International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue 1, January 2015, PP 110-116
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Diagnostic Value of Presepsin (Scd14-St Subtype) Evaluation in the Detection of Severe Neonatal Infections

Stojewska Małgorzata

Department of Pediatrics
Zabrze, Silesian Medical University
Katowice, Poland

Behrendt Jakub, Szymańska Anna, Pukas-Bochenek Anna, Stachurska Aneta, Godula – Stuglik Urszula

Department of Neonatal Intensive Care Units
Zabrze, Silesian Medical University
Katowice, Poland

Mazur Bogdan

Depatrment of Microbiology and Immunology
Zabrze, Silesian Medical University
Katowice, Poland

Abstract: *The clinical usefulness of assesment of presepsin concentration in peripheral vein blood in male and female severe infected newborns, including their birth weight, fetal maturity, gender, birth asphyxia, mode of mother's delivery was studies and compared with C-reactive protein, procalcitonin, hemoglobine, hematocrit and platelet count in a multicenter prospective study. The study population comprised 124 newborns (41 septic, 37 with severe local infections without bacteriemia, 16 without infections, but with clinical symptoms suggesting infection and perinatal risk factors and 30 healthy as control), in which by rapid chemiluminescent enzyme immunoassay, using fully automated PATHFAST TM analyser from Mitsubishi Kagaku Iatron Inc, presepsin concentration in whole blood was measured. The mean presepsin concentration in septic newborns, independently of their gender, fetal maturity, birth asphyxia (1389±861 pg/ml) was significantly ($p<0,001$) higher than in others newborns. In severe local infected newborns (mainly pneumonic, with negative blood cultures) independently of their fetal maturity, birth asphyxia and mode of delivery, significant presepsin increase than in noninfected newborns was stated. Positive correlation between presepsin and CRP concentrations, negative correlation between presepsin and hemoglobin, hematocrit value and platelet count in septic newborns were noted. Positive correlation between presepsin and procalcitonin in local infected newborns was observed. The cutoff value of presepsin for discrimination of neonatal sepsis was determined to be 1066 pg/ml, of which the clinical specyfity and sensivity were 89,2% and 63,4%, respectively. Presepsin determination might be used as a high specyfity biomarker for the early diagnosis of neonatal sepsis and severe local infections, both early and late-onset.*

Keywords: *presepsin, newborn, sepsis, local infection, C-reactive protein, procalcitonin*

1. INTRODUCTION

Despite the recent advanced in method for culturing bacteria, using such biomarkers as C-reactive protein (CRP), procalcitonin (PCT), cytokines, especially interleukin (IL) – 6 and -10, interferon-γ, tumor necrosis factor, and modern treatments, severe neonatal infections remain a major challenge in intensive care unit with considerable morbility and mortality [1,2, 3]. Progress on the pathophysiology and immunopathogenes of neonatal infection have the potential of generating new diagnostics metod focused on looking for molecular and biochemical markers [4, 5].Up till now was not performed for newborns treated in the NICU, which laboratory findings are objective and reliable for early diagnosis of severe infections, to afford for monitoring and to define the prognosis in these diseases. They are results of neonatal immaturity and sometimes infection is misinterpreted as adaptation disorders or noninfectious diseases (mainly congenital

heart defects, intracranial haemorrhages, meconium aspiration syndrom, congenital metabolic and endocrinologic diseases). Some authors emphasize the significance determination of two acute-phase proteins in blood, soluble CD14 subtype (sCD14-ST) called presepsin and lipopolysaccharides (LPS) binding protein in the diagnosis of sepsis and systemic inflammatory response syndrome in an emergency department for adults and in pediatric oncology patients with chemotherapy [6, 7, 8]. They have been previously investigated in several clinical studies using enzyme-linked immunoabsorbent assay (ELISA), but was not convinient and took a long time [9, 10, 11].

