# THE ROLE OF MANNOSE BINDING LECTIN (MBL2) GENE POLYMORPHISM IN INCIDENCE OF NEONATAL SEPSIS

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**Abstract**

**Background:** The mannose-binding lectin (MBL) is a collectin family member that is produced by the liver as an acute-phase protein. MBL activates macrophages, improves phagocytosis, and aids in complement activation. Low serum MBL levels raise the risk of infection, particularly when combined with other conditions such as immune deficiencies of various origins. Because their adaptive immunity has not yet developed, neonates are considered immunocompromised. This study aimed to determine the role of serum MBL levels in the diagnosis of neonatal sepsis, as well as the role of MBL2 gene polymorphism at codon 54 (G/A) in the development of neonatal sepsis.

**Methods:** This case-control study was carried out on 100 neonates classified into two groups: 50 preterm neonates diagnosed with sepsis and 50 healthy preterm neonates with no clinical signs or laboratory evidence of sepsis who were enrolled as a control group. Assessment of history, clinical examination, and investigations (complete blood count, C- reactive protein, blood culture, MBL2 gene polymorphism at codon 54 (G/A), MBL serum level) were performed.

**Results:** Mean serum MBL was significantly lower in septic neonates (45.5 + 10.0 ng/mL) compared to the control group (69.1 + 26.1 ng/mL) (P<0.0001), and had a good predictive value for discrimination between septic neonates and controls with an area under the receiver-operating characteristic (ROC) curve (AUC) of 0.92 (95% CI, 0.849 to 0.965) (p-value <0.001). The normal genotype (GG) of codon 54 was significantly lower in patients (30/50, 60.0%) compared to controls (43/50, 86.0%) (P=0.006), while the heteromutant (GA) genotype of codon 54 was significantly higher in patients (17/50, 34.0%) than in controls (7/50, 14.0%) (P=0.033). The codon 54 alleles were statistically different in patients compared to controls; the G allele was significantly lower in patients (77%) than in controls (93%) (P=0.0025), while the A allele was significantly higher in patients (23%) than in controls (7%) (P=0.0025).

**Conclusions:** The presence of the variant A allele in the MBL2 gene at codon 54 may be associated with a decreased serum level of MBL, and therefore may be associated with an increased risk of neonatal sepsis.

**Keywords:**Mannose-binding lectin, MBL2 polymorphism, neonates, preterm, sepsis

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## 1. INTRODUCTION

Mannose-binding lectin (MBL) is a key component of innate immunity and serves as the first line of defense against infection in newborns. Studies have shown that low serum MBL levels, usually caused by genetic variation, are associated with more frequent and more severe infections [1]. In addition, MBL concentrations can differ in patients of different ethnic groups and different ages [2].

Although serum MBL levels vary with age, they are mainly determined by the exon 1 polymorphism and the promoter region of the MBL2 gene. Three single nucleotide polymorphisms (SNPs) were found in codons 52, 54, and 57 of exon 1. The A allele represents the wild-type allele and the O allele represents the variant alleles [3].

The presence of the O allele has been suggested to impair MBL oligomerization, resulting in lower levels of circulating serum functional proteins. In addition, SNPs in the promoter region at positions -550 and -221 known as H/L (rs11003125) and X/Y (rs7096206) variants, respectively, affect MBL2 expression, although only the X variant affects the MBL serum levels significantly reduced [3]. Thus, the combination of genetic changes in both exon 1 and promoter region yields three expression clusters of the MBL genotype associated with high

(YA/YA, YA/XA), medium (XA/XA, YA/O), and low (XA/O, O/O) levels of MBL [4].

MBL deficiency, defined by low MBL levels and/or low expression genotype (XA/O or O/O) [4], has been associated with reduced opsonizing ability of microorganisms and increased susceptibility to infection, especially in the infancy period and in immune-compromised subjects [3]. Given the interest in the role of MBL in neonatal defense against infection and the existence of inconclusive results in the literature on the clinical significance of MBL deficiency in newborns [4], our study was conducted to determine whether serum MBL deficiency and the presence of MBL2 gene polymorphism in codon 54 (G/A) is associated with an increased risk of neonatal sepsis.

