



PROCALCITONIN AND OTHER BIOMARKERS OF SEPSIS IN NEWBORNS IN THE INTENSIVE CARE UNIT

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Abstract

Neonatal sepsis is one of the most significant causes of mortality and morbidity in infants. Among numerous parameters available to confirm the presence of sepsis in newborns procalcitonin (PCT) has been chosen. The aim of this study was the determination of PCT, C-reactive protein (CRP) serum amyloid A (SAA), plasminogen, protein C, antithrombin III (AT III) and white blood cell count (WBC) in blood sample obtained by puncture of the umbilical vein. Sixty two newborn infants were included in the study: 31 with suspected bacterial infection and 31 healthy babies. Serum procalcitonin was measured using Kryptor analyzer (Brahms Aktiengesellschaft, Germany); serum hsCRP and SAA on the Behring Nephelometer II (Dade Behring Diagnostics GmbH, Marburg, Germany); plasma plasminogen, protein C and AT III on BCT Coagulation system, (Dade Behring Diagnostics GmbH, Marburg, Germany); and WBC count was determined in the whole blood using hematological analyzer ADVIA 120 Hematology System (Bayer, Germany). The obtained mean values of PCT, hsCRP, SAA, WBC, plasminogen, AT III, protein C in newborn's samples with suspected bacterial infection/healthy newborns were: 0.188 ng/L / 0.121 ng/L; 1.20 mg/L/ 1.30 mg/L, 1.28% / 1.70%; $16.0 \times 10^9/L$ / $12.0 \times 10^9 / L$, 61.0% / 59.0%, 52.0% / 64.5%, 39.0% / 41.0%, respectively. Neonates with bacterial infection had significantly higher values of PCT ($p < 0.001$), WBC ($p < 0.001$) and CRP ($p < 0.05$) compared to healthy babies. Based on these results, it may be concluded that procalcitonin is useful for early diagnosis of sepsis in newborns.

INTRODUCTION

Neonatal sepsis is one of the most significant causes of mortality and morbidity among neonates. In comparison to other forms of sepsis, neonatal sepsis has some specificity for several reasons:

- Different modes of spread of infectious agents: a homogeneous horizontal expansion, colonization during birth, transplacental vertical expansion and neonatal exposure to various environment factors,
- Immunological immaturity caused by humoral and cellular immunodeficiency due to poor production of immunoglobulin G (IgG), decreased granulocyte, immature leukocyte function,
- Manifestations are variable, being affected by the exposure duration, type of infectious agents and the immune status and the presence of other illnesses that complicate diagnosis and treatment of neonatal sepsis (1).

Neonatal sepsis is commonly present in infants who were admitted to intensive care units and 10% of them will develop late neonatal sepsis; if premature birth occurred, the percentage would increase significantly and exceed 25%. Early diagnosis of neonatal sepsis is very difficult because the signs are nonspecific (2). The early signs of neonatal sepsis are temperature instability, irritability, lethargy, pale, marbled skin, etc. Tachycardia, apnea, petechiae and purpura with omphalitis are present very often. The most common cause of neonatal sepsis is bacterial and fungal infection. Diagnosis of neonatal sepsis is based on isolation of bacteria and measurement of their endotoxins or bacterial antigens in body fluids (3). Elevated levels of procalcitonin (PCT), C-reactive protein (CRP), cytokines (TNF- α , IL-1, IL-6, IL-8) and their receptors, growth factors, increased number of WBC allow early and rapid diagnosis.

Procalcitonin consists of 116 amino acids (aa) and it is a precursor of prohormone calcitonin. Under normal metabolic conditions, calcitonin is produced by thyroid C-cells after specific intracellular activity of procalcitonin. In conditions such as sepsis, which are accompanied by bacterial infection, intact PCT was found in the blood. This suggests that the origin of PCT is extrathyroidal. Synthesis is complex and begins by translation of precursor peptide, preprocalcitonin composed of 141 amino acids. After transcription of CALC-1 gene, primary transcript is translated into mRNA encoding protein with 141 aa and molecular weight of about 16 kDa. "Precursor protein", prepro-calcitonin, contains a signal sequence, N-terminal procalcitonin (N-PCT), the middle sequence of calcitonin and C-terminal region of procalcitonin, katacalcin (4). Signal sequence is hydrophobic and under the exposure to endopeptidases is degraded in the endoplasmic reticulum (5). Calcitonin precursor is glycosylated and resistant to the effects of the enzyme as a glycoprotein (6). On the basis of the aforesaid, it is assumed that infection-induced PCT is not a glycoprotein. Signal sequence is a place of action of the enzyme prohormone convertase (PC) that produces degradation products: N-PCT, calcitonin and katacalcin and their combination. These products do not arise from the inflammation-induced PCT. Signal sequence is suitable for phosphorylation, which is the reason for PCT to be found intact in plasma during sepsis and infection.

