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Diagnostic values of CD64, C-reactive protein and procalcitonin in ventilator-associated pneumonia in adult trauma patients: a pilot study

DOI 10.1515/cclm-2015-0656
Received July 10, 2015; accepted September 25, 2015; previously published online October 24, 2015

Abstract

Background: Ventilator-associated pneumonia (VAP) is one of the most common nosocomial infections; however, its diagnosis remains difficult to establish in the critical care setting. We investigated the potential role of neutrophil CD64 (nCD64) expression as an early marker for the diagnosis of VAP.

Methods: Forty-nine consecutive patients with clinically suspected VAP were prospectively included in a single-center study. The levels of nCD64, C-reactive protein (CRP), and serum procalcitonin (PCT) were analyzed for diagnostic evaluation at the time of intubation (baseline), at day 0 (time of diagnosis), and at day 3. The receiver operating characteristic curves were analyzed to identify the ideal cutoff values.

Results: VAP was confirmed in 36 of 49 cases. In patients with and without VAP, the median levels (interquartile range, IQR) of nCD64 did not differ either at baseline [2.4 (IQR, 1.8–3.1) and 2.6 (IQR, 2.3–3.2), respectively; p=0.3] or at day 0 [2 (IQR, 2.5–3.0) and 2.6 (IQR, 2.4–2.9), respectively; p=0.8]. CRP showed the largest area under the curve (AUC) at day 3. The optimum cutoff value for CRP according to the maximum Youden index was 133 mg/dL. This cutoff value had 69% sensitivity and 76% specificity for predicting VAP; the AUC was 0.73 (95% CI, 0.59–0.85). The nCD64 and PCT values could not discriminate between the VAP and non-VAP groups either at day 0 or day 3.

Conclusions: The results of this pilot study suggest that neutrophil CD64 measurement has a poor role in facilitating the diagnosis of VAP and thus may not be practically recommended to guide the administration of antibiotics when VAP is suspected.

Keywords: biomarkers; CD64; CRP; procalcitonin; ventilator-associated pneumonia.

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Introduction

Ventilator-associated pneumonia (VAP) remains the second leading type of nosocomial infection and is associated with a substantial increase in mortality and prolongation of the length of intensive care unit (ICU) stay [1, 2]. The clinical diagnosis of VAP is based on the presence of new or progressive radiographic infiltrates and at least two additional criteria of leukocytosis or leukopenia, fever, and purulent respiratory secretions [3]. Although this diagnosis has high sensitivity, it overestimates the incidence of VAP [4], resulting in the use of antibiotics in patients who do not have an infectious process. Microbiological sampling is typically used to confirm the diagnosis of VAP; however, it takes 48–72 h to identify the causative pathogens.

Biomarkers are proteins that have been developed and whose existence correlates with a disease [5]. Ideally, the VAP biomarker should be low when infection is not present and increases in the presence of infection. Thus far, there is no ideal biomarker available for routine use in the diagnosis of VAP [6]. Although PCT and C-reactive protein (CRP) are used frequently to diagnose sepsis in critically ill patients, their diagnostic values for VAP are controversial [7, 8].

CD64, the high-affinity and restricted isotype-specificity FcγRI receptor, is constitutively expressed on

leukocytes and binds to monomeric IgG [9]. The Fc receptors are involved in the innate and adaptive immune response, stimulating either phagocytosis or antibody-mediated cytotoxicity. Several studies have shown that CD64 expression on neutrophils is upregulated during bacterial infection in both adult and pediatric populations [10, 11], indicating that neutrophil CD64 (nCD64) might have potential use in the diagnostic assessment of sepsis or infection [12]. However, none of these studies have examined the potential usefulness of nCD64 for diagnosing VAP in the ICU.

Therefore, we carried out this prospective observational study to establish the diagnostic application of nCD64 expression for the identification of VAP during ICU stay. Furthermore, we investigated whether the sensitivity and specificity of the nCD64 assay in patients with VAP have improved compared with those of CRP and PCT.

Materials and methods

This prospective observational study was carried out in the 12-bed trauma-surgical ICU at Cairo University Hospital. The study protocol was approved by the Research Ethics Committee, and informed consent was obtained from the next of kin of patients before the research began.

