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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 intensive care unit (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 Acute Physiology and Chronic Health Evaluation (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.

1. Introduction

Sepsis remains a major burden worldwide with high incidence (Martin et al., 2003) and prevalence (Vincent et al., 2009). Mortality may be on the decline (Kaukonen et al., 2014), but low awareness, late recognition, and late treatment are still common (Reinhart et al., 2013). C-reactive protein (CRP) (Simon et al., 2004) and procalcitonin (PCT) (Wacker et al., 2013) have shown conflicting results and impact studies are rare (Bouadma et al., 2010). A constant clinical challenge is to make an early distinction between systemic inflammatory response syndrome (SIRS) and sepsis (Kaukonen et al., 2015) 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 (Kumar et al., 2006). Therefore, the need for early biomarkers of infection with sepsis is justified in term of rapid diagnosis, and appropriate management strategies (Samraj et al., 2013).

Neutrophils play an important role in the innate immune response to infection (Kolaczkowska and Kubes, 2013). Cluster of differentiation 64 (CD64, FcγRI) antigen is involved in neutrophil phagocytosis, oxidative burst, and target killing (Hirsh et al., 2001) and has been promising in the early detection of bacterial infection (Cid et al., 2010; Hsu et al., 2011; Icardi et al., 2009; Li et al., 2013; Livaditi et al., 2006; Wang et al., 2015). Monocyte/macrophages are also key players in the innate immune response to infection. CD64 is also constitutively expressed

Abbreviations: 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; HLA-DR, Human Leukocyte Antigen complex; ICU, Intensive Care Unit; LR, Likelihood Ratio; NPV, Negative Predictive Value; PCT, Procalcitonin; PPV, Positive Predictive Value; SIRS, Systemic Inflammatory Response Syndrome; SOFA, Sequential Organ Failure Assessment score; WBC, White Blood Cell count.

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on monocyte/macrophage cells and further upregulated in the context of sepsis (Danikas et al., 2008). CD163 (haptoglobin–hemoglobin complex receptor) expression, restricted to monocyte/macrophages, belongs to the scavenger receptor cysteine-rich domain superfamily class B (Van Gorp et al., 2010). 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 (Fabriek et al., 2009). Human leukocyte antigen complex (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 (Fumeaux and Pugin, 2006). The loss of HLA-DR expression on monocytes reflects impaired monocyte activation, and appears to be correlated with the development of sepsis and outcome (Monneret et al., 2006).

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 (Gros et al., 2012). The combination of CRP with CD64 (Dimoula et al., 2014) and PCT (Cardelli et al., 2008; Gibot et al., 2012) 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.

2. Materials and methods

2.1. 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 (Fig. 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.

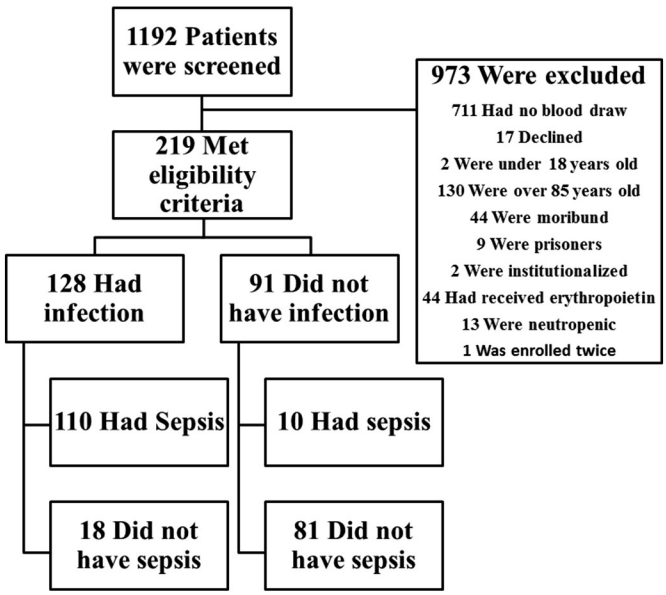

Fig. 1. Flow diagram for screening and eligibility (sepsis was defined by 2 or more SIRS criteria and suspected or present source of infection and included sepsis, severe sepsis, and septic shock criteria).

2.2. Test methods

The reference standard was infection (Bone et al., 1992). The target condition was sepsis based on current consensus definitions (Levy et al., 2003) 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 (Zimmerman et al., 2006), Sequential Organ Failure Assessment (SOFA) score (Vincent et al., 1996), microbiology, treatment, and outcome variables were prospectively collected and managed using REDCap (Research Electronic Data Capture) (Harris et al., 2009). 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 μL 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 1 mL of BD Stain Buffer, resuspended in 500 μL 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.

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