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ORIGINAL ARTICLE

The Diagnostic Value of Neutrophil CD64 in Detection of Sepsis in Children

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ABSTRACT

Background: *Pediatric sepsis is a life-threating condition that requires instant management. The choice of the best biomarker and its best cut-off value is critical for diagnosis of infection in children especially with the delay in obtaining the culture results.* ***Objective:*** *The main objective of this study was to evaluate the diagnostic value of neutrophil CD64 in early diagnosis of sepsis among children.* ***Methodology:*** *This prospective study was conducted on 50 children with suspected sepsis and 50 control children without infection. Blood samples were taken on admission for blood culture. Detection of neutrophil CD64 (nCD64) was carried out by flow cytometry and CRP was measured by ELISA.* ***Results:*** *CD64 was elevated significantly in sepsis compared to the control group. There was a significant difference between the levels of nCD64 in culture positive cases (80%) compared to culture negative ones (20%). Neutrophil CD64% was highly predictive of childhood sepsis with are under the curve (AUC) =1.* ***Conclusion:*** *Neutrophil CD64% is a very good diagnostic marker for early diagnosis of sepsis with a higher sensitivity than CRP.*

INTRODUCTION

Sepsis is a serious problem and time-critical emergency causing worldwide morbidity and mortality in infants and children that needs to be diagnosed as early as possible[1]. The symptoms of sepsis which is defined as systemic inflammatory response syndrome (SIRS) due to an infection are similar to SIRS without infection[2]. Rapid diagnosis and empirical introduction of antibiotics are crucial [3].

Diagnosis is often based on clinical examination, measurement of white blood cell count, blood cultures and measurement of an acute-phase biomarker C-reactive protein (CRP). Early clinical diagnosis is problematic in either adults or children due to minimal signs of infection[4]. Even more, despite blood cultures and other cultures are gold standard for discrimination of infectious causes, they are time-consuming and their results are affected by empirical antibiotic therapy [5]. In addition, culture-negative patients were found to represent 25% to 48% of all septic cases in different clinical studies performed in North America, Europe, and Asia [6, 7].

Several biomarkers have been designated for the diagnosis of sepsis, however; the most reliable biomarkers for accurate diagnosis of patients suffering from severe sepsis or septic shock are still controversial[8] Despite CRP can be used to diagnose sepsis early in the course of infection[9,10], they may be elevated in other unrelated conditions such as autoimmune disease, cancer, tissue necrosis and viral and parasitic infection[11].

CD64 (Fc-gamma receptor 1 (FcγR1) is a membrane glycoprotein, which is expressed mainly on monocytes and macrophages with low concentration on the surface of non-activated neutrophils. Neutrophil CD64 (nCD64) expression can be distinctly elevated at the onset of sepsis[12] and was found to be a better diagnostic marker for sepsis than PCT[13] and CRP [14] in adults as well as children [15].

Due to the fact that most of the studies in animal models were not reproducible in humans, several studies have identified that the best policy is to consider a combination of markers [16, 17]. Markers for detection of sepsis in children are not investigated as much as in the case of neonatal sepsis. In this study we aimed to investigate the value of nCD64 as an early marker of pediatric sepsis. In addition, this study aimed at determining the most appropriate cut-off value of nCD64 in early detection of sepsis among children.

METHODOLOGY

This prospective observational study was carried out at the Microbiology Department, Faculty of Medicine, Minia University and at the Clinical Pathology Department, Faculty of Medicine, Assiut University in the period between June 2013 and May 2015. The study was ethically approved and was

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conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from parents before enrollment in the study. The included children were divided according to the International Pediatric Sepsis Consensus Conference definitions [18] into the following groups:

- Group (1): 50 healthy children.
- Group (2): 50 children diagnosed as having SIRS with sepsis.

The attending physician carried out the classification, unaware of the results of the laboratory data. Infection suspected clinically was combined with the initiation of a diagnostic workup (leukocyte and differential counts, CRP, nCD64%, microbiological cultures and chest radiograph if needed) to rule out infection and empirical antibiotic therapy was prescribed till culture results appeared. The diagnosis of bacterial sepsis was confirmed by positive blood cultures. Bacterial sepsis was also established in patients with negative cultures but with a strong clinical suspicion of bacterial sepsis who received a full course of antibiotic therapy and improved after antibiotic therapy.

