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Evaluation of serum chemokine RANTES concentration as a biomarker in the diagnosis of early-onset severe infections in neonates

Ocena stężenia chemokiny RANTES w surowicy jako biomarkera diagnostyki wczesnych ciężkich zakażeń u noworodków

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Objective: Only a few studies on improving the early diagnosis of severe neonatal infections have focused on the role of serum RANTES concentration (sRC). The aim of the study was to establish sRC in neonates with early-onset infections, according to their gestational age, sex, birth asphyxia, mode of delivery and value of some biochemical and hematological parameters.

Material/Methods: The analysis comprised 129 neonates, including 89 infected (52 preterm, 37 full-term; 43 with sepsis, 39 with congenital pneumonia, 7 with severe urinary tract infection) and 40 healthy (control group, 25 full-term, 15 preterm). The sRC in peripheral vein blood was measured by the ELISA method using Quantikine Set (R & D systems, USA).

Results: The sRC in infected neonates ranged from 10.83 to 122.55 μg/ml, in full-term neonates from 18.28 to 122.55 μg/ml, and in preterm from 10.83 to 118.24 μg/ml. The mean sRCs in full-term septic neonates (73.95±25.99 μg/ml) and with organ infections (58.43±29.24 μg/ml) were significantly higher than healthy ones (28.25±14.06 μg/ml). The mean sRCs in septic preterm neonates (59.17±28.29 μg/ml) and those with organ infections (50.86±28.16) were significantly higher than in healthy preterm neonates (25.61±8.29 μg/ml). Positive correlations between sRC and CRP value (r=0.3014, p=0.004) and between sRC and band cell count (r=0.2489, p=0.019) were found in all infected neonates.

Conclusion: The significant increase of serum RANTES concentration in early-onset infections in neonates, regardless of their gestational age, sex and birth asphyxia, not only proves the presence of an active immunological process but also may be a useful biomarker for diagnosis of severe neonatal infections.

Keywords: **RANTES • sepsis • organ infection • full-term neonate • preterm neonate**

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Introduction

Early-onset infections, especially in preterm neonates, besides respiratory distress syndrome (RDS), are the main cause of bad outcome in neonates [3,9,19,36,49]. Their occurrence not only prolongs hospital stay but also influences the development of bronchopulmonary dysplasia and retinopathy of preterm neonates [18,40,41]. The incidence of early-onset neonatal infections depends on gestational age and birth weight. There are 1-5 cases of 1000 neonates with birth weight >2500 g, approximately 20 cases in neonates weighing between 2000 and 2500 g, 160 cases regarding very low birth weight, and in preterm neonates with birth weight <1000 g up to 75% [31,41,48]. The mortality rate as a consequence of irreversible septic shock ranges from 20 to 50% [5,11,19,34]. The most frequent clinical forms of severe early-onset infections are sepsis, pneumonia, meningitis and urinary tract infections [5,34,48].

