Role of neutrophil CD64 as an emerging biomarker in the evaluation of neonatal sepsis

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Abstract

Background: Early diagnosis and treatment of neonatal sepsis may be helpful in decreasing neonatal mortality. Neonatal sepsis is considered as one of the major causes of morbidity and mortality.

Methods: This cross sectional study was conducted in the Department of Clinical Pathology, Department of Neonatology and Department of Microbiology and Immunology, BSMMU, Dhaka from March 2013 to February 2014. Neonates with signs and symptoms of sepsis, admitted in the Neonatology department in BSMMU and Neonates with no symptoms or signs of sepsis as control were included in the study.

Results: We found high percentage of expression of CD64 on neutrophils in patients (67.73±24.74) when compared with controls (8.19±3.64) and also their percentage of expression was higher in culture positive sepsis (76.25±29.07) than culture negative sepsis (42.21±33.24). In the present study, I/T ratio>0.2 had a sensitivity, specificity, PPV, NPV and accuracy i.e. 70.0%, 72.0%, 33.3%, 92.3% and 71.7% respectively.

Conclusion: Neutrophil CD64 expression is increased in neonates with early and late onset neonatal sepsis and correlated well with other laboratory markers of sepsis.

Introduction

Early diagnosis and treatment of neonatal sepsis may help decreasing neonatal mortality.[1] Neonatal sepsis is considered as one of the major causes of morbidity and mortality. To avoid unnecessary treatment of non-infected neonates, emergence of multidrug resistance organisms, prolonged hospitalization and a considerable economic burden, particularly in developing countries, an early, sensitive and specific laboratory test would be helpful to guide clinicians in neonatal units to decide whether or not to start antibiotics.[2] Blood culture has been considered the gold standard diagnostic test but its analysis takes too long time and lacks sensitivity at early stages.[3] It is also thought that total leukocyte count (TLC), total neutrophil count, immature-to-total neutrophil ratio (I/T), micro-ESR and C-reactive protein (CRP) and platelet count also failed to reach the appropriate sensitivity and specificity in this disease.[4]

Neutrophil CD64 (nCD64) has been reported as a diagnostic marker of neonatal sepsis, because nCD64 expression is stable for 24 h and can be detected rapidly by flow cytometer with minimal blood volumes.[5] However, the diagnostic accuracy of nCD64 remains unclear due to the large range of sensitivity (0.26–0.95) and specificity (0.62–0.97) in different individual studies.[6,7] Although a meta-analysis has been conducted by Jia et al.[8] they combined the results of median monocyte/nCD64 ratio with nCD64 expression, which might be a source of heterogeneity. In addition, recently new individual studies[9,10] on this topic have reported conflicting results with Jia et al.[8] Thus, there is a need to update the exploration. In this study, we performed to systematically evaluate the diagnostic performance of nCD64, neutrophil CD64 expression, white blood cell count, platelet count, IT ratio and C-reactive protein (CRP) in early diagnosis of neonatal sepsis. Evaluation of neutrophil CD64 expression, white blood cell count, platelet count, IT ratio and C-reactive protein (CRP) in early diagnosis of neonatal sepsis was aim of this study.

Materials and methods

This cross sectional study was conducted in the Department of Clinical Pathology, Department of Neonatology and Department of Microbiology and Immunology, BSMMU, Dhaka from March 2013 to February 2014. Neonates with signs and symptoms of sepsis, admitted in the Neonatology department in BSMMU and Neonates with no symptoms or signs of sepsis as control were included. After taking informed written consent from attendant blood sample were obtained from peripheral venipuncture in all neonates within 24hrs of admission. A total 2.5ml venous blood was taken of which 1.5ml was collected in EDTA tube for complete blood count, PBF and for neutrophil CD64 estimation. Another 1.0ml blood was taken for blood culture for the purposes of this study. Samples were remained acceptable for up to 24hours after collection when held at room temperature (18-22ºC) and for 48hours when refrigerated (2-8ºC). Complete blood count with PBF was done in Clinical pathology Department. Flow cytometric analysis and blood culture were done in Microbiology and Immunology department. Data was collected by a pre designed proforma. Blood sample was obtained from patients suspected cases of neonatal sepsis or clinically sepsis. Patient information was obtained through using patient's information sheet which involved questionnaire and clinical findings. Data editing, clearing and analysis was done by statistical package for social science (SPSS) 23.0. Sensitivity, specificity, PPV, NPV of neutrophil CD64

