Journal of Perinatology (2012) 32, 210–217

www.nature.com/jp

ORIGINAL ARTICLE

Serum mannose-binding lectin (MBL) gene polymorphism and low MBL levels are associated with neonatal sepsis and pneumonia

H Özkan[1], N Köksal[1], M Çetinkaya[1], Ş Kılıç[2], S Çelebi[3], B Oral[4] and F Budak[4]

[1]Division of Neonatology, Department of Pediatrics, Uludag University School of Medicine, Bursa, Turkey; [2]Division of Pediatric Immunology, Department of Pediatrics, Uludag University School of Medicine, Bursa, Turkey; [3]Division of Pediatric Infectious Disease, Department of Pediatrics, Uludag University School of Medicine, Bursa, Turkey and [4]Department of Medical Microbiology, Immunology Unit, Uludag University School of Medicine, Bursa, Turkey

Objective: The aim of this study was to determine the serum mannose-binding lectin (MBL) levels and the frequency of MBL gene polymorphisms in infants with neonatal sepsis.

Study Design: Between January 2008 and January 2010, a total of 93 infants were included in this study and 53 of them had neonatal sepsis diagnosis as study group and 40 infants who had no sepsis according to clinical and laboratory findings as control group.

Result: Serum MBL levels were found to be low in 17 of 93 infants. Eleven of them were in the sepsis group and six of them were in the control group. Serum MBL levels were significantly lower in infants with sepsis compared with the control group. Frequencies of genotype AB and BB were also significantly higher in the study group compared with the control group. Most importantly, presence of B allele of MBL exon 1 gene was found to be associated with an increased risk for neonatal sepsis. Additionally, in the study group, the mean serum MBL levels were found to be significantly lower in the premature infants compared with the term infants. Pneumonia, bronchopulmonary dysplasia (BPD) and intraventricular hemorrhage (IVH) were significantly higher in infants with MBL deficiency compared with infants with normal MBL levels.

Conclusion: Low MBL levels and presence of B allele of MBL exon 1 gene were found to be important risk factors for development of both neonatal sepsis and pneumonia, especially in premature infants. Low MBL levels and MBL gene polymorphisms might also be associated with inflammation-related neonatal morbidities such as BPD and IVH.
Journal of Perinatology (2012) **32,** 210–217; doi:10.1038/jp.2011.79; published online 16 June 2011

Keywords: neonatal sepsis; mannan-binding lectin; premature infant; gene polymorphism

Correspondence: Dr M Çetinkaya, Division of Neonatology, Department of Pediatrics, Uludag University School of Medicine Hospital, 16059 Gorukle, Bursa, Turkey.
E-mail: drmerih@yahoo.com
Received 18 January 2011; revised 2 May 2011; accepted 6 May 2011; published online 16 June 2011

Introduction

Innate immune system is the most important defense against infection in newborns and genetic and/or developmental immaturity of innate immune system may predispose to neonatal sepsis.[1] Mannose-binding lectin (MBL) is a plasma protein which is primarily produced by the liver and it has an important role in the innate immune defense by activating the lectin pathway of the complement system by binding various microorganisms. This also leads to opsonization and enhanced phagocytosis.[2]

Circulating plasma MBL levels are associated with structural and genetic variations in promoter region of MBL. The MBL monometer is encoded by the MBL-2 gene on human chromosome 10 and consists of four exons. The structural mutations in exon 1 are called as 'O', whereas normal wild-type allele is called 'A'. Three single-nucleotide polymorphisms in the promoter region of exon 1 may lead to reduced plasma MBL levels and X variant polymorphisms are also associated with low plasma MBL levels.[3] These polymorphisms are common as they are found in one third of the population; however, their frequency show differences between various ethnic groups. Although heterozygous polymorphisms reduce MBL levels 5- to 10-fold, the homozygous mutations may lead to almost undetectable MBL levels.[4,5] It was reported that MBL deficiency might be associated with recurrent and serious infections in certain patient groups, especially in those with immune deficiency and compromised immune functions such as malignancy or neutropenia.[6,7]

The absence of passively derived maternal antibodies, low or suboptimal functioning of acquired immune system and decreased function of neutrophils may be responsible for the host-defense impairment in both term and preterm infants, so newborns might have increased susceptibility to infections.[8,9] Therefore, it may be suggested that innate immune system might provide protection against infections in postnatal period. In recent years, limited number of studies evaluated the effect of defects in the innate immune system, especially the role of MBL levels contributing to

predisposition to neonatal sepsis. However, these studies had conflicting results.[1,10–16]

The aim of this study was to determine serum MBL levels and the frequency of MBL gene polymorphism in both term and premature infants with neonatal sepsis. We also intended to investigate the possible association between MBL levels and inflammation-related morbidities in preterm infants.