The proper and quick diagnosis is still one of the most significant challenges for neonatologists. It is sometimes based on the data from obstetrical and perinatal history, mainly presence of infectious risk factors [2]. The diagnostics of difficulties are the result of non-specific and indistinguishable clinical signs, which are often falsely taken for symptoms of adaptation disorders, noninfective diseases, such as cardiac defect, intracranial hemorrhage, aspiration syndrome and RDS [30,33]. On the other hand, a very rapid course of infection may lead to death within a few days or even hours [11]. To date, none of the infectious biomarkers used in adults is sensitive and specific enough to definitely confirm or exclude the diagnosis of neonatal infection. Various diagnostic and prognostic values of "old" early markers of infections (total white blood cells, immature/total ratio of neutrophil counts, morphologic and degenerative changes in neutrophils, platelet count, activity and plasma concentration of coagulation factors, fibronectin, haptoglobin, lactoferrin, C-reactive protein, procalcitonin) have been widely described [1,8,17,26,32,44,46]. Absolute total immature neutrophils and immature to total neutrophil proportions may have diagnostic value for early-onset sepsis > 6 hours of postnatal life [25]. However, the changes of the values of these parameters are also observed in non-infectious diseases (RDS, meconium aspiration syndrome, birth asphyxia), which limits the effectiveness of these tests as biomarkers of neonatal infection [25,36]. There are tests to indicate cytotoxicity of NK cells, monocytes' capacity for phagocytosis, concentration of GM-CSF, G-CSF, calprotectin and presepsin, CD4+/CD25+, adhesion molecules and surface antigens (CD11b, CD62L, CD64, ICAM-1, VCAM-1) in diagnosis of neonatal infections [12,13,16,22,23,39]. Increased concentrations of pro- and anti-inflammatory cytokines indicated in infected neonates as an effect of the possibility to generate an inflammatory reaction responding to an infectious agent were the subject of research of the new diagnostics methods [24,39,42]. A particular subject of interest of researchers is chemotactic cytokines, one of whose representatives is RANTES (regulation on activation normal T-cell expressed and secreted). It is produced by macrophages, epithelial cells, platelets, megakaryoblasts, T lymphocytes and eosinophils [15,21,38]. It acts through CCR1, CCR3 and CCR5 receptors. It has an effect on chemotaxis of monocytes, T lymphocytes (including memory cells), NK cells, eosinophils, dendritic cells, and mast cells including adhesion molecules VCAM-1 and ICAM-1. Moreover, it activates histamine secretion by mast cells, stimulates lymphocyte proliferation and IgE and IgG production, increases the expression of CD80 on antigen-presenting cells and activates the cytotoxicity of T lymphocytes and NK cells [4,27]. The aim of this study was to evaluate the serum chemokine RANTES concentration in severely infected neonates and to assess the relationship between their gestational age, birth weight, sex, birth asphyxia, mode of delivery, value of some hematological and biochemical parameters and RANTES values in sick neonates.

Material

The study population comprised 129 neonates aged from 3 to 7 days of life, with birth weight > 1000 g, including 89 infected (study group, 37 full-term, 52 preterm) and 40 healthy (control group, 25 full-term, 15 preterm). All the neonates eligible for inclusion in the study had no congenital anomalies, other genetic disorders, hyperbilirubinemia or gestational age < 28 weeks. Clinical characteristics of the study population are shown in table 1 and chosen typical symptoms of 43 cases of early-onset sepsis are presented in Fig. 1. All the 39 neonates with pneumonia, confirmed by X-ray examinations, presented respiratory distress: tachypnea, grunting, dyspnea and cyanosis within 48 hours of life; pulmonary hypertension was diagnosed in two pneumonic full-term

Table 1. Clinical characteristics of the study population

	Infected full term n=37	Infected preterm n=52	Healthy full term n=25	Healthy preterm n=15
Sex m/f	23/14	27/25	13/12	9/6
Gestational age (weeks) mean ± SD min-max	39.7±1.1 38-41	31.6±3.1 28 -36	39.6±0.9 38-41	33.1±1.1 31-36
Birth weight (g)	3434.3±581.1 1520-4460	1695.4±654.0 1100-3150	3440.0±684.6 3000-4250	1805.0 ± 2240.4 1400-2200
Intrauterine growth retardation	1%	13%	0	0
Cesarean section urgent/ elective	5/3	38/2	0/0	0/0
Apgar score at 5 min min-max	2-10	1-8	9-10	8-10
High-risk pregnancy	70%	88%	0	0
PROM > 12 hours	8%	7%	0	0

