Original article

Neutrophil CD64 as a diagnostic marker of sepsis in children

Background: *Sepsis is one of the leading causes of mortality among children worldwide. Reliable evidence was insufficient in pediatric sepsis and many aspects in clinical practice actually depend on expert consensus with some evidence in adult sepsis.* ***Objective:*** *This study aimed to investigate neutrophil expression of CD64 in septic children and in healthy controls. We hypothesized that these receptors are elevated during sepsis and can be used as a diagnostic marker.* ***Methods:*** *This study was carried out on 50children with pediatric sepsis and 40 apparently healthy children as controls. Cases were recruited from the PICU of Al Zahraa University Hospital, Al-Azhar University for Girls in the period from May 2014 to March 2015. All the cases were assessed clinically and by routine laboratory investigations. Expression of neutrophil CD64 was measured by flow cytometry.* ***Results:*** *The mean CD64 expression in children with sepsis (66.49 ± 23.45) was significantly higher than in the control group (9.39 ± 6.17) p<0.001 .CD64 expression had a significant positive correlation with CRP level (r=0.416, p<0.003). ROC curve for CD64 expression showed100% sensitivity and specificity. The most common isolated organisms were gram negative organisms mainly E. coli. A highly significant increase was demonstrated in CRP and TLC values in the culture proven sepsis group compared to clinical sepsis group, while there was no statistical significant difference in CD64 values between the two groups.* ***Conclusion:*** *change in cell surface expression of CD64 on peripheral blood neutrophils can be considered a sensitive marker for the detection of pediatric sepsis.*

Keywords: Neutrophil, CD64, sepsis, children.

Soheir Ibrahim Mohamed, Entisar Raafat Mokhtar*, Amira Mohammed Naguib**

*Department of Pediatrics, and *Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.*

Correspondence:
Soheir I. Mohamed, Ass. Prof of pediatric, Faculty of Medicine for girls, Al-Azhar University, Cairo, Egypt.
E-mail: *elswah.soheir @yahoo.com*

INTRODUCTION

Severe sepsis and septic shock remain leading causes of mortality and morbidity in children. Despite advances in prevention and treatment, children with severe sepsis continue to present significant treatment challenges to clinicians.[1]

Although the diagnosis and management of sepsis in infants and children is largely influenced by studies done in adults, there are important considerations relevant for pediatrics[2].

The CD64 is a membrane glycoprotein that mediates endocytosis, phagocytosis, antibody-dependent cellular toxicity, cytokine release, and superoxide generation. It is constitutively expressed on monocytes and macrophages. It is expressed at low concentration on the surface of non- activated neutrophils but can be markedly upregulated at the onset of sepsis[3].

There are several reports regarding its potential utility for the diagnostic assessment of sepsis or infection in adults[4] and neonates[5], but only a few in children[6]. In our study we investigated neutrophil expression of CD64 in septic children and in healthy controls. We hypothesized that their expression is increased during sepsis and could be a potential diagnostic marker.

METHODS

This was a cross-sectional controlled study carried out on 50children with pediatric sepsis and 40 apparently healthy children as controls. Cases were recruited from among those admitted to the PICU of Al-Zahraa University Hospital, Al-Azhar University for Girls during the period from May 2014 to March 2015. The patients' ages ranged from one month to 14 years, Patient clinically diagnosed as sepsis or septic shock were included in the study according to the international pediatric sepsis consensus conference of 2005[7].Children were excluded if they had chronic systemic disease, degenerative neurological disease, or primary or acquired immunodeficiency diseases, were on corticosteroid therapy, non-steroidal anti-inflammatory drugs, or antibiotics for more than 24 hours, or suffered from trauma or burn, or were in post-operative care. Verbal consents and approval

were obtained from the parents or caregivers after explanation of the subject and procedure.

All children were subjected to complete history taking, complete clinical examination and laboratory investigations including complete blood counts (CBC) using cell counter [Sysmex KX-21N, Sysmex, Kobe, Japan], with examination of Leishman stained peripheral blood (PB) smears for differential leucocyte outcrop measurement immunoturbidmetrically using (Turpox), kidney functions (blood urea and serum creatinine), liver function tests (AST, ALT and albumin), serum electrolytes, blood gases, and blood cultures.