The study included all consecutive patients who were clinically suspected of having developed VAP after 48 h of mechanical ventilation. Patients under the age of 18 years, those who had a change in antibiotics in the preceding 3 days, and those diagnosed with sepsis or septic shock were excluded from the study.

Patients were suspected to have VAP when new, persistent infiltrate was seen on their chest X-rays and at least two of the following were observed: body temperature below 36 °C or above 38 °C; white blood cell count lower than 4000/mm^3 or higher than 11,000/mm^3; and macroscopically purulent tracheal aspirate [13]. Tracheal aspirate was classified as purulent or non-purulent after visual inspection by the clinical treatment team. A definitive diagnosis of VAP was considered only in patients with positive quantitative culture results for bacterial pathogens.

Microbiological analysis

All patients underwent fiber-optic bronchoscopy to collect distal pulmonary secretions. The bronchoscope was advanced into the involved lung segment as evidenced by the chest radio-graph, or into the left lower lobe in patients with diffuse bilateral infiltrates. The BAL was performed using 100 mL of nonbacteriostatic saline in 20-mL aliquots as previously described [14]. The bronchoalveolar lavage (BAL) fluid was transferred immediately to the microbiology laboratory for direct smear examination (Gram, Leishman-Giemsa and Ziehl-Neelsen stains) and culture to detect the presence of bacterial growth and to estimate the colony count before proceeding to antimicrobial sensitivity testing. A positive quantitative culture from minimally contaminated BAL specimens [bacterial growth ≥10^4 colony-forming units (CFU)/mL] confirmed the diagnosis of VAP [15]. For all patients in whom the clinical suspicion of VAP was confirmed, empirical antimicrobial therapy was started. The antibiotic therapy was selected by the critical care or primary care team according to the local guidelines.

Immunologic analysis

Estimates of the nCD64 expression on peripheral blood leukocytes were used for quantitative flow cytometric analysis. EDTA blood was either analyzed immediately or maintained at 4 °C for up to 24 h. Samples kept for up to 24 h were subjected to automated cell counting to ensure the number of neutrophils in it prior to flow cytometry analysis. Briefly, whole blood was incubated for 15 min at room temperature with saturating amounts of fluorescein isothiocyanate (FITC)-conjugated murine monoclonal antibody against 64 (Beckman Coulter, Marseille, France) or isotype control murine antibody, followed by red blood cell lysis with red cell lysis solution. Flow cytometric analysis was done with the use of the EPICS XL flow cytometer (Beckman Coulter). The neutrophils were gated based on their side- and forward-scatter characteristics; 10,000 cells were analyzed in each sample. The CD64 expression of neutrophils in MESF units was corrected for any nonspecific antibody binding by subtracting values for the isotype control. An index of nCD64 expression was calculated by using the ratio of the mean fluorescence intensities (CD64 MFI) of the study population to the fluorescence signal of the isotype control [16]. We evaluated CD64 expression on neutrophils only. Dual color flow cytometry; CD14-PE and CD64-FITC were used. The gated population was CD14- CD64+ cells to eliminate monocytic expression of CD64 from our results.

The procalcitonin (PCT) levels were determined by using the commercially available immunoluminometric assay (Siemens Healthcare Diagnostics, Munich, Germany) with a functional assay sensitivity of 0.1 ng/mL, defined as the lowest value that can be obtained with an inter-assay coefficient of variance (CV) <20%, as analyzed on an Advia Centaur autoanalyzer.

The CRP serum levels were measured by nephelometry (BN ProSpec; Siemens Healthcare Diagnostics, Germany), as routinely carried out at the Cairo University Hospital clinical pathology laboratory. The treating physicians were blind to the biomarker results

Data collection

All immunologic analyses were done at the following time points: at the time of intubation (baseline), at day 0 (time of diagnosis), and at day 3. The severity of the presenting illness was assessed based on the acute physiology and chronic health evaluation II (APACHE II) score calculated within 24 h of ICU admission [17].

The sequential organ failure assessment (SOFA) score was calculated at the time of VAP diagnosis (day 0) [18]. Modifications to the empirical therapy were based on the results of tracheal aspirate cultures and blood cultures.