PROM = premature rupture of membranes

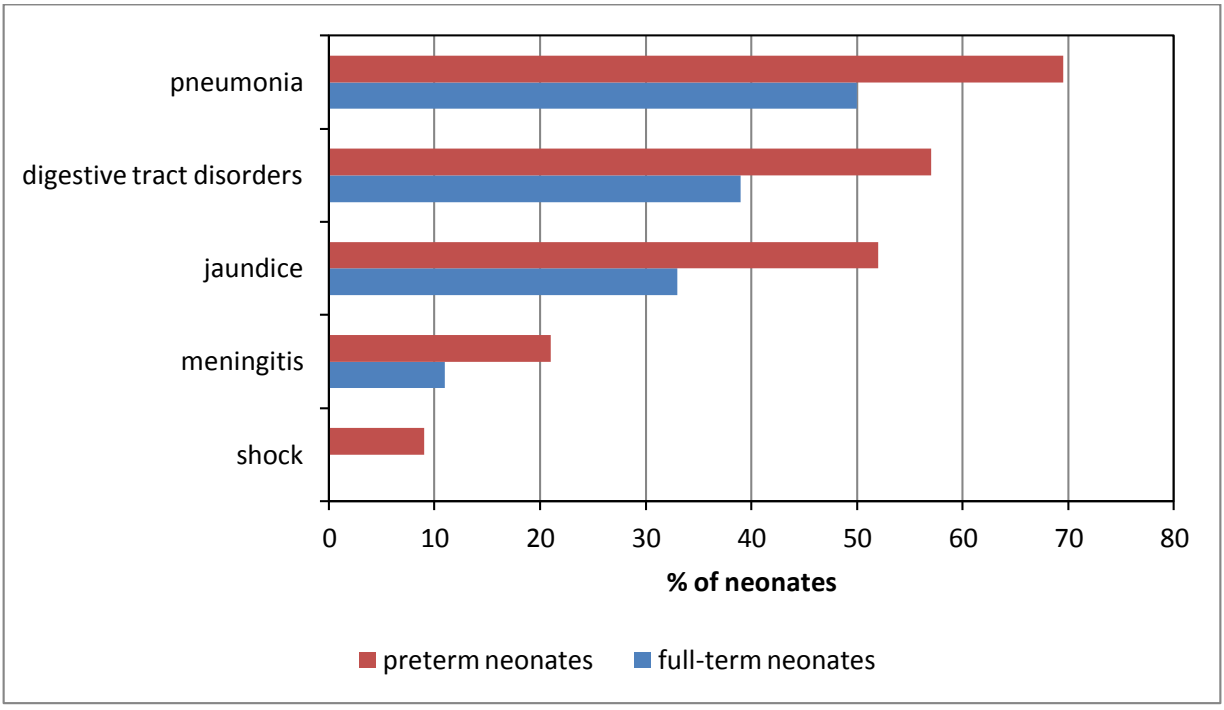

Fig. 1. Typical clinical symptoms of early – onset neonatal sepsis

neonates. Neutrophils in endotracheal aspirates were noted in 28 orotracheally intubated and mechanically ventilated neonates with pneumonia, on the first day of hospitalization, but cultures were negative. Significant bacteriuria (<10^5/ml) due to *E. coli* in 5 cases (2 full-term, 3 preterm) and due to *Klebsiella pneumoniae* in 2 preterm neonates was detected in neonates with pyuria and negative blood culture without congenital defects in kidneys and urinary tracts. The bacteria isolated from blood in septic neonates were meticillin-resistant (MR) *Staphylococcus epidermidis* (22 cases), *Pseudomonas aeruginosa* (9), *Klebsiella pneumoniae* (4), *E. coli* (4), *Staphylococcus aureus* (3) and *Serratia marcescens* (1). Thrombocytopenia occurred in 38%, metabolic acidosis in 35%, increase of serum C-reactive protein concentration >5 mg/l in 47%, hypo- or hyperglycemia in 29%, hyperbilirubinemia (>256 µmol/l) in 27%, hyponatremia (<130 mEq/l) in 27%, leukocytosis >30.0 G/l in 21%, leukopenia <3.5G/l in 12%, and anemia in 11% of infected neonates.

