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Evaluation of CD11b Expression on Peripheral Blood Neutrophils for Early Detection of Neonatal Sepsis

Minoo Adib[1], Vajiheh Ostadi[1], Fakhri Navaei[2], Fereshteh Saheb Fosoul[1], Farzad Oreizi[1], Raheleh Shokouhi[3], and Zahra Bakhshiani[1]

[1] Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[2] Department of Pediatrics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[3] Immunology, Asthma and Allergy Research Institute, Medical Sciences / University of Tehran, Tehran, Iran

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ABSTRACT

Neonatal sepsis is a disease of infants who are less than 1 month of age. These infants are clinically ill, and their blood culture are positive for bacteria. The reported incidence of neonatal sepsis for all infants is 1 to 10 per 1000 live births. The mortality rate is 4.2-26%. The clinical signs are not specific and diagnosis of neonatal sepsis is one of the most difficult tasks in clinical medicine. The aim of this work was determination of CD11b sensitivity and specificity for early detection of neonatal sepsis.

We studied 65 neonates with gestational age of 27 to 38 weeks who were suspected for sepsis within the 28 days of life. Whole blood was obtained from neonates to determine CD11b expression on peripheral blood neutrophils by flow cytometry. C-Reactive protein (CRP) was measured qualitatively. Neonates were divided into two groups. Classification was based on the result of the blood culture.

In the sepsis group all of the neonates (n = 8) showed positive blood culture and clinical symptoms. In the suspected group (n = 57) the neonates showed clinical signs but blood cultures were negative. Sensitivity and specificity of CD11b were 75%, 100% respectively. Also positive and negative predictive values of CD11b were 100% and 86% respectively.

Results of present study and previous studies showed that measurement of neutrophil surface markers can be useful for diagnosis of infection in the early phases. Also, the quantitative measurement of CRP in addition to CD11b further enhances the ability to diagnose infections and improves sensitivity and negative predictive value by 100%.

Key words: CD11b; CR3; Neonatal sepsis; Neutrophils

INTRODUCTION

Neonatal sepsis is an infection of infants who are less than 1 month of age, who are clinically ill, and show positive blood culture.[1] The reported incidence of neonatal sepsis for all infants is 1 to 10 per 1000 live births.[2] The mortality rate is high (4.2-26%), with the higher rates observed in premature infants and in those with early fulminant of clinical signs.[2,3] Diagnosis of neonatal sepsis is one of the most difficult tasks in clinical medicine, because the symptoms and clinical signs are subtle and nonspecific.[3-5] The current gold standard for confirming the diagnosis of neonatal sepsis is isolation of the causal microorganism by blood

Corresponding Authors: Minoo Adib, MD, PhD;
Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. Tel: (+98 311) 7922 529, Fax: (+98311) 6688 597, E-mail: adib@med.mui.ac.ir

culture. However, blood culture results are not available until 24-72 hours after starting the culture, and they are often negative in cases of pneumonia and meningitis, or even in fatal generalized bacterial infection.[4,5]

Previously, white blood cell counts and the determination of C-reactive protein (CRP) have been used to diagnose neonatal sepsis.[6] C-reactive protein is a major acute phase plasma protein which is synthesized by hepatocytes.[7,8] Considering the high mortality associated with neonatal sepsis, a diagnostic marker with a very high sensitivity and negative predictive value approaching 100% is very necessary to reduce antimicrobial unnecessary treatment and hospitalization. Because of the advances in flowcytometric technology, this study paid attention to CD11b, a neutrophil surface antigen, and its sensitivity and specificity in diagnosis of neonatal sepsis. CD11b (Mac-1, CR3) is an α subunit of the β_2 integrin adhesion molecule. It is normally expressed at a very low concentration on the surface of non-activated neutrophils. CD11b increases on the neutrophil surface within 5 minutes of exposure to bacteria or endotoxin (9, 10). The aim of this work was determination of CD11b sensitivity and specificity for early detection of neonatal sepsis.

PATIENTS AND METHODS

Patients

Sixty five at risk neonates in the first 28 days of life who were admitted to the Neonatal Intensive Care Unit (NICU) at the Dr. Beheshti hospital in Isfahan were studied. Inclusion criteria were the presence of at least one clinical sign (temperature instability, grunting, apnea, cyanosis, tachycardia, bradycardia) or perinatal risk factor (prematurity, low birth weight, prolonged rupture of membranes exceeding> 24 hours, maternal peripartum fever or infection, urinary tract infection) suggesting infection. Finally, neonates were classified into two groups: suspected sepsis (n = 57): who showed clinical symptoms but negative blood culture. Proven sepsis (n = 8): with positive blood culture and clinical symptoms. 12 healthy full term neonates with physiologic hyperbilirubinemia were classified in control group. Characteristics of the groups are presented in Table 1.

