0021-7557/10/86-06/509
Jornal de Pediatria

Early-onset neonatal sepsis: cord blood cytokine levels at diagnosis and during treatment

Dulcimar P. Campos,[1] Marcos V. Silva,[2] Juliana R. Machado,[3] Lúcio R. Castellano,[4] Virmondes Rodrigues,[5] Cristina H. C. Barata[6]

Abstract

Objective: To assess clinical and laboratory parameters and serum cytokine levels in 55 neonates who developed early-onset sepsis.

Methods: Clinical parameters associated with early-onset neonatal sepsis were assessed. White blood cell differential and serum C-reactive protein and glucose levels were measured upon diagnosis of sepsis and 48 hours later. IL-1β, IL-10, IL-6, and TNF-α levels were measured in cord blood samples obtained on the day of diagnosis and from samples collected 48 and 96 hours after treatment onset.

Results: Among newborns with early-onset sepsis, the length of hospital stay was inversely correlated with birth weight. Clinical parameters varied widely, especially body temperature. Blood glucose changes – particularly hypoglycemia – were common. Leukopenia, usually due to neutropenia, was the most prevalent change in blood cell count. C-reactive protein levels correlated with the immature-to-total neutrophil ratio. Serum TNF-α and IL-10 levels measured early in the course of sepsis were positively correlated with those detected in cord blood.

Conclusions: Clinical and laboratory parameters varied widely among neonates with sepsis in this sample. In neonates who presented with increased cytokine levels at birth, this abnormality persisted throughout the infectious process.

J Pediatr (Rio J). 2010;86(6):509-514: Early-onset sepsis, neonates, cytokines, TNF-α, IL-10.

Introduction

Sepsis is one of the most common infectious conditions in the neonatal period, and remains a major source of morbidity and mortality despite extraordinary progress in the field of neonatology in recent years. According to the World Health Organization (WHO), approximately 5 million neonatal deaths occur each year worldwide, 98% of which in least developed and developing countries.[1]

Early-onset neonatal sepsis usually occurs in the first 72 hours of life, with 80 to 90% of cases presenting up to 48 hours after birth, and, in the absence of compelling

1. Mestre, Medicina Tropical e Infectologia. Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, MG, Brazil. Médica Pediatra, Unidade de Terapia Intensiva Pediátrica e Neonatal, Hospital das Clínicas, UFTM, Uberaba, MG, Brazil.
2. Mestre, Medicina Tropical e Infectologia. UFTM, Uberaba, MG, Brazil.
3. Mestre, Patologia Geral. UFTM, Uberaba, MG, Brazil.
4. Mestre, Medicina Tropical e Infectologia. UFTM, Uberaba, MG, Brazil. Professor, Escola Técnica de Saúde, Universidade Federal da Paraíba (UFPB), João Pessoa, PB, Brazil.
5. Professor titular, Disciplina de Imunologia, UFTM, Uberaba, MG, Brazil.
6. Professora, Disciplina de Doenças Infecciosas e Parasitárias, UFTM, Uberaba, MG, Brazil.

No conflicts of interest declared concerning the publication of this article.

Financial support: CAPES, CNPq e FUNEPU.

Suggested citation: Campos DP, Silva MV, Machado JR, Castellano LR, Rodrigues V, Barata CH. Early-onset neonatal sepsis: cord blood cytokine levels at diagnosis and during treatment. J Pediatr (Rio J). 2010;86(6):509-514.

Manuscript received Jul 22 2010, accepted for publication Sep 17 2010.

doi:10.2223/JPED.2043

evidence to suggest otherwise, is considered due to infection of maternal origin.[2] Onset is usually insidious, with markedly nonspecific signs and symptoms often mistaken for those of typical neonatal conditions, such as transient tachypnea of the newborn, thermal dysregulation due to environmental factors, apnea of prematurity, and acute exacerbations of bronchopulmonary dysplasia. Studies have also showed that, for every truly septic newborn, many others with no confirmed diagnosis of infection receive treatment based on clinical suspicion alone.[3,4]

Blood cultures are the gold standard for diagnosis of sepsis; however, positivity rates vary widely, ranging from 30 to 87%.[5] Physicians must therefore rely on a variety of nonspecific laboratory tests to assist in and perhaps speed diagnosis of sepsis. Increased C-reactive protein (CRP) levels have also proved useful as a sepsis biomarker in several studies, although the negative predictive value and sensitivity of CRP testing are not enough to allow definitive diagnosis of the condition.[6,7]

