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# Low mannose-binding lectin (MBL) levels in neonates with pneumonia and sepsis

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## Summary

**We investigated whether deficiency of mannose-binding lectin (MBL), a component of innate immunity, is associated with neonatal pneumonia and sepsis during the first 72 h, i.e. early onset, and during the first month after birth. In 88 neonatal intensive care patients (71 premature), *MBL2* genotype and MBL plasma levels at birth were determined prospectively by Taqman analysis and enzyme-linked immunosorbent assay, respectively. Thirty-five neonates (40%) had low, i.e. ≤ 0·7 μg/ml, MBL plasma levels at birth. Median (interquartile range) MBL plasma levels in 32 no early-onset sepsis (EOS) cases, 44 possible EOS cases and 11 EOS cases were 1·57 (0·57–2·67) μg/ml, 1·05 (0·41–1·70) μg/ml and 0·20 (0·10–0·77) μg/ml, respectively ($P < 0·01$). During the first month, 28 neonates (32%) had no infection, 49 (55%) had suspected infection, five (6%) had pneumonia and six (7%) had culture-proven sepsis. Low MBL levels at birth were associated both with an increased risk of developing pneumonia (OR: 12·0; 95% CI: 1·1–126·1; $P = 0·04$) and culture-proven sepsis (OR: 15·0; 95% CI: 1·5–151·3; $P = 0·02$). These results were confirmed by genetic analysis of MBL deficiency. Low MBL levels at birth are associated with an increased risk of early-onset sepsis, culture-proven sepsis and pneumonia during the first month of life.**

**Keywords:** complement, innate immunity, mannose-binding lectin, neonate, sepsis

## Introduction

Despite improved neonatal care over the past decades, infections remain common and sometimes life-threatening in neonates admitted to the neonatal intensive care unit (NICU) [1,2]. Sepsis and pneumonia have the highest morbidity [3]. Early-onset sepsis (EOS) in neonates occurs in the perinatal period, while late-onset infection, especially sepsis and pneumonia, is transmitted in the nursery. For many years, a search has been ongoing to find predictors for neonatal sepsis that identify effectively patients who are at risk of infection [4].

Mannose-binding lectin (MBL) is a plasma protein that plays an important role in the innate immune defence. MBL activates the lectin pathway of the complement system by binding to various microorganisms. This leads to opsonization and enhanced phagocytosis [5]. Circulating MBL plasma levels are determined genetically and may vary between 0 and 10 μg/ml [6,7]. Three structural mutations in exon-1 of the *MBL2* gene interfere with the assembly of the protein and cause decreased functional MBL plasma levels [7]. These variant genotypes are designated *O*, while the normal wild-type allele is called *A* [8]. In addition, three polymorphisms in the promoter region affect the MBL plasma level, but only the *X* variant of one of these polymorphisms is associated with low plasma levels. In contrast, the *Y* variant is associated with high MBL plasma levels [9]. Normal MBL plasma levels are seen in individuals with the *YA/YA* and *YA/XA* wild-type genotypes, whereas the *XA/XA* genotype is associated with both normal and low plasma levels [6,7]. Individuals with variant structural alleles (*YA/O*, *XA/O* and *O/O*) have low functional MBL plasma levels; functional MBL is almost absent in the *XA/O* and *O/O* genotypes [6,10]. In clinical studies, different definitions are used to describe genetic MBL deficiency, but most MBL disease associations are found in the presence of variant structural alleles [7]. Therefore, we will compare neonates with variant *MBL2* structural genotypes (*YA/O*, *XA/O* and *O/O*) to neonates with wild-type *MBL2* structural genotypes (*YA/YA*, *YA/XA* and *XA/XA*).

Variant *MBL2* genotypes and low MBL plasma levels can be found in approximately 40% of the European population

[6,11–13], and MBL deficiency has been associated with an increased susceptibility to infections, especially in children and immunocompromised individuals [14–16]. Very recently, low MBL levels at birth were found in neonates with nosocomial sepsis, in contrast to previous observations by others [17]. Sepsis definitions varied in these studies. In neonates, low MBL levels are associated not only with variant *MBL2* genotype, but also with low gestational age (GA) [10,18–20]. Therefore, detection of MBL deficiency at birth should be based on actual MBL plasma levels rather than on *MBL2* genotype. However, additional genetic analyses are important because we showed that neonates with wild-type *MBL2* genotypes but low MBL levels at birth were able to obtain normal levels within time, in contrast to neonates with variant *MBL2* genotypes [10].

