REVIEW ARTICLE

Neutrophil CD64 a Diagnostic and Prognostic Marker of Sepsis in Adult Critically Ill Patients: A Brief Review

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Abstract

Introduction: Sepsis is a life-threatening organ dysfunction with increased incidence of morbidity and mortality. Early diagnosis and prompt therapeutic intervention is the cornerstone of sepsis care. Biomarkers play an important role in sepsis having both diagnostic and prognostic implications. Neutrophil CD64 (nCD64) is a useful candidate biomarker for sepsis. Neutrophil CD64 also known as Fc receptor 1 (FcR1), is a high-affinity receptor present on neutrophils for Fc part of immunoglobulin-G (IgG) heavy chain. Its expression gets strongly upregulated in response to proinflammatory cytokines of infection within 4–6 hours. Neutrophil CD64 integrates function involving both innate and adaptive immune responses. The aim of this review is to present literature about nCD64 as a diagnostic and prognostic marker in patients with sepsis/septic shock.

Background: The authors searched articles over 13 years, i.e., from 2006 to 2019. They included articles written in English only and further reviewed the reference list of selected articles to obtain potentially relevant articles. Reviews, letters, commentaries, correspondences, case reports, conference abstracts, expert opinions, editorials, and animal experiments were excluded. Articles involving pediatric patients (≤18 years) were also excluded.

Review results: Several studies have indicated that nCD64 is a highly sensitive and specific marker for the diagnosis of sepsis. Various combinations of biomarkers have been used with nCD64 for a better diagnostic value. Neutrophil CD64 as a prognostic marker in critically ill patients needs to be explored more. Most of the existing literatures have highlighted its prognostic utility based on single value at enrolment. There are limited literatures on prognostic implications of serial trend and kinetics of nCD64.

Conclusion: Neutrophil CD64 is a useful diagnostic and prognostic marker of sepsis in critically ill patients. Additional studies are needed on nCD64 in sepsis based on sepsis-3 criteria. Further trials with large sample size are needed to establish prognostic implications of serial nCD64 trend.

Keywords: Fc receptor 1, Immunoglobulin-G, Neutrophil CD64, Sepsis, Septic shock.

Indian Journal of Critical Care Medicine (2020): 10.5005/jp-journals-10071-23558

Background

Sepsis is a major burden for healthcare.[1] Due to its aggressive nature, rapid recognition and appropriate urgent therapeutic interventions are cornerstone for sepsis and septic shock management. Biomarkers have important implications in patients with sepsis including diagnosis, prognosis, and therapeutic guidance.[2] Cell surface receptors are important candidate biomarkers for sepsis. Surface receptors of neutrophils recognize bacterial antigen, thereby activating neutrophils. Activated neutrophils have a capacity for phagocytosis, chemotaxis, oxidative burst, and production of cytokines.[3] Phagocytosis is facilitated by various opsonins like immunoglobulin-G (IgG) and complement factors. Polymorphonuclear leukocytes (PMNs) express receptors for the attachment of opsonin IgG called Fcγ receptor. These receptors are named as Fcγ receptors, as they bind to specific portion of antibody, the Fc (constant) region. Cluster of differentiation 64 (CD64) is the monoclonal antibody which recognizes FcγR1 neutrophilic receptor.[4] In addition to innate immune response stimulating phagocytosis, Fc receptor is also involved in adaptive immune response stimulating antibody-mediated cytotoxicity.[5] FcγR1 receptor is constitutively expressed to a very low extent on resting neutrophils. However, its expression gets strongly upregulated once these become activated by proinflammatory cytokines produced in response to infection like IFN-γ, IL-6, TNF-α, and granulocyte colony-stimulating factor (G-CSF), within 4–6 hours, allowing good discrimination between resting and activated neutrophils. When these stimulation factors are absent, it will substantially decrease within 48 hours and will be back to normal baseline values after 7 days.[6–9] Therefore, CD64 expression is a very early step of immune host response to bacterial infection and has been found to correlate with proinflammatory cytokine levels both in time and quantitatively.

