Differential Role of the Lectin Pathway of Complement Activation in Susceptibility to Neonatal Sepsis

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Background. The incidence of bacterial sepsis during the neonatal period is high. Mannan-binding lectin (MBL), L-ficolin, and H-ficolin recognize microorganisms and activate the complement system via MBL-associated serine proteases (MASPs). This study investigated whether cord blood concentrations of the lectin pathway proteins are associated with neonatal sepsis.

Methods. This was a case-control study including 47 infants with culture-proven sepsis during the first month of life and 94 matched controls. MBL, L-ficolin, H-ficolin, MASP-2, and MASP-3 levels were measured in cord blood with use of enzyme-linked immunosorbent assay and time-resolved immunofluorometric assay. Multivariate logistic regression was performed.

Results. Infants with gram-positive sepsis had significantly lower H-ficolin cord blood concentrations than controls (multivariate odds ratio [OR], 4.00; 95% confidence interval [CI], 1.51–10.56; $P = .005$), whereas infants with gram-negative sepsis had lower MBL cord blood concentrations (OR, 2.99; 95% CI, 0.86–10.33; $P = .084$). When excluding patients with postoperative sepsis, multivariate analysis confirmed that low H-ficolin was associated with a significantly higher risk of gram-positive sepsis (OR, 3.71; 95% CI, 1.26–10.92; $P = .017$) and late-onset sepsis (OR, 3.14; 95% CI, 1.07–9.21; $P = .037$). In contrast, low MBL was associated with a significantly higher risk of gram-negative sepsis (OR, 4.39; 95% CI, 1.10–17.45; $P = .036$) and early-onset sepsis (OR, 3.87; 95% CI, 1.05–14.29; $P = .042$). The concentrations of all the lectin pathway proteins increased with gestational age ($P < .01$).

Conclusions. These preliminary results indicate that low MBL concentrations are a susceptibility factor for gram-negative sepsis, and low H-ficolin concentrations indicate susceptibility to gram-positive sepsis. The decreased expression of lectin pathway proteins in neonates must be considered to be an additional form of neonatal immunodeficiency.

Severe infections represent the main cause of neonatal mortality accounting for >1 million neonatal deaths worldwide every year [1]. Thanks to advances in perinatal and intensive care, the prognosis for infants has improved over the last decade [2]. Implementation of recommendations for antibiotic prophylaxis in mothers carrying group B streptococci (GBS) has lead to a significant decrease in GBS neonatal sepsis [3]. However, even in developed countries, morbidity and mortality due to neonatal sepsis remain high and cause severe long-term sequelae, such as bronchopulmonary dysplasia and cerebral paresis [4, 5].

The adaptive immune system of neonates, particularly of preterm infants, is severely impaired because of immature B and T cell function [6, 7]. After birth, the neonate is exposed to a diversity of potentially lethal pathogens never confronted by its immune system. In the absence of a functional adaptive immunity, protection by innate immune defenses is crucial [8]. Innate immunity is mediated by pattern recognition molecules recognizing conserved pathogen-associated molecular patterns, such as repetitive sugar arrays present on many microorganisms but not on mammalian cells [9]. The complement system, a mainstay of innate immuni-

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ty, eliminates microorganisms and enhances adaptive immune response [9]. Complement activation occurs by the classical, the alternative, and the evolutionary more ancient lectin pathway [10, 11]. The latter consists of soluble pattern recognition molecules containing collagen-like regions, namely mannan-binding lectin (MBL), L-ficolin (ficolin-2), and H-ficolin (ficolin-3 or Hakata-antigen) [12]. Both MBL and ficolins rely on MBL-associated serine proteases (MASPs) to activate the complement system [9]. On binding of MBL-MASP or ficolin-MASP complexes to microbial surfaces, MASP-2 sequentially cleaves C4 and C2, thereby generating the C3 convertase C4bC2b, which leads to opsonization and lysis of pathogens and recruitment of inflammatory cells [13].

MBL recognizes a broad range of pathogens exposing sugar residues, whereas ficolins bind to acetylated molecules on microbial surfaces, such as GlcNAc and GalNAc [9, 14, 15]. Because of single nucleotide polymorphisms within the MBL2 gene and the associated promoter region, MBL deficiency affects ~30% of the white population [9]. Single nucleotide polymorphisms resulting in decreased protein concentrations have been identified in the genes encoding ficolins (FCN1, FCN2, and FCN3) and MASPs (MASP1 and MASP2) [16, 17].

MBL deficiency has been extensively investigated in adult patients and is associated with an increased susceptibility to sepsis [18–21]. In contrast, studies on neonatal sepsis yielded partially conflicting results [22–26]. In spite of the close structural and functional similarities, the role of ficolins and MASPs in sepsis remains largely unknown [9], and no study has assessed the role of the entire lectin pathway of complement in host immunity. The aim of the present study was to investigate whether cord blood concentrations of lectin pathway proteins are associated with neonatal sepsis.

