Evaluation of adhesion molecules CD64, CD11b and CD62L in neutrophils and monocytes of peripheral blood for early diagnosis of neonatal infection

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Brief report

Background: **This study was undertaken to assess the value of neutrophils CD11b, CD64, and CD62L for the early diagnosis of neonatal infection.**

Methods: **Eighty-four neonates who were followed up for a suspected neonatal infection were included in this study. They were assigned into an infection group (*n*=49) and a non-infection group (*n*=35). Healthy neonates served as controls (*n*=35). A full sepsis screening was performed and neutrophil and monocyte expressions of CD11b, CD64 and CD62L were determined by flow cytometry.**

Results: **The expressions of CD64 and CD11b were significantly enhanced in the infection group compared to the non-infective group and the controls.**

Conclusions: **CD64 expression on neutrophils and monocytes is a useful diagnostic marker for the early diagnosis of neonatal infection. Combination of CD64, CD11b and C reactive protein further enhances the sensitivity of the expression and its negative predictive value.**

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Introduction

The clinical signs of neonatal infection are subtle, nonspecific and indistinguishable from those caused by a variety of neonatal non-infectious disorders. Recent advances in flow cytometric technology have provided opportunities for detecting cell surface activation markers on specific cell types in a few hours with a minimal blood volume.[1-6] The present study was undertaken to assess the value of neutrophil adherence molecules CD11b, CD64, and CD62L (L selectin) for the early diagnosis of neonatal infection.

Methods

Neonates

Eighty-four neonates who had been treated at the neonatal intensive care unit at Dr. Behcet Uz Children's Hospital in Izmir, Turkey, between March 2006 and April 2007, for a suspected infection were included in this study. Clinical signs and symptoms suggestive of neonatal infection were temperature instability, tachycardia or bradycardia, poor perfusion, shock, apnea, cyanosis, intercostals retractions, tachypnea, hypotonia, lethargy, seizures, abdominal distension, gastrointestinal bleeding or petechiae. Hematological and biochemical laboratory investigations were performed. Leucopenia was defined as leukocyte count $<5\times10^3/\mu L$; leucocytosis was defined as leucocyte count $>25\times10^3/\mu L$ at birth, $>30\times10^3/\mu L$ at 12-24 hours, and $>21\times10^3/\mu L$ after the second day. Thrombocytopenia was defined as platelet count $<150\times10^3/\mu L$. Normal absolute neutrophil count was taken as $7.8\text{-}14.5\times10^3/\mu L$ in the first 60 hours and $1.75\text{-}5.4\times10^3/\mu L$ after 60 hours. An immature to total neutrophil ratio above 0.20 was considered to be increased. The internationally accepted Rodwell hematologic scoring system was used for the diagnosis of neonatal sepsis.[7] Blood, urine, stool, cerebrospinal fluid cultures, chest and abdominal radiographs were determined at the initial evaluation. Peripheral blood

cultures were taken from two different sides in order to eliminate the risk of contamination. Pneumonia was diagnosed on the basis of respiratory findings in combination with abnormal chest radiographs. Serum C-reactive protein (CRP) was determined by the nephrometric method, and a concentration above 10 mg/L was taken as the cutoff value for neonatal infection. Venous ethylenediaminetetraacetic acid (EDTA) blood specimen was obtained for CD11b, CD64 and CD62L analysis of neutrophils and monocytes by flow cytometry. According to their clinical and laboratory findings, the neonates were assigned into two groups, infection group and non-infection group.

The infection group (group 1) consisted of 49 neonates who had a positive bacterial blood culture plus clinical signs of infection (n=20) or negative bacterial culture but the presence of three or more clinical signs of infection and abnormal laboratory signs consistent with infection (abnormal hematologic values and/or elevated CRP) or chest radiographs suggestive of pneumonia with elevated CRP and/or abnormal hematologic values (n=29). The non-infection group (group 2) comprised 35 neonates. Initially this group of neonates were enrolled in the study because of clinical symptoms suggestive of sepsis. When no source of infection was identified with negative blood cultures and laboratory tests were not suggestive of infection, they were subsequently diagnosed as having seizures and/or perinatal asphyxia (8 neonates), transient tachypnea (8), congenital cardiopathy (5), apnea of prematurity (3), dehydration (2), aspiration syndrome (2), and others (7). Thirty-five healthy neonates whose blood had been taken for other routine examinations were selected as a control group (group 3).

The study was approved by the hospital ethics committee and informed parental consent was obtained.