## 2. METHODS

- **Study population**

Our prospective observational case-control study was carried out at the neonatal intensive care unit (NICU) of Cairo University Children's Hospital from June 2017 to December 2018 and included 50 preterm neonates diagnosed with neonatal sepsis enrolled as the patient group and 50 healthy preterm neonates as the control group. All consecutive preterm (<37 weeks of gestational age) neonates (0-28 days) with early-onset and late-onset sepsis were considered as the patient group. Neonatal sepsis was diagnosed by; (a) At least two clinical signs of sepsis (respiratory rate > 60 breaths/min (tachypnea), grunting, apnea, temperature >37.7°C or <35.5°C (hypothermia), lethargic or unconscious, not able to sustain suckling, tachycardia, convulsion, feeding intolerance, poor reflexes, and poor capillary refill >2 seconds) [5] (b) Two laboratory findings (leucopenia<5.000/mm3, leucocytosis >20.000/mm3, thrombocytopenia <100.000/mm3, elevated C-reactive protein (CRP) > 6 mg/dL and bandemia> 0.2) (c) In addition to recovery of a bacterial pathogen in blood-culture [6]. The entire patient group was diagnosed as proven sepsis by a positive blood culture. Preterm neonates who had no clinical signs or laboratory findings of infection until discharge were considered the control group. Exclusion criteria; perinatal asphyxia, major congenital anomalies, and metabolic diseases.

- **Samples collection**

A complete blood count (CBC) was performed using a Coulter MD 18 (Automated Hematology Counter) [7], CRP titers [8], and blood cultures [venous whole blood samples were inoculated into aerobic and anaerobic BACTEC tubes]. BACTEC Plus vials were used for patients on antibiotic therapy and Standard vials were used for untreated patients. Two sets were collected simultaneously from two different locations. The flasks were incubated in the BACTEC FX "Becton Dickinson" automated blood culture system.

- **MBL2 genotyping by PCR–RFLP**

For all the study subjects; 2 mL of venous blood in ethylene diamine tetraacetic acid (EDTA) Vacutainer tubes stored at -20°C for subsequent DNA extraction using the GeneJET™ Genomic DNA Purification Kit (Thermo Scientific, catalog number: #K0781, USA), followed by detection of MBL2 gene polymorphisms in codon 54 by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) **(Fig.1).**

- **Serum MBL level**

For all the study subjects; Shortly after collection, 2 ml of venous blood was isolated in standard tubes, then aliquoted and stored at -20°C. The serum was coagulated at room temperature for 10-20 minutes and then centrifuged at 2000-3000 rpm for 20 minutes to collect the supernatant. Serum MBL was measured by enzyme-linked immune-sorbent assay (ELISA) using a human MBL kit (Cat. No. BYEK1089, Biospes, China) according to the manufacturer's specifications [9,10].

## STATISTICAL ANALYSIS

Data were analyzed using JMP® version 13.2.1 (SAS® Institute Inc., Cary, NC). Continuous counts are presented as mean and standard deviation, and differences between groups were compared using an unpaired t-test. Biased numbers are presented as a median and interquartile range, and differences between groups were compared using the Kruskal-Wallis test, with Dunn's test applied as needed for post hoc comparisons. Categorical data were presented as ratios or numbers and percentages, and differences between groups were compared using Fisher's exact test or the chi-square test for trend (for ordered data). Correlations were tested using Pearson's and Spearman's rank correlation, respectively. The diagnostic (or predictive) value of MBL measurements was examined by receiver-operating characteristic (ROC) analysis. Multivariate regression was used to examine the effect of MBL levels and different genotypes on sepsis outcomes. P values have been considered statistically significant if < 0.05.

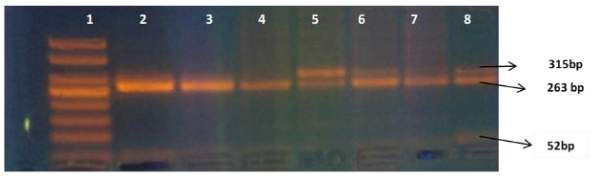


**Fig.1** MBL2 gene polymorphisms in codon 54
Lane 1: Marker-ladder 50 bp (fermentas).
Lanes 2, 3, 4, 7: Wild genotype (GG) with two bands at 263 bp and 52 bp.
Lanes 5, 6, 8: Heteromutant genotype (GA) with three bands at 315 bp, 263 bp and 52 bp.