Methods

A total of 93 newborn infants (53 infants with neonatal sepsis and 40 controls) who were admitted to NICU (Neonatal Intensive Care Unit) of Uludag University, Faculty of Medicine between January 2008 and January 2010 were enrolled to this prospective study. Neonatal sepsis was defined as presence of clinical signs of sepsis with positive blood culture.[17] Temperature instability (fever or hypothermia), apnea, need for supplemented oxygen, need for ventilation, tachycardia/bradycardia, hypotension, feeding intolerance, abdominal distension, bloody stool, convulsion, hypotonia and irritability were considered among clinical signs of sepsis. The neonates were classified into the following three groups according to sepsis type: early-onset sepsis (EOS), late-onset sepsis (LOS) and very late-onset sepsis. EOS, LOS and very late-onset sepsis were distinguished by the time at which sepsis first occurred: in the first 3 days of life, between 4 and 30 days after birth and >30 days after birth, respectively. Infants who had no signs of clinical and laboratory infection were referred as the control group.

The study protocol was approved by the Ethics Committee of Uludag University, Faculty of Medicine. Informed parental consent was obtained for all infants. Exclusion criteria included major congenital abnormalities and refusal of parental consent. Gestational age, birth weight, gender, mode of delivery, Apgar score at 1 and 5 min, prenatal demographics (for example, preeclampsia and diabetes), antenatal steroid administration, premature rupture of membranes and history of chorioamnionitis were all recorded.

Intraventricular hemorrhage (IVH) was evaluated by cranial ultrasound examinations which were performed by the same pediatric radiologist and diagnosed using the Papile classification system.[18] Bronchopulmonary dysplasia (BPD) was defined as oxygen dependency at 36 weeks postconceptional age for <32 gestational age or at 28 days of age for >32 gestational age.[19] Necrotizing enterocolitis was diagnosed using Bell criteria.[20] Retinopathy of prematurity was classified according to the International Classification of Retinopathy of Prematurity.[21]

The changes in the hematologic parameters were processed according to the Manroe and Rodwell scoring systems.[22,23] Leukopenia was defined as leukocyte count <5000 per mm^3; leukocytosis was defined as leukocyte count >25 000 per mm^3 at birth, >30 000 per mm^3 at 12 to 24 h and >21 000 per mm^3 after the second day. Thrombocytopenia was defined as platelet count <150 000 per mm^3. Normal absolute neutrophil count was accepted as 7800 to 14 500 per mm^3 in the first 60 h and 1750 to 5400 per mm^3 after 60 h. Before initiating the antimicrobial therapy, blood samples for whole blood count, C-reactive protein, procalcitonin, serum amyloid A and culture were obtained both from neonates with sepsis and from control patients. Blood samples for MBL levels and MBL genotyping were obtained at time of sepsis diagnosis at sepsis group, whereas they were obtained after exclusion of neonatal sepsis in the control group. Cerebrospinal fluid, urine and tracheal and gastric materials were also sent out for culture, if obtained. Blood smears of all infants were also evaluated for the findings of sepsis when blood samples obtained. Meningitis was diagnosed according to the cell count, glucose and protein levels of cerebrospinal fluid along with cerebrospinal fluid culture.

Blood samples were analyzed by enzyme-linked immunosorbent assay and MBL phenotyping was performed by PCR and restriction fragment length polymorphism methods. Serum MBL levels were measured using an immunoassay (Oligomer ELISA kit, Antibody Shop, Copenhagen, Denmark) according to the instructions of the manufacturer. MBL deficiency was defined as presence of serum MBL levels $<0.7\ \mu g\ ml^{-1}$. For MBL genotyping, DNA was extracted from blood samples by using a commercial available kit (Puregene, Gentra, MN, USA). DNA samples were kept at $-20\ ^{\circ}C$ until use. All genotypes were detected by PCR and restriction enzyme digestion. Exon 1 of MBL gene was amplified by PCR. The primer sequences were 5′-GTA GGA CAG AGG GCA TGC TC-3′ ve 5′-CAG GCA GTT TCC TCT GGA AGG-3′. In all, 349 bp PCR product was digested with *Ban*I and *Mbo*II for codon 54 and codon 57, respectively. The normal allele (allele A) was cut into two fragments with *Ban*I, 260 and 89 bp. The variant allele (allele B) and allele D remained uncut. *Mbo*II cleaved the variant allele (allele C) into 270 and 79 bp fragments. The fragments were visualized by electrophoresis on 2% agarose gel.[24] At electrophoresis, the dual band at the restriction site was defined as a heterozygous mutation, whereas single band was defined as homozygous mutation. As stated, normal structural MBL allele is named A, while allele B (mutation in codon 54), allele B (mutation in codon 57) and allele C (mutation in codon 52) are named as O. A representative gel electrophoresis of MBL gene exon 1 codon 54 polymorphisms is shown in Figure 1.

Whole blood count, procalcitonin, C-reactive protein, serum amyloid A levels and cultures were studied immediately. Whole blood count was performed using an automatic counter, Cell Dyn 3700 (Abbott Diagnostics Division, Santa Clara, CA, USA). C-reactive protein and serum amyloid A were determined by an immunonephelometric method using BN II device (Dade Behring Marburg GMBH, Marburg, Germany). Detection levels were $0.5\ ng\ ml^{-1}$ for procalcitonin and C-reactive protein and $6.8\ mg\ dl^{-1}$ for serum amyloid A. Blood and cerebrospinal fluid cultures were analyzed using fully automatic BACTEC method by BACTEC 9240 device (Becton Dickinson, Heidelberg, Germany).

