



Proinflammatory Cytokine Inhibitors, TNF- α and Oxidative Burst of Polymorphonuclear Leukocytes in the Pathogenesis of Sepsis in Newborns

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Abstract. This study was to evaluate the levels of the proinflammatory cytokine tumor necrosis factor α (TNF- α) and the cytokine inhibitors soluble TNF- α receptor (sTNFR) and interleukin (IL-1) receptor antagonist (IL-1ra), as well as the intensity of oxidative metabolism of peripheral blood polymorphonuclear leukocytes in the course of sepsis in newborns. An increase of TNF- α , sTNFR and IL-1ra concentrations was found in the blood serum of the patients at the time of diagnosis. This was further accompanied by polymorphonuclear leukocyte stimulation and, as a consequence of prolonged bacterial antigen stimulation, functional exhaustion of these cells and their diminished oxidative metabolism was observed. Within the same time period, an enhanced expression of p55 and p75 TNF- α receptors on polymorphonuclear leukocyte cell surfaces was found. It was indicated that the applied pharmacotherapy caused a decrease of the initially elevated concentrations of TNF- α and proinflammatory cytokine inhibitors (sTNFR, IL-1ra). The intensive therapy of sepsis was associated with the increased oxidative burst of polymorphonuclear leukocytes along with the decrease of p55 and p75 expression on their cell surfaces.

Key words: sepsis; TNF- α ; cytokine inhibitors; oxidative burst; polymorphonuclear leukocytes

Introduction

Sepsis is defined as an organism's systemic reaction to an infectious factor. Currently, the risk of sepsis and its complications is increasing due to the growing number of immune-compromised child patients. Sepsis pathogenesis is complex and is still being elucidated. The endotoxin of Gram-negative bacteria elicits humoral

and cellular defense mechanisms: it activates coagulation and the fibrinolysis system, macrophages, neutrophils and the release of endogenous inflammatory mediators. The numerous pathogenic pathways in sepsis involve the remarkable role of the recently recognized proinflammatory cytokines (TNF- α , IL-1, IL-6, IL-8) released by activated macrophages, as well as the reactive oxygen species (ROS) generated in the course

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of the so-called oxidative burst of polymorphonuclear leukocytes^{7, 13, 16, 20}. These cytokines may accelerate septic states and bring about a fatal outcome under conditions of uncontrolled immune response to infectious factors.

Among the proinflammatory cytokines, TNF- α is the first one to appear in response to an infectious factor and it is included among the most important sepsis and endotoxic shock mediators^{1, 4, 11, 13}. Many factors (e.g. lipopolysaccharides of Gram-negative bacteria, staphylococcus enterotoxin A, streptococcus exotoxin A and streptolysin O, antigens of fungi, parasites, viruses) may stimulate monocyte-macrophage TNF- α release. This cytokine, as a major proinflammatory mediator, can induce oxidative burst in polymorphonuclear leukocytes and activate ROS production in patients with systemic infection^{9, 13, 16}. The enzyme responsible for this reaction in polymorphonuclear leukocytes and other phagocyte cells is an oxidase system of NADPH+H⁺, which catalyses one-electron oxygen reduction to the superoxide anion (O₂⁻)¹⁴. ROS, released by phagocyte cells and active, also within these cells, display pronounced bactericidal properties. However, their enhanced extracellular production by activated phagocytes is responsible for tissue damage and multi-organ failure: multiple organ dysfunction syndrome (MODS) in the course of sepsis^{2, 19}.

Some mechanisms evoked in the inflammatory systemic response to infection appear efficient in maintaining system homeostasis. The increased serum level of the soluble type II receptor of IL-1 (sIL-1R type II) in patients with sepsis binds free IL-1, eliminating its proinflammatory action. IL-1, along with TNF- α , is an important sepsis mediator responsible for numerous injurious effects^{17, 20}. However, other authors observed increased concentrations of soluble TNF- α receptor (sTNFR) and IL-1 receptor antagonist (IL-1ra) in an experimental model of meningitis¹². Some recent reports in the literature indicate that polymorphonuclear leukocytes can express antagonists of IL-1 (IL-1ra) that are capable of IL-1 activity inhibition; therefore, it is assumed that they might decrease inflammatory response in this way. Some authors report an increased concentration of IL-1ra in the blood serum of children with sepsis^{3, 8, 15}. These observations suggest that IL-1ra may be a potent regulator of immune response *in vivo*.

