JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Oxidative stress parameters and inflammatory and immune mediators as markers of the severity of sepsis

Isil Bavunoglu[1], Habibe Genc[2], Dildar Konukoglu[3], Hayriye Cicekci[3], Volkan Sozer[4], Remise Gelisgen[3], Hafize Uzun[3]

[1] *Department of Internal Medicine, Medical Faculty of Cerrahpasa, Istanbul University, Istanbul, Turkey*
[2] *Department of Medical Biochemistry, Medical Faculty, Bezmialem Vakif University, Istanbul, Turkey*
[3] *Department of Biochemistry, Medical Faculty of Cerrahpasa, Istanbul University, Istanbul, Turkey*
[4] *Department of Biochemistry, Faculty of Science and Letters, Yildiz Technical University, Istanbul, Turkey*

Abstract

Introduction: Sepsis is a complex inflammatory syndrome with diverse etiology and wide spectrum of severity. The aim of this study was to investigate whether inflammatory mediators, in comparison with oxidative parameters, are associated with severity of sepsis. Methodology: Plasma neopterin, adenosine deaminase (ADA), vascular cell adhesion molecule (VCAM), intracellular adhesion molecule (ICAM), interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF-α), as inflammatory mediators, and serum nitric oxide (NOx), nitrotyrosine (NT), oxidized LDL (oxLDL) levels, serum paraoxonase 1 (PON1) activity, and erythrocyte glutathione (GSH) levels as oxidative stress parameters of 12 patients with mild sepsis, 25 patients with severe sepsis, and 20 healthy control subjects were evaluated. NOx, GSH levels and PON1 activity were determined by colorimetric methods, whereas neopterin, VCAM, ICAM, IL-1, IL-6, TNF-α, NT, and oxLDL levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results: All parameters in mild and severe sepsis were significantly different from those of healthy subjects, except ADA activities. Patients with severe sepsis exhibited higher IL-6, TNF-α, NT, and oxLDL levels than patients with mild sepsis. GSH (98%, 98%), oxLDL (98%, 98%), VCAM-1 (99%, 99%), and ICAM-1 (99%, 99%) have much more sensitivitiy and specificity in sepsis.

Conclusions: Our results suggest that the oxidative stress and inflammatory response in patients with sepsis were increased and that serum IL-6, TNF-α, NT, and oxLDL levels were correlated with the severity of sepsis. Therefore, increases in these parameters may contribute to the dysfunction or failure of one or more organs, or even death, in sepsis.

Key words: Sepsis; cytokine; nitrotyrosine; glutathione; nitric oxide

J Infect Dev Ctries 2016; 10(10):1045-1052. doi:10.3855/jidc.7585

(Received 24 August 2015 – Accepted 07 November 2015)

Introduction

Sepsis is defined as the presence or presumed presence of an infection accompanied by evidence of systemic inflammatory response syndrome. Severe sepsis is defined as the occurrence of sepsis and multiple organ dysfunctions. Organ dysfunction can include acute lung injury; coagulation abnormalities; thrombocytopenia; altered mental status; renal, liver, or cardiac failure; or hypoperfusion with lactic acidosis [1].

One of the key features of sepsis is tissue infiltration by phagocytic cells. Polymorphonuclear leukocytes (neutrophils; PMN) and monocytes/macrophages respond to septic stimulation by producing reactive oxygen species (ROS) (*e.g.*, superoxide, hydrogen peroxide) and reactive nitrogen species (RNS) (*e.g.*, nitric oxide/NO) [2]. Septic stimuli (*e.g.*, lipopolysaccharides, tumor necrosis factor alpha [TNF-α]) initiate activation of transcription factors such as NFκB and AP-1, resulting in transcriptional activation of multiple genes. This leads to the release of pro-inflammatory cytokines (*e.g.*, TNF-α, interleukin (IL)-1β, etc.) and increased expression of adhesion molecules (*e.g.*, E-selectin, vascular cell adhesion molecule [VCAM], intracellular adhesion molecule [ICAM]) and chemokines by endothelial cells. A key role of ROS/RNS in modulation of the endothelial cell pro-inflammatory phenotype has been emphasized [2]. An inflammatory response to septic stimuli is crucial for host defence, because it up-regulates anti-inflammatory mediators (*e.g.*, IL-1 receptor antagonist, IL-4, IL-10), antioxidants (*e.g.*, glutathione, vitamin C, vitamin E) and antioxidant enzymes (*e.g.*, catalase, glutathione peroxidase, manganese superoxide

dismutase) [2]. Oxidative stress, a situation with increased ROS and RNS production and/or decreased antioxidant defence mechanisms, might be important in the pathophysiologic function and prognostic value of sepsis.

An association has been described between inflammation and the progression of sepsis and has been shown with several biochemical parameters. Our aim was to examine whether there is a correlation between inflammatory mediators and oxidative stress parameters in severe sepsis. We determined inflammatory mediators, such as VCAM-1, ICAM-1, IL-1, IL-6, and TNF-α, adenosine deaminase (ADA) and neopterin levels, and oxidative stress markers such as paraoxonase 1 (PON1) activity, NOx, nitrotyrosine (NT), oxidized LDL (oxLDL), and erythrocyte glutathione (GSH) levels. Additionally, we investigated the effect of these parameters in the determination of severity of sepsis and their prognostic value.

