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Biomarker-Based Differentiation of Gram-Negative and Gram-Positive Bacteremia in Intensive Care Unit Patients

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ABSTRACT

Background: Rapid differentiation between gram-negative and gram-positive bacteremia is crucial for optimizing antimicrobial therapy in critically ill patients. This study aimed to evaluate the diagnostic utility of biomarkers, including C-reactive protein (CRP), Interleukin-6 (IL-6), soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), and leukocyte counts, in distinguishing gram-negative bloodstream infections (BSIs) from gram-positive BSIs, which are more common than gram-positive BSIs in ICU patients.

Objectives: The objectives of this study were to evaluate the diagnostic utility of inflammatory biomarkers, including C-reactive protein (CRP), Interleukin-6 (IL-6), soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), and leukocyte counts, in differentiating gram-negative and gram-positive bloodstream infections (BSIs) in ICU patients, individually and in combination.

Methods: A prospective observational study was conducted on 54 ICU patients aged 18–78 admitted to a university-affiliated hospital between September 2023 and August 2024. Blood culture-confirmed BSIs included 31 gram-negative and 23 cases of gram-positive bacteremia. Biomarkers were measured on the second day of ICU admission to minimize the confounding effects of early interventions. Statistical analyses included the Mann-Whitney U test for group comparisons and the receiver operating characteristic (ROC) curve analysis to evaluate diagnostic performance.

Results: CRP, sTREM-1, and leukocyte counts were significantly higher in gram-negative bacteremia compared to gram-positive bacteremia (p=0.023, p=0.007, and p=0.017, respectively), while IL-6 showed no significant difference (p=0.407). ROC curve analysis revealed moderate discriminatory power for sTREM-1 (AUC=0.719), leukocyte counts (AUC=0.676), and CRP (AUC=0.646). IL-6 exhibited poor discrimination (AUC = 0.439). A combined biomarker model achieved an improved AUC of 0.782, highlighting the potential of a multi-marker approach.

Conclusions: Among the biomarkers studied, sTREM-1 and leukocyte counts demonstrated the highest utility in distinguishing gram-negative from gram-positive bacteremia. The combined use of CRP, IL-6, sTREM-1, and leukocyte counts further enhanced diagnostic accuracy, underscoring the value of a multi-marker strategy in ICU settings. Future studies with larger cohorts are recommended to validate these findings and explore their clinical application.

Keywords: Biomarkers; bloodstream infection; C-reactive protein (RP); gram-negative bacteremia; gram-positive bacteremia; intensive care unit (ICU); interleukin-6 (IL-6); leukocyte counts; soluble triggering receptor expressed on myeloid cell-1 (sTREM-1).

BACKGROUND

Bloodstream infections are a significant cause of morbidity and mortality in critically ill patients, particularly those admitted to intensive care units.[1] Rapid identification of the infectious pathogen is crucial for initiating antimicrobial treatment. Delays in administering the appropriate therapy have been linked to higher mortality rates, extended hospitalizations, and the development of antibiotic resistance.[2] One of the key challenges in managing BSIs is distinguishing between gram-negative and gram-positive bacterial infections, as their clinical manifestations often overlap, and culture-based identification methods require 24 to 48 hours, leading to delays in optimal therapy.[3]

Gram-negative and gram-positive bacteria differ in their structural components and pathogenic mechanisms, influencing host immune responses.[4] Gram-negative bacteria possess lipopolysaccharides in their outer membrane, triggering strong inflammatory responses via the Toll-like receptor four pathway.[5] In contrast, gram-positive bacteria contain peptidoglycan and lipoteichoic acids that activate the immune system primarily through Toll-like receptor 2.[6] These differences in pathogen-associated molecular patterns result in distinct immune activation profiles, which can potentially be leveraged for rapid biomarker-based differentiation of bacteremia types.

Several inflammatory biomarkers have been studied for their utility in distinguishing gram-negative from gram-positive infections.[7] C-reactive protein, Interleukin-6, soluble triggering receptor expressed on myeloid cells-1, and leukocyte counts are potential candidates due to their roles in systemic inflammation and immune regulation. CRP is an acute-phase reactant induced by interleukin-6 and is commonly used as a marker of infection and inflammation.[8] IL-6 is a pro-inflammatory cytokine released in response to infection, although its concentration can be influenced by many factors, causing its differentiation ability to vary.[9] Soluble triggering

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receptor expressed on myeloid cell-1 (sTREM-1) is a transmembrane receptor expressed on innate immune cells and is known to be upregulated in bacterial infections, particularly gram-negative sepsis.[10] Leukocyte counts reflect immune activation and may fluctuate based on the severity and type of disease.[11]

Despite their potential, the individual diagnostic performance of these biomarkers remains inconsistent in differentiating between gram-negative and gram-positive bacteremia. Previous studies have reported varying sensitivity and specificity levels, suggesting that a multi-marker approach may improve diagnostic accuracy.[12] By combining biomarkers that reflect different aspects of the immune response, we may enhance early pathogen differentiation, allowing clinicians to tailor antimicrobial therapy more effectively.

