

Assessment of the expression of neutrophil CD11b receptors in preterm infants

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Abstract: The objective of the study was to assess the neutrophil surface expression of CD11b as a marker of early onset infection in preterm infants. The study comprised 16 pre-term infants with gestational age of 26-32 weeks, suspected of infection within 48 hours of life, and 13 healthy term infants who served as the control. A full sepsis screen was performed in both groups. There was no increase in CD11b expression on neutrophil surface even in infants with elevated CRP. None of the infants had positive blood cultures. The results in this study of pre-term infants born before 33 weeks of gestation showed that there is no increase in neutrophil CD11b expression; however, none of the infants had positive blood cultures. The diagnostic utility of neutrophil CD11b expression in pre-term infants needs to be further evaluated.

Key words: neonate, infection, CD11b expression

INTRODUCTION

Maternal infections have long been recognized as risk factors for adverse pregnancy outcomes. Intrauterine infection is often chronic, and is usually asymptomatic until labour begins or the membranes rupture. Even during labour, most women who are later confirmed (by pathology or culture) to have chorioamnionitis are asymptomatic, except for preterm labour [1].

The most advanced and serious stage of ascending intrauterine infection is foetal infection. The overall mortality rate of neonates who have congenital neonatal sepsis is 25% - 90% [2-6]. One study, which focused on infants born before 33 weeks of gestation, reported mortality rates of 33% for infected foetuses, and 17% for non-infected foetuses [7]. Carroll et al. reported foetal bacteremia in 33% of foetuses that had positive amniotic fluid culture, and 4% of those that had negative amniotic fluid culture [8]. Therefore, subclinical foetal infection is far more common than can be recognized by routine laboratory tests.

Although the onset of illness is often inconspicuous, the clinical course may be alarmingly fulminant, leading to septic shock, disseminated intravascular coagulation, and death within hours of the onset of clinical manifestations [9]. Therefore, it is important to identify and differentiate infected neonates from non-infected neonates, and to begin treatment with antibiotics without delay. However, as microbiological culture results and anti-microbial susceptibility data are not usually available until at least 48 hours after the specimen reaches the laboratory, early identification of genuine sepsis is a major diagnostic problem. In addition, anti-microbial treatment based solely on risk factors and clinical conditions will result in overtreatment. Continuation of antibiotics for

presumptive bacterial infection often leads to unnecessary and prolonged treatment.

Very often, the first sign of intrauterine infection is premature labour [1]. Pathological inflammatory changes in the placenta and the membranes correlate with the increased risk of premature labour. These changes were detected in 38% of preterm deliveries that ranged from 22-28 weeks, and in only 10% of term deliveries [9]. There are several mechanisms for the intrauterine infection, and one of the main ones by which the foetus becomes infected is the development of ascending infection. Once in the amniotic cavity, the bacteria may gain access to the foetus by different ports of entry. Aspiration of the infected fluid by the foetus may lead to congenital pneumonia, and further, disseminating of the infection to the foetal circulation may result in foetal bacteremia and sepsis. However, it is worth remembering that such clinical signs as respiratory failure and low muscle tone could be due to immaturity and not infection. Hence, the need exists for a very specific and sensitive marker of the intrauterine infection in premature neonates.

A wide variety of haematological and biochemical markers have been investigated for the evaluation of clinical sepsis in neonates. Due to high morbidity and mortality caused by intrauterine infection in preterm neonates, a reliable method for detecting and monitoring sepsis in this group of infants would therefore be beneficial in terms of patient diagnosis and management. To date, published reports on leucocyte cell surface antigens in preterm infants are limited. Flow cytometric analysis has the advantage over conventional immunological assay methods by being able to localize the activated markers to a specific cell type. In older patients, such serum markers as C-reactive protein (CRP) and neopterin have proved to be useful laboratory tools of infection and inflammation. C-reactive protein in particular is commonly used. This is a marker of acute-phase response in which serum levels of CRP increase rapidly after tissue injury, infection, or other inflammatory processes. CRP is produced by the liver and is normally present at very low levels. Following acute trauma or infection, CRP is