In contrast to the previously published studies on MBL deficiency and neonatal sepsis, to our knowledge we are the first to determine both *MBL2* genotype and MBL plasma levels at birth in neonates admitted to the NICU. The aim of our study was to investigate whether low MBL levels or variant *MBL2* genotypes were associated with the occurrence of EOS during the first 72 h after birth, and with culture-proven sepsis or pneumonia during the first month of life.

## Methods

### Subjects and samples

From July 2002 until June 2003, we performed a prospective cohort study in the NICU of the Academic Medical Center, Amsterdam, the Netherlands. All neonates in whom blood was drawn for routine care within 24 h after birth were eligible. Patients with congenital abnormalities were excluded. Eighty-eight neonates (71 premature: gestational age < 37 weeks) were included consecutively after written informed consent was given by the parents. Recently, we described the prevalence of MBL deficiency in 85 neonates of this cohort [10]. In the remaining three patients, MBL analyses were performed recently in stored blood samples. The study protocol was approved by the local medical ethics committee.

We determined *MBL2* genotype and MBL plasma levels in umbilical cord blood and neonatal blood drawn within 24 h after birth. Previously, we showed that MBL plasma levels in these samples are comparable [10]. When infection was suspected (see below), routine laboratory investigations included total leucocyte and leucocyte differentiation counts, C-reactive protein (CRP) and blood cultures. CRP levels were considered elevated above 10 mg/l [21]. The normal range for total leucocyte count was $5–30 \times 10^9$ cells/l [22]. Chest X-ray and tracheal aspirate cultures were performed when indicated clinically. Specimens were processed according to standard procedures.

### Clinical data and infection classification

Along with general pre- and intrapartum clinical data, infectious signs and symptoms were recorded prospectively. They were divided into five categories: (1) temperature instability (< 37·0°C or > 38·5°C); (2) respiratory distress, e.g. dyspnoea, tachypnoea (> 60 breaths/min), apnoea, ventilation support, oxygen requirement, surfactant use; (3) cardiovascular dysfunction, e.g. tachycardia (> 160 beats/min), bradycardia (< 100 beats/min), decreased peripheral circulation, hypotension (diastolic blood pressure < 40 mm Hg), need for vasopressor support or inotropic medication; (4) neurological irregularities, e.g. hypotonia, lethargy, irritability; and (5) gastrointestinal problems, e.g. milk intolerance, vomiting, abdominal distension, suspicion of necrotizing enterocolitis [21,23]. Maternal risk factors for infection were fever (temperature > 38·0°C) and prolonged rupture of membranes > 24 h before delivery [21,24]. Diabetes gravidarum, maternal hypertension and pre-eclampsia were considered pregnancy-related diseases. Mothers who developed fever received a single shot of amoxicillin and gentamicin. When neonates were transferred to another hospital within 30 days after birth, clinical records and culture results of these hospitals were retrieved.

For our analysis, we used two outcome measures: (1) EOS within 72 h after birth and (2) infectious outcome during the first month of life. Clinical and laboratory records were reviewed and concerning EOS, three physicians (F. F., M. O. and K. D.), blind for MBL values, classified neonates independently as 'no EOS' cases, 'possible EOS' cases and 'EOS' cases (clinical EOS and culture-proven EOS). Discrepancies (seven of a total of 88 neonates) were solved by a consensus meeting.

For this purpose we used strictly defined criteria, which have been used previously in clinical studies on neonatal sepsis [20,21,23]: no EOS cases not had been evaluated for infection and therefore had not received any antibiotic treatment. The remaining neonates had received an EOS work-up because of maternal risk factors or neonatal clinical signs; a blood culture was drawn and antibiotics were administered. Possible EOS cases showed a combination of clinical signs, laboratory abnormalities or maternal risk factors without fulfilling the criteria of EOS. These neonates had received antibiotics (penicillin and gentamicin) for up to 7 days. Culture-proven EOS was defined as a combination of infectious signs and symptoms and a positive blood culture. Neonates were classified as having clinical EOS in the presence of maternal risk factors, increased CRP levels or pathological leucocyte counts accompanied by typical clinical signs of infection from at least three of the five categories [21,23]. EOS cases received antibiotics intravenously for at least 7 days.

