Reactive Hyperemia and Interleukin 6, Interleukin 8, and Tumor Necrosis Factor-α in the Diagnosis of Early-Onset Neonatal Sepsis

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ABSTRACT. Objective. To evaluate the diagnostic value of peripheral circulatory reactive hyperemia and serum levels of interleukin-6 (IL-6), IL-8, and tumor necrosis factor-α (TNF-α) in early-onset neonatal sepsis.

Methods. Reactive hyperemia in the dorsal hand and serum levels of IL-6, IL-8, and TNF-α were studied in newborn infants (n = 32; gestational age 39 ± 3 weeks) who had been admitted to the neonatal unit because of suspected sepsis <48 hours after birth. On admission, reactive hyperemia after a standardized arterial occlusion was measured with laser Doppler technique, and blood samples were taken for cytokine analyses. On the basis of predetermined criteria, the infants subsequently were classified as septic (n = 12) or not (n = 20).

Results. The degree of reactive hyperemia was higher in the group with sepsis (median + 170% perfusion increase) than in that without (+37%). On admission, serum levels of IL-6, IL-8, and TNF-α all were higher in septic (median values: 1620, 331, and 22 pg/mL, respectively) than in nonseptic neonates (median values: 42, 63, and 13 pg/mL, respectively). In the group with sepsis, the degree of reactive hyperemia correlated to log IL-6 (r = 0.80) and log IL-8 values (r = 0.71).

Conclusion. Newborn infants with septicemia have increased reactive hyperemia and elevated cytokine levels very early in their disease. Reactive hyperemia in skin can be analyzed at the bedside and noninvasively and therefore may serve as an additional diagnostic tool in neonatal sepsis. Pediatrics 2001;108(4). URL: http://www.pediatrics.org/cgi/content/full/108/4/e61; neonatal sepsis, newborn infant, interleukin-6, interleukin-8, TNF-α, reactive hyperemia, diagnostic marker.

ABBREVIATIONS. CRP, C-reactive protein; WBC, white blood cell count; IL-6, interleukin 6; TNF-α, tumor necrosis factor-α; IL-8, interleukin 8; LD, laser Doppler; PPV, positive predictive value; NPV, negative predictive value; ROC, receiver-operating characteristic curve; RDS, respiratory distress syndrome; GBS, group B streptococci.

Serious infections are still causing significant morbidity and mortality among newly born infants. Immediately after birth, the clinical picture of neonatal sepsis is difficult to distinguish from more benign conditions. Rapid and reliable diagnostic tools are not yet available at the bedside. Because a delay in instituting antibiotic therapy may have adverse consequences in cases of true neonatal sepsis, in many cases decisions to treat are based on risk factors and nonspecific signs and symptoms.

Experienced neonatologists and nurses recognize that in infants with sepsis, a poor peripheral circulation with a gray skin color often precedes more alarming symptoms, such as arterial hypotension and circulatory failure. Specific deviations in peripheral vascular reactivity have been found in late neonatal sepsis,1 even before traditional markers, such as C-reactive protein (CRP) elevation and changes in white blood cell count (WBC) in peripheral blood, indicate serious infection. The advantages of peripheral circulatory measurements as diagnostic markers are that 1) they can be done rapidly and noninvasively, 2) the measurements are reproducible shortly after birth, and 3) the result of the test is available immediately.2 However, before these observations can become useful in clinical work, they need to be confirmed and validated against other diagnostic markers of neonatal sepsis.

Cytokines, produced by monocytes, macrophages, and endothelial cells in response to infectious stimuli are important proinflammatory mediators in the early phases of the sepsis syndrome. Elevated serum levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and, in recent years, interleukin-8 (IL-8) have been found in both neonatal and adult sepsis.3–6 Several studies have evaluated the role of cytokine determinations as early diagnostic markers in neonatal sepsis.7–13 Although promising data have been published, problems with invasiveness, response time, and specificity remain to be solved.7,8,14–17

In sepsis, cytokines are involved in activating vascular and endothelial defense mechanisms that may cause changes in perfusion and vascular resistance in the microcirculation.18,19 Accordingly, it is possible that cytokine activation is reflected in vascular reactivity. This study was conducted to evaluate the diagnostic use of a vascular reactivity test as compared with that of circulating cytokine levels in infants with suspected sepsis.