## 3. RESULTS

**Table (1):**Comparison of the demographic characteristics between the patients and the controls

| Variable | Patients (n=50) | Controls (n=50) | Difference | 95% CI | P-value* |
|---|---|---|---|---|---|
| **Age at the time of sampling (days),** mean (SD) | 12.6 (6.1) | 11.7 (6.2) | -0.9 | -3.3 to -1.5 | 0.451 |
| **GA (weeks)**, mean (SD) | 31.6 (2.3) | 32.4 (2.3) | 0.7 | -0.2 to 1.7 | 0.113 |
| **BW (kg)**, mean (SD) | 1.8 (0.3) | 1.9 (0.3) | 0.1 | -0.1 to 0.2 | 0.364 |
| **Apgar 1 min**, mean (SD) | 7 (2.0) | 7 (1.0) | 0.0 | -0.6 to 0.6 | 1.000 |
| **Apgar 5 min**, mean (SD) | 8 (1.0) | 8 (1.0) | 0.0 | -0.6 to 0.6 | 1.000 |
| **Gender, n (%)** | | | | | |
| *Female* | 27 (54.0%) | 25 (50.0%) | | | 0.841# |
| *Male* | 23 (46.0%) | 25 (50.0%) | | | |

GA: Gestational age, BW: Birth weight; Data are mean and standard deviation (SD) ornumber (n) and percentage (%); 95% CI = 95% confidence interval; *Unpaired t test unless otherwise indicated; #Fisher's exact test.

Mean serum MBL was significantly lower in septic preterm neonates (45.5 + 10.0 ng/mL) compared to the preterm control group (69.1 + 26.1 ng/mL), (P<0.0001) **(Fig. 2)** and had a good predictive value for the occurrence of neonatal sepsis with an area under the ROC curve (AUC) of 0.92 (95% CI, 0.849 to 0.965) (p-value <0.001) **(Fig. 3).** A cut-off criterion of MBL ≤ 48.5 ng/mL had a sensitivity of 88% and a specificity of 88% for identifying increased susceptibility to neonatal sepsis, a positive predictive value of 88% and a negative predictive value of 88%. The characteristics of the study subjects are provided in **Table 1.**

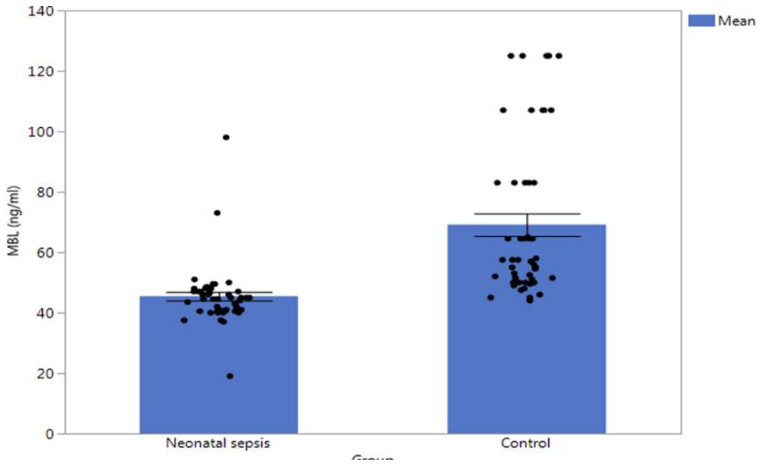


**Fig. 2** Mean serum MBL level in patients and controls

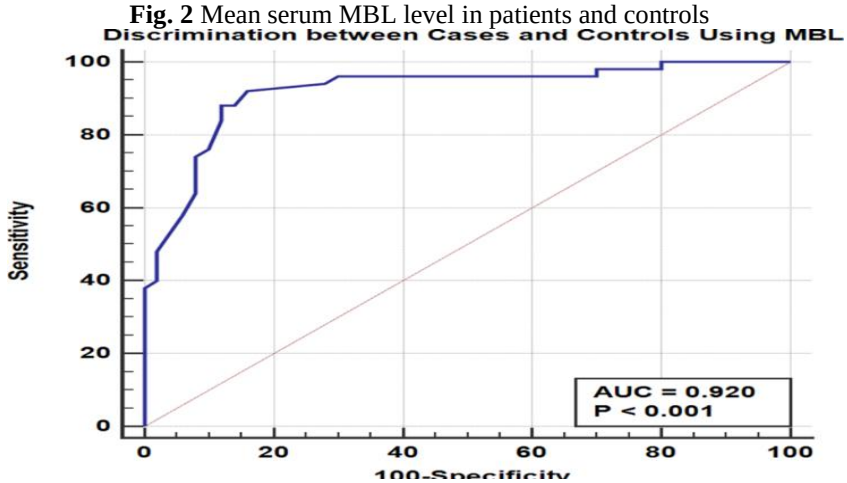


**Fig. 3** Receiver-operating characteristic (ROC) curve for discrimination between patients and controls using serum MBL level

