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Original article

Neutrophil-surface antigens CD11b and CD64 expression: a potential predictor of early-onset neonatal sepsis

Background: *CD11b, an α subunit of the β_2 integrin adhesion molecule, and CD64, the high affinity Fcγ receptor I, are specific neutrophil-surface antigens activated in response to systemic inflammation and, hence, they might potentially help identifying neonatal infections.*

Objective: *We sought to evaluate the time course of expression and diagnostic and prognostic utility of CD11b and CD64 in early-onset sepsis in the suspected newborn.*

Methods: *Sixty newborn infants (28-40 weeks gestation) with antenatal risk factors for sepsis were enrolled and subjected to sepsis work-up including complete blood count, quantification of serum C reactive protein (CRP) and flow cytometric analysis of CD11b and CD64 in cord blood (0 h). These tests were repeated at 8, 24 and 48 h postnatally. Neonates were defined, retrospectively, in two groups: sepsis and no infection, on basis of clinical observation over their first five postnatal days and sepsis work-up results.*

Results: *A significant enhancement of neutrophil CD11b and CD64 expression was demonstrated in the sepsis group as compared to the non-infected group. CD11b over-expression had an onset at 0 h. Its mean value approached two-fold mean level of non-infected neonates by 8-24 h, and declined thereafter. CD64 rising onset was detectable at 8 h and its mean percentage reached four-fold mean value of the non-infected group at 24 h. At 24 h, an optimal cut-off value for CD11b expression of 35% (sensitivity 80%, and specificity 100%), and for CD64 expression of 17% (sensitivity 88%, and specificity 90.3%) had the best performance for prediction of sepsis. Combined use of both markers at 24 h yielded 90% sensitivity and 95% specificity for sepsis prediction. Sepsis survivors showed significantly lower mean expression for CD11b and CD64 as compared to those with fatal outcome. At 24 h, a cut-off value of 88% expression for CD11b and 50% expression for CD64 predicted mortality with sensitivity and specificity of 100%.*

Conclusion: *Enhanced expression of neutrophil-surface antigens CD11b and CD64 could be a promising tool for prediction and therapeutic decision-making in early-onset sepsis indicating the necessity of initiation of antimicrobial therapy and reduction of its unnecessary use in non-infected neonates even before definitive microbiologic identification.*

Key words: sepsis, neonate, early-onset, neutrophil activation, surface antigen, CD11b, CD64

Nehal M. El-Raggal, Mohamed N. El-Barbary, Mona F. Youssef*, Hanaa A. El-Mansy

From the Departments of Pediatrics and Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.*

Correspondence:
Dr. Nehal M. El-Raggal Assistant Prof. of Pediatrics, Faculty of Medicine, Ain Shams University, Abbasiah, Cairo, Egypt.
E-mail: nraggal @hotmail.com

INTRODUCTION

Sepsis neonatorum is a life-threatening disease that remains a major cause of morbidity and mortality in the newborn[1], mainly among preterm and low birth weight (LBW) infants[2]. Early recognition and implementation of appropriate therapy offers the best outcome, and careful assessment of the newborn is essential.

Early-onset sepsis presents a fulminant multi-system illness during the first days of life. It mostly occurs in newborn infants with one or more obstetric complication including premature rupture of membranes (PROM) [3], premature onset of labor[4], chorioamnionitis[5], or peripartum maternal fever[6]. Early detection of neonatal sepsis is difficult as the clinical signs are subtle, non-specific and indistinguishable from those caused by a variety of neonatal non-infective disorders such as aspiration syndrome, maladaptation and respiratory distress syndrome. It is therefore recommended for all neonates who develop these signs to start empirical antimicrobial therapy[1]. This clinical practice,

however, renders many neonates unduly susceptible to side effects of antimicrobial agents, increases hospital costs and promotes development and spread of resistant bacterial strains. Hence, there remains a requirement for an efficient marker that can reliably predict and identify truly infected neonates, and exclude infection in suspected, but non-infected, infants.

An important early event in sepsis is the generation and release of cytokines by immune cells in response to invasion by bacteria and their toxins[7]. These cytokines induce activation of leucocytes, the primary function of which is to eliminate invading microorganisms[8]. During neutrophil activation, surface adhesion molecules including L-selectin[9], CD43, CD44[10] and CD50[11] are expressed and cleaved from neutrophil cell surface, down-regulating their expression on activated neutrophils[11,12]. In contrast, the leucocyte integrin Mac-1 (CD11b/CD18) and the high affinity Fcγ receptor I (CD64) behave as activation antigens on neutrophils, increasing their expression on the surface of the cell after its activation and hence, are considered as specific neutrophil-surface activation markers[10]. Activation of CD11b, an α subunit of the β2 integrin adhesion molecule, and CD64 surface antigens expression had been shown by neonatal neutrophils to an extent similar to those of older children and adults, thus they could potentially be used for identification of neonatal life-threatening infections[13].

