The Utility of Serum Hepcidin as a Biomarker for Late-Onset Neonatal Sepsis

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Objective To assess the utility of hepcidin, a potent regulator of host defense and inflammation, in the diagnosis of late-onset sepsis in very low birth weight infants.

Study design We compared the diagnostic performance of hepcidin with C-reactive protein from the serum concentrations in acute and convalescent blood specimens obtained from 44 infants suspected of late-onset sepsis. The predictive accuracies were assessed from the areas under receiver operating characteristic curves and the cutoffs that differentiated infants with and without sepsis were identified using classification and regression tree analysis.

Results Seventeen of the enrolled infants in this study were bacteremic and/or received antibiotics for neonatal sepsis for ≥5 days (infants with sepsis). The concentrations of hepcidin were increased 4-fold in infants with compared with infants without sepsis ($P < .0001$) and returned to similar levels following therapy. The areas under receiver operating characteristic curves of hepcidin was 0.93 compared with 0.83 for C-reactive protein, $P = .06$. Hepcidin concentration >92.2 ng/mL correctly classified 91% of all infants (positive predictive value: 100%, negative predictive value: 87%, specificity: 100%, and sensitivity: 76%).

Conclusion Serum hepcidin concentration may be a useful adjunct test, in addition to blood culture and other markers of infection, in the evaluation of late-onset sepsis in very low birth weight infants. *(J Pediatr 2013;162:67-71)*.

Sepsis is a major cause of morbidity and mortality in neonates. Adoption of intrapartum antibiotic prophylaxis against *Streptococcus agalactiae* infection has resulted in 80% reduction in the incidence of early-onset neonatal bacterial sepsis caused by group B *Streptococcus*. No similar preventive strategy exists for late-onset sepsis except for meticulous hand hygiene and strict adherence to insertion and maintenance protocols for peripherally-inserted central venous catheters.[1] In very low birth weight infants [(VLBW); birth weight <1500 g], the rates of late-onset infections are as high as 20%-25%.[2] The deleterious consequences of neonatal sepsis are particularly pronounced in VLBW infants, adversely impacting growth, neurodevelopment, pulmonary function, and prolonging hospital stay.[3] Diagnosing late-onset neonatal sepsis is problematic because the clinical signs are nonspecific, especially in low-birth weight infants. Reliance on blood culture as a 'gold standard' presents several challenges in neonates because of long turn-around time for results and frequent falsely negative culture results secondary to low inoculum of bacteria in the small volume of blood sample collections.[4,5] Consequently, various adjunctive diagnostic tests, including biochemical markers, hematological indices, and scoring systems are used to aid decision-making in antibiotic therapy in VLBW infants suspected of sepsis.[6,7]

Hepcidin, a highly conserved antimicrobial peptide, is an acute-phase reactant that plays a critical role in inflammation and iron homeostasis.[8,9] Hepcidin contributes to host defense by depriving microbes access to iron[10] and through direct antimicrobial activity against bacteria and viruses. The utility of hepcidin as an adjunct test for sepsis has not been assessed in preterm neonates. The goal of this study is to compare the diagnostic performance of hepcidin with a well-established marker of neonatal sepsis, C-reactive protein (CRP), in predicting late-onset neonatal sepsis in VLBW infants.

Methods

All VLBW infants evaluated for late-onset neonatal sepsis (at >7 days of life) were eligible for the study. Infants born with congenital anomalies, twin–twin transfusion syndrome, placenta abruption, or immediate postnatal hemoglobin level <10 g/dL on admission were excluded because of the important role of hepcidin in anemia.[11] Sepsis was defined post hoc as a positive blood culture and/or antibiotic therapy for 5 or more days in infants with

CRP	C-reactive protein
CART	Classification and regression tree
CV	Coefficient of variation
ln	Natural logarithmic
NPV	Negative predictive value
PPV	Positive predictive value
VLBW	Very low birth weight

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clinical signs of infection such as persistent cardiorespiratory instability, neutropenia, immature-to-total granulocyte ratio >0.2, and elevated CRP concentrations. Infants who were treated for ≤3 days and with negative blood culture results were classified as nonseptic. This study was conducted at The University Hospital Neonatal Intensive Care Unit in Cincinnati, Ohio, and was approved by the Institutional Review Board of the hospital. The care providers were unaware of hepcidin results at the time of clinical decision to treat with antibiotics.