Determination of cell surface markers by flow cytometry

Freshly collected EDTA blood was maintained at 4ºC until test. EDTA blood sample (100 μL) was stained with mAbs (10 μL for each) and respective isotypic controls. The mAbs (immunotech, Marseille, France) included anti-CD45 (clone: Immu19.2, isotype: IgG1 mouse; fluorescein isothiocyanate [FITC] conjugated), anti-CD14 (clone: RMO52, isotype: IgG2a mouse; phycoerythrin [PE] conjugated), anti-CD64 (clone: 22, isotype: IgG1 mouse; PE), anti-CD11b (clone: bear1, isotype: IgG1 mouse; FITC), and anti-CD62L (clone: DREG56, isotype: IgG1 mouse; FITC). All tubes were incubated in the dark for 20 minutes. Finally, erythrocytes were lysed and leucocytes were stabilized, fixed by TQ-Prep (Coulter, Hialeah, FL), and analyzed by flow cytometry on an Epics XL-MCL (Coulter). The cytometer was routinely optimized using the Flow-Check Fluorospheres (Coulter, Fullerton, California, USA). Neutrophils and monocytes were selected by their light scatter pattern. For each sample, 10 000 events were recorded. Results were expressed as mean fluorescence intensity (MFI) of cells showing expression of the assessed adhesion molecules.

Statistical analysis

The results were expressed as mean ± SD. Differences among the groups were analyzed using the Kruskal-Wallis test and the non-parametric Mann-Whitney U test. The diagnostic specificity and sensitivity were tested using the ROC curve analysis.

Results

The demographic characteristics of the three study groups are summarized in Table 1. In the infection group, 20 neonates (40.8%) were blood-culture positive: 7 for group coagulase negative *Staphylococcus*, 3 for *Candida spp*, 2 for *Escherichia coli*, 2 for *Streptococcus spp*, 1 for *Staphylococcus aureus*, 1 for *Enterococcus spp*, 1 for *Klebsiella spp*, 1 for *Acinetobacter baumannii*, 1 for *Sacharomyces cerevisiae*, and 1 for *Erysipelothis rhusiopathiae*.

CRP, neutrophil and monocyte CD64 and CD11b expressions were significantly elevated in infected neonates as compared with non-infected neonates and control subjects. There was no significant difference between the non-infected neonates and the control subjects. Neutrophil CD64 expressions were increased three-fold, monocyte CD64 expressions were doubled in infected neonates when compared to non-infected neonates. Neutrophil and monocyte CD62L expressions were similar in all groups (Table 2).

During the follow-up, 5 (10.2%) of the 49 patients with infection died. In respect to adhesion molecule expressions, no statistically significant difference was found between the death group and the surviving patients. Also, no significant difference was found between bacterial culture positive and negative patients with infection, and between mature and premature patients with infection.

Tables 3 and 4 summarize the sensitivity, marker specificity and the combination of markers using optimal cutoff values. A comparison of each test showed that CRP and neutrophil CD64 (MFI) had a higher sensitivity (81% and 81%, respectively) and negative predictive value (77% and 75%, respectively) for detecting neonatal infection. Combination of the markers suggested that although its specificity was limited, the use of multiple markers was associated with both higher sensitivity and better negative predictive values.

Table 1. Demographic characteristics of the patients

Variables	Infection (group 1)	Non-infected (group 2)	Control (group 3)
Number of neonates	49	35	35
Gestational age (wk)	37.2±3.9 (27-41)	38.2±3.0 (28-40)	37.5±3.3 (27-40)
Birth weight (g)	2632±901 (980-4500)	3047±818 (1200-4790)	2828±797 (900-3900)
Male/female	31/18	22/13	17/18
Postnatal age (d)	11.4±9.5 (0-30)	9.2±9.3 (0-30)	5.3±4.9 (0-21)

Table 2. C-reactive protein, neutrophil/monocyte CD64 and CD11b expressions

Variables	Infection (group 1)	Non-infected (group 2)	Control (group 3)	*P*
C-reactive protein (mg/L)	50.6±51.0*	4.4±3.0	3.2±0.9	0.001
Neutrophil CD64 (MFI)	7.2±5.5*	2.7±0.9	2.7±0.7	0.001
Monocyte CD64 (MFI)	22.5±11.2*	9.9±4.7	10.8±3.8	0.001
Neutrophil CD11b (MFI)	14.0±4.4†	11.3±2.5	11.5±2.1	0.001
Monocyte CD11b (MFI)	13.1±4.0‡	10.1±2.5	11.2±2.8	0.010
Neutrophil CD62L (MFI)	5.8±1.9	5.1±1.2	5.3±1.9	0.250
Monocyte CD62L (MFI)	6.3±2.1	5.7±2.6	5.9±2.2	0.170

MFI: mean fluorescence intensity. *: $P<0.001$, group 1 *vs.* 2, group 1 *vs.* 3; †: $P=0.02$, group 1 *vs.* 2, group 1 *vs.* 3; ‡: $P<0.001$, group 1 *vs.* 2; $P=0.02$, group 1 *vs.* 3.