The second outcome measure, the occurrence of infections during the first month of life, included the following

outcome groups: (1) no infection at all; (2) suspected infection (including clinical EOS); (3) pneumonia; and (4) culture-proven sepsis. Pneumonia was defined as the presence of clinical respiratory symptoms, abnormalities on chest X-ray and positive tracheal aspirate cultures [25]. Culture-proven sepsis required the presence of typical infectious signs and symptoms in combination with a positive blood culture [26]. Severe infection was defined as the presence of culture-proven sepsis or pneumonia.

### Assays

MBL measurements were performed at Sanquin Research and the Landsteiner Laboratory, Academic Medical Center, Amsterdam. MBL plasma levels were measured by an enzyme-linked immunosorbent assay as described previously [13,27]. Briefly, mannose was coated to the solid phase and after incubation with plasma, biotinylated mouse-anti-human MBL IgG (Tacx *et al.* [27]; 10 μg/ml, Amsterdam) was used as detection antibody. A receiver-operator characteristic curve yielded an optimal cut-off MBL plasma level of 0·7 μg/ml to discriminate between neonates with wild-type *versus* variant *MBL2* genotypes. MBL plasma levels ≤ 0·7 μg/ml will be referred to hereafter as low MBL plasma levels.

Genotyping was performed independently of the clinical data collection, as described previously [10].

### Statistical analysis

The Kruskal–Wallis test was used to determine whether MBL plasma levels differed significantly between the different outcome categories, followed by Mann–Whitney *U*-tests for single-outcome categories comparisons with Bonferroni correction for multiple testing. As numbers in the pneumonia and sepsis categories were small, both were combined into one severe infection outcome category for statistical analysis purposes. To examine the association of low MBL levels (≤ 0·7 μg/ml) and *MBL2* genotypes (variant *versus* wild-type) with EOS and first-month infectious outcome categories, we estimated odds ratios (ORs) and 95% confidence intervals (CIs) from multinominal logistic regression analysis. These analyses were repeated with a cut-off plasma level of 0·2 μg/ml.

Univariate associations of GA, pregnancy-related disease, fetal distress and mode of delivery with EOS (no EOS, possible EOS and EOS) were studied with multinomial logistic regression analysis. Subsequently, we performed explorative multivariate analysis to study the association between MBL deficiency and EOS, adjusted for GA and clinical characteristics associated univariately with EOS, keeping in mind that due to small numbers reliability is limited. To optimize reliability, no more than two variables were added into a model at a time [28]; SPSS 12·0·1 for Windows statistical software was used.

## Results

### Clinical characteristics and MBL

The clinical characteristics of the 88 neonates (47 males) are presented in Table 1. The median GA was $32^{+3}$ (interquartile range (IQR): $29^{+6}$–$36^{+1}$) weeks. The median birth weight was 1620 (IQR: 1200–2605) g. The mothers of 42 neonates showed signs of infection: maternal fever ($n = 19$), prolonged rupture of membranes ($n = 16$), or both ($n = 7$).

In 87 of 88 neonates MBL plasma levels were determined at birth. The remaining neonate had the *YA/YA* genotype and a MBL plasma level of 2·63 μg/ml 1 week after birth. Thirty-five neonates (40%) had low MBL plasma levels (Table 2). Due to the very small size of the blood samples taken, genotypes could be determined in only 70 neonates (80%). Twenty-three neonates (33%) had variant *MBL2* genotypes (Tables 1 and 2). Outcome measures, MBL plasma levels, birth weight, GA and other clinical characteristics did not differ between neonates with and without known *MBL2* genotypes (*P*-values > 0·25). The median MBL plasma level