Several studies have indicated that neutrophil CD64 (nCD64) is a highly sensitive and specific marker for the diagnosis of sepsis of bacterial origin and differentiating sepsis from non-septic conditions. Various combinations of biomarkers have been used in combination with nCD64 for a better diagnostic value in sepsis. Studies comparing nCD64 with C reactive protein (CRP), procalcitonin (PCT), and other biomarkers as diagnostic markers of sepsis have got results in favor of nCD64. Neutrophil CD64 is an

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How to cite this article: Patnaik R, Azim A, Agarwal V. Neutrophil CD64 a Diagnostic and Prognostic Marker of Sepsis in Adult Critically Ill Patients: A Brief Review. Indian J Crit Care Med 2020;24(12): 1242–1250.

Source of support: Nil

Conflict of interest: None

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emerging novel biomarker with prognostic implications in critically ill patients. Serial nCD64 has been found to correlate with clinical course and prognosis. Literature on nCD64 as a prognostic marker in intensive care unit (ICU) is limited.

The aim of this review is to present literature about nCD64 as a diagnostic marker and a prognostic marker in critically ill patients with sepsis. We systemically searched studies using PubMed, Embase, Web of Knowledge, and the Cochrane Library. The search terms were ("CD64, Fc gamma receptor") and (SIRS, sepsis, or septic shock) and (mortality, diagnosis, prognosis, sensitivity, specificity). We searched articles over 13 years, i.e., from 2006 to 2019. We included articles written in English only. We further reviewed the reference list of the selected articles to obtain potentially relevant articles. Reviews, letters, commentaries, correspondences, case reports, conference abstracts, expert opinions, editorials, and animal experiments were excluded. Articles involving pediatric patients (≤18 years) were also excluded.

Review Results

nCD64 for the Diagnosis of Sepsis in Adult Critically Ill Patients

Delay in the diagnosis of sepsis and thereby use of appropriate antibiotic leads to organ dysfunction and increased risk of mortality in sepsis. Though microbiological culture is a gold standard for the diagnosis of infection, it takes time to get culture reports. Diagnostic test with high sensitivity and low specificity yield more number of false positives, thereby promoting irrational use of antibiotics. Conversely, a diagnostic test with low sensitivity and high specificity like microbiological culture can lead to missed diagnosis. Diagnostic test with early diagnosis of infection with biomarkers plays an important role in critically ill patients. Hence, to diagnose sepsis, a biomarker with acceptable sensitivity and specificity should be used. For the diagnosis of sepsis, most widely studied biomarkers are PCT and CRP. Most of the studies have evaluated nCD64 as a biomarker for the diagnosis of sepsis (Table 1). A prospective observational study of 112 patients with suspected sepsis admitted to ICU found that nCD64 was a sensitive and specific test for the early diagnosis of sepsis. Neutrophil CD64 value ≥2398 molecules/cell was able to differentiate between patients with and without sepsis had area under receiver operating characteristic curve (AUROC) of 0.97.[10] In another prospective study, CD64 index of >1.19 was predictive of a clinical and/or culture diagnosis of infection with a sensitivity of 94.6% and a specificity of 88.7%.[11] As described by Gámez-Díaz et al., CD64 cutoff level of 1.7 molecules of equivalent soluble fluorochrome (MESF) showed 65.8% sensitivity and 64.6% specificity and AUROC 0.706 to diagnose sepsis within first 24 hours of emergency department admission.[12] A prospective study published 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 illness as SIRS, sepsis, and severe sepsis/septic shock with higher values in non-survivors.[13] Another prospective trial found that in patients admitted to medical ICU with at least two SIRS criteria, CD64 index >2.2 predicted bacterial infection with good specificity (89%) and AUROC (0.8), at the cost of low sensitivity of 63%. Low sensitivity of nCD64 for the prediction of bacterial infection in this study was attributed to more number of gram-positive infections in their study.[14] Earlier study has described that CD64 expression was more in patients with gram-negative infection.[15] A 2013 prospective observational study found that CD64 index had excellent diagnostic accuracy and high discriminative power to differentiate between sepsis and SIRS in adult ICU patients. To differentiate sepsis from non-sepsis, a cutoff value of CD64 index 1.66 showed a 100% sensitivity, 95% specificity, positive predictive value (PPV) of 96%, and negative predictive value (NPV) of 95%.[16] An observational retrospective study published in 2014 found that CD64 index was a good biomarker for sepsis diagnosis, better than PCT and CRP (AUROC 0.98) with a cutoff 1.15 of CD64 index.[17] Similar results favoring CD64 were obtained in subsequent prospective trials.[18–20]