During systemic bacterial infection that is seen in sepsis, septic shock and multisystem organ dysfunction syndrome, the increased levels of calcitonin precursors were found in plasma, but with the absence of calcitonin secretion (7). PCT is one of the major peptides and its half-life in circulation is 20-24 hours (8,9). It is believed that the targeted proteolytic cleavage of PCT in Golgi apparatus suppressed the effect of cytokines and endotoxin, and therefore, precursor proteins, including procalcitonin and its fragments, are being released into the circulation (7,10).

SUBJECTS AND METHODS

Sixty two newborns (35 females and 27 males) from the Institute of Gynecology and Obstetrics, Clinical Center of Serbia were included in the study during the period from November 2006 to October 2007. Approval for the collection of samples and biochemical testing was obtained from each mother and from the Ethics Committee of the Clinical Center of Serbia. Blood was taken by puncture of the umbilical vein, up to two hours after birth. Blood was collected without anticoagulant for determination of PCT, SAA and CRP, followed by blood with EDTA as anticoagulant for determination of WBC count and blood with sodium citrate for determination of plasminogen, protein C and antithrombin III. Babies were divided into two groups: healthy babies (control group, N = 31), who were born naturally in full term of 36-week gestation and sick babies (infants at potential risk, N = 31), who were born in term, naturally, but placed in the Intensive Care Unit because of the risk factors and the onset of clinical symptoms of sepsis. This group consisted of babies who were born after prolonged rupture of membranes (OVR > 18 hours), whose amniotic fluid was green, yellow-green and cloudy and whose mothers had elevated serum glucose levels during pregnancy and high blood pressure and, therefore, were administered therapy. One baby, born after treated infertility and twin pregnancy (which is itself a possible cause of complications and sepsis) was included in study, too. WBC counts were determined immediately after admission to the Emergency Laboratory Diagnostics Centre. After centrifugation, plasminogen, protein C and antithrombin III were determined, and serum samples for determination of PCT, CRP and SAA were separated, divided into portions, and frozen. They were kept at -20°C up to a month. During this period, the freezing did not affect the concentration of tested parameters (11). After thawing the samples, they were

re-centrifuged to eliminate potential insoluble material and analyzed. Serum procalcitonin was measured using Kryptor analyzer (Brahms Aktiengesellschaft, Germany); serum CRP and SAA on the Behring Nephelometer II (Dade Behring Diagnostics GmbH, Marburg, Germany); plasma plasminogen, protein C and AT III in on BCS Coagulation system, (Dade Behring Diagnostics GmbH, Marburg, Germany); and WBC count was done in the whole blood by hematological analyzer ADVIA 120 Hematology System (Bayer, Germany)(12).

For the control group-healthy babies (A), and for sick babies (B), median (Me), minimum (Min) and maximum (max) values were calculated. Nonparametric t-test (Mann-Whitney) and correlation analysis (Spearman's correlation) were used for statistical analysis.

RESULTS

The study included 31 healthy newborns (control group) and 31 newborns who with their clinical symptoms were allocated to the group which was suspected of developing the sepsis (13). These babies were at potential risk as being separated from their mothers and placed in the Intensive Care Unit, for further monitoring of their condition and performing diagnostic procedures. Table 1 presents the clinical parameters that influenced the selection of study babies:

Table 1. Clinical parameters influencing the selection of studied babies.

Parameters		Studied babies and mothers (%)
Sex (baby)	Male	44
	Female	56
Former pregnancies (mother)	1st pregnancy	71
	2nd pregnancy	24
	1st pregnancy – death	2
	Treated infertility – twin pregnancy	3
Illness of mother	↑ blood pressure (> 120/80)	24
	↓ blood pressure (< 120/80)	16
	glucose intolerance	8
	Rh(D)-	5
Prolonged rupture of membranes (OVR> 18 hours)		8
Amniotic fluid	Clear	44
	Green	32
	Cloudy	24

Median, minimum and maximum values for all measured parameters in control group (A) and sick newborns (B) were presented in Table 2.

Table 2. Median values of measured biochemical parameters in sick and healthy newborns and control group

		Median (min–max)	P <
Procalcitonin (ng/mL)	healthy newborns	0.121 (0.036-0.319)	0.000
	sick newborns	0.188 (0.107-0.462)	
C-reactive protein (mg/L)	healthy newborns	1.20 (0.10-12.10)	0.001
	sick newborns	1.30 (0.30-8.00)	
serum amyloid A (mg/L)	healthy newborns	1.70 (0.70-23.50)	0.552
	sick newborns	1.28 (0.70-4.30)	
white blood cell count (x10 ⁹ /L)	healthy newborns	12.0 (5.0-18.8)	0.001
	sick newborns	16.0 (12.0-23.0)	
Plasminogen (%)	healthy newborns	59.0 (45.0-173.0)	0.297
	sick newborns	61.0 (43.0-92.0)	
protein C (%)	healthy newborns	41.0 (22.0-123.0)	0.847
	sick newborns	39.0 (29.0-65.0)	
antithrombin III (%)	healthy newborns	64.5 (35.0-111.0)	0.073
	sick newborns	52.0 (38.0-75.0)	

Spearman's correlation coefficients between measured biochemical parameters are presented in Table 3. There was a significant correlation ($p < 0.01$) between: CRP and SAA ($r = 0.556$), CRP and ATIII ($r = 0.497$), SAA and WBC count ($r = 0.417$), plasminogen and protein C ($r = 0.463$), plasminogen and ATIII ($r = 0.424$), protein C and ATIII ($p = 0.449$).