**Table 1.** Clinical characteristics of 88 neonates.

| Clinical characteristic | *n* (%) | Median (interquartile range) |
|---|---|---|
| Neonate characteristics | | |
| Birth weight (g) | | 1620 (1200–2605) |
| Gestational age (weeks) | | $32^{+3}$ ($29^{+6}$–$36^{+1}$) |
| Prematurity (< 37 weeks) | 71 (81) | |
| Duration postnatal antibiotic treatment (days) | | 3 (0–5) |
| *MBL2* genotype | | |
| YA/YA | 31 (44) | |
| YA/XA | 15 (22) | |
| XA/XA | 1 (1) | |
| YA/O | 13 (19) | |
| XA/O | 5 (7) | |
| O/O | 5 (7) | |
| Delivery characteristics | | |
| Pregnancy-related disease | 23 (26) | |
| Vaginal culture | | |
| Missing | 43 (49) | |
| No bacterial growth | 29 (33) | |
| Bacterial growth | 16 (18) | |
| Maternal risk factors of infection: | 42 (48) | |
| Maternal fever | 26 (30) | |
| Prolonged rupture of membranes | 23 (26) | |
| Antenatal antibiotic administration | 26 (30) | |
| Mode of delivery | | |
| Vaginal | 35 (40) | |
| Caesarean section | 53 (60) | |
| Fetal distress | 46 (52) | |

**Table 2.** Risks of possible early-onset sepsis (EOS) and EOS compared to no EOS according to mannose-binding lectin (MBL) plasma levels, *MBL2* genotype, gestational age and other selected clinical characteristics.

| | Possible EOS | | | | EOS | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | *n* | OR | 95% CI | *P*-value | *n* | OR | 95% CI | *P*-value |
| Pregnancy-related disease | | | | | | | | |
| No | 37 | Ref | | | 10 | Ref | | |
| Yes | 8 | 0·3 | 0·1–0·8 | 0·02 | 1 | 0·1 | 0·0–1·1 | n.s. |
| Fetal distress | | | | | | | | |
| No | 25 | Ref | | | 4 | Ref | | |
| Yes | 20 | 0·5 | 0·2–1·4 | n.s. | 7 | 1·2 | 0·3–4·9 | n.s. |
| Gestational age (1 week↑) | 45 | 1·0 | 0·9–1·1 | n.s. | 11 | 1·0 | 0·8–1·2 | n.s. |
| Mode of delivery | | | | | | | | |
| Vaginal | 22 | Ref | | | 7 | Ref | | |
| Caesarean section | 23 | 0·2 | 0·1–0·7 | < 0·01 | 4 | 0·1 | 0·0–0·6 | < 0·01 |
| MBL plasma level (1 μg/ml↑) | 44 | 0·7 | 0·4–1·1 | n.s. | 11 | 0·2 | 0·1–0·7 | 0·01 |
| MBL plasma level | | | | | | | | |
| Normal (> 0·7 μg/ml) | 27 | Ref | | | 3 | Ref | | |
| Low (≤ 0·7 μg/ml) | 18 | 1·7 | 0·6–4·5 | n.s. | 8 | 6·8 | 1·5–31·6 | 0·01 |
| MBL plasma level | | | | | | | | |
| Plasma level > 0·2 μg/ml | 38 | Ref | | | 5 | Ref | | |
| Plasma level (≤ 0·2 μg/ml | 7 | 2·8 | 0·5–14·3 | n.s. | 6 | 18·0 | 2·8–115·6 | < 0·01 |
| *MBL2* genotype | | | | | | | | |
| Wild-type (*YA/YA, YA/XA, XA/XA*) | 25 | Ref | | | 3 | Ref | | |
| Variant (*YA/O, XA/O, O/O*) | 13 | 3·3 | 0·8–13·2 | n.s. | 7 | 14·8 | 2·4–91·2 | < 0·01 |

OR, odds ratio; CI, confidence interval; Ref, reference; n.s., not significant. Multinominal logistic regression was used.

was 1·63 (IQR: 0·97–2·03) μg/ml in neonates with the wild-type *MBL2* genotypes and 0·17 (IQR: 0·09–0·46) μg/ml in neonates with variant *MBL2* genotypes ($P < 0·01$).