was calculated using specific formulas that are specified (Figure 1) (Tables 1-7).
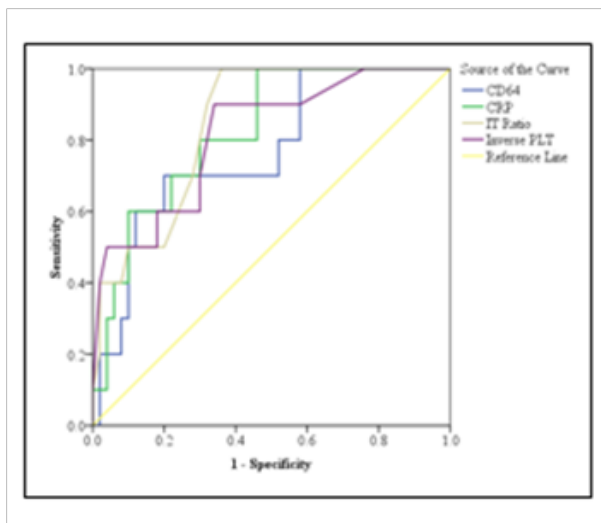

Figure 1 ROC Curve of Neutrophil CD64, CRP, IT ratio & Inverse of PLT level by Blood culture.

Table 1 Demographic characteristics of the study population (n=60)

Demographic characteristics	Study group Cases n=40		Control n=20		p value
Age group	n	%	n	%	
0-7days	26	65.0	16	80.0	0.23ns
> 7days	14	35.0	4	20.0	
Sex					
Male	23	57.5	11	55.0	0.85ns
Female	17	42.5	9	45.0	
Gestational age					
Preterm <37wks	31	77.5	10	50.0	0.03s
Term ≥ 37wks	9	22.5	10	50.0	
Birth weight					
Very low birth weight ≤ 1500gm	12	30.0	0	0.0	
Low birth weight >1500-2499gm	19	47.5	4	20.0	0.001s
Normal weight ≥2500gm	9	22.5	16	80.0	
PROM					
Yes	29	72.5	1	5.0	0.001s
No	11	27.5	19	95.0	

*t test was done to measure the level of significance.

Table 2 Comparison between demographic characteristics with blood culture (n=60)

Demographic characteristics	Blood culture Positive n=10		negative n=50		p value
Age group	n	%	n	%	
0-7days	7	70.0	35	70.0	0.999ns
> 7days	3	30.0	15	30.0	
Sex					
Male	7	70.0	27	54.0	0.491ns
Female	3	30.0	23	46.0	
Gestational age					
Preterm <37wks	9	90.0	32	64.0	0.148ns
Term ≥ 37wks	1	10.0	18	36.0	
Birth weight					
Very low birth weight ≤ 1500gm	5	50.0	7	14.0	
Low birth weight >1500-2499gm	5	50.0	18	36.0	0.004s
Normal weight ≥2500gm	0	0.0	25	50.0	
PROM					
Yes	10	100.0	20	40.0	0.001s
No	0	0.0	30	60.0	

Table 3 Results of neutrophil CD64, IT ratio, PLT & CRP detected by blood culture (n=60)

Parameters	Blood culture Positive Mean±SD	Negative Mean±SD	p value*
Neutrophil CD64	76.25±29.07	42.21±33.24	0.001s
CRP	82.11±59.23	27.31±41.87	0.001s
IT ratio	0.29±0.13	0.16±0.08	0.001s
PLT (x10^9/L)	98.50±79.93	210.40±107.87	0.003s

Table 4 Area under the curve

Test result variables	AUC	p value*	95% Confidence Interval Lower	Upper
CD64	0.768	0.008s	0.613	0.923
CRP	0.822	0.001s	0.698	0.946
IT Ratio	0.844	0.001s	0.736	0.952
PLT	0.815	0.002s	0.672	0.958

* Null hypothesis: true area: 0.5, AUC: Area Under the Curve, S: Significant

Table 5 Distribution of the study population according to neutrophil CD64 with CRP

| CRP | Neutrophil CD64 | | | | p value |
| | Positive n=40 | | Negative n=20 | | |
	n	%	n	%	
Abnormal	31	77.5	00	0	0.004ˢ
Normal	09	22.5	20	100	
Total	**40**	**100**	**20**	**100**	

