РОЛЬ ФАКТОРА НЕКРОЗА ОПУХОЛИ-АЛЬФА В ПРОГНОЗИРОВАНИИ ТЯЖЕСТИ И ИСХОДА СЕПСИСА У ПАЦИЕНТОВ НЕОТЛОЖНОГО ОТДЕЛЕНИЯ С СИСТЕМНЫМ ВОСПАЛЕНИЕМ

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Tumor Necrosis Factor-α In Emergency Department Patients with Systemic Inflammation as a Predictor of Severity and Outcome of Sepsis

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Цель исследования — определить, связаны ли уровни ФНО-α, ключевого медиатора воспаления, у пациентов с сепсисом, поступающих в отделение неотложной помощи и переводящихся в отделение реанимации, с прогрессированием его до тяжелого сепсиса, септического шока и смерти. *Материал и методы.* Ретроспективное обсервационное исследование было выполнено на выборке в 100 взрослых человек, поступивших в неотложное отделение с признаками системного воспаления двух возможных этиологий: тяжелый острый панкреатит (предполагаемый, а затем подтвержденный в отделении реанимации и/или операционной) или общий перитонит. Производили измерения ФНО-α в крови сразу после поступления. ФНО-α измеряли коммерчески доступным ELISA-методом в плазме крови. *Результаты.* Средние уровни ФНО-α при поступлении (день 0, в неотложном отделении) были в 191,5 раз ниже в группе с септическим шоком по сравнению с группой тяжелого сепсиса и в 63 раза выше у выживших (*p*<0,01). Площадь под кривой для ФНО-α была 0,813 (тяжесть состояния) и 0,834 (исход). У пациентов в уровнями ФНО-α ниже 7,95 пг/мл отмечалась в 3,2 раза более высокая вероятность развития септического шока по сравнению с более высокими уровнями (чувствительность 83,9%, специфичность 72,5). При уровне ФНО-α более 10,5 пг/мл была выявлена в 4,8 раз большая вероятность выживания по сравнению с более низкими уровнями ФНО-α (чувствительность 83%, специфичность 77,4%). *Выводы.* Снижение концентрации ФНО- приводит к развитию септического шока и смерти. ФНО- является хорошим инструментом прогнозирования тяжести и исхода сепсиса. *Ключевые слова:* сепсис, фактор некроза опухоли-альфа, неотложные медицинские службы, выживаемость, индекс тяжести заболевания.

Aim of the study was to determine whether the TNF-α levels, proximal inflammatory mediator, in septic patients presenting to the emergency department (ED) and admitted to the intensive care unit (ICU) are associated with progression to severe sepsis, septic shock or death. *Material and Methods.* A retrospective observational study was performed on a sample of one hundred adult subjects presenting to the ED with systemic inflammatory response syndrome of 2 etiologies: presumed (and later confirmed in the ICU and/or operating room) severe acute pancreatitis or generalized peritonitis. Blood TNF-α samples measurements were taken shortly after ED admission. TNF-α was measured by commercial ELISA test in plasma. *Results.* Mean values of TNF-α on admission (day zero, in ED) were 191,5-fold lower in group with septic shock compared to severe sepsis group and were 63-fold higher in survivors (*p*<0.01). The area under the curve (AUC) for the TNF-α plots for severity of clinical status was 0.813 and for outcome 0.834. Patients with TNF-α levels lower than 7.95 pg/mL had a 3.2-fold higher probability of septic shock development than those with higher values, at the cutoff level sensitivity was 83,9% and specificity 72,5%. Patients with TNF-α levels higher than 10.5 pg/mL had a 4.8-fold higher probability to survive than those with lower values, at the cutoff level sensitivity was 83,0% and specificity 77,4%. *Conclusion:* Decreasing in TNF-α concentration leads to the septic shock development and fatal outcome. TNF-α is very good predictor of sepsis severity and outcome. *Key words:* sepsis, tumor necrosis factor-alpha, emergency medical services, survival rate, severity of illness index.