Table 3. Cutoff points, sensitivity and specificity of markers

Variables	Cutoff point	Sensitivity	Specificity	PPV	NPV
C-reactive protein	0.79	0.81	0.88	0.90	0.77
Neutrophil CD64 (MFI)	3.05	0.81	0.77	0.83	0.75
Monocyte CD64 (MFI)	12.70	0.81	0.77	0.83	0.75
Neutrophil CD11b (MFI)	12.60	0.66	0.71	0.76	0.61
Monocyte CD11b (MFI)	11.00	0.70	0.62	0.72	0.61

MFI: mean fluorescence intensity; PPV: positive predictive value; NPV: negative predictive value.

Table 4. Sensitivity, specificity, PPV and NPV of combination of markers

Combination of markers	Sensitivity	Specificity	PPV	NPV
CRP + neutrophil CD64 (MFI)	0.89	0.71	0.81	0.83
Neutrophil CD64 (MFI) + neutrophil CD11b (MFI)	0.95	0.54	0.73	0.90
CRP + neutrophil CD11b (MFI)	0.97	0.60	0.76	0.95
CRP + neutrophil CD64 (MFI) + neutrophil CD11b (MFI)	1.00	0.48	0.71	1.00

CRP: C-reactive protein; MFI: mean fluorescence intensity; PPV: positive predictive value; NPV: negative predictive value.

Discussion

An early event in the inflammatory response is the cytokine-induced activation of leukocytes. Adhesion molecules expressed on leukocytes are involved in inflammatory and immune reactions.[8-10] L-selectin (CD62L) mediates leukocyte rolling and it is cleaved from the surface by a proteolytic mechanism after cell activation.[11,12] Although Bührer et al[11] found that L-selectin expression on umbilical cord blood granulocytes and monocytes significantly decreased in newborns with infection, others[13,14] reported that plasma levels of soluble L-selectin did not change in neonatal infection. Similarly we observed no differences in the expression of L-selectin by neutrophils and monocytes among the study groups.

During the infections, CD11b and CD64 expressions enhance neutrophils and monocytes and trigger various important immune functions.[5,13,15-17] Weirich et al[18] and Nupponen et al[15] reported that CD11b is a highly effective marker for diagnosing early-onset neonatal infection. In our study, neutrophil and monocyte CD11b expressions in infected neonates were significantly elevated when compared to the non-infected neonates and the control subjects. The expression of CD11b by neutrophils in neonatal infection remains controversial and their possible value as a marker of neonatal infection requires further examination.

Layseca-Espinosa et al[13] found that the enhanced expression of CD64 was a highly specific indicator of neonatal infection (96.8%), although its diagnostic sensitivity was low (25.8%). Recently, Ng et al[16,19] reported that the measurement of neutrophil CD64 expression by quantitative flow cytometric analysis is a very sensitive and moderately specific diagnostic

marker for the identification of early and late onset neonatal infection, both in term and preterm neonates. In our study, neutrophil CD64 expression was elevated significantly when compared to the non-infected neonates and the control patients. Neutrophil CD64 expression was also similar in term and preterm infected newborns and bacterial culture positive and negative newborns with infection. CD64, CD11b and CD62L expressions were not predictive for the outcome of newborns with infection.

Recent investigations have focused on the combination of markers ensuring greater diagnostic accuracy.[16,19] Ng et al[19] found that the use of CD64 in combination with other diagnostic markers such as IL-6 or CRP improved the sensitivity and negative predictive value to 100% for late onset neonatal infection. For early-onset neonatal infection, however, the addition of CRP to CD64 enhanced the sensitivity and negative predictive value to 97% and 98%, respectively.[16] Our data indicate that CRP and neutrophil CD64 have a higher sensitivity for detecting neonatal infection and although its specificity is limited, the addition of CD64 and CD11b to CRP can improve the sensitivity and negative predictive value to 100%. These findings need to be confirmed by future studies with a larger number of neonates.

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