Table 6 Distribution of the study population according to blood culture with neutrophil CD64 (n=60)

| Neutrophil CD64 | Blood culture | | | | p value |
| | Positive n=10 | | negative n=50 | | |
	n	%	n	%	
Positive(>20%)	10	100	30	60	0.001ˢ
Negative(≤20%)	0	0	20	40	
Total	**10**	**100**	**50**	**100**	

Table 7 Distribution of the study population according to blood culture with CRP (n=60)

| CRP | Blood culture | | | | p value |
| | Positive n=10 | | negative n=50 | | |
	n	%	n	%	
Positive(>5)	8	80.0	23	46.0	0.001ˢ
Negative(≤5)	2	20.0	27	54.0	
Total	**10**	**100**	**50**	**100**	

Table 8 Performance of diagnostic tests

Diagnostic tests	Sensitivity	Specificity	PPV	NPV	Accuracy
CD64	100.0%	40.0%	25.0%	100.0%	50.0%
CRP	80.0%	54.0%	25.8%	93.1%	58.3%
IT ratio	70.0%	72.0%	33.3%	92.3%	71.7%
PLT	60.0%	68.0%	27.3%	89.5%	66.7%

Discussion

Sepsis is an upward health problem especially among low birth weight neonates.[11] Diagnosis of neonatal sepsis is one of the most difficult tasks for physicians and other medical staff. There is no single reliable test for early diagnosis. Currently blood culture is the most reliable method for detection of bacterial infections. But the sensitivity of blood culture is low, longer time required for report (preliminary 24hours, final 7days) and false negative result may be found. Culture positive sepsis is a small proportion of a larger group of clinical sepsis (with negative blood cultures). So it is clear that to manage neonates with sepsis properly, a single reliable marker of infection is needed, to avoid unnecessary antibiotic therapy. A rapid laboratory test with high specificity for neonatal sepsis help in making a therapeutic decision and avoiding the unnecessary use of antibiotics in patients with clinical signs and symptoms of sepsis but negative blood cultures.[11]

In this study, we tried to determine the neutrophil CD64 expression as an immunological marker for rapid and accurate diagnosis of neonatal sepsis. Several published studies have indicated that neutrophil CD64 is an ideal method for evaluation as a more sensitive and specific marker of infection.[6,11–14] This study included 60patients with a mean age of 5.9±6.49days. There was 40 clinically sepsis neonates and 20 control neonates with no symptoms or signs of sepsis. In sepsis group, early onset was observed more (65%) than that of late onset of sepsis (35%). This observation was constant with the findings of others Noor et al.[15] Khaleda et al.[16] in BSMMU. Among the infected newborns, male were predominant (57.5%). It is due to the factors regulating the synthesis of a globulin situated on the X chromosome. Male has only one X chromosome, so he is immunologically less protected than the females. Predominance of male babies suffering from neonatal sepsis was found in other study.[15–17]

In our study, preterm (77.5%) and low birth weight babies (77.5%) are more susceptible to infection. This study was similar to other study.[18,19] Higher susceptibility of infection in preterm and low birth weight babies might be due to low level of IgG and lower defense mechanism. 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 gestational age and birth weight. Duration of premature rupture of membrane (PROM) for >24hours has to be an important risk factor in neonatal septicemia because PROM poses of ascending infection to the fetus. In our study, PROM was 72.5% in sepsis neonates and 5.0% in control group. This finding was consistent with the study of Khaleda et al.[16] and Kuruvilla et al.[20] in this study, out of 40 clinically diagnosed neonatal sepses, blood culture was found positive in 10(25.0%) cases. According to Shaha et al.[21] and Noor et al.[15] Positive cultures reportedly range from 8-73% in the diagnosis of neonatal sepsis. 12% neonates are culture positive sepsis.[16] In the present study, there was high percentage of exp ression of CD64 on neutrophils in patients (67.73±24.74) when compared with controls (8.19±3.64) and also their percentage of expression was higher in culture positive sepsis (76.25±29.07) than culture negative sepsis (42.21±33.24). These results were consistent with other study.[12] This may be due to faulty sterile technique in collection procedure, insufficient sample volumes, intermittent or low-density bacteraemia, or suppression of bacterial growth by earlier antibiotic administration and delayed arrival of patients. Total leukocyte count (TLC) and absolute neutrophil count is of little clinical use in the diagnosis of neonatal sepsis because of wide variation in values. Neutrogena has been more common in association with sepsis, compared with neutrophilia.[22] Probably due to increased adherence to altered endothelial cells and utilization at the site of infection. I: T ratio is the ratio between immature neutrophil count (band form) and the total neutrophil in a blood smear. As a marker of sepsis in newborn babies, the IT ratio should be>0.2. In the present study, I/T ratio>0.2 had a sensitivity, specificity, PPV, NPV and accuracy of 70.0%, 72.0%, 33.3%, 92.3% and 71.7% respectively. While and I/T ratio>0.2 suggested by Khaleda et al.[16] had a sensitivity of 100% specificity 04%, PPV 13% and NPV of 100%. Similar result was also found by Ghosh et al.[23] Specificity and positive predictive value was low because of large number of false positive results. Therefore this parameter alone should not be evaluated for diagnostic purpose.