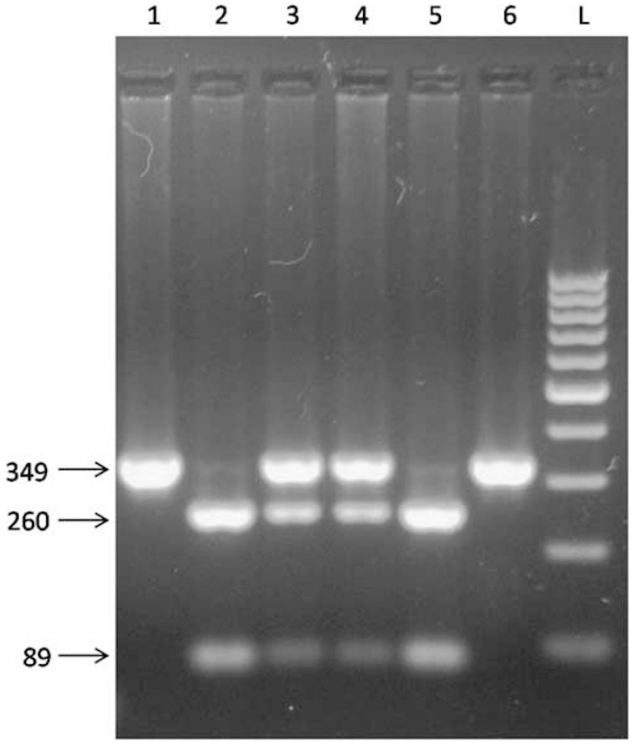

Figure 1 DNA fragments on agarose gel electrophoresis after restriction enzyme digestion of exon 1 of the mannose-binding lectin (MBL) gene codon 54. In all, 349 bp PCR product was digested with *Ban*I for codon 54 polymorphism. The normal allele (allele A) is cut into two fragments with *Ban*I (lanes 2 and 5), 89 and 260 bp. The variant allele (allele B) remains uncut (lanes 1 and 6). Both uncut and digested fragments are seen in AB heterozygote (lanes 3 and 4). L: 100 bp DNA ladder.

Infants were treated with appropriate antibiotic therapies. Neonates who had positive cultures were treated with antibiotics according to the culture antibiogram. The antimicrobial therapy was stopped after clinical and laboratory improvement. All of the groups were compared according to demographic features, clinical and laboratory findings.

SPSS software (SPSS, version 16.0, Chicago, IL, USA) was used for data analyses. Descriptive statistics was given as mean, median, s.d., minimum, maximum and percentage. The Kruskal–Wallis test and Mann–Whitney *U*-test was used to determine the differences between groups as appropriate. Odds ratios and 95% confidence intervals were also calculated in case of that χ^2 or Fisher's exact test was significant. The data were analyzed for appropriateness between the observed and expected genotype values and their fit to Hardy–Weinberg equilibrium, Arlequin Software v. 2000, University of Geneva, Switzerland. Values of $P<0.05$ were considered to be significant.

Results

A total of 93 term and premature infants (53 with sepsis and 40 as control) were included in this study. Fifty of them (54%) were born before 37 week of gestational age. Table 1 shows the demographic features of the study population.

Table 1 Neonatal and maternal characteristics of 93 infants included in this study

Neonatal characteristics	
Birth weight (g), mean ± s.d.	2441 ± 1099 (900–4700)
Gestational age (week), mean ± s.d. (min–max)	35 ± 4.5 (26–40)
Male gender, *n* (%)	53 (57)
Prematurity (<37 gestational age), *n* (%)	50 (53)
Apgar score at minute 1, mean ± s.d.	6.7 ± 2.6
Apgar score at minute 5, mean ± s.d.	8.5 ± 1.8
Mean MBL levels ($\mu g\,ml^{-1}$), mean ± s.d. (min–max)	2.4 ± 1.8 (0.0017–12.1)
MBL deficiency (MBL level<0.7 $\mu g\,ml^{-1}$), *n* (%)	17 (18.1)
Maternal characteristics	
Maternal age, mean ± s.d.	28 ± 5
Cesarian delivery, *n* (%)	56 (60)
Maternal preeclampsia, *n* (%)	22 (24)
Maternal diabetes, *n* (%)	10 (11)
Premature rupture of membranes, *n* (%)	4 (4)
Maternal infection, *n* (%)	4 (4)
Antenatal steroid, *n* (%)	8 (9)

Abbreviation: MBL, mannose-binding lectin.