This study is aimed to delineate the time course and pattern of expression of the neutrophil-surface activation markers CD11b and CD64 as immunological indicators of inflammatory response to infection in neonates suspected for early-onset sepsis, and to establish their diagnostic and prognostic utilities during the first 48 hours of postnatal life.

METHODS

The study series comprised 60 newborn infants with gestational ages of 28 to 40 weeks and birth weights between 1500 and 4100 gm, delivered at the Maternity Hospital of Ain Shams University, Cairo, Egypt. They were transferred to the Neonatal Intensive Care Unit (NICU) of the hospital because of having at least one risk factor for suspicion of neonatal sepsis (prolonged premature rupture of membranes >24 h, premature onset of labor, chorioamnionitis or peripartum maternal fever). Clinical symptoms and signs suggesting infection in the early neonatal period (first 5 postnatal days), as recorded by medical and nursing staff members in the NICU, were divided into the following categories:

- Temperature instability (hypothermia, hyperthermia)
- Respiratory distress (grunting, intercostals retractions, apnea, cyanosis)
- Cardiovascular (tachycardia, bradycardia, poor perfusion, shock)
- Neurologic (hypotonia, lethargy, sluggish Moro reflex)
- Gastrointestinal (feeding intolerance, abdominal distension)

Blood samples were withdrawn immediately at birth from cord blood (0 h) and were repeated at 8, 24 and 48 h of postnatal age from peripheral blood for determination of:

- Complete blood counts (CBC) using Coulter Max M Counter (Coulter Corporation, Miami, Florida 33196, USA)
- Semi-quantitative measurement of serum CRP concentration, using kits supplied by Behring Diagnostics (GmbH, Marburg, Germany)
- Expression of CD11b and CD64 on neutrophil surface by flow cytometry (Epics profile II Coulter) using DAKO monoclonal antibodies (Glosture, Denmark)

One sample for each neonate was obtained for blood culture (requested at the age of 0-48 hours) applying the BACTEC 9050 system (Becton Dickenson Microbiology System, 7 Loventon circle, Sparks, Maryland 21152)

The neonates were assigned *retrospectively*, on the basis of clinical observation over their first 5 postnatal days and sepsis work-up results, into 2 groups:

1. *Sepsis group (n=29):* clinical sepsis developed (3 or more clinical signs of infection, hematological scoring for sepsis[14] ≥3, CRP >12 mg/L, with or without positive blood culture results). They were 15 males and 14 females; 18 (62%) were delivered vaginally and 11 (38%) by cesarean section. They had a mean gestational age of 35.3±3.54 weeks, and a mean birth weight of 2700±680 gm.
2. *No infection (non-septic) group (n=31):* infection was ruled out on both clinical and laboratory basis. They were 17 males and 14 females; 25(81%) were delivered vaginally, and 6(19%) by cesarean section. Their mean gestational age was 37.9±0.95 weeks; and mean birth weight was 3100±390 gm. They served a control group

Procedure of blood sampling:
for each neonate of the study, two milliliters of blood were collected at birth (0 h) and at 8, 24, and 48 h of postnatal life. Each sample was divided in two tubes. The first tube contained K-EDTA as an anticoagulant. Blood in this tube was used for complete blood count and flow cytometric analysis of neutrophil surface markers. The second tube was a plain tube in which blood was left to clot and serum was separated for CRP determination.

Procedure of Flow cytometric analysis:
Flow cytometric analysis was performed on EPICS–XL Coulter Flow Cytometer using flourescein isothiocyanate (FITC)-labeled anti-CD64 and phycoerythrin (PE)-labeled anti-CD11b together with their specific isotypic control reagents within 24 hours of collection. All monoclonal antibodies were purchased from Becton Dickinson (Mountain View, CA., USA). The collected blood was incubated with each of the two monoclonal antibodies for 30 minutes at room temperature. Erythrocytes were lysed by adding a lysing solution (ammonium chloride 0.85 % buffered with potassium bicarbonate pH 7.2) for 5 minutes at 37^{o}C. Finally, the samples were washed with phosphate buffer saline (PBS) prior to flow cytometric analysis. The neutrophils were specifically analyzed by selective gating based on the parameters of forward and side scatter (Fig I and II).