Despite some results with new therapies, approximately 750 000 cases of sepsis occur in U.S. annually with a mortality of 50% which makes sepsis the disease with the higher mortality than any other major disease. Sepsis is also a leading cause of death in patients admitted through emergency department (ED). Sepsis is now defined as the presence of systemic inflammatory response syndrome (SIRS) associated with a confirmed infectious process. In the

absence of infection, the presence of one or more of the following criteria is suggestive of sepsis: 1) significant edema or positive fluid balance (20 mL/kg over 20 h); 2) hyperglycemia (plasma glucose higher than 120 mg/L) in the absence of diabetes; 3) inflammatory variables: plasma C-reactive protein higher than 50 mg/L or plasma procalcitonin higher than 1.5 ng/mL; mixed venous oxygen saturation (SvO_2) >70%; and 5) cardiac index >3.5 L/min/m2. Severe sepsis is defined as sepsis-associated organ dysfunction, hypoperfusion, or hypotension. Organ dysfunction variables include arterial hypoxemia (PaO_2/fraction of inspired oxygen − FiO_2 ratio of <300); acute oliguria (urine output <0.5 mL/kg/h); creatinine >2 mg/dL; coagulation abnormalities (international normalized ratio − INR >1.5 or activated partial thromboplastin time − aPTT >60 sec); thrombocytopenia (platelet count <100 000); hyperbilirubinaemia (plasma total bilirubin >2 mg/dL; tissue perfusion variable: hyperlactataemia (>2 mmol/L); haemodynamic variables: arterial hypotension (systolic blood pressure decrease >40 mmHg). Septic shock is defined as acute circulatory failure induced by sepsis with hypotension despite adequate fluid resuscitation [1]. Currently the most recent definition of the International Consensus Conference, Paris, France, April 2006, does not require the presence of hypotension. Instead, the definition of shock as «failure to deliver and/or utilize adequate amounts of oxygen» may include, but is not limited to the presence of hypotension. Shock is defined as a circulatory and cellular dysfunction, manifested by markers of hypoperfusion such as elevated blood lactate, decreased central venous oxygen saturation ($ScvO_2$) or SvO_2, with or without hypotension [2]. Multiorganic dysfunction syndrome (MODS) is defined as the presence of two or more altered organ functions in an acutely ill patient, when homeostasis cannot be maintained without intervention.

Frequently, the presentation and clinical course of infected ED patients is not as distinct as the definitions of severe sepsis and septic shock would suggest. Many patients with severe sepsis and septic shock present to the ED with long delays in transfer to an intensive care unit (ICU). Some of the new approaches to management of severe sepsis and septic shock (early goal directed therapy − EGDT) appear to be time dependent, suggesting a «golden hour» and «silver day» perspective to the management of this disorder, giving the ED a more important role in the care of these patients [3]. Most recent studies of pathophysiology of sepsis-induced MODS support EGDT approach and reveal that a critical limitation of tissue oxygenation delivery due to macrocirculatory or microcirculatory failure may play a role, but only in the early phase of the disease process before resuscitation has been initiated, in the first several hours. Nonetheless, a growing body of evidence suggests that MODS may develop during sepsis mainly as a consequence of impaired cellular oxygen utilization as a result of mitochondrial dysfunction [4−7].

Cytokines are key family of effector molecules that coordinate the innate and acquired host antimicrobial defense responses in sepsis. The cytokine family of messenger molecules comprises tumor necrosis factor (TNF), interleukins, chemokines, interferons and colony stimulating factors. TNF-α, a secreted 17 kDa cytokine, is produced by a broad range of cells including monocytes, macrophages, dendritic cells and neutrophils. It is an early-onset proinflammatory cytokine. It also triggers the synthesis of proinflammatory mediators such as cytokines (including TNF-α itself), eicosanoids, nitric oxide (NO), platelet activating factor (PAF), and free radicals. TNF-α recruits immune cells to inflammatory and infectious sites through induction of endothelial adhesion molecules and chemokines. TNF-α also plays an important role in the synthesis of hepatic acute phase proteins. It is a potent inducer of apoptosis of inflammatory cells, fibroblasts and myocytes. TNF-α is pyrogenic cytokine; it causes anorexia and can induce shock by decreasing vascular resistance, causing capillary leak and depressing myocardial function [8].

The purpose of this retrospective study was to determine whether the levels of TNF-α, proximal inflammatory mediator, in patients presenting to the ED and admitted to the ICU with sepsis are associated with the progression to severe sepsis, septic shock or death.