DISCUSSION

Among the most common possible causes of neonatal sepsis, maternal factors assuming various illnesses of mother (diabetes mellitus, high or low blood pressure, kidney disease) and social

behavior of mothers before and during pregnancy are singled out. Prolonged rupture of membranes lasting more than 18 hours is also one of the major factors of neonatal sepsis. Amniotic fluid, which is not colorless, but milky, yellow or green, is sufficient warning to clinicians that sepsis may be developed in these infants. Sastre et al (14) pointed out that this is the reason why doctors administer antibiotics immediately after birth or even as early as in the intrauterine period (15).

Serum amyloid A (SAA) is a parameter that may be also used for verifying the bacterial infection (16). Its disadvantage is that it is neither sufficiently specific nor sufficiently sensitive, because its values increase only with longer duration of infection. In this study, there was no significant difference of SAA between the two groups, healthy and sick infants. Serum amyloid A was significantly correlated with CRP ($r = 0.556$) and AT III ($r = 0.417$), as shown in Table 3.

Table 3. Correlation between measured biochemical parameters

Parameters	PCT	CRP	SAA	WBC count	plasminogen	protein C	antithrombin III
PCT	-	0.264	0.104	0.180	0.311	0.047	0.270
CRP	0.264	-	0.556*	0.206	0.165	0.309	0.497*
SAA	0.104	0.556*	-	0.417*	0.325	0.168	0.205
WBC count	0.180	0.206	0.417*	-	0.008	-0.033	-0.164
Plasminogen	0.311	0.165	0.325	0.008	-	0.463*	0.424*
Protein C	0.047	0.309	0.168	-0.033	0.463*	-	0.449*
Antithrombin III	0.270	0.497*	0.205	-0.164	0.424*	0.449*	-

* $p < 0.01$.

According to many studies, determination of procalcitonin in the umbilical vein blood could be a parameter of sufficient specificity and sensitivity for diagnosis of neonatal infection. In addition, procalcitonin exhibits good positive and negative predictive values. In clinical practice, the determination of procalcitonin in the umbilical cord blood obtained after birth may be helpful for early diagnosis of neonatal infection. Good negative predictive value of procalcitonin could prevent unnecessary use of antibiotics in treatment (17).

The concentration of procalcitonin were higher in the group of sick babies, which was confirmed by statistical analysis. There was a significant difference between the groups ($p = 0.000$). Correlation analysis found no significant correlation between procalcitonin and other measured parameters (CRP, WBC, SAA, protein C, plasminogen and AT III).

C-reactive protein has been the most analyzed parameter for detection of bacterial infection for years (18,19). This protein acts as a "scavenger" because it leads to opsonization of bacteria and activation of the complement system and facilitates phagocytosis in the inflammatory response. The advantage of PCT compared to C-reactive protein is that the increase of the further in bacterial infection and its restoration to normal is more rapid (20). In our study, the

data obtained for CRP in the control group were significantly lower than those obtained for sick babies ($p = 0.001$). Significant correlation between CRP and SAA ($r = 0.556$) and AT III ($r = 0.497$) was also observed (Table 3).

Regarding the number of leukocytes (20, 21), there was a significant difference between the two groups, healthy and sick infants ($p = 0.001$) (Table 2). In addition, there was a significant correlation between WBC and SAA ($r = 0.417$), as shown in Table 3.

The values of parameters of hemostasis were, as expected, similar (plasminogen, protein C) or slightly lower (antithrombin III) in the group of sick infants, but there was no statistically significant difference between the groups (Table 2). A significant correlation between plasminogen and AT III ($r = 0.424$) and protein C ($r = 0.436$) were observed (Table 3). Antithrombin III also significantly correlated with CRP ($r = 0.497$) and plasminogen ($r = 0.424$).

The obtained values for the studied parameters are only a guideline for further investigation as the low quantity of cases was the limitation of the study. Higher number of studied cases could provide more reliable conclusions about the significance of determination of procalcitonin as an early marker of neonatal sepsis, especially when it is the question of performing assays in specimens obtained from the umbilical vein. Significant difference found between the study groups for procalcitonin and white blood cell count could suggest that simultaneous determination of these two parameters may be significant for the diagnosis of bacterial infection. The difference was found for CRP between these two infant groups, however, the values in cases with bacterial infection were much lower than expected (Table 2). This may mean that the period just after the birth is too short for detection of increase of this parameter. Determination of parameters of hemostasis in blood samples from the umbilical vein is of little diagnostic significance, because the values of these parameters change with the development of bacterial infection after a few days. Determination of SAA concentrations just after birth proved to be insufficiently sensitive, since there was no difference in values between the groups.

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