Evaluation of CD64 Expression on Peripheral Blood Neutrophils for Early Detection of Neonatal Sepsis

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ABSTRACT

Background: Neonatal sepsis is a life-threatening disease with an incidence of 1 to 10 per 1000 live births and a mortality rate of 15% to 50%. The clinical signs are non-specific and indistinguishable from those caused by a variety of neonatal non-infectious disorders. **Objective:** The aim of this study was to determine the importance of CD64 expression (FcγRI), a neutrophil surface marker, in early diagnosis of neonatal sepsis. **Methods**: The studied population comprised of 65 neonates with gestational ages of 27 to 38 weeks, suspected of having sepsis in the first 28 days of life and 12 healthy neonates with physiologic hyperbilirubinemia. One ml of whole blood was obtained to determine CD64 expression on peripheral blood neutrophils by flow cytometry. **Results**: CD64 expression was significantly higher in the group with sepsis than the control groups ($P < 0.001$). Sensitivity and specificity of CD64 were 92.3% and 100%, respectively. The negative and positive predictive values of CD64 for identifying sepsis were 100% and 88%, respectively. **Conclusion:** A change in cell surface expression of CD64 on peripheral blood neutrophils may be considered as a sensitive marker for detection of neonatal sepsis if used in combination with other laboratory parameters.

Keywords: Neonatal Sepsis, CD64, FcgRI

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INTRODUCTION

Neonatal sepsis is a disease with an incidence of 1 to 10 per 1000 live births, and a mortality rate of 15 to 50% (1). Clinically, neonatal sepsis occurs in either of two settings: early onset sepsis, presenting within the first 4 days of life and late onset sepsis, presenting thereafter (2). Early diagnosis of sepsis in neonates is difficult because of subtle and nonspecific clinical signs, which are indistinguishable from those caused by a variety of non-infectious disorders, such as aspiration syndromes, maladaptation, and respiratory distress syndrome (1,3,17). The gold standard test in diagnosis of neonatal sepsis is isolation of the causal microorganisms by blood cultures. However, blood culture results are not available until 24-48 hours and are often negative in cases of pneumonia and meningitis, or even in fatal generalized bacterial infection (3). Previously, white blood cell count and the acute phase reactants such as C-reactive protein (CRP) were used in diagnosis of neonatal sepsis (4). CRP, a major acute phase plasma protein, is synthesized by hepatocytes in response to infections or tissue injuries (5, 6). Considering the high mortality rate of neonatal sepsis, a diagnostic marker with a high sensitivity and negative predictive value close to 100% is highly needed to reduce unnecessary antimicrobial treatment and hospitalizations. With advancements in flowcytometry technology, CD64, a neutrophil surface antigen, was assessed as a sensitive and specific marker in the diagnosis of neonatal sepsis. CD64 (FcγRI) is constitutively expressed only by monocytes and to a very low extent by neutrophils. It is a high affinity receptor that binds monomeric IgG. During bacterial infections, the FcγRI (CD64) expression increases on neutrophils and triggers various important biological functions in cells, such as phagocytosis, activation of the oxidative burst, degranulation, and antibody dependent cytotoxicity (7).

MATERIALS AND METHODS

Patients. In current study 65 at-risk neonates in the first 28 days of their life who were admitted to the Neonatal Intensive Care Unit (NICU) at Dr. Beheshti Hospital, Isfahan-Iran, were studied. Selection criteria were the presence of at least 1 clinical sign (temperature instability, grunting, apnea, cyanosis, tachycardia or bradycardia) or perinatal risk factor (prematurity, low birth weight, prolonged ruptured membranes more than 24 hours, maternal peripartum fever or infection or urinary tract infection) suggesting infection. Selected neonates were classified into two groups: sepsis suspected (n=57): who had clinical symptoms/signs but negative blood cultures, proven sepsis (n=8): who had positive blood cultures and clinical symptoms/signs. 12 healthy term neonates with physiologic hyperbilirubinemia were selected as the control group.

Assay for CD64. Flow cytometric determination of neutrophil CD64 expression was performed at 4ºC to minimize neutrophil activation (1). Five µl of fluorescent monoclonal antibody CD64 (R-phycoerythrin, Serotec, UK) was added to 50µl of whole blood in a polystyrene tube and incubated for 25 minutes at 4ºC in darkness. Then, 2 µl of a 1/10 diluted cold FACS lysing solution (Serotec, UK) was added and incubated for another 5 minutes at 4ºC. After centrifugation, the cells were resuspended in 2 ml of FACS lysing solution and incubated for 5 minutes at room temperature. After centrifugation, leukocytes were resuspended in 0.2 ml PBS and for the acquisi-

tion and analysis of the data FACS flow cytometer and CellQuest analysis were used (Figure 1).

