Evaluation of neutrophilic CD64 in adult sepsis as a novel diagnostic biomarker

Running title: Neutrophilic CD64 in Adult Sepsis as a Novel Diagnostic Marker

Abstract

Background: Sepsis is one of the most common causes of mortality among patients who are critically diseased and in Intensive Care Units (ICU). Bacterial infection or sepsis leads to an increase in Neutrophilic CD64(nCD64) expression on activated polymorphonuclear leukocytes (PMNs). Early diagnosing of sepsis is very important in order to start timely and specific treatment. The availability of a rapid laboratory test with high specificity for sepsis in adult patients could support in therapeutic decision making and reduce unnecessary antibiotic use.

Methods: Total 40 patients of sepsis diagnosed as per sepsis-3 definition were included in this study. 2 mL blood sample was collected in EDTA and plain vial each for evaluation of nCD64, Procalcitonin (PCT) and high sensitivity C-reactive protein(hS-CRP). The samples were run on Flow cytometer, Nephelometer and Chemiluminescence for nCD64, hS-CRP and PCT respectively.

Result: The Positive Predictive Value (PPV) of nCD64 for prediction of sepsis was 92.68% and the Negative Predictive value (NPV) was 94.87%. Receiver operating curve (ROC) was plotted for indicating the diagnostic accuracy of nCD64(≥1.8), hS-CRP (≥3 mg/L) and PCT (≥0.4 ng/mL). Area under the curve (AUC) for nCD64 was highest [0.938(95%CI=0.876-0.999)] followed by hS-CRP [0.888(95%CI=0.807-0.968)] and PCT [0.850(95%CI =0.759-0.941)].

Conclusion: These findings are suggestive of the possibility that nCD64 determination was a useful tool for diagnosing infection in patients with septic syndrome, with a performance higher to that of hS-CRP and PCT.

Keywords: nCD64, PCT, hS-CRP, Adult sepsis, Novel diagnostic marker

Introduction

Sepsis is one of the most common causes of mortality among the patients who are critically diseased and in Intensive Care Units (ICU). Sepsis is a medical emergency in which the body's systemic immune response to an infectious process may lead to end-stage organ dysfunction and death (1).

The septic response is a complex chain of events that involves inflammatory and anti-inflammatory processes, humoral and cellular reactions and circulatory abnormalities. In order for the host to respond to pathogens, innate immune cells such as neutrophils, macrophages, monocytes, and natural killer cells must first be activated. There is even release of proinflammatory cytokines like tumor necrosis factor-α, Interleukin-1 and Interleukin-6. These cytokines cause up-regulation of endothelial adhesion molecules, activation and proliferation of leucocytes, activation of complement system, tissue factor production and induction of hepatic acute phase reactants. Thus, exaggeration of these immune responses leads to collateral damage of the host tissue and organs (1). There is simultaneous activation of inflammatory and coagulative cascades and this interaction may lead to mild thrombocytopenia or even disseminated intravascular coagulation (DIC) (2). Hypoperfusion occurs as a result of decreased delivery and utilization of oxygen by cells and is the major reason for tissue damage and organ dysfunction. Hypoperfusion also occurs because of cardiovascular dysfunction which is commonly observed in sepsis (3). Thus, sepsis leads to systemic inflammation and organ dysfunction.

The early diagnosis and stratification of the severity of sepsis is very important as it increases the possibility of starting timely and specific treatment (4). Blood culture is the gold standard for diagnosing sepsis; however, they require 24 to 48 hours to complete (5). Biomarkers play an important role in identification of severity of sepsis as well as to differentiate bacterial from viral and fungal infection. Various other potential uses of biomarkers include roles in prognostication, guiding antibiotic therapy, evaluating the response to therapy, differentiating Gram-positive from Gram-negative microorganisms as the cause of sepsis and predicting complications of sepsis. It is stated that high sensitivity C-reactive protein(hS-CRP) and Procalcitonin (PCT) have been extremely useful in diagnosing infection. However, they are unable to differentiate between infection and inflammatory conditions (6).