The other data collected included: smoking status, history of congestive heart failure, history of malignancy, immunosuppression, corticosteroid use, need for dialysis, cause of ICU admission, duration of mechanical ventilation, and duration of ICU stay before VAP.

Statistical analysis

Previous study has shown that the area under receiver operating curve (ROC) of nCD64 was 0.8 [19]. Assuming that the prevalence of disease was 47% [20], a sample size of 38 patients was needed to yield a diagnostic accuracy with an area under the ROC of 0.75, power of 80%, and alpha error of 5%.

The data are expressed as medians (interquartile range, IQR) unless otherwise specified. Continuous variables were compared by using the Mann-Whitney U-test, and categorical variables by applying the χ^2-test. For repeated measures, a linear mixed models procedure with restricted maximum likelihood estimation was used. Bonferroni correction was applied in post hoc comparisons. To compare the performances of CD64, CRP, and PCT in predicting pneumonia, the ROC curves and their areas under the curve (AUC) were constructed. The software generated the biomarker value with the highest sensitivity and specificity (Youden index) to conclude that a patient had VAP. The significance level was set at $p<0.05$ for two-tailed tests. The statistical analysis was done by using MedCalc version 12.1.4.0 (MedCalc Software, Mariakerke, Belgium).

Results

Over 1-year period ending February 2014, 100 trauma patients needed mechanical ventilation. Only 49 met the clinical criteria for VAP; their demographic and other characteristics are shown in Table 1. The median age in the VAP group was 50 years (IQR, 38-59), which is higher than that in the non-VAP group (38 years; IQR, 24–55). In the VAP group, the median APACHE II score was 20 (IQR, 16–24), which is significantly higher than in the non-VAP group (Table 1).

A diagnosis of VAP was established in 36 patients (36%), of which 24 had Gram-negative bacilli, and 12 had Gram-positive cocci. *Acinetobacter baumannii* was the most common species (n=19), followed by methicillin-resistant *Staphylococcus aureus* (n=11), *Pseudomonas aeruginosa* (n=3), *Escherichia coli* (n=2), and *Proteus* (n=1).

In patients with and without VAP, the median levels (IQR) of nCD64 did not differ either at baseline [2.4 (IQR, 1.8–3.1) and 2.6 (IQR, 2.3–3.2), respectively; p=0.3] or at day 0 [2 (IRQ, 2.5–3.0) and 2.6 (IQR, 2.4–2.9), respectively; p=0.8] (Figure 1).

The CRP level did not differ in patients with and without VAP at day 0. However, at day 3, the CRP level increased significantly in the VAP group compared with the non-VAP group [163 (IQR, 122–198) versus 148 (IQR, 31–148), respectively; p=0.013] (Figure 1A). The time course of PCT in patients with and without VAP did not vary significantly throughout the study protocol (Figure 1B and C).

Table 2 shows the ROC curves for the data on the VAP group at days 0 and 3. The CRP level did not discriminate between the two groups at day 0; however, the predictive ability of CRP to diagnose VAP increased at day 3. The optimum cutoff value for CRP according to the maximum Youden index was 133 mg/dL. This cutoff value had 69% sensitivity and 76% specificity for predicting VAP; the AUC was 0.73 (95% CI, 0.59–0.85). The CD64 and PCT values could not discriminate between the VAP and non-VAP groups either at day 0 or day 3 (Tables 2 and 3; Figures 2 and 3).

Table 1: Patient characteristics at ICU admission.