Sampling:

Three milliliters of venous blood were aseptically collected and divided in to three tubes:

- One milliliter of venous blood was dispensed into a tube containing K-Ethylene Diamine Tetra Acetic acid (K-EDTA) at a concentration of 1.2mg/ml, to be used fresh for CBC and for the flow cytometric analysis of neutrophils expressing CD64.
- One milliliter of venous blood was dispensed into a plain tube, to be used for CRP, AST, ALT, urea and creatinine, determination.

One milliliter of venous blood was added to BACTEC PEDS Plus/F culture vials (soybean-Casein Digest Broth with Resins) and incubated in BACTEC (9050) blood culture instrument Beckton-Dickenson, for early detection of CO_2, and or pH changes, then subcultures on blood agar plate, nutrient agar plate and MacConkey media (incubate at 37°C for 24 hours), on the next day, gram stained film for isolated colonies were done.

Flow cytometric analysis of neutrophil CD64 expression in PB samples was carried out on coulter EPICS-XL. Monoclonal antibodies for CD64 were supplied by BECKMAN COULTER company, USA. Data acquisition and analysis were performed on cell quest program of the coulter EPICS XL flow cytometry. Gating on neutrophil, 1000 events were acquired, and statistical analysis was done by cell quest software, results were expressed as percentage (%) and mean fluorescence intensity (MFI).

Interpretation: The positivity was expressed as a percentage with a cut off >20% over the corresponding isotopic control.

Statistical methods

The collected data were analyzed using statistical package for social science (SPSS) version for windows (version18.0.). All data were expressed as mean values ± SD. Comparisons of parameters among groups were made using paired t test. Comparisons between two qualitative variables were performed using chi-square and fisher's exact tests. A p value ≤ 0.05 was considered significant. Pearson's correlation coefficient (r) test was used for correlating data. Receiver operating characteristic (ROC) curve analysis was used to find the overall predictivity of parameter and the best cut-off value with detection of sensitivity and specificity.

RESULTS

The mean CD64 expression in children with sepsis (66.49 ± 23.45%)was significantly higher than those in the control group (9.39 ± 6.17) p<0.001 as in Table1. CD64 expression had a significant positive correlation with CRP level; r = 0.416, p<0.003 (Table 2 and figure1). ROC curve For CD64 expression showed 100% sensitivity and specificity, the cut-off point is 19.6 % (Table 3). In this study, bacterial cultures were positive in68% and negative in 32% of blood cultures (Table 4). E coli was the most common organism isolated from septic patients (38.2%) as in figure 2. The present study demonstrated a highly significant increase in CRP and TLC values in patients with culture proven sepsis compared to those with clinically diagnosed sepsis, while there was no statistical significant difference in CD64 values between the two groups (Table 5).

Table 1. Comparison between studied groups regarding neutrophil CD64.

	CD64 %		Independent t-test	
	Mean ± SD	**Range**	**T**	**p-value**
Control group	9.39 ± 6.17	0.69 – 19.6	14.620	0.001*
Patients group	66.49 ± 23.45	26.4 – 99.4		

* Means significant

Table (2): Correlation between neutrophil CD64 and laboratory parameters

	CD64	
	R	**p-value**
Hb(g/dl)	-0.066	0.656
TLC ($\times 10^9$/L)	0.049	0.741
HCO_3 (mEq/l)	0.003	0.985
CRP (mg/l)	0.416	0.003*
Urea (mg/dl)	-0.146	0.312
AST (U/L)	-0.169	0.241
ALT (U/L)	-0.249	0.081
Alb (g/dl)	-0.148	0.311