This study is aimed at evaluating TNF- α and the proinflammatory cytokine inhibitors (sTNFR and IL-1ra) as well as the oxidative burst of polymorphonuclear leukocytes in the progression of sepsis in children.

Materials and Methods

The study included 17 patients with sepsis hospitalized at the Department of Intensive Care and Anesthesiology of the Pediatrics Institute and Clinical Department of Pediatrics, Institute of Medical University in Łódź. These children were born after 24 to 40 weeks of a gestation, with birth masses of 580 to 4300 g and Apgar scores of 1 to 10 points. The children's ages at diagnosis were between 8 and 88 days, mean 24 days. Sepsis developed after the first week of life. Sepsis was diagnosed based on clinical symptoms and laboratory test results: blood morphology, bacteriological blood, urine, stool, intubation catheter discharge tests, and the indices of acute phase: C-reactive protein (CRP) and procalcitonin (PCT). Symptoms of respiratory and circulatory failure, lack of oral nutrition tolerance, abdominal distension, hypothermia, apathy, hepatosplenomegaly and hyperbilirubinemia should be included among the sepsis symptoms that were noted in our patients. Endotoxic shock was diagnosed in cases of apnea, paleness, anuria, metabolic acidosis and values of mean blood pressure below the age-normal range. In 4 children with MODS, the disease outcome was fatal.

At the beginning of therapy, a wide spectrum antibiotic therapy (aminoglycosides, synthetic penicillin), modified with glicopeptides and cephalosporines in accordance with the results of the bacteriological blood tests and antibiotic resistance of bacterial species, was applied. Moreover, cytokine inhibitors (pentoxifylline – PTXF – at a dose of 5 mg/kg/h for 24 h) were applied in the therapy of all patients for three consecutive days. In endotoxic shock-complicated cases, 1 g of Pentaglobin (Biotest) from the first day of therapy for three consecutive days, the catecholamines dopamine and dobutamine at doses over 5 μ g/kg/min, as well as norepinephrine, epinephrine at normotension-sustaining doses, were administered.

The concentrations of TNF- α and the inhibitors of proinflammatory cytokines: sTNFR1 55 kDa and IL-1ra, were assayed in the blood sera of all patients. Further, an evaluation of peripheral blood polymorphonuclear leukocyte oxidative burst and TNF- α receptors p55 and p75 on polymorphonuclear leukocyte cell surfaces was performed in all patients. The levels of TNF- α , sTNFR and IL-1ra were assayed twice in 13 out of the 17 patients. The first assay was performed at diagnosis establishment, prior to antibiotic and pentoxifylline therapy introduction, the second after antibiotic therapy termination and positive outcome, that is, 12–24 h after the last dose. In the 4 children with multiorgan failure

and death in the course of disease, TNF- α and proinflammatory cytokine inhibitor assays were performed only once. A similar schedule of assays (twice in the 13 children who survived and once in the 4 children that died) was performed to evaluate the oxidative burst of polymorphonuclear leukocytes, the expressions of p55 and p75 receptors, as well as the indices of acute phase (leukocytosis, CRP, PCT).

TNF- α , sTNFR, IL-1ra assay. The levels of TNF- α , sTNFR and IL-1ra in blood sera of the patients were assayed with an immunoenzymatic method (ELISA) with assay sets for cytokines (Quantikine human TNF- α , Quantikine human sTNFR, Quantikine human IL-1ra, R&D Minneapolis, MN, USA). The entire procedure was performed according to the manufacturer's instructions. The concentrations were calculated based on the obtained standard curves.

The assay of polymorphonuclear leukocyte oxidative burst. The oxidative burst of human polymorphonuclear leukocytes was evaluated with the Bursttest reagent kit (Orpegen Pharma, Heidelberg, Germany) using flow cytometry. For this purpose, a FACSCalibur cytometer with argon laser 488 nm (Becton Dickinson) was applied. The results were analyzed with the CELLQuest program (Becton Dickinson). The results were expressed as the mean fluorescence intensity (MFI) of polymorphonuclear leukocytes activated with *E. coli*. The assay of oxidative burst was performed indirectly with dihydrorhodamine 123 (DHR 123). This reagent is oxidized with an enzyme system involving NADPH+H⁺ to rhodamine 123, which can be measured with flow cytometry.