Three meta-analyses have been published on nCD64 as a diagnostic marker of bacterial infection and sepsis. Two of which[21,22] evaluated CD64 as a diagnostic marker of bacterial infection. Both meta-analyses included studies which were heterogeneous and included both adult and pediatric patients. Meta-analysis by Cid et al. including 13 studies suggested that pooled sensitivity and specificity for nCD64 expression as a marker for bacterial infection was 79 and 91%, respectively. Area under curve for summary receiver operating curve was 0.94. Neutrophil CD64 was suggested as a useful diagnostic test for bacterial infection. Subgroup analysis revealed that pooled sensitivity and specificity was higher in adults (sensitivity 0.9, specificity 0.95) than children (0.71, 0.87). But, the published studies in this meta-analysis showed low methodological quality.[21] Another meta-analysis by Li et al. found that nCD64 was a reliable biomarker for the early diagnosis of bacterial infection with a higher diagnostic accuracy for rheumatoid arthritis and systemic lupus erythematosus (SLE) patients. Total 26 studies with 3,944 patients were included with both adult and pediatric population. The overall pooled sensitivity, specificity, positive, and negative likelihood ratios were 0.76, 0.85, 6.67, and 0.24, respectively. Summary of diagnostic odds ratio was 34.29 and area under summary AUROC was 0.92. But, the studies cited in the meta-analysis were heterogeneous and the cutoff value varied in studies.[22]

Meta-analysis by Wang et al. evaluated the diagnostic utility of nCD64 for sepsis in critically ill adult patients. It included 8 studies (7 of ICU, 1 of emergency department) with 1,986 patients. This meta-analysis showed good sensitivity and excellent specificity of nCD64 to diagnose sepsis. Pooled sensitivity and specificity was 0.76 and 0.85, respectively, with area under summary AUROC was 0.95. The positive and negative likelihood ratio and diagnostic odds ratio were 8.15, 0.16, and 60.41, respectively. The authors suggested that nCD64 was a helpful marker for the early diagnosis of sepsis as in accordance with previous meta-analysis by Cid et al. and Li et al. But, there was significant heterogeneity between studies and different studies used different assays for nCD64 with no ideal cutoff point. It was suggested that nCD64 should not be used alone to diagnose sepsis and should be interpreted with clinical correlation and other test results.[23]

Recently, a meta-analysis consisting of 14 studies [3 in emergency department (ED) and 11 in ICU] with a total of 2,471 patients, compared the diagnostic accuracy of CD64 with PCT and CRP for sepsis identification. The pooled sensitivity and specificity of CD64 was 0.87 and 0.89, respectively, with summary AUROC of 0.94. Cluster of differentiation 64 was found to be better than PCT and CRP for sepsis identification (AUROC of both 0.84). Subgroup analysis of this study revealed that no difference in diagnostic accuracy of CD64 according to source of patient (ED or ICU) or assay method (in-house method or Leuko64 kit). This meta-analysis has its limitations of significant heterogeneity among included studies.[24]

Table 1: Characteristic of studies on neutrophil CD64 for diagnosis of sepsis

Author	Cardelli	Icardi	Gámez-Díaz	Hsu	Gros	Gerrits	Rogina	Righi	Tan	Dal Ponte
Year	2008	2009	2011	2011	2012	2013	2014	2014	2016	2018
Place	Italy	Iowa	Columbia	Taiwan	France	Netherlands	Slovenia	Italy	Malaysia	Brazil
Clinical setting	ICU	In hospital	ED	Respiratory ICU	Medical ICU	ICU	ICU	ICU	ED	ED
Study population	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
Sample size	112	109	631	66	293	31: sepsis 20: postsurgical SIRS	88	93	42	109
Disease severity	Suspected sepsis	Suspected sepsis	Suspected sepsis	SIRS, severe sepsis, septic shock	SIRS, sepsis	SIRS, sepsis	SIRS, sepsis, severe sepsis, septic shock	SIRS, sepsis, severe sepsis, septic shock	SIRS, sepsis	SIRS, sepsis
Control	50 healthy control	24 healthy control	No	19 healthy control	No	24 out-clinic patients	No	No	No	No
Measurement time	Within 6 hours of suspected sepsis	Within 36 hours of blood culture event	Within 24 hours of admission	Admission to ICU	Admission to ICU	Admission to ICU	Admission to ICU	Within 24 hours of infection onset	Admission	Within 6 hours of admission then after 48 hours
Number of times measured	Single time	Single time	Single time	Single time	Single time	Single time	Single time	Single time	Single time	Twice
CD64 assay	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry
Unit of measurement	CD64 molecules/ neutrophil	CD64 index	MESF	CD64 molecules/ neutrophil	CD64 index	CD64 index	CD64 index	Antibody binding capacity (ABC)	CD64 antigen bound cell (abc)	CD64 index
To diagnose sepsis cutoff	≥2398 CD64 molecules/ neutrophil	≥1.19	1.7 MESF	>4300 molecules/ neutrophil	>2.2	>1.66	>1.15	>2000 antibody binding capacity	45 abc	>1.45
AUROC	0.97		0.706	0.928	0.8		0.982	0.93	0.88	0.832
Sensitivity	96%	94.6%	65.8%	89.1%	63%	100%	97.6%	90.2%	81%	85%
Specificity	95%	88.7%	64.6%	95.9%	89%	95%	95.9%	96.9%	89%	75%
PPV	91%	89.8%	78.5%	98%	85.3%	96%	95%	98.2%	97%	96%
NPV	98%	94%	49.1%	62.5%	70.1%	95%	93%	83.8%	50%	38%
PLR	–	8.36	1.85	9.80	5.7	–	–	–	–	0.38
NLR	–	0.06	0.52	0.12	0.4	–	–	–	–	0.34