Methodology

Patient selection and definitions

A total of 37 patients with sepsis and 20 healthy subjects as the control group were included in the study. All participants were informed about the survey and voluntarily signed and dated the consent form. Two patients with severe sepsis were transferred from the emergency room to the intensive care unit (ICU), and four patients with severe sepsis died during observation in the emergency unit. The average duration of hospitalization for 19 severe sepsis patients followed in the emergency service was 15.5 days. One of the two patients who was transferred to the ICU died, whereas the other was intubated and monitored for 30 days in the ICU. Patients with mild sepsis were treated in the emergency service. All of the patients survived, and the average duration of hospitalization was 8.9 days.

Bacterial species in blood cultures were evaluated. The species that were produced from those blood cultures are as follows: Methicillin Resistant Coagulase Negative Staphylococcus (MRCNS) in 5 severe sepsis patients, methicillin-resistant *Staphylococcus aureus* (MRSA) in 3, *Pseudomonas aeruginosa* in 2, extended-spectrum beta-lactamase (ESBL)-positive *E. coli* in 1, and *Acinetobacter baumannii* in 1. Simultaneous MRCNS and *Pseudomonas aeruginosa* growth was determined in one patient. Each blood culture of patients with mild sepsis, MRCNS, *E. coli*, ESBL-positive *E. coli*, and *Candida* growths were observed. Blood cultures obtained from 12 patients with severe sepsis and 8 patients with mild sepsis did not reveal growth of any bacteria.

Septic patients were included in the study as soon as they met at least two of the criteria for systemic inflammatory response syndrome (SIRS) defined by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee [3]: temperature > 38°C or < 36°C; heart rate > 90 beats/minute; respiratory rate > 20 breaths/minute or arterial carbon dioxide tension < 32 mmHg; and leukocyte count > 12×10^9 cells/L or < 4×10^9 cells/L. Mild sepsis is defined as the presence of probable or documented infection together with systemic manifestations of infection [4]. To distinguish severe sepsis, at least one of following conditions was required: hypoxemia (arterial oxygen tension/fractional inspired oxygen ratio < 250); oliguria (urine output < 0.5 mL/kg body weight for 2 hours); lactic acidosis (lactate concentration > 2 mmol/L); thrombocytopenia (platelet count < 100×10^9/L); and a recent change in mental status without sedation [5]. The Acute Physiology and Chronic Health Evaluation (APACHE) II score [6] and Sepsis-related (or Sequential) Organ Failure Assessment (SOFA) score [7] were employed to determine the initial severity of illness.

The patients' initial blood samples were drawn as soon as the diagnosis of sepsis was made clinically. Blood cultures were studied along with biochemical tests. The duration of hospital stay and/or mechanical ventilation was recorded. Survival was defined as being alive at hospital discharge. All patients were periodically followed up by the same intensivists. Patients with trauma, pregnant and breast-feeding women, patients with cardiopulmonary arrest who required basic cardiac life support measures, and those who received intravenous fluid resuscitation, antibiotics, catecholamines, or intravenous corticosteroids before enrolment were excluded.

Individuals without any known illness residing in the same area as the other study patients were selected as a control group. These controls were people presenting to the outpatient clinic of the internal medicine department for routine check-up, without any history or signs of active disease and no signs of infection. Smokers and healthy subjects taking any drug known to affect carbohydrate and lipid metabolism, or oxidant and antioxidant status, were excluded. All patients and controls were of Turkish descent.

Specimen collection and processing

Blood samples were collected from an indwelling arterial or venous catheter, in one tube with anticoagulant (with EDTA) and another tube without anticoagulant. After immediate centrifugation (3,000 g,

10 minutes, 4°C), plasma and serum were stored at -80°C until the final analysis. Erythrocytes were washed three times in 5 mL saline, hemolyzed by diluting fourfold with water, and GSH was studied in erythrocytes on the same day. Before the assays, all samples were thawed to room temperature and mixed by gentle swirling or inversion. All parameters from all samples were analyzed in a single batch after completion of patient enrolments.

Biochemical analysis

Total nitric oxide (NOx) was measured as its stable metabolites nitrate (NO3-) and nitrite (NO2-). Nitrate was first reduced by nitrate reductase to nitrite, and then nitrite was determined spectrophotometrically by the Griess reaction (Nitric Oxide Colorimetric Assay, Roche Diagnostics GmbH, Sandhoferstr, Mannheim, Germany). The coefficients of intra- and inter-assay variations were 3.7% (n = 10) and 4.1% (n = 10), respectively. Reduced GSH concentration was determined according to the method of Beutler *et al*. [8] using metaphosphoric acid for protein precipitation and 5-5'-ditiobis-2- nitro benzoic acid for color development. Hemoglobin concentration was determined by the cyanmethemoglobin method [9]. Serum PON1 activity was assayed using synthetic paraoxon (diethyl-p-nitrophenyl phosphate) as substrate [10].