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rapidly synthesized by hepatocytes in response to cytokines released into the circulation by activated leukocytes. This acute inflammation-dependent response can lead to a 1,000-fold or greater increase in serum CRP levels. As these markers are regulated by cytokines, it has been proposed that cytokines themselves could be used to monitor immune activation and infection. Although, the circulating concentrations of cytokines may not necessarily reflect their biological activities, assessing the cellular response to cytokines can be a better way of identifying an early immunological response to bacterial invasion. In newborns, cell surface antigens have been studied in connection with congenital, early and late onset sepsis [11]. Neutrophil CD11b and CD64 have been found to be promising markers for the diagnosis of early and late infections, respectively [10, 11].

CD11b is an β_2 integrin adhesion molecule. It is normally expressed at a very low concentration on the surface of non-activated neutrophils [10, 11]. Expression of CD11b, however, increases considerably within a few minutes after the inflammatory cells come into contact with bacteria and endotoxins [12, 13]. These unique characteristics of CD11b enables it to be used as a potential early warning marker for the prediction of bacterial infection. Nupponen *et al.* and Weirich *et al.* have shown that in infants under 29 weeks, the sensitivity and specificity of CD11b for diagnosing early onset neonatal sepsis are very high - 96–100% and 100% in 2 studies [11, 14].

An evaluation of systemic inflammatory status in preterm infants by using cellular markers of systemic inflammation may be helpful in the clinical decision-making process. Therefore, we studied prospectively neutrophil CD11b expression in preterm neonates in a very poor condition, suspected of infection in first 48 hours of life.

METHODS

This study was conducted at the Polish Mother's Health Center Research Institute in Łódź, Poland, between January - December 2004. The study protocol was approved by the institutional review board and informed consent was obtained from the parents. The study group consisted of 16 premature, very-low-birth-weight neonates, while the control group consisted of 13 healthy, term neonates born after uncomplicated pregnancy and delivery.

The gestational age of the 16 neonates in the study group ranged from 26-32 weeks, mean 30.3 weeks. The Apgar score at 5 minutes ranged from 2-10, mean 6.8. Birth weight ranged from 750 g - 2,880 g, mean 1,550 g. Five neonates were born by normal spontaneous vaginal delivery (NSVD). Eleven were born by c-section. The study group had 7 female and 9 male neonates. Four cases had premature rupture of membranes (PROM) after 12 hours. No other risk factors for the intrauterine infections were found in the remaining cases.

The gestational age of the 13 healthy, term neonates in the control group ranged from 37 - 40 weeks, mean 38.8 weeks. The birth weight in this group of neonates ranged from 2,850 g - 4,200 g, mean 3,200 g. The Apgar score at 5 minutes ranged from 7-10, mean 8.2. The mothers of the neonates in the control group had no risk factors for intrauterine infection.

In all neonates in the study and the control groups, a sepsis screen was performed, which included blood cultures,

complete blood count (CBC) and C-reactive protein (CRP). Blood specimens were also obtained for evaluation of CDB11. For the study and the control group, the first sample was taken from cord blood. For the study group, 2 additional samples were obtained at 24 and 48 hours after birth.

Blood samples. Blood samples were taken in the study group from the umbilical arterial catheter soon after birth, and at 24 and 48 hours thereafter. These samples were in addition to the samples taken intermittently for standard laboratory tests (c-reactive protein (CRP), white blood cells (WBC), I/T ratio (immature/total neutrophils ratio), platelet count). In the control group (healthy, term neonates), blood samples were taken only from the cord blood after birth. The volume of each blood sample was 100 μ L.

