Association Between Mannose - binding Lectin and Serum Parameters of Neonatal Sepsis

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The aim of this study was to evaluate the MBL values and the associations and correlations between MBL and different serum parameters of neonatal sepsis (procalcitonin, interleukin-6, C-reactive protein) in preterm neonates. The results are in concordance with the studies that show the utility of procalcitonin, CRP, IL-6 or MBL as diagnostic markers of neonatal sepsis.

Keywords: MBL, neonatal sepsis, procalcitonin, interleukin-6, C-reactive protein

Sepsis is normally defined as bacteremia combined with systemic inflammatory response syndrome, but there is no widely accepted definition for neonatal sepsis [1]. Neonatal sepsis is a severe and potentially dangerous disease defined on the basis of clinical and laboratory study which can develop rapidly and lead to increased morbidity and mortality without a promptly and correctly treatment [2]. Although it follows the discovery of rapid diagnostic techniques, the basic problem still prevails. Many children, both term and preterm have vague nonspecific symptoms and the doctor must decide empiric antibiotic treatment [3]. This decision must be taken quickly because currently available tests are inaccurate and time consuming [4]. However, since the discovery of penicillin, this dilemma has remained the same. Several hematological markers such as white blood cell count (WBC) and neutrophil counts were proposed and evaluated as diagnostic tests for neonatal sepsis [5]. The interpretation of these tests is quite complicated, because normal values are affected by certain conditions, such as post-gestational age, asphyxiation or maternal factors such as fever and hypertension [6]. Therefore, a real challenge is now to identify a specific and sensitive marker for early detection of neonatal sepsis to avoid prolonged antibiotic therapy, numerous days of hospitalization and mother-child separation.

C-reactive protein is an acute phase reactant discovered in 1930. The classical pentraxins CRP belongs to the family of calcium-dependent plasma proteins [7]. There is a rapid increase in the concentration of IL-6 after exposure to the bacteria that may precede even the increase in CRP. IL-6 in cord blood was consistently shown to be a sensitive marker for the diagnosis of early onset neonatal sepsis (EONS), with a sensitivity of 87-100 and 93-100% predictive values [8]. IL-6 has the highest sensitivity (89%) and the predictive value (91%) at the onset of infection, compared with other biochemical markers, including CRP, IL-1, TNF-α, and E-selectin, but the sensitivity is reduced from 24 to 48 h (67 and 58%), as the serum IL-6 levels decreased rapidly and become undetectable after 24 h [10]. Thus, CRP can be regarded as a specific marker for LONS extremely useful, but repeated measurements may be useful in the treatment of neonatal sepsis especially in the first 48 h of birth. Furthermore, it was suggested that CRP is a marker for late onset neonatal sepsis (LONS) if determined in combination with IL-6 [11].

Procalcitonin (PCT) is a protein with 116 amino acids and molecular weight of 13 kDa, discovered 25 years ago as a prohormone of calcitonin, produced by the thyroid C cells and cleaved by intracellular proteolytic enzymes in the PCT. It was suggested that postnatal physiological values of PCT are most likely an endogenous synthesis attributed to direct stress during the perinatal period or adaptation to the environment [12]. Even physiological maximum value of PCT is useful in diagnosing ectopic and monitoring of the newborn at risk for infection. It has been shown that PCT is useful not only in the diagnosis of sepsis, but also in monitoring treatment response and prognosis of newborns with neonatal sepsis [13].

Mannose-binding lectin (MBL), also named mannan-binding protein (MBP) or mannose-binding protein is a serum protein with an important role in the immune response pattern recognition of pathogens [14]. A recent study has shown that MBL had a sensitivity of 97% and a specificity of 97% for the diagnosis of neonatal sepsis in preterm infants.

Experimental part

Material and method

This prospective study included all infants admitted to the Emergency County Hospital Timisoara, Department of Neonatology in the period 2012 - 2013. The group of 37

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Parameters	Group 1 with positive cultures (n=20)	Group 2 with negative cultures (n=20)	CON Group (n=29)
Weight (g)	640,99 ± 143,33	1600,50 ± 445,96	3193,45 ±434.20
C reactive protein	53.43 ±29.55	14.06 ± 2.76	4.18 ±1.85
Procalcitonin (ng/ml)	26.49 ± 15.06	1.43 ± 0.66	0.20 ± 0.11
Interleukin-6 (pg/ml)	35.33 ±11.51	21.12. ± 6.48	7.44 ± 2.86
MBL (µg/ml)	0.37 ± 0.08	1.10 ± 0.34	1.64 ± 0.46

Table 1
BASELINE CHARACTERISTICS OF THE PREMATURE NEONATES IN THE THREE GROUPS

preterm was divided than into two groups, depending on blood cultures. The first group (group 1) comprised 20 premature infants with positive cultures and other signs or symptoms suggestive of neonatal sepsis (sepsis shown). The second group (group 2) included 17 premature infants with negative cultures and two or more clinical signs suggestive of neonatal sepsis and abnormal hematological values (suspected sepsis). Both groups were than compared with a control group which included 18 premature infants with hyperbilirubinemia without physiological and clinical data and biological infection (without sepsis).