	All patients (n=49)	VAP (n=36)	Non-VAP (n=13)	p-Value
Age, years	47 (34.5–56.5)	50 (38–59)	38 (24–55)	0.065
Male, %	35 (71.4%)	25 (69.4%)	10 (76.9%)	0.6
APACHE II	19 (14–24)	20 (16–24)[a]	15 (12–20)	0.04
SOFA score at day 0	4 (4–5)	4.5 (4–5)	4 (4–5)	0.16
Reason for mechanical ventilation				0.137
Polytrauma	38 (77.6%)	26 (72.2%)	12 (92.3%)	
Head trauma	11 (22.4%)	10 (27.8%)	1 (7.7%)	
History of smoking	26 (53%)	18 (50%)	8 (61.5%)	0.45
Hypoalbuminemia	12 (24.5%)	8 (22.2%)	4 (30.8%)	0.53
Steroid use	32 (65.3%)	22 (61%)	10 (76.9%)	0.38
Dialysis	5 (10%)	4 (11%)	1 (7.7%)	0.7
H2 blocker	39 (79.6%)	28 (77.8%)	11 (84.6%)	0.6
Proton pump inhibitor	10 (20.4%)	8 (22.2%)	2 (15.4%)	0.8
Duration of mechanical ventilation, days	14 (10–18)	14 (11–18)	14 (9–23)	0.37
Mortality, %	19 (38.8%)	12 (33.3%)	7 (53.8%)	0.2

Data are presented as medians (IQR) or numbers (percentages). APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment. [a]Denotes significance; $p<0.05$.

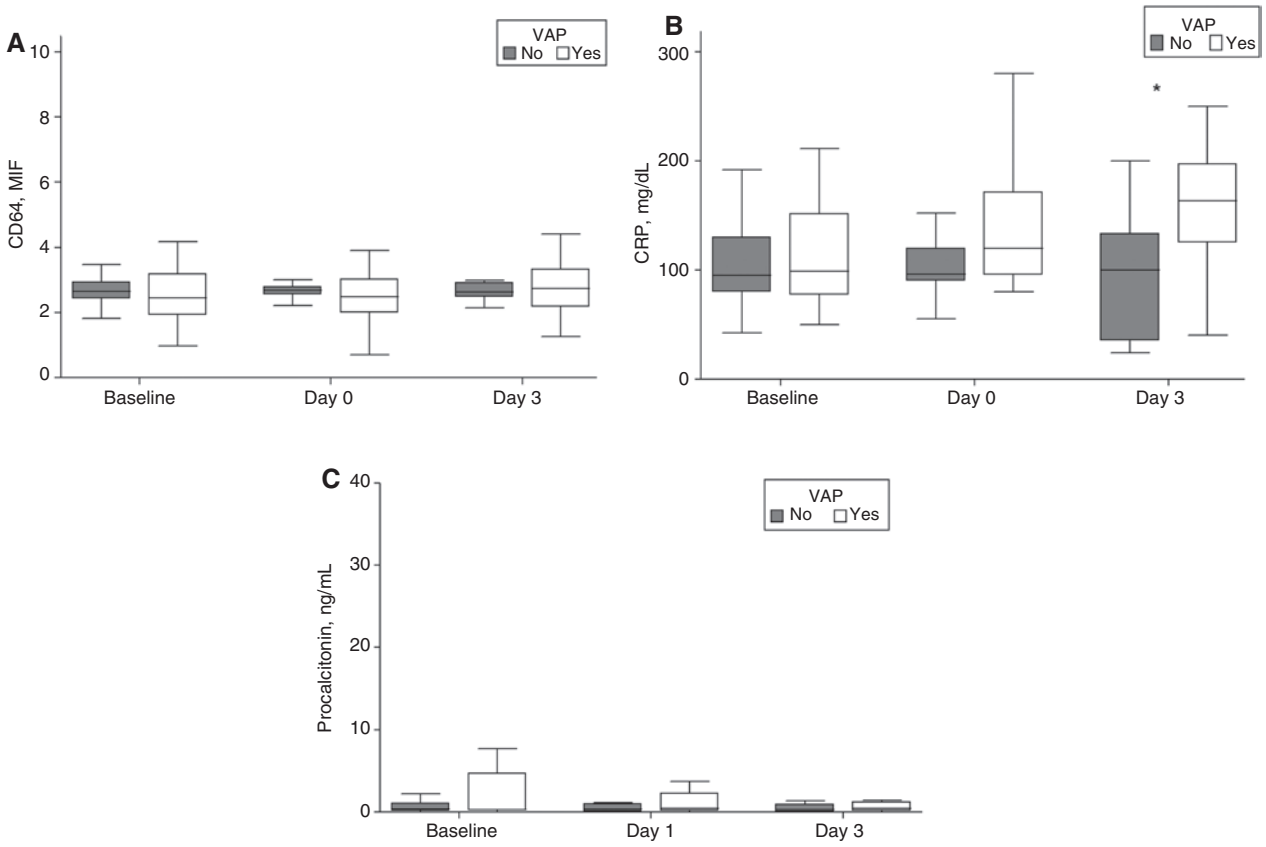

Figure 1: nCD64 (A), CRP (B), and procalcitonin (C) levels at baseline, at the time of intubation, (day 0), at the time of VAP diagnosis, (day 3), and 3 days after the initiation of antibiotics.
*Denotes significance; $p<0.05$.

