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Neutrophil CD64, C-reactive protein, and procalcitonin in the identification of sepsis in the ICU — Post-test probabilities

Joel Jämsä [a,b,*], Tero Ala-Kokko [a], Virva Huotari [c], Pasi Ohtonen [a], Eeva-Riitta Savolainen [c], Hannu Syrjälä [d]

[a] Department of Anesthesiology, Division of Intensive Care Medicine, Research Group of Surgery, Anesthesiology and Intensive Care Medicine, Oulu University Hospital, Medical Research Center Oulu, University of Oulu, Oulu, Finland
[b] Department of Internal Medicine, Lapland Central Hospital, Rovaniemi, Finland
[c] NordLab Oulu, Oulu University Hospital, Medical Research Center Oulu, Department of Clinical Chemistry, University of Oulu, Oulu, Finland.
[d] Department of Infection Control, Oulu University Hospital, Research Group of Surgery, Anesthesiology and Intensive Care Medicine, Medical Research Center Oulu, University of Oulu, Oulu, Finland.

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ABSTRACT

Purpose: We were interested in whether C-reactive protein (CRP) and procalcitonin (PCT) distinguish sepsis from non-septic controls and whether a combination of CRP, PCT, and neutrophil CD64 improves identification of sepsis in the intensive care unit (ICU).
Materials and methods: We analyzed the CRP and PCT concentrations from 27 patients with sepsis and 15 ICU controls. In addition, CD64 on neutrophils was measured using quantitative flow cytometry. We present a multiple marker analysis for sepsis diagnostics combining neutrophil CD64, CRP, and PCT using post-test analysis.
Results: The CRP and PCT values separated sepsis and non-septic ICU patients. In post-test analysis, CRP provided a positive probability of 0.48 and a negative probability of 0.053 for sepsis in the ICU; while, the corresponding values were 0.35 and 0.0059, respectively, for PCT and 0.62 and 0.0013, respectively, for neutrophil CD64. When neutrophil CD64 was analyzed with PCT and CRP, the probabilities were 0.98 and <0.001, respectively.
Conclusions: Neutrophil CD64 expression was superior to PCT and CRP for the identification of sepsis in ICU. Positive post-test probability for any combinations of simultaneously analyzed CRP, PCT and CD64 showed improved diagnostic accuracy for sepsis. This approach may be useful for guiding antibiotic treatment in ICU.

1. Introduction

Sepsis is a major cause of death in intensive care units (ICU) [1]. Early diagnosis and timely antimicrobial treatment are crucial to improve the outcome of sepsis patients [1,2]. Early diagnosis is challenging because inflammation caused by infection and other causes may present with similar signs and symptoms and there currently are no reliable laboratory tests to distinguish these conditions rapidly [3]. Furthermore, it was recently shown that the widely used systemic inflammatory response syndrome (SIRS) criteria for sepsis miss a number of septic patients [4].

Different combination of biomarkers, leukocyte surface molecules, and clinical scores may improve sepsis diagnostics [5], detection of positive blood cultures [6], and the prediction of outcome [7]. However, to date, there is no feasible standardized combination for early sepsis diagnostics in the ICU. The most commonly used laboratory tests in sepsis diagnostics are for C-reactive protein (CRP) and procalcitonin (PCT), as was recently thoroughly reviewed [8]. As CRP and PCT are produced in both inflammation and infection, they are rather controversial for sepsis diagnostics [8]. Leukocyte surface molecules have been suggested as possible markers of sepsis and bacterial infection and may be promising in monitoring patients' immune system functions during ICU treatment [8,9].

We have previously demonstrated that leukocyte surface molecule expression in the critically ill is highest in the early phase of ICU treatment in sepsis [10,11]. In these studies, we showed that CD64 is superior to other leukocyte markers for identifying sepsis patients. In the current sub-study, we were interested in comparing the diagnostic accuracy of using neutrophil CD64, CRP, and PCT concentrations obtained at admission in sepsis. We used a multiple marker approach to define

Abbreviations: AUC, area under the curve; CRP, C-reactive protein; ICU, intensive care unit; LR, likelihood ratio; MESF, molecules of equivalent soluble fluorochrome; OPCAB, off-pump coronary artery bypass; PCT, procalcitonin; ROC, receiver operating characteristics; SIRS, systemic inflammatory response syndrome.

