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Diagnostic Accuracy and Clinical Relevance of an Inflammatory Biomarker Panel for Sepsis in Adult Critically Ill Patients

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Abstract

The objective of this study was to assess the diagnostic accuracy of C-reactive protein (CRP), procalcitonin (PCT) and cellular immune markers levels in sepsis.

This was a prospective observational study in adult ICU patients, between 2012 and 2014. The 8-color flow cytometric biomarker panel included CD64, CD163, and HLA-DR. Index test results were compared with sepsis, using receiver operating characteristic curve analyses. Multivariate logistic regression assessed the relationship of sets of markers with the probability of sepsis.

Of 219 enrolled patients, 120 had sepsis. C-statistic was the highest for CRP (0.86) followed by neutrophil CD64 expression (0.83), procalcitonin (0.82), and APACHE IV (0.72). After adjustment for APACHE IV, the combination of CRP, PCT, and neutrophil CD64 measure remained a significant predictor of sepsis with an excellent AUC (0.90).

In a targeted ICU population at increased risk of sepsis, CRP, PCT and neutrophil CD64 combined improve the diagnostic accuracy of sepsis.

Graphical Abstract

Keywords

Study of diagnostic accuracy; Sensitivity and specificity; Sepsis; C-reactive protein; Procalcitonin; CD64

Introduction

Sepsis remains a major burden worldwide with high incidence [1] and prevalence [2]. Mortality may be on the decline [3], but low awareness, late recognition and late treatment are still common [4]. C-reactive protein (CRP) [5] and procalcitonin (PCT) [6] have shown conflicting results and impact studies are rare [7]. A constant clinical challenge is to make an early distinction between systemic inflammatory response syndrome (SIRS) and sepsis [8] where infection is complicated by dysregulation of the host immune response. A delay in recognizing infection can impact outcome since there is a direct relationship between survival and early appropriate therapy [9]. Therefore, the need for early biomarkers of infection with sepsis is justified in term of rapid diagnosis, and appropriate management strategies [10].

Neutrophils play an important role in the innate immune response to infection [11]. Cluster of differentiation 64 (CD64, Fc γ RI) antigen is involved in neutrophil phagocytosis, oxidative burst and target killing [12] and has been promising in the early detection of bacterial infection [13-18]. Monocyte/macrophages are also key players in the innate immune response to infection. CD64 is also constitutively expressed on monocyte/macrophage cells and further upregulated in the context of sepsis [19]. CD163 (haptoglobin-hemoglobin complex receptor) expression, restricted to monocyte/macrophages, belongs to the scavenger receptor cysteine-rich domain superfamily class B [20]. CD163 functions as a macrophage sensor, and binds to intact Gram-positive and Gram-negative bacteria, triggering a local pro-inflammatory cytokine production without prominent phagocytosis [21]. HLA-DR is constitutively expressed on the cell-surface of monocytes/macrophages. It plays an active role in antigen-presentation and subsequent activation of T cells and thus, facilitates the adaptive immune response to infection [22]. The loss of HLA-DR expression on monocytes reflects impaired monocyte activation, and appears to be correlated with the development of sepsis and outcome [23].

Thus, CD64 expression on neutrophils and presence of CD163 and loss of HLA-DR expression on monocytes have the potential to be robust diagnostic and prognostic biomarkers of infection and sepsis, although additional data is required to delineate their exact role in the diagnostic workup of sepsis [24]. The combination of CRP with CD64 [25] and PCT [26, 27] also seems promising. Therefore, the main goal of this study was to compare the diagnostic accuracy of CRP and PCT to neutrophil CD64, monocyte CD163 and monocyte HLA-DR expression in early sepsis.