In the current study, the normal genotype (GG) of codon 54 was significantly lower in patients (30/50, 60.0%), than in controls (43/50, 86.0%) (P=0.006), while the heteromutant (GA) genotype of codon 54 was significantly higher in patients (17/50, 34.0%) than in controls (7/50, 14.0%) (P=0.033). Even though the homomutant (AA) genotype of codon 54 was found in 3 patients (3/50, 6.0%) compared to none of the controls, the difference was not statistically significant (p=0.242) **(Fig. 4).** The codon54 alleles, were statistically different in patients compared to controls; the G allele was significantly lower in patients (77%) than in controls (93%) (P=0.0025), while the A allele was significantly higher in patients (23%) than in controls (7%) (P=0.0025) **(Fig. 5).**

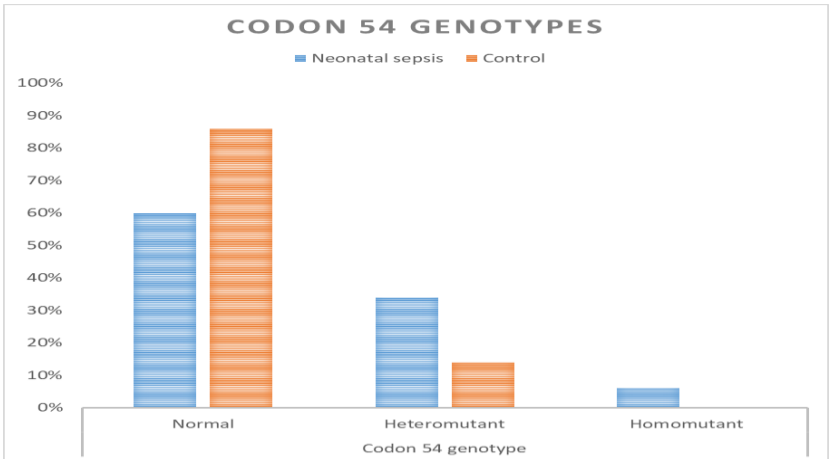


**Fig. 4** Codon 54 genotypes in patients and controls

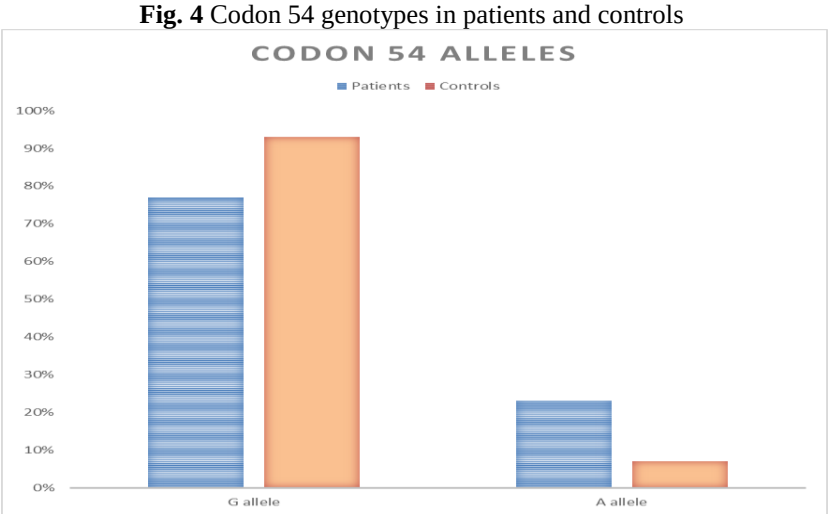


**Fig. 5** Codon 54 alleles in patients and controls

**Table (2):**Relation between codon 54 genotypes and MBL level in patients, controls, and the whole study population

| Variable | Group | GG | GA | AA | Ht (df) | P-value* |
|---|---|---|---|---|---|---|
| **MBL (ng/mL)** | **Patients, n, (med.), IQR** | (n =30)<br>46.0<br>44.0 – 48.0 | (n =17)<br>41.0<br>40.5 – 46.5 | (n =3)<br>40.0<br>37.5 – 45.0 | 5.854 (2) | 0.054 |
| | **Controls, n,(med.), IQR** | (n =43)<br>57.0<br>50.0 – 83.0 | (n =7)<br>83.0<br>51.5 – 107.0 | n =0 | 1.522 (1) | 0.217 |
| | **All the study population, n,(med.), IQR** | (n =73)<br>50.0 #<br>46.0 – 58.0 | (n =24)<br>45.8<br>40.8 – 53.3 | (n =3)<br>40<br>37.5 – 45.0 | 9.539 (2) | **0.008** |

Data are median (med.) and interquartile range (IQR); n = number, Ht = Kruskal Wallis statistic, df = degree of freedom; *Kruskal Wallis test; #Statistically significant difference versus AA genotype (P-value <0.05 by Dunn test).

