The CD64 Expression on Neutrophils as a Diagnostic Biomarker of Bacterial Infection in Critically Ill Children

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Abstract

Background: Bacterial infections are an important cause of hospital morbidity and mortality worldwide. The quantification of CD64 expression on the surface of neutrophils by flow cytometry has recently been proposed as a diagnostic test for sepsis. The aim of this study to assess the role of CD64 expression on neutrophils as diagnostic marker of bacterial infection. **Patients and Methods:** A cross-section study included 70 cases with evidence of sepsis in age of 2month to 13 years and conducted in pediatric intensive care unit in Zagazig Universty Hospital. All patients in this study had been subjected to complete history taking and clinical examination. Laboratory investigation**. Results:** The present study showed mean temperature of the studied patients was 37.717 °c and mean PsO2 was 97.27 %. Pneumonia occurred in 81.4% of the studied patients and UTI occurred 10% of the studied patients. CNS infection occurred in 5.7%, and gastroenteritis occurred in 2.9% of the studied patients. Mean hemoglobin level among the studied patients was 9.723 g/dL. Mean RBCs and hematocrit were 3.811 (10^6/mm^3) and 28.704 respectively. Mean TLC was 16.657 10^3/mm^3. Platelet count ranged from 21 to 777 10^3/mm^3 with median182.5 10^3/mm^3. Concerning differential leucocytic count, median neutrophil, lymphocyte, monocyte, eosinophil, and basophil were 8, 3.3, 1.2, 0.15 and 0 respectively. Positive CRP occurred in100% and Median CRP among the studied patients was 29 mg/L. Positive PCT occurred in 88.6% and median was 0.6. Neutrophil CD 64 ranged from 69.4 to 97.13 with mean 85.916**.** There is statistically non-significant relation between diagnosis and neutrophil CD64 levels**. Conclusion:** Use of CD64 can be included for early and more accurate diagnosis of pediatric bacterial infection at PICU.

Keywords: CD64 Expression; bacterial infection; PICU ; Children

Introduction

Infectious diseases are a main cause of admission in pediatric intensive care unit (PICU). Bacterial and viral infections are the principal causes in healthy children. Added to the supportive therapies used in PICU, to start a correct and prompt antimicrobial treatment is critical in order to avoid complications and minimize morbidity **(1).** The inexistence of accurate and precocious biomarkers of viral or bacterial infection forces the physician to initiate broad spectrum therapies that maybe are not indicated, in acute or critical context, early Etiological recognition of infection remains a matter of concern **(2).**

There is no doubt about how the etiological diagnosis of infection has been improved in recent years. The introduction of molecular diagnosis tools, such reactive polymerase chain reaction or rapid immunological test, has collaborated in this new status. But, gold standard, as the blood culture or other body fluid cultures, requires at least 24-48 h to offer its results. Also, negative cultures do not completely exclude the presence of suspected bacterial infection **(3)**.

Biomarkers that may facilitate early diagnosis and the assessment of therapeutic responses are in focus and widely explored. These new molecules have high sensibility and specificity and also inform about the inflammatory status, the presence of bacteremia or the respond to the therapy initiated **(4).** The fragment crystallizable (Fc) portion of human immunoglobulin G has three classes of receptors: FcgRI (CD64), FcgRII (CD32), and FcgRIII (CD16), Among them, CD64 is the only high-affinity receptor and is constitutively expressed on macrophages, monocytes, and eosinophil's, in healthy individuals, the expression of CD64on neutrophils (nCD64) is at very low levels, However when microorganisms and some inflammatory cytokines such as interferon-gamma (IFN-γ) and granulocyte colony-stimulating factor (G-CSF) are present, those stimulating factors can induce significant expression of CD64 on neutrophils **(5).**

Expression of the Fc receptor CD64 on their surface has been shown to correlate with complications in sepsis, infectious diseases, and even in solid organ transplanted patients, CD64, also

called FcγRI (Fc γ receptor I), is a class of plasma membrane receptors on human myeloid cells. It contains three extracellular immunoglobulin-like domains that represent binding sites for the Fc portion of IgG **(6).**

The present study aimed to assess the role of CD64 expression on neutrophils as diagnostic marker of bacterial infection.