Table 2: Comparison of the areas under the ROC curves for predicting VAP.

	ROC area	95% CI	Cutoff value	Sensitivity, %	Specificity, %	p-Value
Day 0						
CRP	0.64	0.49–0.7	96	77	39	0.15
CD64	0.61	0.46–0.75	2.54	58	77	0.19
Procalcitonin	0.57	0.42–0.7	1.12	36	84	0.46
Day 3						
CRP	0.74[a]	0.59–0.85	133	69	77	0.009
CD64	0.514	0.38–0.65	2.4	39	84	0.8
Procalcitonin	0.6	0.45–0.7	0.1	80	46	0.3
Kinetics from day 0 to day 3						
CRP	0.64	0.49–0.77	15	83	46	0.13
CD64	0.5	0.4–0.69	0.26	83	15	0.5
Procalcitonin	0.57	0.43–0.7	0.26	33	92	0.37

Day 0: at the time of VAP diagnosis. Day 3: 3 days after the initiation of antibiotics. CRP, C-reactive protein. [a]Denotes significance; $p<0.05$.

Discussion

The main finding of this pilot study was that CD64 is a poor marker for VAP diagnosis. Additionally, CRP was shown to be superior to both CD64 and PCT as a marker for the diagnosis of VAP. To the best of our knowledge, this is the first study that tested the diagnostic ability of CD64 in patients with VAP.

Several studies have evaluated the ability of CD64 expression to diagnose sepsis in the critical care setting

Table 3: Different cut offs values for predicting VAP at time of diagnosis (day 0).

Level	Sensitivity, %	Specificity, %	PPV	NPV
CRP				
80	94	15	76	50
100	66	53	80	37
120	47	76	85	34
CD64				
2.0	27	92	90	31
2.5	52	76	86	37
3.0	75	15	71	18
Procalcitonin				
0.25	55	38	71	23
0.5	44	69	80	31
1.0	36	82	85	31

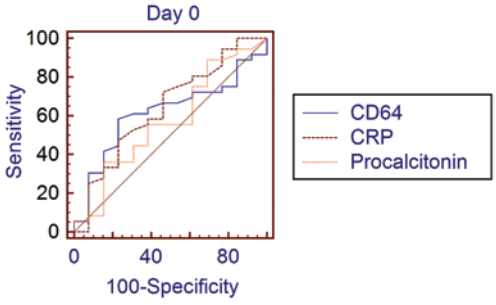

Figure 2: ROC curves comparing the abilities of nCD64, CRP, and procalcitonin to discriminate ventilator-associated pneumonia at day 0 (time of diagnosis).
ROC, receiver operating characteristic; nCD64, neutrophil CD64; CRP, C-reactive protein.

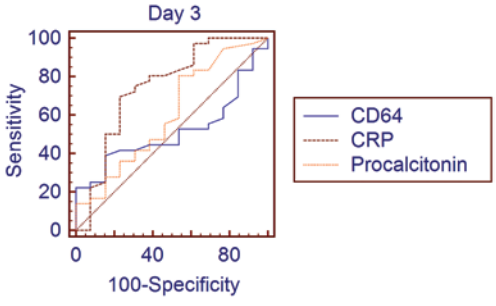

Figure 3: ROC curves comparing the abilities of nCD64, CRP, and procalcitonin to discriminate ventilator-associated pneumonia at day 3.
ROC, receiver operating characteristic; nCD64, neutrophil CD64; CRP, C-reactive protein.