Neonates with sepsis develop thrombocytopenia, possibly because of disseminated intravascular coagulation (DIC) and the damaging effects of endotoxin on platelets. In this study, we found thrombocytopenia with cut off value<150x10⁹/L had sensitivity of 60.0%, specificity 68.0%, PPV 27.3% and NPV 89.5%. This parameter could be used as an early but nonspecific marker for sepsis. These results were consistent with other study.[16,17] Considering high mortality and morbidity associated with neonatal sepsis, tests with high sensitivity and NPV are most desirable because all genuinely infected newborns should be identified and treated. Flow cytometric analysis has the advantage over conventional immunological assay methods which being able to localize the activated markers to a specific cell type.

CD64 is normally expressed in very low concentrations by unstimulated neutrophils, whereas it is considerably up regulated on the trigger of bacterial invasion. CD64 is the activation marker for neutrophils. Neutrophils react within one hour of acute inflammation. CD64 has already been identified as a high-affinity Fc-gamma receptor of IgG antibody in the process of phagocytosis and intracellular killing of opsonized microbes. The pathogens that require opsonization are encapsulated bacteria, so that CD64 expression is not related to viral infection.[13] In addition, it has been shown that neutrophils of preterm infants express CD64 antigen to a similar extent comparing to older children and adults.[24] Thus, this specific marker can be used for the identification of life threatening infections in preterm infants. The present study showed high statistically significant difference (P=<0.001) between culture proven sepsis and culture unproven sepsis regarding percentage of expression of CD64 on neutrophils. Similarly Azza et al.[12] found that there was a highly significant difference between patients with positive blood cultures and those with negative blood cultures regarding the percentage of CD64 positive neutrophils.

There are many advantages of using neutrophil CD64 expression as an indicator of neonatal sepsis, as the quantitation of neutrophil CD64 is rapid (<60minutes) and only minimal blood volume (100µl) is used, which is a real advantage in neonates.[25] In fact, for the present study, no extra blood was obtained from the neonates to perform this test; the samples sent for the complete blood count is adequate. In the present study neutrophil CD64 showed high sensitivity 100%, specificity 40%, PPV 25% and also high NPV 100%. Specificity and PPV were low because of large number of false positive result. This may be due to small sample size and blood culture was found positive only in 16.7% cases of neonatal sepsis. The results of our study also showed significantly elevated levels of CD64 in septic neonates when compared to healthy controls. These findings coincided with the outcome of numerous studies done for the diagnostic performance of neutrophil CD64 in neonatal sepsis in view of the high sensitivity and negative predictive values.[6,11–14] The results of present study and previous studies show that measurement of neutrophil surface marker (CD64) can be useful for diagnosis of neonatal sepsis in early phase.

An accurate inflammatory marker with high diagnostic sensitivity and negative predictive value for neonatal sepsis would be a valuable tool for therapeutic decision-making and avoidance of unnecessary use of antibiotics. In the current study, CD64 as a leukocyte surface antigen was compared to the conventional and routine markers. Our investigation showed that evaluation of neutrophil CD64 was a superior diagnostic marker for the early detection of neonatal sepsis. This study was done for evaluation of neutrophil CD64 expression in the diagnosis of neonatal sepsis. In conclusion, flow cytometric assessment of neutrophil CD64 may be considered as a valuable marker for diagnosis of bacterial neonatal sepsis. Neutrophil CD64 expression is increased in neonates with early and late onset of neonatal sepsis and correlated well with other laboratory markers of sepsis.

Acknowledgements

Conflict of interest

The author declares no conflict of interest.

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