Codon 57 polymorphism was not detected in any of the subjects from either group (data not shown). MBL codon 54 genotype and allele frequencies are given in Table 2. The frequency of MBL exon 1 BB genotype was higher in the sepsis group in comparison with the control group (15.1 vs 0.0%, $P=0.01$), whereas AA genotype was more common in healthy controls when compared with the infants with sepsis (87.5 vs 67.9%, $P=0.03$). Also, B allele frequency was significantly higher in the sepsis group (23.6%) compared with the control group (6.2%) ($P=0.002$, odds ratio (95% confidence interval) = 4.63 (1.62 to 16.17)).

Seventeen infants (18.1%) were found to have low serum MBL levels. The mean serum MBL levels were found to be significantly lower in infants who had AB ($1.6 \pm 3.0\,\mu g\,ml^{-1}$) and BB ($0.84 \pm 0.93\,\mu g\,ml^{-1}$) genotypes compared with those who had AA genotype ($2.7 \pm 1.4\,\mu g\,ml^{-1}$) ($P=0.03$). MBL deficiency was determined in 9, 42 and 51% of infants with AA, AB and BB genotypes, respectively, and the differences between AB genotype and AA genotype and BB genotype and AA genotype were found to be statistically significant ($P=0.03$ and $P=0.02$, respectively).

Fifty-three infants (54%) had at least one sepsis episode. Of these 53 infants with sepsis, 3 (6%) had EOS, 33 had (62%) LOS and 17 (32%) had very late-onset sepsis. The mean serum MBL levels were found to be significantly lower in infants with sepsis compared with those who did not have sepsis. In the sepsis group, premature infants had significantly lower MBL levels compared with term infants. Although serum MBL levels were also lower in

the premature infants in the control group, this difference was not statistically significant (Table 3). Also, there was no significant association with allele and genotype frequencies of MBL exon 1 gene when preterm and term infants were compared in sepsis and control groups.

Table 2 Comparison of demographic features, MBL levels and MBL genotyping of infants in sepsis and control groups

Demographic features	*Infants with sepsis (n = 53)*	*Infants as control (n = 40)*	*P*
Gestational age (week), mean ± s.d.	34.5 ± 5.0	37.7 ± 2.4	0.06
Birth weight (g), mean ± s.d.	2071 ± 1193	2530 ± 721	0.06
Male gender, *n* (%)	30 (56.6)	23 (57.5)	0.90
Maternal age, mean ± s.d.	28 ± 5	29 ± 4	0.27
Cesarian delivery *n* (%)	32 (60)	24 (60)	0.95
MBL levels			
Mean MBL levels ($\mu g\,ml^{-1}$),	2.0 ± 2.1	2.5 ± 2.1	**0.04**
mean ± s.d. (min–max)	(0.0017–12.1)	(0.063–4.4)	
MBL deficiency ($<0.7\,\mu g\,ml^{-1}$)	11/53 (21%)	6/40 (15%)	0.48
MBL exon 1 genotypes, n (%)			
AA	36 (68)	35 (87)	**0.03**
AB	9 (17)	5 (13)	0.55
BB	8 (15)	0 (0)	**0.01**
MBL exon 1 alleles, n (%)			
A	81 (76)	75 (94)	**0.002**
B	25 (24)	5 (6)	

Abbreviation: MBL, mannose-binding lectin.
Bold values show *P*-values <0.05, which are statistically significant.

When the infants in the study group were classified into four categories according to their gestational age, the infants <37 weeks had also significantly lower MBL levels compared with those born >37 weeks. When MBL levels were evaluated according to birth weight, although 80% of infants <1000 g had low MBL levels, only 8% of infants >2500 g had low MBL levels (Table 4).

There were no significant differences between infants with or without low serum MBL levels in terms of neonatal inflammation-related morbidities such as retinopathy of prematurity and necrotizing enterocolitis. However, BPD and IVH were significantly higher in premature infants with low MBL levels compared with the premature infants with normal MBL levels.

In both term and preterm infants, neonatal sepsis and pneumonia were diagnosed significantly more often compared with infants who had normal MBL levels. Although neonatal meningitis and mortality rates were found to be higher in infants with low MBL levels, this difference did not reach to a statistical significant level (Table 5). Although MBL levels were in normal levels in infants with EOS, they were found to be lower in 18 and 29% of infants with LOS and very late-onset sepsis, respectively. Coagulase-negative staphylococci (CONS) (62%) were found to be the primary causative agent in sepsis group, which was followed by fungi (20%) and gram-negative pathogens (7.5%). No significant difference was determined between low serum MBL levels and pathogens isolated from blood culture.