PATIENTS AND METHODS

Patients. Infants born from November 2002 through November 2007 at the Department of Obstetrics, University of Bern, Switzerland, were eligible for this study if cord blood serum had been retrieved and stored. Sepsis cases were defined as infants fulfilling all of the following criteria: (1) clinical signs of sepsis (temperature instability, irritability, apathia, feeding difficulties, prolonged capillary refill, apnea, tachycardia, or tachypnea); (2) elevated infectious parameters (C-reactive protein level, >20 mg/L; leukocyte level, $<5 \times 10^9$ leukocytes/L, immature/total neutrophil ratio, >0.2); (3) recovery of pathogens in blood-culture within the first 30 days of life; and (4) treatment for at least 7 days with intravenous antibiotics. Blood cultures yielding either coagulase-negative staphylococci or *Staphylococcus aureus* were considered to be contaminants if the infant was not fulfilling all the above-mentioned criteria, or if the attending physician had considered the bacterium as a contaminant. The study was approved by the institutional review board.

Controls and matching criteria. For each patient, 2 controls who did not have infections during the neonatal period were matched for the following criteria: (1) gestational age (± 1 week); (2) sex; and (3) chorioamnionitis, defined as maternal fever, elevated maternal C-reactive protein level, fetal tachycardia, prolonged rupture of membranes, and/or placental histology indicative of chorioamnionitis [27]. Infants were not eligible to be controls if they had developed proven or probable neonatal infection or if they had received antibiotic treatment for suspected neonatal infection for >72 h.

Measurements of proteins of the lectin pathway. Cord blood is routinely collected and stored at our institution to determine *Toxoplasma gondii* serology. After coagulation and centrifugation, cord blood serum was frozen in sterile tubes at −80°C. MBL, MASP-2, and L-ficolin concentrations were measured using commercially available enzyme-linked immunosorbent assays, according to manufacturers' instructions (MBL oligomer ELISA kit, Antibodyshop; MASP-2 HK326 ELISA kit and L-ficolin HK 336 ELISA kit, HyCult Biotechnology).

The concentrations of H-ficolin were measured by time-resolved immunofluorometric assay, as described elsewhere [28]. In brief, microtiter plates were coated with monoclonal anti–H-ficolin antibody (4H5; HyCult Biotechnology), and serum samples diluted 1000-fold were added to the wells. After incubation and wash, the wells were incubated with biotinylated monoclonal anti–H-ficolin antibody and were finally developed by incubation with europium-labeled streptavidin followed by measurement of the bound europium by time-resolved fluorometry. Normal human standard serum with known content of H-ficolin was used to construct the standard curve. In the assay, we included 3 different control sera for test of interassay reproducibility (coefficients of variation, 9.6% for 9800 ng/mL, 8.2% for 16,800 ng/mL, and 11.8% for 24,100 ng/mL).

For quantification of MASP-3, microtiter wells were coated with 0.2 μg anti-MASP-1/3 antibody (MAb 1E2, subclass IgG1, Hycult Biotechnology, reacting with an epitope within the N-terminal domains shared by MASP-1 and MASP-3) in phosphate-buffered saline [29]. The wells were blocked with human serum albumin (1 mg/mL 0.14 mol/L NaCl, 10 mmol/L Tris, 15 mmol/L NaN_3, pH 7.4; TBS) and washed; next, samples were added, diluted 50-fold in MASP-3 binding buffer (1 mol/L NaCl, 10 mmol/L Tris-HCl, 5 mmol/L $CaCl_2$, 15 mmol/L NaN_3, pH 7.4, 0.05% [v/v] Triton X-100, 100 μg heat aggregated human IgG/mL [added to block the signals caused by rheumatoid factor if present in the samples]). A standard plasma pool with 5330 ng/mL of MASP-3 (estimated by comparison with dilutions of purified rMASP-3) was used to construct the standard curve. The standard plasma was diluted 1:10 followed by 2.5-fold dilutions (8 times). Following incubation overnight, the wells were washed with TBS (5 mmol/L $CaCl_2$, 0.05% Tween 20; TBS/Tw/Ca]) and were incubated with 1 μg of biotinylated

Table 1. Baseline Characteristics of Patients and Controls

Characteristic	Patients (n = 47)	Controls (n = 94)	P[a]
Male sex[b]	20 (43)	40 (43)	>.99
Gestational age,[b] median weeks (IQR)	31 (28–34)	32 (29–35)	.46
Birth weight, median g (IQR)	1500 (1095–2430)	1645 (1119–2193)	.79
SGA	8 (17)	18 (19)	.76
Prenatal steroids	34 (72)	72 (77)	.58
Maternal chorioamnionitis[b]	19 (40)	38 (40)	>.99
Maternal fever	3 (6)	2 (2)	.22
PROM	8 (17)	24 (26)	.26
Elevated maternal CRP	13 (28)	24 (26)	.79
Cesarean section	33 (70)	60 (64)	.45
Apgar 1 min, median score (IQR)	6 (3–7)	6 (5–8)	.06
Apgar 5 min, median score (IQR)	8 (7–9)	8 (7–9)	.32
Apgar 10 min, median score (IQR)	9 (8–9)	9 (8–9)	.49
Umbilical artery pH, median pH (IQR)	7.29 (7.23–7.33)	7.29 (7.25–7.34)	.09
Mechanical ventilation[c]	19 (40)	29 (31)	.26