TNF- α receptors p55 and p75 expressions on the cell surfaces of neutrophils. The expressions of p55 and p75 receptors for TNF- α on the surfaces of polymorphonuclear leukocytes were determined with monoclonal antibodies conjugated with fluorescein (Fluorochrome-anti-human TNFR1 monoclonal antibody, R&D, Minneapolis, USA) and phycoerythrin (Fluorochrome-anti-human TNFR2 monoclonal antibody, R&D, Minneapolis, USA) using flow cytometer FACSCalibur (Becton-Dickinson). The results were analyzed with the CELLQuest program and expressed as MFI in relation to cell population with expressed p55 and p75 receptors.

The results were expressed as arithmetical means in the case of the cytokine and the inhibitors' concentrations, and MFI of *E. coli*-stimulated polymorphonuclear leukocytes as well as in the case of the mean for MFI of cells with p55 and p75 receptor expression. Because the data distribution was not normal (which had been determined with the Shapiro-Wilk test), fur-

ther analysis was based on nonparametric tests. The dependent data were analyzed with Wilcoxon's test. The relations between the measured data were determined with Spearman's correlation coefficient (ρ). The differences were considered significant at $p < 0.05$.

Results

Statistical analysis with Wilcoxon's test revealed that the initially elevated serum concentrations of TNF- α , sTNFR and IL-1ra (at the moment of sepsis diagnosis) decreased with applied therapy and the values observed after successful outcome were significantly decreased ($p=0.0037$, $p=0.0019$ and $p=0.0018$, respectively) compared with the onset of the disease (mean concentration of TNF- α before treatment (1.) – 19.35 pg/ml compared with concentration after treatment (2.) – 4.7 pg/ml, mean concentration 1. of sTNFR – 440.7 pg/ml compared with concentration 2. – 162.0 pg/ml, mean concentration 1. of IL-1ra – 5734.2 pg/ml compared with concentration 2. – 965.9 pg/ml) (Fig. 1).

The assay results of polymorphonuclear leukocyte oxidative burst intensity indicated a significant ($p=0.028$) increase of these values after the termination of antibiotic therapy (mean MFI after *E. coli* stimulation value 1. – 30.06 compared with value 2. – 54.69) (Fig. 2). An individual analysis showed that the oxygen metabolism of polymorphonuclear leukocytes was critically low in the 4 patients who died of sepsis: mean MFI of neutrophils stimulated with *E. coli* in these patients was 11.4 at sepsis diagnosis.

The comparison of the p55 and p75 TNF- α receptor expressions on the surface of polymorphonuclear leukocyte cells revealed significant decreases of p55 ($p=0.002$) and p75 ($p=0.016$) expression after therapy termination compared with the initial values (mean MFI of cells with expressed p55 receptors: value 1. – 15.46 compared with value 2. – 5.02, mean MFI of cells with expressed p75 receptor: value 1. – 52.99, compared with value 2. – 17.47) (Fig. 3).

The indices of acute inflammatory reaction (CRP, PCT) were significantly decreased after therapy termination ($p=0.002$ and $p=0.0015$, respectively) compared with the initial values (mean CRP prior to the therapy was 5.45 mg/dl in comparison with the value after the therapy 0.28 mg/dl; mean PCT concentration prior to the therapy 14.64 ng/ml in comparison with the concentration after therapy of 0.17 ng/ml).

The value of the Spearman coefficient indicated a moderate but insignificant correlation between the TNF- α serum concentration in patients at sepsis diag-