As per neonatal intensive care unit protocol, infants suspected of sepsis are evaluated routinely with complete blood count, 1 blood culture, and CRP prior to initiation of empiric antibiotic therapy. For this study, contemporaneous blood specimens were assessed for hepcidin (designated acute specimens). Blood specimens were also obtained 2 weeks (denoted as convalescent specimens) after the initial evaluation to compare hepcidin and CRP concentrations in acute vs convalescent specimens. Hepcidin and CRP concentrations were measured in healthy full-term infants to generate normative data for comparison with reference values reported for adults. Duration of antibiotic therapy was at the discretion of the providers who were not involved in the study. Medical records were abstracted for demographic data, hematocrit, reticulocyte counts, CRP, duration of antibiotic therapy, and blood culture results.

Hepcidin concentrations were assessed by capture enzyme-linked immunosorbent assay, which detects the 25-amino acid mature form of hepcidin. The intra-assay coefficient of variation (CV) was 5%-19% and the inter-assay reproducibility had an average CV of 12%.[12]

Statistical Analyses

The natural logarithmic (ln) transformations of hepcidin and CRP were used in order to stabilize the variance of these measures. Results are reported as ln (hepcidin) values with respect to statistical testing and hepcidin values on the original scale when nonparametric tests were used or when transformed back to the original scale. Continuous demographic and clinical variables are reported as means with SD if normally distributed, means with CV if back transformed, or as medians with IQRs if non-normally distributed. Correlations were tested between continuous variables with Pearson or Spearman rank correlation coefficient. Differences in continuous variables between infants with and without sepsis were tested using an independent t test. An ANOVA with Tukey–Kramer's test for multiple comparisons was used to evaluate differences in ln (hepcidin) between infants without sepsis, infants with culture-negative sepsis, and infants with culture-positive sepsis. Prediction models for neonatal sepsis were developed using logistic regression. Hepcidin and CRP were tested separately and in combination as predictors, with the following potential covariates: gestational age, birth weight, age at sample collection, hematocrit, mode of delivery, sex, nutrition (enteral/parenteral), respiratory support (mechanical ventilation/continuous positive airway pressure/room air), and blood transfusion. Covariates with $P \leq .2$ in bivariate analysis were retained in the regression models. Manual backward elimination was used to determine a parsimonious final model, considering Akaike's information criterion among models. Results are reported as adjusted OR with 95% CI. In order to assess the utility of serum hepcidin as a biomarker for late-onset neonatal sepsis, we compared the areas under receiver operating characteristic curves between hepcidin and CRP using a χ^2 test. Cutoff points of hepcidin concentrations that differentiated infants with and without sepsis were determined using classification and regression tree (CART) analysis. The inherent nonparametric properties of CART render it an appropriate method for determining cutoff points. To assess the pathophysiologic relevance of hepcidin, we compared concentrations in acute and convalescent sera using a paired t test. Statistical significance was determined at $\alpha = 0.05$. CART analysis was performed using DTREG software v. 10.0.1 (Phillip Sherrod). All other analyses were performed using SAS v. 9.2 (SAS Institute, Cary, North Carolina).

Results

A total of 44 VLBW and 21 term infants were enrolled in this study between October 2008 and September 2011. Mean (SD) birth weight was 885 (245) g and mean gestational age was 26.2 (1.7) weeks among VLBW infants and 3297 (348) g and 38.8 (1.2) weeks among term infants. Seventeen of the VLBW infants had positive blood cultures and/or were treated with antibiotics for >5 days. The mean SD age at blood collection was 22 (11.4) days. There were no significant differences between infants with and without sepsis in the study with respect to birth weight or Apgar scores. Bacteria recovered from blood cultures included *Escherichia coli* (n = 2), *Streptococcus agalactiae* (2), coagulase-negative staphylococci (5), and *Serratia marcencens* (1). Two infants in the septic group died of *E. coli* sepsis and necrotizing enterocolitis before completion of antibiotic therapy, and 1 infant in the nonseptic group died of intractable respiratory failure beyond the study period. On average, infants with sepsis were 1 week older by post-menstrual age and blood samples were collected at an earlier age (**Table**). Otherwise, there were no significant correlations between hepcidin and gestational age, birth weight, or hematocrit, all $P > .05$. There was a significant but weak negative correlation between hepcidin and age at sample collection ($\sigma = -0.34$, $P = .02$) and positive correlation between hepcidin and CRP ($\sigma = 0.39$, $P = .008$).

The range of hepcidin concentration was 5.3-89.8 ng/mL in infants without sepsis and 26.8-67.7 ng/mL in healthy term infants. There was a significant stepwise increment in mean hepcidin concentration from infants without sepsis (43.9 ng/mL, CV 13.7%) to infants with sepsis with negative blood cultures (99.2 ng/mL, CV 22.4%) to infants with sepsis with positive blood cultures (244.8 ng/mL, CV 10.7%; $P < .0001$).

Logistic regression models adjusted for gestational age demonstrated that individually, hepcidin and CRP were