Determination of CD11b expression by flow cytometry. Expression of the CD11b receptors was determined by flow cytometry with monoclonal antibodies anti-CD11b (BD Pharmingen, Cowley, UK), conjugated with phycoerythrine. A sample of 100 μ L of heparinized full blood was incubated with 20 μ L of anti-CD11b antibodies for 30 minutes at room temperature. After erythrocyte removal with lysis fluid (BD) and washing out, the cells were suspended in 200 μ L of fixating fluid (CelFix by BD). Then, 10,000 cells were scanned and analyzed with CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, USA) and a FACS Calibur (Becton Dickinson Bioscience, San Jose, USA) flow cytometry device (BD) to determine the percentage of neutrophils with CD11b receptor expression. The density of this receptor was determined by assessment of the mean fluorescence intensity (MFI).

CRP determination. CRP as a standard marker of the inflammation was assessed by the immunoturbidimetric method with latex particles amplification with COBAS INTEGRA 400 Roche device (F. Hoffmann-La Roche Ltd., Switzerland) in the serum with normal range of < 1.0 mg/dL.

Data Analysis. A total of 29 neonates were studied. Statistical comparison between healthy term neonates in the control group and preterm sick neonates in the study group was performed. Statistical analysis comprised of determination of the mean value, standard deviation (SD), and standard error of the mean (SEM) for all the parameters assessed for the neonates in the study. The following tests were used for statistical verification:

1. Student's *t* test - for connected variables in order to compare the differences of the given parameters assessed in time periods in the same patients,
2. χ^2 tests (Fisher's precise equitation, χ^2 test, χ^2 test with Yates correction) to assess the border CRP solution.

Statistical analysis was performed using STATISTICA 5.0 PL software.

The level of significance was set at 5% in all comparisons.

RESULTS

Clinical characteristics of the patients in the control and the study groups are presented in Tables 1 and 2, respectively.

The main diagnosis in the study group was respiratory failure. Seven neonates in this group had to be intubated due

Table 1 Demographic data of the 16 pre-term neonates in the study group.

Mean gestational age – weeks (range)	30.3 (26-32)
Sex	Male 9, Female 7
Mean body birth weight – g (range)	1,550 (750-2,880)
Mean Apgar score (range)	6.8 (2-10)
Number of ventilated neonates	7
Number of neonates requiring nasal continuous positive airway pressure (nCPAP)	9
Spontaneous vaginal delivery (SVD)	5
Caesarean section	11

Table 2 Demographic data of the control group of neonates.

Mean gestational age – weeks (range)	38.8 (37-40)
Sex	Male 6, Female 7
Mean body birth weight – g (range)	3,300 g (2,850-4,200 g)
Mean Apgar score (range)	8.9 (7-10)
Spontaneous vaginal delivery (SVD)	13

to respiratory failure after delivery, and required mechanical ventilation. They also received surfactant within the first 2 hours of life because of radiographic signs of respiratory distress syndrome (RDS). Nine neonates of the study group required nasal CPAP. Chest x-rays in the study group performed after 12-24 hours were consistent with pneumonia.

In the study group, 1 baby had elevated CRP from day 1, and 5 others had elevated CRP between 1-3 days of life. Two neonates in the study group had elevated CRP on day 2, and 2 others showed elevated CRP on day 3 of life. Six neonates in the study group had normal CRP despite persistent clinical signs of respiratory insufficiency, and positive chest x-rays for pneumonia. Similarly, the mean value of WBC, I/T ratio, platelets (PLT) in the first 3 days of life in the study group were in the range considered as normal. Blood cultures were also negative in all neonates in the study group. However, neonates in the study group showed decreased expression of the neutrophil's CD11b receptors in the first 3 days of life.

There was no statistically significant difference between the expression of the neutrophil's CD11b receptors from the cord blood ($p = 3930$) assessed for both the control and study groups. However, there was a statistically significant decrease in expression of the neutrophil's CD11b receptors in the study group between day 1 and day 2 of life ($p=0.0000004$), and day 1 or day 3 of life ($p=00015$). Such a statistically significant difference was also seen between expression of the neutrophil's CD11b receptors on day 2 or 3 of life in the study group, and day 1 of life in the control group. The results are presented in Tables 3 and 4.