Material and Methods

A retrospective observational study was performed on a sample of one hundred adult subjects presented to the ED with SIRS of 2 etiologies: presumed (and later in ICU and/or operating room confirmed) severe acute pancreatitis or generalized peritonitis. Levels of TNF-α were determined and associated with clinical severity and outcome. Any patient of 18 y. o. or older presenting to the ED and meeting the criteria for SIRS (2 or more of the following: body temperature of >38°C or <36°C; heart rate (HR) of >90 beats/min; tachypnea, as manifested by a respiratory rate of >20 breaths/min or hyperventilation, as indicated by a $PaCO_2$ of <35 mmHg; an alteration of the white blood cell (WBC) count of >12000 cells/mm³, <4000 cells/mm³, or the presence of >10% immature neutrophils) due to severe acute pancreatitis or generalized peritonitis was eligible for the study. Survivors were patients who were alive 30 days after taking the blood sample for cytokine measurement. As described in introduction, severity of sepsis was determined to be either severe sepsis or septic shock. Blood sample for cytokine measurement was drawn immediately after admission to ED of Military Medical Academy (academic tertiary center). TNF-α was measured by a commercial ELISA test in plasma (MILENIA BIOTEC, Germany). Concentration of cytokine is displayed in pg/ml. Blood was drawn for routine laboratory analyses daily. All 100 patients were treated with appropriate antibiotics, vasopressors and inotropic agents, adequate circulatory volume replacement with fluids and mechanical ventilatory support was performed when needed. They underwent surgical procedures if required for management of severe acute pancreatitis or generalized peritonitis.

Data analysis

All results are reported as mean SD. Statistical analysis of results was performed with Mann-Whitney U − Wilcoxon Rank Sum W test, Pearson's 2, Fisher's Exact Test, Logistic Regression and nonparametric Spearman's correlations. Receiver operator curves were generated to determine the cutoff values for optimal sensitivity and specificity for the cytokine mediator levels for progression to severe sepsis, septic shock and death. Statistical Package SPSS for Windows (version 7.2; Chicago, Illinois) was used. Differences between groups were considered to be significant at $p<0.05$ and highly significant at $p< 0.01$.

Descriptive parameters of patients and comparison of mean values of TNF-α according to severity and outcome

	TNF-α (pg/mL)			
	Severity		Outcome	
	Severe sepsis	Septic shock	Survivors	Nonsurvivors
n	69 (69%)	31 (31%)	53 (53%)	47 (47%)
Mean	865.4	4.52	1113.5	17.7
Median	99	4.3	232.5	5.2
SD	2122.7	3.48	2370.5	53.5
SE	255.5	0.62	325.6	7.8
Minimum	0	0	0	0
Maximum	11596.3	12.1	11596.3	340.5
z	-4.995		-5.747	
df	98		98	
p	<0.01		<0.01	

Results

This clinical study involved 100 patients (average age was 50,2 years; range: 19 — 85 years; 37 females, 63 males) with either severe sepsis (less severe clinical status) or septic shock (more severe clinical status) due to severe acute pancreatitis or generalized peritonitis. Descriptive parameters of patients and comparison of mean values of TNF-α regarding severity of clinical status and outcome are presented in Table and Fig. 1.

When comparing the group of patients who developed severe sepsis with the group who developed sepsis shock, we found statistically highly significant difference ($p<0.01$) in TNF-α concentrations; mean values of TNF-α on admission (day zero, in ED) were 191,5-fold lower in the group with septic shock.

When comparing nonsurvivors with survivors, we found statistically highly significant difference ($p<0.01$) in TNF-α concentrations; mean values of TNF-α on admission (day zero, in ED) were 63-fold higher in survivors.

When cross-tabulation was performed on severity of clinical status and outcome, we found a statistically high significant difference (Pearson's Chi-Square = 33.851, df = 1, $p<0.01$) in survival between patients with severe sepsis and patients with septic shock. In the severe sepsis group the number of survivors was 2.6-fold higher than number of nonsurvivors while in the septic shock group the number of nonsurvivors was 9,3-fold higher than the number of survivors (Fig. 2).

Decrease in TNF-α concentration leads to the development of septic shock and fatal outcome. That is confirmed by nonparametric correlation analysis with statistically highly significant difference. The Spearman's correlation coefficient between TNF-α concentration and survival was 0.578 ($p<0.01$) and Spearman's correlation coefficient between TNF-α concentration and more severe clinical status (septic shock) was -0,502 ($p<0.01$).