* Means significant

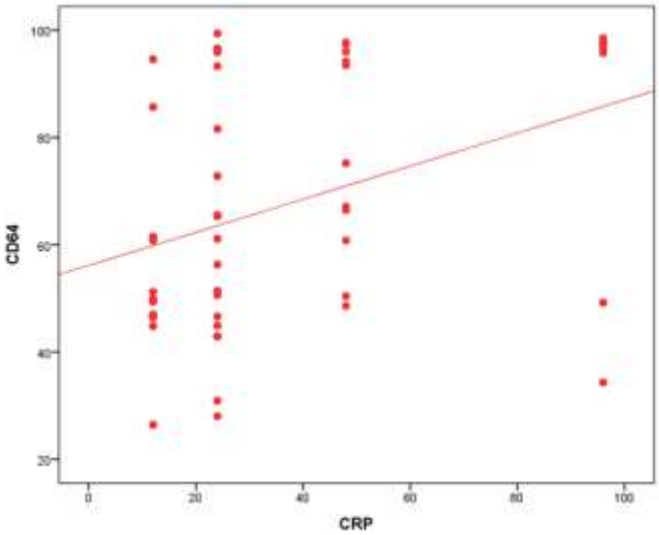

Figure 1. Scattered diagram showing positive correlation between neutrophil CD64 and CRP

Table 3. Cut off point,sensitivity and specificity of neutrophil CD64 for diagnosis of sepsis.

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
>19.6%	1.000	100.00	100.00	100.0	100.0

Table 4. Culture results in patient group

	No.	%
Negative culture	16	32.0%
Positive culture	34	68.0%

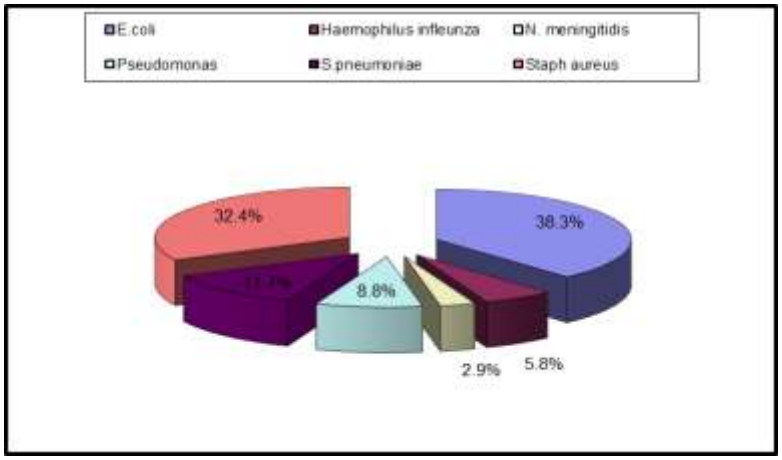

Figure 2. Blood culture results.

Table 5. Comparison between clinical sepsis and culture proven sepsis groups regarding CBC, CRP and neutrophil CD64

	clinical sepsis		culture proven sepsis		Independent t-test	
	Mean	**SD**	**Mean**	**SD**	**t**	**p-value**
Hb(g/dl)	12.93	1.29	13.01	1.50	-0.206	0.838
TLC ($\times 10^9$/L)	20.36	4.00	22.12	6.58	-0.522	0.025*
Plt Count ($\times 10^9$/L)	288.76	122.49	273.52	110.64	0.460	0.648
CRP (mg/l)	25.14	14.15	44.69	31.57	-2.646	0.011*
CD64%	73.75	22.22	63.29	23.62	1.532	0.132

* Means significant

DISCUSSION

The diagnosis of sepsis remains one of the most difficult tasks for physicians and other medical staff. Blood cultures often remain negative in the presence of pneumonia, meningitis and even fulminant blood born septicemia. A rapid laboratory test with high specificity for pediatric sepsis would be a valuable tool in therapeutic decision making and avoiding the unnecessary use of antibiotics .[8]The high affinity CD64 is mainly involved in phagocytosis and intracellular killing of pathogens, but it is also expressed at very low levels on the surface of unstimulated neutrophils.[9] Upregulation of CD64 on neutrophils is thought to be a very early step of host's immune response to bacterial infection, increasing approximately one hour after invasion**.**[10]

In this study, determination of the CD64 expression as an immunological marker for diagnosis of pediatric sepsis was done. The mean CD64 expression in children with sepsis was significantly higher than those in the control group. These findings are in agreement with previous studies.[11,12]Similar results have been reported in adults by Cid et al.[13] who revealed that patients with sepsis had a greater number of circulating CD64 positive PMNs (mean 71%) than in healthy controls (mean 19%).