The clinical criteria of preterm neonates with neonatal sepsis for inclusion in the study were:

- maternal risk factors, such as fever or rupture of membranes> 24 h;

- neonatal history: low birth weight (<2500 g), preterm delivery (<37 weeks);

- signs and symptoms of sepsis: food intolerance, lethargy, temperature instability, apnea, respiratory distress syndrome, seizures, tachypnea, bradycardia, abdominal distension and vomiting.

Data were collected on standard forms which detailed the medical history, nine clinical signs known to be associated with neonatal sepsis and laboratory values. Personal data: age, gender. Physical examination of premature neonates consisted of measuring length, weight, Apgar score, thoracic and cranial perimeter. Because clinical signs are considered subjective, we collected data only from the Department of Neonatology County Hospital Timisoara to ensure the accuracy of the data.

Laboratory diagnosis for all groups included blood culture system (Biomerieux) blood count, C-reactive protein (immunoturbidimetry), procalcitonin (immunochromatography), the Il-6 (ELISA) and MBL (ELISA) determination.

The study was conducted with the approval of the Research Ethics Committee of the University of Medicine and Pharmacy *Victor Babes* Timisoara, Romania. It was obtained a written consent from the families of prematures neonates investigated in this study.

Results and discussions

The baseline characteristics of the subject are summarized in table 1.

It was observed that CRP values were significantly increased in the group of positive cultures compared with preterm premature and negative-culture group (p<0.001). Also, the group with negative cultures has statistically insignificant increased values of CRP compared to the control group. Also, CRP values were significantly increased in the group of preterm with positive culture compared to the negative ones, observing that premature infection influenced the increase of CRP values (table 1).

The values of procalcitonin values were low, but statistically insignificant for the control group compared to the group with negative cultures (p = 0.872) and significantly lower than the group of preterm positive cultures (p <0.001). Also procalcitonin values were significantly lower for the group of preterm with negative cultures, compared to those with positive cultures (p<0.001), suggesting that premature infections influenced the increased values of procalcitonin (table 1).

It has been noticed that IL-6 values were significantly lower for the control group compared with the other 2 groups (p <0.001), also for lot with negative cultures from that with positive cultures (p <0.001), suggesting that infant infection influenced the increase of IL-6 (table 1).

The MBL values were significantly increased in the control group compared to the group with negative culture and compared to the group of preterm with positive cultures (p <0.001). Furthermore, the values of MBL were significantly higher for the group with negative cultures from that of positive cultures (p <0.001), which indicated that premature sepsis influenced the decline of MBL levels (table 1).

It has been observed that the values of MBL were strongly correlated with the IL-6 (r = 0.609, p < 0.001) (fig. 1) and procalcitonin (r = 0.758, p < 0.001) values (fig. 2).

In this study the values of MBL = 0.7 mg/mL is considered to be low and the optimal cut-off value for MBL deficiency was found 0.7 mg / mL. It was found that the area under the ROC curve is 0.842. It was found that MBL values below