1. Blood samples:

Blood was drawn and collected in EDTA-containing tubes for a complete blood count using Cell Dyne 3500 automated cell counter. Serum was separated for detection of C-reactive protein (CRP) by High Sensitivity C-Reactive Protein enzyme immunoassay test kit (Immunospec, USA). Renal and liver functions; including levels of creatinin, total and direct bilirubin, alanine aminotransferase (ALT) were assessed in all patients using BM Hitachi 911 Chemistry Analyzer.

2. Processing of Blood Cultures:

Blood cultures were taken by sterile venipuncture and processed using the Bact/Alert FA (bio- Mérieux, Marcy l'Etoile, France). Bacteremia was identified by the development of microbial growth in one blood culture bottle. Sub-sequent sub-cultures on blood agar, chocolate agar, and MacConkey agar media were carried out. Identification of isolated organisms was achieved by colony morphology and microscopic examination of a Gram-stained smear. Gram-positive bacteria were further subjected to conventional biochemical reaction including: catalase test, culture on mannitol salt agar, slide coagulase test, Novobiocin susceptibility test, and DNase test, while Gram-negative bacteria were identified by triple sugar iron, citrate test, urease test, indole test, and oxidase test and API 20E identification system.

3. Neutrophil CD64 %:

Flow cytometry was performed on a FACSCalibur flow cytometer (Becton Dickinson, NY, USA) with 50µl EDTA-anticoagulated whole blood. Whole blood was mixed with FITC-CD64 monoclonal antibody (AbD Serotec, Bio-Rad, UK) and incubated for 20 minutes at room temperature in the dark. FITC mouse IgG1 was used as an isotype control (BD, USA)**.** Red cell lysis was performed afterwards using the ammonium-chloride-based lysis solution. Flow cytometry was performed on at least 50,000 leukocytes. Differentiation of cell type was carried out using the right-angle side scatter and forward scatter method. A gate was set around various cell populations and the percentage of CD64 positive population (nCD64) within the gate was analyzed using the CellQuest Software (Becton Dickinson, NY, USA). MFI is defined as the geometric mean of the logarithmic fluorescence intensity emitted by the cell population.

4. Statistical Analysis:

Data management and analysis were performed using Statistical Package for Social Sciences version 19 (SPSS Inc., Chicago, IL). Comparisons between the 2 groups were done using the Kruskal-Wallis and Fisher exact test. Comparison between positive and negative blood culture was done using Mann-Whitney test. The Rceiver Operating Characteristic (ROC) curve was used to calculate the area under the curve (AUC) as well as the sensitivity, specificity, PPV, and NPV of each marker. P value less than 0.05 was considered significant.

RESULTS

Children enrolled in the study were classified into 2 groups, for whom clinical and laboratory data are presented in table 1.

Table 1: Comparison of the Demographic, Clinical, and Laboratory Data among the Studied Groups:

Variables	*Control N=50*	*Sepsis N=50*	*P value*
Blood pressure			
SBP	85±19.5	99.4±4.5	0.001*
DBP	50±15.4	60±4.8	0.001*
Respiratory rate	35.6±2.7	37±8.6	0.06
Temperature	25.3±19.5	39±0.6	0.02*
Serum creatinine (mg/dl)	0.6±0.1	1.02±0.4	0.001*
ALT (IU/L)	20±6.5	23.3±6.4	0.001*
Serum Bilirubin (mg/dl)	0.6±0.1	0.8±0.1	0.001*
WBCs x109/L	6.4±1.7	20.2±11.1	0.001*
NE x109/L	3.9±1.1	13.01±1.5	0.001*
LY x109/L	2.2±0.8	4.5±2.1	0.001*
MO x109/L	0.2±0.1	1.4±1.02	0.001*
Platelets x109/L	300.8±73.8	207.2±79.8	0.001*
Neutrophil CD64%	5.1±3.4	64.2±34.6	0.001*
CRP (mg/L)	1.3±0.7	22.2±13.9	0.001*

Quantitative data presented by mean ±SD and qualitative data are presented by frequency distribution.

Blood cultures were positive in 80% of the sepsis cases. The following micro-organisms were isolated from their blood cultures: *Staphylococcus aureus* in 14 cases (35%); *Klebsiella pneumoniae* in 12cases (30%); *Escherichia coli* in 8 cases (20%); each of *Candida albicans*, *Enterococcus faecalis* and *Streptococcus pneumoniae* were isolated in only 2 case (5%).