NOTE. Data are no (%) of persons, unless otherwise indicated. CRP, C-reactive protein; IQR, interquartile range; PROM, prolonged rupture of membranes (>18 h); SGA, small for gestational age (birth weight <10th percentile for gestational age).

[a] P value determined by univariate logistic regression.

[b] Matching criteria.

[c] Intubation for respiratory distress syndrome before onset of sepsis.

anti–MASP-3 antibody (MAb 38:12–3) in 100 μL of TBS/Tw/Ca containing 1% (v/v) bovine serum. The wells were washed, incubated with europium-labelled streptavidin, and measured as described above. Three internal controls were added to each assay plate. The means and interassay coefficient of variations, determined from 15 individual assays, were 7%, 6%, and 8% for the 3 internal controls of 510 ng/mL, 2280 ng/mL, and 4950 ng/mL, respectively. The sensitivity for MASP-3 of the assay (ie, the concentration yielding a signal 2 standard deviations above the background) was 1000 ng/mL.

Statistical analysis. Outcomes were occurrence of sepsis, gram-positive and gram-negative sepsis, and early-onset sepsis (EOS, <72 h of life) versus late-onset sepsis (LOS, >72 h of life). Because no data are available for normal values of lectin pathway proteins in neonates, we used receiver operating characteristic curve analysis with sepsis as outcome to define cutoffs for low concentrations, resulting in the following categorizations: (1) low MBL <300 ng/mL versus normal MBL ⩾300 ng/mL, (2) low H-ficolin <12,000 ng/mL versus normal H-ficolin ⩾12,000 ng/mL, (3) low L-ficolin <1000 ng/mL versus normal L-ficolin ⩾1000 ng/mL, (4) low MASP-2 <30 ng/mL versus normal MASP-2 ⩾30 ng/mL, and (5) low MASP-3 <3000 ng/mL versus normal MASP-3 ⩾3000 ng/mL.

Patients and controls were compared using univariate and multivariate logistic regression with sepsis or type of sepsis as the dependent variable. The concentration of lectin pathway proteins, gestational age, chorioamnionitis, mode of delivery, and mechanical ventilation after birth were included as covariates. Spearman's rank correlation was used to assess correlation between gestational age or birth weight and lectin pathway parameters. Two-sided tests were used throughout, and P values <.05 were considered to be significant. SPSS, version 18.0 (SPSS) software was used for all analyses.

RESULTS

During the study period, 72 infants for whom cord blood serum was available developed blood culture–positive sepsis within the first 30 days of life. Twenty-four (33%) cases were considered to be due to contaminants. One infant who died of meningococcal sepsis was excluded because cord blood was not available in sufficient quantities. Thus, 47 infants with a median gestational age of 31 weeks (range, 24–41 weeks) were enrolled as patients in the study. Baseline characteristics between the case patients with sepsis (n = 47) and the matched controls (n = 94) did not differ significantly (Table 1). Infants developed sepsis at a median age of 7 days (range, 0–27 days) with 13 (28%) classified as EOS and 34 (72%) as LOS (Table 2). Six infants (13%) required treatment with catecholamines because of septic shock, and 5 infants (10%) died during sepsis. Maximum C-reactive protein levels during sepsis was at median 62 mg/L (range, 21–246 mg/L). Thirty-one episodes (66%) were due to gram-positive and 15 (32%) to gram-negative organisms; 1 episode (2%) was due to fungal infection. Eleven (23%) infants developed sepsis after surgery for congenital malformation or necrotizing enterocolitis (Table 2).

Table 2. Pathogens Recovered in Blood Cultures

Pathogen	Type of sepsis			Total, no (%)
	EOS	LOS	Surgery[a]	
Gram positive				
Staphylococcus aureus	1	15	4	16 (34)
Coagulase-negative staphylococci	0	10	4	10 (21)
Group B streptococci	2	1	0	3 (6)
Streptococcus viridans	1	0	0	1 (2)
Listeria monocytogenes	1	0	0	1 (2)
All gram-positive bacteria	5	26	8	31 (66)
Gram negative				
Escherichia coli	6	3	0	9 (19)
Enterobacter cloacae	0	3	2	3 (6)
Haemophilus influenzae	1	0	0	1 (2)
Proteus mirabilis	1	0	0	1 (2)
Acinetobacter baumanii	0	1	1	1 (2)
All gram-negative bacteria	8	7	3	15 (32)
Fungal				
Candida albicans	0	1	0	1 (2)
All fungal septicemias	0	1	0	1 (2)
Total, no (%) of isolates	13 (28)	34 (72)	11 (23)	47 (100)

NOTE. Data are no of isolates, unless otherwise indicated. EOS, early-onset sepsis (<72 h after birth); LOS, late-onset sepsis (>72 h after birth).