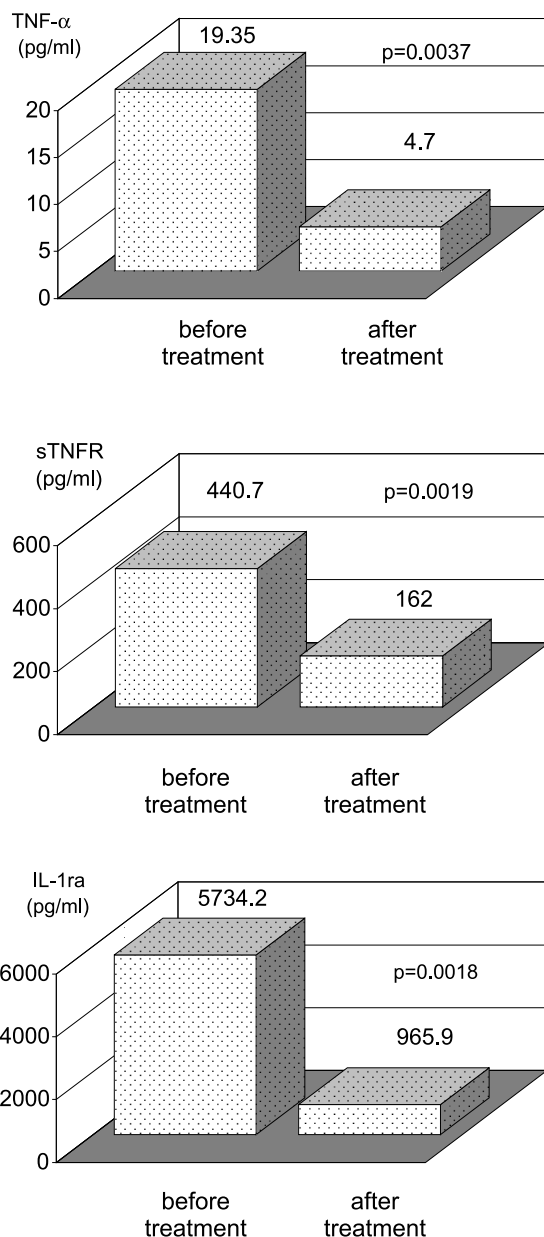


Fig. 1. The level of TNF- α , sTNFR, IL-1ra in blood serum of children with sepsis

nosis and the intensity of the oxidative metabolism of polymorphonuclear leukocytes ($\rho=0.2585$, $p=0.3936$). Similarly, an insignificant negative correlation was found between the oxidative metabolism of polymorphonuclear leukocytes and TNF- α receptor p75 expression on the surfaces of these cells ($\rho=-0.3516$, $p=0.2387$) and sTNFR serum concentration before therapy commencement ($\rho=-0.4780$, $p=0.0984$).

However, a significant negative correlation was found between IL-1ra serum concentration at the time of diagnosis and the activity of polymorphonuclear leukocyte oxidative metabolism ($\rho=-0.6749$, $p=0.0113$) (Fig. 4). Moreover, there was a significant negative

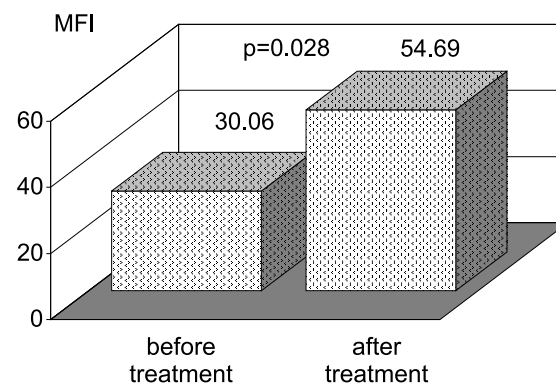


Fig. 2. Values of arithmetical mean MFI of polymorphonuclear leukocytes activated with *E. coli* (Bursttest) in children with sepsis

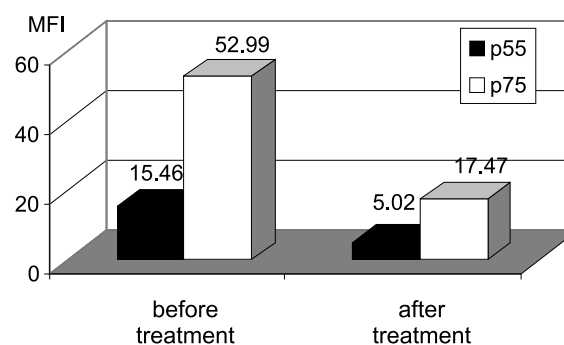


Fig. 3. Values of arithmetical mean MFI of polymorphonuclear leukocytes with p55 and p75 receptor expression in children with sepsis

correlation between CRP serum concentration after sepsis therapy termination and the oxidative metabolism of polymorphonuclear leukocytes ($\rho=-0.7180$, $p=0.0057$).

