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Low Serum Levels of Mannose Binding Lectin Are a Risk Factor for Neonatal Sepsis

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ABSTRACT: Mannose binding lectin (MBL) is a soluble pattern recognition receptor of innate immunity that binds a wide range of pathogens and exerts opsonic effects. We investigated the association between serum MBL levels and development of sepsis in infants admitted to neonatal intensive care units (NICUs). Serum MBL levels on admission were measured by enzyme-linked immunosorbent assay (ELISA) in 206 neonates consecutively admitted to an NICU of whom 138 did not develop hospital-acquired sepsis and 68 did. Of these 68, 40 had confirmed sepsis with positive blood cultures, 19 clinically suspected sepsis, with negative blood cultures, and nine had clinically suspected sepsis with blood culture yielding coagulase-negative staphylococci (CoNS). Serum MBL levels on admission were significantly lower in infants with sepsis [0.45 μg/mL; interquartile range (IQR) 0.09–1.68], particularly in those with confirmed sepsis (0.17 μg/mL; IQR 0.05–0.96), compared with infants without sepsis (1.45 μg/mL; IQR 0.43–3.52), and infants with CoNS-positive blood culture (1.70 μg/mL: IQR 0.85–3.60). After adjusting for duration of exposure gestational age (GA) and birth weight (BW), the association of low MBL levels with development of sepsis was maintained [odds ratio (OR) = 0.52; 95% confidence interval (CI): 0.36–0.75]. The measurement of serum MBL levels on admission in NICU may help to identify neonates at higher risk of developing sepsis. **(*Pediatr Res* 61: 325–328, 2007)**

Hospital-acquired infections are an important cause of morbidity and mortality in neonates admitted to NICUs (1–4).

Physiologic immaturity of the adaptive immune response, low GA, and invasive procedures are major risk factors for neonatal infections (1–4). Because the innate immune response represents an important first-line defense mechanism against infections in newborns, it is possible that genetic and/or developmental variations in the innate immune response play a significant role in modulating the predisposition to severe infections.

MBL, a member of the collectin family, is produced by the liver as an acute phase protein and its blood levels increase significantly in response to infections. MBL is a soluble pattern recognition receptor of innate immunity, capable of recognizing and binding glycoproteins, ending with mannose and *N*-acetyl-glucosamine, present on the surface of a wide variety of pathogens, including bacteria, viruses, and fungi (5,6). Upon binding, MBL exerts opsonic and inflammatory effects: it activates macrophages, facilitates phagocytosis, and stimulates the third pathway of complement activation, therefore contributing to contain invading organisms (6,7).

Serum MBL levels show a remarkable interindividual variability, which is partly due to genetic variants in both the promoter and the coding sequence of the gene (7). Approximately 15%–20% of the white populations carry MBL genotypes conferring low serum MBL levels. Low serum MBL levels appear to have clinical significance (8) and have been reported to be associated with an increased risk of infection in at least three situations: (i) in association with another immune deficiency, such as primary defects of antibody responses (9); (ii) in immunosuppressed patients with systemic lupus erythematosus (10), malignancies (11,12), or after bone marrow transplantation (13); (iii) in patients exposed to unusual infectious challenges, such as patients with cystic fibrosis (14,15) or those needing intensive care (16,17). In otherwise healthy children and adults, the association of low MBL levels with susceptibility to infections has been reported by some authors (18,19), but is still controversial (6,7).

In this study, we investigated the association between MBL serum levels and susceptibility to hospital-acquired sepsis in newborns admitted to NICUs.

MATERIALS AND METHODS

Patients. Two hundred six neonates, consecutively admitted to an NICU within the first 24 h of life, were studied. These infants were part of a larger cohort included in a 28-mo prospective multicenter surveillance study on the epidemiology of hospital-acquired infections in the NICU (Auriti C et al 2005 Determinants of nosocomial infection (NI) in six Italian intensive care units. Pediatr Res 58:356). The study was reviewed and approved by the hospital ethics committee. Informed consent was obtained.

Because MBL behaves as an acute phase protein, with a significant increase in blood concentration in response to infection, only patients in whom a serum sample collected on admission was available were included in the present study.