CD14 is a glicoprotein expressed on the surface membranes of monocytes, granulocytes and macrophages and serves as a high-affinity receptor for complexes of LPS and LPS binding protein [7, 8, 12]. The complex of LPS-LPBP-CD14 is released into circulation by shedding of CD14 from the cell membrane yielding soluble CD14. Plasma protease activity generates also another sCD14 molecule called sCD14 subtype or presepsin, whose increase in the blood of septic patients, is more sensitive marker for the sepsis diagnosis than IL-6 and PCT and increased faster than CRP, PCT and D-dimer [7, 13]. Some clinical studies showed significantly elevated sCD14-ST concentration in inflammatory process, such as liver disease, rheumatoid arthritis, systemic lupus erythromatosus, atopic dermatitis [14, 15, 16, 17]. The studies in newborns are only a few and in local infections without bacteriemia were not performer till now [12, 18, 19, 20]. In the present study, we apply a new, high-sensitive, fully-automatic chemiluminescent enzyme immunoassay (CLEILA), which need small sample of whole blood for quantitative measurement of presepsin concentration as a rapid diagnostics method for neonatal severe infections.

2. MATERIAL AND METHODS

All of the sick newborns in this study were inpatients at the Neonatal Intensive Care Units in Zabrze University Hospital of Silesian Medical University in Katowice. Healthy newborns served as control subject born in Regional Obstetric-Gynecology Unit in Gliwice. We obtained voluntary consent to participation in this study from the parents of all newborns as well as the approval of the Bioethics Committee of Silesian Medical University in Katowice (Poland).

The study comprised 124 newborns, among them 41 septic (19 with early-onset, 22 with late-onset), 37 with local infections without bacteriemia, 16 without of infections, but with clinical symptoms suggesting infection and with perinatal risk factors and 30 healthy, full-term (16 boys, 14 girls), breast-fed, born vaginally with Apgar score 8-10 and without congenital malformations newborns (control group). The septic group (21 boys, 20 girls, 26 born by cesarean section, 15 through natural passages) had mean birth weight 2,495±0,83 kg (range 1,07-3,90), mean gestational age 36,2±3,3 of weeks (range 29-41 weeks) and fulfilled three of the diagnostic criteria : positive blood cultures (gram-positive-33, gram-negative – 6), inflammatory signs in two organs, mainly pneumonia, purulent meningitis and urinary tract infection and biochemical/haematological disorders as metabolic acidosis, hyperglycemia, thrombocytopenia, anaemia, hiperbilirubinemia and increase of CRP, PCT and D-dimers concentrations.

Congenital bilateral pneumonia (14 cases), purulent meningitis (4 cases), urinary tract infections (7 cases), pneumonia and purulent meningitis (2 cases), pneumonia and urinary tract infection (3 cases), systemic dermatitis (4 cases) and purulent omphalitis (3 cases) were diagnosed in newborns with negative blood cultures. The mean birth weight of these newborns was 3,038±0,84 kg (range 1,2 -4,4 kg) and mean gestational age 37,8±2,7 of weeks (range 30-41), 18 born by cesarean section and 17 through natural passages.

Birth weight of 16 newborns without of infection ranges from 1,95 to 3,96 kg (3,25±0,49) and gestational age from 36 to 41 of weeks (38,7±1,6). They presented such clinical disturbances: respiratory (tachypnoe, dyspnoe, apnoe), circulatory (bradycardia, tachycardia, arrhythmia, hypotension), gastrointestinal (meteorism) or neurological (convulsiones, hypotonia, hipertonia) as an effect of intracranial haemorrhage (4 cases), congenital heart defect (2 cases), transient tachypnoe (5 cases), respiratory distress syndrom (4 cases) and ovarian cyst (1 case).