NeutrophilicCD64(nCD64) is an Fcγ receptor which is expressed principally on monocytes and on resting polymorphonuclear leukocytes (PMNs). Bacterial infection or sepsis leads to an increase in nCD64 expression on activated PMNs. It has been proposed as a potential diagnostic and prognostic biomarker for sepsis in hospitalized adults, neonates and children and can be used as a novel diagnostic marker in adult sepsis (7). This study is based on the hypothesis that the expression of nCD64, serum- PCT and hS-CRP can further prove to be useful indicators of sepsis. This study was carried out with the aim to evaluate nCD64 as a diagnostic marker in adult sepsis. The primary aim was to measure the percentage expression of nCD64, serum PCT & hS-CRP levels in newly clinically diagnosed cases of adult sepsis. Moreover, diagnostic accuracy of percentage expression of nCD64 with serum PCT and hS-CRP were compared for predicting adult sepsis.

Methods

This study was conducted in the Department of Pathology and General Medicine at Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi after obtaining Institutional ethical committee approval. This cross-sectional study was carried out for a period of 18 months from July 2021 to December 2022. This study included clinically diagnosed cases of sepsis as per sepsis-

3 criteria defined by European Society of Intensive Care Medicine – Society of Critical Care Medicine (8). All these adult patients with age 27-70 years were new patients admitted in ICU with no initiation of antibiotic therapy.

Cases with known history of malignancy, hematological disease, after commencement of antibiotic therapy and severe liver/kidney disease were excluded from this study. According to operational feasibility and after applying the inclusion and exclusion criteria, 40 cases and 40 age and gender matched normal controls were included in this study. Organ Dysfunction or Failure Severity of organ dysfunction has been evaluated by various scoring systems that measure abnormalities according to clinical findings, laboratory data, or therapeutic interventions. The main score in current use is the Sequential Organ Failure Assessment (SOFA). A higher SOFA score is associated with an increased probability of mortality (8). All 6 systemic variables namely Glasgow coma scale (GCS), Mean arterial pressure (MAP), PaO2/FiO2, Platelets count, serum Bilirubin values and serum Creatinine included in SOFA score were recorded from the medical records.

2 mL blood sample was collected in EDTA vial for performing Flow cytometry for nCD64. Flow cytometry was performed on Beckman Coulter, Model Navios. nCD64 was obtained by evaluating nCD64 expression on neutrophils which were gated by CD45 versus side scatter graph as shown in Figure 1.

2 mL of blood was collected in plain vial for quantitative evaluation of serum PCT & hS-CRP levels respectively. hS-CRP was evaluated by using Nephelometer (Biocell Medicare BNII) and serum PCT by Chemiluminescence (ADVIA Centaur CP Immunoassay).

Primary data was collected using paper based CRF (Case Report Form) and the data was then entered in Microsoft Excel spreadsheets 2016. Statistical analysis was done on IBM SPSS Statistics version 2020. The Continuous variables were described as Means± standard deviation. Mean comparison was done using Kruskal Wallis test. The non-parametric tests were used as data was not distributed normally. The median values were represented on column or bar graphs.

The categorical variables are taken in the form of frequencies and proportions and cross tabulations are done for the chosen parameters. P-Value < 0.05 was considered significant and P-Value < 0.01 was considered highly significant. Based upon the cut off values identified for true and false positive and negative cases, sensitivity, specificity, positive likelihood ratio, negative likelihood ratio was obtained. By using sensitivity and specificity, ROC (Receiver operator curve) was plotted and AUC (Area under curve) were compared among nCD64, PCT and hS-CRP respectively.

Ethics code number- IEC/VMMC/SJH/Thesis/2020-11/CC-240

Results

New clinically diagnosed 40 cases of Sepsis as per Sepsis-3 criteria (8) and 40 age and gender matched controls were studied.

There was male preponderance with total 68.75% males and 31.25% females. The gender ratio male and female (M:F) was found to be 2.2:1. The average age of the patients was 50 years (27-70 years) and median age was 51 years. The eldest patient of sepsis was 70 years old while the youngest was 27 years old.

The minimum SOFA score in sepsis was 2 and maximum was 5. The median and the average score in patients was 3 and 2.72±0.7 respectively. The controls had median score of 1. The difference in mean rank between cases and controls was highly significant(p-value<0.001).

Descriptive Statistics and Mean Rank Comparison of different Sepsis parameters like PAO2/FIO2, platelet count, MAP, GCS Score, Serum Bilirubin and Serum Creatinine are shown in Table 1.