Discussion

This study showed that the presence of B allele of MBL exon 1 gene and low MBL levels might be important risk factors for sepsis development in both term and preterm infants. This risk was found

Table 3 MBL levels and MBL genotypes and alleles in preterm and term infants in both sepsis and control groups

	Sepsis group		*Control group*		*P-values*
	Preterm (n = 34)	*Term (n = 19)*	*Preterm (n = 16)*	*Term (n = 24)*	
Mean MBL levels ($\mu g\,ml^{-1}$), mean ± s.d.	1.8 ± 1.6	2.5 ± 2.5	2.1 ± 1.2	3.6 ± 1.3	0.03[a], 0.06[b]
MBL deficiency, *n* (%)	9 (27)	2 (11)	4 (25)	2 (8)	0.17[a], 0.16[b]
MBL genotypes, n (%)					
AA	24 (70)	12 (63)	13 (81)	22 (92)	0.43[a], 0.3[b]
AB	6 (18)	3 (16)	3 (19)	2 (8)	0.7[a], 0.17[b]
BB	4 (12)	4 (21)	0 (0)	0 (0)	0.1[a]
MBL alleles, n (%)					
A	54 (80)	27 (71)	29 (91)	46 (96)	0.33[a], 0.34[b]
B	14 (20)	11 (29)	3 (9)	2 (4)	

Abbreviation: MBL, mannose-binding lectin.
[a]Comparison of preterm and term infants in the sepsis group.
[b]Comparison of preterm and term infants in the control group.

Table 4 MBL levels in premature infants in terms of gestational age and birth weight

	Gestational age (week)					*Birth weight (g)*				
	⩽28 (n=8)	*29–32 (n=21)*	*33–37 (n=21)*	*>37 (n=43)*	P-*value*	*<1000 (n=5)*	*1000–1500 (n=25)*	*1500–2500 (n=21)*	*>2500 (n=42)*	*P-value*
MBL levels[a]	1.7 ± 1.8	1.9 ± 1.6	1.9 ± 1.1	3.0 ± 2.0	0.03[b], 0.04[c,d]	0.8 ± 1.4	2.1 ± 1.7	2.0 ± 1.3	2.9 ± 1.9	0.02[e,f], 0.01[g]
MBL deficiency[h]	3 (37)	5 (24)	5 (24)	4(9)	0.03[b], 0.04[c,d]	4 (80)	5 (20)	5 (24)	3 (7)	0.03[e,f], 0.001[g]

Abbreviation: MBL, mannose-binding lectin.
[a]Mean ± s.d..
[b]Comparison of infants with a gestational age of ⩽28 and >37 weeks.
[c]Comparison of infants with a gestational age of 29–32 and >37 weeks.
[d]Comparison of infants with a gestational age of 33–37 and >37 weeks.
[e]Comparison of infants with a birth weight of <1000 and 1000–1500 g.
[f]Comparison of infants with a birth weight of <1000 and 1500–2500 g.
[g]Comparison of infants with a birth weight of <1000 and >2500 g.
[h]*n* (%).

Table 5 Comparison of infants with low and normal serum MBL levels in terms of neonatal comorbidities

Neonatal comorbidities	*Infants with low MBL levels*			*Infants with normal levels*			*P-value*
	Preterm, n (%)	*Term, n (%)*	*Total, n (%)*	*Preterm, n (%)*	*Term, n (%)*	*Total, n (%)*	
RDS	6 (46)	0 (0)	6 (35)	14 (38)	0 (0)	14 (18)	0.68[a]
ROP	8 (61)	0 (0)	8 (47)	19 (51)	0 (0)	19 (25)	0.08[a]
NEC	8 (61)	0 (0)	8 (47)	16 (43)	1 (2)	17 (22)	0.1[a]
İVH	7 (53)	0 (0)	7 (41)	11 (30)	0 (0)	11 (14)	**0.01**[a]
BPD	10 (77)	0 (0)	10 (59)	20 (54)	0 (0)	20 (26)	**0.03**[a]
PDA	3 (23)	0 (0)	3 (18)	1 (3)	0 (0)	1 (1)	**0.04**[a]
Sepsis	9 (69)	2 (50)	11 (65)	25 (67)	17 (43)	42 (55)	>0.05[a,b], **0.04**[c]
Meningitis	2 (15)	1 (25)	3 (18)	4 (11)	5 (13)	9 (12)	>0.05[a,b,c]
Pneumonia	8 (61)	2 (50)	10 (59)	17 (46)	5 (13)	22 (29)	**<0.05**[a,b,c]
Mortality	4 (30)	0 (0)	4 (23)	7 (19)	1 (2)	8 (10)	>0.05[a,b,c]

Abbreviations: BPD, bronchopulmonary dysplasia; IVH, intraventricular hemorrhage; MBL, mannose-binding lectin; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity.
[a]Comparison of preterm infants with low and normal serum MBL levels.
[b]Comparison of term infants with low and normal serum MBL levels.
[c]Comparison of all infants with low and normal serum MBL levels.
Bold values show *P*-values <0.05, which are statistically significant.

to be more predominant in premature infants, especially in infants with small gestational age and birth weight. The incidence of neonatal pneumonia was found to be higher in infants with low MBL levels. We also determined that the presence of MBL gene polymorphisms and low MBL levels might contribute to the development of inflammation-related morbidities such as BPD and IVH in premature infants.