Plasma IL-1, IL-6, TNF-α, VCAM-1, and ICAM-1 levels (R&D Systems Inc., Minneapolis, USA), serum NT (Cell Biolabs, San Diego, USA), ox-LDL (Mercodia Oxidized LDL ELISA, Uppsala, Sweden), neopterin (IBL Immuno Biological Laboratories, Hamburg, Germany) levels were measured in duplicate aliquots, using a human enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions. Serum ADA activity was measured in duplicate aliquots, using a commercial colorimetric assay kit with a cut-off value for positive test of 30 U/L in accordance with the manufacturer's instructions (Diazyme, General Atomics, San Diego, CA, USA).

The other biochemical parameters were measured by routine methods with commercial kits. High-sensitivity C-reactive protein (hsCRP) measurements were performed using a nephelometric method (BN II nephelometer; Dade Behring Holding GmbH, Liederbach, Germany). Erythrocyte sedimentation rate (ESR) measurements were performed using the Westergren method.

Statistical analysis

All results are expressed as the mean ± standard deviation (SD). All parameters were tested using the Mann-Whitney U test. For correlation analysis, Spearman's rho correlation coefficient was determined. A receiver operator characteristic (ROC) curve was formed and the area under the curve (AUC) was used to compare the performance of various tests. The optimal cut-off point of testing values was selected as the point on the curve that had the shortest distance to the top-left corner (sensitivity = 1 and false positive = 0) of the graph. The sensitivity and specificity with 95% confidence intervals in the prediction of sepsis were calculated. The null hypothesis was rejected for $p < 0.05$. Data were analyzed using SPSS version 17 (SPSS, Chicago, USA).

Ethical approval

The study protocol was approved by the ethics committee of Cerrahpasa Medical Faculty and was conducted in accordance with the Helsinki Declaration.

Results

Demographics characteristics of the patients with sepsis and controls are described in Table 1. The reported analysis included 37 patients with sepsis. Of these 37 patients, 25 were defined as severe sepsis and 12 as mild sepsis. A total of 18 patients with sepsis (5 mild and 13 severe sepsis) had respiratory infection,

Table 1. Demographic characteristics of patients with mild and severe sepsis and healthy controls.

	Controls (n = 20)	Mild sepsis (n = 12)	Severe sepsis (n = 25)	p
Age	55 ± 15	58.9 ± 15.7	63.4 ± 17.5	0.178
Sex (female/male)	11 / 9	7/5	12/13	0.812
APACHE	-	19 (18–23)	24 (18–26)	< 0.01
SOFA	-	11 (10–12)	13 (10–14)	0.011
Respiratory infection	-	5 (41.7%)	13 (52%)	
Urinary infection	-	5 (41.7%)	10 (40%)	
Other infection	-	2 (16.6%)	2 (8%)	

APACHE: acute physiology and chronic health evaluation; SOFA: sepsis-related organ failure assessment.

and 15 patients with sepsis (5 mild and 10 severe sepsis) had a urinary infection. Non-parametric tests were used for comparison of the study parameters. Results of immune, inflammation, and oxidative marker levels in sepsis and in controls are detailed in Table 2. Erythrocyte GSH and serum PON1 activity levels were significantly lower in patients with mild and severe sepsis compared to those in the control group ($p < 0.001$ and $p < 0.001$, respectively). Patients with mild and severe sepsis had significantly higher plasma NT ($p < 0.001$), IL-1 ($p < 0.001$), IL-6 ($p < 0.001$), TNF-α ($p < 0.001$), NOx ($p < 0.002$), oxLDL ($p < 0.001$), neopterin ($p < 0.001$), VCAM-1 ($p < 0.001$), ICAM-1 ($p < 0.001$), and CRP ($p < 0.001$) levels than the control subjects. Plasma ADA levels were similar in both groups. There was a significant difference in plasma NT, IL-6, TNF-α, and oxLDL in the severe sepsis group compared to the mild sepsis group ($p < 0.002$, $p < 0.002$, $p < 0.003$, and $p < 0.02$, respectively). Correlation analysis results and Spearman's rho correlation coefficient are presented in Table 3. Erythrocyte GSH levels were negatively correlated with plasma levels of NT ($p < 0.01$), IL-1 ($p < 0.01$), IL-6 ($p < 0.01$), TNF-α ($p < 0.01$), NOx ($p < 0.01$), oxLDL ($p < 0.01$), neopterin ($p < 0.01$), VCAM-1 ($p < 0.01$), ICAM-1 ($p < 0.05$), and CRP ($p < 0.01$), and they were positively correlated with serum PON1 activities ($p < 0.001$). Serum PONl activities were negatively correlated with plasma NT ($p < 0.01$), IL-1 ($p < 0.01$), IL-6 ($p < 0.01$), TNF-α ($p < 0.01$), oxLDL ($p < 0.01$), ICAM-1 ($p < 0.01$), and CRP ($p < 0.001$) levels. There was also a significant relationship between plasma oxLDL levels and IL-1 ($p < 0.01$), IL-6 ($p < 0.01$), TNF-α ($p < 0.01$), NOx ($p < 0.01$), VCAM-1 ($p < 0.01$), and CRP ($p < 0.05$) levels. Correlation analysis results between plasma NT and plasma IL-1, IL-6, TNF-α, oxLDL, and CRP levels were found to be statistically significant ($p < 0.01$, $p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.01$, respectively). Plasma neopterin levels were correlated with IL-1 ($p < 0.05$), IL-6 ($p < 0.01$), TNF-α ($p < 0.01$), VCAM-1 ($p < 0.05$), ICAM-1 ($p < 0.05$), and CRP ($p < 0.05$) levels. There was also a significant relationship among inflammatory markers such as IL-1, IL-6, TNF-α, VCAM-1, ICAM-1, or CRP. The sensitivities and specificities of erythrocyte GSH and serum PON1 activities, plasma IL-1, IL-6, TNF-α, NOx, oxLDL, neopterin, VCAM-1, and ICAM-1 levels to detect sepsis are shown in Table 3. For the sepsis group, the areas under the curve were