[a] Surgery indicates infants with postoperative sepsis (all LOS).

When analyzing cord blood concentrations of the lectin pathway proteins in the whole study population (patients and controls, $n = 141$), median concentrations were as follows: MBL, 1439 ng/mL (range, undetectable to 7166 ng/mL), H-ficolin, 12,573 ng/mL (range, 4434–34,655 ng/mL), L-ficolin, 2251 ng/mL (range, 313–16,836 ng/mL), MASP-2, 55 ng/mL (range, undetectable to 494 ng/mL), and MASP-3, 3233 ng/mL (range, 724–8569 ng/mL). Forty eight infants (34%) had MASP-2 concentration below the detection limit of 12.5 ng/mL. MBL, H-ficolin, L-ficolin, MASP-2, and MASP-3 concentrations were correlated with gestational age ($P < .01$ for all, by Spearman's rank test; Figure 1) and birth weight ($P < .01$ for all).

When comparing sepsis case patients and controls, H-ficolin concentrations in cord blood were significantly lower in infants with sepsis (odds ratio [OR], 2.17; $P = .032$), whereas no difference was found for the other lectin pathway proteins (Figure 2). Twenty (65%) of 31 infants with gram-positive sepsis and 21 (62%) of 34 infants with LOS, compared with 36 (38%) of 94 controls, had low H-ficolin levels, defined as <12,000 ng/mL ($P < .05$ for both; Figure 3 and Table 3). In a multivariate analysis adjusted for gestational age, chorioamnionitis, mode of delivery, and mechanical ventilation, low H-ficolin cord blood concentration was associated with a significantly increased OR of 4.00 for gram-positive sepsis (95% confidence interval [CI], 1.51–10.56; $P = .005$) and an OR of 2.97 for LOS (95% CI, 1.21–7.31; $P = .018$).

Six (40%) of 15 infants with gram-negative sepsis and 6 (46%) of 13 infants with EOS, compared to 17 (18%) of 94 controls had low MBL levels, defined as <300 ng/mL ($P = .062$ for gram-negative sepsis and $P = .028$ for EOS; Figure 4 and Table 3). In multivariate analysis, low MBL level was associated with an OR of 2.99 for gram-negative sepsis (95% CI, 0.86–10.33; $P = .084$) and an OR of 3.87 for EOS (95% CI, 1.05–14.29; $P = .042$).

Because infants with postoperative sepsis are exposed to particular risk factors, we then excluded all infants who had undergone surgery during the first month of life ($n = 11$), leaving 36 case patients and 94 controls. In univariate and multivariate analyses, low H-ficolin cord blood concentration was associated with a significantly increased risk of gram-positive sepsis (multivariate OR, 3.71; 95% CI, 1.26–10.92; $P = .017$; Table 4) and LOS (OR, 3.14; 95% CI, 1.07–9.21; $P = .037$), confirming the main results. Again, low MBL cord blood concentration was associated with a significantly increased risk of gram-negative sepsis (OR, 4.39; 95% CI, 1.10–17.45; $P = .036$) and EOS (OR, 3.87; 95% CI, 1.05–14.29; $P = .042$).

DISCUSSION

The results of this preliminary study indicate differential roles of lectin pathway proteins in susceptibility to neonatal sepsis. Low H-ficolin cord blood concentration was associated with significantly increased risk of gram-positive sepsis and LOS. In contrast, low MBL was associated an increased risk of gram-