Considering the whole study population, serum MBL level was highest among neonates with the GG genotype, followed by those with the GA genotype, and was lowest in neonates with the AA genotype. The difference between the groups was statistically significant (P=0.008), while among the patient group, serum MBL was higher in those with the GG genotype, followed by the GA genotype, and lowest in patients with the AA genotype but the difference did not reach statistical significance (P=0.054). Among the control group, none of the controls had the AA genotype and there was no statistically significant difference in the serum MBL between those with the GG genotype and those with the GA genotype (P=0.217) as shown in **Table 2 and Fig. 6.**

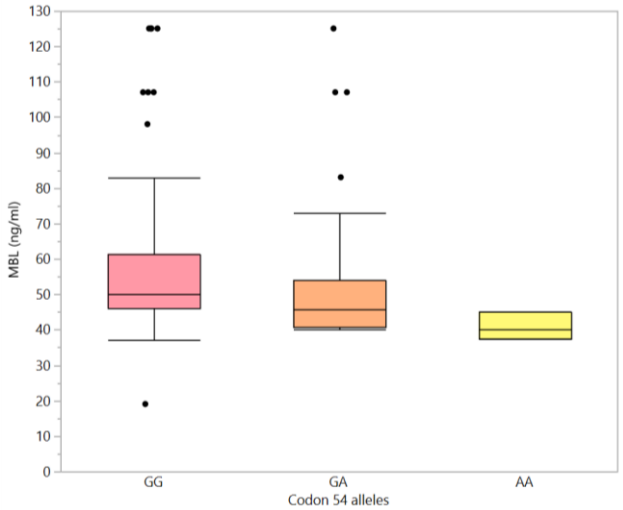


**Fig. 6** Box plot showing the relation between the codon 54 genotypes and the MBL level in the whole study population. [Box represents the interquartile range, line inside the box represents the median. Error bars represent the minimum and maximum values excluding outliers (dots)].

**Table (3):**Multivariable regression analysis for the relation between sepsis and MBL2 codon 54 genotype and the level of MBL

| Independent variables | Regression coefficient (B) | Standard error (SE) | t-statistic | P-value | r (partial) | r (semipartial) |
|---|---|---|---|---|---|---|
| (Constant) | 1.777 | | | | | |
| Sepsis (=1) * | -0.168 | 0.025 | -6.656 | **<0.0001** | -0.562 | 0.554 |
| GG (=1) # | 0.033 | 0.072 | 0.460 | 0.460 | 0.047 | 0.038 |
| GA (=1) # | 0.058 | 0.074 | 0.782 | 0.436 | 0.080 | 0.065 |

*Referenced to no sepsis (=0); #Referenced to AA (=0).

**Table 3** shows that, after adjustment to the effect of the other variables, sepsis was independently related to the level of MBL (B = -0.168, SE = 0.025, t = -6.656, P-value <0.0001). However, there was no statistically significant association between codon 54 genotype and the level of MBL (B = 0.033, SE = 0.072, t = 0.460, P-value = 0.460, and B = 0.058, SE = 0.074, t = 0.782, P-value = 0.436 for the GG and GA genotype, respectively).