Hospital-acquired sepsis was defined according to the Centers for Disease Control and Prevention definition of nosocomial infection (3,20). The diag-

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Abbreviations: BW, birth weight; **CoNS,** coagulase-negative staphylococci; **DOE,** duration of exposure; **MBL,** mannose binding lectin; **VLBW,** very low birth weight

nosis was based on the presence of at least two clinical symptoms and/or biologic signs suggestive of infection, developing 48 h or later after admission to the NICU. Clinical signs suggestive of septicemia were fever (rectal temperature >38°C) or hypothermia (rectal temperature <36°C), tachycardia or bradycardia, apnea episodes, lethargy, gastric biliary stasis, skin water marks, convulsions, and hypotonia. Laboratory abnormalities included leukopenia (<5000/mm^3) or leukocytosis (>20,000/mm^3), low platelet count (<100,000/mm^3), C-reactive protein level >1.5 mg/dL, and metabolic acidosis (3). According to this definition, patients were divided into two groups: (a) infants without sepsis who did not develop clinical and/or laboratory signs of infection during their hospital stay and (b) infants with sepsis who developed at least one episode of sepsis. Infants with sepsis were further subdivided into three groups: those in whom sepsis was confirmed by a positive blood culture (infants with confirmed sepsis); those who showed clinical and laboratory signs of sepsis but in whom repeated (more than two) blood cultures did not lead to the identification of the infecting pathogen (infants with suspected sepsis); those with suspected sepsis and blood cultures positive for CoNS (infants with suspected sepsis and CoNS-positive cultures). The latter group was kept separate to exclude possible cases secondary to contamination with skin commensal bacteria. Being part of a multicenter study on hospital-acquired infections, all infants underwent the same protocol for antibiotic administration, *i.e.* netilmicin and ampicillin/sulbactam for prophylaxis and vancomycin and BBK8 when sepsis was suspected.

Measurement of MBL levels. Serum MBL was measured using an immunoassay (MBL oligomer ELISA, Antibody Shop, Copenhagen, Denmark) according to the instructions provided by the manufacturer. The linear range was 0.25–40 μg/mL. Sera were diluted 1:300 in the sample diluent provided by the manufacturer. The lowest detectable MBL concentration was 0.075 μg/mL. For subsequent statistical analysis, results below the limit of detection were allocated a value of 0.05 μg/mL.

Statistical analysis. Quantitative data were expressed as medians and IQRs. Between-group comparisons were made with the Mann-Whitney *U* test.

Because the chances of acquiring a nosocomial infection may increase with the length of NICU stay, duration of exposure was also calculated and was defined as the time (days) from admission to the development of infection in infants with confirmed and suspected sepsis and as the length of stay from admission to discharge in those who did not acquire infection.

A multivariate logistic regression analysis was performed, including as relevant variables: BW (≤1500 g, >1500 g), GA (≤32 wk, >32 wk), duration of exposure (DOE) (in days), and MBL serum levels on admission (in μg/mL). The relative contribution of each variable to the risk of developing sepsis was expressed as the OR with a 95% CI.

To define a cutoff value of serum MBL for the risk of neonatal sepsis and the associated specificity and sensitivity levels, a receiver operating characteristic (ROC) analysis was also performed, limited to patients with confirmed sepsis and noninfected controls.

RESULTS

One hundred thirty-eight infants did not develop sepsis and 68 did. Among these, 40 infants had at least one episode of sepsis with positive blood culture (infants with confirmed sepsis). The isolated pathogens included *Klebsiella pneumoniae* ($n = 19$), *Pseudomonas aeruginosa* ($n = 8$), *Escherichia coli* ($n = 4$), *Candida albicans* ($n = 4$), *Staphylococcus aureus* ($n = 3$) and *Enterococcus* ($n = 2$). Ten of 40 developed a second episode, and the isolated pathogens included *K. pneumoniae* ($n = 4$), *P. aeruginosa* ($n = 3$), *C. albicans* ($n = 2$) and *E. coli* ($n = 1$). In 19 infants with sepsis, repeated blood cultures did not lead to the identification of the infecting pathogen (infants with suspected sepsis), and in nine infants, blood cultures were positive for CoNS (infants with suspected sepsis and CoNS-positive cultures).

Neonates with confirmed or suspected sepsis, but not those with CoNS-positive blood cultures, had a median GA and a median BW that were significantly lower than those of noninfected infants. There were no significant differences among groups in male/female ratio, in the intrauterine growth rate, in the maternal complications (including premature membrane rupture, fever, stained amniotic fluid, and diabetes), in the mean age at admission to the NICU, and in the median duration of exposure (Table 1).