3. LABORATORY ASSAYS

3.1. Determination of Presepsin (sCD14-ST) Concentrations

Presepsin concentrations were measured by a rapid, commercially available chemiluminescent enzyme immunoassay (CLEIA) using analyzer PATHFAST TM (Mitsubishi Kagaku Iatron Inc,

Japan). An endotoxin-free syringe containing EDTA was used to collect the 100 µl volume of whole peripheral vein blood. During incubation with alkaline phosphatase- labeled antipresepsin polyclonal and monoclonal antypodies coated with magnetic particles, sample presepsin binds to the antibodies forming an immunocomplex with enzyme - labeled antibodies and antibodies - coated magnetic particles. After removing the unbound substances by Magritation technology (technology of B/F separation, where magnetic particles are washed in pipette tip and its registered trademark of Precision System Science), a chemiluminescent substrat was added. After a short incubation, the luminescence intensity generated by the enzyme reaction was measured. The luminescence intensity was related to the presepsin concentration of the sample, which calculated by means of a standard curve. We used immunoassay analyzer PATHFAST TM (Mitsubishi Chemical Medience Corporation, Japan) integrated with computer and printer, operated via touchscreen monitor. The barcode of the samples was read with a scanner. The results were obtained within 17 minutes.

3.2. Determination of CRP and PCT concentrations

The serum CRP concentrations were measured by the commercially available immunoturbidometric assay on the Roche Modular P-system with Cobas 6000 analyzer (Roche Diagnostics, Mannheim, Germany) using neonatal serum from peripheral vein blood. Results were expressed in milligrams/litr. The analytical sensivity was 0,15 mg/l, according to manufacturer information.

PCT was measured by commercially available immunoassay on the Roche Modular E170 analyzer (Roche Diagnostic, Mannheim, Germany) using neonatal serum from peripheral vein blood . Results were expressed in milligrams/litr and analitycal sensivity was 0,1 µg/l, according to manufacturer information.

Other biochemical and haematological analyses were performer according to laboratory standards and internal quality control protocols.

4. STATISTICAL ANALYSIS

Normality of the data was tested using the D'agostino-Pearson test. Mann-Whitney and Kruskall-Wallis tests were used for comparisons of continous variables between the four groups of newborns. Spearman rank correlation was utilized to assess correlations between presepsin and other biomarkers of infection, birth weight, gestational age, Apgar score. P values less than 0.05 were considered significant. ROC analyse was used to examine the capability of presepsin to diagnose sepsis [21].

5. RESULTS AND DISCUSSION

The blood presepsin concentration in all examinated newborns ranged from 194 to 4150 pg/ml. In septic newborns mean value of presepsin (1389,5±861,9, range 294-4150) was significantly ($p<0,001$) higher than in newborns with other infections (717,3 ±382,2, range 209 – 1939), than in newborns without infections (530,0±180,3, range 269 – 953) and than in control group (391,3±83,6, range 194 – 579) – $p<0,0001$. The mean value of presepsin in newborns with local infections was significantly ($p<0,005$) higher than in control group. These data demonstrated, that the PATHFAST presepsin assay, performed using small volume of whole blood is valuable, fast new method for the early diagnosis not only for bacterial sepsis, but also for local severe neonatal infections without bakteriemia. It is especially important for very preterm born infants treated in the NICU. Results obtained within 17 minutes are earlier than in other diagnostics methods, which showed significantly elevated sCD14 and LBP levels in samples from septic newborns [6, 20]. Yeagashi et al. [6] observed that the sCD14-ST concentrations in septic patients were significantly higher than those in patients with SIRS or healthy control. The results of our analyses in newborns seem to confirm this findings in adults [6, 22, 23]. Palmiere et al. [24] indicated in postmortem serum femoral blood and pericardial fluid studies, that serum sCD14-ST and PCT levels, individually considered, allowed sepstic cases to be indentified. Shozushima T el al. [25] noted in 8 cases of sepsis, that adult patients with local infection or sepsis had significantly higher presepsin levels than the patients, who did not have infection.We observed similar significantly higher presepsin concentrations in severe infected newborns, both septic and

local infected, than not only in healthy control, but also in newborns suffering from noninfections diseases (respiratory distress syndrom, transient tachypnoe, intraventricular hemorrhages, heart defect).

No statistically significant differences (p>0,05) were observed between mean values of CRP and PCT in septic and with local infections newborns. No statistical differences (p >0,05) in mean presepsin concentrations between sick boys and girls, between born by cesarean section and through natural passages. Figures 1 and 2 show positive correlations between presepsin concentrations and CRP in septic newborns and PCT in newborns with local infections, without bacteremia.