Statistical analysis:
Data were statistically analyzed using SPSS software version under Windows 98. The results are expressed as mean values ± standard deviation (SD). Student t test was applied for comparisons between groups. For non-parametric data, Wilcoxon rank-sum test was applied. Correlation of different parameters was done using Spearman correlation coefficient (r) taking the probability (p) level of significance at <0.05. The diagnostic performance of CD11b and CD64 was assessed using the receiver operator characteristic (ROC) curve analysis with determination of the optimal cut-off values.

RESULTS

There was no significant difference between sepsis and no infection groups in terms of sex distribution and delivery modalities (p>0.05). Septic neonates, however, were found to have a significantly lower mean gestational age (p<0.01) and mean birth weight (p<0.05) as compared to the non-septic group.

In the group of sepsis, 93% had hematological scores of ≥3 over the study period. Positive blood cultures for bacteria were encountered in 22 (75.8%) of cases. Most of the blood cultures demonstrated the presence of *E.coli* (27.6%), *Klebsiella pneumoniae* (24.1%) or mixed infection with both microbes (7%).
Serial determinations of CRP levels revealed that all 29 neonates who developed early-onset sepsis had negative initial CRP in cord blood. Peak CRP concentrations remained ≤12 mg/L in 27(93%) at 8 h, and in 15(51.7%) at 24 h with corresponding diagnostic sensitivities of 3.57% and 40% respectively. By 48 h, CRP diagnostic sensitivity for sepsis was 85.7%.

Early significant increase in mean percentage of CD11b expression was detected in cord blood samples (0 h) from neonates who developed sepsis as compared to the non-infected group (p<0.05). Progressive increase in mean CD11b expression in sepsis group was subsequently observed in samples obtained at 8 and 24 h (p<0.001, respectively) and started to decline thereafter (Table 1 & Fig 1A).

On the other hand, mean percentage of neutrophil CD64 expression did not show significant difference between sepsis and no infection groups in initial samples obtained from cord blood (p>0.05). Progressive significant increases in mean CD64 expression in sepsis group in comparison to non-infected neonates, were observed in subsequent blood samples obtained at 8 and 24 hr, and peaked at 48 hr (p<0.001, respectively) (Table 1 & Fig 1B).

Non-significant difference was found between mean values of expression of either CD11b or CD64 in neonates with culture proven sepsis and those with sepsis but negative blood culture results: (at 24 hr, mean CD11b expression: 61.3±22.51% vs. 58.3±16.31%, respectively, p>0.05; mean CD64 expression: 42.05±19.67% vs. 34.57±19.36%, respectively, p>0.05).

Correlation study, at the 4 serial measurements, revealed that among study neonates the expression of both neutrophil-surface antigens was not significantly correlated (p>0.05) with either gestational age or birth weight. Similarly, non-significant correlations were also found between each of CD11b and CD64, and the corresponding hematological scores and serum CRP levels at the 4 serial measurements (p>0.05, respectively).

Diagnostic performance test results for determination of the best cut-off value of CD11b and CD64, at 8, 24 and 48 h, for prediction of early-onset sepsis are presented in Table (2) & Fig (2). Expression of CD11b at a cut-off value of 35% at

24 h was the best for prediction of early-onset sepsis with 80% sensitivity, 100% specificity and 91% efficacy. CD64 expression at a cut-off value of 17% at 24 h had also the best diagnostic performance for sepsis prediction with 88% sensitivity, 90.3% specificity and 89.3 % efficacy. Combined use of both markers, at 24 h, resulted in improvement of prediction value with 90% sensitivity, 95% specificity and 93 % efficacy (Fig 3).

Time course of expression of mean percentages of CD11b and CD64 during the first 48 hours in relation to sepsis outcome are shown in Table (3) & Fig (4). Significantly lower mean CD11b expression was predictive of favorable outcome (survival) as early as 8 h and onwards compared to mean values in those who had fatal outcome (p<0.001, respectively). Significantly lower mean CD64 expression was evident in survivors at 24 h, as compared to non-survivors (p<0.001). *Data were missing for non-survivors at 48 h.*

Diagnostic performance test results for determination of the best cut-off value of CD11b and CD64 for prediction of mortality are shown in Table 4. At 24 h, expression of CD11b at a cut-off value of 88% and CD64 at a cut-off value of 50% had the best utility for prediction of mortality with 100% for both sensitivity and specificity.