AUROC, area under receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; MESF, molecules of equivalent soluble fluorochrome; ED, emergency department; ICU, intensive care unit; SIRS, systemic inflammatory response syndrome

Combination of nCD64 and Other Biomarkers to Diagnose Sepsis in Adult Critically Ill Patients

Various biomarkers have been used in combination with nCD64 for a better diagnostic value in sepsis (Table 2**)**. In a prospective observational study conducted in 300 adult patients of ICU, a biological score was constructed combining biomarkers (range 0–3, all below threshold to all above) PCT, soluble triggering receptor expressed on myeloid cell-1 (sTREM-1), and CD64 index. Bioscore had the highest area of the curve (0.95) followed by CD64 index (0.93). Probability of sepsis was 3.8% with bioscore of 0 and 100% with bioscore of 3.[25] A large prospective observational study including 548 critically ill patients of a medico-surgical ICU found that nCD64 identified sepsis with sensitivity of 89% and specificity of 87% with AUROC 0.94. While combining CRP and nCD64 expression, an abnormal result for both was associated with a 92% probability of sepsis, whereas sepsis was ruled out with a probability of 99% if both were normal.[26] A prospective observational study conducted in 219 patients of a 24-bedded medical ICU found that combination of CRP, PCT, and nCD64 measure remained a significant predictor of sepsis with an excellent AUROC (0.90) and improved diagnostic accuracy of sepsis.[27] A prospective study evaluated the diagnostic power of biomarkers CRP, PCT, and nCD64 individually and in combination to distinguish sepsis from non-septic adult ICU patients. Neutrophil CD64 was found to be better than other two biomarkers alone and

Table 2: Characteristic of studies using combination of neutrophil CD64 and other biomarkers for diagnosis of sepsis

Author	Gibot	Dimoula	Bauer	Jamsa
Year	2012	2014	2016	2017
Place	France	Belgium	USA	Finland
Clinical setting	ICU	ICU	ICU	ICU
Study population	Adult	Adult	Adult	Adult
Sample size	79	548	219	27
Disease severity	Sepsis, sever sepsis, septic shock	Sepsis, sever sepsis, septic shock	SIRS, sepsis	SIRS, sepsis
Control	No	No	99 (no SIRS, no infection source)	15 healthy controls
Measurement time	Within 12 hours of admission then on day 2	Within 24 hours of admission then daily	At enrolment	At admission
Number of times measured	Twice	Daily till death or discharge	Single time	Single time
CD64 assay	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry
Unit of measurement	CD64 index	MFI	CD64 molecules per neutrophil and CD64% positive neutrophils	MESF
Cutoff	1.62	CD64: 230 MFI CRP: ≥3.5 mg/dL	≥1040.5 CD64 molecules/neutrophil and ≥49.96% positive neutrophils	9172 MESF
Other biomarkers	PCT, sTREM-1	CRP	CRP, PCT, APACHE IV	CRP, PCT
AUROC	Combination: 0.95, CD64: 0.93	CD64: 0.94	Combination: 0.90, CD64 molecules/neutrophil: 0.83, CD64% positive neutrophils: 0.81	
Sensitivity	CD64: 84.4%	Combination: 76%, CD64: 89%	CD64: 76.4%	
Specificity	CD64: 95.2%	Combination: 98%, CD64: 87%	CD64: 76.7%	
PPV	CD64: 94.9%	Combination: 92%, CD64: 66%	CD64: 80.8%	
NPV	CD64: 85.3%	Combination: 93%, CD64: 97%	CD64: 71.7%	
PLR	CD64: 17.6	Combination: 38.1, CD64: 6.8	CD64: 3.28	Combination: 0.98, CD64: 0.62
NLR	CD64: 0.16	Combination: 0.24, CD64: 0.1	CD64: 0.31	Combination: <0.001, CD64: 0.0013