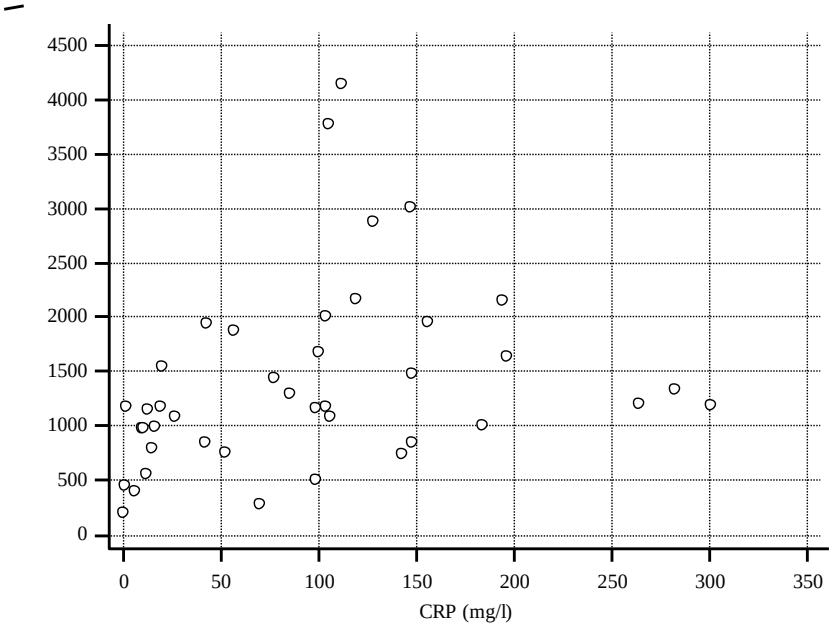

Fig1. *Positive correlation between presepsin and CRP concentrations in septic newborns (p = 0,0004; r = 0,531)*

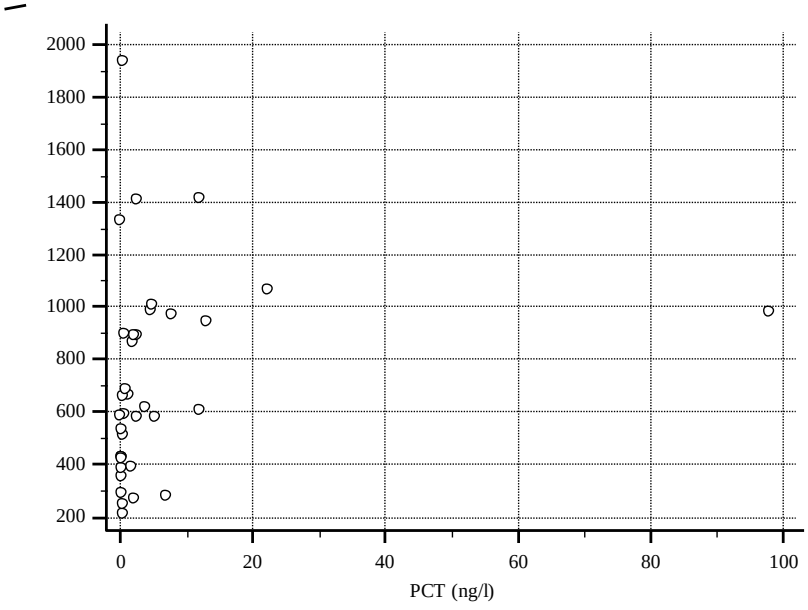

Fig2. *Positive correlation between presepsin and PCT concentrations in local infected newborns (p=0,0095; r= 0,426)*

Negative correlations between presepsin concentrations and hemoglobin (p=0,002; r= - 0,46), count of platelet (p=0,015; r= - 0,377; Fig. 3) and hematocrit (p =0,008; r= - 0,41) only in septic newborns were stated.