Assay for CD11b

Flow cytometric determination of neutrophil CD11b performed at 4ºC to minimize neutrophil activation.[3] To 50 λ of whole blood was added 5 λ of fluorescent anti CD11b antibody (R-phycoerythrin) (Serotec, UK) into the tube and incubated for 25 min at 4ºC in the dark. 2 ml of a 1/10 diluted cold lysing solution (Serotec, UK) were added and incubated for 5 min at 4ºC. After centrifugation, the cells were resuspended in 2 ml of FACS lysing solution and incubated for 5 min at room temperature.

After centrifugation, leukocytes were resuspended in 0.2 ml PBS for the acquisition and analysis of the data FACS (Fluorescent Activated cell sorter) flow cytometer and Cell Quest analysis soft ware were used.

CRP Assay

Plasma CRP measured qualitatively by commercial kit (kimiapazhooh, Iran).

RESULTS

Neonates in Sepsis group (n=8) showed positive blood culture, including 5 for antrobacter, 2 for Escherichia coli and 1 for Coagulase negative staphylococcus. Four neonates were CRP positive and 4 were CRP negative. In suspected group (n = 53) neonates were CRP negative and 4 were CRP positive [1 neonate (+++) and 3 neonates (+)]. There were significant differences in mean gestational age, birth weight, apgar score 1 and 5 between groups (Table 1). There were no significant differences in the mean CD11b expression level between groups. However the mean of CD11b level was high in sepsis and suspected groups compared with control group (Table 2 and figure 1). Sensitivity and specificity of CD11b were 75% and 100% respectively. Also positive and negative predictive values of CD11b were 100% and 86% respectively.

DISCUSSION

Sepsis is a growing problem among low birth weight infants. The reported incidence of neonatal sepsis for all infants is 1-10 per 1000 live births. In one recent large National Institute of Child Health and Human Development Neonatal Research Network (NICHHD-NRN) consortium study of 7861 Very Low Birth Weight (VLBW) infants; the incidence of early onset sepsis with proven positive blood culture in VLBW infants was 13-27 per 1000 live births (1.9%).

Added to this affected population of VLBW infants, is late onset sepsis (nasocomial/horizontal acquisition),

Table 1. Clinical Characteristics of groups.

Features	Control group	Suspected group	Sepsis group	P-value
No. of neonates	12	57	8	-
Gastational age (week)	36.9 ± 1.2	33 ± 2.7	30.7 ± 1.8	< 0.001
Birth weight (gr)	2647 ± 363.5	1940 ± 578	1518 ± 397	< 0.001
Apgar scor 1	8.9 ± 0.51	7.2 ± 1.8	5.8 ± 1.5	0.001
Apgar scor 5	9.83 ± 0.38	8.5 ± 1.3	8.2 ± 1.6	0.005

Table 2. Neutrophil CD11b expression levels in three groups.

Marker	Control group	Suspected group	Sepsis group	P-value
CD11b	199.6±54.9	293±140	285.7±65.3	0.069

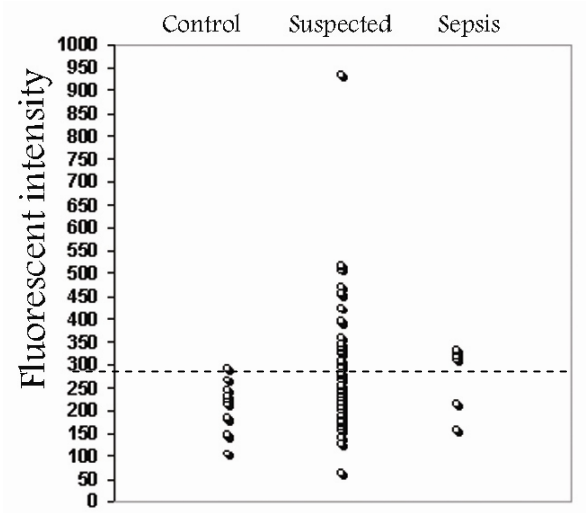

Figure 1. Expression of CD11b in different groups. The dashed line corresponds to the cut-off value of the percentage of $CD11b^+$ cells found in normal neonates.