Table 2. Erythrocyte GSH, plasma and serum immune, inflammation, oxidative marker levels in patients with mild and severe sepsis and controls.

	Controls (n = 20)	Mild sepsis (n = 12)	Severe sepsis (n = 25)	P[a]	P[b]
Erythrocyte GSH (%mg /gHb)	3.91 ± 0.28	1.21 ± 0.67[a]	1.01 ± 0.42[a]	< 0.001	Ns
NT (nmol/L)	1.11 ± 0.95	121.5 ± 82.1[a]	340.4 ± 212.2[a]	< 0.001	< 0.002
IL-1 (pg/mL)	2.61 ± 0.94	5.95 ± 1.67[a]	5.73 ± 2.35[a]	< 0.001	Ns
IL-6 (pg/mL)	3.20 ± 1.19	73.1 ± 25.8[a]	113.5 ± 37.4[a]	< 0.001	< 0.002
TNF-α (pg/mL)	3.36 ± 1.57	102.1 ± 30.8[a]	142.5 ± 38.3[a]	< 0.001	< 0.003
NOx (µmol/L)	6.8 ± 4.5	18.9 ± 13.3[a]	13.7 ± 8.9[a]	< 0.002	ns
oxLDL (U/L)	15.8 ± 4.8	77.1 ± 25.6 [a]	103.8 ± 33.6 [a]	< 0.001	< 0.02
PON1 (U/mL)	117.4 ± 29.7	25.2 ± 10.7[a]	23.8 ± 12.5[a]	< 0.001	Ns
Neopterin (nmol/L)	2.4 ± 1.2	41.6 ± 31.1[a]	50.8 ± 35.9[a]	< 0.001	Ns
ADA (U/L)	1.61 ± 1.23	2.27 ± 2.24	1.13 ± 0.73	Ns	Ns
VCAM-1 (ng/mL)	1.25 ± 0.79	4.04 ± 1.64[a]	3.29 ± 0.75[a]	< 0.001	Ns
ICAM-1 (ng/mL)	0.29 ± 0.08	2.37 ± 0.66[a]	2.52 ± 0.65[a]	< 0.001	Ns
CRP (mg/L)	1.12 ± 0.33	102.5 ± 59.3[a]	128.6 ± 48.5[a]	< 0.001	Ns

Statistical significance at $p < 0.05$; P: plasma; Hb: hemoglobin; GSH: glutathione; NT: nitrotyrosine; IL-1: interleukin-1; IL-6: interleukin-6; TNF-α: tumor necrosis factor alpha; NOx: nitric oxide; oxLDL: oxidized-LDL; PON1: paraoxonase 1; ADA: adenosine deaminase; VCAM: vascular cell adhesion molecule; ICAM: intracellular adhesion molecule; CRP: C-reactive protein; Ns: not significant; P[a]: statistical difference from control group; P[b]: statistical difference from mild sepsis group.

Table 3. Spearman's rho correlation coefficient (r) for studied oxidative and inflammatory markers in the sepsis and control groups (n = 57).

	GSH	NT	IL-2	IL-6	TNF-α	NOx	OxLDL	PON1	Neopterin	VCAM-1	ICAM-1	CRP
GSH	1.000	-0.681**	-0.536**	-0.727**	-0.690**	-0.661**	-0.623**	0.640**	-0.502**	-0.486**	-0.359*	-0.690**
NT	-0.681**	1.000	0.546**	0.800**	0.790**	0.510**	0.758**	-.690**	0.380*	0.318*	0.348*	0.793**
IL-1	-0.536**	0.546**	1.000	0.477**	0.512**	0.534**	0.649**	-.523**	0.340*	0.485**	0.365*	0.566**
IL-6	-0.727**	0.800**	0.477**	1.000	0.735**	0.534**	0.733**	-.719**	0.482**	0.343*	0.233	0.746**
TNF-α	-0.690**	0.790**	0.512**	0.735**	1.000	0.442**	0.699**	-.709**	0.280	0.183	0.281	0.805**
NOx	-0.661**	0.510**	0.534**	0.534**	0.442**	1.000	0.648**	-.410**	0.226	0.634**	0.300	0.427**
OxLDL	-0.623**	0.758**	0.649**	0.733**	0.699**	0.648**	1.000	-.669**	0.154	0.402**	0.274	0.741**
PON1	0.640**	-0.690**	-0.523**	-0.719**	-0.709**	-0.410**	-0.669**	1.000	-0.220	-0.200	-0.398**	-0.785**
Neopterin	-0.502**	0.380*	0.340*	0.482**	0.280	0.226	0.154	-.220	1.000	0.311*	0.387*	0.374*
VCAM-1	-0.486**	0.318*	0.485**	0.343*	0.183	0.634**	0.402**	-.200	0.311*	1.000	0.413**	0.391*
ICAM-1	0-.359*	0.348*	0.365*	0.233	0.281	0.300	0.274	-.398**	0.387*	0.413**	1.000	0.376*
CRP	-0.690**	.0793**	0.566**	0.746**	0.805**	0.427**	0.741**	-.785**	0.374*	0.391*	0.376*	1.000

