



Proinflammatory Cytokines (IL-6, IL-8), Cytokine Inhibitors (IL-6sR, sTNFRII) and Anti-Inflammatory Cytokines (IL-10, IL-13) in the Pathogenesis of Sepsis in Newborns and Infants

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Abstract. The levels of the proinflammatory cytokines interleukin 6 (IL-6) and IL-8, and the anti-inflammatory cytokines IL-10 and IL-13 were studied in child patients with sepsis. The changes of the cytokine inhibitors soluble IL-6 receptor and soluble p75 TNF- α receptor were also investigated in the patients' sera. An increase of pro- and anti-inflammatory cytokine levels was demonstrated at the time of diagnosis. Pharmacotherapy was accompanied by a decrease of the elevated concentrations of both cytokines and their inhibitors. The time pattern of changes in cytokine and cytokine inhibitor serum concentrations along with the time course of acute phase indices, including procalcitonin and C-reactive protein, allows for an evaluation of the system inflammatory response and may support diagnostic and prognosis methods.

Key words: sepsis; cytokines; cytokine inhibitors; children.

Introduction

Sepsis is a systemic inflammatory reaction against active septic process in the host organism that involves the release of endogenous mediators of inflammation. Sepsis pathogenesis is complex and its mechanisms are still being elucidated.

The key role in sepsis pathogenesis has been recently attributed to proinflammatory cytokines released by numerous cells, including lipopolysaccharide-activated macrophages. Tumor necrosis factor α (TNF- α) and interleukins (IL-1, IL-6, IL-8) are the most important

factors. IL-6, IL-1 and TNF- α are responsible for the release of acute phase proteins (including C-reactive protein – CRP) during system infection. The increased serum levels of these cytokines and interferon γ (IFN- γ) were found to correlate with the severity and mortality in the course of sepsis^{1, 4, 9, 18}.

The anti-inflammatory mechanisms associated with the control of immune response, elicited to maintain system homeostasis, involve the release of anti-inflammatory cytokines (IL-4, IL-10, IL-13) and the decreased expression of cytokine receptors, and the release of soluble cytokine receptors or receptor

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antagonists^{12, 16, 22}. Particular attention is focused on IL-10 and IL-13, which inhibit the release of proinflammatory cytokines, such as TNF- α , IL-1, IL-6, IL-8, by activated macrophages^{13–15}. Along with other proinflammatory cytokines, IL-10 levels were significantly higher in meningococcus septic shock compared with other pathogens⁵. DOUGHTY et al.⁶ reported increased IL-10 levels in the blood serum of children with multiorgan failure in the course of sepsis, which correlated with IL-6 levels. VAN DER POLL et al.¹⁹ found an increased concentration of the soluble type II receptor for IL-1 in sepsis patient blood. This receptor, released into the circulation, bound free IL-1, thereby abolishing its activity¹⁹. Other authors demonstrated increased levels of soluble p55 TNF- α receptor (sTNFRI) and soluble p75 TNF- α receptor (sTNFRII) and IL-1 receptor antagonist IL-1 (IL-1ra), which were found in experimentally elicited infection^{12, 16}.

The aim of this study was to evaluate the involvement of proinflammatory cytokines (IL-6, IL-8) and cytokine inhibitors (soluble IL-6 receptor (IL-6sR), sTNFRII) as well as anti-inflammatory cytokines (IL-10, IL-13) in the progress of child sepsis.

Materials and Methods

The study included 20 children with sepsis (12 girls and 8 boys), born after 25 to 41 weeks of gestation, with birth weights of 850 to 4100 g and Apgar scores from 1 to 10. Thirteen patients were newborns, and 7 were infants 1.5 to 9.5 months old. These children were hospitalized at the Department of Critical Care and Anesthesiology and the Department of Pediatric Propedeutics, Institute of Pediatrics, Medical University of Łódź. The control group included 20 healthy, full-term newborns with body weights exceeding 2500 g. Control blood samples for immune tests were collected simultaneously with the routine blood tests.