Materials and Methods

a. Participants

All adult patients consecutively admitted to the 24-bed medical Intensive Care Unit (ICU) of a 2200-bed academic tertiary center were prospectively screened and recruited according to their presenting symptoms concerning for sepsis within the first 24 hours following admission. A control group was made of patients also in need for ICU during the same period but with no immediate concern for sepsis. Exclusion criteria included age, vulnerable

population and treatment with erythropoietin; no specific blood draw for research purpose was allowed (Figure 1). Sepsis work up was at the discretion of the clinical team who was not directly involved in the study. One set of blood cultures (anaerobic and aerobic bottles) was drawn as part of the study design if not clinically indicated (control group). Written consent was obtained from patients or their legal representative. This study was conducted in accordance with the amended Declaration of Helsinki and approved by the Mayo Clinic (10-008831) institutional review board.

b. Test methods

The reference standard was infection [28]. The target condition was sepsis based on current consensus definitions [29] that is two or more Systemic Inflammatory Response Syndrome (SIRS) criteria with suspected or present source of infection and included sepsis, severe sepsis and septic shock. The control group included patients who could have none or up to four SIRS criteria but no source of infection. Two set of clinicians reviewed the final diagnosis of infection (PRB and JGP) and sepsis (PRB and RK). In case of disagreement, a consensus was obtained. A sensitivity analysis evaluated the target condition as confirmed infection only. Blood samples, consisting of 1×4 mL sodium heparin (placed on wet ice) and 1×3 mL EDTA (room temperature) were collected upon enrollment. Those samples were handled by a mobile Clinical Research Unit and sent immediately to the Cellular and Molecular Immunology Laboratory after de-identification and coding for blinding and confidentiality purpose. Demographics, co-morbidities, sepsis screening, Acute Physiology and Chronic Health Evaluation (APACHE) IV score [30], Sequential Organ Failure Assessment (SOFA) score [31], microbiology, treatment, and outcome variables were prospectively collected and managed using REDCap (Research Electronic Data Capture) [32]. Patients were followed up until hospital discharge.

C-reactive protein (CRP) was measured by particle enhanced immunoturbidimetric assay (Roche Diagnostics™) and procalcitonin (PCT) by homogeneous automated immunofluorescent assay (BRAHMS Kryptor Compact or Compact PLUS™, Thermo Scientific).

Determination of HLA-DR and CD163 expression on monocytes and CD64 expression on neutrophils and monocytes was performed using 100 μ L Sodium Heparin whole blood stained for 1 hour at room temperature in three separate tubes: tube 1: QuantiBRITE™ Anti-HLA-DR PE*/Anti-Monocyte PerCP-Cy5.5 (BD Biosciences, San Jose, CA), CD163 APC (R&D Systems, Minneapolis, MN), and CD45 Krome Orange (Beckman Coulter, Miami, FL); Tube 2 (isotype control for Tube 3): CD14 FITC and IgG1 Pacific Blue (Beckman Coulter, Miami, FL), IgG1 PE and CD45PerCP (BD Biosciences, San Jose, CA); Tube 3: CD14 FITC and CD15 Pacific Blue (Beckman Coulter, Miami, FL)) and QuantiBRITE™ CD64PE*/ CD45 PerCP (BD Biosciences, San Jose, CA). All tubes were lysed with BD FACSlyse, washed with 1mL of BD Stain Buffer, resuspended in 500 μ L of BD Stain Buffer, and run on a Gallios® cytometer (Beckman Coulter, Miami, FL). BD QuantiBRITE™ PE* tubes were used per the package insert to calculate molecules/cell and were run using the instrument settings for each part of the assay. Data analysis for all assays

was performed using Kaluza® v1.2 software (Beckman Coulter). Standard curve graphs and molecules/cell data were generated in MicroSoft Excel 2003.

For control values, blood samples from 173 normal subjects, age 23 years and older were used. Each of these parameters was analyzed individually and collectively to determine their performance singly or in combination. Intra- and inter-assay precisions were performed using five normal donor samples in replicates of four, showing, for instance, a mean coefficient variation of 1.92% and 2.87% (neutrophil CD64 expression), 13.83% and 25.18% (CD163 MFI monocytes), and 2.66% and 8.16% (HLA-DR molecules/monocyte) respectively.

The clinical investigators (PRB, RK, JGP and OG), the laboratory investigators (RSA and SCL), and the biostatisticians (SMJ and CYS) remained blinded to each other assessment until the data analysis of the biomarker panel was completed.

c. Statistical methods

Based on preliminary data using neutrophil-CD64 expression to predict sepsis, we predicted that a sample size of 100 per group (septic and non-septic) would give a sensitivity of 90% $\pm$ 4-7% margin of error and a specificity of 70% $\pm$ 8-10% margin of error. Furthermore, we would have more than 99% power to detect a difference in mean CD64 expression on neutrophils between the two groups.

