



ORIGINAL RESEARCH PAPER

Medical Science

A STUDY OF NEUTROPHIL CD64 EXPRESSION BY FLOWCYTOMETRY IN CASES OF SEPSIS- A HOSPITAL BASED STUDY

KEY WORDS: CD64, Flowcytometry and Sepsis

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ABSTRACT

Diagnostic indicators of the systemic acute inflammation response to infection or sepsis have shown few innovations in the past few decades. The usual laboratory tests employed to diagnose systemic infections such as leukocyte count, presence of immature leukocyte forms (bands), C reactive protein (CRP), and erythrocyte sedimentation rate (ESR), have poor sensitivity and specificity. Microbiological cultures are time consuming, often negative in those who are receiving antibiotics, and depend on various technical factors, such as the timing and technique of specimen collection and the transport time to the laboratory.

CD64 is the Fc receptor (Fcγ receptor I Fcγ RI) on Neutrophils which is constitutively expressed on macrophages, monocytes, and eosinophils. It is up regulated on neutrophils as a physiological response to microbial wall components and some cytokines. Up regulation of CD64 occurs within four to six hours after stimulation with interferon or granulocyte colony stimulating factor 4 and other activators, such as lipopolysaccharides

**Aims:** Our study aimed to demonstrate CD64 expression in cases of Sepsis.

**Settings and Design:** This is a Prospective Hospital based study in a Tertiary Hospital.

**Methods and Material:** We carried out the study in 100 suspected cases of sepsis with an equal number of age and sex matched control using fluorochrome APC H7 for CD45, PE for CD64 and FITC for CD14 by multiparametric flowcytometry.

**Statistical analysis used:** P Value was calculated statistically by using CHISQUARE TEST.

For Statistical convenience, MFI 80 was taken as cut off value.

**Results:** Out of 100 cases we found True Positive of 89% and False Negative of 11%. Out of 100 controls we found False Positive of 10% and True Negative of 90% which showed accuracy of 93.72%.

**Conclusions:** Our study demonstrates that neutrophil CD64 expression by Flowcytometry can be a diagnostic tool in cases of sepsis. The neutrophilic CD64 (nCD 64) expression was measured by estimating the median flurochrome intensity (MFI).

INTRODUCTION:

Sepsis is a significant health problem with an estimated 750,000 new cases increasing at the rate of 1.5% annually. Sepsis is among the 10 leading causes of death and the leading cause of mortality in the noncoronary intensive care unit with a mortality rate of 30% to 50% leading to yearly economic burden of a country. Thus, an improved diagnostic test for infection and sepsis would have both economic and therapeutic health care benefits.(1)

A collaborative effort of the European Society of Intensive Care Medicine, the Society of Critical Care Medicine, and the International Sepsis Forum, estimates that the number of sepsis cases in the world has reached 18 million annually. With a mortality rate of almost 30%, sepsis is considered a leading cause of death worldwide.(1) The frequency of sepsis has increased more than 135% in the most recent decade and is predicted to continue to rise due in part to the continued increase in antibiotic resistance.(2,3) Ironically, although treatment of sepsis has evolved in the last decades with newer therapeutic options, little has changed to improve diagnosis or therapeutic monitoring. Thus, an improved diagnostic test for infection and sepsis would have both economic and therapeutic health care benefits.(4-8)

Although efforts are being continuously made to diagnose SIRS or sepsis; none of the diagnostic test are satisfactory in terms of sensitivity and specificity. For example leukocyte count, presence of immature leukocyte forms (bands), C reactive protein (CRP), and erythrocyte sedimentation rate (ESR), have poor sensitivity and specificity.(9,10) Microbiological cultures are time consuming, often negative in those who are receiving antibiotics, and depend on various technical factors, such as the timing and technique of specimen collection and the transport time to the laboratory. A highly sensitive and specific laboratory test that has a short turn-around time would be extremely useful in these circumstances. One such candidate is the Fc receptor on Neutrophils because these cells form an important component of the innate immune

system and are activated early in the process.10-15 The Fc receptors, members of the immunoglobulin gene super family, are found on white blood cells, where they function to integrate responses involving both the innate and acquired immune systems. They are important for effective phagocytosis of bacteria and immune complexes. One of the Fc receptors for IgG is Fcγ receptor I (Fcγ RI or CD64). It is constitutively expressed on macrophages, monocytes, and eosinophils. It is upregulated on neutrophils as a physiological response to microbial wall components such as lipopolysaccharides and other inflammatory mediators. The aim of our study was to demonstrate CD64 expression in cases of Sepsis.

