Prognostic value of neutrophil CD64 in low birth weight neonates with sepsis

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Abstract

Introduction: Neonatal sepsis represents a major health problem with high mortality and morbidity rates. Although early diagnosis of neonatal sepsis is very important for proper management yet it remains a difficult task. Neutrophil CD64 (nCD64) is used as a marker for the diagnosis of sepsis, requiring a small sample volume, short turnaround time.

Objectives: In this study we aimed to study the diagnostic performance of nCD64 against routine markers in low birth weight neonates (LBWN) with sepsis.

Patients and methods: A case control study was conducted on 40 LBWN suspected clinically to have early onset neonatal sepsis against 20 neonates clinically free of sepsis as control. Investigations included CBC, CRP, blood culture and nCD64 expression.

Results: Among the studied markers of sepsis; immature neutrophil count, immature/mature ratio, immature/total ratio, CRP and nCD64 were significantly higher in suspected group than control (p value 0.007, 0.001, 0.002, 0.001, and 0.001 respectively). Among the group of neonates with suspected sepsis, blood culture of 11 cases (27.5%) did not show growth. nCD64 showed the highest sensitivity and specificity; 100% each. Immature neutrophil count and total leucocytic count showed the lowest sensitivity 40% and mature neutrophil showed the lowest specificity 45%. The expression of nCD64 in those neonates who died as a complication of sepsis was significantly higher than those who survived (p value 0.001).

Conclusion: nCD64 is a reliable marker for the diagnosis of early onset neonatal sepsis in LBWN with a significant predictive value for disease course.

Key words: Respiratory Low birth weight neonates; Neonatal sepsis; Neutrophil CD64.
Background

Early onset neonatal sepsis (EONS) is a life threatening condition for neonates during their first 72 hours of life. In general, neonatal sepsis, sepsis neonatorum or neonatal septicemia are synonyms that being used to describe a systemic response of a neonate to infection [1]. Multiple maternal, neonatal and environmental factors play a role in the development of neonatal sepsis[2].

The associated risk factors for EONS include: preterm delivery, premature rupture of membranes (PROM), maternal urinary tract infection (UTI), maternal fever, maternal chorioamnionitis, group B streptococcal (GBS) infections rectovaginal colonization, foul smelling liquor, multiple per vaginum examinations, difficult or prolonged labour, aspiration of meconium, very low birth weight (VLBW), prematurity, asphyxia, low Apgar score and male sex.[3]

The micro-organisms most commonly associated with EONS are GBS, E.coli, haemophilus influenzae and listeria monocytogenes. [4]

In Egypt, rates of neonatal sepsis (EONS) exceeding 50% especially in neonatal intensive care unit (NICU) with a mortality rate of 51% for proven EONS and 42.9% for proven LONS.[5,6,7]

The lack of a well-established laboratory marker for an early diagnosis of neonatal sepsis increases the challenge for management as rapid definitive diagnosis is required. Although blood culture is the gold standard technique it has a long turnaround time (TAT) in addition to its poor positive and negative predictive values.[8] On the other hand, white blood cell (WBC) counts, absolute neutrophil count (ANC), immature neutrophil count, the ratio of immature: Total neutrophils (I:T) and immature : mature neutrophils (I:M) are commonly used parameters as screening tests for the diagnosis of neonatal sepsis,(9,10)yet they have poor positive predictive value (PPV) and poor diagnostic accuracy in terms of sensitivity and specificity .[4]

Cytokines can be used for screening of sepsis yet they have poor specificity and their levels are linked to the immune status of the neonate.[11]

Inflammatory markers as C-reactive protein (CRP) and procalcitonin (PCT) are commonly used as routine markers for the diagnosis of sepsis. However, CRP requires 6-8 hours for being synthesized after stimulation and 24 hours to reach the peak. Its quantitative assay has no superiority over WBC counts or ratios except in monitoring the effect of treatment if measured serially [12].Although it is more accurate than CRP for the diagnosis of neonatal sepsis, procalcitonin has moderate accuracy for the diagnosis of neonatal sepsis. [13]

Neutrophils CD64 (nCD64) is a surface marker expressed in a very low concentration in resting condition however its level increases 5-10 folds after infectious stimulations and the level is correlated with the process of phagocytosis.[14] It is considered a sensitive laboratory marker for diagnosing neonatal sepsis. It is superior to CRP as it is activated even before CRP starts to rise’ [15] The assessment of nCD64 is relatively simple and fast; it requires a small blood volume with no special precautions and no effect of previous antibiotic use.[16]
In this study we aimed to evaluate the nCD64 for early diagnosis of EONS against other markers and to evaluate its value in predicting disease course in LBWN.