METHODS

Study Population

We studied 32 consecutive infants who were admitted to our neonatal unit because of suspected sepsis <48 hours after birth, all of whom had fulfilled previously used inclusion criteria9–20: 1) at least 1 risk factor for infection; 2) signs of respiratory or circulatory dysfunction (tachypnea >60 bpm, recurrent apnea >20 seconds, tachycardia >160 bpm, or bradycardia <100 bpm); and 3) at least
1 of the following symptoms: feeding intolerance, lethargy, irritability, temperature instability, or jaundice. The risk factors for early infection included rupture of membranes >24 hours, maternal body temperature of >38°C, chorioamnionitis, maternal colonization with group B streptococci, or preterm delivery. Infants who were >48 hours old at onset of suspected sepsis, unstable children with respiratory and/or circulatory failure, and infants with neonatal asphyxia (Apgar scores <7 at 5 minutes) or congenital malformations were excluded.

Parental informed consent was obtained for every patient before admission to the study. The protocol was approved by the Ethics Committee at Karolinska Hospital.

Protocol
On admission, skin perfusion was measured, followed by blood sampling for cytokine and CRP analyses, peripheral blood count, and arterial blood gas analyses. Cultures from blood and upper airways were obtained from all infants, and spinal fluid cultures were done when clinically indicated. Intravenous antibiotic treatment was started immediately thereafter. Chest radiographs were taken for infants with signs of respiratory dysfunction. Additional data recorded for study infants included gender, birth weight, gestational and postnatal ages, mode of delivery, Apgar scores, heart and respiratory rates, blood pressure, rectal temperature, oxygen requirements, and O2 saturation. A second blood sample for analyses of cytokines, CRP, and peripheral blood count was obtained 24 hours after the first sample.

Skin Perfusion Measurements
A laser Doppler (LD) instrument (Periflux PF 2B; Perimed AB, Järfälla, Sweden) was used to measure skin perfusion in the left dorsal hand skin. The LD signal is proportional to the number and velocity of moving blood cells in illuminated (wavelength 633 nm) superficial microvessels in a skin area of approximately 1 cm² (integrating probe PF 313, Perimed AB). The LD output is semi-quantitative and expressed in perfusion units of output voltage (1 PU = 10 mV).21,22 The mean skin perfusion was measured for at least 2 minutes, after which arterial occlusion for 1 minute was produced by inflating a pneumatic cuff to a supra systolic pressure. The postocclusive reactive hyperemia was evaluated in terms of peak perfusion, time-to-peak perfusion after cuff release, and relative perfusion change (peak/basal ratio, %). The complete recording sequence was performed twice, and data are presented as mean values. Only sequences that were free from movement artifacts were analyzed.

Cytokine Determinations
Blood samples for cytokine and CRP determinations were centrifuged within 30 minutes of collection, and sera were transferred to plastic tubes. Sera then were stored at −70°C pending simultaneous assay at the end of the study. The serum TNF-α, IL-6, IL-8, and CRP levels were measured with a chemiluminescence immunoassay (Immulite; Diagnostic Products Corporation, Los Angeles, CA). According to the manufacturer, the detection limits of the assays for IL-6 was 5.0 pg/mL, for IL-8 was 2.0 pg/mL, for TNF-α was 1.7 pg/mL, and for CRP was 0.1 mg/L.

Statistical Analysis
Values are given as means ± 1 SD, median (range), or as the number of subjects and proportions. The Student t test was used for group comparisons of normally distributed variables, and the Mann-Whitney U test and Wilcoxon signed-rank test were used for comparisons of variables with skewed distribution. The χ² test was used to compare proportions. Correlation coefficients were used to describe associations between variables, and multiple regression analysis was used to detect any relationships between the variables. P < .05 was considered significant.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to analyze IL-6, IL-8, TNF-α, CRP, and reactive hyperemia as predictors of neonatal sepsis. The receiver operator characteristic (ROC) method was used to determine the best possible cutoff values; the curves were obtained by plotting sensitivity on the y axis against the false-positive rate (1 − specificity) on the x axis for all possible cutoff values of the tests.