Patients and Methods:

A cross-section study included 70 cases with evidence of sepsis in age of 2month to 13 years and conducted in pediatric intensive care unit in Zagazig Universty Hospital. Children admitted to pediatric intensive care unit because of infectious disease as the main cause of their critical illness from January 2020 to July 2020.This study was approved by the Zagazig medical institutional review board (IRB).Informed consent will be taken from all parent and confidentiality of information is assured.

Inclusion criteria:

All children with evidence of sepsis as the main cause of PICU admission in age between 2month to 13 years.

Exclusion criteria:

Patients admitted due to non-infectious causes and immunodeficiency patient.

All patients in this study had been subjected to complete history taking and clinical examination. Laboratory investigation were done in the form of: Complete blood count (CBC), CRP, blood culture, PCT sample, Flow cytometric analysis: of neutrophils expressing CD64 in PB sample using (coulter EPICS-XL) flow cytometry &Kit (E-AB-F1082C) FITC Anti-Human CD64 Antibody.

Sample collection:

Samples were collected in sterile EDTA at room temperature or refrigerated at 4 °C, used for CD45+ cells marking, and analyzed by flow cytometry in a time period shorter than 24 h. CD64 surface expression was measured by BD FACS Canto II flow cytometer (Becton Dickinson, New York, USA). It was measured neutrophils (nCD64) staining a blood sample with a CD64 antibody from Biolegend®, San Diego (clone 10.1). 20,000 events were recorded for each sample. The intensity of CD64 surface expression was measured as mean fluorescence intensity (MFI) in arbitrary units. The positive CD64 cells were expressed as percentage. Results were blinded for clinicians.

Statistical Analysis:

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20 (SPSS Inc. Chicago, IL, U.S.A). Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies and werecompared using chi square test. Data were handled using appropriate statistical tests of significance such as: Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests.To compare quantitative data between more than two groups, one way ANOVA test (for normally distributed data) was used. Pearson and Spearman rank correlation coefficient were used to assess strength and direction of a linear relationship between two continuous variables. The level statistical significance was set atP<0.05. Highly significant difference was present if p≤0.001.

Results:

The present study showed about 56% of patients were males. Their age ranged from 2 months to 156 months with median 11 months **(Table 1)**. The mean Temperature of the studied patients was 37.717 °c and mean PsO2 was 97.27 %. All Vital signs and weight of the studied patients was shown in **Table (2)**. Pneumonia occurred in 81.4% of the studied patients and UTI occurred 10% of the studied patients. CNS infection occurred in 5.7%, and gastroenteritis occurred in 2.9% of the studied patients **(Table 3)**.

Regarding laboratory examination, mean hemoglobin level among the studied patients was 9.723 g/dL. Mean RBCs and hematocrit were 3.811 (10^6/mm^3) and 28.704 respectively. Mean TLC was 16.657 10^3/mm^3. Platelet count ranged from 21 to 777 10^3/mm^3 with median182.5 10^3/mm^3. Concerning differential leucocytic count, median neutrophil, lymphocyte, monocyte, eosinophil, and basophil were 8, 3.3, 1.2, 0.15 and 0 respectively **(Table 4)**. Positive CRP occurred in100% and Median CRP among the studied patients was 29 mg/L. Positive PCT occurred in 88.6% and median was 0.6 **(Table 5)**. Neutrophil CD 64 ranged from 69.4 to 97.13 with mean 85.916 **(Table 6)**. About 44% of the studied patients had Klebseilla growth on blood culturing. Acinobacter, E.coli,

Pseudomonas and Staph aureus prevailed in 4.3%, 11.4%, 28.6% and 11.4% of patients respectively **(Table 7)**. There is statistically non-significant relation between diagnosis and neutrophil CD64 levels **(Figure 1)**.