Sepsis was diagnosed based on clinical data, bacteriological results (blood, urine, stool, cerebrospinal fluid, discharge from trachea intubation), the indices of acute inflammation (procalcitonin (PCT), CRP) and blood morphology. The clinical symptoms of sepsis included respiratory and circulatory failure, intolerance of oral feeding, abdominal distension, apathy, hypothermia, hepatosplenomegaly and hyperbilirubinemia. Among the 4 children with a diagnosis of septic shock (apnea, anuria, paleness of skin surface, metabolic acidosis, mean blood pressure < 20 mmHg), only one child died, demonstrating multiorgan failure syndrome. Children with systemic infection were treated with anti-

biotics of wide band activity confirmed by antibiotics sensitivity tests (semisynthetic penicillins, aminoglycosides, glycopeptides, cephalosporines). In particularly serious cases of septic shock, additional pharmacotherapy was applied: catecholamines (dopamine, dobutamine at doses >5 μ g/kg/min), intravenous immunoglobulin preparations (Sandoglobin, Biomed) or Pentaglobin (Biotest) in cases of Gram-negative sepsis at a dose of 1 g) and pentoxifylline (Pentilin, KRKA) at a dose of 5 mg/kg/h for 24 h for 3 consecutive days.

The serum levels of the following cytokines and their inhibitors: IL-6, IL-8, IL-6sR, sTNFRII, IL-10, IL-13 and the indices of acute phase infection (PCT and CRP) were evaluated. These immune indices of the 20 children with sepsis were performed twice: the first assay was made at the time of diagnosis (prior to the pharmacotherapy) and the other assay after complete recovery and the termination of antibiotic therapy (12–24 h after the last dose). In the cases of the patient who died due to septic shock accompanied by multiorgan failure and the 20 healthy newborns included into control group, a single assay of cytokine levels and cytokine inhibitor concentration was performed.

Assays of IL-6, IL-8, IL-6sR, sTNFRII, IL-10, IL-13 and PCT and CRP levels

The concentrations of IL-6, IL-8, IL-6sR, sTNFRII, IL-10 and IL-13 in serum samples were evaluated with immunoenzymatic methods (ELISA), with commercially available test kits (Quantikine human IL-8, Quantikine human IL-6sR, Quantikine human sTNFRII, Quantikine human IL-10, Quantikine human IL-13, R&D Minneapolis, MN, USA and Cytelisa human IL-6, Cytimmune Maryland, USA). The procedures followed the manufacturer's instructions. Cytokine levels were calculated based on standard curves.

The concentration of PCT was evaluated with a luminometric method using the LUMitest PCT test kit, Brahms Berlin, Germany.

CRP concentration was evaluated with the turbidimetric method based on the kit from OUSN Turbi-quant CRP, Dade Behring Marburg, Germany.

Study results were presented as arithmetic mean ($\bar{x}$) $\pm$ SD for cytokines and their inhibitors. Because the variable distribution was not normal (based on the Shapiro-Wilk test), further statistical analysis followed the guidelines of non-parametric analysis. The dependent variables were compared with Wilcoxon's test, whereas independent variables were analyzed with Mann-Whitney's test. The analysis of correlation between two values was performed with the correlation

coefficient of Spearman (ρ). The differences were significant at a p level < 0.05 .