Over the past few years, cytokines have been widely studied as reliable markers of neonatal infection. IL-6 has been associated with maternal chorioamnionitis and used to provide early diagnosis of early-onset neonatal sepsis when high levels are detected in cord blood.[8] Increased TNF-α and IL-1β levels are apparently associated with greater disease severity, although some studies have failed to confirm any correlation.[9,10] When combined with IL-6 measurement, sensitivity may be as high as 98.5%.[11] IL-1β has also been described and used as an isolated marker of neonatal sepsis, although it is inferior to IL-6 and TNF-α in diagnostic accuracy.[12] IL-10 has also been associated with septic shock in adults and children alike. High levels correlate with poor prognosis in sepsis, and may be a predictive factor of septic shock severity and death.[13] In inflammatory conditions, IL-10 inhibits TNF-α activity and the restoration of homeostasis.[14] Achieving normal IL-10 levels is key to the treatment of these conditions, as higher levels also favor progression to multiple organ dysfunction syndrome.[15] Research has shown that adequate IL-10 response may protect against systemic inflammatory response syndrome (SIRS) and that elevated IL-6/IL-10 ratio is associated with poor prognosis.[16]

The objective of this study was to assess cord blood cytokine levels at diagnosis of early-onset neonatal sepsis and after the onset of therapy.

Methods

The study sample comprised all 55 neonates admitted to the Hospital das Clínicas da Universidade Federal do Triângulo Mineiro (UFTM) Neonatal/Pediatric Intensive Care Unit between March 2008 and January 2009 who presented with clinical or laboratory evidence of sepsis in the first 72 hours of life, survived at least 7 days after birth, and did not meet any of the exclusion criteria. Sepsis was defined by the following criteria: SIRS in the presence of suspected or proven infection (in full-term neonates)[17] or risk factors such as clinical signs and laboratory tests (for premature newborns).[18] Antibiotic therapy was immediately begun upon diagnosis of sepsis and was discontinued after clinical improvement was detected and relevant laboratory values were within normal limits. Although neonates were not receiving antibiotics when the first blood samples were collected (on the day of diagnosis), all were receiving antimicrobial therapy when further assessments were conducted at 48 and 96 hours after diagnosis. Exclusion criteria were defined as those capable of interfering with clinical and laboratory findings, particularly those associated with SIRS: perinatal asphyxia; Apgar scores < 5; signs of hypoxia in the first 72 hours of life; maternal infection, positive serological testing, or corticosteroid use; rupture of membranes more than 6 hours before delivery; and presence of congenital malformations.

Clinical parameters assessed included gender, birth weight, delivery type, and newborn heart rate, respirations, peripheral perfusion, body temperature, oxygen saturation, and activity. Laboratory tests included blood glucose, CRP level, complete blood count with differential, blood cultures, and serum cytokine assay.

Cord blood samples were obtained from all neonates at delivery, at the time sepsis was diagnosed, and 48 and 96 hours after diagnosis. Blood samples were collected into an EDTA-containing tube and partially used for blood counts. Serum was separated for quantitative CRP measurement using a standard biochemical kit, according to manufacturer instructions (Human do Brasil, Itabira, Brazil) and blood glucose measurement with an Accu-Chek® Performa glucose meter (Roche Diagnostics, USA). The remainder of the sample was kept frozen at -70 ºC for later cytokine measurement.

Automated complete blood counts were performed using samples collected on the day of diagnosis and 48 hours post diagnosis. CRP and blood glucose levels were measured by quantitative methods. Blood cultures were performed in brain heart infusion (BHI) media, using at least 1 mL of blood collected on the day sepsis was diagnosed.

IL-6, IL-10, IL-1β, and TNF-α levels were determined by ELISA (BD Pharmingen, USA), following the protocol recommended by Pissetti et al.[19]

Statistical analysis

Data were analyzed with the Microsoft Office Excel 2007 for Windows (Microsoft Corp., Redmond, WA, USA) and StatView (Abacus Concepts, Berkeley, CA, USA) software programs. Nominal variables were analyzed when necessary by chi-square testing, whereas continuous variables were assessed with the Mann-Whitney U and repeated measures

ANOVA as appropriate. Correlations were assessed with Spearman's rank test. The significance level was set at $p < 0.05$.