RESULTS
Classification and Basic Characteristics
At discharge from the hospital, the 32 infants received a final diagnosis of septic or not. Twelve subsequently were classified as septic, whereas the remaining 20 infants were classified as nonseptic. Those assigned to the sepsis group had a positive blood and/or spinal fluid culture or all of the following predetermined criteria: 1) CRP >50 on admission and/or 24 hours (ie, above the level reported in healthy newborn infants immediately after birth),23 2) WBC less than 9 × 10⁹ or more than 30 × 10⁹ on admission and/or 24 hours, and 3) at least 1 of the following symptoms in the sepsis syndrome20: oliguria, metabolic acidosis, or hypoxemia.

The final diagnoses in nonseptic infants were transient tachypnea of the newborn (n = 12), minimal respiratory disease (n = 4), persistent pulmonary hypertension of the newborn (n = 1), pneumothorax (n = 1), neonatal hyperbilirubinemia (n = 1), and anemia of the newborn (n = 1). None of the infants included had respiratory distress syndrome (RDS).

The mean gestational age was 39 ± 2 weeks in the sepsis group and 39 ± 3 weeks in the no sepsis group (P = .95), and the mean postnatal age did not differ between the groups (11 ± 6 vs 12 ± 8 hours; P = .70). The distributions of preterm newborns (<37 weeks of gestational age) were similar in the 2 groups (3 of 12 in the sepsis group and 5 of 20 in the no sepsis group). There were no differences in gender, birth weight, Apgar score at 5 minutes, or mode of delivery between the groups. The frequency of antenatal maternal antibiotic treatment (4 of 12 in the sepsis group and 6 of 20 in the no sepsis group) and prolonged rupture of the membranes (>24 hours) were similar in both groups (7 of 12 in the sepsis group and 12 of 20 in the no sepsis group).

At study entry, we found no differences in body or hand skin temperatures, mean arterial blood pressure, respiratory and heart rates, or oxygen requirement between the 2 groups (Table 1). On admission, CRP values did not differ between the sepsis group (median: 19 mg/L) and the no sepsis group (median: 7.3 mg/L; P = .67), whereas 24 hours after inclusion, the CRP values were significantly higher in the sepsis group compared with the no sepsis group (median: 92 vs 17.5 mg/L; P < .001). Total WBC at inclusion was lower in the sepsis group (median: 5.9 × 10⁹/L) than in the no sepsis group (17.4 × 10⁹/L; P < .001).

<table>
<thead>
<tr>
<th>Value</th>
<th>On Admission</th>
<th>Sepsis (n = 12)</th>
<th>No Sepsis (n = 20)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td>37.1 ± 0.5</td>
<td>37.0 ± 0.5</td>
<td>.63</td>
</tr>
<tr>
<td>Hand skin temperature (°C)</td>
<td></td>
<td>33.2 ± 1.1</td>
<td>33.1 ± 1.4</td>
<td>.88</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td></td>
<td>44 ± 4</td>
<td>48 ± 8</td>
<td>.17</td>
</tr>
<tr>
<td>Respiratory rate (min⁻¹)</td>
<td></td>
<td>79 ± 19</td>
<td>74 ± 20</td>
<td>.45</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td></td>
<td>132 ± 17</td>
<td>130 ± 17</td>
<td>.73</td>
</tr>
<tr>
<td>FIO₂ (%)</td>
<td></td>
<td>21 (21–95)</td>
<td>21 (21–97)</td>
<td>.99</td>
</tr>
</tbody>
</table>

Data given as mean ± 1 SD or median (range). MAP indicates mean arterial blood pressure; FIO₂, fraction of inspired oxygen.