Innate immune system is the first-line defense mechanism against infections in newborns and MBL, a plasma protein, has an important role in the innate immune system. MBL is a Ca^{+2}-dependent lectin and it can recognize and bind to repeating pattern structures of polysaccharides that were found on the surfaces of several microorganisms. It also opsonizes these microorganisms before the production of specific IgM.[25] Subsequently, MBL-associated serine protease activates the complement cascade which leads to killing these microorganisms.[4] Newborns, especially premature infants, are more vulnerable to infections and sepsis. The innate immune system is the primary defense as the adaptive immune responses are not entirely developed. It was suggested that MBL deficiency in neonates might be associated with increased sepsis incidence as low MBL levels might contribute to sepsis development in both children and immunocompromised individuals.[12,26] Although it was suggested that a substantial decrease in MBL levels as a result of consumption from sepsis might unlikely cause to the association between low MBL levels and sepsis, it was also shown that MBL was consumed in gram-negative sepsis.[27,28]

In recent years, increasing number of studies were performed with the aim of exploring the association between MBL levels and sepsis incidence in both term and preterm infants. Kielgast *et al*.[29] reported that low cord MBL levels were associated with childhood infections and hospitalizations due to viral infections. In another study, low MBL levels were found in 41% of premature infants and it was suggested that it might be responsible for sepsis development in premature infants.[30] The same group also determined that low MBL levels at birth were associated with increased risk of EOS, culture-proven sepsis and neonatal pneumonia.[13] Low MBL levels at birth in premature infants were reported to render premature infants to increased infection risk.[10] A strong association was determined between low MBL levels and confirmed neonatal sepsis compared with suspected sepsis and the authors also reported that low MBL levels at birth might be associated with hospital-acquired sepsis in NICU.[1] In a recent study, lower MBL levels were found to be associated with neonatal sepsis, especially with culture-proven sepsis in preterm infants.[31] In agreement with previous data, our study showed that low MBL levels lead to susceptibility to neonatal sepsis in both term and preterm infants and it was more predominant in premature neonates.

Some studies reported that serum MBL levels were lower in preterm infants compared with term infants and gestational age was found to be more likely associated with lower MBL levels than birth weight.[16,28,32] Lau *et al*.[32] studied MBL levels in 885 longitudinally collected serum samples from 168 preterm infants and 63 were genotyped for codon 54 mutation in MBL gene. They found that for infants with codon 54 mutation, there was a significant difference between preterm and term infants. Therefore, they suggested that there might be a maturation process for MBL levels in preterm infants with codon 54 mutation, which was presumably completed by full-term gestation. Dzwonek *et al*.[10] also determined that serum MBL levels were correlated with MBL genotyping, gestational age and birth weight. They found that premature infants who were born <1000 g or born <28 gestational week with low MBL levels more likely suffered from neonatal sepsis. In concordant with these data, we determined that infants born <28 gestational age or born <1000 g had significantly lower MBL levels. All these data might support the hypothesis of possible developmentally regulation of MBL gene expression. This regulation may be elucidated with future studies.

Although Dzwonek *et al*.[10] found CONS as the most common causative agent, they could not find an association between serum MBL levels and causative pathogens. CONS were also found to be the most common blood stream infection pathogen in NICU and no relationship was established between low-MBL producing genotypes and nosocomial infection risk.[32] Similarly, CONS were the primary causative pathogen in our sepsis group, which was followed by fungi and gram-negative pathogens. As MBL binds poorly to CONS, complement activation through MBL binding is not effective; and therefore, MBL may not be protective in CONS infections.[15]

Three single-point mutations in exon 1 were found to be associated with absence or low levels of MBL.[3] The frequency of MBL gene polymorphisms may show differences between different ethnics and geographic areas. In a recent study from Turkey, the most common genotype was found to be AA (63.5%) genotype which was followed by AB (35.4%) and BB (1%) genotypes in 99 premature infants.[12] Our study also showed similar but slightly different results as the total frequency of AA, AB and BB genotypes in both sepsis and control groups were 76, 15 and 9%, respectively. In previous studies, mutations in MBL gene and associated low MBL levels were reported to be associated with increased incidence of skin, respiratory and gastrointestinal infections.[7,33,34] Koroglu *et al*.[12] reported that the frequency of sepsis in infants with AB and BB genotypes were significantly higher than those with AA genotypes. Similarly, in this present study, neonatal sepsis was diagnosed significantly higher in infants with AB and BB genotypes compared with normal genotype (AA). In our study, analysis of allele frequencies demonstrated that the presence of B allele was associated with an increased risk for sepsis in neonates.

It was suggested that the presence of MBL gene polymorphisms and low MBL levels were associated with increased number of respiratory infections in both neonates and children.[35–37] Frakking *et al*.[13] found that low MBL levels were associated with pneumonia in the first month of life in premature infants. Similarly, Hilgendorff *et al*.[16] also suggested that low MBL levels might contribute to both pulmonary and systemic infection development in preterm infants. Our findings were in good agreement with these studies.

Several studies reported conflicting data about the association between MBL deficiency and meningococcal meningitis in adults.[38,39] To our knowledge, there is no study that evaluated the association between neonatal meningitis and serum MBL levels. Although neonatal meningitis was higher in infants with MBL deficiency and presence of MBL genotyping, the difference was not significant. This might be associated with small number of infants in our study.