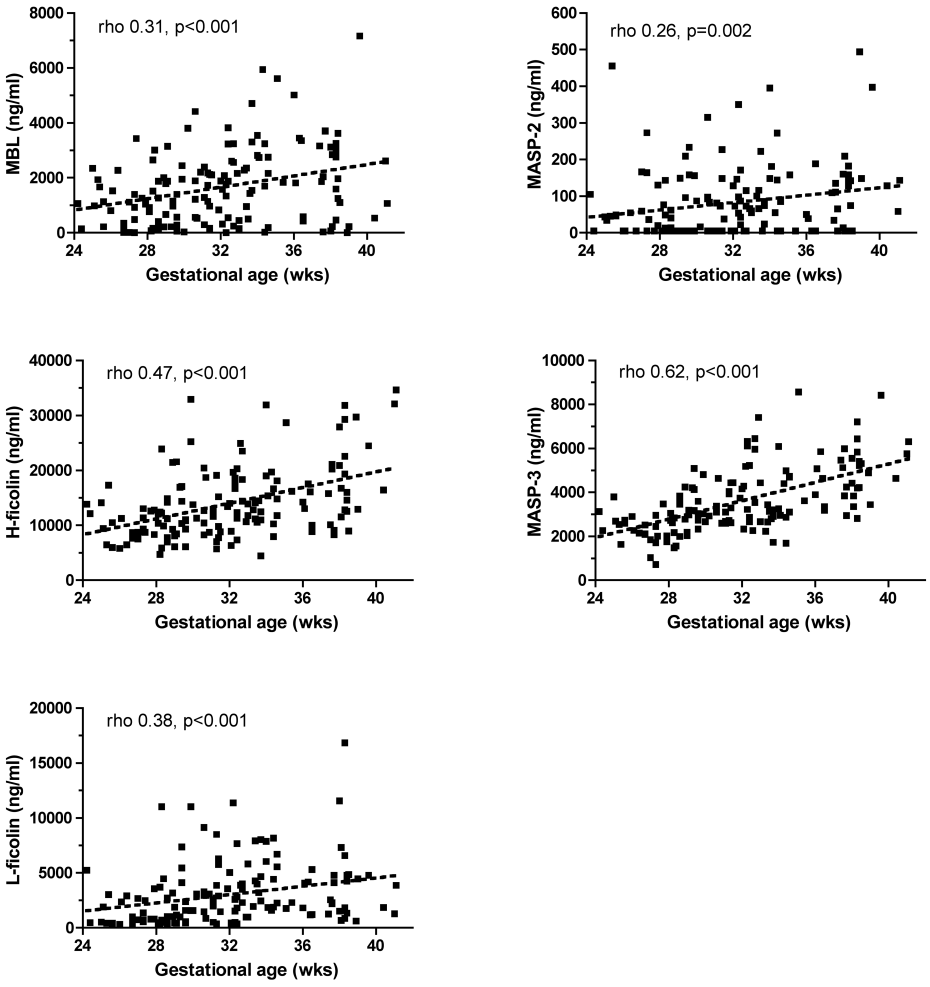

Figure 1. Concentrations of mannan-binding lectin (MBL), H-ficolin, L-ficolin, MBL-associated serine protease (MASP)-2, and MASP-3 in cord blood and gestational age in the whole cohort (patients and controls). The regression line is shown (*dotted line*). *P* values and correlation coefficients determined by Spearman's rank test are shown.

negative sepsis and EOS. In addition, we show that the expression of the lectin pathway of complement activation is very immature in neonates.

Overall the concentrations of the lectin pathway proteins measured in this cohort were lower, compared with children and adults, and were strongly correlated with gestational age [28, 30–34]. We have previously shown that MASP-2, L-ficolin, and H-ficolin concentrations increase over the first 6 months of life [31, 32], when they reach adult levels. These findings indicate that the lectin pathway of complement activation is not fully functional at birth. Decreased expression of lectin pathway proteins during the neonatal period, in particular, in premature infants, may thus contribute to the extraordinary susceptibility of newborns to invasive infections. This must be considered to be an additional form of neonatal immunodeficiency.

Infants with low H-ficolin cord blood concentration had a significantly increased risk to develop gram-positive sepsis, compared with infants with normal H-ficolin levels. Multivariate analysis adjusted for several potential confounders confirmed that low H-ficolin level was associated with a 4-fold increased risk of gram-positive sepsis. Because most gram-positive infections occurred after day 3 of life, LOS occurred significantly more often in infants with low H-ficolin levels. It is thus highly unlikely that consumption of H-ficolin in the course of chorioamnionitis influenced this association. Importantly, when patients with postoperative sepsis were excluded, the association between low H-ficolin and gram-positive sepsis remained essentially unchanged, indicating the robustness of this finding.

To date, the role of H-ficolin in health and disease remains largely unknown. In contrast to other lectin pathway members, H-ficolin is present only in humans, and severe H-ficolin deficiency is extremely rare [9], suggesting an important role for H-ficolin in human immune defense. Clinical studies