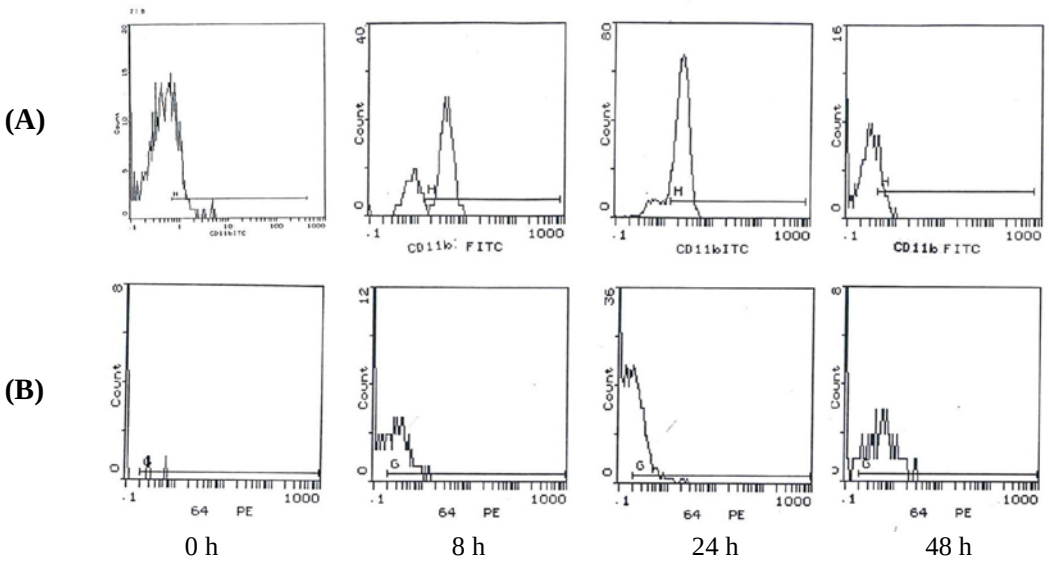


Fig I : Serial flow cytometric analyses of CD11b (A) and CD64 (B) expression by peripheral blood neutrophils of a septic neonate at 0, 8, 24 and 48 h.

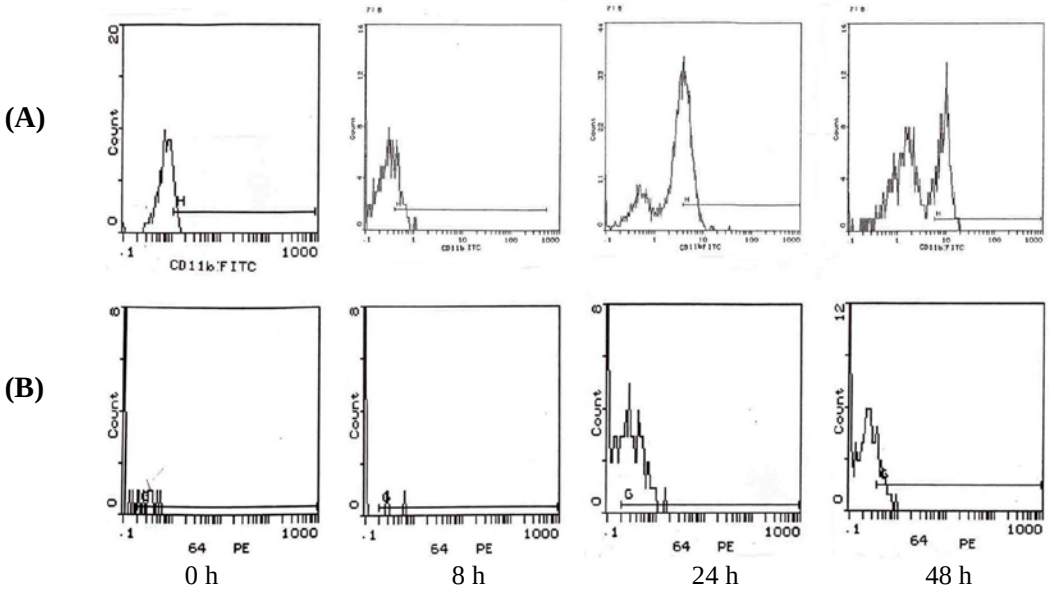


Fig II : Serial flow cytometric analyses of CD11b (A) and CD64 (B) expression by peripheral blood neutrophils of a non-infected (non-septic) neonate at 0, 8, 24 and 48 h.