Neutrophil CD64 percentage increased significantly in sepsis compared to the control group ($p \leq 0.001$*). In addition, CRP increased significantly in comparison to the control group (P = 0.001*). Moreover, nCD64% was significantly higher ($p \leq 0.05$) in patients with positive blood cultures (75.6±29.3) than those with negative blood cultures (39.1±37.7).

ROC curves were created for both nCD64% and CRP (Figure 1). Neutrophil CD64% showed a high AUC (1±0.00) (p=0.001), specificity (100%), PPV (100%), NPV (100%) and sensitivity (100%). On the other hand, CRP showed an AUC of 0.85±0.09 (p= 0.002), 100% specificity, 100% PPV, while it showed a lower sensitivity (83.3%) and NPV (94.3%) than nCD64%. The best cutoff value for nCD64% was ≥14.1, while that of CRP was ≥ 2.9mg/L.

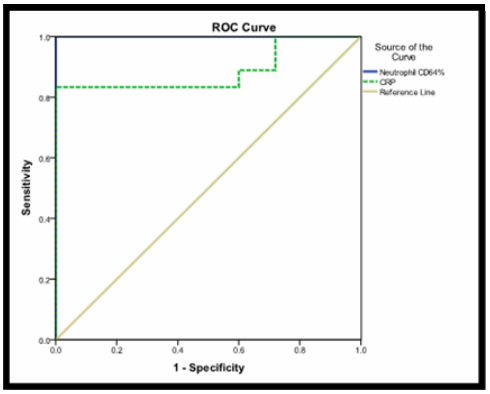

Fig. 1: ROC Curves of nCD64% and CRP between sepsis versus Control Groups

DISCUSSION

The definition of sepsis differs between children and adults especially in regards to the age-specific cut-offs or physiologic and organ system-related laboratory parameters as the child's immune system is remarkably different from adults in terms of innate and adaptive immune function[19]. Early diagnosis of sepsis is mandatory for tailoring the treatment and improving the diagnosis, given the necessity for timely initiation of appropriate antibiotics[20]. A pivotal role in rapid diagnosis is attributed to biomarkers, which are necessary for early diagnosis, monitoring of treatment and evaluation of prognosis of sepsis.

The purpose of the current study was to evaluate the diagnostic value and determine the best cut-off levels for nCD64 in early diagnosis in childhood sepsis.

Both nCD64% and CRP increased significantly in sepsis compared to the control group. nCD64% showed a higher AUC than CRP with a better predictive value. Owing to the fact that standard microbiologic cultures can lack sensitivity with an inherent delay in obtaining microbiologic culture results, biomarkers can serve a critical role in providing the necessary information to help clinicians in rapid diagnosis in the absence of culture results or even in cases with culture negative sepsis. nCD64% was markedly increased in cases of sepsis with positive cultures compared to those with negative bacterial cultures.

Several characteristics in neutrophil CD64 make it a suitable biomarker for clinical use: its expression is low in resting neutrophils, which increases significantly after activation and returns to baseline level within a few days after removal of the cause [21]. In this study, the expression of CD64 increased significantly in the presence of sepsis. This is similar to several studies done on neonates and children[22 15, 23]. The cut-off value of nCD64 was 14.1 in the case of sepsis. Relatively different cut-off values were reported in a different

study [24], probably due to different flow cytometry measurement methods. However, similar sensitivity and specificity was observed in a study carried by Groselj-Grenc and colleagues [15]. CRP has been used as a biomarker for sepsis for more than 30 years now. Several studies have demonstrated that with CRP cut-off values ≥1 mg/L, statistical outcomes ranged between 70-90% sensitivity; 97%-99% NPV; 41-98% specificity and 6-83% PPV[25, 26]. In the current study, the best cut-off value for CRP was 2.9 mg/L for sepsis. In the case of sepsis, the sensitivity was 83.3%; NPV 92.6%; specificity 100%; and PPV 100%. The lower sensitivity of CRP could be attributed to the gradual increase of CRP during the first 24 hours of infection. The specificity of CRP is known to be less than other markers, due to its elevation in non-infectious inflammatory conditions; however, in this study the high specificity could be due to the absence of such cases in the study as all the cases are sepsis cases.

It is most favorable for the diagnostic biomarker to have 100% sensitivity (infected children being positive for the test) and NPV, taking into consideration the high mortality and serious morbidity of sepsis, and severe sepsis. Moreover, a100% specificity and a similar PPV are recommended to minimize the use of antibiotics in false positive cases [27]. Based on the results, it can be concluded that nCD64% can be used for prediction of sepsis in children as it is well used in neonatal sepsis.

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