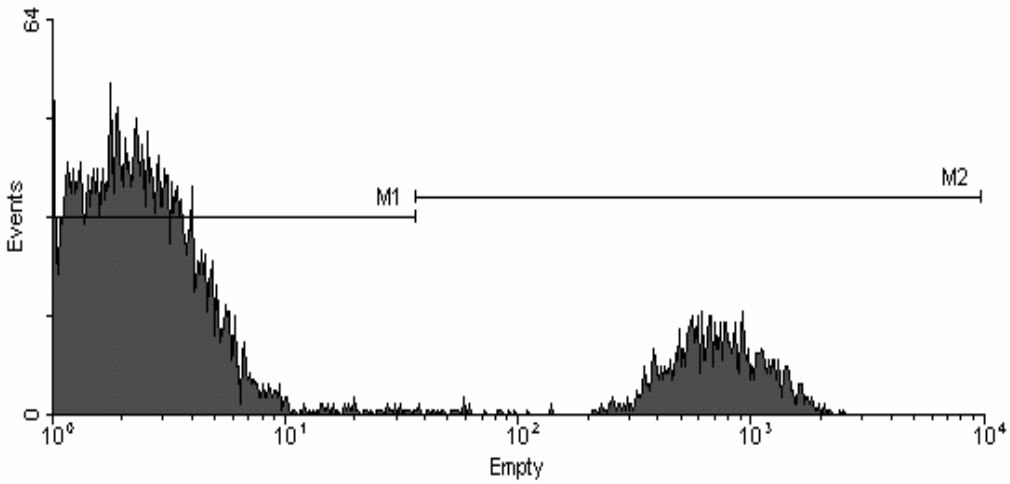

Figure 1. Flow cytometric analysis of CD64 expression on neutrophils in an infected neonate.

CRP Assay. Plasma CRP was measured qualitatively by a commercial kit (Kimiapajooh, Iran).

Statistical Analysis. The data were analyzed statistically by Chi-square and one way ANOVA test.

RESULTS

Neonates with positive blood cultures were classified into three groups (5 Antrobacter, 2 E.coli and 1 coagulase negative Staphylococcus). Characteristics of each group are shown in table 1.

Table 1. Clinical characteristics of groups

	Control group (n=12)	Suspected group (n=57)	Sepsis group (n=8)	P value
Gestational 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
Appgar score 1	8.9 ± 0.51	7.2 ± 1.8	5.8 ± 1.5	0.001
Appgar score 5	9.83 ± 0.38	8.5 ± 1.3	8.2 ± 1.6	0.005

In the sepsis group, 4 neonates were CRP positive (+) and 4 were CRP negative whereas in suspected sepsis group, 53 were CRP negative and 4 were CRP positive [1 neonate (+++) and 3 neonates (+)].

There were significant differences in mean gestational age, birth weight and Appgar

score (1st and 5th minute) of the two groups. There were significant differences in mean CD64 expression in sepsis and control group (Table 2).

Table 2. Neutrophil CD64 expression level in three groups

Marker	Control group	Suspected group	Sepsis group	P value
CD64	111.6 ± 61.4	108.5 ± 70.3	435.9 ± 201.5	< 0.001

Sensitivity and specificity of CD64 was 100% and 92.3%, respectively. In addition, positive and negative predictive values of CD64 were 88% and 100%, respectively (Table 3).

Table 3. Sensitivity, specificity, NPP and PPV of CD64

Marker	Sensitivity	Specificity	NPP	PPV
CD64	100%	92.3%	100%	88%

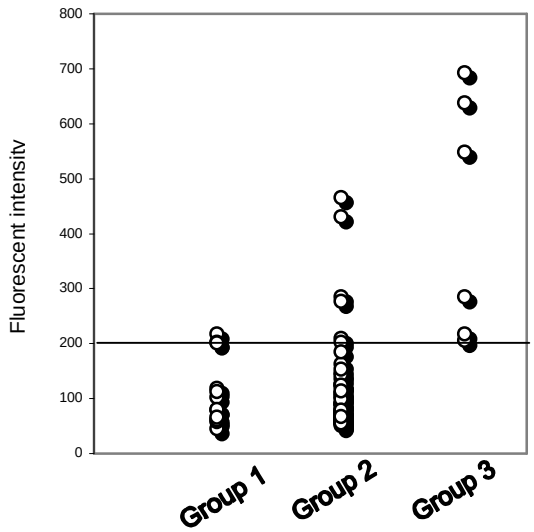

Figure 2. Expression of CD64 in different groups. The line corresponds to the cut-off value of the percentage of $CD64^+$ cells found in normal neonates.