In our research, there was a negative significant correlation between the serum MBL levels and the serum CRP (correlation coefficient; -0.345 and P=0.014) in the patient group. There was no correlation between serum MBL levels and each of the following; postnatal age at the time of sampling, gestational age (GA), birth weight (BW), immature/total neutrophils ratio (I/T ratio), absolute neutrophilic count (ANC), platelets count, pH, PaCO2, HCO3-, base excess (BE), NICU length of stay (LOS).

No statistically significant difference was found between survivors and non-survivors in patients with neonatal sepsis regarding the codon 54 genotypes [normal (GG), heteromutant (GA), and homomutant (AA)], and regarding the codon 54 alleles (G allele and A allele) (P>0.05).

## 4. DISCUSSION

Mannose-binding lectin is an important component of innate immunity, which is the newborn's first line of defense against infection. Several researchers have found conflicting associations between MBL2 gene polymorphisms, circulating MBL levels, and sepsis susceptibility and outcome [1]. In addition, MBL concentrations can differ in patients of different ethnic groups and different ages [11].

The mannose-binding lectin (MBL2) gene has 5 exons and is located on chromosome 10q11.2. Variations in the MBL2 gene alter the structure of the peptide chain and affect the formation and stability of the oligomeric MBL. Five well-studied polymorphisms of the MBL2 gene have been associated with lower serum levels of MBL. These polymorphisms include three structural variants of exon 1: codon 52 (rs5030737 C/T, also called A/D), codon 54 (rs1800450 G/A, also called A/B), and codon 57 (rs1800451 G/A, also like called air conditioning). The normal wild-type allele of the exon 1 polymorphisms is termed the A allele, while the three abnormal variants of the alleles (B, C, and D) are collectively termed the O allele [12].

There are two promoter variants in the promoter region: -550 (rs11003125 G/C, also called H/L) and -221 (rs7096206 G/C, also called Y/X). Both alleles B and C can inhibit the formation of helical structures in the collagen-like region (CLR); the D allele can cleave a disulfide bond in a peptide chain; and promoter polymorphisms can affect MBL concentration at the transcriptional level [12]. The aim of this study was to verify whether the lack of MBL, a component of innate immunity, is associated with an increased risk of neonatal sepsis and to verify whether there is a correlation between the polymorphism of the MBL2 gene at codon 54 (G/A) and MBL serum levels.

In the current study, MBL was significantly lower in patients compared to controls, and a low serum MBL level was positively correlated with neonatal sepsis. These findings were in agreement with several studies [1,2,4] [13-19] that reported that neonates with neonatal sepsis had lower serum MBL levels than controls. In a meta-analysis done by **Ma et al.,** [13] the MBL levels decreased with increased risk of nosocomial sepsis as the median MBL on admission was significantly lower among the 104 infected than 261 non-infected Italian neonates (540 ng/mL; interquartile range (IQR) 0.11–1.70 vs 1500 ng/mL; IQR 0.36–3.30) (P <0.001). Similarly, **Auriti et al**. [15] determined a strong association between low MBL levels and confirmed neonatal sepsis compared with suspected sepsis and the authors also observed that low MBL levels at birth might be associated with hospital-acquired sepsis in the NICU. **Szala-Poździej et al.** [4] explained that MBL deficiency was associated with an opsonization defect and was associated with recurrent infections, especially in immunocompromised individuals.

Because adaptive immunity has not yet developed, neonates are considered immunocompromised, and immune defense relies on maternal antibodies and innate immunity. As a result, neonates, particularly premature admitted to the NICU, are vulnerable to infections that can be fatal [4]. In the studies done by **Szala-Poździej et al.** [4] and **El-Shimi et al.,** [14] the combination of prematurity and low MBL levels increased the risk of sepsis to 70%. They explained that low MBL levels could be linked to a reduced ability to phagocytose or opsonize microorganisms. They also discovered that preterm neonates had significantly lower mean MBL serum levels than full-term neonates.

In our research, serum MBL level had a good predictive value for the occurrence of neonatal sepsis. This was in accordance with the results described by **Nosier et al.,** [18] who demonstrated that serum MBL level had an excellent predictive value to detect neonatal sepsis in their study with an area under the ROC curve (AUC) of 0.918 (95% CI, 0.971, p-value <0.001). They found that a cut-off criterion of MBL ≤ 42 ng/mL had a sensitivity of 94.1% and a specificity of 100% in the prediction of neonatal sepsis.