DISCUSSION

The results show that in premature infants born less than 33 weeks of gestation, neutrophil's CD11b expression could not serve as a promising marker of the early-onset sepsis. No statistical difference was observed between the expression of the neutrophil CD11b receptors in pre-term infants born less

Table 3 Mean value and range of WBC, neutrophils, I/T, PLT and CRP in days 1, 2 and 3 of life in the study group of neonates.

Day of life	1	2	3
WBC (in mm^3)	10.783 (3.850-30.090)	12.213 (5.540-33.330)	13.838 (4.680-31.800)
Neutrophils (%)	33 (9-75)	59 (29-76)	59 (22-81)
I/T	0.03 (0.03-0.13)	0.03 (0.01-0.10)	0.04 (0.02-0.08)
PLT (in mm^3)	222.666 (159.000-317.000)	176.687 (27.000-282.000)	207.785 (89.000-420.000)
CRP (mg/dL)	0.69 (0.6-2.61)	0.86 (0.6-3.32)	0.84 (0.6-2.9)

Table 4 Expression of neutrophil's CD11b receptors pronounced as MFI in the study and control group, and comparison between the groups.

Study Group:						
Day of life	Day 1 (n=17)		Day 2 (n=16)		Day 3 (n=14)	
	Percent of Cells	MFI	Percent of Cells	MFI	Percent of Cells	MFI
Measured value	93.58	1783.24	94.28	1106.75	96.01	870.86
SD	6.851	490.444	5.534	555.391	5.514	353.554
SEM	1.662	118.950	1.384	138.848	1.474	94.491
Control Group:						
Day of life	Day 1 (n=11)					
	Percent of Cells		MFI			
Measured value	94.21		1837.18			
SD	7.270		488.943			
SEM	2.192		147.422			

than 33 weeks of gestation, and who subsequently developed respiratory distress and the expression of the neutrophil CD11b receptors in healthy, term neonates born without any risk factors for intrauterine infection. Moreover, in the study group of preterm neonates, a statistically significant decrease of the expression of the neutrophil CD11b receptors was observed in the first 3 days of life, i.e., between day 1 – day 2, and day 1 – day 3.

Contrary to our expectations based on prior studies [2, 3, 6], the expression of neutrophil CD11b receptors in pre-term infants born less than 33 weeks of gestation had a statistically significant decrease in the first 3 days of life. Considering that there was no significant difference of expression of the neutrophil CD11b receptors on day 1 of life between study and control groups, neutrophil CD11b could not serve as a marker of systemic inflammation in preterm neonates. The small difference between the expression of the neutrophil's CD11b receptors in preterm infants less than 33 weeks of gestation, and in the term, healthy neonates, could be because of the delivery process itself, and similar to the observed increase in WBC in term neonates in the first 24 hours of life.

The statistically significant decrease in the expression of the neutrophil CD11b receptors observed in our investigations in pre-term infants in the day 2 and day 3 of life could be a physiological decrease similar to the decrease of WBC in the term, healthy neonates. Furthermore, the activity of the cells of immune system in premature neonates is less pronounced than that in term neonates [5]. Hence, the decrease could not necessarily be considered as an expression

of the decrease of the inflammatory process in the premature neonates. Note also that the preterm neonates in the study group showed persistent signs of inflammation, observed by clinical respiratory insufficiency and pathological changes in the lungs seen in CXR. Consequently, the neonates in the study group were treated for inflammation, and the decrease of the expression of the neutrophil CD11b receptors in day 2 and day 3 day of life could be due to the progress in treating inflammation in these babies.

The expression of the neutrophil CD11b receptors in preterm infants less than 33 week gestation in our study failed to be a sufficient marker of infection in the first 24 hours of life and thereafter. The mechanism of the immunological response for intrauterine infection in premature infants is probably much more complex than that of secondary infections. Unlike in the term neonates for whom the CD11b expression serves as a promising marker, its clinical usefulness in pre-term infants exposed to sub-clinical intrauterine infection requires further studies. The need to find an objective marker of the inflammation for intrauterine infection in premature neonates continues to be a challenge.

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