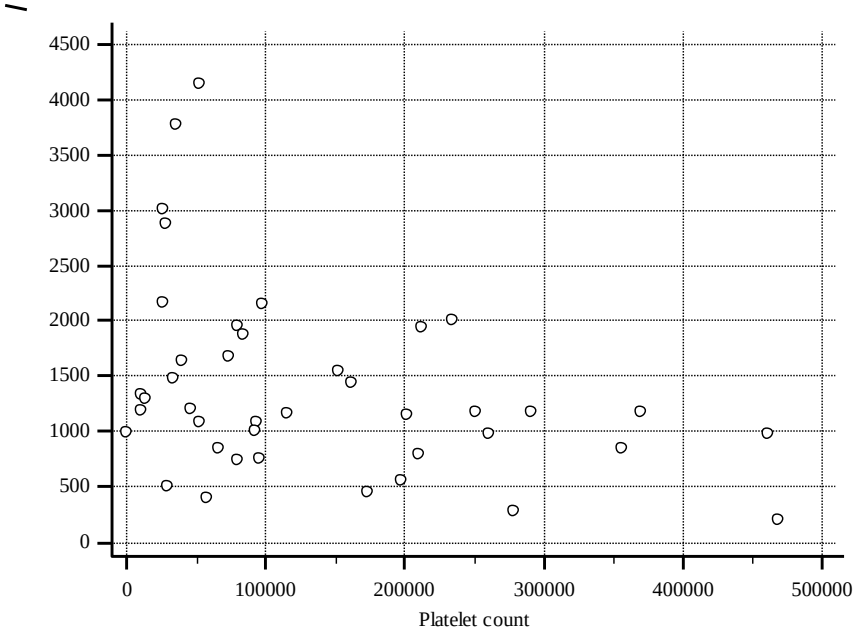

Fig3. *Negative correlation between presepsin concentrations and platelet count in septic newborns (p = 0,0151; r= 0,377)*

Any correlations between presepsin values and gestational age and birth asphyxia in infected neonates were observed. Mussap et al. [18] clearly suggest, like we noted in our studies, no correlation between gestational age and sCD14-ST presepsin blood level in critically ill preterm newborns between 26 and 36 weeks. We confirmed by ROC analysis (Youden coefficient 0,526, cut off point 1066 pg/ml) sensitive (64%) and high specifity (89,2%) of blood presepsin measurement as a diagnostic biomarker for the sepsis (Fig. 4).

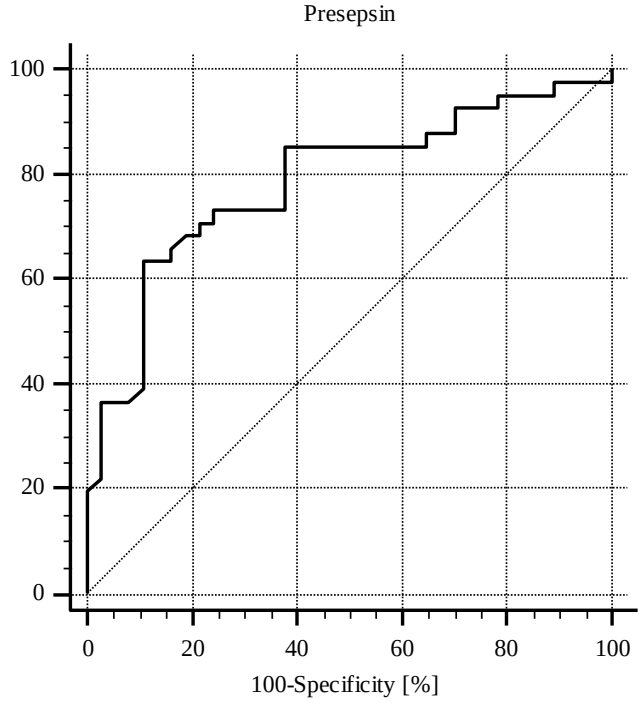

Fig4. *ROC analysis for presepsin (sCD14-ST) concentrations in septic newborns*

6. CONCLUSIONS

(1) In the newborns, both septic and with local infections, independently of their gender, fetal maturity, birth asphyxia and mode of mother's delivery, in comparison to healthy newborns,