Patients and Methods
This prospective study was conducted in the Neonatal intensive care unit& Department of Clinical Pathology, at Minia University Hospital for Obstetrics and children. Written informed consent was signed by the parents of neonates enrolled in the study. The protocol of the study follows the principles outlined in the Declaration of Helsinki at World Medical Association. (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects).

Low birth weight neonates, who were admitted to the NICU were included in the study during the period from June 2016 to January 2017. They were classified into the following two groups; group I included 40 neonates having three or more risk factors for EONSaddition to strong clinical suspicion of sepsis. Their gestational age ranged from (35-40 wks.), their weights ranged from (1.4 – 2.5 kg, mean±SD of 2.16±0.28), 26 were males and 14 females, this group was subdivided into two subgroups; those who survived which included 22 neonate and the other for those who died as a complication of sepsis and included 18 neonate.A control group, group II, included 20 apparently healthy neonates with matched gestational age whom blood samples were taken for other routine investigations as ABO grouping and thyroid functions.

Ethylenediaminetetraacetic acid (EDTA) samples were used for hematological studies on Celtac Es, NIHON KOH DEN CORPORATION, AUTOMATED HEMATOLOGY ANALYSER, Japan and for flowcytometric study of n-CD64 expression using BD FACS cantotm II USA according to the following protocol:
One hundred μl of EDTA blood were used for the evaluation of nCD64 expression. For each sample, 2 tubes were labeled, one for fluorescein iso-thiocyanate (FITC) mouse anti-human CD64 monoclonal antibody (BD-Bioscience), the other tube for negative isotypic control (FITC) Mouse IgG1 κ Isotype control. Fifty μl of samples were delivered in each tube. Four μl of monoclonal antibodies were added to respective tubes. Then both tubes were vortexed, incubated for 15 minutes. Three ml of lysing solution was added to each tube then the tubes were vortexed and incubated for just 10 minutes followed by centrifugation. The supernatant was discarded and phosphate buffered saline (PBS) was added to the sediment and mixed thoroughly, then centrifuged. The supernatant was discarded and cells were suspended in 300 μl PBS to be ready for acquiring data by the flowcytometric analysis.

In flowcytometry, cell surface expression of nCD64 was determined at 468 nm wavelength laser excitation and the emitted fluorescence was monitored with a detector optimized to collect peak emissions at 504 – 541 nm. Neutrophils phenotyping was done by gating according to forward scatter (size) and side scatter (granularity) strategy. Results were expressed molecules of equivalent soluble fluorochrome (MESF).

Serum samples were used for CRP assay quantitatively using Human C-Reactive Protein (CRP) ELISA Kit, the Cell Biolabs, Inc. San Diego, CA 92126. USA, according to the manufacturer instructions.

Statistical Methods:
The data were encoded, entered and processed on computer using Graph Pad prism 4. Data were presented as mean ± standard deviation.
(SD). Statistical analysis was carried out using paired sample t test and Mann–Whitney test. Positive predictive value, negative predictive value, sensitivity, and specificity were obtained using optimal cutoff levels. Correlations were calculated by the Pearson and Spearman rank methods. Probability values <0.05 were considered to be significant.

Results

Results for leucocyte counting

Total leucocytic count in group with suspected sepsis ranged from 10000 to 32300cell/mm³ with a mean of 16600 and SD ±5800, while the number of TLC count in control group ranged from 10000 to 25000cell/mm³ with a mean of 17700 and SD ±4400. There was no statistically significant difference between the two groups regarding to the TLC count (p-value 0.190).

The absolute neutrophil count (ANC) count in group with suspected sepsis ranged from 4900 to 16907cell/mm³ with a mean of 6019.7 and SD ±1781, while the number of ANC in control group ranged from 5705 to 12152cell/mm³ with a mean of 7542.5 and SD ±1695. There was no statistically significant difference between the two groups regarding to the ANC (p-value 0.371).

The count of mature neutrophil in group with suspected sepsis ranged from 3220 to 13398 cell/mm³ with a mean of 7985.4 and SD ±2216, while the number of mature neutrophil count in control group ranged from 3912 to 10388cell/mm³ with a mean of 6019.7 and SD ±1520. There was no statistically significant difference between the two groups regarding to the mature neutrophil count (p-value 0.987).