GSH: glutathione; NT: nitrotyrosine; IL-1: interleukin-1; IL-6: interleukin-6; TNF-α: tumor necrosis factor alpha; NOx: nitric oxide; oxLDL: oxidized-LDL; PON1: paraoxonase 1; VCAM: vascular cell adhesion molecule; ICAM: intracellular adhesion molecule; CRP: C-reactive protein; Statistical significance: * $p < 0.05$, **$p<0.01$.

equal to 1.000, 1.000, 0.993, 0.991, 0.993, 0.995, 1.000, 0.986, 1.000, and 1.000 for GSH and PON1, and IL-1, IL-6, NT, TNF-α, NOx, oxLDL, neopterin, VCAM-1 and ICAM-1 levels, respectively. ROC curve analysis was done to detect cut-off points to determine septicemia; the best cut-off values of GSH, PON1, IL-1, IL-6, TNF-α, NOx, oxLDL, neopterin, VCAM-1, and ICAM-1 levels were 2.92% mg/gHb, 55.6 U/L, 2.50 pg/mL, 5.73 pg/mL, 6.25 pg/mL, 2.97 µmol/L, 36.4 U/L, 6.51 nmol/L, 2.43 ng/mL, and 1.22 ng/mL, respectively (Table 3). The sensitivities and specificities of IL-6, NT, TNF-α, and oxLDL measurements to detect the severity of sepsis are shown in Table 4.

Discussion

Despite intensive basic research and clinical studies, the pathophysiology of sepsis is still poorly understood. Various mediators are involved in the pathology of sepsis, some of which (*e.g.*, IL-6, ICAM, VCAM, TNF-α, macrophage migration inhibitory factor [MIF]) may be considered to be at the core of the inflammatory process. Oxidative stress (oxidant/antioxidant imbalance) also plays an important role in the pathophysiology of sepsis. Previous human and animal studies confirmed severe oxidative stress in patients with sepsis, demonstrating increased oxidant markers and decreased antioxidant capacity [11-16]. Our results are consistent with the literature. Serum PON1 activity was significantly lower in patients with sepsis than in controls, and erythrocyte GSH levels were positively correlated with serum PON1 activities. Serum PONl activities were also negatively correlated with plasma NT, IL-1, IL-6, TNF-α, oxLDL, ICAM-1, and CRP levels. Novak *et al*. [17] supposed that monitoring of PON1 activity during infection in critically ill patients offers a potentially useful marker of sepsis progress and recovery. A similar pattern was characterized by very low PON1 activities and high CRP concentrations. PON1 that decreases during the inflammatory response is classified among the negative acute phase proteins. In an experimental animal model of sepsis [18], low PON1 activities were very similar to those found in our study. The cause-effect relationship

Table 4. Screening efficiency of GSH, PON1, IL-1, IL-6, TNF- α, NOx, neopterin, ox-LDL, VCAM, and ICAM levels based on receiver-operator characteristic curve in sepsis.

Test result variable(s)	Cut-off value	Area under the curve	Sensitivity (%)	Specificity (%)
GSH	2.92% mg /gHb	1.000	98	98
PON1	55.6 U/mL	1.000	97	95
IL-1	2.50 pg/mL	0.993	75	80
IL-6	5.73 pg/mL	0.991	97	84
TNF-α	6.25 pg/mL	0.993	97	84
NOx	2.97 µmol/L	0.995	97	84
OxLDL	36.4 U/L	1.000	98	98
Neopterin	6.51 nmol/L	0.986	92	83
VCAM-1	2.43 ng/mL	1.000	99	99
ICAM-1	1.22 ng/mL	1.000	99	99

GSH: glutathione; PON1: paraoxonase 1; IL-1: interleukin-1; IL-6: interleukin-6; TNF-α: tumor necrosis factor alpha; NOx: nitric oxide; oxLDL: oxidized-LDL; VCAM: vascular cell adhesion molecule; ICAM: intracellular adhesion molecule.

between decreased PON1 activity and increased oxidative stress is not established; most likely it is a dynamic bi-directional relationship [19]. Sepsis occurs as a result of complex host-pathogen interactions, leading to release of inflammatory mediators, as well as reactive oxygen intermediates and reactive nitrogen intermediates. It is likely that the oxidizing environment induced by sepsis could result in an increased binding of free radicals to the PON1, leading to the decrease of PON1 activity in the circulation [17].