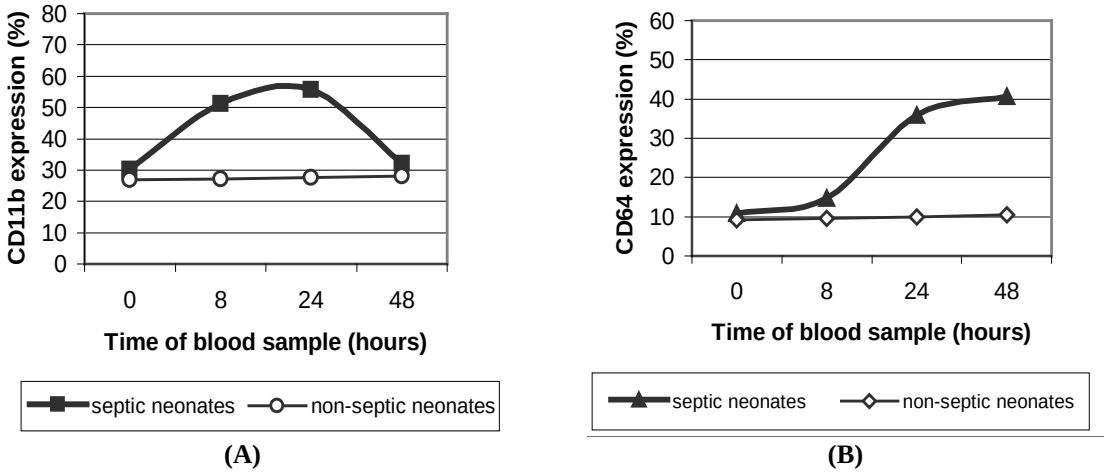
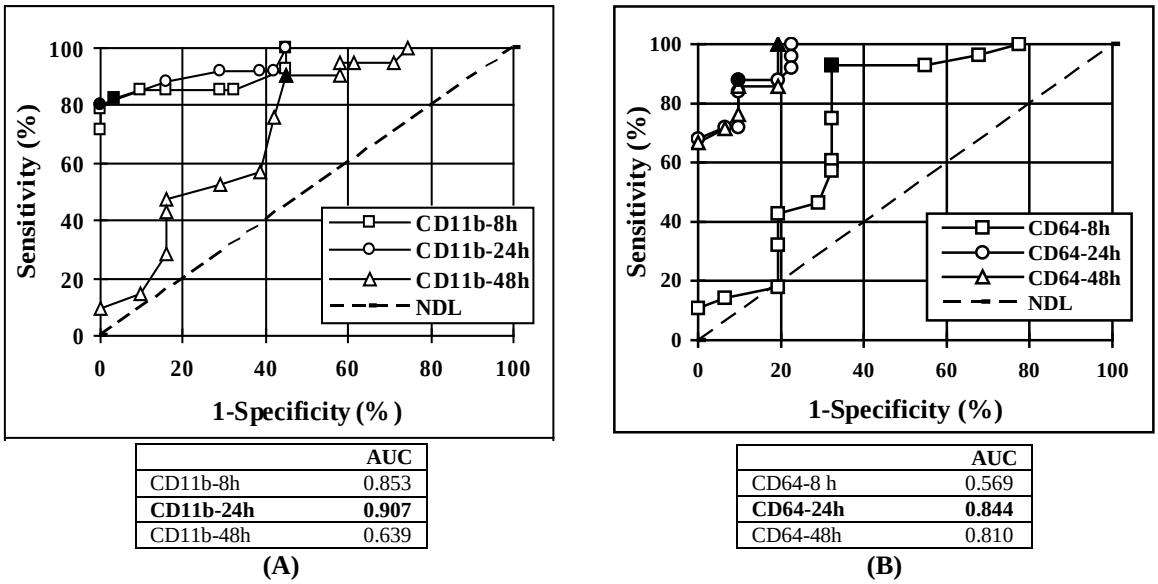


Fig 1: Mean levels of expression of CD11b (A) and CD64 (B) in septic versus non-septic neonates during the first 48 hours of postnatal life.

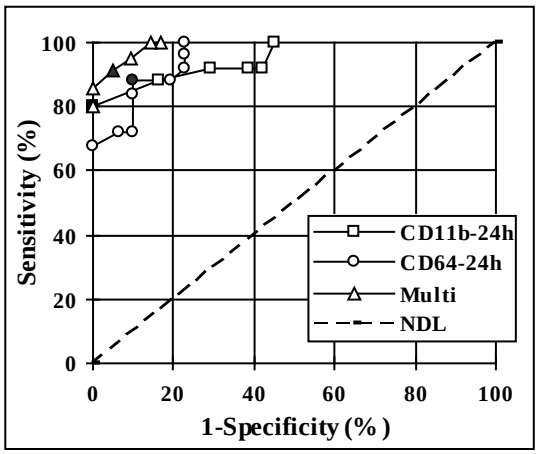


	AUC
CD11b-8h	0.853
CD11b-24h	**0.907**
CD11b-48h	0.639

	AUC
CD64-8 h	0.569
CD64-24h	**0.844**
CD64-48h	0.810

AUC: area under curve
Best cut-off is indicated by the shaded mark.