CRP, a globulin produced by the liver during any generalized inflammatory process, as a result of stimulation by IL-1 and IL-6, increases only after 12-24 hours from the onset of infection. This limits its use in the initial evaluation of the septic infants, but serial measurements of CRP are useful in monitoring the progress of infection[14]**.** In the current study, CD64 expression had a significant correlation with CRP levels pointing to its usefulness as an additional marker of sepsis. This is in agreement with previous studies[14,15]. Our data demonstrated a sensitivity and specificity of 100% each for CD64 expression in pediatric sepsis, higher than those reported by Ng et al.,[16] (97% and 89% respectively), and other investigators.[17,18]

The positive and negative predictive values of CD64 observed in the current study (both 100%) were also higher than those obtained by Dilliet al.,[11] and Chan and GU[18] in early onset sepsis. In addition, Streimish et al.,[12] found that the high sensitivity of raised levels of CD64 for neonatal sepsis is achieved through a single determination and that the high percentage of CD64+ cells seen in early onset neonatal sepsis is maintained in these patients for at least 6 hours.

In this work, ROC curve shows an area under the curve (accuracy) for CD64, AUC for CD64 =1. This implies the greater discriminating power for CD64 for early detection of pediatric sepsis. This is in agreement with previous studies done by Streimish et al.[19] and another study by Hsu et al.[20] who concluded that CD64 expression had a remarkable discriminating power.

Bacterial cultures were positive in 68 % and negative in 32 % of blood culture samples obtained from the studied patients. The organisms isolated were E. coli (38.2%), staph aureus (32.3%), strept pneumoniae (11.7%), hemophilus infleunza (5.8%), pseudomonas (8.8%), and N. meningitidis (2.9%). This contrasts with an earlier Egyptian study[21] in which the most commonly isolated microorganism was Klebsiella species. In a Japanese study, gram negative pathogens were also the most frequent (44% of positive cultures) followed by gram positive ones (31%)**.**[22]

The present study demonstrated a highly significant increase in CRP and TLC values in patients with culture proven sepsis group compared to the clinically diagnosed sepsis group, whereas there was no statistical difference in CD64 values between the two groups. This is in agreement with a previous study on neonates with sepsis[20]. This might be due to the earlier expression of CD64 in response to infection compared to some delay in CRP elevation, and the fact that the studied patients were at different stages of the infectious process.

CONCLUSION

Neutrophil CD64 is a highly sensitive and specific marker for the diagnosis of pediatric sepsis. Further studies are needed to highlight its role as an early predictor of pediatric sepsis.

