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Inflammatory and oxidative cord blood parameters as predictors of neonatal sepsis severity

Marcadores inflamatórios e oxidativos em sangue de cordão umbilical como preditores de gravidade em sepse neonatal

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ABSTRACT

Objectives: Neonatal sepsis is a complex syndrome involving an uncontrolled systemic inflammatory response associated with an infection. It may result in the dysfunction or failure of one or more organs or even death. Given its high incidence in premature neonates, the identification of prognostic factors to optimize the early diagnosis and therapeutic interventions are highly desirable. This objective study determine the relationship between inflammatory markers and oxidative parameters as prognostic factors in early neonatal sepsis.

Methods: We conducted a prospective observational study by collecting data from 120 patients in the maternity unit of a university hospital. Preterm (<37 weeks of pregnancy) infants with at least one additional risk factor for neonatal sepsis were included. The levels of interleukin (IL)-6, IL-10, thiobarbituric acid reactive species (TBARS) and protein carbonyls and their association with sepsis severity were determined in the cord blood.

Results: Levels of IL-6 and TBARS, but not IL-10 and protein carbonyls, demonstrated a mild to moderate correlation with the SNAPPE-II severity score (r=0.435, p=0.02 and r = 0.385, p = 0.017, respectively). No correlations were found between these markers and mortality.

Conclusion: TBARS and IL-6 have a mild to moderate correlation with SNAPPE-II, but none of the studied markers were able to predict mortality in our sample.

Keywords: Sepsis/blood; Infant, newborn; Fetal blood/chemistry; Intensive care units, neonatal; Oxidative stress; Interleukin-6; Interleukin-10

INTRODUCTION

Sepsis is a complex syndrome related to a systemic inflammatory response with multiple manifestations that may cause the dysfunction or failure of one or more organs or even death.[1] Early neonatal sepsis occurs within the first six days of life, whereas late neonatal sepsis occurs after the first week of life (>6 days). Differentiating early from late neonatal sepsis is clinically important because in early neonatal sepsis, the infectious organisms are acquired during the delivery, whereas in late sepsis the infecting organisms are mostly acquired after birth, from either hospital or community sources.[2,3]

The identification of patients who are at increased risk is challenging in critically ill populations.[4,5] Most of the clinical decisions in caring for patients with severe sepsis are based on clinical and laboratory data,[4] which are often not very accurate. In addition, the pathophysiology of systemic inflammation

could be better understood; therefore, identifying more accurate predictors of severity is important.[4,6]

Several parameters have been suggested to be diagnostic of early sepsis, including the levels of some cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10), tumor necrosis factor (TNF) and its soluble receptor (TNF receptor), acute phase proteins (C-reactive protein) and procalcitonin.[7-9] We have recently shown a correlation between oxidative parameters and the development of neonatal sepsis;[10] however, the medical literature has little information on the early predictors of severity in neonatal sepsis. Therefore, this study aimed to determine the correlation between inflammatory and oxidative parameters in cord blood and the severity of early neonatal sepsis.

METHODS

This cross-sectional case-control, retrospective study collected cord blood from 120 consecutive premature newborns in the maternity ward of Hospital Nossa Senhora da Conceição, Tubarão, SC, Brazil, from February to September, 2005, as previously described.[10] The data were primarily collected to determine the correlation of biomarkers with the diagnosis of neonatal sepsis.[10] For the present study, the data from the original study were used. This study was approved by the ethics committee of Universidade do Extremo Sul Catarinense - UNESC, under the protocol number 168/2005.

Samples collection

Cord blood samples were collected by pressing the blood from the umbilical cord into a glass tube after the delivery and prior to discarding the placenta. The samples were centrifuged at 1000 *g* for 10 minutes, and liquid nitrogen was added. The samples were stored at -80ºC until the analysis.