Patients with severe sepsis had significantly higher plasma oxLDL than did patients with mild sepsis in the current study. There was also a significant relationship between oxLDL levels and inflammatory mediators. oxLDL can participate in local or systemic inflammation; it is upregulated in inflammation. Additionally, oxLDL had the highest sensitivity (98%) and specificity (98%) in sepsis. Behnes *et al*. [20] demonstrated that ox-LDL levels increase in patients with severe sepsis. Our study and other ones [20-21] confirm a better understanding of the development of oxidative stress in this type of sepsis. In severe sepsis, tissue damage represents a main consequence of inflammation, and in particular, increased oxLDL leading to endothelial cell damage. Increased NT immunoreactivity was found in human biopsy specimens with viral myocarditis and sepsis, suggesting a pathogenetic role of peroxynitrite formation and/or protein nitration in these diseases [22]. Patients with mild and severe sepsis had significantly higher plasma NT and NOx levels than did the control subjects in the current study. NT levels were also positively correlated with plasma IL-1, IL-6, TNF-α, oxLDL, and CRP levels. Constantin *et al*. [23] demonstrated that septic animals have increased levels of NT and NO, and that NT plays a prominent role in sepsis. However, it seems that NT levels are more effective in determining the severity of sepsis than are NOx levels, as NT levels were significantly higher in patients with severe sepsis than in patients with mild sepsis. Furthermore, increased levels of NT were accompanied by an increase in inflammatory mediators. Peroxynitrite can directly oxidize low-molecular-weight thiols, most notably reduced GSH. Erythrocyte GSH levels were significantly lower in patients with mild and severe sepsis than in controls in the current study. Erythrocyte GSH levels were also positively correlated with serum PON1 activity and negatively correlated with plasma levels of NT, IL-1, IL-6, TNF-α, NOx, oxLDL, neopterin, VCAM-1, ICAM-1, and CRP. The susceptibility of cells to NT and NOx toxicity largely depends on the amount of intracellular GSH. GSH depletion enhances toxicity and tissue injury during sepsis, and a relationship between decreased GSH and enhanced NT and NOx toxicity has also been proposed to contribute to the exacerbation of sepsis. Patients with sepsis have significantly higher IL-1, IL-6, TNF-α, VCAM-1, and ICAM-1 levels than the control subjects. In the current study, reported cytokine values are total plasma levels and of unknown cellular origin. There was also significant relationship among inflammatory markers such as IL-1, IL-6, TNF-α, VCAM-1, ICAM-1, or CRP levels. ICAM and VCAM are inducible glycoproteins that are expressed during inflammatory reactions. The up-regulation on the surface of endothelial cells mediates stable leucocyte adhesion to the vascular endothelium. This process leads to leukocyte extravasations into sites of infection or injury and, therefore, plays a pivotal role in the development of organ injury in sepsis [24]. Increased adhesion molecules have been found in different stages of disease, especially inflammation and infection. For the acute phase of sepsis, increased plasma IL-6 and TNF-α levels were harmful and correlated with severity of sepsis in our study. Patients with severe sepsis had significantly higher plasma IL-6 and TNF-α levels than did patients with mild sepsis. High plasma IL-6 and TNF-α levels have high sensitivity (97%) and specificity (84%) in the diagnosis of sepsis. ICAM-1 and VCAM-1 have the highest sensitivity (99%) and specificity (99%) in sepsis. However, these levels are not effective in determining the severity of sepsis. The systemic effect of sepsis includes the release of inflammatory cytokines. Sepsis seems to result from overwhelming systemic inflammation, which is caused by excessive release of cytokines into the systemic circulation. In human and animal models of sepsis induced by injection of bacterial endotoxin, TNF-α production is quickly activated and can be detected in plasma [25-29]. However, Lvovschi *et al*. [29] showed that there are no typical cytokine profiles associated with systemic inflammatory response syndrome (SIRS), severe sepsis, septic shock, and bacterial infection among febrile patients. Their main finding is that previous associations commonly defined between sepsis and individual cytokine levels were not confirmed using these multi-parameter techniques.

We found that serum neopterin levels were higher in patients with sepsis than in the controls. However, no significant difference regarding serum neopterin levels was found in between mild and severe sepsis cases. We also did not observe any significant difference in ADA activity levels between neither patient and control group nor severe and mild sepsis. In the literature, there

are limited studies regarding plasma ADA activities in patients with sepsis. Its prognostic value is very limited, probably because of the late release of ADA in the mediator cascade. Current results of this study could be related to non-homogenous distribution of the clinical characteristics of our patient groups. Our study shows that neopterin levels were increased in mild sepsis and severe sepsis as well, which are directly correlated with the accompanying infection.

There is also growing evidence that inflammation markers are raised in sepsis. Procalcitonin (PCT), which is a novel marker of inflammation and infection, has been widely investigated for its prognostic value in septic patients. Although studies have produced conflicting results [30-36], Tasdelen Fisgin *et al*. [30] and Hensler *et al*. [32] demonstrated that neopterin was a better prognostic factor than PCT in patients with sepsis.

CRP is a nonspecific marker of inflammation and has been shown to be increased in patients with sepsis compared to controls; however, in our study, this marker failed to discriminate mild sepsis from severe sepsis. CRP is produced within 4–6 hours after onset of tissue injury or inflammation; it doubles every 8 hours before peaking at around 36 hours [37]. The increased severity of sepsis does not seem to change CRP levels.