Results

The results of statistical analysis based on Wilcoxon's test indicated that the originally high blood serum concentrations of both proinflammatory cytokines (IL-6, IL-8), anti-inflammatory (IL-10, IL-13) and cytokine inhibitors (IL-6sR, sTNFRII) at the time of sepsis diagnosis decreased gradually during pharmacotherapy, as the values on recovery were significantly lower than the initial levels ($p = 0.0022$, $p = 0.0004$, $p = 0.0003$, $p = 0.0005$, $p = 0.0112$, $p = 0.0043$, respectively). Arithmetical means and standard deviations at the initial sepsis diagnosis (concentration in the 1st assay) if compared with the results after therapy (concentration in the 2nd assay) were: IL-6 – 158.72 ± 374.09 pg/ml compared with 3.79 ± 3.25 pg/ml; IL-8 – 233.77 ± 506.39 pg/ml compared with 21.74 ± 15.29 pg/ml; IL-10 – 46.29 ± 112.99 pg/ml compared with 6.31 ± 5.51 pg/ml; IL-13 – 28.73 ± 9.67 pg/ml compared with 21.87 ± 3.21 pg/ml (Fig. 1); IL-6sR – 659.76 ± 332.45 pg/ml compared with 560.67 ± 164.75 pg/ml; and sTNFRII – 858.10 ± 515.42 pg/ml compared with 515.33 ± 185.87 pg/ml (Fig. 2).

Similarly, significantly higher levels of acute-phase indices (PCT, CRP) were found at the time of diagnosis in comparison with the values found after therapy termination ($p = 0.00015$, $p = 0.00098$, respectively). The values of the mean concentrations prior to pharmacotherapy when compared with the values after the therapy were: PCT – 8.67 ± 14.22 ng/ml compared with 0.50 ± 1.12 ng/ml, whereas in the case of CRP – 4.15 ± 4.25 mg/dl compared with 0.85 ± 1.52 mg/dl.

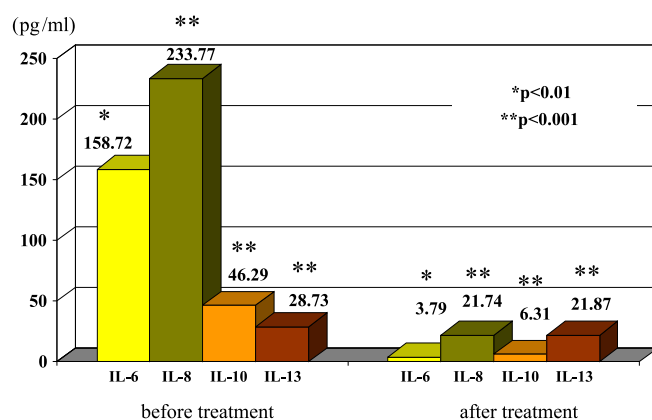


Fig. 1. The levels of IL-6, IL-8, IL-10, IL-13 in blood serum of septic children

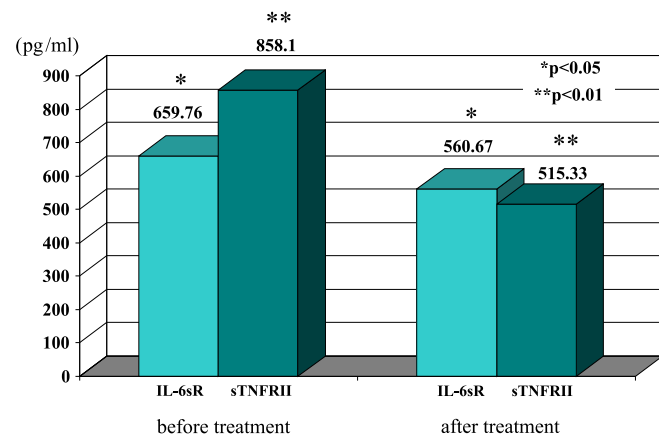


Fig. 2. The levels of IL-6sR, sTNFRII in blood serum of septic children

The Mann-Whitney test was used to evaluate cytokine and cytokine inhibitor concentrations in the group of children with sepsis at the time of diagnosis (concentration in the 1st assay) and in the controls. It demonstrated increased levels in the sick children when compared with the healthy controls. The differences were significant ($p = 0.0001$) in the case of IL-10 (the mean concentration prior to therapy was 46.29 ± 112.99 pg/ml compared with the mean concentration in the healthy controls – 2.14 ± 1.96 pg/ml) (Fig. 3). The level of sTNFRII, however, was within the range of statistical significance ($p = 0.0583$), as it was 858.1 ± 515.42 pg/ml in the septic children compared with 532.93 ± 122.55 pg/ml in the healthy controls (Fig. 4). The levels of the other cytokines (IL-6, IL-8, IL-13) and inhibitors (IL-6sR) were not significant (Fig. 3 and 4).