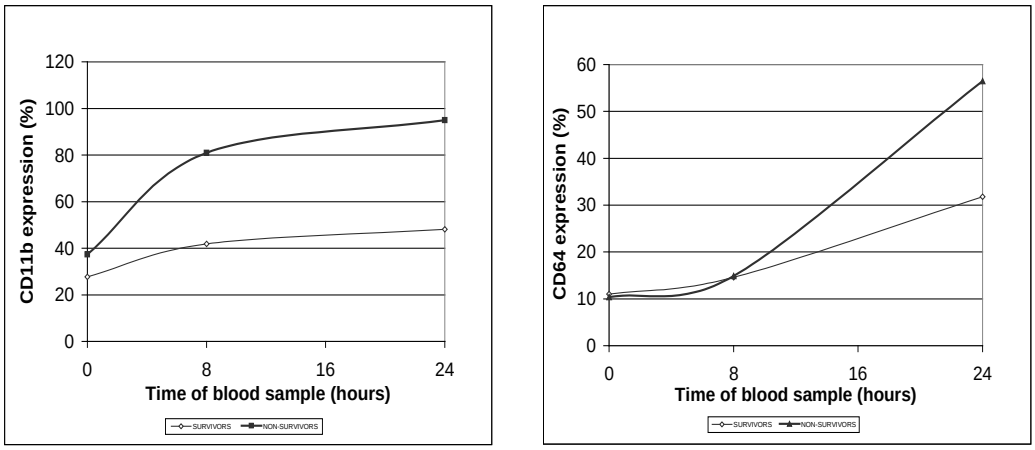
Fig 2: Receiver operator characteristic (ROC) curve analysis for diagnostic performance of CD11b (A) and CD64 (B) at 8, 24 and 48 h for septic versus non-septic cases.



	AUC
CD11b-24h	0.907
CD64-24h	0.844
Multi	**0.920**

AUC: area under curve
Best cut-off is indicated by the shaded mark

Fig 3: Multi-ROC curve analysis for the diagnostic performance of combined CD11b and CD64 at 24 h for septic versus non-septic cases.



(A) **(B)**

Data were missing for non-survivors at 48 h.

Fig 4: Mean levels of expression of CD11b **(A)** and CD64 **(B)** in survivors versus non-survivors during first 24 hours of postnatal life.

Table 1: Levels of neutrophil cell-surface antigen expression at 0, 8, 24 and 48 h: comparison between septic and non-septic neonates

Variable	**Sepsis group**		**No infection group**		**Z**	***p***
	n	mean±SD (range)	n	mean±SD (range)		
CD11b (%)						
0 h	29	30.3±6.46 (19-46)	31	27.0±5.21 (19-36)	2.004	<0.05
8 h	28	51.5±20.98 (29-86)	31	27.1±5.07 (20-35)	5.761	<0.001
24 h	24	55.6±21.89 (30-98)	31	27.6±6.05 (19-35)	5.744	<0.001
48 h	21	32.1±3.84 (24-41)	31	28.1±4.92 (20-36)	2.676	<0.001
CD64 (%)						
0 h	29	10.8±2.88 (5-16)	31	9.2±5.90 (1-20)	1.509	>0.05
8 h	28	14.7±5.43 (5-35)	31	9.6±5.66 (2-19)	3.180	<0.001
24 h	24	35.8±15.35 (13-62)	31	9.9±5.46 (1-21)	5.835	<0.001
48 h	21	40.7±18.17 (16-67)	31	10.4±6.11(2-22)	5.557	<0.001

p<0.05: significant; *p* <0.01& *p* <0.001:highly significant; *p* >0.05: non-significant; SD: standard deviation.

Table 2: Diagnostic performance test results of CD11b and CD64 for prediction of early-onset neonatal sepsis *(sepsis or no infection).*

	CD11b			**CD64**		
Time	**8 h**	**24 h**	**48 h**	**8 h**	**24 h**	**48 h**
Best cut-off value (% of cells)	34	**35**	28	10	**17**	15
Sensitivity (%)	82.14	**80.0**	90.48	92.86	**88**	100
Specificity (%)	96.77	**100**	54.84	67.74	**90.32**	80.65
Positive prediction (%)	95.83	**100**	57.58	72.22	**88**	77.78
Negative prediction (%)	85.71	**86.11**	89.47	91.30	**90.32**	100
Efficacy (%)	89.83	**91.07**	69.23	79.66	**89.29**	88.46

Table 3: Levels of neutrophil cell-surface antigen expression at 0, 8 and 24 h*: comparison between sepsis survivors and non-survivors.