Inclusion and exclusion criteria

Premature neonates (<37 weeks of pregnancy) with at least one additional risk factor for neonatal sepsis (premature rupture of membranes >18 hours before delivery, maternal colonization with group B streptococci, intrapartum fever with an axillary temperature above 37.5ºC, or chorioamnionitis) were included. Some of the parameters assessed in this study may be increased in children born to mothers with preeclampsia and/or diabetes and in babies with restricted intrauterine growth. Therefore, these cases were excluded.

Diagnosis of sepsis

Based on the *2002 Consensus Conference on Pediatric Sepsis: definitions for sepsis and organ dysfunction in pediatrics*, sepsis was considered to be proven when a blood culture or culture from a normally sterile site was positive for a likely pathogen, and sepsis was considered to be likely if the bacterial cultures were negative but a clinical and biological infection-associated syndrome was present.

The clinical and biological evidence of sepsis includes the following: hyper-leukocytosis, defined as a blood leukocyte count >25,000/mm^3; leukopenia, defined as a blood leukocyte count < 4,000/mm^3; neutropenia, defined as a blood neutrophil count < 1,300/mm^3; thrombocytopenia, defined as a platelet count < 150,000/mm^3; hyperglycemia, defined as blood glucose > 7 mmol/L and a C-reactive protein > 20 g/L. Forty children met the inclusion criteria (proven sepsis, n = 20; likely sepsis, n = 20) and were included in the sepsis group.

The severity of sepsis was determined based on five variables measured at the time of diagnosis: C-reactive protein, lactate, bicarbonate, blood glucose and SNAPPE-II severity score.[11] In addition, the patients were followed until death or discharge from the ICU to determine the mortality rate in the intensive care unit.

Inflammatory and oxidative parameters in cord blood

Oxidative stress was determined based on the levels of thiobarbituric acid reactive substances (TBARS) and protein carbonyl. To measure the TBARS, the samples were mixed with 1 mL 10% trichloroacetic acid and 1 mL 0.67% thiobarbituric acid (Sigma, St. Louis, MO) and then heated in a boiling water bath for 15 minutes. Malondialdehyde equivalents were determined by the absorbance at 535 nm, using 1,1,3,3-tetramethoxypropane (Sigma, St. Louis, MO) as the external standard. BHT was added to the samples to prevent malondialdehyde production during the analysis.[12] The results were expressed as malondialdehyde equivalents (nmol) per protein milligram.

Oxidative protein damage was assessed by the determination of carbonyl groups based on the dinitrophenylhydrazine reaction. In summary, proteins were precipitated by adding trichloroacetic acid 20% and dissolved in dinitrophenylhydrazine (Sigma, St. Louis, MO). Absorbance was read at 370 nm,[13] and the results are shown as nmol/mg protein.

The analysis of inflammatory parameters was

performed using commercial enzymatic immunosorbent kits for the detection of IL-6 and IL-10 (Quantikine, R & D Systems Europe, Abingdon, UK); the results are expressed as pg/mg protein.

The correlation between the parameters and mortality was performed using the Student's t test. The correlation between the parameters was assessed with Pearson's correlation. The normal distribution of the variables was tested with the Kolmogorov-Smirnov test. P values of 0.05 or less were considered statistically significant. The sample's statistic power was calculated *a posteriori* (as the study was originally designed for a different purpose). A sample with 10 patients in the non-surviving group, with an alpha = 0.05 and 0.80 power, would allow the detection of a mean 20% difference, projecting a sigma of 0.15 for the assessed parameters.