* Corresponding author at: Oulu University Hospital, Department of Anesthesiology, ICU 1, P.O. Box 21, FI-90029 OYS Oulu, Finland.
E-mail address: joel.jamsa@student.oulu.fi (J. Jämsä).

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the diagnostic performance of the combination of parameters (CD64 and laboratory tests) for sepsis diagnostics in ICU using post-test probabilities.

2. Materials and methods

2.1. Study subjects

This is a sub-study of our earlier research conducted from April 2009 to March 2012 where we evaluated the kinetics of leukocyte surface molecule expression in sepsis and non-septic patients [10,11]. This current sub-study evaluated the diagnostic accuracy of CRP, PCT and neutrophil CD64, obtained at admission in sepsis. The patients have been described in more detail elsewhere [10]. Briefly, the subjects were critically ill sepsis patients treated at the mixed tertiary level ICU of Oulu University Hospital, and a heterogeneous group of non-septic ICU controls, who were postoperative off-pump coronary artery bypass (OPCAB) surgery patients and ICU patients without systemic inflammatory response syndrome (SIRS) criteria [12] in the beginning of the ICU treatment (non-SIRS ICU). The local ethics committee approved the study protocol (The Regional Ethics Committee of the Northern Ostrobothnia Hospital, protocol number: 54/2008, approved: 9.6.2008), and written informed consent was obtained from the patients or their family.

Sepsis in ICU treated patients was based on the existence of two or more SIRS-criteria and suspected or confirmed infection with signs of at least one organ dysfunction (formerly called severe sepsis [13]). For septic shock, the use of vasopressors was required [14]. Exclusion criteria for all the patient groups were age < 18 years, malignant tumor with metastases, hematological malignancy, and the use of biological medication. In addition, in sepsis, surgery other than that related to the current episode of sepsis during the past 6 months was not allowed.

2.2. Laboratory measurements

The CRP and PCT measurements were analyzed by commercially available laboratory methods in our accredited central laboratory (NordLab, Oulu University Hospital) for sepsis patients at admission, and the peak value from days 0–2 in non-septic ICU patients was chosen. The flow cytometry [FACSCalibur™ flow cytometer and CellQuest software (BD Biosciences, San Jose, CA, USA) was performed using regular equipment calibrations with CaliBRITE beads (BD Biosciences)], according to the manufacturer's instructions and the median fluorescence intensity was converted into molecules of equivalent soluble fluorochrome (MESF) values as explained previously [10,15]. For this sub-study, we chose CD64 on neutrophils (CD64-FITC [clone 22(FCγR1)] from Beckman Coulter [Brea, CA, USA]), which had the highest area under the curve (AUC, 0.99) for detecting sepsis in our previous studies [10,11]. In sepsis, the admission flow-cytometric sample was taken at a median of 19 h after ICU admission, and in non-septic ICU patients, the peak value of days 0–2 was chosen [10]. Blood culture samples were obtained from all the patients (BacT ALERT 3D, bioMerieux, Marcy l'Etoile, France).

2.3. Data analysis

Statistical analyses were performed with SPSS (version 22.0, SPSS Inc., Chicago, IL) software. The results were expressed as medians with 25th–75th percentiles, unless otherwise stated. Two-tailed p-values were reported and a p-value of <0.05 was considered significant in all analyses. The Mann-Whitney test and Chi-squared test or Fisher's exact test were used for between group comparisons. The diagnostic accuracy of neutrophil CD64 and laboratory tests were investigated using post-test analysis. Receiver operating characteristics (ROC) analyses were used and sensitivities, specificities, and cut-off values were calculated according to Youden's index. The sensitivities and specificities were further used to calculate positive and negative likelihood ratios (LRs) and to calculate post-test probabilities for neutrophil CD64 and laboratory tests, and their combinations. With the post-test probability we get a chance (or a probability) for a patient having the disease given the known test results. Sepsis was diagnosed in 10.5% of ICU admissions in a Finnish nationwide study [16], and this was used as a pre-test probability. The following equations were used in the calculations: pre-test ODDS × LR (parameter 1) × LR (parameter 2) = post-test ODDS, post-test probability (P) = ODDS / (ODDS + 1), ODDS = P / (1 − P).