Statistical significance was recognized when the levels of the cytokines (IL-6, IL-8, IL-10) were compared in children after therapy termination (mean double assays) and the control group ($p = 0.00027$, $p = 0.00016$,

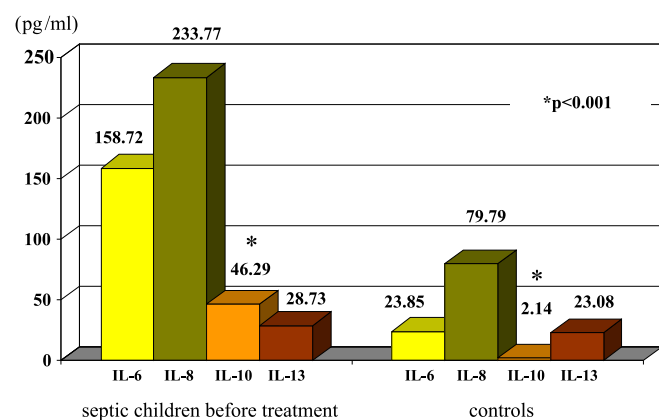


Fig. 3. The levels of IL-6, IL-8, IL-10, IL-13 in blood serum of septic children and controls

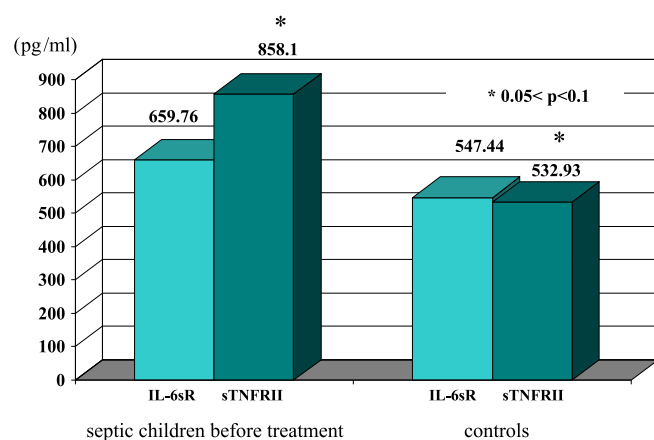


Fig. 4. The levels of IL-6sR, sTNFRII in blood serum of septic children and controls

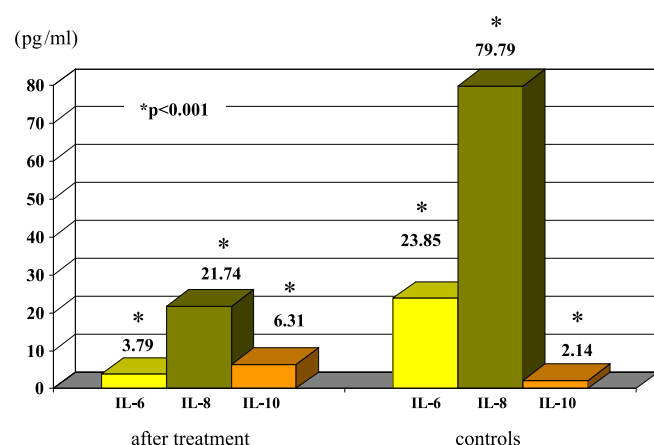


Fig. 5. The levels of IL-6, IL-8, IL-10 in blood serum of septic children and controls

$p = 0.00064$, respectively), indicating decreased proinflammatory cytokine concentrations in the children with sepsis when compared with the healthy controls in the cases of IL-6 (3.79 ± 3.25 pg/ml compared with 23.85 ± 30.86 pg/ml) and IL-8 (21.74 ± 15.29 pg/ml compared with 79.79 ± 89.62 pg/ml). The reverse relations were found in the case of IL-10, as its levels were increased in the sepsis group when compared with the healthy controls (6.31 ± 5.51 pg/ml compared with 2.14 ± 1.96 pg/ml) (Fig. 5).