There are limited number of studies that investigated the effects of low MBL levels or presence of MBL gene polymorphisms on development of inflammation-related morbidities. MBL gene polymorphisms leading to low MBL levels were found to be associated with BPD diagnosis in preterm infants.[40] In contrast, presence of two MBL-2 gene variants (-550G$>$C and R52C) were reported to have opposite effect on pulmonary outcomes and BPD.[41] This present study showed that low MBL levels and presence of MBL gene polymorphism might be a risk factor for BPD development. Similarly, low serum MBL levels were associated with IVH development in preterm infants in our study. To our knowledge, there is no study in neonates that evaluated the association between MBL levels and IVH development. Although

patent ductus arteriosus was also found to be higher in infants with low serum MBL levels, the number of infants with patent ductus arteriosus diagnosis was too small to make a suggestion. As stated before, pneumonia and sepsis were significantly higher in infants with low serum MBL levels. No significant differences were determined between MBL gene polymorphisms and MBL deficiency and other neonatal morbidities such as respiratory distress syndrome, retinopathy of prematurity and necrotizing enterocolitis. We suggest that future prospective studies including more number of premature infants are warranted for exploring the possible association with MBL gene polymorphisms and inflammation-related neonatal morbidities.

In conclusion, presence of low MBL levels and MBL gene polymorphism may be important risk factors for development of neonatal sepsis and pneumonia in both term and premature infants. This risk is especially predominant in preterm infants compared with term infants. There may also be an association between presence of low MBL levels and/or MBL gene polymorphisms and inflammation-related neonatal morbidities. After confirming these data with large, prospective, multicentre and controlled studies, substitution therapy with MBL might be a promising treatment in future in infants, especially in premature infants who were admitted to NICU with neonatal sepsis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

This study was supported by Uludag University Scientific Research Project with a project number T-2006/52.

References

1 de Benedetti F, Auriti C, D'Urbano LE, Ronchetti MP, Ravà L, Tozzi A *et al.* Low serum levels of mannose binding lectin are a risk factor for neonatal sepsis. *Pediatr Res* 2007; **61**: 325–328.

2 Neth O, Jack DL, Dodds AW, Holzel H. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect Immun* 2000; **68**: 688–693.

3 Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP *et al.* Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. *J Immunol* 1995; **155**: 3013–3020.

4 Selander B, Martensson U, Weintraub A, Holmström E, Matsushita M, Thiel S *et al.* Mannan-binding lectin activates C3 and the alternative complement pathway without involvement of C2. *J Clin Invest* 2006; **116**: 1425–1434.

5 Kilpatrick DC. Mannan-binding lectin: clinical significance and applications. *Biochim Biophys Acta* 2002; **1572**: 401–413.

6 Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. *Lancet* 2001; **358**: 614.

7 Roy S, Knox K, Segal S, Griffiths D, Moore CE, Welsh KI *et al.* MBL genotype and risk of invasive pneumococcal disease: a case-control study. *Lancet* 2002; **359**: 1569–1573.

8 Sandberg K, Fasth A, Berger A. Preterm infants with low immunoglobulin G levels have increased risk of neonatal sepsis but do not benefit from prophylactic immunoglobulin G. *J Pediatr* 2000; **137**: 623–628.

9 Geelen SP, Fleer A, Bezemer AC. Deficiencies in opsonic defense to pneumococci in the human newborn despite adequate levels of complement and specific IgG antibodies. *Pediatr Res* 1990; **27**: 514–518.

10 Dzwonek AB, Neth OW, Thiébaut R, Gulczynska E, Chilton M, Hellwig T *et al.* The role of mannose-binding lectin in susceptibility to infection in preterm neonates. *Pediatr Res* 2008; **63**: 680–685.

11 Israëls J, Frakking FN, Kremer LC, Offringa M, Kuijpers TW, van de Wetering MD. Mannose-binding lectin and infection risk in newborns: a systematic review. *Arch Dis Child Fetal Neonatal Ed* 2010; **95**: F452–F461.

12 Koroglu OA, Onay H, Erdemir G, Yalaz M, Cakmak B, Akius M *et al.* Mannose-binding lectin gene polymorphism and early neonatal outcome in preterm infants. *Neonatology* 2010; **98**: 305–312.

13 Frakking FN, Brouwer N, van Eijkelenburg NKA, Merkus MP, Kuijpers TW, Offringa M *et al.* Low mannose-binding lectin (MBL) levels in neonates with pneumonia and sepsis. *Clin Exp Immunol* 2007; **150**: 255–262.

14 Ahrens P, Kattner E, Kohler B, Hartel C, Seidenberg J, Segerer H *et al.* Mutations of genes involved in the innate immune system as predictors of sepsis in very low birth weight infants. *Pediatr Res* 2004; **55**: 652–656.

15 van der Zwet WC, Catsburg A, van Elburg RM, Savelkoul PH, Vandenbroucke-Grauls CM. Mannose-binding lectin (MBL) genotype in relation to risk of nosocomial infection in pre-term neonates in the neonatal intensive care unit. *Clin Microbiol Infect* 2008; **14**: 130–135.