### Early-onset sepsis

Thirty-two (36%) neonates were not suspected of EOS (no EOS cases; median GA: $32^{+6}$ weeks). Forty-five (48%) neonates were classified as possible EOS cases (median GA: $30^{+3}$ weeks). Eleven (16%) neonates were classified as EOS cases (median GA: $32^{+5}$ weeks). Of these, one had a positive blood culture (*Haemophilus parainfluenzae*); the remaining EOS cases were clinical EOS cases. *Listeria monocytogenes* was cultured in the amniotic fluid of three clinical EOS cases. The CRP level was elevated in one no EOS case, in eight possible EOS cases and in five EOS cases ($P = 0·01$). The median duration of antibiotic treatment started within 72 h after birth was 5 (IQR: 2–7) days in neonates with low MBL plasma levels compared to 3 (IQR: 0–4) days in neonates with normal MBL plasma levels ($P = 0·01$).

Neonates with low MBL plasma levels had an increased risk of EOS compared to neonates with normal levels (OR: 6·8; 95% CI: 1·5–31·6; $P = 0·01$; Table 2). A cut-off MBL plasma level of 0·20 μg/ml yielded similar results (Table 2). Median MBL plasma levels were 1·57 (IQR: 0·57–2·67) μg/ml in no EOS, 1·05 (IQR: 0·41–1·70) μg/ml in possible EOS and 0·20 (IQR: 0·10–0·77) μg/ml in EOS cases (Kruskal–Wallis test, $P < 0·01$; Fig. 1). Only median MBL plasma levels of EOS cases were decreased significantly compared to no EOS cases (Bonferroni-corrected Mann–Whitney *U*-test, $P < 0·01$). The MBL plasma level was 0·20 μg/ml in the culture-proven EOS case.

*MBL2* genotypes were detected in 22 no EOS cases, 38 possible EOS cases and 10 EOS cases. Neonates with variant *MBL2* genotypes had a 15-fold higher risk of EOS compared to neonates with wild-type *MBL2* genotypes (OR: 14·8; 95% CI: 2·4–91·2; $P < 0·01$; Table 2).

### Explorative multivariate analysis EOS

The risk of EOS was decreased in neonates born by caesarean section (OR: 0·1; 95% CI: 0·0–0·6; $P < 0·01$, Table 2). GA and the remaining selected clinical characteristics were not associated with EOS. Explorative multivariate analyses suggested that the association between low MBL levels and EOS was maintained when adjusted for GA (adjusted OR of low MBL: 7·0; 95% CI: 1·5–32·9, $P = 0·01$) or mode of delivery (adjusted OR: 7·3, 95% CI: 1·5–36·0, $P = 0·02$, Table 3). The association between variant *MBL2* genotypes and EOS was also maintained after adjustment for GA and mode of delivery, respectively (Table 3).

### First-month infectious outcome

During the first month of life, 28 neonates (32%) did not show any signs of infection at all, 49 (55%) had a suspected