[10, 20, 21]. Although some studies have found CD64 to have a high predictive ability to diagnose sepsis in the critical care setting [10, 20], others have shown that it has limited value in such diagnosis [21]. More recently, Dimoula and colleagues showed that CD64 was a useful marker for diagnosing sepsis at ICU admission in a large cohort of patients, with 89% sensitivity, 87% specificity, and an AUC of 0.94 (95% CI, 0.92–0.97) [12].

In our study, CD64 seemed to be poorly sensitive to VAP diagnosis, with 58% sensitivity and 77% specificity at day 0. The limited ability of CD64 to diagnose VAP may be explained by several factors. First, our cohorts consisted mostly of polytrauma patients. Thus, the contribution of VAP to increasing the expression of CD64 was limited compared with the preexisting massive systemic inflammatory response that was typically present in these patients. Second, a recent study showed that CD64 expression was increased on the surface of dendritic cells in response to pneumonia [22]. Dendritic cells are important for the activation of T cells and the initiation of pulmonary immune responses to pathogens [23]. However, the circulating dendritic cells in the peripheral blood are reduced in response to pneumonia, which might indicate pulmonary recruitment of these cells [23, 24]. Consequently, further studies are needed in patients with pneumonia to determine whether or not alveolar neutrophil CD64 expression is a better biomarker for diagnosing VAP. The value of measurement of inflammatory cytokines in the BAL instead of peripheral blood has been recently highlighted by Hellyer et al. In their study, several biomarkers were measured, including interleukin-1β, interleukin-8, matrix metalloproteinase 8, 9 and human neutrophil elastase. All five of the biomarkers were significantly higher in the patients with confirmed VAP [25].

The nCD64 cutoff value of 2.54 obtained in this study is difficult to be compared with others, who used different techniques to standardize their results [10, 12]. This is another add-on difficulty to implement nCD64 in daily practice because of lack of standardized flow cytometry protocols among different laboratories [26].

The results of the current study show that only a CRP level >133 mg/L at day 3 can be a useful marker for VAP diagnosis; however, this has a low sensitivity of 69% and a specificity of 77%. A limited number of studies have examined serum CRP as a marker for lower respiratory tract infection, with contradictory findings [27–30]. One of these studies showed that CRP had 73% sensitivity and 65% specificity for pneumonia identification but only with a CRP cutoff value >100 mg/L. Although speculative, the high CRP levels observed in our study may be explained by the fact that the CRP correlates with the severity of bacterial infection but it has limited value as a predictor of the presence or absence of pneumonia. Thus, CRP levels can be used mainly to follow up on pneumonia, as well as to evaluate the response to therapy, rather

than to establish its diagnosis [28]. Several studies have reported the use of serum PCT as a biomarker for VAP but with contradictory results [7, 31, 32]. Most of these studies have suggested that serum PCT is not a good biomarker for VAP.

The incidence of VAP in the present study is 36%. This high incidence was comparable to other studies which demonstrated that the incidence of VAP may be ranged from 40% to 60% in trauma patients [33, 34]. The high incidence of VAP in trauma patients may be related to impaired consciousness, the need of emergency tracheal intubation, and the frequent use of invasive procedures [35].

The present study has several limitations. First, the sample size was relatively small, which may have affected the diagnostic capability of CD64. Thus, further studies with a larger sample size are required to verify the results. Second, we used bronchoalveolar lavage as a diagnostic tool for VAP; the gold standard for diagnosis of pneumonia is still tissue histology [36], which is not practical in the critical care setting. Therefore, misclassification of some patients with non-documented bacterial infection might have occurred. Nevertheless, all patients diagnosed to have VAP met all clinical and microbiological criteria. Third, our ICU specializes in caring for critically ill trauma patients, which limits the generalizability of our results to other settings with different populations. Finally, the possibility of including some patients with concurrent occult infection that were not clinically diagnosed could not be ruled out.

In conclusion, this study is the first to evaluate the use of CD64 to diagnose patients with VAP. Despite the small sample size, our results indicate that CD64 is a poor biomarker for VAP diagnosis. Further studies with larger samples are warranted to confirm our findings.

Acknowledgments: We thank the residents, nursing staff, and data collection personnel of our unit for drafting this work.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. This work was carried out at Cairo University Hospital.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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