16 Hilgendorff A, Schmidt R, Bohnert A, Merz C, Bein G, Gortner L. Host defence lectins in preterm neonates. *Acta Paediatr* 2005; **94**: 794–799.

17 Sankar JM, Agarwal R, Deorari AK, Paul VK. Sepsis in the newborn. *Indian J Pediatr* 2008; **75**: 261–266.

18 Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500gm. *J Pediatr* 1978; **92**: 529–534.

19 Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001; **163**: 1723–1729.

20 Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am* 1986; **33**: 179–201.

21 International Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity revisited. *Arch Ophthalmol* 2005; **123**: 991–999.

22 Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. *J Pediatr* 1979; **95**: 89–98.

23 Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematological scoring system. *J Pediatr* 1988; **112**: 761–767.

24 Vardar F, Pehlivan S, Onay H, Atlihan F, Güliz N, Ozkinay C *et al.* Association between mannose binding lectin polymorphisms and predisposition to bacterial meningitis. *Turk J Pediatr* 2007; **49**: 270–273.

25 Epstein J, Eichbaum Q, Sheriff S, Ezekowitz RAB. The collectins in innate immunity. *Curr Opin Immunol* 1996; **8**: 29–35.

26 Koch A, Melbye M, Sørensen P, Homøe P, Madsen HO, Mølbak K *et al.* Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. *JAMA* 2001; **285**: 1316–1321.

27 Eisen DP, Dean MM, Boermeester MA, Fidler KJ, Gordon AC, Kronborg G *et al.* Low serum mannose-binding lectin level increases the risk of death due to pneumococcal infection. *Clin Infect Dis* 2008; **47**: 510–516.

28 Dumestre-Pérard C, Doerr E, Colomb MG, Loos M. Involvement of complement pathways in patients with bacterial septicemia. *Mol Immunol* 2007; **44**: 1631–1638.

29 Kielgast S, Thiel S, Henriksen TB, Bjerke S, Olsen J, Jensenius JC. Umblical cord mannan-binding lectin and infections in early childhood. *Scand J Immunol* 2003; **57**: 167–172.

30 Frakking FN, Brouwer N, Zweers D, Merkus MP, Kuijpers TW, Offringa M *et al.* High prevalence of mannose-binding lectin (MBL) deficiency in premature neonates. *Clin Exp Immunol* 2006; **145**: 5–12.

31 Ozdemir O, Dinleyici EC, Tekin N, Colak O, Aksit MA. Low-mannose-binding lectin levels in susceptibility to neonatal sepsis in preterm neonates with fetal inflammatory response syndrome. *J Matern Fetal Neonatal Med* 2010; **23**: 1009–1013.

32 Lau YL, Chan SY, Turner MW. Mannose-binding protein in preterm infants: developmental profile and clinical significance. *Clin Exp Immunol* 1995; **102**: 649–654.

33 Vallinoto AC, da Silva RF, Hermes RB, Amaral IS, Miranda EC, Barbosa MS *et al.* Mannose-binding lectin gene polymorphisms are not associated with susceptibility to hepatitis C virus infection in the Brazilian Amazon region. *Hum Immunol* 2009; **70**: 754–757.

34 Herpers BL, Yzerman EP, de Jong BA, Bruin JP, Lettinga KD, Kuipers S *et al.* Deficient mannose binding lectin-mediated complement activation despite mannose binding lectin-sufficient genotypes in an outbreak of Legionella pneumophila pneumonia. *Hum Immunol* 2009; **70**: 125–129.

35 Eisen DP, Minchinton RM. Impact of mannose binding lectin on susceptibility to infectious diseases. *Clin Infect Dis* 2003; **37**: 1496–1505.

36 Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. *BMJ* 1997; **314**: 1229–1232.

37 Schlapbach LJ, Latzin P, Regamey N, Kuehni CE, Zwahlen M, Casaulta C *et al.* Mannose-binding lectin cord blood levels and respiratory symptoms during infancy: a prospective birth cohort study. *Pediatr Allergy Immunol* 2009; **20**: 219–226.

38 Hibbert ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. *Lancet* 1999; **353**: 1049–1053.

39 Garred P, Michaelsen TE, Bjune G, Thiel S, Svejgaard A. A low serum concentration of mannan-binding protein is not associated with serogroup B or C meningococcal disease. *Scand J Immunol* 1993; **37**: 468–470.

40 Hilgendorff A, Heidinger K, Pfeiffer A, Bohnert A, König IR, Ziegler A *et al.* Association of polymorphisms in the mannose-binding lectin gene and pulmonary morbidity in preterm infants. *Genes Immun* 2007; **8**: 671–677.

41 Capoluongo E, Vento G, Rocchetti S, Giardina E, Concolino P, Sinibaldi C *et al.* Mannose-binding lectin polymorphisms and pulmonary outcome in premature neonates: a pilot study. *Intensive Care Med* 2007; **33**: 1787–1794.