Discussion

The proinflammatory cytokines and reactive oxygen species are thought to be crucial mediators in the course of sepsis. These cytokines display pleiotropic activities in their cooperation with immune cells in response to systemic infection. Reactive oxygen species, generated due to cytokine preexposition, may damage tissues and organs in the course of so-called oxidative stress, which may be observed in cases of sepsis accompanied by an altered equilibrium state between their production and deactivation^{10, 19}.

The aim of this study was to evaluate blood serum concentrations of TNF- α , sTNFR and IL-1ra in children with serious sepsis and accompanying changes in polymorphonuclear leukocyte oxidative metabolism. The literature data considering these issues are some-

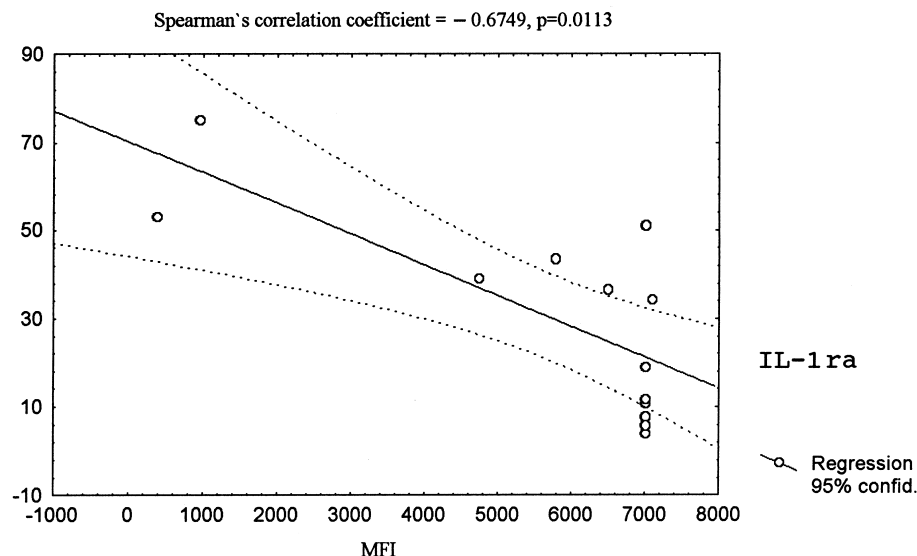


Fig. 4. The level of IL-1ra (pg/ml) in blood serum in relation to respiratory burst of polymorphonuclear leukocytes in children with sepsis

times contradictory^{5, 6, 9, 11, 13, 16, 18}. This study was focused on the pattern of changes of these immune parameters prior to and after sepsis therapy in our patients. The significant decrease of blood serum TNF- α , sTNFR and IL-1ra in the course of sepsis therapy (Fig. 1) is supported by other investigators' reports^{1, 4, 8, 12}. We could also identify a correlation between the presence of TNF- α in blood serum at the moment of diagnosis and the intensity of the oxidative metabolism of polymorphonuclear leukocytes: the lack of statistical significance of the described correlation might be explained by both the small number of investigated children (13 patients) and the short half-life of this cytokine, which may make short-lasting high levels difficult to detect in serum. On the other hand, it is also known that not only TNF- α , but also other proinflammatory cytokines such as IL-1, IL-6 and IL-8, may contribute to activation of neutrophil oxidative burst^{7, 13}. The evaluated TNF- α receptor p55 and p75 expression on the surfaces of polymorphonuclear leukocytes significantly decreased in the course of sepsis therapy (Fig. 3), which may further confirm TNF- α involvement in sepsis pathogenesis.