Results are expressed as median and interquartile ranges for continuous data (except otherwise specified) and number and percentage for categorical data. Comparison between groups used analysis of variance (or Kruskal Wallis test), two sample t-test (or Wilcoxon rank sum test) and chi-square test (or Fisher's exact test) as appropriate. Kappa statistic was used to check agreement on assessing infection and sepsis between reviewers. Sensitivity and specificity were illustrated using the receiver operating characteristic curve (ROC). Area under the curve (AUC) was reported along with the cut-off that maximized the sensitivity and specificity while minimizing the difference between them. The diagnostic ability (AUC) of selected sets of screening tests for sepsis was compared using the method from DeLong [33]. Multivariate logistic regression was used to assess the relationship of sets of markers with the probability of sepsis, adjusting for other patient characteristics. The AUCs from the multivariate models were adjusted using a method of bootstrapping for internal validation [34]. The goodness of fit of the multivariate models was assessed using the Hosmer-Lemeshow test. To account for multiple testing, p-values less than 0.001 were considered statistically significant for unadjusted comparisons between sepsis vs. non-sepsis and infection vs. non-infection. For comparing selected AUCs and assessing the multivariate logistic regression models, p-values less than 0.05 were considered statistically significant. All analyses were conducted using SAS version 9 (Cary, NC) and R (<http://www.r-project.org/>).

Results

Between March 2012 and March 2014, 1192 adult ICU patients were screened, and 219 patients were eligible and included (Figure 1). Patients in the septic group were older, and

admitted more frequently to the ICU because of respiratory or cardiovascular issues (Table 1). The predominance of digestive and metabolic reasons for admission in the ICU in the non-septic group was explained by a greater proportion of gastro-intestinal bleeding and diabetic ketoacidosis. Severity score (APACHE IV), organ failure (SOFA), and length of hospital stay was greater in the sepsis group than the control group. ICU and hospital mortality was not significantly different. Infection was present in 7% non-septic cases, and 70% septic cases (Table 1S). Pneumonia, bacteremia and urinary tract infections were the most frequent causes of infections in septic patients. Septic patients received more fluid boluses, vasopressors, corticosteroids, antibiotics and control of the source of infection. Transfusion was greater in the non-septic group. The degree of kappa agreement on sepsis was good: 0.69 (95% CI 0.60-0.79), $p < 0.0001$, as was the degree of agreement on infection: 0.67 (95% CI 0.59-0.75), $p < 0.0001$.

By univariate analysis, lowest or highest white cell counts (WBC) within the first 24 hours, CRP, PCT, CD64 (either the number of molecules expressing CD64 per neutrophil or the percentage of CD 64+ neutrophils) were higher in cases of sepsis (Table 2) (Figure 2). In contrast, the absolute lymphocyte count by flow cytometry (CD45+ ALC) and HLA-DR molecules per monocytes were lower with sepsis though CD163 expression on monocytes was similar between the groups. The AUC was the highest for CRP, followed closely by CD64 expressing molecules per neutrophil, PCT and the %CD64+ neutrophils indicating that each of these parameters is independently a reliable marker for sepsis (Table 3). A model that included all four measures (CRP, PCT, CD64, and APACHE IV) performed better than CRP alone (AUC 0.90 vs 0.86, $p = 0.03$) and also performed better than CRP + PCT together (AUC 0.90 vs 0.88, $p = 0.04$) (Table 2S). Although the markers moderately correlated with each other (Pearson correlations: CRP vs log PCT: 0.54, CRP vs log CD64: 0.69, log PCT vs log CD64: 0.63), there was no evidence for model instability. The model fit the data well. Findings were similar when only infection that was microbiologically proven (confirmed infection) was used instead of sepsis, where CD64 expression appeared to be a better discriminator of infection with a diagnostic odds ratio (DOR) of 16. Finally, further review of the 18 patients with elevated CRP (> 43 mg/L) but no evidence of sepsis or infection (false-positive results) showed the presence of underlying inflammatory conditions, including vasculitis, connective tissue diseases, vascular, thrombo-embolic and metabolic abnormalities (Table 3S). Of these 18, 15 had CD64 available: 9/15 (60%) had high CD64 (both molecules expressing CD64 per neutrophil and the percentage of CD 64+ neutrophils) and 6/18 (33.3%) had high PCT. Of the 9 that were high on CD64, 5 were also high on PCT.