Ethical aspects

This study was approved by the UFTM Research Ethics Committee with protocol number 1146. The legal guardians of all newborns included in the study sample provided written informed consent.

Results

Of the 55 neonates in the study sample, 28 (51%) were male. Mean maternal age was 23.7 years. Length of hospital stay for the neonates ranged from 5 to 285 days (mean ± SD, 39.9 ± 5.4 days).

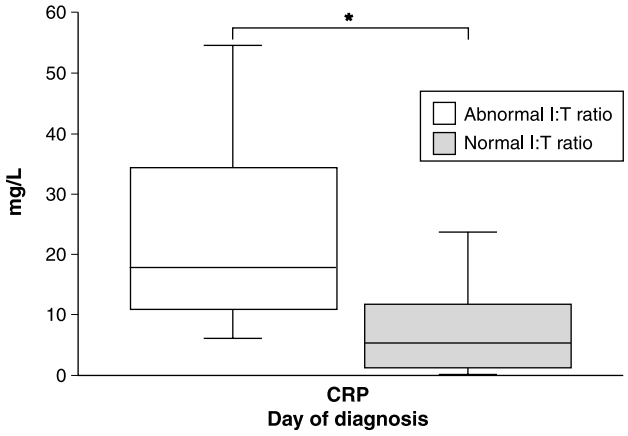
Most neonates (85.3%) were preterm (gestational age < 37 weeks). Only 14% had normal birth weight (> 2,500 g), 22% had low birth weight (1,500 to 2,500 g), 51% had very low birth weight (1,000 to 1,500 g), and 14% were extremely low birth weight infants (< 1,000 g).

Clinical parameters varied widely among the study sample on the day of diagnosis. Hypothermia and hyperthermia were present in 33 and 31% of neonates respectively, and bradycardia and tachycardia in 5 and 11% respectively. Peripheral perfusion and O_2 sats were normal in 67 and 78% of neonates respectively, whereas 94% showed a decreased level of activity.

On laboratory assessment, blood glucose changes were found in 99% of neonates, with hypoglycemia the most common abnormality (58%). Blood cultures were positive in a mere six neonates (10.9%). Organisms isolated included *Klebsiella pneumoniae* in four and *Enterobacter cloacae* and *Staphylococcus* sp. in one patient each.

The most common white blood cell change on the day of diagnosis was leukopenia (54.5%). Neutropenia was the most prevalent finding on differential (52%). After 48 hours of treatment, white blood cell counts were quite similar, with the only significant difference being an increased percentage of leukopenic neonates (69%), most likely indicating progression of sepsis. Furthermore, total white blood cell counts were associated with neutropenia; leukopenic patients had statistically lower absolute neutrophil counts than non-leukopenic neonates on the day of diagnosis and at 48 hours from treatment onset ($p < 0.0001$ and $p = 0.0009$ respectively).

Although only 12.7% of neonates had an increased immature-to-total neutrophil (I:T) ratio, changes in this ratio were accompanied by changes in CRP levels (Figure 1). Furthermore, a positive correlation was detected between both on the day of diagnosis (Spearman's rank correlation; $r = 0.3$, $p = 0.029$) and 48 hours after the start of treatment (Spearman's rank correlation; $r = 0.31$, $p = 0.022$).



CRP = C-reactive protein;
I = T ratio, immature-to-total neutrophil ratio.
* Mann-Whitney U ($p < 0.05$).

Figure 1 - C-reactive protein levels in neonates with normal and increased I:T ratio

Serum cytokine levels were assessed at different points in time in all septic neonates in the sample. Measurements were obtained at the time of delivery (from cord blood) and from peripheral blood samples collected on the day of sepsis diagnosis and 48 and 96 hours after treatment onset. There were no statistically significant differences in cytokine levels over time, although IL-10 and TNF-α levels behaved in a broadly similar manner, with little change in mean levels on the various days of sampling (Figure 2A and 2B); likewise, IL-1β and IL-6 levels showed a downward trend over the first 48 hours, followed by an increase at 96 hours post treatment onset (Figures 2C and 2D). Interestingly, cord blood TNF-α and IL-10 levels correlated with those measured on the day of diagnosis ($r = 0.71$, $p < 0.001$ and $r = 0.55$, $p < 0.001$ respectively), at 48 hours ($r = 0.55$, $p < 0,001$ and $r = 0.42$, $p = 0.026$ respectively) and at 96 hours of treatment onset ($r = 0.5$, $p = 0.004$ and $r = 0.48$, $p = 0.006$ respectively). There were no statistically significant differences in cytokine levels between preterm and full-term neonates, nor was any difference associated with Cesarean versus vaginal delivery. In another important finding, no differences in cytokine levels occurred in association with birth weight at any point in the study.