Also, in a study done by **Xue et al.,** [2] low serum MBL level was a novel marker and had a good predictive value for neonatal sepsis in the Chinese Han population with an area under the ROC curve (AUC) of 0.844 (95% CI, 0.775 - 0.913, P = 0.041). A cut-off value of MBL ≤384.60 ng/mL had a sensitivity of 80.6% and a specificity of 77.7% for predicting sepsis in their study.

Additionally, **Dima et al.** [19] showed that serum MBL level had a good predictive value for the occurrence of neonatal sepsis with an area under the ROC curve of 0.842, odds ratio (OD) = 2.14 (95% CI, 1.49 to 3.05). A cut-off value of MBL ≤ 700 ng / mL had a sensitivity of 50% and a specificity of 93.10%.

Mannose-binding lectin is an important component of the innate immune system that serves as the first line of defense against infection in newborns. MBL can bind and opsonize a variety of pathogens, including bacteria, viruses, and parasites, and it plays an important role in infection resistance [2].

In the current study, the normal genotype (GG) of codon 54 was significantly lower in patients compared to controls (P=0.006), while the heteromutant (GA) genotype was significantly higher in patients compared to controls (P=0.033). The homomutant (AA) genotype was found in 3 patients (3/50, 6%) compared to none of the controls, but the difference did not reach statistical significance (P=0.242). This may be due to our study population's small number of neonates with this genotype (AA). The normal G allele of codon 54 was significantly lower in neonates with sepsis compared to controls (P=0.0025), while the A allele was significantly higher in neonates with sepsis compared to controls (P=0.0025).

Our results are in concordance with **Ma et al.** [13] who found that neonatal sepsis was diagnosed significantly higher in infants with GA and AA genotypes compared to normal genotype (GG) in Turkish neonates. In their study, the AA genotype was higher in the sepsis group compared to the control group (15.1 vs 0.0%, P=0.01), whereas the

GG genotype was more common in healthy controls when compared to the neonates with sepsis (87.5 vs 67.9%, P=0.03).

A study done by **SljivancaninJakovljevic et al.** [16] also reported that the frequency of sepsis in infants with GA and AA genotypes was significantly higher than those with the GG genotype in the Turkish population [16]. Similarly, Liu and Ning [12] also found that the variant allele carriers (GA) were more frequently discovered in the sepsis group than in the control group (P= 0.013, OR= 2.090, 95% CI= 1.163-3.753) and that the GA genotype was significantly associated with the onset of sepsis but the AA genotype had no significant association with the occurrence of sepsis.

Consistent with our results, **Ma et al.** [13] showed that the A allele was a risk factor for sepsis in Turkish patients. The frequency of the A allele was significantly higher in the sepsis group (23.6%) compared to the control group (6.2%) in their study (P= 0.002, OD= 4.63, 95% CI= 1.62 to 16.17). **Badawy et al.** [1] also found that the frequency of the A allele was higher in the septic group than in the control group, but the difference did not reach statistical significance (P= 0.282). Liu and Ning [12] showed that the A allele might increase the risk of sepsis by decreasing the MBL serum level (P= 0.007, OR =1.979, 95% CI = 1.200-3.262). In addition, **Mashaly et al.** [20] found that the A allele, both heterozygous (GA) and homozygous (AA), was the commonest mutant allele encountered in patients with neonatal sepsis (39.4%). **Świerzko and Cedzyński,** [21] **SljivancaninJakovljevic et al.** [16] also revealed that the A allele was the commonest mutant allele in neonatal sepsis.

In contrary to these findings, **Klostergaard et al.** [22] and **Behairy et al.** [23] found that the codon 54 polymorphisms (G/A) had no significant association with sepsis risk. Additionally, **Ma et al.** [13] showed no significant difference in the frequency of MBL2 genotypes between infected and non-infected Italian neonates (P >0.1).

This disparity in results could be explained by different sample sizes as well as the variable distribution of MBL2 genotypes in different populations, as the frequency of MBL gene polymorphisms may differ between ethnicities [20]. The MBL2 polymorphism at codon 54 (rs1800450) causes the Gly54Asp variation, which results in the formation of non-functional monomers that interfere with the formation of higher-order oligomers of MBL, resulting in changes in the protein's functional activity and circulating levels. Thus, the link between MBL2 gene polymorphisms and the occurrence of sepsis remains debatable. Furthermore, gene polymorphisms differ across regions and ethnicities [24].