Discussion

In this prospective, single center, double blind study of diagnostic accuracy for sepsis in patients hospitalized in ICU, we demonstrated that CRP, CD64 expression on neutrophils and PCT were all good discriminators of sepsis. The CD45+ ALC and the frequency of monocytes expressing HLA-DR as well as HLA-DR expression on monocytes while significantly lower were not sufficiently good as discriminators for sepsis; and the same was true for the WBC. The expression of CD163 on monocytes was similar between the groups and this held true when the target condition, sepsis, was restricted to confirmed infection.

Overall, CRP, CD64 and PCT separately were good indicators for sepsis and infection and neutrophil CD64 expression added modestly to the accuracy when only confirmed infection was included.

The definition of sepsis remains challenging and new criteria are being introduced [35]. Currently, patients have sepsis if they have infection and at least two SIRS criteria. This may exclude patients who are otherwise similar in term of infection and outcome [8]. Moreover, the current sepsis definition requires the presence or the suspicion of a source of infection which may not always been confirmed microbiologically. This may in turn lead to either delay in administration or overtreatment with antibiotics. The host response to infection is a key player in sepsis but the use of biomarkers remains uncertain [36]. Here we show that, in ICU patients with sepsis according to the current criteria, and using a control group of patients also in need of ICU admission but not *a priori* in septic state, CRP, a classical marker of inflammation and always readily available, is a good discriminator of sepsis. Clinical implication of our findings suggest that in patients in whom sepsis or infection is in doubt, a readily available inflammatory biomarker (CRP) can meaningfully improve post-test probability of sepsis/infection diagnosis early in the course of critical illness. The small number of false positive results was explained by known inflammatory conditions (vasculitis etc) with variable responses to CD64 and PCT; given the small number, no firm conclusion could be drawn in this very limited subset. The combination of CRP, PCT and CD64 appears promising, but the need for flow cytometry may reduce its immediate impact in critical situation

This study has several strengths. First, the laboratory tests show relevance to clinical practice. The selection criteria included sites of infection typical in sepsis, representative of the continuum of sepsis severity and also included patients with SIRS without infection or infection without sepsis. Second, to guarantee the validity of this study, we followed both Standards for Reporting of Diagnostic Accuracy (STARD) [37] and the Quality Assessment of Diagnostic Accuracy Studies checklist [38]. We chose infection as reference standard and sepsis as target condition, knowing that infection would not be confirmed in every septic case. To compensate for this lack of confirmation of infection on every patient, those who were not *a priori* infected underwent one blood culture coupled with the index test. To prevent verification bias, clinicians, immunologists and biostatisticians were blinded to the individual sets of data until study completion. Before unmasking the results, both diagnoses of sepsis and infection were reviewed by independent clinicians until a consensus was obtained. The reference standard (e.g. blood culture for infection) was part of the clinical routine, independent and blinded to the immunologist performing the index test. To limit spectrum bias, we screened every new admission in ICU within 24 hours and enrolled not only patients with sepsis but also patients symptomatic enough to be admitted to ICU but without evidence of sepsis. Sample size was adequately powered.

This study has also some limitations. The diagnostic accuracy was higher with proven infection, reflecting the lack of a gold standard for sepsis [29]. Samples were collected after initiation of antibiotic therapy which may have accounted for a lower diagnostic performance when compared to the literature [14, 18]. Neutrophil CD64 expression may be affected by use of antibiotics. Since almost all the patients in the septic group received

antibiotics compared to the less than half of them nonseptic group, it is unclear as to what extent prior antibiotic use may have affected CD64 expression, especially in the “borderline” cases, though it appears there can be a difference in CD64 expression on neutrophils in the context of relevant versus empirical antibiotic therapy [25].