TABLE 1. Clinical Findings in Newborn Infants Admitted Because of Suspected Sepsis and 24 Hours Later

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In the sepsis group, a correlation was found between log IL-6 and log IL-8 levels (r = 0.89, P < .001).
No other correlations were found between the cytokines studied.

Relation Between Skin Perfusion and Cytokine Serum Levels

In the sepsis group, the degree of reactive hyperemia correlated to log IL-6 (r = 0.80, P < .001) and log IL-8 values (r = 0.71, P < .001). Variations in basal skin perfusion correlated weakly to log IL-6 (r = 0.65, P < .001) and log IL-8 values (r = 0.39, P < .05). No other correlations were found between circulatory measurements and cytokines.

Relation Among Cytokine Serum Levels, WBC, and CRP

In the group with sepsis, a negative correlation was found between WBC and log IL-6 levels on admission (r = −0.57, P < .05). The values of CRP at 24 hours after admission correlated to log IL-6 (r = 0.65, P < .05) and log IL-8 values (r = 0.77, P < .01) obtained at study entry in the sepsis group. No other correlations were found among cytokine levels, WBC, and CRP in the sepsis group. In the no sepsis group, no correlations were found among cytokine levels, WBC, and CRP.

Evaluation of IL-6, IL-8, TNF-α, and Reactive Hyperemia as Diagnostic Tests

The ideal cutoff point for IL-6, IL-8, and TNF-α serum concentrations is the point that allows detection of as many true-positive findings as possible (high sensitivity) and with few false-positive results (high specificity). In our study, the optimal cutoff point obtained with the ROC method was 160 pg/mL for IL-6, 70 pg/mL for IL-8, and 20 pg/mL for TNF-α (Table 3). The optimal cutoff point for peripheral circulatory measurements was a reactive hyperemia of 75% or more (Table 3). At these cutoff points, the PPV for early-onset neonatal sepsis for IL-6 was 67%, for IL-8 was 65%, for TNF-α was 73%, and for reactive hyperemia was 86%. The NPV for IL-6 was 100%, for IL-8 was 93%, for TNF-α was 81%, and for reactive hyperemia was 100%. Combining reactive hyperemia with 1 or more cytokines studied did not improve sensitivity, specificity, or the predictive values for neonatal sepsis.

DISCUSSION

In neonatal sepsis, the need for an early diagnosis and speedy, appropriate antibiotic treatment must be weighed against the aim to avoid unnecessary antibiotics and hospitalization of newborn infants. At early onset in particular, the clinical symptoms are nonspecific and difficult to differentiate from transient problems with extrauterine adaptation. This study showed that the degree of reactive hyperemia in skin may be helpful as a diagnostic marker in newborn infants with early-onset neonatal sepsis.

Although cytokine analyses are far from being diagnostic for bacterial sepsis, serum levels of IL-6,
IL-8, and TNF-α were suggested previously as early markers of neonatal sepsis. In our study, the sensitivity and specificity of reactive hyperemia were equal to or even higher than those of IL-6, IL-8, and TNF-α found by us and others. Similar to our findings, reactive hyperemia has been reported to discriminate late-onset neonatal sepsis as well, before other laboratory tests could. Irrespective of skin region, a deviating reactive hyperemia was found in neonates with sepsis. Taken together, these studies suggest that systemic vascular changes are induced rapidly in sepsis and that early systemic alteration can be detected at different local, skin microvascular beds.