Discussion

In the present study, cases of early-onset sepsis were distributed equally among male and female newborns. The existing literature suggests that male gender predisposes to development of neonatal sepsis.[20] Mean maternal age in our sample was 23 years. A study by Goulart et al.[21] found a 1.5-fold risk of sepsis in neonates born to mothers under the age of 25. Research has also showed that very low birth weight infants are more susceptible to sepsis,[22]

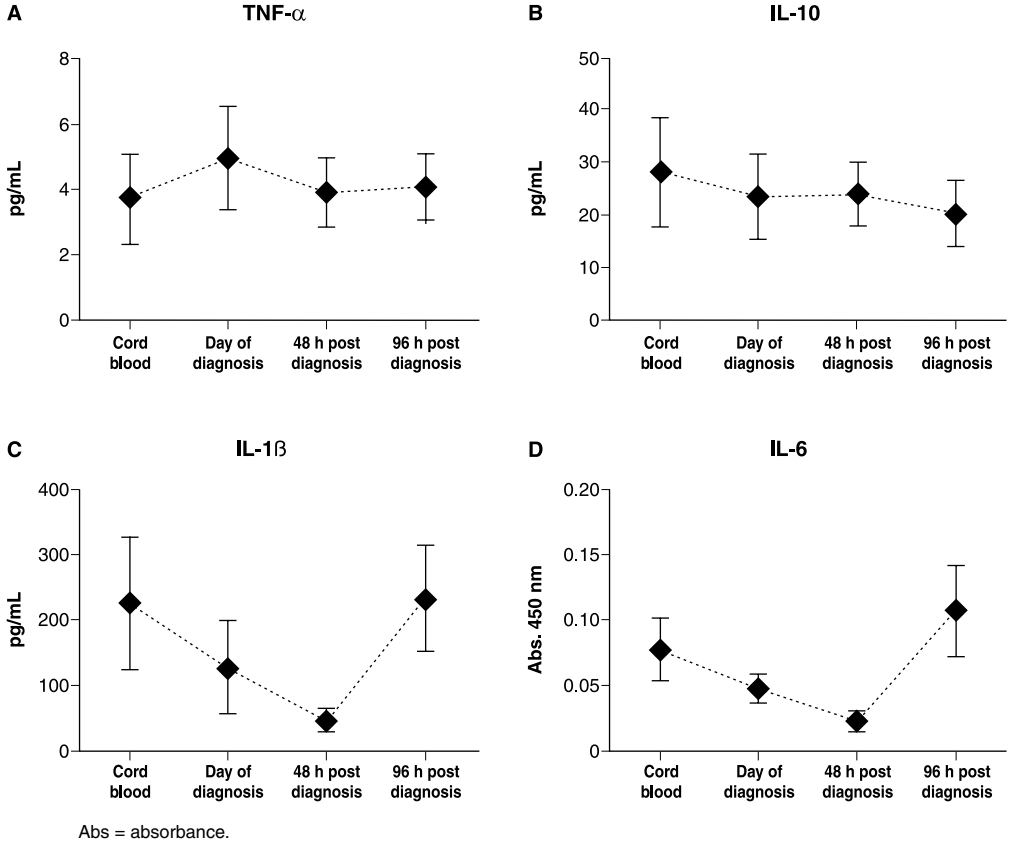


Figure 2 - Serum cytokine levels at birth, on diagnosis, and 48 and 96 hours after treatment onset: A) TNF-α, B) IL-10, C) IL-1β, D) IL-6. Diamonds represent mean values, and whiskers, the standard error. Repeated measures ANOVA

which stresses the potential nature of this condition as an aggravating factor for sepsis. Studies have shown that very low birth weight infants are at a 25-fold risk of developing sepsis when compared with normal birth weight infants.[23]

Despite extensive efforts to isolate causative pathogens, blood cultures are, on average, positive in only 34% of "septic" patients; positivity ranges wildly, from 9 to 64%.[24] Administration of antimicrobial therapy to all preterm neonates or those with clinical evidence of sepsis is part of our unit's treatment protocols, and this may account for the low culture positivity rates reported.