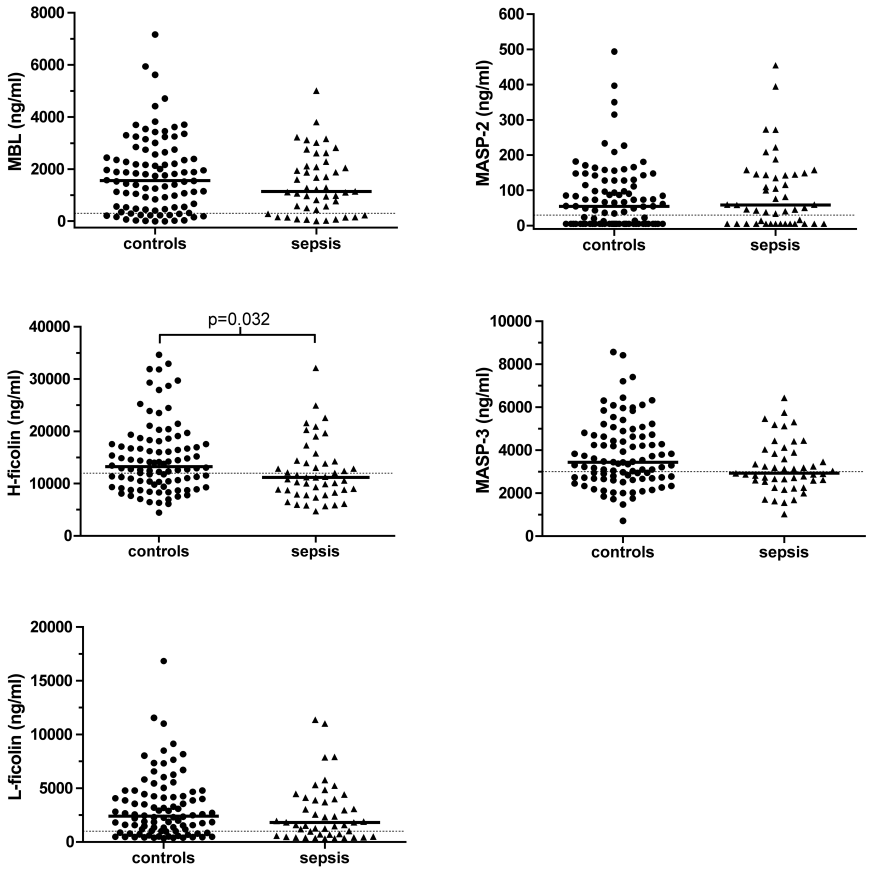

Figure 2. Comparison of mannan-binding lectin (MBL), H-ficolin, L-ficolin, MBL-associated serine protease (MASP)-2, and MASP-3 concentrations in cord blood between patients ($n = 47$) and controls ($n = 94$). *P* values determined by univariate logistic regression (if $P < .05$) and median values are shown. The *dotted lines* indicate low concentrations.

on H-ficolin are scarce. In a recent study including oncologic children, patients with low H-ficolin levels experienced chemotherapy-related infections and bacteremia significantly more often [28]. Only recently, a case of H-ficolin deficiency was reported in a 32-year-old man who had experienced repeated respiratory tract infections since early childhood and brain abscess due to gram-positive bacteria [35].

The bacterial specificity of H-ficolin remains to be determined [9]. H-ficolin recognizes acetylated surface structures such as GlcNAc and GalNAc, which are exposed by many gram-positive bacteria, but it also reacts with other acetylated compounds [14]. Strong H-ficolin binding has thus far only been demonstrated for *Aerococcus viridans* [14, 36]. Although binding of L-ficolin to lipoteichoic acid, a cell wall component of gram-positive bacteria, particularly GBS, has been demonstrated [14, 37], L-ficolin was not associated with particular pathogens in the present study, which had a very low incidence of GBS sepsis.

Infants with low MBL cord blood concentration had an increased risk of developing gram-negative sepsis and EOS, compared with infants with normal MBL levels. In multivariate analysis, low MBL concentration was associated with a 3- to 4-fold increased risk of gram-negative sepsis. Importantly, when excluding patients with postoperative sepsis, low MBL concentration was even stronger associated with gram-negative sepsis. In-

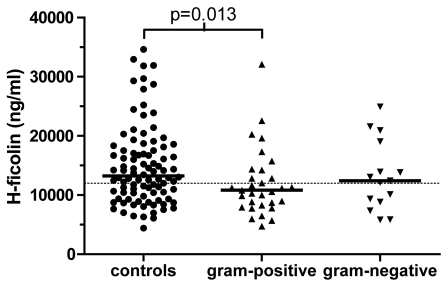

Figure 3. H-ficolin concentrations in cord blood of patients with gram-positive and gram-negative sepsis, compared with controls. *P* values determined by univariate logistic regression and median values are shown. The *dotted line* indicates low H-ficolin concentration (<12,000 ng/mL).

Table 3. Associations of Mannan-Binding Lectin (MBL), H-Ficolin, L-Ficolin, MBL-Associated Serine Protease (MASP)-2, and MASP-3 Cord Blood Concentration with Sepsis, Gram-Positive Sepsis, Gram-Negative Sepsis, Early-Onset Sepsis, and Late-Onset Sepsis