RESULTS

A total of 40 patients were included, 20 with proven neonatal sepsis and 20 with likely neonatal sepsis. Of these patients, 30 patients survived and 10 died. The relevant clinical variables have been previously published.[10]

The TBARS level had a mildly significant correlation with the SNAPPE-II severity score (r = 0.385 / p = 0.017) but not with the serum blood glucose (r = 0.288 / p = 0.08), C-reactive protein (r = 0.027 / p = 0.87), lactate (r = -0.233 / p = 0.49) or bicarbonate (r = 0.10 / p = 0.95). IL-6 had a moderate correlation with the SNAPPE-II severity score (r = 0.435 / p = 0.02) but not with bicarbonate (r = 0.341 / p = 0.09), C-reactive protein (r = 0.124 / p = 0.21), lactate (r = 0.221 / p = 0.25) or blood glucose (r = 0.12 / p = 0.32). No significant correlation was found between carbonylated protein levels and any of the assessed severity parameters, as was found for the IL-10 levels.

None of the assessed parameters were associated with mortality in these patients (Figure 1).

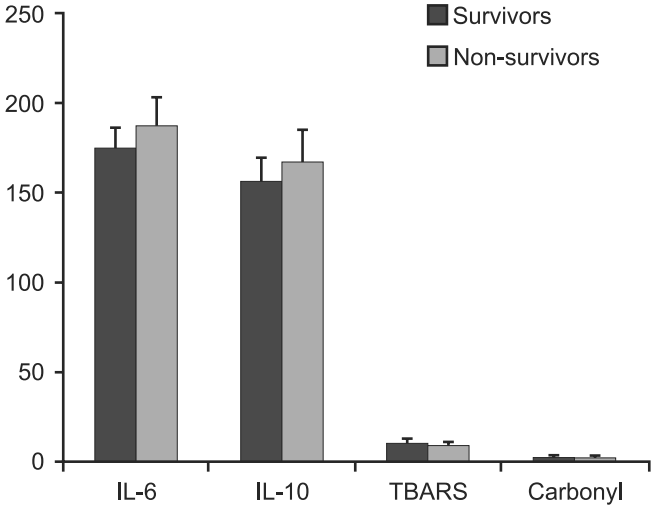

Figure 1 - Levels of Inflammatory and oxidative stress markers collected from the cord blood and their correlation with mortality in patients with neonatal sepsis.

IL-6: interleukin-6; IL-10: interleukin-10; TBARS - thiobarbituric acid reactive species.

DISCUSSION

The quest for an ideal marker that is highly sensitive and specific is a challenge for healthcare professionals. This study was unable to determine a relevant role for the assessed parameters in terms of prognosis and severity in patients with early neonatal sepsis. Although a significant correlation was found for IL-6 and TBARS with the SNAPPE-II score, this correlation was mild to moderate, and none of the markers were able to predict the patients' outcome.

Early markers of neonatal sepsis have been studied in recent years, and cord blood interleukins are promising. Increased cord blood levels of IL-6 and IL-1β were neither able to confirm nor preclude, with a high level of confidence, neonatal sepsis.[10,14-17] Recently, an algorithm including IL-6 levels was shown to be highly effective in identifying newborns at a high probability of developing early neonatal sepsis.[18] IL-6 is considered an important systemic inflammatory response marker and is characterized as an "alarm" cytokine as its serum concentrations quickly peak during infections. IL-6 levels increases up to two to four days before symptoms manifest, and its levels remain stable during the first hours, dropping 24 hours after sepsis is established.[17] Maternal IL-6 levels are able to predict premature delivery and unfavorable outcomes in this population, especially intraventricular hemorrhage.[19] Although cord blood IL-6 is apparently promising as a diagnostic marker,[10] our results do not support its use as an outcome predictor. As some of the parameters have up to a moderate correlation with classical severity markers, they may be used to anticipate the disease severity even before outright clinical or blood chemistry signs of infection.

IL-10 is the main negative regulator of the innate immune response. It acts as an inflammatory cytokine by negative feedback, i.e., by inhibiting proinflammatory cytokine synthesis. High IL-10 concentrations reduce monocyte production of TNF-α, IL-6 and IL-8 while activating IL-1Ra production (positive feedback). IL-

10 suppression results in increased serum TNF-α and IL-6 levels, suggesting that this cytokine is highly anti-inflammatory; indeed, when endogenous IL-10 is neutralized by monoclonal antibodies, mortality is increased.[14,16,17] The magnitude of the IL-10 response apparently correlates with the severity of the inflammatory process and the concentration of proinflammatory cytokines, leading to TNF-α activation. Increased IL-10 and IL-6 levels in a newborn are indicative of an infection, and IL-10 remains high for 48 hours after the infection manifests itself.[18] However, our results do not point to a role of cord blood IL-10 determination as a prognostic marker in neonatal sepsis.