AUROC, area under receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; MFI, median fluorescence intensity; MESF, molecules of equivalent soluble fluorochrome; PCT, procalcitonin; sTREM-1, soluble triggering receptor expressed on myeloid cell-1; CRP, C-reactive protein; APACHE, acute physiology and chronic health evaluation; SIRS, systemic inflammatory response syndrome; ICU, intensive care unit

there was improved diagnostic accuracy when nCD64 was analyzed simultaneously with positive CRP and PCT.[28]

nCD64 as a Prognostic Marker in Adult Critically Ill Patients

Literature on nCD64 as a prognostic marker in critically ill ICU patients is scarce. Due to the availability of few literature on CD64 as a prognostic marker, no meta-analysis has yet been formulated. Most of the existing studies on CD64 for prognosis have used single value at admission/inclusion for prognostication aspects (Table 3). Very few studies have measured serial levels of this biomarker and evaluated its prognostic utility (Table 4).

In a prospective observational study of adult ICU patients with sepsis, nCD64 level was measured single time within 24 hours of onset of sepsis. Neutrophil CD64 expression significantly increased in sepsis than healthy controls and higher levels correlated with worsening severity of sepsis (sepsis, severe sepsis, septic shock) and increased 28-day mortality. It correlated with severity of organ failure via sequential organ failure assessment (SOFA) score and severity of sepsis via Acute Physiology And Chronic Health Evaluation II (APACHE II).[15] In one prospective study of ICU patients with sepsis, expression of the CD64 on polymorphonuclear cells was higher on day of admission in patients who survived than non-survivors. Increased CD64 expression correlated with polymorphonuclear phagocytic activity and patients' survival. Significant decrease in level of this biomarker from admission value was noted in patients who survived to day of discharge.[29] Diagnostic accuracy and prognostic value of nCD64 expression for bacterial infection in febrile adult patients presenting to hospital emergency department was evaluated in another prospective trial.

Table 3: Characteristic of studies using single value of neutrophil CD64 and prognosis of sepsis

Author	Livaditi	Cid	Chen	Olivgeris	Muzlovic
Year	2006	2011	2014	2015	2016
Place	Greece	Spain	China	Greece	Slovenia
Clinical setting	ICU	ED	ICU	ICU	ICU
Study population	Adult	Adult	Adult	Adult	Adult
Sample size	47	132	797	67	32
Disease severity	Sepsis, severe sepsis, septic shock	With (115) and without (17) bacterial infection	Infectious (381) and non-infectious disease (416)	SIRS (infectious and non-infectious)	VAP with or without sepsis
Control	12 healthy controls	No	No	No	No
Measurement time	First 24 hours of sepsis onset	One day after admission	First day within admission	Day 1 of SIRS	When temperature rises
CD64 assay	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry
Unit of measurement	CD64 molecules per cell	CD64 index	Relative CD64 ratio {MFI (mean fluorescence intensity) on granulocytes ÷ MFI on lymphocytes}	Neutrophils expressing CD 64% and MFI (mean fluorescence intensity)	CD64 index
Cutoff	Severe sepsis prediction: 2566. Septic shock: 6512. 28-day mortality prediction: 6252	Survival prediction: CD64 index ≥1.5	For predicting ICU mortality value ≥1.835	Predicting infection in SIRS patient CD64%: >8, MFI of CD64 expression on neutrophils: >1.39	1.58 for possible bacterial infection
AUROC	Severe sepsis: 0.98, septic shock: 0.92, 28-day mortality: 0.75	0.71	0.752		0.92
Sensitivity	Severe sepsis: 94.6%, septic shock: 100%, 28-day mortality: 66.7	85%	For predicting ICU mortality: 60.55%	Predicting infection in SIRS patient CD 64%: 83%, MFI: 83%	100%
Specificity	Severe sepsis: 100%, septic shock: 86.7%, 28-day mortality: 73.9%	33%	For predicting ICU mortality: 80.23%	CD64%: 68%, MFI: 92%	85.7%
PPV	Severe sepsis: 100%, septic shock: 81%, 28-day mortality: 72.7%	–	–	CD64%: 67%, MFI: 89%	83.3%
NPV	Severe sepsis: 83.3%, septic shock: 100%	–	–	CD64%: 83%, MFI: 87%	100%
LR	–	1.27	–	–	–
Accuracy	–	–	–	CD64%: 75%, MFI: 87%	–