Concerning outcome of the studied patients, twenty patients died by the end of study, five still in ICU and forty five transferred to ward **(Table 8)**.

Table (1): Demographic data of the studied patients:

	N=70	**%**
Gender:		
Female	31	44.3
Male	39	55.7
Age (year):		
Median	0.92 (11 months)	
IQR	2months – 13 year	

Table (2):Vital signs and weight of the studied patients:

	N=70
Temperature(°c):	
Mean ± SD	37.717 ± 0.372
Heart rate (b/m):	
Mean ± SD	115.54 ± 19.796
Systolic blood pressure (mmHg):	
Mean ± SD	98.63 ± 20.185
Diastolic blood pressure (mmHg):	
Mean ± SD	55.33 ± 15.725
Respiratory rate (/minute):	
Mean ± SD	43.951 ± 8.703
PsO2 (%):	
Mean ± SD	97.27 ± 1.541
Weight (kg):	
Median	8.3
IQR	2.6 – 32

Table (3): Diagnosis of the studied patients:

	N=70	**%**
Diagnosis:		
Pneumonia	57	81.4
CNS infection	4	5.7
Gastroenteritis	2	2.9
UTI	7	10

Table (4): CBC findings of the studied patients

| Mean ± SD/ Median (range)	N=70
Hemoglobin(g/dL):	9.723 ± 1.716
RBCs ($10^6/mm^3$):	3.811 ± 0.8
Hematocrit:	28.704 ± 5.055
TLC ($10^3/mm^3$):	16.657 ± 3.1
Neutrophils count ($10^3/mm^3$):	8 (4.1 – 24)
Monocytic count ($10^3/mm^3$):	1.2 (0.2 – 7)
Basophils count ($10^3/mm^3$):	0 (0 – 0.1)
Eosinophilic count ($10^3/mm^3$):	0.15 (0 – 1.4)
Lymphocytic count ($10^3/mm^3$):	3.3 (0.8 – 9.6)
Platelet count ($10^3/mm^3$):	182.5 (21 – 777)

Table (5): Acute phase reactants of the studied patients:

	N=70 (%)
C reactive protein (mg/L):	
Positive	70 (100)
Median (range)	29 (4.23 – 338.4)
Procalcitonin (ng\mL):	
Negative	8 (11.4)
Positive	62 (88.6)
Median (range)	0.6 (0.01 – 86)

Table (6):Neutrophil CD64(%) of the studied patients:

Neutrophil CD 64%:	
Mean ± SD	85.916 ± 8.121
Range	69.4 – 97.13

Table (7): Result of culture and sensitivity of the studied patients:

	N=70
Culture:	
Acinobacter	3 (4.3)
E. coli	8 (11.4)
Klebseilla	31 (44.3)
Pseudomonas	20 (28.6)
Staph aureus	8 (11.4)
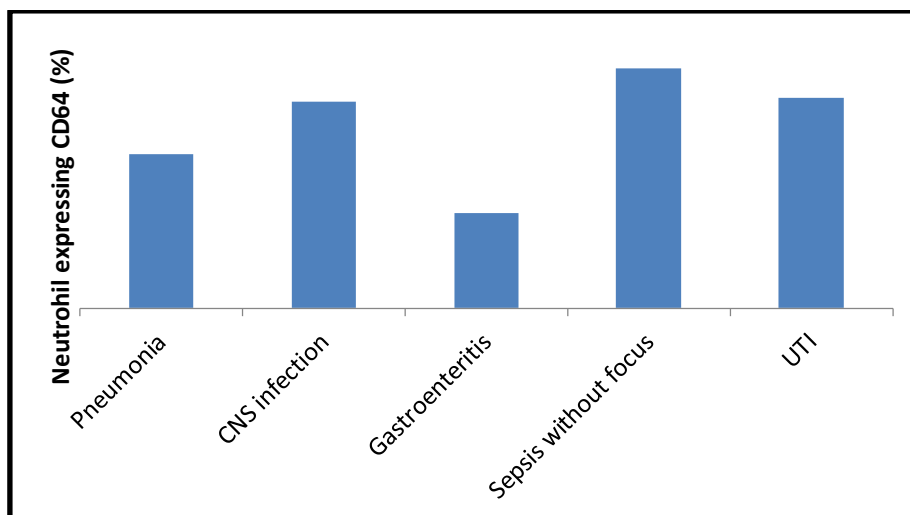
Figure (1): Simple bar chart showing neutrophil CD64(%) level among patients with different diagnoses