Additionally, sepsis promotes the unbalanced production of oxidant and anti-oxidant substances, causing an excess of free oxygen radicals. These molecules are meant to destroy the infective agent but are not specific substances and may instead harm the patient.[20] The production of reactive oxygen species (ROS) is increased during sepsis, which may lead to tissue damage.[21] The production of ROS during neonatal sepsis has been documented, with an evident imbalance between pro- and anti-oxidants,[10,20,22] and the levels of these molecules may be related to the development of neonatal sepsis.[10] Notwithstanding, we were unable to demonstrate a relationship between the severity of neonatal sepsis and oxidative stress markers.

The interpretation of our results should take into consideration some limitations. We had no information on the time between the delivery and the first signs of sepsis. As these parameters can change quickly, this timing may have influenced the interpretation of our results. Second, this sample included patients with both proven and likely sepsis; therefore, the results should be analyzed in the light of this limitation. As we have previously shown,[10] the studied population had significant differences in birth weight and one-minute APGAR scores; therefore, this is a heterogeneous population, which may have influenced the results. Third, our sample had the power to detect a 20% difference in the assessed parameters; because the differences detected were below this level, this sample size could explain our negative results in terms of the outcome. However, we believe that changes less than 20% in these parameters lack biological meaning; therefore, we consider this limitation to be of a minor importance.

CONCLUSION

Thiobarbituric acid reactive substances and IL-6 have mild and moderate correlations with the SNAPPE-II severity score, respectively, but not with mortality. In this context, these factors can be early warning markers of severity but are not useful for determining prognosis, even before the manifestation of the infection.

RESUMO

Objetivos: Sepse neonatal corresponde a uma síndrome complexa, causada por resposta inflamatória sistêmica descontrolada, associada a um foco infeccioso que pode determinar disfunção ou falência de um ou mais órgãos ou mesmo a morte. Apresenta incidência elevada em neonatos prematuros, sendo importante correlacionarmos fatores prognósticos para otimizar nosso diagnóstico precoce e resposta a terapêutica nestes pacientes. Este estudo teve por objetivo determinar a relação entre marcadores inflamatórios e parâmetros oxidativos com fatores prognósticos em sepse neonatal precoce.

Métodos: Foi realizado um estudo observacional, prospectivo e foram coletados os dados de 120 pacientes, da maternidade de hospital universitário. Foram incluídos na pesquisa neonatos prematuros (< 37 semanas de gestação) com pelo menos um outro fator de risco para sepse neonatal. Foram determinados os níveis de interleucina (IL)-6, IL-10, substâncias reativas ao ácido tiobarbitúrico e de proteínas carboniladas em sangue do cordão umbilical e sua relação com gravidade de sepse.

Resultados: Os níveis das substâncias reativas ao ácido tiobarbitúrico e IL-6, mas não IL-10 e proteínas carboniladas, apresentaram correlação significativa com o escore de gravidade SNAPPE-II (r=0,385, p=0,017 e r=0,435 / p=0,02, respectivamente). Não houve relação dos marcadores com a mortalidade dos pacientes.

Conclusão: Substâncias reativas ao ácido tiobarbitúrico e IL-6 têm uma correlação de média a moderada com o escore de gravidade SNAPPE-II, mas não com mortalidade.

Descritores: Sepse/sangue; Recém-nascido; Sangue fetal/química; Unidades de terapia intensiva neonatal; Estresse oxidativo; Interleucina-6; Interleucina-10

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