The count of immature neutrophil (bands, metamyelocytes, myelocytes and promyelocytes) in group with suspected sepsis ranged from 1144 to 3876cell/mm³ with a mean of 1665.7 and SD ±618, while the number of immature neutrophil count in control group ranged from 900 to 2000cell/mm³ with a mean of 1522.9 and SD ±318.8. Immature neutrophil count was statistically significant low in group with suspected sepsis when compared to control group (p-value 0.007).

The I/M ratio in group with suspected sepsis ranged from 0.15 to 1.5 with a mean of 0.34 and SD ±0.2, while I/M ratio in control group ranged from 0.1 to 0.4 with a mean of 0.24 and SD ±0.07. I/M ratio were statistically significant higher in group with suspected sepsis when compared to control group (p-value 0.001).

The I/T ratio in group with suspected sepsis ranged from 0.13 to 0.8 with a mean of 0.26 and SD ±0.1, while I/T ratio in control group ranged from 0.14 to 0.3 with a mean of 0.2 and SD ±0.04. I/T ratio was statistically significant higher in group with suspected sepsis when compared to control group (p-value 0.001).
diagnosis of septicemia in newborns.[26] Total leucocytic counts are particularly unreliable indicator of infection during the first several hours of early-onset (within 48 h of birth) sepsis because their high values are initially normal.[27] The inadequate specificity of mature neutrophil count and the poor sensitivity of immature neutrophil count as markers of EONS were reported previously. During sepsis, a ‘left shift’ of neutrophils happens because of immature neutrophils released from marrow which increases the ratio of immature to total neutrophils.[28] Our results revealed that the I/T ratio, I/M ratio of neutrophils and immature neutrophils are higher in septic neonates compared to normal neonates. These results were in agreement with Mondal et al., [29] who found that the hematologic profiles of neonates with septicemia were characterized by higher I/T ratio. Bhandari et al., found that the hematologic profiles of neonates with septicemia were characterized by higher ANC, I/T ratio and immature neutrophil.[30] Moreover, TLC and differential counts lacks the proper specificity as their automatic assessment is affected by the presence of nucleate red blood cells while manual count and blood film examination requires special skills.[31] This explains the inadequate sensitivity and specificity obtained in this work for blood culture as a marker of sepsis in LBWN.

CRP, a peptide synthesized by the liver in response to infection or inflammatory processes[32]. Our result revealed that CRP was statistically significant in septic neonates compared to control; this was in accordance with other researches. [33,34,12]. However, a positive CRP result does not differentiate between systemic inflammatory response and sepsis, neither between bacterial infection and non-bacterial infections[35]. Moreover, the latency between infection and synthesis of CRP till reaching the peak level affects the sensitivity and specificity as reported in this work [36].

The high affinity antibody receptor CD64 is expressed at a very low level on the surface of neutrophils in the absence of an infection. The expression of CD64 on activated neutrophils markedly increases after an episode of bacterial infection [37]. The results of this study showed a significantly higher expression level of nCD64 in LBWNs with suspected sepsis when compared to control as reported previously[38,39]. Shi and his colleagues reported in a meta-analysis a lower pooled sensitivity and specificity for nCD64 in neonatal sepsis than other markers[40], this disagrees with our results. The higher frequency gram positive organisms in this work is accompanied by higher expression of neutrophils in addition to the selection LBWN as candidates may be a reason for this discrepancy in results. The high sensitivity and specificity of CD64 in our study also high positive and negative predictive values of the test making it of great value in diagnosing neonatal sepsis. Moreover, upregulation of CD64 in the group of died neonates added prognostic importance. Until now, there is no reliable marker that can be used alone to predict
the outcome of neonatal sepsis, yet our work may provide preliminary results for a single marker that can predict disease outcome in LBWN in environment with high prevalence of gram positive organisms. Further studies on a larger scale at different environments are required.

Conclusions
nCD64 is a reliable marker for the diagnosis of early onset neonatal sepsis in LBWN with a significant predictive value for disease course.

Competing interests: The authors declare that they have no competing interests.

Authors’ contributions: SA and AE conceived the study, carried out its designing, coordinated the implementation. EH conducted the statistics of this work. NO conducted and revised the laboratory data of the study. AA participated in the design of the study, analysis and interpretation of data and revised the statistics and final draft of the manuscript. All authors read and approved the final manuscript.

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