significantly increase of blood presepsin concentration are founded. (2) Increase of presepsin concentration in septic newborns significantly correlate with increase of CRP concentration and decrease of hemoglobin, hematocrit value and platelet count. (3) Observed in local infected newborns marked increase of presepsin concentration correlates with increase of procalcitonin value. (4) Measurement of blood presepsin concentration is high sensitive and specifive biomarker for the early diagnosis of neonatal bacterial sepsis.

REFERENCES

[1] Hofer N., Zacharias E., Müller W., Resch B., An update on the use of C-reactive protein in early-onset neonatal septicemia: current insight and new tasks, Neonatology. 102(1), 25-36 (2012).

[2] Vouloumanou E.K., Plessa E., Karageorgopoulos D.E., Mantadakis E., Falagas M.E., Serum procalcitonin as a diagnostics marker for neonatal sepsis: a systematic review and metaanalysis, Intensive. Care. Med. 37(5), 747-62 (2011).

[3] Chiesa C., Natale F., Pascone R., Osborn JF., Pacifico L., Bonci E., De Curtis M., C-reactive protein and procalcitonin reference intervals for preterm and term newborns during the early neonatal period, Clin. Chim. Acta. 412 (11-12), 1053-9 (2011).

[4] Natarajan G., Shankaran S., Mc Donalds S.A., DAS A., Stoll B.J., Higgins R.D., Thorsen P., Skogstrand K., Hougaard D.M., Carlo W.A., NICHD neonatal research network, Circulating beta chemokine and MMP 9 as markers of oxidative injury in extremely low birth weight infants, Pediatr. Res. 67(1), 77-82 (2010).

[5] Manoura A., Goiurgiotis D., Galanakis E., Matalliotakis E., Hatzidaki E., Marmarinos A.S., Giannakopoulou C., Circulating concentrations of α- and β-chemokines in neonatal sepsis, Int. J. Infect. Dis. 14(9), e806-9 (2010), doi: 10. 1016/j.ijid.2010.03.015.

[6] Yaegashi Y., Shirakawa K., Sato N., Suzuki Y., Kojika M., Imai S., Takahashi G., Miyata M., Furusako S., Endo S., Evaluation of a newly indentified soluble CD14 subtype as a marker for sepsis, J. Infect. Chemother. 11(5), 234 – 8 (2005).

[7] Wright S.D., Ramos R.A., Tobias P.S., Ulevitch R.J., Mathison J.C., CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein, Science. 249 (4975), 1431-3 (1990).

[8] Urbonas V., Eidukaite A., Tamuliene I., The predictive value of soluble biomarkers (CD14 subtype, interleukin-2 receptor, human leukocyte antygen-G) and procalcitonin in the detection of bacteriemia and sepsis in pediatric oncology patient with chemotherapy induced febrile neutropenia, Cytokine. 62(1), 345 -7 (2013).

[9] Grunwald U., Krüger C., Westerman J., Lukowsky A., Ehlers M., Shütt C., An enzyme-linked immunoabsorbent assay for the quantification of solubilized CD14 in biological fluids, J. Immunol. Methods. 155, 225-32 (1992).

[10] Landmann R., Zimmerli W., Sansano S., Link S., Hahn A., Glauser M.P. et al., Increased circulating soluble CD14 is associated with high mortality in gram-negative septic shock, J. Infect. Dis. 171(3), 639-44 (1995).

[11] Lien E., Aukrust P., Sundan A., Muller F., Froland S.S., Espevik T., Elevated levels of serum-soluble CD14 in human immunodeficiency virus type 1 (HIV-1) infection: correlation to disease progression and clinical events, Blood. 92: 2084-92 (1998).