The evaluation of polymorphonuclear leukocyte oxidative burst during sepsis was a subject of controversy; these cells display enhanced oxidative metabolism if isolated from patient with bacterial infection^{9, 13, 20}. LLOYD et al.⁹ were the first to demonstrate an increased hydroxyl radical generation *in vivo* in sepsis patients. Considering this fact, it was recognized that polymorphonuclear leukocytes may be primed *in vitro* (and probably *in vivo*) for enhanced reactivity following

their preexposure to chemotactic factors and some (proinflammatory) cytokines, and increase their production of large amounts of reactive oxygen species. However, the studies performed by VESPASIANO et al.¹⁸ implied that a decrease of neutrophil oxidative burst and decreased ROS generation is observed in particularly serious courses of sepsis, often complicated with endotoxemic shock. Further, DRISCOLL et al.⁵ and DROSOU et al.⁶ also observed decreased oxidative metabolism in polymorphonuclear leukocytes in children with serious infection. Based on own material, the evaluation of neutrophil oxidative burst indicated decreased ROS generation in the study patients at sepsis diagnosis as well as increased ROS production by these cells after successful termination of the applied therapy (Fig. 2). The individual case studies revealed that the 4 children who died due to sepsis complicated by endotoxemic shock (excluded from the statistical analysis) presented especially low MFI values after stimulation with *E. coli*. The decreased oxidative burst of neutrophils prior to the sepsis therapy, observed in our study correlated with increased serum concentration of sTNFR before the treatment and with the increased expression of the p75 TNF- α receptor, should be explained by the prolonged stimulation with bacterial antigens and the functional exhaustion of polymorphonuclear leukocytes in the course of serious systemic infection.

The negative correlation between IL-1ra serum concentration before sepsis therapy and the activity of oxidative metabolism (Fig. 4) in neutrophils may confirm the thesis of IL-1ra as a potent regulator of immune response *in vivo* in children with a serious course of

sepsis with a decrease of oxidative metabolism in neutrophils. Our results stay in agreement with other reports on the effects of natural cytokine inhibitors, such as IL-1ra, sTNFR, sIL-1R type II, in sepsis pathogenesis^{3, 8, 11, 17}. According to some reports, IL-1ra may be applied in early laboratory diagnostics in sepsis, as it appears in serum two days prior to clinical symptom onset⁸. Moreover, the initially high serum concentrations of proinflammatory cytokines (sTNFR, IL-1ra) revealed in this study may be explained by system defense mechanism mobilizing against the injurious effects of these cytokines. The assays of CRP activity, simultaneous with neutrophil oxidative metabolism studies, indicated that the increase of neutrophil ability to initialize oxidative burst in patients with sepsis correlated significantly with the decrease of CRP concentration after therapy termination.

In summary, it should be emphasized that a decrease of serum levels of TNF- α as well as the cytokine inhibitors (sTNFR, IL-1ra) was observed in the course of sepsis therapy. It was accompanied by a decrease of p55 and p75 TNF- α receptor expression on human polymorphonuclear leukocytes and an enhanced oxidative metabolism in these cells. The intensive sepsis pharmacotherapy (wide-spectrum antibiotics therapy, catechol amines, immunoglobulines, TNF- α inhibitors – pentoxifylline) obviously caused the observed changes in the immune indices presented above. These observations indicate the substantial role of TNF- α , sTNFR, IL-1ra and ROS in sepsis pathogenesis. Simultaneous evaluation of the acute phase indices of inflammation state, concentration of proinflammatory cytokines and their normally occurring inhibitors as well as the oxidative metabolism of neutrophils may be useful in patients with serious generalized infection for the evaluation of the inflammatory process progression, with further determination of the value of new methods of therapy supporting the standard mode of therapeutic approach. Moreover, the evaluation of peripheral blood polymorphonuclear leukocyte oxidative burst in patients with sepsis (Bursttest) may be relevant in prognosis.