Infants with sepsis had median serum MBL levels on admission significantly lower than those of infants without sepsis. Serum MBL levels were significantly decreased only in infants with confirmed or suspected sepsis, whereas patients with CoNS-positive blood culture had MBL levels comparable with those of infants without sepsis (Table 1). Among infants with confirmed sepsis, those in whom more than one episode developed had MBL levels (0.10 μg/mL; IQR 0.06–0.41; $n = 10$) lower ($p < 0.05$) than those who had a single episode (0.30 μg/mL; IQR 0.06–1.24; $n = 30$).

As expected, in-hospital mortality was higher among infected infants (Table 1). However, low levels of MBL on admission did not appear to be associated with the risk of death in infants with sepsis: median MBL levels on admission in infants with confirmed sepsis who died ($n = 11$) were 0.10 μg/mL (IQR 0.05–0.84) and 0.20 μg/mL (IQR 0.10–0.73) in those who survived ($n = 29$) ($p > 0.1$).

In agreement with previous reports (21–23), we found that MBL levels were related to GA: neonates with a GA ≥32 wk ($n = 125$) had MBL levels on admission (1.5 μg/mL; IQR 0.43–3.6) significantly ($p = 0.005$) higher than those with a GA <32 wk ($n = 81$) (0.59 μg/mL; IQR 0.10–2.03).

Univariate analysis showed that the risk of confirmed sepsis decreased with increasing BW, GA, and MBL serum level on

Table 1. *Clinical characteristics and serum MBL levels on admission of the infants studied*

	Without sepsis	Sepsis	Confirmed sepsis	Suspected sepsis	Suspected sepsis and CoNS positive
No.	138	68	40	19	9
Males, %	48.2	51.6	52.5	47.4	55.6
Gestational age, wk	35 (31–38)	32 (28–36)*	32 (28–35)*	30 (28–37)*	35 (29–38)
Birth weight, g	2215 (1773–2835)	1500 (1055–2487)*	1500 (988–2420)*	1210 (1080–2325)*	2350 (1240–3025)
IUGR: SGA-AGA-LGA, %	16.2-76.5-7.4	20.3-72.4-7.2	20.0-72.5-7.5	15.8-77.6-10.5	11.1-88.9-0
Maternal complication, %	28.2	39.7	45.0	36.8	22.2
Duration of exposure, d	14 (8–24)	10 (6–15)	11 (7–14)	10 (6–18)	8 (6–14)
Deaths, no. (%)	12 (8.7)	15 (22.1)†	11 (27.5)‡	3 (15.8)	1 (11.1)
Serum MBL on admission, μg/ml	1.45 (0.43–3.52)	0.45 (0.09–1.68)*	0.17 (0.05–0.96)*	0.65 (0.07–1.6)†	1.70 (0.85–3.6)

Values are expressed as medians and IQRs. The *p* values of differences between groups were calculated with the Mann-Whitney *U* test.
* $p < 0.001$ *versus* infants without sepsis; ** $p < 0.05$ *versus* infants without sepsis; † $p < 0.01$ *versus* infants without sepsis.
IUGR, intrauterine growth rate; SGA, small for gestational age; AGA, adequate for gestational age; LGA, large for gestational age.

Table 2. *Association of GA, BW, DOE, and serum MBL on admission with risk of confirmed sepsis*

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*	OR (95% CI)	*p*
GA >32 wk	0.43 (0.21–0.89)	0.022	2.26 (0.62–7.19)	NS
BW >1500 g	0.18 (0.08–0.37)	<0.001	0.07 (0.02–0.24)	<0.001
DOE	0.98 (0.97–1.01)	NS	0.98 (0.96–1.00)	NS
MBL	0.58 (0.42–0.80)	0.001	0.52 (0.36–0.75)	0.001

NS, not significant.

admission, whereas it was not affected by DOE (Table 2). In the multivariate logistic analysis, adjusting for GA, BW, and DOE, the association between MBL levels on admission and the subsequent development of confirmed sepsis was maintained, with an OR = 0.52 (95% CI: 0.36–0.75; $p = 0.001$) (Table 2).

ROC analysis of the data showed that the best cutoff MBL value for the risk of confirmed sepsis was 0.7 μg/mL (sensitivity = 70.0%; specificity = 68.1%; area under the curve = 0.718; 95% CI: 0.631–0.805), a level that is within the range reported as indicative of MBL insufficiency.

DISCUSSION

Our results suggest that MBL is protective toward the development of neonatal sepsis and that low MBL levels at birth are associated with an increased risk of hospital-acquired sepsis in infants admitted to an NICU.

