Lipopolysaccharide binding protein in preterm infants

D Behrendt, J Dembinski, A Heep, P Bartmann


Objective: To assess serum concentrations of lipopolysaccharide binding protein (LBP) in preterm infants with neonatal bacterial infection (NBI).

Methods: Blood samples were analysed of 57 preterm (28 to 36 weeks gestation) and 17 term infants admitted to the neonatal intensive care unit within the first 72 hours of life with suspicion of NBI. Samples were obtained at first suspicion of sepsis and after 12 and 24 hours. Diagnosis of NBI was confirmed by raised concentrations of C reactive protein and/or interleukin 6. The influence of gestational age and labour was analysed.

Results: Maximum LBP concentrations in infants with NBI were greatly increased compared with infants without NBI (13.0–46.0 μg/ml (median 20.0 μg/ml) vs 0.6–17.4 μg/ml (median 4.2 μg/ml)). LBP concentrations in infected infants were not yet significantly raised when NBI was first suspected. The LBP concentrations of preterm infants were comparable to those of term infants. Regression analysis revealed no significant effect of labour or gestational age on LBP.

Conclusions: Raised LBP concentrations indicate NBI in preterm and term infants. Preterm infants of > 28 weeks gestation seem to be capable of producing LBP as efficiently as term infants. Neonatal LBP concentrations are not influenced by labour. LBP may be a useful diagnostic marker of NBI in preterm infants.
of sampling. CRP concentrations were analysed by latex immune nephelometry (N Latex CRP mono; Dade Behring, Liederbach, Germany; detection limit 0.2 mg/l), and IL6 and LBP concentrations by enzyme immunoassay (Immulite; DPC Bierrmann GmbH, Bad Nauheim, Germany; detection limit for IL6 5.0 pg/ml; detection limit for LBP 0.5 mg/ml). Blood cultures were taken before initiation of antibiotic treatment (Bactec Peds Plus; Becton Dickinson, Shannon, Ireland).

Definition of NBI
NBI was suspected if infants had at least one clinical sign of NBI or a perinatal history of amniotic infection syndrome. Clinical signs of NBI were apnoea, tachypnoea, dyspnoea, cyanosis, tachycardia, bradycardia, pallor, greyish skin colour, capillary refill time more than three seconds, temperature instability, arterial hypotension, muscular hypotonia or hypertonia, irritability, lethargy, seizures, abdominal distension, and poor feeding ability. NBI was defined as (a) positive blood culture with at least three clinical signs of NBI or (b) CRP > 5 mg/l and/or IL6 > 25 pg/ml within the first 24 hours and at least three clinical signs of NBI.

Statistical analysis
Data were analysed by using SPSS statistical software (SPSS for Windows, version 10.0.7; SPSS Inc, Chicago, Illinois, USA). Statistical significance was tested by the Mann-Whitney U test. The influence of labour and gestational age was also tested by multivariate analysis of variance. p < 0.05 was considered significant.

RESULTS
A total of 21 infants (17 preterm and four term) were diagnosed positive for NBI; 53 infants (40 preterm and 13 term) were negative. Two infants with NBI had positive blood cultures.

Analysis of LBP concentrations
Table 1 shows LBP concentrations for all the infants.

Table 1 Lipopolysaccharide binding protein (LBP) concentrations (mg/ml) according to sampling time in all infants

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>NBI positive</th>
<th>NBI negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16</td>
<td>41</td>
</tr>
<tr>
<td>12</td>
<td>19</td>
<td>17.6</td>
</tr>
<tr>
<td>24</td>
<td>18</td>
<td>17.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>21</td>
<td>20.0</td>
</tr>
</tbody>
</table>

NBI, Neonatal bacterial infection; LBP, lipopolysaccharide binding protein.