DISCUSSION

Sepsis is a growing health problem especially among low birth weight neonates. The reported incidence of neonatal sepsis for all infants is 1 to 10 per 1000 live births. National Institute of Child Health and Human Development-Neonatal Research Network (NICHHD-NRN), in a recent large consortium study on 7861 Very Low Birth Weight (VLBW) infants, showed that the incidence of early onset sepsis with proven positive blood cultures was 13 to 27 per 1000 live births (1.9%). Proven culture positive sepsis is a small proportion of a larger group of clinical sepsis (with negative blood cultures). Added to this affected population of VLBW infants, is late onset

sepsis (nosocomial/horizontal acquisition), which increases the risk for sepsis in these infants throughout their initial hospitalization by another 16% to 24%. Sepsis increases neonatal morbidity and mortality greatly. The mortality rate is high (4.2-26%) (8).

The inability to exclude the diagnosis of neonatal sepsis results in prolonged and unnecessary use of antibiotics, in addition to unnecessary infant hospitalization and family separation. In the NICHHD-NRN consortium study, 47% of the VLBW infants with negative blood cultures were given antibiotics for 5 days or more. Antibiotic therapy for more than 72 hours has been shown to increase the chance of colonization with gram negative organisms and the resultant drug resistance. Early diagnosis and treatment of sepsis in neonates will effectively reduce mortality and morbidity seen in this disorder (8).

The diagnosis of sepsis remains one of the most difficult tasks for physicians and other medical staff. Blood cultures often remain negative in the presence of pneumonia, meningitis and even fulminant bloodborn septicemia. Capturing the specific organism in a small sample of venous blood remains elusive. A rapid laboratory test with high specificity for neonatal sepsis would be a valuable tool in therapeutic decision making and avoiding the unnecessary use of antibiotics in patients with clinical signs and symptoms of sepsis but negative blood cultures (3,8).

In this study, we tried to determine the CD64 expression as an immunological marker for rapid diagnosis of neonatal sepsis.

The high affinity FcγRI (CD64) is mainly involved in phagocytosis and intracellular killing of pathogens, but it is also expressed at very low levels on the surface of unstimulated neutrophils (7, 10). In addition, it has been shown that neutrophils of preterm infants express CD64 antigen to a similar extent comparing to older children and adults (7). Thus, this specific marker can be used for the identification of life-threatening infections in preterm infants.

In this study we chose at-risk 28-day old neonates who were admitted to the neonatal-ICU.

There were significant differences in means of gestational age and birth weight between neonates. These findings showed that prevalence of infection in neonates is inversely related to gestaional age and birth weight (11).

When CRP was measured qualitatively, in sepsis group, 4 neonates were CRP negative and 4 were positive (+). In suspected group, 53 neonates were negative, 3 were positive (+) and 1 was (+++). According to our results, qualitative measurement of CRP is not a sensitive marker for diagnosis of sepsis. Previous studies suggest that CRP is particularly useful in managing late-onset nosocomial bacterial or systemic fungal infections and necrotising enterocolitis. However, as the concentration of CRP increases rather slowly in the initial phase, its sensitivity at the time of sepsis evaluation is only 60%. Serial quantitive measurements of CRP at 24 and 48 hours after the onset of sepsis considerably improve the sensitivity to 82% and 84%, respectively (11). The specificity and positive predictive value of CRP levels ranged from 93% to 100% throughout the study period. Thus, CRP can be considered as a specific but late marker for neonatal infection (12).

There were significant differences in mean CD64 levels among sepsis group, suspected and control groups ($p<0.001$), but there were no significant difference in the means of CD64 levels between suspected and control groups.

These findings were in agreement with some previous studies. Espinosa et al.

showed raised percentages of $CD64^+$ cells in proven or clinical sepsis group (3). Also, they showed that CD64 has a high specificity and positive predictive value for sepsis (96.8% and 88.8%, respectively), although with a low sensitivity (25.8%) and an intermediate negative predictive value (57.4%) (3). In our study, we found 100% sensitivity and 92.3% specificity for CD64. On the other hand, Ng et al. showed very high sensitivity and specificity for CD64 in late onset sepsis, about 97% and 89%, respectively (13). Another study, showed that sensitivity and specificity of CD64 in early onset sepsis is 97% and 72%, respectively if measured 24 hours after clinical onset of the sepsis (13, 14).

In addition, we obtained the Positive and Negative Predictive Value (PPV and NPV) of CD64 as 88% and 100%, respectively.

The results of present study and previous studies show that measurement of neutrophil surface marker can be useful for diagnosis of infection in early phase. Also, the quantitative measurement of CRP in addition to CD11b and CD64 further enhances the ability to diagnose infections and to improve sensitivity and to increase negative predictive value to 100%.

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