Descriptive Statistics and Mean Rank Comparison of different sepsis biomarker like nCD64, PCT & hS-CRP are shown in Table 2.
The sensitivity, specificity, PPV, NPV and diagnostic accuracy for nCD64, PCT & hS-CRP were, respectively as shown in Table 3. ROC was plotted for indicating the diagnostic accuracy of nCD64(≥1.8), hS-CRP(≥3mg/L) and PCT(≥0.4ng/mL), AUCs was 0.938(95%CI=0.876-0.999), 0.888(95%CI=0.807-0.968) and 0.850(95%CI=0.759-0.941) respectively as shown in Figure 2.

Discussion

The diagnosis of sepsis remains one of the most difficult tasks for clinicians. The availability of a rapid laboratory test with high specificity for adult sepsis could aid in therapeutic decision making and reduce unnecessary antibiotic use.
The common finding of preponderance of sepsis in males, as seen in our study also, may be due to male sex hormones, i.e. androgens, which are supposed to have a suppressive effect on cell-mediated immune responses (9,10).
On evaluating different systemic variables of SOFA score in our study the minimum and maximum SOFA score in sepsis was 2 & 5 respectively with AUC of 0.96, which was statistically significant (P-value<0.001). Kilinc Toker et al and Liu C et al also found SOFA score highly significant for sepsis prediction with an AUC of 0.89 & 0.80 respectively (11,12). On analyzing systemic variables of SOFA score, GCS, serum creatinine and platelet were not found to be significantly different in control and patient group (p-value >0.05). However, serum bilirubin, PAO2/FIO2 and MAP were significantly lower among cases as compared to controls (p-value<0.001). Our findings were different from the study done by Liu et al. who found negative correlation with total bilirubin and serum creatinine and significantly positive correlation with MAP, PAO2/FIO2, PLT, and GCS (12). This contradiction could be attributed due to assessment of patients in different stages of sepsis. All newly diagnosed cases of sepsis were enrolled in our study irrespective of the stage of initial presentation.
In this study, the effectiveness of biomarkers hS-CRP, PCT and nCD64 in the diagnosis of early detection of sepsis in adult patients were analyzed, which showed variable results.
In the present study hS-CRP was 87.5% sensitive and 90% specific for prediction of sepsis, keeping the cut off value of 3mg/L with AUC of 0.88. Our results were similar to the study by Wang et al who reported that elevated baseline hS-CRP was associated with increased risk of future sepsis events (13). Lin CT et al. also found hS-CRP as predictor of sepsis however AUC was low which could be due to higher cut off of hS-CRP which they considered as 8mg/L (14).
In this study PCT has 82.50% sensitivity and 87.50% specificity for the prediction of sepsis with AUC of 0.85. Zhang et al who studied diagnostic value and prognostic significance of PCT combined with hS-CRP in patients with bacterial bloodstream infection found PCT highly accurate for the diagnosis of sepsis (15). L Simon et al and Hiromi Toh et al found PCT as a useful biomarker for diagnosis of sepsis with high sensitivity and specificity (16,17). Contrastingly, Benjamin MP Tang et al observed that PCT cannot reliably differentiate sepsis from other non-infectious causes of systemic inflammatory response syndrome (18). They found low sensitivity and specificity (approximately 70%) of PCT which could be due to study population comprising only of critically ill patients.
Sensitivity and specificity of nCD64 in this study was 95% and 92.50% which was similar to study by Cardelli P et al who found sensitivity and specificity 96% and 95% (19). Li et al & Cid et al found low sensitivity 79% & 76% respectively. They suggested that the reason for low sensitivity could be due to the use of a low methodological quality, however specificity in their studies were

comparable to our study (20,21). Use of flow cytometry and inclusion of adult sepsis in our study might have resulted in higher sensitivity & specificity. Study by A Gros et al. and O Livaditi et al in patients admitted to medical ICU found low sensitivity of nCD64 for gram positive infection and high for gram negative infection (22,23). In our study, we have not categorized the patients based on different types of pathogens.