3. Results

3.1. Patients

Twenty-seven patients with sepsis and 15 non-septic ICU controls (7 OPCAB and 8 non-SIRS ICU patients) were included in the study. In both groups, the median age was 66 years. There were 15 males and 12 females in the sepsis group and 10 males and 5 females in the non-septic ICU group. Sepsis patients had higher ICU scores [Acute physiology and chronic health evaluation II: 21 (15–24) vs. 14 (11–16), $p = 0.008$; Sequential organ failure assessment (peak) 9 (6–13) vs. 5 (2–6), $p < 0.001$] and a longer length of stay in the ICU and hospital than the 15 non-septic ICU controls. Twenty-five of the sepsis patients (93%) had septic shock, and eleven patients (41%) had a positive blood culture. The 6 month mortality was 26%. None of the patients in the control group died or developed sepsis. A more detailed description was published previously [10].

3.2. Laboratory test results

Both CRP and PCT distinguished sepsis and non-septic ICU patients (Figs. 1 and 2). The AUC for PCT was 0.92 (0.84–1.00) and was 0.81 (0.68–0.94) for CRP (Fig. 1, Table 1). The corresponding sensitivities and specificities were 0.96 and 0.79, respectively, for PCT and 0.56 and 0.93, respectively, for CRP (Table 1). For comparison, the AUC for neutrophil CD64 was 0.99 (0.97–1.00) with sensitivity of 1.00 and specificity of 0.93 [10].

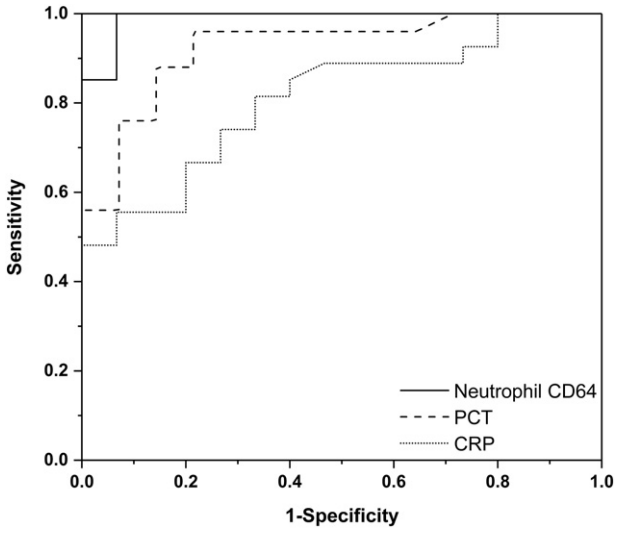

Fig. 1. The results of receiver operating characteristics (ROC) analyses of procalcitonin (PCT), C-reactive protein (CRP) and neutrophil CD64 for identifying sepsis. The ICU admission values of the sepsis patients [$n = 27$ (CRP, CD64), $n = 25$ (PCT)] were compared with the peak values of the non-septic ICU patients [$n = 15$ (CRP, CD64), $n = 14$ (PCT)].