which increases the risk for sepsis in these infants throughout their initial hospitalization by another 16% to 24%. The neonatal mortality rate is high (4.2-26%).[12]

The inability to adequately exclude the diagnosis of neonatal sepsis may result in prolonged and unnecessary use of antibiotics, adding to the infant's hospitalization and further family separation. Antibiotic therapy for more than 72 hours has been shown to increase the colonization of gram negative organisms and to encourage the evolution of drug resistant strains. Early diagnosis and treatment of sepsis in infants help to reduce the high mortality and morbidity seen in this disorder.[12]

The diagnosis of sepsis remains one of the most difficult diagnostic tasks for neonatal nurses, neonatal practitioners and physicians. Blood cultures often remain negative in the presence of pneumonia, meningitis and even fulminant bloodborne septicemia. Capturing the specific organism in a small sample of peripheral venous blood remains a very difficult task. An early laboratory and specificity test for neonatal sepsis would be a valuable tool for therapeutic decision- making, thus avoiding the unnecessary use of antibiotics in those patients without infection but in whom sepsis is suspected on a clinical basis.[4,11]

In this study, we attempted to determine the CD11b as one immunological marker for early detection of neonatal sepsis. CD11b is a cell surface antigen of neutrophil and is normally expressed at a very low level on the surface of non-activated cells.[9,10] Its expression on neutrophil cell surface, however, increases substantially within a few minutes after the cell comes into contact with bacteria or endotoxins.[12,13] This unique property enables CD11b to be used as a potential early warning marker for detection of bacterial infection. Thus, this specific marker could potentially be used for identifying life-threatening infection in preterm infants.

In this study we included risk neonates at 28 days who were admitted to the NICU. There were significant differences in means of gestational age and birth weight between groups. These findings showed that prevalence of infection in neonates was inversely related to gestational age and birth weight.[14]

In present study, CRP was measured qualitatively. These results showed that qualitative measurement of CRP was not sensitive in diagnosis of sepsis. Previous studies suggest that CRP is particularly useful in managing late onset nosocomial bacterial or fungal systemic infection and necrotising enterocolitis.

However, since the concentration of CRP increases rather slowly in the initial phase, its sensitivity at the time of sepsis evaluation is only 60%. Serial quantities measurements at 24 and 48 hours after the onset of sepsis considerably improve the sensitivity about 82% and 84% respectively.[14] The specificity and positive predictive value of CRP range were from 93% to 100% throughout the study period. Thus, CRP can be considered as a specific but late marker of neonatal infection.[15] CD11b expression levels between three groups showed no significant difference (P value = 0.069), but mean of CD11b expression levels were elevated in suspected and sepsis groups compared to the control group. However, Weirich et al. and Nupponen et al. have shown that expression of CD11b was much higher than control group in neonates with confirmed sepsis, but Cui et al have shown that expression of CD11b was lower in neonates with sepsis than control group.[10,3,16] In contrast, Weinschenk et al. and Espinosa et al did not demonstrate a high elevation of CD11b in neonates with sepsis. [4]

We found the sensitivity and specificity of CD11b 75% and 100% respectively, although Weirich et al and Nupponen et al. have shown very high sensitivity and specificity for CD11b, 96% and 100%, 100% and 100%, respectively.[3,10] On the other hand, Cui et al and NG et al showed sensitivity and specificity of CD11b about 86.3% and 100%, 70% and 72% respectively.[16,17] Probably there could be a reason for these controversial results. Since activation of CD11b expression on neutrophils occurs early in the disease process, a delay in recognition of sepsis and consequently in obtaining the blood sample might result in missing the peak of CD11b expression. On the other hand, increased peak of expression of CD11b for sepsis evaluation is initiation of clinical onset.[10] Therefore, accuracy of CD11b in diagnosing late onset infection is variable. The discrepancy of results between studies may be related to different infant populations being evaluated.[9] Moreover we obtained Positive and Negative Predictive Value (PPV and NPV) of CD11b 100% and 86% respectively. The results of present study and previous studies showed that the measurement of neutrophil surface markers can be useful for diagnosis of infection in the early phases. Also, the quantitative measurement of CRP in addition to CD11b further enhances the ability to diagnose infections and improves sensitivity and negative predictive value to 100%.

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