SUBJECTS AND METHODS:

The study was carried out in the Clinical Service laboratory and DBT Health Care Flowcytometry Laboratory, Department of Pathology, Assam Medical College and Hospital, Assam, India for a period of one year from from July, 2014 to June, 2015. A total of 200 subjects were taken out of which 100 were clinically suspected cases of sepsis and 100 healthy controls.

Each case and control were examined and evaluated by taking proper history which was followed by Sepsis workup that involved complete blood count along with hematological score, biochemical test such as CRP and microbial culture. Complete blood count was done by using Sysmex XS-800i, peripheral blood film were stained by Leishman and Giemsa stains and ESR by Westergren method.

CRP in serum was estimated by CRP Latex Agglutination slide method. Quantitative estimation of CRP greater than 0.6 mg/dl was considered as positive [AGAPPE diagnostics (p) Ltd. India].

For Blood Culture, one mL of blood was inoculated in conventional blood culture bottle containing 10 mL of glucose broth and; Blood Agar and Mc Conkey's Agar plates were used. The colonies were

identified macroscopically and microscopically and biochemical tests were performed to confirm the organisms. Finally antibiotic sensitivity was tested for the cultured organism. Cases with positive blood cultures were considered to have proven sepsis while the others were still considered as clinically suspected case of infection.

For Flowcytometry, sample preparation was done using Stain-Lyse-Wash method for immunofluorescence staining with different antibodies viz. CD45, CD64 & CD14 which were conjugated with fluorochrome viz. APC H17, PE and FITC. Cell washing was done with phosphate buffer saline (PBS) (NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O<sub>4</sub> and NaCl). When whole blood is added to the monoclonal antibody reagent, the fluorochrome labeled antibodies in the reagent bind specifically to leucocyte surface antigens. The stained samples were then treated with FACS lysing solution (NH<sub>4</sub>CL) which lyses erythrocytes under gentle hypotonic conditions while preserving the leucocytes. Data acquisition and analysis were performed on a FACS Canto 2 flow cytometer (Becton Dickinson San Jose, USA) using BD FACS Diva Software. Identification of CD64 positive Neutrophils was performed using side scatter (SSC) versus CD45 intensity and SSC versus forward scatter(FSC) parameter for dot plots. The neutrophilic CD64 (nCD 64) expression was measured by estimating the median fluoro intensity (MFI). Different flowcytometer uses different fluoro-chrome which result in variable cutoff value in MFI. During the study, clinically suspected cases fulfilling the criteria of international definition of sepsis, we found 89 % cases are with MFI more than 80 and healthy subject taken as control showed a MFI less than 80. Hence for statistical convenience MFI 80 was taken as cut off value.

**RESULTS:**

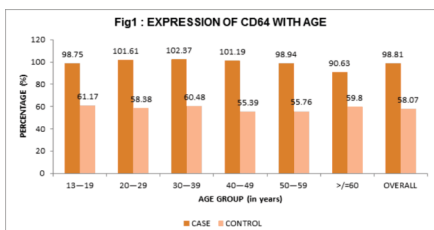
The study was conducted in the Department of Pathology, Assam Medical College & Hospital, Dibrugarh from June 2014 to July, 2015. Cases suspected to have sepsis attending Medicine OPD and admitted in Medicine Ward, Assam Medical College & Hospital, Dibrugarh were enrolled in this study. Sample size of 100 consecutive suspected cases of sepsis admitted in Medicine unit of Assam Medical College & hospital shall be recruited in the study and equal number of age and sex matched control shall also be included.

In the present study the mean nCD64 expression in different age groups among the cases were evaluated and it was found to be highest in the age group of 30-39 years and least in the 60yrs and above category. And mean nCD64 expression in different age groups among the controls was seen highest in age group of 30-39 years and least in the 40-49 years age group as Table1 and Figure1.

**Table 1: EXPRESSION OF CD64 WITH AGE**

Table 1: EXPRESSION OF CD64 WITH AGE			
AGE GROUP (in years)	CASE	CONTROL	p value
	Mean ± S.D.	Mean ± S.D.	
13—19	98.75± 27.03	61.17± 18.90	< 0.001
20—29	101.61± 27.71	58.38± 11.45	< 0.001
30—39	102.37± 17.96	60.48± 16.85	< 0.001
40—49	101.19± 21.09	55.39± 10.73	< 0.001
50—59	98.94± 22.18	55.76± 16.26	< 0.001
>=60	90.63± 17.79	59.80± 15.22	< 0.001
TOTAL	98.81 ± 21.73	58.07 ± 15.11	< 0.001