The full logistic regression model for predicting outcome was performed using TNF-α and severity of clinical status. Both TNF-α and severity of clinical status are shown to be predictors of outcome with statistically highly significant difference ($p<0.01$). Severity of clinical status may be more important predictor of outcome (Wald statistic 9.942) than TNF-α concentration (Wald statistic 6.243).

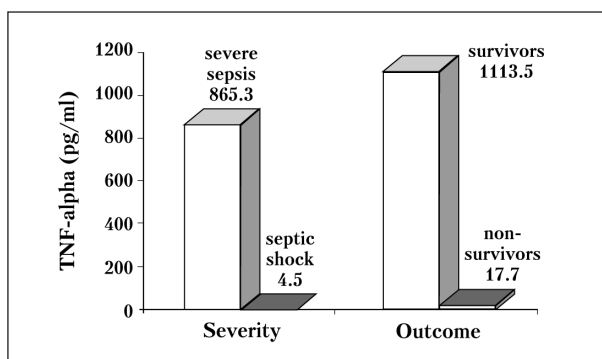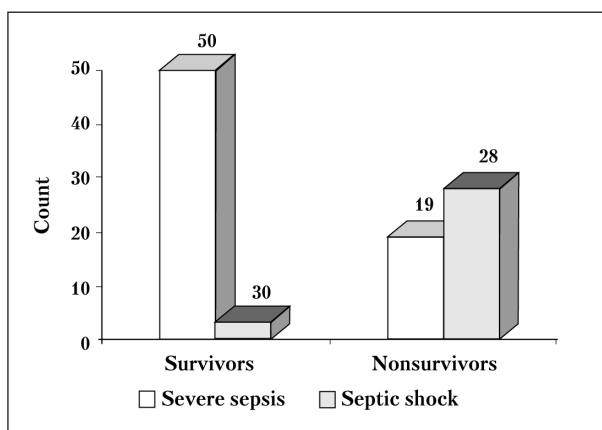Fig. 1. Mean values of TNF-α according to severity and outcome.

Fig. 2. Cross-tabulation of severity of clinical status and outcome.

Receiver operator curves were generated to determine cutoff values for optimal sensitivity and specificity for the TNF-α levels for progression to severe sepsis, septic shock and death. The area under the curve (AUC) for the TNF-α plots for severity of clinical status was 0.813 (Fig. 3).

TNF-α is a very good predictor of severity of clinical status. At cutoff level of 7.95 pg/mL sensitivity was 83,9% and specificity was 72,5%. Patients with TNF-α level lower than 7.95 pg/mL had 3.2-fold higher probability to develop septic shock than those with higher values.

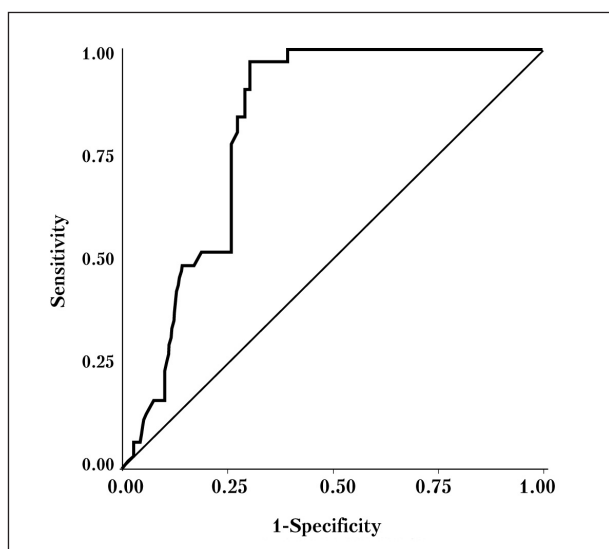The area under the curve (AUC) for the TNF-α plots for outcome was 0.834 (Fig. 4).

Fig. 3. Receiver operator curves for TNF-α and severity of clinical status.

Fig. 4. Receiver operator curves for TNF-α and outcome.

TNF-α is a very good predictor of severity of clinical status. At cutoff level of 10.5 pg/mL sensitivity was 83,0% and specificity was 77,4%. Patients with TNF-α level higher than 10.5 pg/mL had 4.8-fold higher probability to survive than those with lower values.

Discussion

Researchers estimate that 750 000 cases of severe sepsis occur each year, more than congestive heart failure, or breast cancer, colon cancer and AIDS combined. Emergency departments could be used to stop the deadly spread of sepsis, because time is of the essence for a patient with sepsis. Although multiple studies of acute myocardial infarction, trauma and stroke have been translated into improved outcomes by applying diagnosis and therapy at the most proximal stage of hospital presentation (before ICU arrival), this approach to the sepsis patient has been lacking [9].