AUROC, area under receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; MFI, median fluorescence intensity; VAP, ventilator-associated pneumonia; ICU, intensive care unit

Table 4: Characteristic of studies using serial neutrophil CD64 monitoring for prognosis of sepsis

Author	Danikas	Dimoula	Djordjevic	De Jong	Ghosh
Year	2008	2014	2015	2016	2018
Place	Greece	Belgium	Serbia	Netherlands	India
Clinical setting	ICU	ICU	ICU	ICU	ICU
Study population	Adult	Adult	Adult	Adult	Adult
Sample size	31	548	102	155	51
Disease severity	Severe sepsis, septic shock	Sepsis, septic shock	Severe sepsis, severe trauma	Sepsis, severe sepsis, septic shock	Sepsis, septic shock
Control	30 healthy control	No	No	No	No
Number of times measured	Twice (at admission, at discharge)	Daily till death or discharge from admission	Three times, at admission (day 1), day 2 and 3	Daily till death or discharge	Three times, at admission, day 4 and 8
CD64 assay	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry
Unit of measurement	Mean MIF	MFI (median fluorescence intensity)	CD64 index	CD64 index	% of neutrophils positive for CD64
Cutoff CD64 value	At admission, survival prediction: 3.75, non-survivor: 1.08, control: 0.285	Survivor: 155, non-survivor: 232, inappropriate antibiotic use at day 3: ≥260, non-septic patients developing hospital acquired infection: maximum variation of CD64 expression before event ≥40 MFI	To predict outcome (survival and non-survival) CD64 index at day 1 with cutoff CD64 index 2.8	CD64 index day 1; sepsis: 1.48, severe sepsis: 1.93, septic shock: 2.89, Positive culture: 2.26, negative culture: 1.49, survivor: 1.51, non-survivor: 1.81	% CD64 at admission; sepsis: 38%, septic shock: 67%, % CD64 day 4: survivor: 56.5, non-survivor: 70% CD64 day 8; survivor: 31, non-survivor: 74
AUROC	AUC of admission CD64 for predicting survival: 0.892	AUC of admission CD64 for predicting hospital death: 0.65, AUC to predict inappropriate antibiotic use at day 3: 0.71, maximum variation of CD64 expression before event to predict non-septic patients developing hospital-acquired infection: 0.77	AUC of admission CD64 to predict outcome (survival and non-survival): 0.727	–	AUC of admission CD64 to predict septic shock: 0.747, AUC of CD64 on day 8 to predict septic shock: 0.679
Remark	Increased CD64 expression correlate with survival	Serial CD64 measurement has prognostic implications (predict mortality, inappropriate antibiotic use and non-septic patients developing hospital acquired infection) during ICU stay.	CD64 index at day 1 and 2 was higher in non-survivors. CD64 index: good discriminator power to predict hospital mortality	CD64 index on day 1 significantly correlate with sepsis severity and higher in culture positive cases	Increased CD64 in septic shock than sepsis and survivors had improving trend of CD64

AUROC, area under receiver operating characteristic curve; MFI, median fluorescence intensity; MIF, mean intense fluorescence; ICU, intensive care unit

To detect bacterial infection, CD64 index showed good sensitivity but low specificity and higher expression of CD64 correlated to survival of the patients.[30]

In a large prospective study conducted in critically ill patients of medico-surgical ICU, nCD64 was found to be a prognostic marker during ICU stay. It correlated with severity of sepsis. Level was higher in hospital non-survivors than survivors. Cluster of differentiation 64 >260 median fluorescence intensity (MFI) at day 3 identified inappropriate antibiotic treatment with sensitivity of 93% and specificity of 48%. Septic patients receiving inappropriate empirical antibiotics had persistently elevated CD64 expression, whereas it decreased overtime in patients receiving appropriate antibiotics. In non-septic patients, an increase in C64 expression ≥40 MFI predicted ICU acquired infection (n = 29) with a sensitivity of 88% and specificity of 65%.[26]