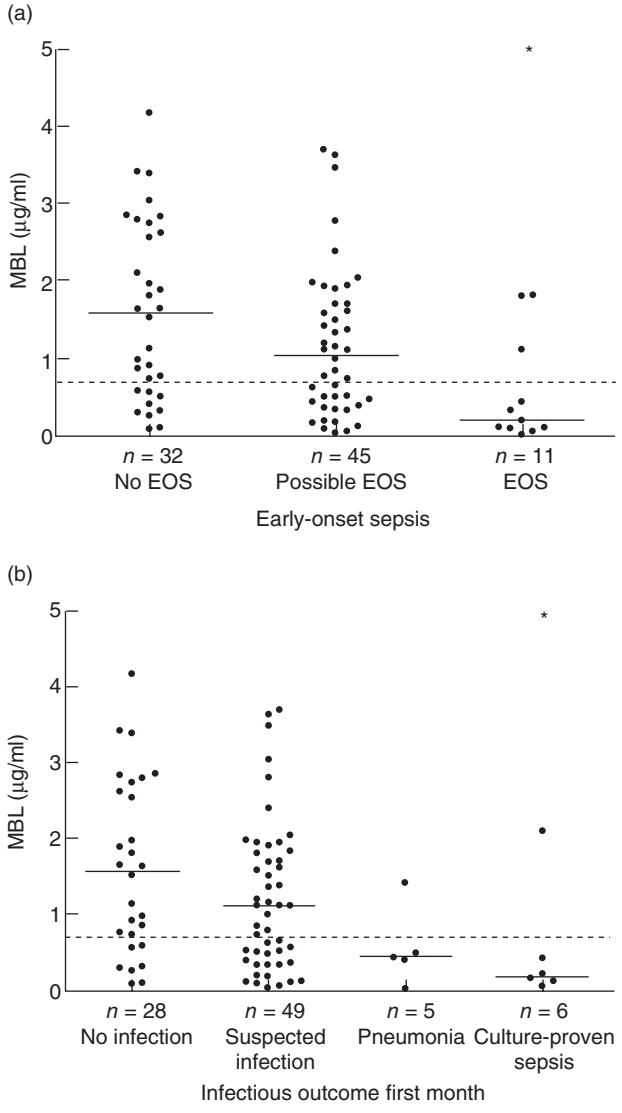


**Fig. 1.** (a) Early-onset sepsis (EOS). Mannose-binding lectin (MBL) plasma levels in 32 no EOS cases, 45 possible EOS cases and 11 EOS cases. *P*-value (Kruskal–Wallis test) between groups < 0·01. (b) First month infectious outcome. MBL plasma levels in 28 no infection cases, 49 suspected infection cases, five pneumonia cases and six culture-proven sepsis cases. *P*-value (Kruskal–Wallis test) between groups is 0·03. MBL plasma level of one neonate missing. Dotted lines represent MBL plasma level of 0·7 μg/ml **P*-value < 0·01 compared to reference (no EOS or no infection) group; Mann–Whitney *U*-test, adjusted for multiple comparisons.

infection at least once, five (6%) had pneumonia and six (7%) had culture-proven sepsis (including the culture-proven EOS case). The microorganisms cultured in the tracheal aspirates of the neonates with pneumonia were *Citrobacter*, coagulase-negative *Staphylococcus epidermidis* (CNS), *Ureaplasma urealyticum*, *Klebsiella pneumoniae* and *Stenothrophomonas maltophilia*. CNS was cultured in the blood of the five neonates with late-onset culture-proven sepsis. Only one neonate died, but this was not due to infectious complications.

During the first month, neonates with low MBL levels had an increased risk of severe infections (pneumonia or culture-proven sepsis) (OR: 13·5; 95% CI: 2·3–78·1; $P < 0·01$) compared to neonates with normal levels (Table 4). Four of five neonates with pneumonia and five of six neonates with culture-proven sepsis had low MBL levels at birth (Fig. 1). A cut-off MBL plasma level of 0·2 μg/ml yielded similar results (data not shown). Median MBL plasma levels were 1·57 (IQR: 0·62–2·70) μg/ml in the no infection group, 1·11 (IQR: 0·37–1·81) μg/ml in the suspected infection group and 0·41 (IQR: 0·14–0·46) μg/ml in the severe infection group (Kruskal–Wallis, $P < 0·01$). After Bonferroni correction, only the median MBL plasma level of the severe infection compared to the no infection group was decreased significantly ($P < 0·01$). Median plasma levels in the pneumonia and culture-proven sepsis group separately were 0·43 (IQR: 0·21–0·95) μg/ml and 0·18 (IQR: 0·10–0·85 μg/ml), respectively (Fig. 1).

Neonates with variant *MBL2* genotypes had a statistically significant increased risk of developing any severe infection during the first month of life (OR: 10·0; 95% CI: 1·5–65·7; $P = 0·02$), compared to neonates with wild-type *MBL2* genotypes (Table 4). Of the 11 neonates with a severe infection, five had variant, three had wild-type and three had unknown *MBL2* genotypes. The separate risks of pneumonia (OR: 9·0; 95% CI: 1·0–78·6, $P = 0·05$) and culture-proven sepsis (OR: 12·0; 95% CI: 0·8–177·4, $P = 0·07$) also tended to be increased (Table 4).

