODS. Similarly as reported in previous studies, a significant correlation between the concentration of sFas and APACHE II score was obtained.

The prognostic significance of sFas levels for patient survival comes from the study which demonstrated significantly higher concentrations of sFas in patients with sepsis who died and MODS, compared with survivors and from the finding that an increase in these concentrations over the time was inversely associated with the probability of survival [23]. Our results are in agreement with those, since we showed that patients with a fatal outcome had significantly higher levels of sFas in relation to the surviving patients. In addition, we observed unmeasurable concentrations of sFas only in surviving patients, suggesting that sFas concentrations may be a good prognostic parameter for outcome of sepsis. However, there were opposite conclusions resulted from a prospective cohort study in patients with bacteremia, that there were no association between maximum sFas, sFas/Fas-L ratio or minimum Fas-L levels during days 1–4 after positive blood culture had been available with increased death [42].

Conclusion

Initial sFas plasma concentrations in patients with sepsis were elevated in the majority of patients, especially in patients with complications of sepsis and positively correlated with APACHE II score values. These concentrations were significantly higher in patients with septic shock, multiple organ dysfunction syndrome, and those who died. Therefore, determination of sFas in sepsis as a parameter of apoptosis induction together with other immune-inflammatory markers might be of clinical significance.

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