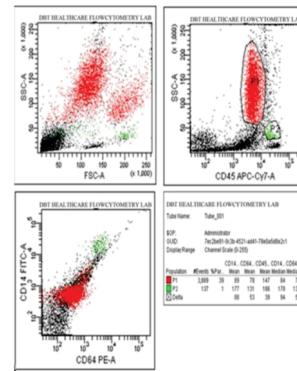
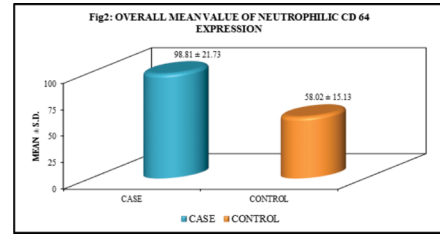
The overall mean value of neutrophilic CD 64 expression in cases was found to be 98.81 ± 21.73 and of controls 58.02 ± 15.13 with 95% confidence interval as shown in Table2 and Figure2.



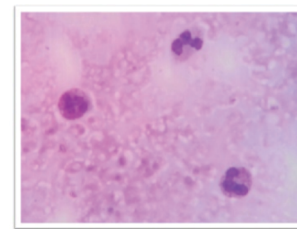
The overall mean value of neutrophilic CD 64 expression in cases was found to be 98.81 ± 21.73 and of controls 58.02 ± 15.13 with 95% confidence interval as shown in Table2 and Figure2.

**Table 2 : OVERALL MEAN VALUE OF NEUTROPHILIC CD 64 EXPRESSION**

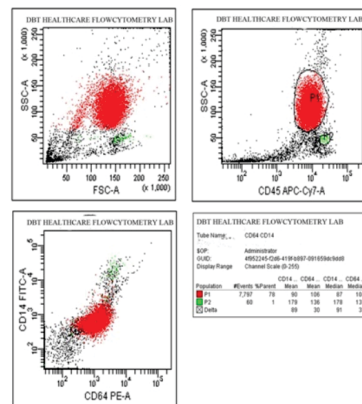
Table 2 : OVERALL MEAN VALUE OF NEUTROPHILIC CD 64 EXPRESSION				
	MEAN ± S.D.	RANGE	95% CI	p value
CASE	98.81 ± 21.73	53–147	94.5–103.1	< 0.001
CONTROL	58.02 ± 15.13	24–121	55.02–61.02	



**FIGURE-10: FLOWCYTOMETRIC PHOTOGRAPHS SHOWING HIGHLIGHTED CELLS WITH MoAb CD45,CD14 and CD64. MFI of nCD64 SHOWING HERE IS 75**



**FIGURE-11: NEUTROPHILIC LEUCOCYTOSES WITH TOXIC GRANULATIONS (Leishman Stain; Oil Immersion 100X)**



**FIGURE-11: FLOWCYTOMETRIC PHOTOGRAPHS SHOWING HIGHLIGHTED CELLS WITH MoAb CD45,CD14 and CD64. MFI of nCD64 SHOWING HERE IS 104**

**DISCUSSION:**

In the present study, nCD64 expression has a sensitivity and specificity of 89% and 90% respectively which was similar to other studies as shown in the Table below..

**COMPARISON OF SENSITIVITY AND SPECIFICITY IN DIFFERENT STUDIES ON NEUTROPHIL CD64 EXPRESSION**

Serial No	STUDY	SENSITIVITY (%)	SPECIFICITY (%)
1.	Davis et al 2005 <sup>[12]</sup>	94	85
2.	Livaditiet al 2006 <sup>[16]</sup>	95	100
3.	Cardelliet al 2008 <sup>[17]</sup>	96	95
4.	Lobreglioet al 2008 <sup>[18]</sup>	92	100
5.	Hsu et al 2011 <sup>[20]</sup>	89	96
6.	Present study (2014–15)	89	90

In the present study, nCD64 expression has positive and negative predictive value of 89.9 % and 89.1 % which correlates with Davis et al[13] and Hoffmann et al.[19] Further in the present study nCD64 expression showed an accuracy of 93.72%.

The quantification of nCD64 expression is a useful biomarker for bacterial infection and sepsis. CD64 expression is low on resting neutrophils, and it is rapidly dysregulated after activation. When the stimulations are removed, nCD64 expression will dramatically decrease within 48 hours and be back to normal levels within 7 days.[13] Additionally, nCD64 expression is relatively stable in blood samples for more than 30 hours, and the assay method is accurate, fast, and simple.[21] Moreover, nCD64 expression represents a pathophysiologic process that plays a key role in the innate immune response: neutrophils acting as phagocytes.[19] Therefore, nCD64 is one of the most useful markers for bacterial infections and sepsis.

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ETHICAL APPROVAL: Ethical Approval was given by the institutional ethical committee for conducting the study..

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