ROC was plotted for indicating the diagnostic accuracy of nCD64 (≥1.8), which has AUC of 0.938. Similar findings were observed by Cid et al, Li et al & Cardelli P et al who reported similar area under the ROC curve 0.94, 0.92 and 0.97 respectively (21,20,19). Study by Patnaik et al demonstrated nCD64 as not only a useful diagnostic marker but also has prognostic significance in the critically ill patients of sepsis (24)

When comparing between two biomarkers i.e. hS-CRP with PCT, most of the studies found PCT as a better predictor of sepsis than hS-CRP, however H Zhang et al found hs-CRP similar to PCT in the diagnosis of sepsis and septic shock, which could be due to study population comprising exclusively of older age group (25,26). In our study too, we also found hS-CRP as a better biomarker predictive of adult sepsis. In our study, we have not categorized patient based on different age groups and stages of sepsis.

We have found very few studies comparing these three parameters together to predict sepsis; however, no such study found in adults. In a study by Yin et al nCD64 was found to be better than PCT and CRP for diagnosing infection, though we included hS-CRP in place of crp (27). KH Hsu et al published a prospective study in 2011 found that nCD64 was better than PCT for differentiating systemic inflammatory response syndrome (SIRS) from severe sepsis and septic shock and correlated with severity of SIRS, sepsis (28). CF Yeh et al also found nCD64 as a better biomarker than PCT for diagnosis of sepsis (29). Likewise, in this study too it was found that nCD64 as a better marker than hS-CRP & PCT.

In developing countries like India, cost-effectiveness of a diagnostic test is a major concern which may lead to an additional financial burden on the morbid patients and their attendants. In Indian settings, in a routine laboratory, approximate cost of hS-CRP is 800 rupees, PCT is 3100 rupees and nCD64 is 2000 rupees per test. Thus, nCD64 can emerge out as an independent and cost-effective diagnostic test for the prediction of sepsis in adults.

There are few limitations to this study. There are no established standards for determining nCD64, and many techniques have been employed to express nCD64. The ideal cut-off for nCD64 is still up for debate, and different studies use different cut-offs to distinguish between sepsis and non-sepsis.

Conclusion

nCD64 levels along with clinical parameters like SOFA is useful as an early diagnostic marker of sepsis in adult patients. In this study, nCD64 was found to be a relatively simple, cost effective and better biomarker with high sensitivity and specificity than hS- CRP and PCT for diagnosis of adult sepsis. However, the nCD64 assay should be standardized with appropriate cut-off levels to differentiate sepsis from non-sepsis.

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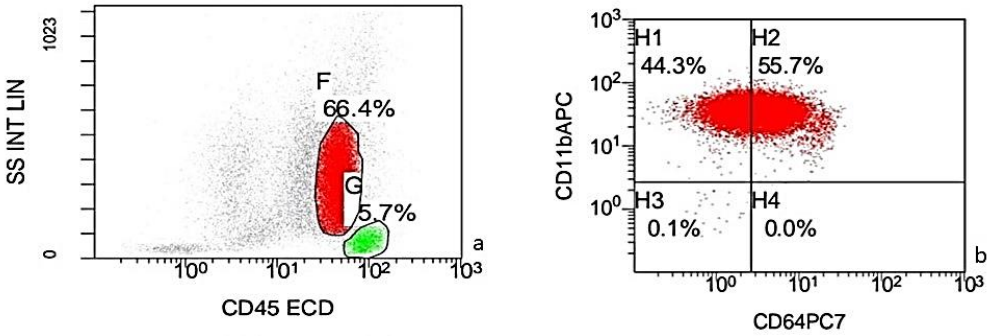
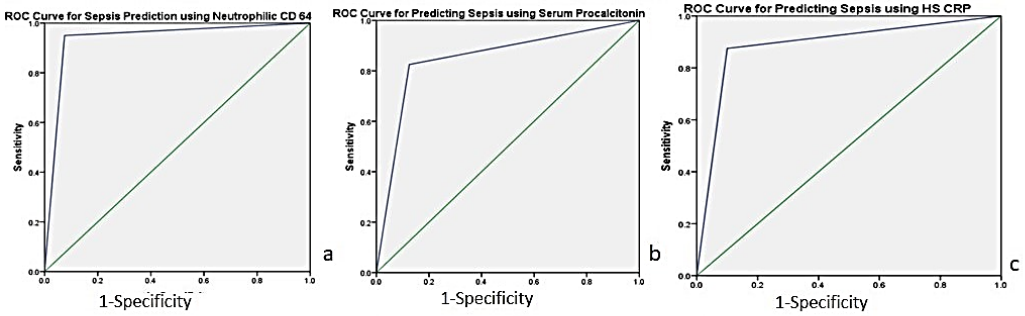


Figure 1. a) The events were gated on CD45 versus Side scattered (SS), Granulocytes (F population) were 66.4% of the total events and lymphocytes (G population) were - 5.7% of the total events. b) Granulocytes (F population) showed 55.7% of CD11b and CD64 showing positively.