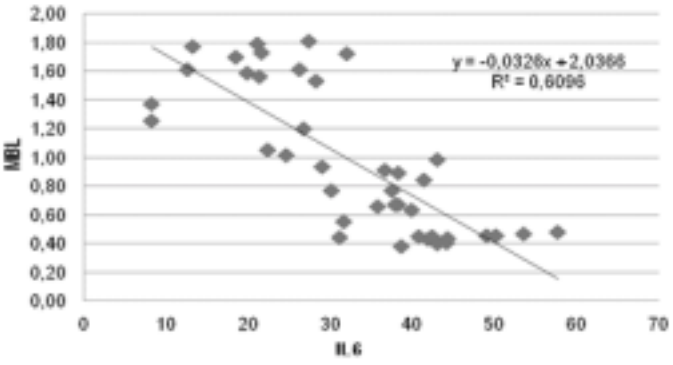

Fig. 1. Correlation between MBL and IL-6

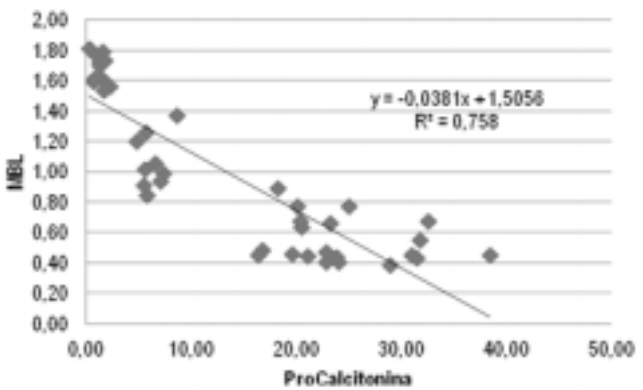

Fig. 2. Correlation between MBL and procalcitonin

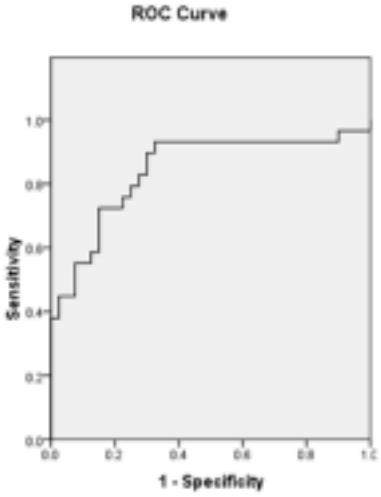

Fig. 3. The specificity and sensitivity analysis of MBL

0.7 mg/ml were considered a risk factor for the occurrence of neonatal sepsis (OR = 2.14 index) and the 95% confidence interval belongs (1.49, 3.05). MBL has sensitivity was 50%, the specificity of 93.10% and the positive predictive value of 90.91% (fig. 3).

In neonatology units is essential a rapid diagnosis of neonatal sepsis and the causes of its appearance, using modern methods and rapid clinical diagnosis, in order not to lose valuable time and start appropriate treatment. The delay in recognizing neonatal sepsis can cause multi-organ dysfunction and death invariably. There are various diagnostic tests used for rapid diagnosis of neonatal sepsis. It is absolutely necessary the differentiation of premature without infection or with infection using serum markers such as procalcitonin, CRP, IL-6 or MBL. This study showed that the values of procalcitonin, IL-6 and CRP were significantly increased in premature infants with positive cultures as compared to the group of premature infants with negative cultures and control group. On the contrary, the highest values of MBL were observed in control group compared to the groups of premature infants with negative and positive cultures. Our results are in concordance with difference studies that showed the utility of procalcitonin, CRP, IL-6 or MBL as diagnostic markers of neonatal sepsis. Moreover, the cytokines and chemokines, such as interleukin IL-6 and IL-8 have demonstrated that they can have good utility as markers in diagnosis of early neonatal sepsis, while the acute phase reactants, such as C-reactive protein and procalcitonin have superior diagnostic properties during late sepsis [15].

A systematic review and meta-analysis of 29 studies suggested that serum PCT has a very good diagnostic accuracy (AUC = 0.87) for the diagnosis of neonatal sepsis [16]. In infants with very low birth weight was observed that serum PCT> 2.4 ng/mL require empiric antibiotic treatment established quickly, while infants with normal weight and value of PCT d" 2, 4 ng/mL low risk of neonatal sepsis [17]. Other studies have claimed that serum procalcitonin is superior to CRP for early diagnosis of neonatal sepsis in detecting and assessing the severity of response to therapy [18, 19].

The results of our study were in conformity with different other studies which have shown that neonates with sepsis had significantly higher IL-6, CRP, procalcitonin compared with infants who have sepsis. Combining measuring PCT and IL-6 increased sensitivity to 88%, and the combination PCT and CRP increased sensitivity to 82% [18, 20-22]. Therefore, the combination of simultaneous determination of IL-6, CRP and PCT appears to be predictive for the diagnosis of early-onset sepsis.

IL-6 values were significantly increased in the group of positive cultures compared with preterm premature lot of negative cultures ($p < 0.001$), and compared to the control group, which indicated that infection of the newborn might increase IL-6. Similar to this study other studies have shown that in infants with large numbers of leukocytes and suspected sepsis, IL-6 level was high, with a greater sensitivity (85.71%) and a negative predictive value (95%). IL-6 has been shown to have high sensitivity (76.9%), specificity (73.68%), positive predictive value (80%) and negative predictive value (70%) in cases of suspected sepsis with elevated CRP values . Therefore, it can be considered that IL-6 is an extremely useful marker for early detection of sepsis [22, 23].

The MBL values were significantly increased compared in the control group compared to the group with negative culture and compared to the group of preterm with positive cultures. Furthermore, the values of MBL were significantly higher for the group with negative cultures from that of positive cultures, which indicated that premature sepsis influenced the decline of MBL levels. However genetic factors might influence the values of MBL. A new meta-analysis further indicated that MBL polymorphisms could be associated with the susceptibility to sepsis [24]. Another meta-analysis which evaluated the association between serum levels of MBL and the development of sepsis observed a crucial contribution of serum MBL in infants with sepsis [25].

Conclusions

MBL values were inversely strongly and significantly correlated with the IL-6 and procalcitonin values, suggesting that combined determination of these markers increase the rapidity of diagnostic. A better understanding of the pathogenesis of neonatal sepsis will help to develop therapeutic strategies for reducing mortality of this age group.

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