Conclusions

The finding demonstrating that a marker of inflammation or immune activation is elevated in patients with progressive sepsis is consistent with previous reports, showing that disease activity may be present despite clinical stability. Our results suggest that neopterin, ICAM-1, VCAM-1, TNF-α, IL-1, and IL-6 being released after infection play important roles in the initiation of the inflammatory process in sepsis. Oxidative stress (increased NT, NOx, and oxLDL and decreased GSH and PON1) is an important contributor to the pathophysiology of sepsis. However, it seems that the serum oxLDL levels are better biomarkers of lipid peroxidation in sepsis than are the other markers. This may be related to their high specificity and sensitivity in sepsis. Additionally, we should emphasize that oxLDL levels increase in severe sepsis. Increased oxidative stress and higher inflammation levels may also reflect disease severity and organ dysfunction. Although these parameters are unable to distinguish mild sepsis and severe sepsis, and they are unlikely to be useful in outcome measures, they may function as early predictors of survival in patients with severe sepsis. Therefore, increases in these parameters may contribute to the dysfunction or failure of one or more organs, or even death, in sepsis. Although the number of our cases is limited, our results indicate that the plasma levels of inflammation and oxidative stress markers may be important to discriminate sepsis. Further studies should be planned to ascertain the clinical importance of these parameters as early predictors of sepsis.

References

1. Nguyen HB, Rivers EP, Abrahamian FM, Moran GJ, Abraham E, Trzeciak S, Huang DT, Osborn T, Stevens D, Talan DA; Emergency Department Sepsis Education Program and Strategies to Improve Survival (ED-SEPSIS) Working Group (2006) Severe sepsis and septic shock: review of the literature and emergency department management guidelines. Ann Emerg Med 48: 28-54.
2. Cepinskas G, Wilson JX (2008) Inflammatory response in microvascular endothelium in sepsis: role of oxidants. J Clin Biochem Nutr 42: 175-184.
3. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (1992) Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 20: 864-874.
4. Schorr CA, Dellinger RP (2014) The Surviving Sepsis Campaign: past, present and future. Trends Mol Med 20: 192-194.
5. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb SA, Beale RJ, Vincent JL, Moreno R, Surviving Sepsis Campaign Guidelines, Committee; Pediatric, Subgroup (2013) Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012. Crit Care Med 41: 580-637.
6. Knaus WA, Draper EA, Wagner DP, Zimmerman JE (1985) APACHE II: a severity of disease classification system. Crit Care Med 13: 818-829.
7. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, Reinhart CK, Suter PM, Thijs LG (1996) The SOFA (sepsis-related organ failure assessment) score to describe organ dysfunction/failure. Intensive Care Med 22: 707-710.
8. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 51: 882-888.
9. Fairbanks V, Klee GG (1986) Biochemical aspects of hematology. In Tietz NW, editor. Textbook of Clinical Chemistry. Philadelphia: WB Saunders Company. 1532-1534.
10. Uzun H, Karter Y, Aydin S, Curgunlu A, Simşek G, Yücel R, Vehiyd S, Ertürk N, Kutlu A, Benian A, Yaldiran A, Oztürk E, Erdine S (2004) Oxidative stress in white coat hypertension; role of paraoxonase. J Hum Hypertens 18: 523-528.
11. Brenner T, Hofer S, Rosenhagen C, Steppan J, Lichtenstern C, Weitz J, Bruckner T, Lukic IK, Martin E, Bierhaus A, Hoffmann U, Weigand MA (2010) Macrophage migration inhibitory factor (MIF) and manganese superoxide dismutase (MnSOD) as early predictors for survival in patients with severe sepsis or septic shock. J Surg Res 164: e163-e171.
12. Gül M, Ayan M, Seydanoğlu A, Cander B, Girişgin S, Erayman I, Erdem S (2011) The effect of N-acetyl cysteine on serum glutathione, TNF-alpha and tissue malondialdehyde