## Discussion

Our findings suggest that MBL deficiency increases the susceptibility to infections in premature and term NICU-patients. Both the presence of low ($\leq$ 0·7 μg/ml) MBL plasma levels at birth and variant *MBL2* genotypes appeared to be associated with EOS. In addition, neonates with low MBL plasma levels appeared to have an increased risk of severe infections during the first month of life compared to neonates with normal plasma levels. Although our study was hampered by small numbers, these results are in agreement with a recently reported increased risk of nosocomial sepsis with low MBL levels in a cohort of 206 neonates [17]. Furthermore, variant *MBL2* genotypes have been associated with an increased susceptibility to develop sepsis and septic shock in critically ill children and adults previously [11,29,30]. Low MBL levels might increase the susceptibility for sepsis by a decreased capacity of phagocytosis or opsonization of microorganisms.

The present study on the association between MBL deficiency and neonatal infections is, to our knowledge, the first to involve both MBL plasma level and *MBL2* genotype. This is very important in studies on MBL in neonates, as premature neonates without *MBL2* gene mutations can have only

**Table 3.** Multivariate analysis of the association between early-onset sepsis (EOS) and low mannose-binding lectin (MBL) levels and variant structural *MBL2* genotypes, respectively.

| | Possible EOS | | | EOS | | |
|---|---|---|---|---|---|---|
| Model | Adjusted OR | 95% CI | *P*-value | Adjusted OR | *P*-value | 95% CI |
| Model 1 | | | | | | |
| Gestational age (1 week↑) | 1·0 | 0·9–1·1 | n.s. | 1·0 | 0·8–1·2 | n.s. |
| MBL plasma level | | | | | | |
| Normal (> 0·7 μg/ml) | Ref | | | Ref | | |
| Low (≤ 0·7 μg/ml) | 1·7 | 0·6–4·6 | n.s. | 7·0 | 1·5–32·9 | 0·01 |
| Model 2 | | | | | | |
| Mode of delivery | | | | | | |
| Vaginal | Ref | | | Ref | | |
| Caesarean section | 0·2 | 0·1–0·7 | < 0·01 | 0·1 | 0·0–0·6 | 0·01 |
| MBL plasma level | | | | | | |
| Normal (> 0·7 μg/ml) | Ref | | | Ref | | |
| Low (≤ 0·7 μg/ml) | 1·8 | 0·6–4·9 | n.s. | 7·3 | 1·5–36·0 | 0·02 |
| Model 3 | | | | | | |
| Gestational age (1 week↑) | 1·0 | 0·9–1·2 | n.s. | 1·0 | 0·8–1·2 | n.s. |
| *MBL2* genotype | | | | | | |
| Wild-type (*YA/YA, YA/XA, XA/XA*) | Ref | | | Ref | | |
| Variant (*YA/O, XA/O, O/O*) | 3·3 | 0·8–13·2 | n.s. | 14·9 | 2·4–92·3 | < 0·01 |
| Model 4 | | | | | | |
| Mode of delivery | | | | | | |
| Vaginal | Ref | | | Ref | | |
| Caesarean section | 0·2 | 0·1–0·8 | 0·03 | 0·1 | 0·0–0·8 | 0·02 |
| *MBL2* genotype | | | | | | |
| Wild-type (*YA/YA, YA/XA, XA/XA*) | Ref | | | Ref | | |
| Variant (*YA/O, XA/O, O/O*) | 3·1 | 0·7–13·0 | n.s. | 13·5 | 2·1–89·2 | < 0·01 |

OR, odds ratio; CI, confidence interval; NS, not significant. Multinominal logistic regression was used.