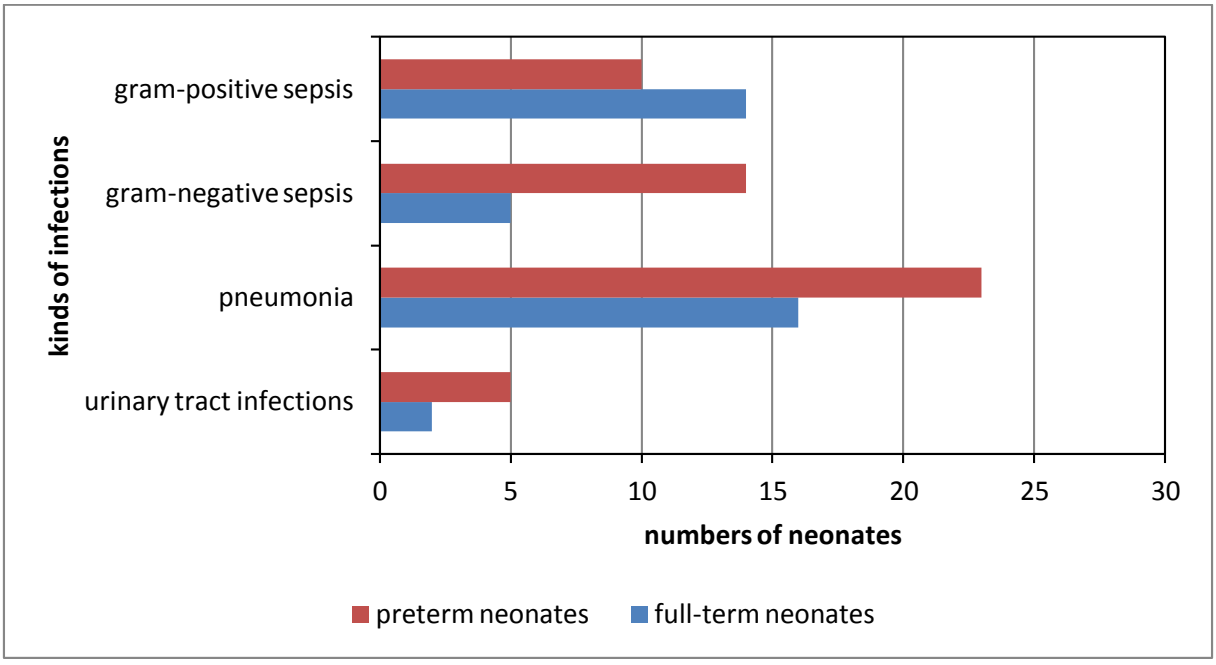

Fig. 2. Kinds of neonatal infections

Types of infections are shown in Fig. 2

All infected neonates were treated with broad spectrum antibiotics, mainly aminoglycosides (netilmicin or amikacin) with ampicillin. It was necessary to administer vancomycin in all cases of staphylococcal sepsis due to MR strains. In 53 (25 with sepsis and 28 with pneumonia) neonates, mechanical ventilation from the first day of life, in 44 (49%) catecholamines, in 44 (49%) phototherapy, in 38(43%) total parenteral nutrition, in 10 (11%) concentrated erythrocytes, in 9 (10%) concentrated platelets and in 4 (4.5%) filgrastim were applied.

Control subjects

The control group included 25 full-term and 15 preterms, 21 boys and 19 girls, born vaginally by healthy mothers, with an Apgar score > 8 points, breast-fed from birth, without signs of infection, adaptation disorders, jaundice or perinatal risk factors.

Methods

a) Collection of blood samples and serum RANTES measurement

Blood samples were obtained by venipuncture from infected neonates immediately after admission to the Neonatal Intensive Care Unit and from the control group, between the second and third day of life. The total white blood cell count was measured in neonates with suspected infection with the Cell Dyne 1600 Analyser, and CRP concentration by dry chemistry assay (Kodak Ektachem; Eastman Kodak, Rochester, NY, USA). The chemokine RANTES was determined by the ELISA method using Quantikine Set (R & D systems – Minneapolis, USA). All samples were assayed in duplicate. Intra- and interassay variation coefficients were 3.3 and 8.3%. The detection limit was 8 pg/ml. The investigations were granted permission from the Bioethical Commission of the Silesian Medical University in Katowice (L.dz. NN-013-175/03)

b) Statistical analysis

The following statistical methods were applied: analysis of variance ANOVA followed by the post-hoc RIR Tukey, Mann-Whitney U or Kruskal-Wallis test. $P < 0.05$ was considered statistically significant. Spearman correlations were used to analyze correlation between RANTES concentration and CRP, procalcitonin concentration, white blood cells, band cells, monocytes, neutrophils and thrombocytes count.