levels in the treatment of sepsis. Ulus Travma Acil Cerrahi Derg 17: 293-297.
13. Ribeiro Nogueira C, Ramalho A, Lameu E, Da Silva Franca CA, David C, Accioly E (2009) Serum concentrations of vitamin A and oxidative stress in critically ill patients with sepsis. Nutr Hosp 24: 312-317.
14. Goode HF, Cowley HC, Walker BE, Howdle PD, Webster NR (1995) Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. Crit Care Med 23: 646-651.
15. Seija M, Baccino C, Nin N, Sánchez-Rodríguez C, Granados R, Ferruelo A, Martínez-Caro L, Ruíz-Cabello J, de Paula M, Noboa O, Esteban A, Lorente JA (2012) Role of peroxynitrite in sepsis-induced acute kidney injury in an experimental model of sepsis in rats. Shock 38: 403-410.
16. Lange M, Szabo C, Traber DL, Horvath E, Hamahata A, Nakano Y, Traber LD, Cox RA, Schmalstieg FC, Herndon DN, Enkhboatar P (2012) Time profile of oxidative stress and neutrophil activation in ovine acute lung injury and sepsis. Shock 37: 468- 472.
17. Novak F, Vavrova L, Kodydkova J, Novak F Sr, Hynkova M, Zak A, Novakova O (2010) Decreased paraoxonase activity in critically ill patients with sepsis. Clin Exp Med 10: 21-25.
18. Draganov D, Teiber J, Watson C, Bisgaier C, Nemzek J, Remick D, Standiford T, La Du B (2010) PON1 and oxidative stress in human sepsis and an animal model of sepsis. Adv Exp Med Biol 660: 89-97.
19. Roberts RA, Laskin DL, Smith CV, Robertson FM, Allen EM, Doorn JA, Slikker W (2009) Nitrative and oxidative stress in toxicology and disease. Toxicol Sci 112: 4-16.
20. Behnes M, Brueckmann M, Liebe V, Liebetrau C, Lang S, Putensen C, Borggrefe M, Hoffmann U (2008) Levels of oxidized low-density lipoproteins are increased in patients with severe sepsis. J Crit Care 23: 537-541.
21. Al-Banna N, Lehmann C (2013) Oxidized LDL and LOX-1 in experimental sepsis. Mediators Inflamm 2013: 761-789.
22. Kooy NW, Lewis SJ, Royall JA, Ye YZ, Kelly DR, Beckman JS (1997) Extensive tyrosine nitration in human myocardial inflammation: evidence for the presence of peroxynitrite. Crit Care Med 25: 812-819.
23. Constantin L, Gonçalves RC, Giombelli VR, Cristiane DT, Vuolo F, Kist LW, Oliveira GMT de, Pasquali MA de B, Bogo MR, Mauad T, Horn Jr A, Melo KV, Fernandes C, Moreira JCF, Ritter C, Dal-Pizzo F (2014) Regulation of lung oxidative damage by endogenous superoxide dismutase in sepsis. Intensive Care Med Exp 2: 17.
24. Parrillo JE (1993) Pathogenetic mechanisms of septic shock. N Engl J Med 328: 1471.
25. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, Cerami A, Wolff SM, WilmoreDW (1988) Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 318: 1481-1486.
26. Cannon JG, Tompkins RG, Gelfand JA, Michie HR, Stanford GG, van der Meer JW, Endres S, Lonnemann G, Corsetti J, Chernow B (1990) Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. J Infect Dis 161: 79-84.
27. Hesse DG, Tracey KJ, Fong Y, Manogue KR, Palladino MA Jr, Cerami A, Shires GT, Lowry SF (1988) Cytokine appearance in human endotoxemia and primate bacteremia. Surg Gynecol Obstet 166: 147-153.
28. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, parrillo JE (1989) The cardiovascular response of normal humans to the administration of endotoxin. N Engl J Med 321: 280-287.
29. Lvovschi V, Arnaud L, Parizot C, Freund Y, Juillien G, Ghillani-Dalbin P, Bouberima M, Larsen M, Riou B, Gorochov G, Housfater P (2011) Cytokine profiles in sepsis have limited relevance for stratifying patients in the emergency department: a prospective observational study. PLoS One 6: e28870.
30. Tasdelen Fisgin N, Aliyazicioglu Y, Tanyel E, Coban AY, Ulger F, Zivalioglu M, Esen S, Leblebicioglu H (2010) The value of neopterin and procalcitonin in patients with sepsis. South Med J 103: 216-219.
31. Pourakbari B, Mamishi S, Zafari J, Khairkhah H, Ashtiani MH, Abedini M, Afsharpaiman S, Rad SS (2010) Evaluation of procalcitonin and neopterin level in serum of patients with acute bacterial infection. Braz J Infect Dis 14: 252-255.
32. Hensler T, Sauerland S, Lefering R, Nagelschmidt M, Bouillon B, Andermahr J, Neugebauer EA (2003) The clinical value of procalcitonin and neopterin in predicting sepsis and organ failure after major trauma. Shock 20: 420-426.
33. Sauer U, Domnanich P, Preininger C (2011) Protein chip for the parallel quantification of high and low abundant biomarkers for sepsis. Anal Biochem 419: 46-52.
34. Liu D, Su L, Han G, Yan P, Xie L (2015) Prognostic Value of Procalcitonin in Adult Patients with Sepsis: A Systematic Review and Meta-Analysis. PLoS One 10: e0129450.
35. Enguix-Armada A, Escobar-Conesa R, La Torre AG, De La Torre-Prados MV (2015) Usefulness of several biomarkers in the management of septic patients: C-reactive protein, procalcitonin, presepsin and mid-regional pro-adrenomedullin. Clin Chem Lab Med 17: 163-168. doi: 10.1515/cclm-2015-0243.
36. Que YA, Guessous I, Dupuis Lozeron E, Alves de Oliveira CR, Ferreira Oliveira C, Graf R, Seematter G, Revelly JP, Pagani JL, Liaudet L, Nobre V, Eggimann P (2015) Prognostication of mortality in critically ill patients with severe infections. Chest 148: 674-682.
37. Du Clos TW (2000) Function of C-reactive protein. Ann Med 32: 274-278.

Corresponding author

Isil Bavunoglu, Associate Professor, MD
Department of Internal Medicine, Medical Faculty of Cerrahpasa, Istanbul University
Istanbul, Turkey
Phone: +90 212 4143000/21937
Fax: +90 212 4143528
Email: isilbavunoglu@gmail.com

Conflict of interests: No conflict of interests is declared.