Variable	Frequency, no (%) of persons		Univariate analysis[a]		Multivariate analysis[a,b]	
	Case patients	Controls (n = 94)	OR (95% CI)	P	OR (95% CI)	P
Sepsis (n = 47)						
MBL <300 ng/mL	11 (23)	17 (18)	1.38 (0.59–3.26)	.457	1.45 (0.60–3.51)	.404
H-ficolin <12,000 ng/mL	27 (57)	36 (38)	2.17 (1.07–4.43)	.032[c]	2.12 (0.99–4.55)	.053
L-ficolin <1000 ng/mL	15 (32)	22 (23)	1.53 (0.71–3.34)	.281	1.33 (0.56–3.18)	.516
MASP-2 <30 ng/mL	16 (34)	39 (41)	1.37 (0.66–2.85)	.394	0.68 (0.32–1.44)	.311
MASP-3 <3000 ng/mL	24 (51)	34 (36)	1.84 (0.91–3.75)	.092	2.03 (0.88–4.71)	.098
Gram-positive sepsis (n = 31)						
MBL <300 ng/mL	4 (13)	17 (18)	0.67 (0.21–2.17)	.505	0.82 (0.24–2.74)	.745
H-ficolin <12,000 ng/mL	20 (65)	36 (38)	2.93 (1.26–6.82)	.013[c]	4.00 (1.51–10.56)	.005[d]
L-ficolin <1000 ng/mL	8 (26)	22 (23)	1.14 (0.45–2.90)	.786	1.14 (0.45–2.90)	.786
MASP-2 <30 ng/mL	11 (35)	39 (41)	0.78 (0.33–1.80)	.554	0.78 (0.32–1.87)	.572
MASP-3 <3000 ng/mL	15 (48)	34 (36)	1.65 (0.73–3.76)	.229	2.39 (0.87–6.57)	.091
Gram-negative sepsis (n = 15)						
MBL <300 ng/mL	6 (40)	17 (18)	3.02 (0.95–9.62)	.062	2.99 (0.86–10.33)	.084
H-ficolin <12,000 ng/mL	6 (40)	36 (38)	1.07 (0.35–3.27)	.900	0.81 (0.24–2.79)	.743
L-ficolin <1000 ng/mL	7 (47)	22 (23)	2.86 (0.93–8.79)	.066	2.90 (0.77–10.97)	.116
MASP-2 <30 ng/mL	5 (33)	39 (41)	0.71 (0.22–2.23)	.551	1.44 (0.43–4.85)	.558
MASP-3 <3000 ng/mL	9 (60)	34 (36)	2.65 (0.87–8.08)	.087	2.04 (0.54–7.69)	.293
Early-onset sepsis (n = 13)						
MBL <300 ng/mL	6 (46)	17 (18)	3.88 (1.16–13.02)	.028[c]	3.87 (1.05–14.29)	.042[c]
H-ficolin <12,000 ng/mL	6 (46)	36 (38)	1.38 (0.43–4.44)	.588	1.00 (0.27–3.68)	.998
L-ficolin <1000 ng/mL	5 (38)	22 (23)	2.05 (0.61–6.89)	.248	2.31 (0.50–10.64)	.284
MASP-2 <30 ng/mL	4 (31)	39 (41)	0.63 (0.18–2.18)	.463	1.88 (0.49–7.25)	.357
MASP-3 <3000 ng/mL	8 (62)	34 (36)	2.82 (0.86–9.32)	.088	2.15 (0.48–9.59)	.316
Late-onset sepsis (n = 34)						
MBL <300 ng/mL	5 (15)	17 (18)	0.78 (0.26–2.31)	.655	0.83 (0.27–2.57)	.753
H-ficolin <12,000 ng/mL	21 (62)	36 (38)	2.60 (1.16–5.83)	.020[c]	2.97 (1.21–7.31)	.018[c]
L-ficolin <1000 ng/mL	10 (29)	22 (23)	1.36 (0.57–3.28)	.489	1.12 (0.42–3.00)	.822
MASP-2 <30 ng/mL	12 (35)	39 (41)	0.77 (0.34–1.74)	.528	1.40 (0.59–3.30)	.441
MASP-3 <3000 ng/mL	16 (47)	34 (36)	1.57 (0.71–3.47)	.266	1.94 (0.75–5.05)	.174

NOTE. CI, confidence interval; OR, odds ratio.

[a] Results of binary logistic regression.

[b] Including gestational age, chorioamnionitis, mode of delivery, and mechanical ventilation after birth as covariates.

[c] $P < .05$.

[d] $P < .01$.

fants undergoing major surgery during the neonatal period are exposed to very different risk factors, compared with neonates not undergoing surgery, such as invasive procedures, open wounds, and prolonged presence of central catheters. Under these circumstances, the impact of innate immunity on systemic infections may be outweighed by the mentioned risk factors.

The association between low MBL concentration and early-onset sepsis could be the result of MBL consumption in the course of severe intrauterine infection. Dumestre-Perard et al [38] observed MBL consumption in patients with gram-negative sepsis. However, in our study, no association between chorioamnionitis and MBL was found. We thus believe it more likely that low MBL cord blood concentration predisposes infants to gram-negative bloodstream infections. Binding of MBL has been demonstrated in isolates from immunocompromised children including *S. aureus, Listeria monocytogenes, Haemophilus influenzae, Escherichia coli,* and *Candida albicans,* whereas only minimal binding was found for GBS and coagulase-negative staphylococci [14, 15].