Based on the values of Spearman's coefficient, a significant correlation between PCT and IL-8 serum concentration in the septic children was demonstrated at the beginning of septic shock ($p = 0.4797$, $p = 0.0323$), and also between CRP and IL-13 levels (significant negative correlation $\rho = -0.5236$, $p = 0.0214$) in patients after therapy. Similar significant correlations were found in patients prior to therapy in the cases of IL-6 and IL-8 and sTNFRII serum concentration ($p = 0.5714$,

$p = 0.0085$ and $p = 0.6030$, $p = 0.0049$, respectively). A significant correlation was also found between the serum concentrations of the proinflammatory cytokines IL-6 and IL-8 ($\rho = 0.4739$, $p = 0.0349$) in septic patients prior to pharmacotherapy. Moreover, the correlations between the proinflammatory cytokines (IL-6 insignificant) and IL-8 ($\rho = 0.4977$, $p = 0.0255$) and the concentration of IL-10 in blood sera of children with sepsis at the time of sepsis diagnosis were observed. Further, prior to the pharmacotherapy, there was a correlation between the serum levels of IL-10 and sTNFRII in patients with systemic infection ($\rho = 0.4120$, $p = 0.071$ – within the range of statistical significance).

Discussion

Proinflammatory cytokines (TNF- α , IL-1, IL-6, IL-8) are endogenous mediators with immunomodulating properties which are involved in sepsis pathogenesis and take part in the systemic response to infection^{1, 7, 9, 14, 22}. An excessive release of mediators by activated macrophages may stimulate polymorphonuclear leukocytes to enhanced generation of reactive oxygen species, which further leads to tissue damage and multiorgan failure^{21, 22}. However, there are some counteracting mechanisms that bring about homeostasis in the human body. After their activation, cellular receptors of proinflammatory cytokines are released from cell surfaces and then appear in the blood serum in patients with sepsis as soluble fragments of cytokine receptors. Conjunction with the soluble receptors decreases the activity of the proinflammatory cytokines and attenuates the immune response. The literature has reported the identification of the inhibitors of TNF- α , IL-1 and IL-6 along with inhibitors of anti-inflammatory cytokines in patients with systemic infection^{5, 7, 10, 12, 13, 15, 16, 19}. The release of anti-inflammatory cytokines seems to be an initial stage for the further control and limitation of the immune response in these patients^{5, 7, 13}.

This study was aimed at assessing the levels of proinflammatory cytokines (IL-6, IL-8) and their inhibitors (IL-6sR, sTNFRII) along with anti-inflammatory cytokines (IL-10, IL-13) in the blood serum of children with serious sepsis. The results demonstrated significant decreases of both pro- and anti-inflammatory cytokine as well as inhibitor concentrations after the pharmacotherapy of systemic infection compared with their initially high levels at the time of sepsis diagnosis (Fig. 1 and 2). These observations may be confirmed with other reports^{4, 7, 8, 12, 19}. Individual analysis proved that, in the case of the child who died of multi-

organ failure and septic shock, the levels of all cytokines studied, and the inhibitors, were particularly high (e.g. IL-6 – 1062 pg/ml, IL-10 – 500 pg/ml, sTNFR II – 2000 pg/ml).