Standardizing a quantitative flow cytometry assay for clinical use may be challenging. Minor fluctuations in instrument performance, that may not have a significant effect on semi-quantitative assays, could have a larger impact on a quantitative assay. Absolute quantitation was only used for major cell subsets, such as lymphocytes (T cells, B cells and NK cells) and monocytes using a standard and well-validated method of bead-based direct quantification. For the CD64 and the HLA-DR quantitation on neutrophils and monocytes respectively, a standard curve was used for calculating the molecules per cell, which was generated each time the assay was run, similar to the diagnostic application of standard immunoassays. One of the components required to standardize clinical flow cytometers is the use of application settings, which ensures the mean fluorescence intensity (MFI) of any signal measured is consistent from day to day independent of minor variations in instrument performance thereby ensuring consistency of analytical data even during serial monitoring of patients. The difference in neutrophil CD64 expression was quite large between the septic and nonseptic group: 1945.8 molecules/neutrophil, with a median (IQR) of 2486.2 (1084-5775.2) when sepsis was present and of 540.4 (387.6-957.5) when sepsis was absent. By taking an approximate coefficient variation of 2%, for a value close to the cut-off, we might expect a level of 1040.5 $\pm$ 20 molecules/neutrophil (1020.5 to 1060.5); this variation is relatively modest and should have a comparatively small contribution to either false positive or false negative cases.

The direct impact on patient management, outcome and cost-effectiveness of this panel [39, 40] was not studied since the goal here was to define which biomarker (s) and/or combination of markers would be the most accurate to diagnose sepsis.

Conclusions

In a targeted population at increased risk of sepsis, CRP improves the diagnosis accuracy compared to clinical findings alone or to PCT and different markers of activation of the innate immune response (e.g. CD64 expression on circulating neutrophils). In the presence of sepsis where infection has not yet been confirmed, an immediate CRP, which is readily available, combined with PCT, and neutrophil CD64 measured by flow cytometry, which would usually be available 4-6 hours after sample collection, may lead to a more accurate diagnosis and thus a more appropriate and individualized care of critically ill septic patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Sarah M. Jenkins contributed to conception, design, statistical analysis, and interpretation of data, review, and final approval of the manuscript.

Carin Y. Smith contributed to statistical analysis, and interpretation of data, review, and final approval of the manuscript.

Roshini S. Abraham contributed to conception, design, analysis, and interpretation of data, draft, review, and final approval of the manuscript. She is also a guarantor of the paper, taking responsibility for the integrity of the work as a whole, from inception to published article.

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Abbreviation List

APACHE IV	Acute Physiology and Chronic Health Evaluation IV score
AUC	Area Under the Curve
CD45+ ALC	Absolute lymphocyte count by flow cytometry
CD64	Cluster of Differentiation 64 antigen
CD163	Cluster of Differentiation 163 antigen
CREST syndrome	Limited scleroderma
CRP	C-Reactive Protein
DOR	Diagnostic Odds Ratio
G-CSF	Granulocyte Colony-Stimulating Factor receptor
HLA-DR	Human Leukocyte Antigen complex
ICU	Intensive Care Unit
LR	likelihood Ratio
NPV	Negative Predictive Value
PCT	Procalcitonin
PPV	Positive Predictive Value

ROC	Receiver Operating Characteristic curve
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment score
WBC	White Blood Cell count

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Key point

- C-reactive protein, procalcitonin and neutrophil CD64 individually accurately discriminate early sepsis and infection in the critically ill patients.
- The combination of C-reactive protein, procalcitonin and neutrophil CD64 improves the diagnostic accuracy of sepsis.

Highlights

- This was a prospective, single center, double blind observational study of diagnostic accuracy of an inflammatory and immune panel in sepsis.
- C-reactive protein, procalcitonin and neutrophil CD64 individually accurately discriminated early sepsis and infection in the critically ill patients.
- The absolute lymphocyte count by flow cytometry (CD45+) and the frequency of monocytes expressing HLA-DR as well as HLA-DR expression on monocytes while significantly lower were not sufficiently good as discriminators for sepsis.
- The combination of C-reactive protein, procalcitonin and neutrophil CD64 improved the diagnostic accuracy of sepsis.

Figure 1.