Our findings are in line with the results of several studies involving adults, which indicate that MBL deficiency is associated with an increased susceptibility to sepsis [18–21]. Sim-

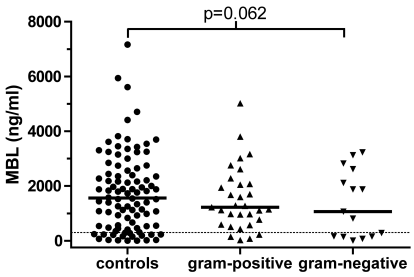

Figure 4. Mannan-binding lectin (MBL) concentrations in cord blood in patients with gram-positive and gram-negative sepsis, compared with controls. *P* values determined by univariate logistic regression and median values are shown. The *dotted line* indicates low MBL concentration (<300 ng/mL).

ilarly, 3 recent phenotype-based studies identified MBL deficiency as a risk factor for neonatal sepsis [22–24]. However, none of these adjusted for precise gestational age and chorioamnionitis. In contrast, 2 studies assessing MBL genotype reported no association between mutations in the MBL2 gene and neonatal sepsis [25, 26]. Several problems in these studies need to be addressed. First, definition of sepsis was variable, and several studies were based on clinical definitions of infection. Second, a high proportion of blood cultures yielding coagulase-negative staphylococci were included, which could represent contaminations.

We believe that the present study has several advantages. The definition of sepsis required the presence of both positive blood cultures and clinical signs of infection. In the absence of strong clinical signs of infection, potential contaminations were excluded. Furthermore, patients were carefully matched with controls for gestational age, sex, and chorioamnionitis, which strongly influence the risk of sepsis. Multivariate analyses adjusting for several potential confounders and sensitivity analyses excluding patients with postoperative sepsis confirmed the main results.

Some limitations need to be addressed. Only infants for whom cord blood was available were included. A selection bias, however, seems unlikely, because cord blood was routinely taken for determining *T. gondii* serostatus, a condition which is highly unlikely to affect lectin pathway concentrations or sepsis risk. Similar to other studies on MBL and neonatal sepsis [22–26], sample size is a major limitation. Due to the exploratory nature of the analyses, confirmation by future prospective cohorts is needed.

Because of significant variability in genotype-phenotype correlation and posttranscriptional events, genotype may not be a sufficient predictor of function in individual patients [25]; therefore, we decided to determine the concentrations of the proteins. Because data on normal concentrations in the neonatal period are lacking, we used cut-offs based on receiver operating characteristic curve analysis to define low lectin pathway concentrations.

In conclusion, we report that low MBL cord blood concentrations are an important susceptibility factor for gram-negative sepsis, and low H-ficolin concentrations indicate susceptibility for gram-positive sepsis. In addition, we demonstrate that neonates, in particular preterm infants, have an immature expression of the entire lectin pathway, which is likely to contribute to their extraordinary susceptibility to invasive infections. Our preliminary findings indicate differential pathogen

Table 4. Associations of Low Mannan-Binding Lectin (MBL) and Low H-Ficolin Cord Blood Concentration with Sepsis, Gram-Positive Sepsis, Gram-Negative Sepsis, Early-Onset Sepsis, and Late-Onset Sepsis in Infants without Surgery

	Frequency, no (%) of persons		Univariate analysis[a]		Multivariate analysis[a,b]	
Variable	Case patients	Controls (n = 94)	OR (95% CI)	*P*	OR (95% CI)	*P*
Sepsis (n = 36)						
H-ficolin <12,000 ng/mL	22 (61)	36 (38)	2.53 (1.15–5.57)	.021[c]	2.04 (0.88–4.73)	.096
Gram-positive sepsis (n = 23)						
H-ficolin <12,000 ng/mL	16 (70)	36 (38)	3.68 (1.38–9.82)	.009[d]	3.71 (1.26–10.92)	.017[c]
Gram-negative sepsis (n = 12)						
MBL <300 ng/mL	6 (50)	17 (18)	4.53 (1.30–15.77)	.018[c]	4.39 (1.10–17.45)	.036[c]
Early-onset sepsis (n = 13)						
MBL <300 ng/mL	6 (46)	17 (18)	3.88 (1.16–13.02)	.028[c]	3.87 (1.05–14.29)	.042[c]
Late-onset sepsis (n = 23)						
H-ficolin <12,000 ng/mL	16 (70)	36 (38)	3.68 (1.38–9.82)	.009[d]	3.14 (1.07–9.21)	.037[c]

NOTE. CI, confidence interval; OR, odds ratio.

[a] Results of binary logistic regression.

[b] Including gestational age, chorioamnionitis, mode of delivery, and mechanical ventilation after birth as covariates.

[c] $P < .05$.

[d] $P < .01$.

specificity within the lectin pathway of complement activation. Considering that therapeutical studies with MBL replacement are already underway for children with cancer [39], future prospective studies should include ficolins and MASPs, because MBL alone may not adequately reflect the impact of the lectin pathway in susceptibility to infection.

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