Variable	**Survivors**		**Non-survivors**		**Z**	***p***
	No.	mean±SD (range)	No.	mean±SD (range)		
CD11b (%)						
0 h	21	27.7±4.91 (19-35)	8	37.3±4.71 (31-46)	3.489	<0.001
8 h	21	41.8±13.82 (29-78)	7	80.9±2.61 (78-86)	3.873	<0.001
24 h	21	48.1±14.3 (30-78)	4	95.0±4.24 (89-98)	3.113	<0.001
CD64 (%)						
0 h	21	11.0±3.18 (5-16)	8	10.4±1.99 (8-13)	0.683	>0.05
8 h	21	14.6±6.03 (5-35)	7	14.9±3.39 (11-20)	0.371	>0.05
24 h	21	31.8±13.31 (13-50)	4	56.5±4.51 (51-62)	3.113	<0.001

**Data were missing for non-survivors at 48 h.*

p<0.05: significant; *p* <0.01&*p* <0.001:highly significant; *p* >0.05: non-significant; SD: standard deviation..

Table 4: Diagnostic performance test results of CD11b & CD64 for prediction of sepsis outcome *(survivors versus non-survivors)*

	CD11b		**CD64**	
Time	**8 h**	**24 h**	**8 h**	**24 h**
Best cut-off value (% of cells)	77	**88**	19	**50**
Sensitivity (%)	100	**100**	14.29	**100**
Specificity (%)	95.24	**100**	90.48	**100**
Positive prediction (%)	87.5	**100**	33.33	**100**
Negative prediction (%)	100	**100**	76	**100**
Efficacy (%)	96.43	**100**	71.43	**100**

DISCUSSION

An early laboratory marker that can reliably predict neonatal sepsis with high sensitivity and specificity remains a continuing challenge to the clinician[15]. There is a growing interest in the use of granulocytic surface markers for the diagnosis of some inherited and acquired disorders[16,17]. Data concerning their use as markers for prediction of neonatal sepsis are infrequently reported in the literature.

Our findings revealed an enhanced expression of both CD11b and CD64 antigens by peripheral blood neutrophils within the first 48 hours of postnatal life in neonates with early-onset sepsis in comparison to non-infected neonates. These results were in agreement with those obtained by several other investigators[18,19,20]. They assumed that these markers can predict sepsis by providing a means to distinguish infected neonates, at a very early time point of infection before clinical evidence, from suspected, but non-infected, neonates.

In our study population, prematurity associating prolonged premature rupture of membranes (PROM) was the dominant risk factor for the development of early-onset neonatal sepsis. This was revealed by the lower mean gestational age and birth weight of septic neonates compared to non-septic neonates. PROM specially if associated with prematurity had been previously mentioned as the most important risk factor of sepsis, thus neonatal infection results from vertical transmission of microorganisms from infected maternal birth canal to the preterm who had relatively deficient immune responses[3,4].

It has been previously described that the expression of cell-surface activation markers by neutrophils is under developmental control, being down-regulated in preterm when compared with term infants and adults[21,22]. If this is the case, the dominant prematurity of the septic group in our study would have resulted in lower, rather than the encountered significantly higher, expression level of both neutrophil surface activation markers. It is worth mentioning, however, that gestational age was unlikely related to the observed variations in the expression of CD11b and CD64 by neutrophils as the levels of expression of both markers were not correlated with the gestational age. Similar observation of lack of influence of gestational age on CD11b expression was previously reported[23]. Moreover, it was mentioned that the increased expression of neutrophil-surface antigens occurs only upon cell activation[24].

An early onset of enhanced CD11b expression, in septic neonates of the present study, was detectable in cord blood (0 h). CD11b approached near two-fold its mean level in non-infected neonates at 8 h, was sustained at 24 h, and started to decline thereafter. The enhanced CD11b expression in response to infection was previously mentioned by several investigators[10,25] who explained that in the resting neutrophils 95% of their total content of CD11b occurs as membrane components of specific granules and secretory vesicles, i.e. as intracellular storage granules, with only 5% of CD11b content expressed on cell surface. When stimulated, exocytosis of these secretory vesicles takes place resulting in enhanced CD11b expression. The subsequent decline demonstrated at 48 h may reflect the early down-regulation of neutrophil activation prior to the resolution of an ongoing infection. Weirich et al[18] and Nupponen et al[20] found an increased expression of CD11b in neonatal sepsis that seemed superior to CRP in the detection of inflammation at its early stage. The same findings were also confirmed by El-Kerdani et al[23] and Khazbak et al[26] who suggested that CD11b is normally expressed at a low level on the surface of neonatal non-activated neutrophils. Its expression on neutrophil cell surface, however, increases substantially within a few minutes after the cell comes in contact with bacteria or endotoxins thus it could be superior to CRP and the

hematological scoring system in the early detection of neonatal sepsis.