Most neonates in our sample had blood glucose changes, with hypoglycemia being most prevalent. Waeschle et al. [25] made a major contribution to the association between poor prognosis in septic patients and blood glucose changes, as well as the role of fluctuations between hypoglycemia and hyperglycemia during sepsis as predisposing factors for death. Among white blood cell count changes, neutropenia is the most reliable predictor of neonatal sepsis; it reflects the severity of sepsis and represents depletion of neutrophil reserves, and requires specific therapeutic measures for management.[26]

CRP has been used as an important early indicator of sepsis development, and declining CRP levels in the presence of clinical improvement is used as a parameter for discontinuing antibiotic therapy in our service, as has been described elsewhere in the literature.[27] Sabel & Wasworth[28] found that 85% of newborns diagnosed with sepsis and meningitis had positive C-reactive protein levels in the first hours after onset of clinical signs of infection, suggesting a strong correlation between presence (and rapid synthesis) of CRP and infection. Furthermore, they found that 16 of a sample of 18 septic newborns (89%) with a gestational age of 27 to 36 weeks had positive C-reactive protein.

Like CRP, the immature-to-total neutrophil ratio (I:T ratio) has been suggested as a good indicator of neonatal

sepsis.[18] We found an interesting association between CRP levels and I:T ratio: the former were statistically higher in neonates with abnormal I:T ratios, whether on the day of diagnosis or 48 hours after treatment on set. If, on the one hand, this shows that the pathological changes in some septic neonates affect both indicators, it also shows that neonates with a normal I:T ratio will have lower CRP values, thus hampering the use of both tests.

Cytokines, particularly IL-6, TNF-α, IL-1β, have been studied as reliable markers of neonatal infection. Due to their systemic inflammatory nature, attempts have been made to correlate increased proinflammatory cytokine or decreased anti-inflammatory cytokine levels with the clinical course of sepsis. Although we did not detect changes in cytokine levels during the study period in our sample, neonates with the highest cytokine levels during full-blown sepsis already had increased levels in cord blood, and had persistently high levels of IL-10 and TNF-α up to 96 hours after diagnosis.

In a multicenter trial to assess inflammatory markers in neonatal sepsis, Küster et al.[29] concluded that IL-1RA and IL-6 levels were already elevated 2 days prior to onset of sepsis manifestations, and that changes were detectable as early as 48 hours before diagnosis. A study conducted by Silveira & Procianoy[11] showed that combined IL-6 and TNF-α testing had a sensitivity of 98.5% and a negative predictive value of 90%, thus providing a high likelihood of accurate diagnosis (or exclusion of suspected) infection. Ceccon[30] note that IL-6 was superior to CRP and IL-8 on the day of diagnosis, whereas CRP was most accurate at 24 hours post diagnosis.

Most studies aim to establish and detect ever-earlier indicators of sepsis, although there is still no consensus on the real-world applicability of these signs. Routine cytokine assays entail increased cost; furthermore, there are no reliable established reference ranges, as cytokine levels vary widely from person to person and are highly influenced by external factors.

The substantial heterogeneity in cytokine production at delivery found in the septic neonates in our sample may pose a significant challenge to the use of cytokine levels as reliable diagnostic markers, as it suggest that not all septic neonates will meet a definite clinical picture of low or high cytokine levels. On the other hand, measurement of circulating cytokine levels in cord blood showed that neonates with early-onset sepsis already display immune response behavior at delivery, which suggests that the causative infection may already be underway at birth.

Acknowledgements

The authors would like to thank UFTM, The Neonatal and Pediatric ICU Service at the Hospital de Clínicas da UFTM, and the Graduate Program in Tropical Medicine and Infectious Diseases.