Table (8): Outcome of the studied patients:

	N=70	%
Outcome :		
Die	20	28.6
Still in ICU	5	7.1
Transferred to ward	45	64.3

Discussion:

As it is known, several methods are used and studied to identify or anticipate bacterial infections. Improve the rational use of antibiotics is a worldwide priority and the interest of new methods of precocious bacterial diagnosis is rising. At PICU admission, the initiation of antibiotic is mainly influenced by clinical sings and analytical biomarkers (**4**).

About the flow cytometry, it is a laboratory technique based on marking leukocyte populations with monoclonal antibodies. This technique allows to evaluate in a dynamic way immune status and response of a patient both under basal and disease conditions. Its use results in a novel approach with great interest for these patients (**7**).

Mainly in the context of an infection, its use and interpretation may provide unique information letting know immunological changes in real time. These properties would help to substantiate diagnostic suspicion, anticipate the evolution, and modify therapeutic attitudes dynamically (**7**).

In the present study, we describe the CD64 expression on neutrophils in previously healthy children admitted to PICU because of an infectious disease.

In the current study, about 56% of patients were males. This came in agreement with **Stoll (8) and Leal with his colleagues (9)** who supposed that the gender could influence the sepsis process, they mentioned that the male are at a higher risk for sepsis rather than their age-matched female, which could be explained by theprobability of sex linked factor affecting in the host susceptibility.

In the present study, concerning the need for the respiratory support about 41% were on MV. This came in agreement with **Qazi and Stoll (10), &Hadzimuratovic with his colleagues (11)** who found that MV was much higher among the sepsis patients.

In the present study, median CRP among the studied patients was higher (29 mg/L) with 100% of patients had positive CRP. This came in agreement with **Jan with his colleagues (12)** who founded that CRP values were much higher among sepsis patients. While, **Choo and his colleagues (13)** results didn`t agree with our current results as they reported that CRP values were less than 1.0 mg/dL. These differences could be explained by the difference of the methodology used between both

the studies, much more sensitive results could be achieved in our current study by using the immuneturbidimetric assay of CRP (hs-CRP) compared to the conventional latex agglutination methods.

In the current study, 28.6% of patients died by the end of study, 7.1% still in ICU and 64.3% transferred to ward. In fact, we can`t attribute this high mortality to sepsis alone because most of such patients had other major clinical conditions, however sepsis still on the top of the list of the morality causes and in reality, septicemia was usually the final step that brought these patients to the end.

Our current results were concordant with **Shehab and his colleagues (14)** who conducted their prospective analytical study in three Egyptian ICUs in Mansoura Hospitals, they reported that the mortality rate from septicemia was very high reaches up to 50%.

The WHO and UNICEF conducted an updated systematic analysis for the child mortality causes with time trends since 2000 till 2010, they reported that sepsis accounts for 33% of the total childhood deaths **(15)**. This came in agreement with **Hashem et al. (16)** who found that one third of sepsis patients died from sever sepsis and its complications.

Conclusion:

Use of CD64 can be included for early and more accurate diagnosis of pediatric bacterial infection at PICU.

No conflict of interest.

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