The aim of the present study was to distinguish between infants with and without sepsis, all having nonspecific symptoms on admission. We previously studied healthy control subjects, and the degree of reactive hyperemia among subjects without sepsis did not differ from that found in age-matched, healthy newborn infants. Our findings point to the possibility that a decreased basal skin blood flow and a longer time to peak hyperemia explain the elevated reactive hyperemia in septic infants. Because of the semiquantitative nature of LD perfusion measurements, basal skin blood flow data are not completely reliable in interindividual comparisons. The predictive values for sepsis using time to peak was lower (PPV: 77%; NPV: 89%) than for reactive hyperemia, which limits the use of the former variable as a diagnostic marker. Hemodynamic changes during sepsis involve regional differences in blood flow mediated by early vasoconstriction and, in later stages of the disease, general vasodilation and shock. The microcirculation is particularly important in the vascular response to sepsis because microvascular disturbances affect blood pressure and tissue perfusion. Our findings suggest early peripheral vasoconstriction in sepsis. Besides vasoconstriction, the accumulation and adherence of leukocytes to the vascular wall seen in sepsis may add to the impaired microcirculation. Possible mediators of these early microvascular changes include cytokines, which have been reported to increase vascular tone, and endothelial expression of adhesion molecules in early stages of sepsis. In later stages of the disease, the microvascular tone and reactivity, as well as their correlation to cytokine levels, may well differ from the findings in our study. Absent reactive hyperemia has been found in adult patients in septic shock.

Other clinical methods of studying skin blood flow or skin vascular reactivity in neonates include transcutaneous PO₂ measurements and capillary refill time. Neither of the methods has been evaluated in neonatal sepsis. In comparison with the LD method, an advantage of the transcutaneous PO₂ method is that many neonatal units have this instrument and are familiar with its use. It has been evaluated for postocclusive reactive hyperemia at 37°C of electrode temperature and shown to have some correlation with LD measurements of skin perfusion in healthy children and adults. Capillary refill time can be used clinically, but marked interobserver variability exists.

**TABLE 3. Sensitivity, Specificity, and Predictive Values of IL-6, IL-8, TNF-α, and Reactive Hyperemia in Early-Onset Neonatal Sepsis**

<table>
<thead>
<tr>
<th></th>
<th>IL-6 (≥160 pg/mL)</th>
<th>IL-8 (≥70 pg/mL)</th>
<th>TNF-α (≥20 pg/mL)</th>
<th>Reactive Hyperemia (&gt;75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>92</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>70</td>
<td>70</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>67</td>
<td>65</td>
<td>73</td>
<td>67</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>100</td>
<td>93</td>
<td>81</td>
<td>100</td>
</tr>
</tbody>
</table>

Cutoff values are given in parentheses.
ability has been reported even with experienced observers. It was assessed recently in pediatric intensive care patients but showed only a weak correlation to indicators of hemodynamic status. Additional studies are needed to pursue its usefulness and relation to measurements of reactive hyperemia in suspected sepsis.

Because of frequent use of intrapartal antibiotics to the mother and only 1 blood culture obtained from the infant, we found few infants with positive blood/spinal fluid cultures (17% in the sepsis group). A similarly small proportion of culture-proven infection also was found in other recent and larger studies of neonatal sepsis, and this problem may limit the conclusions that can be drawn from the present data. However, the strict diagnostic criteria for clinical sepsis used in this study ensured that the infants assigned to this group were diagnosed correctly. The reactive hyperemia and cytokine levels in the 2 infants with culture-proven sepsis did not differ from the data found in infants with clinical sepsis.

Three nonseptic neonates had CRP values above 50 at study entry (n = 2) or 24 hours later (n = 1). They were deemed not to have sepsis because they did not fulfill the other diagnostic criteria for clinical sepsis. In addition, upper airway cultures were negative, and these infants were discharged from the neonatal unit within 3 days. All had a final diagnosis of transient tachypnea, complicated in 1 case by a small pneumothorax.

Although not an exclusion criterion, our sample did not include any infant with RDS. This is another group of neonates who should be studied in the future, as distinguishing clinically between RDS and GBS sepsis may be very difficult.

CONCLUSION

Newborn infants with early-onset neonatal sepsis showed increased postocclusive reactive hyperemia and elevated cytokine levels even on admission to the neonatal unit. We suggest that reactive hyperemia, a fast, noninvasive skin test, could be used as an additional diagnostic marker in neonatal sepsis.

ACKNOWLEDGMENTS

This study was supported by grants from the research foundations of the Karolinska Institute, Stockholm; the Samaritan Foundation; Swedish Society for Medical Research; General Maternity Hospital Foundation; the Solstician Foundation; and the First Mayflower Annual Campaign, Sweden.

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