REFERENCES

1. **Farrell D, Nadel S.** What's New in Paediatric Sepsis. Curr Pediatr Rep 2016; 4: 1–5.
2. **Randolph AG, McCulloh RJ.** Pediatric sepsis: important considerations for diagnosing and managing severe infections in infants, children, and adolescents; Virulence 2014;5(1):179-89.
3. **Davis BH.** Improved diagnostic approaches to infection/sepsis detection. Expert Rev MolDiagn2005; 5:193–207.
4. **Qureshi SS, Lewis SM, Gant VA, Treacher D, Davis BH, Brown KA.** Increased distribution and expression of CD64 on blood polymorphonuclear cells from patients with the systemic inflammatory response syndrome (SIRS) Clinical & Experimental Immunology 2001;125(2):258–265.
5. **Ng PC, Li G, Chui KM, Li K, Wong RP, Fok TF.** Quantitative measurement of monocyte HLA- DR expression in the identification of early-onset neonatal infection. Biology of the Neonate 2006; 89(2):75–81.
6. **Fjaertoft G, Håkansson LD, Pauksens K, Sisask G, Venge P.** Neutrophil CD64 (FcγRI) expression is a specific marker of bacterial infection: a study on the kinetics and the impact of major surgery. Scand J Infect Dis2007; 39(6-7):525-35.
7. **Goldstein B, Giroir B, Randolph A.** International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005 ;6(1) :2–8.
8. **Layseca-Espinosa E, Pérez-González LF, Torres-Montes A, Baranda L, de la Fuente H, Rosenstein Y, González-Amaro R.** Expression of CD64 as a potential marker of neonatal sepsis. Pediatr Allergy Immunol 2010; 13:319-27.
9. **Fjaertoft G, Pauksen K, Hankansson L, Xu S, Venge P.** Cell surface expression of CD64 on neutrophils and monocytes in patients with influenza A with or without complications. Scand J Infect Dis2012;37 (11-12): 882-9.
10. **Van der Meer W, Pickkers P, Scott CS, van der Hoeven JG, Gunnewiek JK.** Hematological indices, inflammatory markers and neutrophil CD64 expression: comparative trends during experimental human endotoxemia. J Endotoxin Res2007; 13: 94–100.
11. **Dilli D, Oğuz ŞS, Dilmen U, Köker MY, Kizilgün M.** Predictive values of neutrophil CD64 expression compared with interleukin-6 and C-reactive protein in early diagnosis of neonatal sepsis. J Clin Lab Anal 2010; 24 (6): 363-70.
12. **Streimish I, Bizzaro M, Northrup V , Wang C, Renna S, Koval N.** Neutrophil CD 64 with Hematologic Criteria for Diagnosis of Neonatal Sepsis. Am J Perinato2014;31(1):21-30.
13. **Cid J, García-Pardo G, Aguinaco R, Sánchez R, Llorente A.** Neutrophil CD64: diagnostic accuracy and prognostic value in patients presenting to the emergency department. Eur J Clin Microbiol Inf Dis 2011; 30(7):845-52.
14. **Faix JD.** Established and novel biomarkers of sepsis. Biomarkers Med 2011; 5:117-30.
15. **Kantar M, Kultursay N, Kutukculer N, Akisu M, Cetingul N, Caglayan S.** Plasma concentrations of granulocyte-macrophage colony stimulating factor and interleukin-6 in septic and healthy preterms. Eur J Pediatr2000;159(3):156-7.
16. **Ng PC, Li G, Chui KM, Chu WC, Li K and Wong RP.** Neutrophil CD64 is a sensitive diagnostic marker for early-onset neonatal infection. Pediatr Res2009; 56 (5): 796-803.
17. **Lino S, Ravetch R, Kint JP, Perussia F and Bazil V.** Cell adhesion molecules in inflammatory diseases. Drugs 2009; 56: 977-88.
18. **Chan T, Gu F.** Early diagnosis of sepsis using serum biomarkers. Expert Rev Mol Diagn 2012; 11:487-96.
19. **Streimish I, Bizzarro M, Northrup V, Wang C, Renna S, Koval N, Bhandari V.** Neutrophil Cd64 as a Diagnostic Marker in Neonatal Sepsis. The Pediatric Infectious Disease Journal2012; 31(7), 777–781.20)Hsu KH, Chan MC, Wang JM, Lin LY, Wu CL. . Comparison of Fc gamma receptor expression on neutrophils with procalcitonin for the diagnosis of sepsis in critically ill patients. Respirol 2011;16(1):152-60.
20. **Hsu KH, Chan MC, Wang JM, Lin LY, Wu CL.** Comparison of Fc gamma receptor expression on neutrophils with procalcitonin for the diagnosis of sepsis in critically ill patients. Respirol 2011;16(1):152-60.
21. **Gadallah MA, AboulFotouh AM, Habil IS, Imam SS, Wassef G.** Surveillance of health care-associated infections in a tertiary hospital neonatal intensive care unit in Egypt: 1-year follow-up. Am J Infect Control 2014 ;42(11):1207-11.
22. **Shime N, Kawasaki T, Saito O, Akamine Y, Toda Y, Takeuchi M, et al.** Incidence and risk factors for mortality in pediatric severe sepsis: results from the national pediatric intensive care registry in Japan. Intensive Care Med 2012;38:1191-1197.