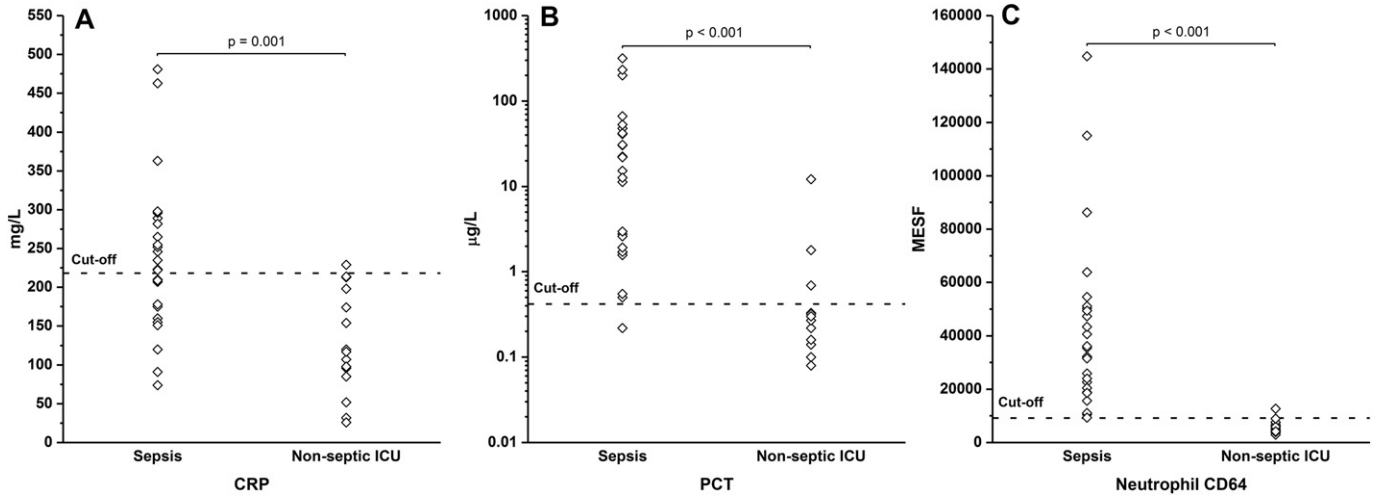

Fig. 2. C-reactive protein (CRP), procalcitonin (PCT) and neutrophil CD64 results in individual patients [D0 for sepsis ($n = 27$; $n = 25$, PCT), peak values for non-SIRS ICU patients ($n = 15$; $n = 14$, PCT)]. The dashed line represents the cut-off value for sepsis received from the ROC analyses. p values were obtained from Mann-Whitney test.

3.3. Post-test analysis for sepsis diagnostics

The positive post-test probability for a sepsis diagnosis was 0.48 for CRP and the negative post-test probability was 0.053; while, the corresponding values were 0.35 and 0.0059, respectively, for PCT (Table 1). The simultaneous analyses of positive CRP and PCT provided a post-test probability of 0.81, and if both were negative, the post-test probability was 0.0028. The positive post-test probability for sepsis diagnosis for neutrophil CD64 was 0.62 and the negative post-test probability was 0.0013 (Table 1). The simultaneous analysis of positive neutrophil CD64 and positive CRP provided a post-test probability of 0.93, while positive neutrophil CD64 and positive PCT provided a post-test probability of 0.88 for sepsis. Analyzing positive neutrophil CD64 simultaneously with both positive CRP and PCT provided a post-test probability of 0.98 for sepsis (Table 1).

4. Discussion

Our prospective observational ICU study showed that combining flow cytometric analyses of CD64 expression with CRP or PCT increased the diagnostic accuracy of sepsis in ICU. In the present study, both CRP and PCT distinguished sepsis from non-septic controls with PCT having a higher AUC of 0.92. Similarly, PCT was better than CRP in sepsis diagnostics in the emergency room [17] and ICU [18]. However, the AUC value found for neutrophil CD64 expression in our previous study was higher than those for CRP and PCT reported in this study [10]. Controversial results have been obtained regarding the diagnostic accuracy of PCT compared with CD64. Similar to our results, neutrophil CD64 expression was better than PCT for differentiating SIRS from sepsis in mechanically ventilated patients [19]. However, in another study, only PCT could determinate SIRS severity in sepsis patients [20]. In our study, CRP had a low sensitivity for sepsis; whereas, the combination with CD64 increased both sensitivity and specificity. Similarly, in an earlier study using flow cytometry analyses combining CRP and CD64, an abnormal result for both gave a 92% probability of sepsis; while, it was ruled out with a probability of 99% if both measurements were normal [21].

In a recent meta-analysis, the sensitivity and specificity of neutrophil CD64 for bacterial infection were 76% and 85%, which were lower than in our series [22]. However, in the meta-analyses, the case mix was heterogeneous ranging from neonates to adults in the ICU and types of infection varied. Some studies have criticized the low sensitivity of neutrophil CD64 in sepsis diagnostics in the ICU or emergency department patient populations [23,24], but because of its high specificity, it could be useful when combined with a more sensitive marker [24]. Recently, there have been two different ways of reporting neutrophil CD64 expression: for example, in a commercial method for CD64 index, an index is calculated by dividing neutrophil CD64 fluorescence intensity by the fluorescence intensity of the beads in commercial CD64 kit [23-25], and quantitative flow cytometry [21], as in our present study. Using the CD64 kit provides simplified analyzing process. However, according to meta-analyses, the diagnostic performance was better in studies reporting quantitative flow cytometry [22,26].