Test Result Variable(s)	Area under curve	Std. Error[a]	P value.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
nCD 64 (≥1.8)	.938	.031	.000	.876	.999
HS CRP (≥3 mg/L)	.888	.041	.000	.807	.968
PCT (≥0.4 ng/mL)	.850	.046	.000	.759	.941
The test result variable(s): CD 64 (≥1.8), HS CRP (≥3 mg/l), PROCALCITONIN (≥0.4 ng/ml) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.					
a. Under the nonparametric assumption					
b. Null hypothesis: true area = 0.5					

a) NeutrophilicCD64(Ncd64) b)Proalcitonin(PCT) c) High Sensitivity CRP(hS-CRP)

Figure 2. Receiver operater curve showing area under curve (AUC) for neutrophilic CD64, Procalcitonin and hs-CRP

Table 1. Descriptive statistics and mean rank comparison of different sepsis parameter

	Groups	Mean	Standard deviation	Median	Minimum	Maximum	Range	P-value
Pao2/Fio2	Control	426.28	11.79	425.00	410.00	450.00	40.00	<0.001
	Sepsis Case	377.55	65.46	409.50	200.00	430.00	230.00	
Platelets count (10^3/dL)	Control	277.63	64.99	273.00	165.00	416.00	251.00	0.07
	Sepsis Case	232.43	106.77	221.00	69.00	417.00	348.00	
Mean Arterial Pressure (mm Hg)	Control	80.18	5.43	81.00	70.00	88.00	18.00	<0.001
	Sepsis Case	76.60	3.14	77.00	71.00	81.00	10.00	
GCS Score	Control	15.00	0.00	15.00	15.00	15.00	0.00	1.00
	Sepsis Case	15.00	0.00	15.00	15.00	15.00	0.00	
Serum Bilirubin (mg/dL)	Control	0.84	0.24	0.85	0.40	1.20	0.80	<0.0001
	Sepsis Case	1.63	0.53	1.60	0.90	3.40	2.50	
Serum Creatinine (mg/dL)	Control	1.12	0.22	1.15	0.80	1.40	0.60	0.23
	Sepsis Case	1.42	0.77	1.10	0.40	4.20	3.80	

Abbreviations: GCS- Glasgow Coma Scale, dL- Deciliter, mg/dL-Milligram per Deciliter, mm Hg-Millimeter of Mercury.

Table 2. Descriptive statistics and mean rank comparison of different sepsis biomarkers

	Groups	Mean	Standard deviation	Median	Minimum	Maximum	Range	P-value
nCD64 (molecules/cel)	Control	1.15	0.78	1.20	0.03	4.80	4.77	<0.0001
	Sepsis Case	16.17	15.67	9.45	1.32	55.70	54.38	
Procalcitonin (ng/mL)	Control	0.14	0.13	0.095	0.01	0.53	0.52	<o.ooo1
	Sepsis Case	1.38	1.89	0.790	0.05	9.90	9.85	
hS-CRP (mg/L)	Control	1.21	1.45	0.80	0.40	7.50	7.10	<0.0001
	Sepsis Case	26.99	35.71	15.50	1.20	154.00	152.80	

Abbreviations: nCD64- Neutrophilic CD64, hS-CRP- High Sesitive C-Reactive Protein, ng/mL-Nanogram per Milliliter, mg/L-Milligram per Liter.

Table 3. Descriptive statistics and mean rank comparison of different sepsis biomarkers

	nCD64	PROCALCITONIN	hS-CRP
Sensitivity	95.00%	82.50%	87.50%
Specificity	92.50%	87.50%	90.00%
PPV	92.68%	86.84%	89.74%
NPV	94.87%	83.33%	87.80%
Accuracy	93.75%	85.00%	88.75%

Abbreviations: nCD64-Neutrophilic CD64, hS-CRP-High Sensitivity C-Reactive Protein, PPV-Positive Predictive Value, NPV-Negative Predictive Value.