References

1. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110:285-91.
2. Haque KN. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med. 2005;6:S45-9.
3. Da Silva O, Ohlsson A, Kenyon C. Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review. Pediatr Infect Dis J. 1995;14:362-6.
4. Ng PC. Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed. 2004;89:F229-35.
5. Gerdes JS. Diagnosis and management of bacterial infections in the neonate. Pediatr Clin North Am. 2004;51:939-59, viii-ix.
6. Gerdes JS. Clinicopathologic approach to the diagnosis of neonatal sepsis. Clin Perinatol. 1991;18:361-81.
7. Caldas JP, Marba ST, Blotta MH, Calil R, Morais SS, Oliveira RT. Accuracy of white blood cell count, C-reactive protein, interleukin-6 and tumor necrosis factor alpha for diagnosing late neonatal sepsis. J Pediatr (Rio J). 2008;84:536-42.
8. Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, et al. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. Am J Obstet Gynecol. 2000;183:1124-9.
9. Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet. 1987;1:355-7.
10. Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest. 1993;103:565-75.
11. Silveira RC, Procianoy RS. Evaluation of interleukin-6, tumour necrosis factor-alpha and interleukin-1beta for early diagnosis of neonatal sepsis. Acta Paediatr. 1999;88:647-50.
12. Meadow W, Rudinsky B. Inflammatory mediators and neonatal sepsis. Rarely has so little been known by so many about so much. Clin Perinatol. 1995;22:519-36.
13. Gogos CA, Drosou E, Bassaris HP, Skoutelis A. Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options. J Infect Dis. 2000;181:176-80.
14. Bone RC, Grodzin CJ, Balk RA. Sepsis: a new hypothesis for pathogenesis of the disease process. Chest. 1997;112:235-43.
15. Doughty L, Carcillo JA, Kaplan S, Janosky J. The compensatory anti-inflammatory cytokine interleukin 10 response in pediatric sepsis-induced multiple organ failure. Chest. 1998;113:1625-31.
16. Taniguchi T, Koido Y, Aiboshi J, Yamashita T, Suzaki S, Kurokawa A. Change in the ratio of interleukin-6 to interleukin-10 predicts a poor outcome in patients with systemic inflammatory response syndrome. Crit Care Med. 1999;27:1262-4.
17. Goldstein B, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005;6:2-8.
18. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr. 1988;112:761-7.
19. Pissetti CW, Correia D, Braga T, Faria GE, Oliveira RF, Ribeiro BM, et al. Associação entre os níveis plasmáticos de TNF-α, IFN-γ, IL-10, óxido nítrico e os isotipos de IgG específicos nas formas clínicas da doença de Chagas crônica. Rev Soc Bras Med Trop. 2009;42:425-30.

20. Oddie S, Embleton ND. Risk factors for early onset neonatal group B streptococcal sepsis: case-control study. BMJ. 2002;325:308.
21. Goulart AP, Valle CF, Dal-Pizzol F, Cancelier AC. Fatores de risco para o desenvolvimento de sepse neonatal precoce em hospital da rede pública do Brasil. Rev Bras Ter Intensiva. 2006;18:148-53.
22. Stoll BJ. The global impact of neonatal infection. Clin Perinatol. 1997;24:1-21.
23. Benitz WE. Perinatal treatment to prevent early onset group B streptococcal sepsis. Semin Neonatol. 2002;7:301-14.
24. Carvalho PR, Trotta Ede A. Avanços no diagnóstico e tratamento da sepse. J Pediatr (Rio J). 2003;79 Suppl 2:S195-204.
25. Waeschle RM, Moerer O, Hilgers R, Herrmann P, Neumann P, Quintel M. The impact of the severity of sepsis on the risk of hypoglycaemia and glycaemic variability. Crit Care. 2008;12: R129.
26. Silveira RS, Procianoy RS. Sepse neonatal precoce: diagnóstico e conduta. PRORN - ciclo 2, módulo 3. Porto Alegre: Artmed/ Panamericana Editora; 2003.
27. Bomela HN, Ballot DE, Cory BJ, Cooper PA. Use of C-reactive protein to guide duration of empiric antibiotic therapy in suspected early neonatal sepsis. Pediatr Infect Dis J. 2000;19:531-5.
28. Sabel KG, Wadsworth C. C-reactive protein (CRP) in early diagnosis of neonatal septicemia. Acta Paediatr Scand. 1979;68:825-31.
29. Küster H, Weiss M, Willeitner AE, Detlefsen S, Jeremias I, Zbojan J, et al. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet. 1998;352:1271-7.
30. Ceccon ME. Análise do uso das interleucinas 6 e 8 e proteina C reativa para diagnóstico e seguimento terapeutico de recem-nascidos com sepse tardia internados na unidade de cuidados intensivos neonatais [tese livre docência]. São Paulo: Universidade de São Paulo; 2002.

Correspondence:
Juliana R. Machado
Getúlio Guaritá, s/nº
CEP 38025-440 – Uberaba, MG – Brazil
Tel.: +55 (34) 3318.5299
Fax: +55 (34) 3318.5651
E-mail: julianareismachado@yahoo.com.br