Results

The serum RANTES concentrations in infected neonates ranged from 10.83 to 122.55 µg/ml, in full-term neonates from 18.28 to 122.55 µg/ml, and in preterm neonates from 10.83 to 118.24 µg/ml. The mean RANTES concentrations in full-term septic neonates (73.95±25.99 µg/ml) and with organ infections with negative blood culture (58.43±29.24 µg/ml) were significantly higher ($p<0.05$) than in healthy ones (28.25±14.06 µg/ml) – Fig 3.

Mean values of RANTES in septic preterm neonates (59.17±28.29 µg/ml) and those with organ infections (50.86±28.16 µg/ml) were significantly ($p<0.05$) higher than in healthy preterm neonates (25.61±8.29 µg/ml) – Fig 4.

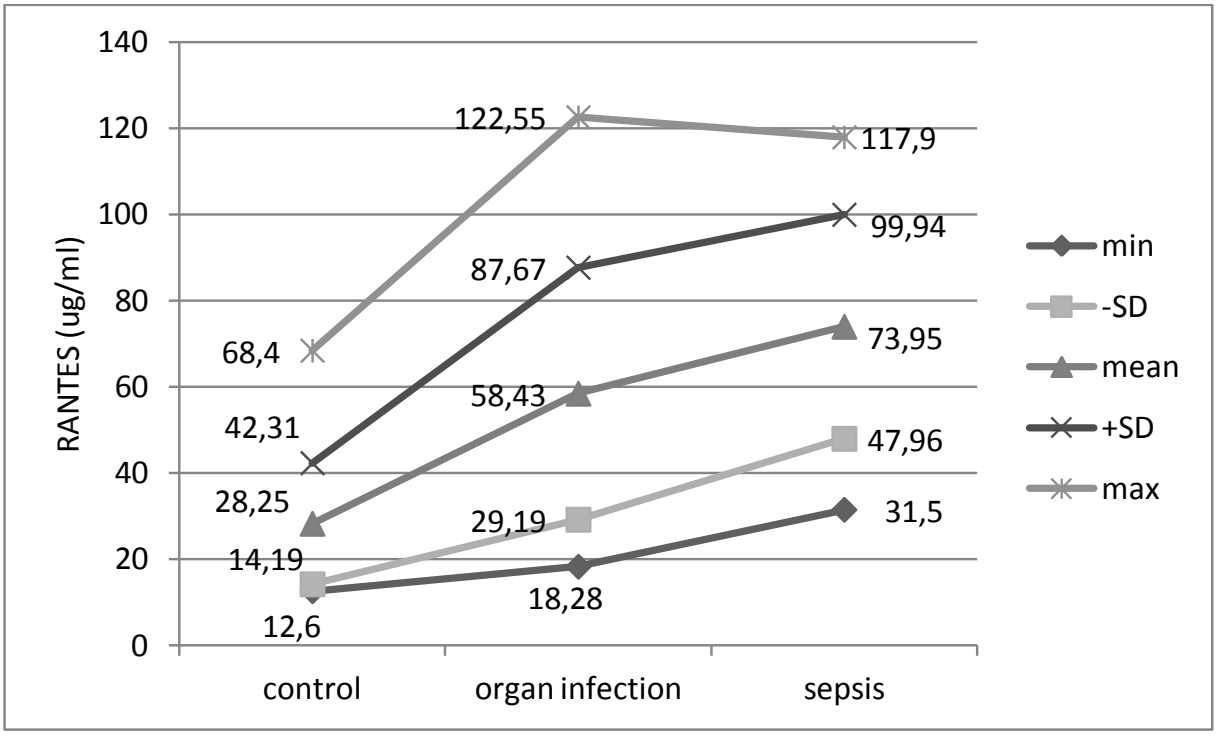

Fig. 3. Statistical analysis of mean serum RANTES concentrations in full-term neonates (control : neonates with sepsis $p<0.05$; control : neonates with organ infection $p<0.05$)