Sepsis is essentially an exaggerated inflammatory response. It is best understood in relation to endotoxin, component of the cell walls of Gram negative bacteria. Events may also be triggered by exotoxin release from Gram positive bacteria, by other microbial products or by elements of the complement system. A syndrome identical to severe sepsis may occur in other conditions such as trauma, burns, pancreatitis, peritonitis. Only half of the patients with clinical and pathological evidence of severe SIRS have positive blood cultures. Bacterial cell wall products are recognized by the monocytes and macrophages via so-called Toll-like receptors within their own cell walls. As a result of this interaction, pro-inflammatory cytokines such as TNF-α and the interleukins are released from the activated mononuclear cells. The cytokines are responsible for majority of clinical features of sepsis [10].

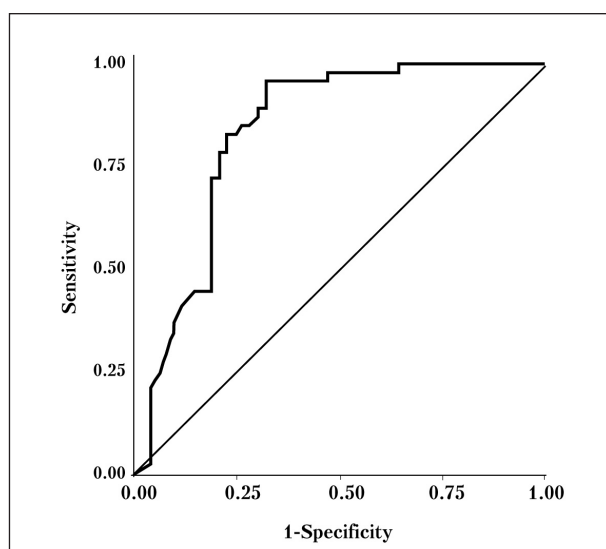More than 80 biological markers of sepsis (e.g., C-reactive protein, interleukin-6, procalcitonin, protein C) were investigated both for their diagnostic and prognostic capabilities. In general, presence of these markers has been associated with increasing morbidity and mortality. However, lack of availability, long result turnaround times and nonstandardized assays and cutoff values limit practical use for most of them. Cytokines were associated with sepsis and its prognosis in critical care patients but were not extensively studied in ED patients.

Several studies have produced conflicting results regarding the circulating levels of cytokines and severity and outcome of sepsis in ICU patients. Contrary to some authors [11], who found that high serum TNF-α levels correlate positively with the severity of sepsis and fatal outcome, we showed in our investigation that patients with septic shock and fatal outcome had very low serum TNF-α levels. One of our previous studies has shown that there is correlation between TNF-α and severity and outcome in combat casualties from blast or explosive trauma with or without secondary sepsis [12]; mean values of TNF-α were 17-fold higher in trauma and sepsis group; 2.2-fold higher in survivors ($p<0.01$) but were 43,5-fold higher in MODS group ($p<0.01$) contrary to our findings in this study that patients with septic shock had serum TNF-α 191,5-fold lower than those in group with severe sepsis. Patient population in that study was different, they were combat casualties with or without secondary sepsis and TNF-α was determined in ICU and not in ED. For the purpose of comparison, MODS corresponds to septic shock in our present study.

Florence Riche with co-authors found, as did we in our study, that in patients with abdominal septic shock high serum TNF levels were associated with increased survival [13]. The high serum level of TNF may reflect the efficacy of peritoneal inflammatory response against abdominal sepsis.

In several other studies, low levels of TNF-α were detected in various patients in ED [14, 15]. The timing of

measurement of the proinflammatory cytokines from the onset of the disease is of great importance. In sepsis, TNF-α is known to be released in the circulation within hours and then rapidly disappear. Because our patients presented to the ED at various stages of SIRS, in some cases the sample collection may have occurred after this early TNF-α peak. Also, presence of soluble TNF receptors can interfere with the measurement of unbinded TNF. One explanation for low TNF-α levels might be the phenomenon of desensitization. Although whole-body inflammation is frequently initiated by endotoxin, a single sublethal injection of endotoxin also renders the host temporarily refractory to subsequent endotoxin challenge as well as to other inflammatory stimuli. This phenomenon, referred to as endotoxin tolerance may be at least partially due to a reduced capacity of monocytes/macrophages to synthesize cytokines upon reexposure to endotoxin. Tolerance of monocytes/macrophages to endotoxin can be induced both in vivo and in vitro by endotoxin itself. Our results may be explained with TNF-α synthesis functional block that occurs in spite of continuously high endotoxin levels. Many septic patients do not have elevated, or even detectable, TNF-α levels.