In agreement with our results regarding the relation between codon 54 genotypes and MBL level, **Ma etal.** [13] demonstrated that, in Turkish patients, the mean and the median serum MBL level in the normal genotype GG were significantly higher than the heteromutant GA and homomutant GA genotypes (P=0.03 & P < 0.01 respectively).

In accordance with our research, **Nosier et al.** [18] concluded that there was a negative correlation between the serum MBL level and the serum CRP level (r = -0.677, P<0.001). They also found a significant positive correlation between the serum MBL level and hemoglobin level, platelet count, and total leucocyte count (TLC) (r = 0.622, 0.592, and 0.588 respectively, P<0.001). The authors also concluded that there was no correlation between the serum MBL level and either gestational age or birth weight. Similar to our findings, **El-Shimi et al.** [14] demonstrated that there was no correlation between the serum MBL level and each of the following; gestational age, birth weight, respiratory distress syndrome, maternal pre-eclampsia, gender, and mode of delivery.

On the opposite results, **Badawy et al.** [1] showed that birth weight and head circumference were negatively correlated with the serum MBL levels (r = -0.248 and -0.344, respectively, and p = 0.048 and 0.005, respectively). A link between serum MBL levels and both gestational age and birth weight was shown in a study by **SljivancaninJakovljevic et al.** [16]. They discovered that premature infants born at 1000 g or at 28 gestational weeks with low MBL levels were more likely to suffer from neonatal sepsis. Furthermore, **Ma et al.** [13] showed that infants born at 28 weeks of gestation or at 1000 g had significantly lower MBL levels. These findings point to the possibility of developmental regulation of MBL gene expression and a maturation process for MBL levels in preterm infants with codon 54 mutations, presumably completed by full-term gestation.

In accordance with our research, **Hartz et al.** [25] demonstrated that there was no significant difference in mortality between preterm infants regarding the codon 54 genotypes [normal (GG), heteromutant (GA), and homomutant (AA)]. **Ma et al.** [13] also did not find a significant association between MBL2 genotypes and mortality in Italian neonates. **Del Vecchio et al.** [26] concluded that the overall mortality was not found to be related to MBL genotypes, it is likely that some SNPs by themselves are not the cause of increased susceptibility to or outcome from sepsis but rather are makers for an extended haplotype for genetic variations.

## STUDY LIMITATIONS

There were some difficulties in our study; the number of neonates with the AA genotype in our population was small, sepsis is a complex disease

that is affected by various genetic and environmental factors, other polymorphisms in the MBL2 gene and other risk factors are confounding factors which could have affected the risk for neonatal sepsis and the outcome.

## 5. CONCLUSION

Serum MBL level could be considered a sensitive and specific marker for increased susceptibility to neonatal sepsis and was negatively correlated with the CRP level. Serum MBL level was lower in preterm septic neonates and was lower in neonates with the homomutant (AA) genotype compared to neonates with the heteromutant (GA) or normal (GG) genotypes. The abnormal GA and AA genotypes, and the abnormal A allele of codon 54 were higher in preterm neonates with sepsis. No significant difference was found between survivors and non-survivors of neonatal sepsis regarding the codon 54 genotypes and alleles.

## ETHICAL APPROVAL

The study was approved by ethics committee, Faculty of Medicine, Cairo University, Egypt.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ANC | : absolute neutrophilic count |
| AUC | : area under the receiver-operating characteristic curve |
| BE | : base excess |
| BW | : birth weight |
| CBC | : complete blood count |
| CI | : confidence interval |
| CLR | : collagen-like region |
| CRP | : C-reactive protein |
| EDTA | : ethylene diamine tetraacetic acid |
| GA | : gestational age |
| $HCO_3^-$ | : blood bicarbonate |
| IQR | : interquartile range |
| I/T ratio | : immature: total neutrophils count ratio |
| LOS | : length of stay |
| MBL | : mannose-binding lectin |
| MBL2 | : mannose-binding lectin gene |
| NICU | : neonatal intensive care unit |
| OD | : odds ratio |
| $PaCO_2$ | : arterial carbon dioxide pressure |
| PCR-RFLP | : polymerase chain reaction-restriction fragment length |
| pH | polymorphism |
| ROC | : hydrogen ion concentration |
| rpm | : receiver-operating characteristic |
| SNPs | : revolutions per minute |
| | : single nucleotide polymorphisms |

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