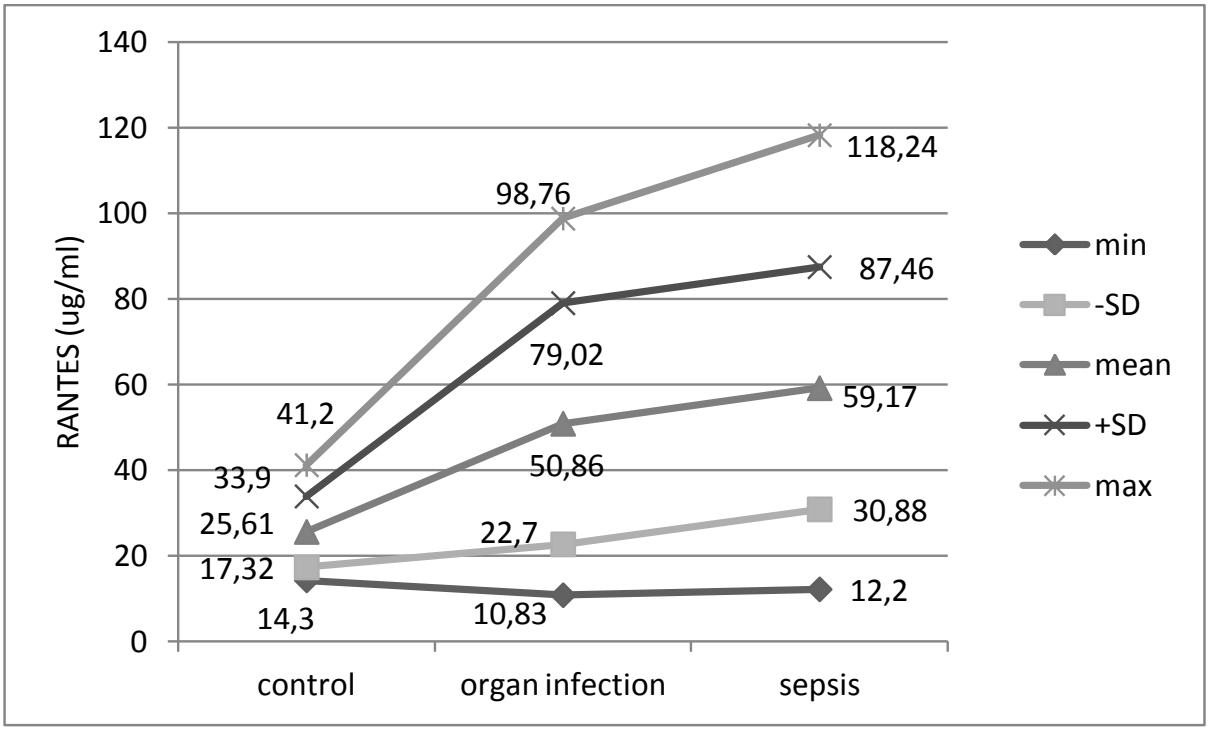

Fig. 4. Statistical analysis of mean serum RANTES concentrations in preterm neonates (control : neonates with sepsis $p<0.05$; control : neonates with organ infection $p<0.05$)

There was no statistically significant difference between the mean RANTES concentration in neonates with Gram-positive sepsis (64.5±22.0; range 12.2-96.3 µg/ml) and with Gram-negative sepsis (67.3±33.9; range 22.9–118.2 µg/ml).

No statistically significant differences ($p>0.05$) were noted between mean serum RANTES concentration and neonatal sex and mode of delivery in their mothers (cesarean section or spontaneous) and low Apgar score (<6 points in 5 minutes of life of neonates).

Positive correlations between serum RANTES concentration and CRP value (r= 0.3014, p= 0.004) and between RANTES concentration and band cell count (r= 0.2489, p= 0.019) were found in all infected neonates. No correlations between RANTES and procalcitonin concentrations, and number of white blood cells, neutrophils, monocytes and thrombocytes were noted.

Discussion

The early diagnosis of neonatal infection, especially sepsis in preterm neonates, before clinical signs, is important in order to start antimicrobial therapy and prevent unfavorable complications, such as intraventricular hemorrhage, periventricular leukomalacia, bronchopulmonary dysplasia and long-term consequences, mainly cerebral palsy. Clinical symptoms of infection are nonspecific, and for this reason early diagnosis is based on the conventional sepsis biomarker panel: microbiological (blood culture), biochemical (mainly CRP, procalcitonin) and hematological test (leukocyte and platelet counts, immature/total ratio of neutrophils and cytokines. Serological tests and isolation of bacteria do not give immediate results and can sometimes be difficult to interpret [1,8,36,44].