[12] Mussap M., Noto A., Fravega M., Fanos V., Soluble CD14 subtype presepsin (sCD14-ST) and lipopolysaccharide binding protein (LBP) in neonatal sepsis: new clinical and analytical perspectives for two old biomarkers, J. Matern. Fetal. Neonatal. Med. Suppl 2, 12-4 (2011).

[13] Okamura Y., Yokoi H., Development of point-of case assay system for measurement of presepsin (sCD14-ST), Clin. Chim. Acta. 412 (23-24), 2157-61 (2011).

[14] Oestreicher C., Pfeffel F., Peterman D., Müller C., Increased in vitro production and serum levels of the soluble lipopolysaccharide receptor sCD14 in liver diseases, J. Hepatol. 23 (4), 396 - 402 (1995).

[15] Horneff G., Sack U., Kalden J.R., Emmrich F., Burmester G.R., Reduction of monocyte-macrophage activation markers upon anti-CD14 treatment. Decreased levels of IL-1, IL-6, neopterin and soluble CD14 in patients with rhematoid arthritis. Clin Exp Immunol. 91(2), 207-13 (1993).

[16] Egerer K., Feist E., Rohr U., Pruss A., Burmester G.R., Dö ner T., Increased serum soluble CD14, ICAM-1 and E-selectin correlate with disease activity and prognosis in systemie lapus erythematosus, Lupus. 9(8), 614-21 (2000).

[17] Würthrich B., Kaegi M.K., Joller-Jemelka H., Soluble CD14 but not interleukin-6 is a New marker for clinical activity in atopic dermatitis, Arch. Dermatol. Res. 284 (6), 339-42 (1992).

[18] Mussap M., Paxeddu E., Burrai P., Noto A., Cibecchini F., Testa M., Puddu M., Ottonello G., Dessi A.,., Irmesi R., Gassa E.D., Fanni C., Fanos V., Soluble CD14 subtype (sCD14-ST) presepsin in critically ill preterm newborns: preliminary reference ranges, J. Matern. Fetal. Neonatal. Med. Suppl 5, 51-3 (2012).

[19] Kwiatkowska – Gruca M., Behrendt J., Sonsala A., Wisniewska-Ulfig D., Mazur B., Godula-Stuglik U., Presepsin (soluble CD14-ST) as a biomarker for sepsis in neonates, Ped. Pol. 88(5), 392-7 (2013).

[20] Berner R., Fürll B., Stelter F., Droese J., Müller H.P., Schütt C., Elevated levels of lipopolysaccharide-binding protein and soluble CD14 in plasma in neonatal early-onset sepsis. Clin. Diagn. Lab. Immunol. 9, 440-5 (2002).

[21] R. Core Team (2014), R: language and environment for statistical computing. R. Foudation for statistical computing, Vienna, Austria, URL: /www. R-project. org/.

[22] Endo S., Suzuki Y., Takahashi G., Shozushima T., Ishikura H., Murai A.,Nishida T., Irie Y., Miura M, Igushi H., Fukui Y., Tanaka K., Nojima T., Okamura Y., Presepsin as a powerful monitoring tool for the prognosis and treatment of sepsis: a multicenter prospective study, J. Infect. Chemother. 20 (1), 30-4 (2014)

[23] Ishikura H., Nishida T., Murai A., Nakamura Y., Irie Y., Tanaka J., Umemura T., New diagnostics strategy for sepsis-induced disseminated intravascular coagulation: a prospective single-center observational study, Crit. Care. 18: R 19 (2014).

[24] Palmiere C., Mussap M., Bardy D., Cibecchini F., Mangin P., Diagnostic value of soluble CD14 subtype (sCD14-ST) presepsin for the postmortem diagnosis of sepsis-related fatalities, Int. J. Legal. Med. DOI 10.1007/s00414-012-0804-5 (2012)

[25] Shozushima T., Takahashi G., Matsumoto N., Kojika M., Okamura Y., Endo S., Usefulness of presepsin (sCD14-ST) measurements as a marker for the diagnosis and severity of sepsis that satisfied diagnostics criteria of systemic inflammatory response syndrome, J. Infect. Chemother. 17 764-9 (2011).