Table 2 Maximum lipopolysaccharide binding protein (LBP) concentrations (mg/ml) according to gestational age

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>NBI positive</th>
<th>NBI negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;28–32</td>
<td>9</td>
<td>20.4</td>
</tr>
<tr>
<td>&gt;32–37</td>
<td>8</td>
<td>24.8</td>
</tr>
<tr>
<td>&gt;37</td>
<td>4</td>
<td>17.5</td>
</tr>
</tbody>
</table>

NBI, Neonatal bacterial infection; LBP, lipopolysaccharide binding protein.
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Table 3 Maximum lipopolysaccharide binding protein (LBP) concentrations (µg/ml) for all infants born before or after the onset of labour

<table>
<thead>
<tr>
<th>NBI</th>
<th>With labour</th>
<th>Without labour</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>17.0</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>4.4</td>
<td>38</td>
</tr>
</tbody>
</table>

Figure 4 Maximum lipopolysaccharide binding protein (LBP) concentration in infants without neonatal bacterial infection born before or after the onset of labour.

NBI, Neonatal bacterial infection; LBP, lipopolysaccharide binding protein.

In the multivariate analysis of variance, there was no significant independent effect on LBP concentrations of labour (p = 0.36) or gestational age (p = 0.51).

**DISCUSSION**

LBP concentrations are significantly higher in preterm infants of > 28 weeks gestation with NBI than infants without NBI. Other data have shown peak serum concentrations of LBP 6–12 hours after the first suspicion of NBI. It is therefore not surprising that the increase in LBP at 0 hours in infants positive for NBI is not yet significant. At 12 and 24 hours, the difference between LBP concentrations of NBI positive and negative preterm infants is highly significant. LBP therefore does not indicate NBI as early as IL6 and IL8, but its raised concentrations persist for longer. There are data indicating that IL6 production is influenced by labour. Labour did not have an independent effect on LBP concentrations. CRP concentrations do not increase before 12–24 hours after exposure to endotoxin. Therefore a combination of IL6 and LBP for the early diagnosis of NBI could yield a higher sensitivity and specificity than IL6 alone or in combination with CRP. CRP is a valuable guide for antibiotic treatment in NBI. We would suggest that a combination of IL6 and LBP for early diagnosis, with CRP to guide antibiotic treatment, would close the diagnostic gap between IL6 and CRP and be the most promising way to manage preterm infants with NBI with the greatest sensitivity and specificity. If LBP is analysed by an automatic enzyme immunoassay such as the Immulite system used here, it can be performed at the same time as IL6 analysis and requires only 45 minutes and an extra sample volume of 10 µl. This is especially important for the diagnosis of NBI in preterm infants, who have a small total blood volume and higher NBI associated morbidity.

Gestational age did not have an independent effect on LBP concentration. In preterm infants of > 28 weeks gestation, LBP concentrations were significantly raised in the presence of NBI. We also analysed data for nine preterm infants of < 28 weeks gestation. Seven were NBI positive and had raised LBP concentrations. As only two patients in this group were negative for NBI, the number was not sufficient to calculate statistical significance. We therefore excluded these patients retrospectively from the study, but we believe that LBP would also be a valuable marker for diagnosis of NBI in this group of patients. Further studies are needed to confirm this.

One infant classified as NBI negative had a maximum LBP concentration within the range of the NBI positive infants. This was a preterm infant with clinical chorioamnionitis who was born by emergency caesarean section after maternal antibiotic treatment for several days. Postnatal CRP and IL6 concentrations were not raised and the blood culture was negative, and the infant was therefore considered to be NBI negative. Because of early antenatal antibiotic treatment, IL6 and CRP concentrations may already have been decreased, while LBP remained high because of its long half life.

A positive blood culture is still the “gold standard” in diagnosis of NBI. In Europe the detection rate is 5–15% in infants with proven NBI. In our study population, only two of 21 NBI positive infants had a positive blood culture (9.5%). We therefore did not differentiate between NBI positive infants with or without positive blood cultures. Improvements should be made to increase the detection rate of blood cultures in our laboratory as in most laboratories in Europe.

**CONCLUSIONS**

LBP concentrations in preterm infants of > 28 weeks gestation can be used to diagnose NBI. However, they are not significantly raised before 12 hours after the first suspicion of NBI. LBP concentration alone therefore is not sufficiently sensitive for the early diagnosis of NBI, but could be a valuable marker in combination with IL6 and IL8. Neonatal LBP concentrations are not influenced by labour or gestational age. Preterm infants of > 28 weeks gestation seem to have the same capacity to produce LBP as term infants. LBP is not affected by the stress of labour and therefore may be more specific in the diagnosis of NBI compared with cytokines. The sensitivity compared with CRP remains to be assessed.
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REFERENCES