Regarding neutrophil CD64 surface antigen, an enhanced expression was first detectable in the sepsis group of the present study at 8 h with high specificity, but at a very low sensitivity. However, the mean percentage of CD64 expression in the sepsis group approached four-fold the corresponding mean in non-infected group at 24 h where the best diagnostic sensitivity and specificity were obtained by that time. Similar results of enhanced expression CD64 in bacterial infection were previously obtained[19]. It was explained that CD64, the high affinity Fcγ receptor I, which is involved in the process of phagocytosis and intracellular killing of pathogens[27] is also expressed at a low level on the surface of un-stimulated neutrophils[19]. During bacterial infection, the expression of CD64 on activated neutrophils is markedly increased[28,29].

Fjaertoft and associates[19] found that in a small series, the expression of CD64 was significantly enhanced in infected newborns as compared to healthy counterparts. It was also reported that neonates with proven late-onset sepsis or clinical signs of sepsis had an increased percentage of CD64 positive cells, indicating that the level of expression of this antigen may be a specific in vivo marker of neutrophil activation and strongly suggest the presence of infection which would indicate the necessity of antibacterial administration[30]. Ng and coworkers[31] had also raised the high sensitivity value of neutrophil CD64 expression in the diagnosis of late-onset nosocomial infection in the very low birth weight (VLBW) infants. Another group of investigators found that CD64 expression was elevated in most survivors and non-survivors of septic shock as compared to non-septic adult patients[32].

Diagnostic performance tests of CD11b and CD64 for prediction of early-onset sepsis revealed that both markers had their best performance at 24 h. By that time, CD11b expression at a cut-off value of 35% (80% sensitivity, 100% specificity and 91% efficacy) and CD64 expression at a cut-off value of 17% (88% sensitivity, 90.3% specificity and 89.3% efficacy) were the best for prediction of early-onset sepsis. Combined use of both markers, at 24 h also, resulted in improvement of prediction value with 90% sensitivity, 95% specificity and 93% efficacy.

The enhanced expression of CD11b and CD64 neutrophil markers in our series did not correlate with the serum CRP concentration. Both markers were superior to the latter in prediction of early-onset sepsis as serial concomitant measurements of serum CRP revealed a very low early diagnostic value with a sensitivity of only 3.6% at 8 h and 40% at 24 h for sepsis prediction. A gradually increasing diagnostic sensitivity of CRP during the coarse of septicemia was also shown previously.[33] Kuster et al[34] had emphasized that CRP is of limited use in early detection of sepsis. It remains normal at the onset and after 8 hours of invasion by pathogenic bacteria, but may become apparent within 24 hours, which goes with the findings of our study. This lag period between the triggering factors and the onset of a positive CRP result, renders a single measurement of very limited early diagnostic value. Serial CRP measurements are useful for monitoring the course of sepsis and the response to antibiotics[35].

An additional question of importance was that whether the enhanced expression of these neutrophil-surface markers is related to the severity of systemic inflammation and sepsis outcome. It was previously mentioned that the enhanced CD11b expression, serving as an activation marker of neutrophils, is related to the severity of systemic inflammatory response syndrome in acutely ill adults[36,37,38] and may predict organ failure in patients with septic shock[37]. It was also shown that newborn infants who had the highest levels of CD11b expression in a setting of early-onset streptococcal sepsis, died with fulminant sepsis at the age of 3 days[20]. In concert, our findings also revealed that CD11b and CD64 are related to the severity of systemic inflammation and, subsequently, to the outcome. Septic neonates who demonstrated the highest levels of expression of both markers at 8 and 24 h died shortly-after within the age of 48 h. In this respect, CD11b was superior to CD64 as a predictor of the outcome as early as 8 h with 100% sensitivity and 95.2% specificity. CD64 demonstrated the best prognostic utility as an indicator of mortality at 24 h with 100% value for both sensitivity and specificity. On the contrary, Muller and coworkers[39] found that poor prognosis in critically ill patients with sepsis was associated with a lower expression of some activation markers on monocytes and neutrophils suggesting that poor outcome may be due to a compensatory anti-inflammatory response.

Finally, it could be concluded that the expression of the neutrophil activation markers CD11b and CD64 could be a reliable tool for early prediction and prognosis of early-onset sepsis in the suspected neonate, irrespective to other laboratory results and before the evolution of clinical signs. Although CD11b showed earlier predictive and prognostic values, both markers had their best

utility, especially when combined, at 24 hours of life. This may be of a significant importance for therapeutic decision-making in neonates suspected for early-onset infection. Thus, they might indicate the necessity of early initiation of antibiotic treatment for sepsis, with corresponding improvement of the outcome, and reduce the unnecessary use of antimicrobials in non-infected neonates, without waiting for definitive microbiologic results.

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