**Table 4.** Risks of infectious outcome during the first month of life.

| | MBL plasma level | | | | |
|---|---|---|---|---|---|
| Outcome measure | Normal (> 0·7 μg/ml) *n* | Low (≤ 0·7 μg/ml) *n* | OR | 95% CI | *P*-value |
| (a) According to mannose-binding lectin (MBL) plasma level (*n* = 88) | | | | | |
| Severe infection | 2 | 9 | 13·5 | 2·3–78·1 | < 0·01 |
| Culture-proven sepsis | 1 | 5 | 15·0 | 1·5–151·3 | 0·02 |
| Pneumonia | 1 | 4 | 12·0 | 1·1–126·1 | 0·04 |
| Suspected infection | 30 | 19 | 1·9 | 0·7–5·3 | n.s. |
| No infection | 21 | 7 | Ref | – | |
| | *MBL2* genotype | | | | |
| | Wild-type | Variant | | | |
| (b) According to *MBL2* genotype (*n* = 70) | | | | | |
| Severe infection | 3 | 5 | 10·0 | 1·5–65·7 | 0·02 |
| Culture-proven sepsis | 1 | 2 | 12·0 | 0·8–177·4 | n.s. |
| Pneumonia | 2 | 3 | 9·0 | 1·0–78·6 | 0·05 |
| Suspected infection | 26 | 15 | 3·5 | 0·9–13·7 | n.s. |
| No infection | 18 | 3 | Ref | – | |

Multinominal logistic regression was used. The odds ratio (OR) can be interpreted as an estimation of the relative risk for the infectious outcome category compared to the 'no infection' category. CI, confidence interval; n.s., not significant.

temporary low MBL levels at birth [10]. De Benedetti *et al.* [17] might have overestimated the infection risk because they determined MBL serum levels only once on admission to the NICU. Premature neonates with low MBL levels at birth that had wild-type *MBL-2* genotypes might have developed normal MBL levels before the onset of sepsis [10,19]. However, our genetic analyses support the findings of de Benedetti *et al.* [17]. Therefore, persistently low MBL levels indeed appear to play a role in susceptibility to neonatal infections during the first weeks of life. Ahrens *et al.* [4] did not find an increased infection risk in premature neonates with exon-1 mutations, but they probably underestimated the infection risk because the effect of low MBL plasma levels due to prematurity and the *X* promoter polymorphism were not investigated [4]. Hilgendorff *et al.* [20] did not find an increased EOS risk in neonates with low MBL levels, but they had a smaller cohort.

Comparison of studies on MBL and neonatal sepsis is also hampered by different sepsis definitions used in different studies. For instance, Ahrens *et al.* [4] and De Benedetti *et al.* [17] used culture-proven sepsis at any time during hospital stay, while Hilgendorff *et al.* [20] studied congenital sepsis diagnosed clinically within 72 h of life. Although culture-proven infections are preferred in clinical research, it is now accepted widely that early-onset neonatal sepsis is often a clinical diagnosis because blood cultures can be false-negative after antenatal administration of antibiotics [20–23]. By using generally accepted criteria for clinical EOS, similar to previous reports on the subject, exclusion of other explanations of the clinical symptoms is pursued [20–23]. An advantage of our study is that in contrast to the three previously published studies in neonates, we investigated the presence of early-onset sepsis and infectious outcome during the first month separately. In this way we overcome sepsis definition difficulties.

Our cohort consisted of primarily premature neonates. Given the fact that low gestational age is associated with low MBL levels and increased risk of infection [10,24], the selection of younger children may lead to an overestimation of the observed association between MBL and infection risk. As a consequence, these results may not be applicable to term neonates. However, as the association between low MBL plasma levels and risk of EOS was maintained after adjustment for GA in multivariate analysis, we do not expect that maturational MBL plasma level differences affected our results. The association between low MBL plasma levels and increased risk of EOS is also independent of mode of delivery. However, because our study number was small, a larger prospective study is needed to confirm these findings. The most likely explanation for the univariate association between mode of delivery and EOS is that children born by uncomplicated vaginal delivery were admitted to the NICU only in case of (suspected) infection.

In conclusion, neonates with low MBL plasma levels appear to be at increased risk of severe neonatal infections, both of early- and late-onset. Therefore, MBL measurements might be used to identify which neonates are prone for infections. Furthermore, in the near future substitution of plasma-purified or recombinant MBL may protect neonates with low MBL plasma levels from sepsis or pneumonia [31]. However, a large, well-designed prospective multi-centre study should confirm the association between low MBL plasma levels and both early-onset and late-onset sepsis, taking into account the complicated developmental profile of MBL in neonates. This study must include serial MBL level measurements during the first weeks after birth and at the onset of and during infection. In addition, *MBL2* genetic polymorphisms are required to validate these measurements.

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