Flow diagram for screening and eligibility (sepsis was defined by 2 or more SIRS criteria and suspected or present source of infection and included sepsis, severe sepsis and septic shock criteria)

Figure 2. Neutrophil CD64 expression [log scale] and sepsis. Note the reference line at the cutoff (1040.5 molecules/neutrophil)

Table 1

Clinical characteristics and sepsis (ns, not statistically significant)

Characteristics	Sepsis absent N=99	Sepsis present N=120	P-value
Demographics			
Age, years, median (IQR)	59 (49-67)	66 (56-76)	0.0006
Male (%)	54 (55)	65 (54)	ns
White (%)	92 (93)	114 (95)	ns
Not Hispanic or Latino (%)	95 (96)	115 (96)	ns
BMI, median (IQR)	26 (22-34)	31 (26-38)	ns
Smoking, ever (%)	70 (71)	74 (62)	ns
Comorbidities			
COPD (%)	13 (13)	24 (20)	ns
Cancer (%)	13 (13)	22 (18)	ns
Metastatic cancer (%)	3 (3)	11 (9)	ns
Hematological cancer (%)	2 (2)	8 (7)	ns
Insulin-dependent diabetes mellitus (%)	25 (25)	17 (14)	ns
Heart failure (NYHA III-IV) (%)	9 (9)	10 (8)	ns
Chronic renal failure (%)	20 (20)	23 (19)	ns
Cirrhosis (%)	7 (7)	8 (7)	ns
Hepatic failure (%)	2 (2)	4 (3)	ns
Immunosuppression (%)	9 (9)	27 (23)	ns
Steroid therapy prior to admission (%)	9(9)	27 (23)	ns
Reason for ICU admission			
Surveillance/monitoring only (%)	7 (7)	2 (2)	ns
Respiratory (%)	26 (26)	60 (50)	0.0003
Cardiovascular (%)	30 (30)	77 (64)	<0.0001
Renal (%)	13 (13)	36 (30)	ns
Hematological (%)	5 (5)	3 (3)	ns
Digestive/liver (%)	45 (45)	29 (24)	0.0009
Metabolic (%)	26 (26)	10 (8)	0.0004
Trauma (%)	0 (0)	4 (3)	ns
APACHE IV on admission, median (IQR)	57 (43-66)	70 (57-83)	<0.0001
SOFA on admission, median (IQR)	7 (5-9)	8 (7-11)	<0.0001
ICU mortality (%)	4 (4)	4 (3)	ns
Hospital mortality (%)	4 (4)	13 (11)	ns
ICU LOS, days, median (IQR)	1 (1-2)	2 (1-3)	ns
Hospital LOS, days, median (IQR)	4 (2-7)	6 (4-9)	0.0001
Discharge home (%)	63 (64)	60 (50)	ns

Table 2

Biomarkers and sepsis (ns, not statistically significant)

Biomarkers	Median (IQR)		P-value
	Sepsis absent N=99	Sepsis present N=120	
<i>Time from admission to blood draw (hours)</i>	12.6 (9.4-19)	13 (9.4-17.8)	ns
WBC, lowest, 0-24 hours ($\times 1,000/\text{mm}^3$)	8 (6.4-10)	10.8 (7.4-14.7)	0.0002
WBC, highest, 0-24 hours ($\times 1,000/\text{mm}^3$)	10.3 (8.6-14.2)	15.4 (10.4-20)	<0.0001
C-reactive Protein (mg/dL)	14.3 (2.8-38.7)	136.7 (46.1-201.8)	<0.0001
Procalcitonin, ng/mL	0.2 (0.1-0.7)	3.7 (0.6-16)	<0.0001
CD45, absolute lymphocytes (10^3 cells/ μL)	1129.3 (790.6-1665.8)	595.4 (383.2-915.6)	<0.0001
CD64, molecules/neutrophil	540.4 (387.6-957.5)	2486.2 (1084-5775.7)	<0.0001
CD64, % positive neutrophils	25.3 (8.7-50.6)	90.8 (48.2-98.2)	<0.0001
CD163, monocyte MFI	4.5 (1.4-12.2)	7.4 (1.8-20.7)	ns
HLA-DR, molecules/monocyte	17273.5 (11243.2-25116.6)	12127.6 (7148.5-18458.7)	0.0009

Table 3

Measures of diagnostic accuracy for predicting sepsis, severe sepsis and septic shock (sepsis) and presence of source of infection microbiologically proven (confirmed infection) (AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; LR, likelihood ratio; DOR, diagnostic odds ratio)