Previous studies have also suggested biomarker combinations to improve diagnostic performance [5]. In the present study, we used a multiple marker analysis to define post-test probabilities, for CRP and PCT with or without neutrophil CD64, in an ICU population with a 10.5%

Table 1
Results of the ROC analysis of sepsis diagnostics and the post-test probability.

Variable	AUC	Cut-off (≥)	Sens.	Spec.	LR+	LR−	Post-test probability					
									CD64+		CD64-	
							Test +	Test −	Test +	Test −	Test +	Test −
PCT (µg/l)	0.92	0.42	0.96	0.79	4.6	0.051	0.35	0.0059	0.88	0.078	0.0057	<0.001
CRP (g/l)	0.81	218	0.56	0.93	8.0	0.47	0.48	0.053	0.93	0.44	0.010	<0.001
CD64 (MESF)	0.99	9172	1.00	0.93	14.1	0.011	0.62	0.0013				
PCT + CRP							0.81	0.0028	0.98	0.038	0.044	<0.001

The ICU admission values of the sepsis patients [$n = 27$ (CRP, CD64), $n = 25$ (PCT)] were compared with the peak values of non-septic ICU patients [$n = 15$ (CRP, CD64), $n = 14$ (PCT)]. Cut-off values, sensitivities, and specificities were calculated according to Youden's index. Positive and negative likelihood ratios (LR, calculated from sensitivities and specificities; for value 1.00, the value 0.99 was used in calculations) were used to asses post-test probability values for PCT, CRP, CD64 and their combinations (pre-test probability 0.105). The ROC curves are shown in Fig. 1. The cut-off, sensitivity, and specificity for neutrophil CD64 were reported also previously [10].

incidence of sepsis [16]. A post-test analysis could help to assess the risk of sepsis from a panel of diagnostic parameters by calculating the post-test probabilities. For example, according to this study, if the PCT was ≥0.42 μg/l and CRP ≥218 g/l (both positive), the probability for sepsis is 0.81, and if both were negative, the probability is 0.0028. Accordingly, 19% of patients would receive unnecessary sepsis antibiotic treatment if only positive CRP and PCT test results are considered. To improve the diagnostic accuracy, positive CD64 was analyzed simultaneously with positive CRP and PCT tests, and the probability of sepsis increased to 0.98 and decreased to 0.044 with a negative CD64 test result. Consequently, with a positive CD64 result, the risk for unnecessary antibiotic treatment is only 2%, and after a negative CD64 and positive PCT, only 0.57% of patients would not receive adequate antibiotic treatment.

The limitation of the study is the single center setting with a small sample size. Our study setting could overestimate the diagnostic performance of the laboratory tests; thus, the post-test probabilities could be overestimated since our sepsis patients had higher ICU scores than the controls, and post-test probabilities cannot directly be adapted to a mixed ICU population. However, significantly elevated CRP levels in the non-septic ICU controls confirm that all patients represent a major challenge with respect to the initiation of antibiotic treatment. We introduced combining neutrophil CD64 with PCT and CRP for identifying sepsis and used a control group of mixed ICU patients. In the future, post-test analysis may provide improved diagnostics in larger patient samples. We evaluated prospectively collected material with special emphasis on quantitative surface molecule analysis [10,15] and demonstrated that CD64 on neutrophils is comparable to or even better than the widely used biomarkers used to diagnose sepsis, and this study encourages a wider use of neutrophil CD64 for sepsis diagnostics in ICU. However, the post-test analyses support the idea that CD64 should be analyzed simultaneously with CRP or PCT for a more reliable diagnosis of sepsis. Whether our preliminary findings are clinically applicable, further studies with larger patient groups and multicenter approaches are needed. The post-test analysis is easy to calculate in other studies reporting sensitivities and specificities to define how the positive or negative test results impact the probability of the disease.

5. Conclusion

Neutrophil CD64 expression was superior to PCT and CRP for the identification of sepsis in ICU. Post-test probability of CD64 showed improved diagnostic accuracy for sepsis when it was analyzed simultaneously with positive CRP or PCT results. This approach may be useful for guiding antibiotic treatment in ICU.

Conflicts of interest

The authors declare that they do not have any conflicts of interest. The results were partly presented at ESICM 2013 in Paris.

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