The significantly increased level of IL-10 and sTNFR II in patients with sepsis at the initial stage compared with healthy controls may indicate additional anti-inflammatory mechanisms involved in the course of infection (Fig. 3 and 4). Similarly, increased serum levels of IL-10 and sTNFR II in patients with systemic infections have been previously reported^{5, 7, 12, 19, 20}. Based on literature data, IL-10 blood serum levels in sepsis were apparently increased in cases of fatal outcome^{19, 20}. There are only scarce data on IL-13 involvement in *in vivo* infections. Experiments on a mouse model confirmed that IL-13 decreased mortality in sepsis by a decrease of CD14 receptor expression and attenuation of IL-1 and TNF- α release¹⁵. Preliminary experiments with healthy volunteers demonstrated that IL-10 also decreased production of other proinflammatory cytokines, such as TNF- α , IL-1 β , IL-6, IL-8^{2, 3}. Prolonged stimulation with bacterial antigens may elicit an excessive release of cytokines (including anti-inflammatory cytokines), which might explain the significantly increased IL-10 levels and decreased levels of proinflammatory cytokines (IL-6, IL-8) in children with a serious course of sepsis after pharmacotherapy if compared with healthy controls (Fig. 5).

The search for associations between the indices of acute phase (PCT, CRP) and pro- or anti-inflammatory cytokines demonstrated a significant correlation between PCT and IL-8 and a negative correlation between CRP and IL-13. As may be concluded from the data analysis of patients with systemic infections in our study, the high PCT concentrations were accompanied by the increased IL-8 levels. However, after recovery from sepsis, the decrease of the CRP level was accompanied by significantly increased levels of anti-inflammatory IL-13. The study results confirm the involvement of pro- and anti-inflammatory cytokines in the sepsis pathogenesis in newborns and infants and their concentrations seem to maintain a dynamic equilibrium. Other authors reported similar data and this study may also conclude that the increase of proinflammatory cytokine levels after antigen stimulation induces anti-inflammatory mechanisms as a systemic response to infection.

Not only TNF- α and IL-1, but also IL-6 and IL-8 are regarded as important mediators of the inflammatory reaction in sepsis, and similarly to other proinflammatory cytokines, an increase of their levels was observed in the blood serum of children with systemic

infection (4,9). There was a positive correlation between IL-6 levels and mortality and prognosis. Moreover, increased IL-6 levels were detected two days before clinical symptoms appeared^{11, 18}. Further, IL-6 was reported to affect the release of IL-1ra and sTNFR¹⁷. The significant correlation between IL-6, IL-8 and sTNFR II found in our study in the group of children with sepsis prior to pharmacotherapy may imply the participation of cytokines in the inhibitor release in the course of system infection. The nearly significant correlation between IL-10 and sTNFR II in the blood serum of septic patients at the initial diagnosis might confirm the influence of IL-10 on TNF- α synthesis, which in *in vivo* conditions does not inhibit endogenous release of sTNFR, which is confirmed by other studies³. The correlation found between IL-6 and IL-10 (insignificant) as well as that between IL-8 and IL-10 ($p=0.0255$) may suggest the involvement of these pro-inflammatory cytokines in IL-10 production that apparently takes part in homeostasis under the conditions of generalized infection¹⁰.

In conclusion, the serum levels of proinflammatory cytokines (IL-6, IL-8) increases in the course of sepsis in newborns and infants and their significant elevation can be found as early as at the initial stage of the disease. In response to these increases, adaptation mechanisms associated with the release of cytokine inhibitors (IL-6sR, sTNFR II) and anti-inflammatory cytokines (IL-10, IL-13) are evoked. It was demonstrated that these elevated concentrations of both pro- and anti-inflammatory cytokines were lowered in the course of the applied pharmacotherapy. Similar kinetics was observed when cytokine inhibitor concentrations were compared. These findings prove the involvement of proinflammatory (IL-6, IL-8) and anti-inflammatory (IL-10, IL-13) cytokines as well as cytokine inhibitors (IL-6sR, sTNFR II) in sepsis pathogenesis. The assessment of blood serum levels of cytokines and their inhibitors along with acute phase indices (PCT, CRP) allows an evaluation of the activity of the generalized inflammatory response and it may be an additional diagnostic and prognostic tool.

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