Our study demonstrates that regardless of gestational age and sex of neonates and magnitude of neutrophil and thrombocyte counts, early-onset neonatal severe infections was closely associated with the increase of serum RANTES concentration, and the highest value of this parameter was noted in septic neonates. It needs to be emphasized that the tests were done in neonatal peripheral blood, not in cord blood. Neonatal cord blood differs from the peripheral one in RANTES production. Previous studies suggested that the highest levels of the chemokine are detected after 12 hours of life and then cord blood seems to be inappropriate for early diagnosis of perinatal infection [28,37,44]. Shalak et al. [37] discovered higher RANTES level in full-term neonates born by mothers with chorioamnionitis in the sixth hour of their life, not just after delivery. Dammann et al. [10] measured RANTES concentration along with the other inflammatory markers in cord and peripheral blood, between the second and fifth day of life in neonates and observed higher chemokine concentration in preterm than in full-term neonates. Different results were obtained by Sullivan et al. [43] and Królak-Olejnik el al. [20]. The differences might reflect various assays used to determine chemokine concentration: Dammann et al. [10] used recycling immunoaffinity chromatography of whole blood, while Sullivan et al. [43] and Królak-Olejnik et al. [20] used enzyme-linked immunosorbent assay for umbilical serum. Moreover, Dammann et al. [10] evaluated only extremely low gestational age prematures. Our results are similar to the effects obtained by Sullivan et al. [43]. Królak-Olejnik et al. [20] reported that non-infected preterm neonates had lower RANTES concentrations than healthy term neonates and infected preterm neonates did not have a higher RANTES value in cord blood than noninfected ones. Hariharan et al. [14] showed that cord blood lymphocytes are not capable of constitutive secretion of RANTES, which was normal for peripheral blood lymphocytes, and found a decrease in the ability of cord blood lymphocytes to secrete RANTES even after lipopolysaccharide stimulation. Sato et al. [35] claimed that differences between the lymphocytes are the effect of a stronger chemotactic response of RANTES to CD45RO than to CD45RA in the peripheral blood. They also emphasized that on the surface of peripheral blood cells there are CCR1, CCR2, CCR5 and CCR6 receptors, while on the surface of cord blood cells there are only CXR4 receptors, which may reflect the differences in chemokine activity regulation between cord and peripheral blood.

Some authors have reported an association of poor recovery in sepsis and meningitis with lower RANTES concentration [6,7,29]. They hypothesized a correlation between low RANTES concentration and disseminated intravascular coagulation (DIC) as a result of a low number of platelets, which secrete RANTES. In the opinion of Ng et al. [29], neonates with sepsis and hematological disorders have greater changes in plasma inflammatory mediator concentrations compared with neonates with mild infection without DIC. Moreover, preterm neonates are capable of eliciting chemotactic and pro- and anti-inflammatory responses to invading pathogens.

In our study we confirmed the correlation between RANTES and CRP concentration and band cell count. Carrol et al. [6] found a positive correlation between RANTES and platelet count but no correlation with granulocyte count. They did not analyze other biochemical and hematological parameters.

An overall evaluation of dynamic changes in RANTES concentrations in response to infection and other stimulating factors faces a lot of difficulties, not only methodological, but mainly ethical, both in term and preterm neonates. Thorough knowledge of interrelations between them should, in each case, take into account the individual good of a neonate rather than knowledge for knowledge's sake. This fact can explain the low number of studies concerning RANTES evaluation in the diagnosis of neonatal infections, despite the fact that according to the studies done so far, it seems be a very promising biomarker for early-onset infection. It seems especially difficult to determine whether RANTES synthesis and their mutual relations are developed in preterm neonates well enough to consider their common use in the diagnosis of infections. This requires further research.

Conclusion

The significant increase of serum RANTES concentration in early-onset infections in neonates, regardless of their gestational age, sex and birth asphyxia, not only proves the presence of an active immunological process but also may be a useful biomarker for diagnosis of severe neonatal infections.

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