Literature has highlighted nCD64 as predictor of ICU mortality similar to APACHE II score.[31] Another prospective cohort study conducted in a medico-surgical ICU showed that MFI of nCD64 expression on neutrophils had high sensitivity, specificity, and accuracy in the diagnosis of sepsis but not for the prediction of

survival.[32] Study by Djordjevic et al. evaluated nCD64 levels of 102 critically ill ICU patients at admission, then on day 2 and 3. They pointed higher levels of nCD64 at admission and on day 2 in non-survivors.[33] A similar study prospectively analyzed that longitudinal measurements of nCD64 discriminated between culture positive and negative sepsis, correlated with severity of disease as sepsis or septic shock, but not as a good predictor for 28-day mortality.[34]

Diagnostic accuracy of CD64 index and other biomarkers to predict ventilator-associated pneumonia (VAP) induced sepsis in ICU was evaluated by an observational pilot study. Cluster of differentiation 64 index on neutrophils showed the highest diagnostic accuracy to predict VAP-induced sepsis (AUROC: 0.929) and found to be predictor of survival.[35] A prospective study evaluated nCD64 expression in patients with community-acquired pneumonia admitted to emergency department where CD64 sample was sent before antibiotic administration. Patients with levels ≥2,700 MFI had more clinical deterioration and more ICU admission. To determine clinical deterioration and ICU admission, nCD64 had excellent specificity of >90% but low sensitivity. It was found that addition of nCD64 to pneumonia severity index and CURB-65 score did not improve the accuracy to predict outcome.36

In a recent prospective study on 51 patients with sepsis/septic shock in a mixed adult ICU, CD64 was measured serially at inclusion then on day 4 and 8 of ICU stay. Median values of CD64 percentage were significantly higher in patients with septic shock than sepsis at inclusion and on day 8. At day 8, higher value of CD64 was noted in non-survivors than survivors. Significantly decreasing trend of this biomarker over time periods was noted in patients who survived than who did not.[37]

To summarize, CD64 as a biomarker has important utility of prognosis in critically ill patients in various aspects. Neutrophil CD64 has been found to correlate with sepsis severity,[15,26,34,37] correlate with illness severity like APACHE II and SOFA score,[15] and predict survival/mortality during ICU stay.[15,21,31,35,37] For prognosis, CD64 has been found to be better than PCT and/or CRP.[15,33,35] Some of the studies where CD64 levels measured serially, described added advantage in addition to above aspects. Three studies described about significant decrease in level of this biomarker in patients who survived than their admission value.[29,33,37]

Five studies have described admission/inclusion value of CD64, and highlighted its prognostic significance in critically ill patients with sepsis/septic shock (Table 3). When a biomarker is evaluated for its prognostic utility, serial trend has more impact than mere single value at admission/inclusion. Five studies are there, where this biomarker levels have been measured serially **(**Table 4**).** In two of these studies though this biomarker levels were measured serially, only admission value of CD64 at day 1 was used for prognosis.[29,34] Study by Djordjevic et al. and Ghosh et al. compared median values of CD64 on serial days between survivor/non-survivors and/or sepsis/septic shock. Study by Dimoula et al. with largest number of included patients and daily assessment of CD64 has some limitations too. They used single day median value of CD64 for predicting hospital death and inappropriate antibiotic use.

Discussion

Neutrophil CD64 is determined using direct immunofluorescence method with flow cytometry in a small volume of ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood sample and results can be available within 20 minutes.[4] Cluster of differentiation 64 levels on surface of neutrophils can be evaluated with Leuko64 kit, which uses premixed fluorescein labeled anti-CD64 monoclonal antibodies (results are expressed as CD64 index, calculated as ratio of mean fluorescence intensity of cell population to that of the beads) or by in-house staining with fluorochrome-labeled anti-CD64 antibodies [results reported as percentage of CD64-positive cells and mean fluorescence index (MFI), with or without calculating number of molecules per cell]. Cluster of differentiation 64 expression is stable with EDTA-anticoagulated blood samples for at least 36–72 hours at ambient temperature.[7,38]