The diagnosis of sepsis in the neonatal period is often difficult and at times uncertain. Clinical signs and laboratory findings may be nonspecific, particularly in very low birth weight (VLBW) infants. Furthermore, blood cultures may result falsely negative, due to the small amounts of blood available, or erroneously positive, due to contamination from skin commensals. Therefore, we divided infants with sepsis into three groups: those with clinical and laboratory signs suggestive of sepsis and a positive blood culture (confirmed sepsis); those with negative blood cultures, notwithstanding the presence of clinical and laboratory signs of sepsis (suspected sepsis); and those with suspected sepsis and blood cultures positive for CoNS (CoNS positive), with a higher possibility of contamination. The strength of the association between low MBL serum levels and sepsis was not uniformly distributed among these three groups of infants: it was more evident in those with confirmed sepsis, less so in infants with suspected sepsis, and absent in CoNS-positive infants.

There are various possible explanations for this finding. Patients with suspected sepsis had the lowest GA and BW, and it is known that clinical and laboratory signs of infection are less specific in very preterm infants. Therefore, it is possible that some of these infants have been erroneously classified as septic, thus weakening the association between low MBL levels and infection. The CoNS-positive group is heterogeneous in that it most likely includes at least two different populations of patients: those who are truly infected by CoNS (*i.e.* confirmed sepsis) and those in whom the positivity of the blood culture represents a mere contamination by skin commensals (noninfected). Therefore, it is not surprising that the median value of MBL (as well as the range) is similar to that of noninfected infants. Nevertheless, the possibility also exists that MBL may not confer protection against CoNS. In this respect, *Staphylococcus epidermidis* has been reported to have low binding to MBL (24). However, these data should be interpreted with great caution because the *in vitro* binding characteristics to MBL of several pathogens change with the growth phase of the organism; moreover, epidemiologic reports have shown inconsistencies between *in vitro* binding capacity and susceptibility to a particular microbe (25).

In adults and older children, the variability of serum MBL levels is largely, but not completely, accounted for by genetic variants involving the coding sequence and the promoter of the MBL gene (6,7). In neonates, MBL levels are related to GA and increase after birth in term and preterm infants (21,22,26). This maturational pattern has been confirmed by our study: 53% of our neonates with a GA <32 wk had serum MBL levels on admission <0.7 μg/mL compared with only 33% of those with a higher GA ($p = 0.005$). Therefore, low MBL levels in neonates may also be secondary to a maturational defect, possibly involving the liver secretory capacity. However, it should be stressed that the association between serum MBL level on admission and the risk of confirmed sepsis was maintained after adjusting for GA in the multivariate logistic analysis. A recent study in VLBW infants did not find a significant association between genetic variants of the coding region of the MBL gene and the development of sepsis (27). In the latter study, serum MBL levels were not measured and therefore differences in the relative contribution of maturational delay and genetic variability in MBL to the susceptibility to infections may explain the apparent discrepancy between our quantitative study on serum MBL levels and the genetic study by Ahrens *et al.* (27) on MBL genotypes in VLBW infants.

As expected, the univariate analysis showed that GA, BW, and MBL serum level on admission were all significantly associated with the risk of sepsis, whereas DOE was not. A subsequent multivariate analysis showed that the association between serum MBL level on admission and the risk of confirmed sepsis was maintained, indicating that high serum MBL levels protect from sepsis, independently of GA, BW, and DOE.

Among infants with confirmed sepsis, the MBL levels on admission were lower in those who died than in those who survived, but the difference did not reach statistical significance. This could also be due to the small number of subjects in the two groups of infants, and the possible prognostic value of MBL initial levels in relation to survival in infected infants will have to be investigated in a larger number of subjects.

In our study, we could not take into consideration other factors, known to be directly or indirectly associated with the risk of neonatal infections and mortality, such as the clinical conditions at birth or before NICU admission (Apgar and clinical severity scores), the indication for NICU admission (besides prematurity), the type and duration of invasive procedures used *(e.g.* indwelling catheters, mechanical ventilation). A larger, prospective, multicenter study is therefore needed to confirm our findings and also to describe the

changes in MBL levels in response to infection and their possible relationship with outcome.

Although preliminary, our results suggest that the simple measurement of serum MBL levels on admission may help to identify neonates at high risk of developing sepsis in the NICU. Because substitution therapy with MBL is now possible and its safety has been recently reported (28), confirmation of the association between low MBL levels and neonatal sepsis may provide the rationale for a controlled trial to evaluate the efficacy of early administration of MBL in preventing the development of sepsis in neonates, particularly in VLBW infants, admitted to intensive care units.

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