The production of cytokines at various tissue sites depends, in part, on the proximity of the site to the injurious stimulus. Trends have been observed suggesting that cytokine concentrations increase with the magnitude of the injury. However, it has been difficult to correlate plasma concentration of a particular proinflammatory cytokine with the overall extent of tissue damage in clinical trials. The difficulty in finding a correlation occurs because cytokine are a component of a paracrine system that is designed to signal the local presence of inflammation to adjacent somatic tissue. Also, cytokines exist both as free secreted and cell-associated forms. TNF-α exists as a high-molecular-weight, cell-associated membrane form in inflammatory cells. This form of TNF-α acts by direct cell-to-cell contact. This dichotomous nature of this cytokine also helps to explain why systemic concentrations of circulating TNF-α may not be reflective of the degree of local TNF-α activity.

Three important variables must be considered in any discussion of the role of mediators in sepsis: the clinical status of the patient before the onset of sepsis, the length of time the patient has been ill, and innate variations in patient's ability to secrete mediators. Severe sepsis and septic shock do not develop in healthy persons; in most instances, they develop in persons with preexisting severe disease or in persons who have suffered catastrophic acute illness or trauma. Patients at greatest risk of dying of sepsis are the elderly; those receiving immunosuppressive drugs; and those with malignancies, cirrhosis, asplenia, or multiple underlying disorders (such diabetes mellitus, renal failure, or heart disease). However, these patients often already have elevated levels of one or more inflammatory mediators. They may also have activated macrophages, neutrophils, and T-cells, and thus are «primed» for the release of additional mediators. Production of other mediators, however, may sometimes be reduced in such patients. Increased production of some mediators coupled with decreased production of others may make it difficult for the body to restore homeostasis.

The length of the patient's illness may also alter the «mix» of mediators. Synthesis of cytokines may be decreased after continued infection, downregulation of the relevant receptor may occur, or inhibitors may eventually be generated. This may help to explain why high circulating levels of some mediators in a susceptible patient do not produce the massive toxic response that occurs in a healthy volunteers.

There may also be innate, genetic differences in patient's response to (or ability to produce) TNF-α and other mediators. After injection of endotoxin, human subjects show marked variation in TNF-α levels and response to fever. The finding of increase cytokine levels in patients with various arthritic disorders or inflammatory bowel disease further supports the concept that some patients may be inherently more susceptible to cytokine-induced damage.

The amount of endotoxin or mediator released initially does not need to be large; small amounts of TNF-α, for example, may be more than capable of causing sepsis in the setting of neutrophil activation and endothelial damage. Further, the continued presence of the instigating mediator is not required for sepsis to develop. Once organ damage occurs, however, local release of mediators can be quite extensive. Bronchoalveolar lavage fluid specimens from patients with the acute respiratory distress syndrome, for example, have been shown to have TNF-α concentrations greater than 500 U/mL (12500 pg/mL), a level dramatically higher than the highest serum levels usually detected in patients with septic shock.

Conclusion

Any treatment plan for sepsis must take into account the fact that at any given time, a patient will have multiple, frequently persistent sites of inflammation, each at a different stage. Thus, by the time a patient has a severe sepsis or septic shock, a complex therapy is likely to be effective provided it was started early enough. If the treatment is not given promptly, sepsis cascade can eventually become self-perpetuating, independent of the original mediator or mediators. The reticuloendothelial system eventually becomes hyperstimulated; macrophages lose an ability to secrete TNF-α. The net result is a state of metabolic anarchy in which the body can no longer control what it has created. Unless effective treatment is given, in most cases the patient will die.

The earlier therapy is started, the more likely it will be effective. Thus in our opinion, it is important to have, in patients treated in the ED and admitted to the ICU with sepsis, a reliable predictor of progression to severe sepsis, septic shock or death such as proximal inflammatory mediator TNF-α. Decreasing in TNF-α concentration leads to development of a septic shock and fatal outcome.

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