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References

1. ATICI A., SATAR M., CETINER S. and YAMAN A. (1997): Serum tumor necrosis factor-alpha in neonatal sepsis. *Am. J. Perinatol.*, **14**, 401–404.
2. CHĘCIŃSKI P., KRAUSS H. and KOZLIK J. (1998): Oxygen free radicals and their role in tissues damage. In ZAPALSKI S.: Clinical aspects of ischemia and reperfusion. α -Medica Press, Białsko-Biała, 24–30.
3. DE BONT E. S., DE LEIJ L. H., OKKEN A., BAARSMA R. and KIMPEN J. L. (1995): Increased plasma concentrations of interleukin-1 receptor antagonist in neonatal sepsis. *Pediatr. Res.*, **37**, 626–629.
4. DE BONT E. S., MARTENS A., VAN RAAN J., SAMSON G., FETTER W. P., OKKEN A., DE LEIJ L. H. and KIMPEN J. L. (1994): Diagnostic value of plasma levels of tumor necrosis factor alpha (TNF α) and interleukin-6 (IL-6) in newborns with sepsis. *Acta Paediatr.*, **83**, 696–699.
5. DRISCOLL M. S., THOMAS V. L. and RAMAMURTHY R. S. (1990): Longitudinal evaluation of polymorphonuclear leukocyte chemiluminescence in premature infants. *J. Pediatr.*, **116**, 429–434.
6. DROSSOU V., KANAKOUDI F., TZIMOULI V., SARAFIDIS K., TAPARKOU A., BOUGIOUKLIS D., PETROPOULOU T. and KREMENOPOULOS G. (1997): Impact of prematurity, stress and sepsis on the neutrophil respiratory burst activity of neonates. *Biol. Neonate*, **72**, 201–209.
7. KRAGSBJERG P., HOLMBERG H. and VIKERFORS T. (1996): Dynamics of blood cytokine concentrations in patients with bacteremic infections. *Scand. J. Infect. Dis.*, **28**, 391–398.
8. KÜSTER H., WEISS M., WILLEITNER A., DETLEFSEN S., JEREMIAS I., ZBOJAN J., GEIGER R., LIPOWSKY G. and SIMBRUNER G. (1998): Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. *Lancet*, **352**, 1271–1277.
9. LLOYD S. S., CHANG A. K. and TAYLOR F. B. (1993): Free radicals and septic shock in primates: the role of tumor necrosis factor. *Free Radic. Biol. Med.*, **14**, 233–236.
10. MARISCALCO M. M. (1998): Infection and the host response. In FUHRMAN B. P.: *Pediatric critical care*. Mosby, St. Louis, 979–994.
11. MODZELEWSKI B. (1997): Release of tumor necrosis factor α and its soluble receptor p55 in clinical septic syndrome. *Arch. Immunol. Ther. Exp.*, **45**, 213–219.
12. PARIS M. M., FRIEDLAND I. R., EHRETT S., HICKEY S. M., OLSEN K. D., HAUSEN E., THONAR M. A. and MCCracken G. H., JR. (1995): Effect of interleukin-1 receptor antagonist and soluble tumor necrosis factor in animal models of infection. *J. Infect. Dis.*, **171**, 161–169.
13. PASCUAL C., KARZAI W., MEIER-HELLMANN A., BREDLE D. L. and REINHART K. (1997): A controlled study of leukocyte activation in septic patients. *Intensive Care Med.*, **23**, 743–748.
14. ROSSI F. (1986): The O₂⁻-forming NADPH oxidase of phagocytes: nature, mechanisms of activation and function. *Biochim. Biophys. Acta*, **853**, 65–69.
15. SAMSON L. M., ALLEN V. D., CREERY W. D., DIAZ-MITOMA F. and SINGH R. N. (1997): Elevated interleukin-1 receptor antagonist levels in pediatric sepsis syndrome. *J. Pediatr.*, **131**, 587–591.
16. TRAUTINGER F., HAMMERLE A. F., POSCHL G. and MICKSCHE M. (1991): Respiratory burst capability of polymorphonuclear neutrophils and TNF-alpha serum levels in relationship to the development of septic syndrome in critically ill patients. *J. Leukoc. Biol.*, **49**, 449–454.
17. VAN DER POLL T., DE WAAL MALEFYT R., COYLE S. M. and LOWRY S. F. (1997): Antiinflammatory cytokine responses during clinical sepsis and experimental endotoxemia: sequential

- measurements of plasma soluble interleukin (IL)-1 receptor type II, IL-10, and IL-13. *J. Infect. Dis.*, **175**, 118–122.
18. VESPASIANO M. C., LEWANDOSKI J. R. and ZIMMERMAN J. J. (1993): Longitudinal analysis of neutrophil superoxide anion generation in patients with septic shock. *Crit. Care Med.*, **21**, 666–672.
19. VLESSIS A. A., GOLDMAN R. K. and TRUNKEY D. D. (1995): New concepts in the pathophysiology of oxygen metabolism during sepsis. *Br. J. Surg.*, **82**, 870–876.
20. ZIMMERMAN J. J. (1998): Sepsis/septic shock. In FUHRMAN B. P.: *Pediatric critical care*. Mosby, St. Louis, 1088–1100.

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