

ORIGINAL ARTICLE

Lipopolysaccharide binding protein in preterm infants

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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⁺¹ to 36⁺⁶, median 33⁺² 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) v 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.

Neonatal bacterial infection (NBI) in preterm infants is associated with high morbidity and mortality.¹ Clinical signs of NBI are non-specific.² Early diagnosis can be established by measurement of proinflammatory serum markers. C reactive protein (CRP) has been shown to be a specific variable in the diagnosis of NBI in preterm and term infants.^{3–6} Maximum serum concentrations of CRP are found 12–24 hours after exposure to endotoxin. Therefore, CRP has limited sensitivity in the early diagnosis of NBI.^{7–9} Negative CRP may be used as a guideline to terminate antibiotic treatment. Proinflammatory cytokines, such as interleukin (IL) 6 and IL8, which already show peak serum concentrations two to three hours after exposure to endotoxin *in vivo*, are useful early diagnostic variables in NBI.^{10–14} Although persistent raised concentrations may indicate insufficient disease control, serum concentrations of IL6 and IL8 generally decrease to normal values after four to six hours. As the decrease in interleukin concentration occurs before the increase in CRP concentration, there is a diagnostic gap between these serum markers. Lipopolysaccharide binding protein (LBP) is an acute phase protein synthesised by hepatocytes, which has an essential role in presentation of bacterial antigen to the innate immune system.¹⁵ Lipopolysaccharide is captured by LBP within the circulation and binding of the lipopolysaccharide–LBP complex to CD14 receptors of immune effector cells initiates the inflammatory response.

Peak plasma concentrations of LBP have been observed 6–12 hours after clinical suspicion of sepsis,¹⁶ and its half life time in serum is 12–24 hours. LBP could therefore fill the diagnostic gap between the decrease in interleukin and the increase in CRP.

Raised LBP concentrations have been shown in adult patients with systemic inflammatory response syndrome in both Gram positive and Gram negative sepsis.^{17–19} In a recent study, Berner *et al*²⁰ found raised LBP concentrations in term infants with NBI. There are no data on LBP in preterm infants.

Studies on the capacity of preterm infants to produce proinflammatory cytokines compared with term infants have produced conflicting results.^{21–26} Also, findings on the effect of labour on concentrations of proinflammatory cytokines in neonates are contradictory.^{27, 28} Production of cytokines as well as acute phase proteins may be triggered by non-specific stimuli such as stress.

In this prospective study, we investigated the diagnostic value of LBP in preterm infants with NBI and evaluated the influence of labour and gestational age.

METHODS AND MATERIALS

The study protocol was approved by the ethical committee of the University of Bonn, Germany. Written informed parental consent was obtained on the infant's admission.

Patients

All patients admitted to our neonatal intensive care unit with suspicion of NBI (onset of sepsis before 72 hours of postnatal age) between January and June 2002 were included. One patient was excluded because of chromosomal aberration, one because of perinatal asphyxia, and 16 because of insufficient sampling. Nine patients of gestational age < 28 weeks were excluded retrospectively, because the number was insufficient for analysis.

Blood samples of 57 preterm (17 of > 28 to < 32 weeks gestation, 40 of > 32 to < 37 weeks gestation) and 17 term infants were analysed. Fifty two infants were delivered by elective caesarean section, 12 by emergency caesarean section, eight by vaginal delivery, and two by vacuum extraction. Infants delivered by elective caesarean section were delivered before the onset of labour.

Sample analysis

Blood was obtained at 0, 12, and 24 hours after the first suspicion of NBI. Samples were analysed within three hours

Abbreviations: CRP, C reactive protein; IL, interleukin; LBP, lipopolysaccharide binding protein; NBI, neonatal bacterial infection

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 Biermann GmbH, Bad Nauheim, Germany; detection limit for IL6 5.0 pg/ml; detection limit for LBP 0.5 µg/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. NBI negative infants had lower LBP concentrations than NBI positive infants. At 0 hours (immediately after NBI suspected) the difference did not reach significance (p = 0.376). However, after 12 and 24 hours, the difference was highly significant (p < 0.0001).

The maximum LBP concentrations of all 74 infants were measured. The difference between NBI positive and NBI negative infants was highly significant (p < 0.0001) (fig 1).