Target and Analyte	N	AUC	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	+ LR	- LR	DOR
Sepsis										
C-reactive Protein (mg/L)	208	0.86	43.00	76.9% (68.5-83.6)	76.9% (67.3-84.4)	72.2% (62.6-80.1)	81.1% (72.8-87.3)	3.33	0.30	11.08
CD64 Molecules/Neutrophil	196	0.83	1,040.5	76.4% (67.7-83.4)	76.7% (66.7-84.4)	71.7% (61.8-79.9)	80.8% (72.2-87.2)	3.28	0.31	10.66
Procalcitonin	216	0.82	0.74	73.1% (64.5-80.3)	74.2% (64.7-81.9)	69.2% (59.8-77.3)	77.7% (69.1-84.4)	2.83	0.36	7.82
%CD64+ Neutrophils	196	0.81	49.96	74.5% (65.6-81.7)	74.4% (64.3-82.4)	69.6% (59.6-78.1)	78.8% (70.0-85.6)	2.91	0.34	8.49
CD45+ Lymphocytes	194	0.74	835.3	67.9% (58.7-75.9)	68.2% (57.7-77.1)	62.4% (52.2-71.6)	73.3% (63.9-81.0)	2.14	0.47	4.54
WBC, Highest, 0-24 hours ($\times 1000/\text{mm}^3$)	219	0.68	12.60	65.8% (56.9-73.7)	66.7% (56.9-75.2)	61.7% (52.2-70.4)	70.5% (61.5-78.2)	1.98	0.51	3.85
WBC, Lowest, 0-24 hours ($\times 1000/\text{mm}^3$)	219	0.65	9.10	61.7% (52.8-69.9)	62.6% (52.8-71.5)	57.4% (48.0-66.3)	66.7% (57.5-74.8)	1.65	0.61	2.70
HLA-DR Molecules/Monocyte	196	0.64	14,472	59.1% (49.8-67.8)	60.5% (49.9-70.2)	53.6% (43.7-63.2)	65.7% (55.9-74.3)	1.50	0.68	2.21
CD163 Monocyte MFI	196	0.57	5.90	55.5% (46.2-64.4)	55.8% (45.3-65.8)	49.5% (39.8-59.3)	61.6% (51.8-70.6)	1.26	0.80	1.57
Infection (confirmed)										
C-reactive Protein (mg/L)	172	0.88	49.90	78.2% (68.4-85.6)	77.6% (67.7-85.2)	77.6% (67.7-85.2)	78.2% (68.4-85.6)	3.49	0.28	12.43
CD64 Molecules/Neutrophil	165	0.86	1,084.0	79.8% (70.0-87.0)	80.2% (70.2-87.4)	79.3% (69.3-86.7)	80.7% (70.9-87.8)	4.03	0.25	16.00
Procalcitonin	179	0.84	0.84	74.4% (64.5-82.3)	74.2% (64.2-82.2)	74.2% (64.2-82.2)	74.4% (64.5-82.3)	2.88	0.35	8.36
%CD64+ Neutrophils	165	0.85	53.83	77.4% (67.4-85.0)	77.8% (67.6-85.5)	76.8% (66.6-84.6)	78.3% (68.3-85.8)	3.49	0.29	12.00
CD45+ Lymphocytes	164	0.76	844.3	67.9% (57.3-76.9)	67.5% (56.6-76.8)	66.7% (55.9-76.0)	68.7% (58.1-77.7)	2.09	0.48	4.39

Target and Analyte	N	AUC	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	+ LR	– LR	DOR
WBC, Highest, 0-24 hours ($\times 1000/\text{mm}^3$)	182	0.65	12.80	62.6% (52.3-71.8)	63.7% (53.5-72.8)	63.0% (52.8-72.2)	63.3% (53.0-72.5)	1.72	0.59	2.94
WBC, Lowest, 0-24 hours ($\times 1000/\text{mm}^3$)	182	0.60	9.20	58.2% (47.9-67.8)	58.2% (47.9-67.8)	58.2% (47.9-67.8)	58.2% (47.9-67.8)	1.39	0.72	1.94
HLA-DR Molecules/Monocyte	165	0.64	13.965	60.7% (50.0-70.5)	60.5% (49.6-70.4)	59.8% (49.0-69.7)	61.4% (50.6-71.1)	1.54	0.65	2.37
CD163 Monocyte MFI	165	0.58	5.90	56.0% (45.4-66.1)	55.6% (44.8-65.9)	54.9% (44.2-65.2)	56.6% (45.9-66.7)	1.26	0.79	1.59