Extensive literature search has revealed that nCD64 is a valuable marker for the early diagnosis of patients with sepsis both in emergency department and ICU. Prospective studies and meta-analysis have documented that CD64 has a very good sensitivity and specificity for the diagnosis of sepsis (80–90%). Its diagnostic accuracy is definitely better than conventional biomarkers for sepsis including the most widely used biomarker PCT and CRP, with better AUROC in most of the studies. Apart from a useful established diagnostic marker, it has its own prognostic implications. Neutrophil CD64 has been found to be a predictor of outcome during ICU stay in the form of survival or mortality and an early predictor of impending clinical deterioration. It distinguishes the stages of sepsis severity and predicts appropriate antibiotic use. However, these studies have their own limitations, being small sample size and heterogeneous patient populations. In most of the studies, serial level of this biomarker has not been assessed. Most of them highlighted prognostic utility of nCD64 to predict outcome based on single value at enrolment. Clinical status of critically ill patients with sepsis can change in the initial few days of admission to ICU due to resuscitation, appropriate antibiotic use, and other therapeutic interventions. Cluster of differentiation 64 as a biomarker for prognosis needs to be explored more. None of the existing literature have highlighted the kinetics of serial trend of this biomarker for prognosis in critically ill ICU patients. No literature is there on combination of CD64 with other biomarkers for prognostic utility in critically ill patients with sepsis/septic shock. Cluster of differentiation 64 needs to be evaluated as a combination of biomarkers for prognosis in critically ill patients and its kinetics of serial trend for different aspects of prognosis.

After sepsis-3 definition put forward in 2016, no prospective study is available about CD64 where sepsis-3 criteria were used for the diagnosis and the prognosis of sepsis. According to this criterion, sepsis is defined as life-threatening organ dysfunction caused by dysregulated host response to infection. For clinical operation, this is based on change in SOFA score in patients with known or suspected infection. In a recent retrospective study,[39] diagnostic accuracy of CD64, PCT, and CRP was evaluated for sepsis identification in 35 adult ICU patients and compared with 27 controls (underwent coronary bypass surgery). Sepsis 3 criteria were used for sepsis identification. Cluster of differentiation 64 index showed better diagnostic accuracy than PCT and CRP with sensitivity, specificity, and AUROC of 83%, 88%, and 0.923, respectively. But, this study has limitations of small sample size and retrospective in nature.[39] Till now, no literature is there describing CD64 as a prognostic marker or sepsis as per sepsis 3 criteria.

Limitations

Though nCD64 has been found to have improved sensitivity and specificity for sepsis diagnosis than conventional sepsis biomarkers like PCT and CRP, it has its own limitations. Some studies have also pointed out the low sensitivity of nCD64 to diagnose sepsis.[14,23,36]

No sepsis biomarker is entirely specific for the diagnosis of sepsis. Biomarkers are a better tool to rule out rather than ruling in sepsis. Though CD64 is a promising biomarker for bacterial sepsis, its role in fungal and viral sepsis needs to be evaluated more. It has not been validated in larger trials for the de-escalation of antibiotics and role in antibiotic stewardship. Determination of nCD64 lacks standardization and various methods have been used in expressing CD64. Optimal cutoff for nCD64 is still unknown and cutoff values to differentiate sepsis and non-sepsis vary in different studies. There are limited literature on serial monitoring of nCD64. Cluster of differentiation 64 analysis needs a laboratory with flow cytometry facility.

Clinical Significance

Neutrophil CD64 is a relatively simple test and can be performed in a short period of time in most of the laboratories. Combination of nCD64 with other biomarkers increases the diagnostic accuracy of sepsis. Despite being a diagnostic marker, it is a reliable prognostic marker of sepsis itself. It correlates well with sepsis severity and predicts outcome in critically ill patients. Neutrophil CD64 levels along with clinical parameters is a useful diagnostic and prognostic marker of sepsis in critically ill patients. Neutrophil CD64 assay should be standardized with appropriate cutoff levels to differentiate sepsis from non-sepsis. Additional studies are needed on nCD64 for both diagnosis and prognosis of sepsis diagnosed based on sepsis-3 criteria. In this literature review, the authors have tried to highlight the existing literature on nCD64 in critically ill patients with sepsis/septic shock with respect to its diagnostic and prognostic implications. Further prospective trials with large sample size are needed to establish prognostic implications of serial nCD64 trend and combination of CD64 with other biomarkers for prognosis in critically ill patients.

Acknowledgments

Entire department of the Critical Care Medicine.

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