Analysis of maximum LBP concentration by gestational age group

Infants were divided into three groups according to gestational age, and maximum LBP concentrations in NBI positive and NBI negative infants were compared. Maximum LBP concentrations were lower in NBI negative infants than in

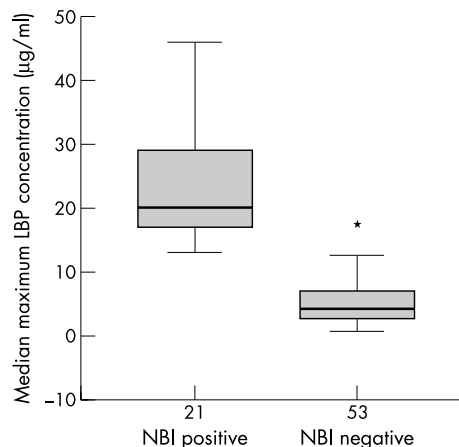


Figure 1 Maximum lipopolysaccharide binding protein (LBP) in infants who were positive or negative for neonatal bacterial infection (NBI). *Significantly different from NBI positive (p < 0.0001).

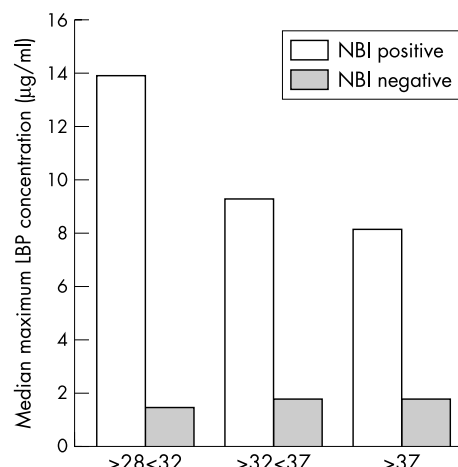


Figure 2 Maximum lipopolysaccharide binding protein (LBP) concentration by gestational age group.

NBI positive infants (fig 2, table 2). In all three groups, the difference was highly significant.

Analysis of maximum LBP concentrations according to birth before or after onset of labour

Infants were divided into two groups according to birth before or after the onset of labour, and maximum LBP concentrations of NBI positive and NBI negative infants were compared (table 3). In both groups, the difference did not reach significance: NBI positive infants, p = 0.40 (fig 3); NBI negative infants, p = 0.42 (fig 4).

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

Time (h)	NBI positive		NBI negative		p Value
	No	Median LBP	No	Median LBP	
0	16	4.1	45	2.9	0.376
12	19	17.6	19	6.0	<0.0001
24	18	17.8	14	6.0	<0.0001
Maximum	21	20.0	53	4.2	<0.0001

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

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

Gestational age (weeks)	NBI positive		NBI negative		p Value
	No	Median LBP	No	Median LBP	
>28<32	9	20.4	8	3.9	<0.001
>32<37	8	24.8	32	4.3	<0.0001
>37	4	17.5	13	3.9	<0.001

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

Table 3 Maximum lipopolysaccharide binding protein (LBP) concentrations ($\mu\text{g/ml}$) for all infants born before or after the onset of labour

NBI	With labour		Without labour		p Value
	No	Median LBP	No	Median LBP	
Positive	7	17.0	14	21.7	0.40
Negative	15	4.4	38	4.1	0.42

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.

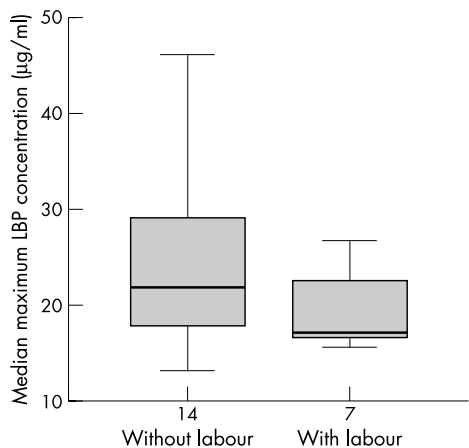


Figure 3 Maximum lipopolysaccharide binding protein (LBP) concentration in infants with neonatal bacterial infection born before or after the onset of labour.

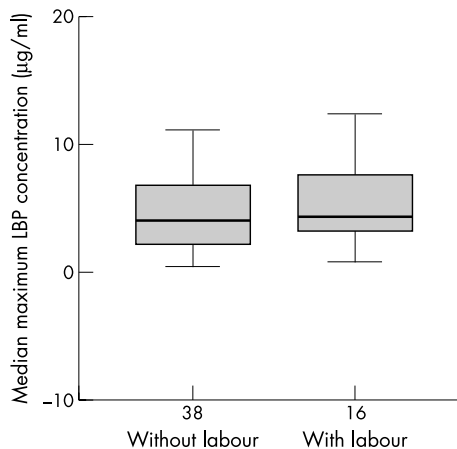


Figure 4 Maximum lipopolysaccharide binding protein (LBP) concentration in infants without neonatal bacterial infection born before or after the onset of labour.

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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