This study explores the diagnostic utility of CRP, IL-6, sTREM-1, and leukocyte counts to distinguish gram-negative from gram-positive BSIs in ICU patients. By assessing their individual and combined performance, we seek to determine whether a biomarker-based strategy can help with the early differentiation of bloodstream infections. If successful, this approach would optimize empirical antibiotic selection, reduce inappropriate antimicrobial exposure, and improve patient outcomes in critically ill individuals.

METHODS

This prospective observational study was conducted at a university-affiliated hospital over 12 months, from September 2023 to August 2024. The study enrolled 54 adult patients admitted to the intensive care unit (ICU), aged between 18 and 78 years, who were clinically suspected of sepsis and met the criteria for systemic inflammatory response syndrome (SIRS) or sepsis. Blood culture results confirmed the presence of bloodstream infections (BSIs), with 31 patients identified as having gram-negative bacteremia and 23 as having gram-positive bacteremia.

Biomarkers evaluated in this study included C-reactive protein (CRP), Interleukin-6 (IL-6), soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), and leukocyte counts. These biomarkers were chosen for their known roles in systemic inflammation and their potential to differentiate between gram-negative and gram-positive infections.

To ensure consistency and minimize the effects of early ICU interventions, biomarker measurements were taken on the second day following ICU admission. This timing reduced confounding influences from treatments such as fluid resuscitation, antibiotic administration, and mechanical ventilation, providing a more precise reflection of the infection-related immune response.

Patients were excluded if they met any of the following: Diagnosed hematological malignancies or other hematological diseases that could alter biomarker levels, Ongoing chemotherapy, ongoing corticosteroid therapy, and HIV, Hepatitis B virus (HBV), and Hepatitis C virus (HCV) infection.

This study design ensured the inclusion of patients with well-defined clinical presentations of gram-negative or gram-positive BSIs while reducing confounding factors that might influence biomarker levels. By combining biomarkers with distinct roles in systemic inflammation and immune activation, the study aimed to evaluate their individual and combined diagnostic utility in differentiating these two types of bacteremia in critically ill patients.

Biomarker analysis

The analysis of biomarkers included CRP, IL-6, and sTREM-1. CRP levels were assessed routinely through standard biochemical assays in clinical care. IL-6 concentrations were measured using an electrochemiluminescence method on the Cobas e411 analyzer (Elecsys IL; Roche Diagnostics GmbH, Mannheim, Germany), with a minimum detection threshold of 1.5 pg/mL. sTREM-1 levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine ELISA Test, Wuhan Fine Biotech, Wuhan, China), with a detection limit of 18.75 pg/mL.

Blood cultures were monitored using the Oxoid Signal™ manual blood culture system (Thermo Fisher Scientific). Bacterial and fungal isolates were identified at the species level using the API identification system (Biomerieux, France). ICU-acquired bloodstream infections (BSIs) were defined as infections diagnosed in patients more than 48 hours after ICU admission, confirmed by one or more blood cultures positive for pathogenic microorganisms. Blood culture specimens were ordered by attending physicians based on clinical suspicion of sepsis, septic shock, or other infections identified during patient evaluations and clinical rounds.

The date of the blood sample collection was considered the onset of BSI. Bacteremia was defined as isolating bacterial species from blood cultures, while candidemia referred to Candida species in the blood sample.

Statistic analysis

Statistical analyses were performed to compare biomarker levels (CRP, IL-6, sTREM-1, and leukocyte counts) between gram-negative and gram-positive bacteremia groups and evaluate their diagnostic performance. Due to the non-normal data distribution, the Mann-Whitney U test was used to summarize continuous variables using mean ranks and compare between groups. P-values less than 0.05 were considered statistically significant.

The diagnostic ability of each biomarker to differentiate gram-negative from gram-positive bacteremia was assessed using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) was calculated individually for CRP, IL-6, sTREM-1, leukocyte counts, and their combined predictive model. The combined biomarker model was analyzed to evaluate the potential improvement in diagnostic accuracy over individual biomarkers. All statistical tests were conducted using SPSS version 27.0.

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RESULTS

The study included 54 ICU patients with bloodstream infections (BSIs), including 31 gram-negative bacteremia and 23 cases of gram-positive bacteremia. Biomarker levels were compared between the two groups using the Mann-Whitney U test, and their diagnostic performance was assessed through receiver operating characteristic (ROC) curve analysis.

The Mann-Whitney U test demonstrated significant differences in C-reactive protein (CRP) levels and soluble triggering receptors expressed on myeloid cells-1 (sTREM-1). Leukocyte counts between gram-negative and gram-positive bacteremia (Fig.1 and Tabl.1). CRP levels were significantly elevated in patients with gram-negative bacteremia compared to those with gram-positive bacteremia (p=0.023). Similarly, sTREM-1 levels were considerably higher in the gram-negative group (p=0.007), indicating a stronger immune activation response associated with gram-negative infections. Leukocyte counts also showed a statistically significant increase in gram-negative bacteremia compared to gram-positive cases (p=0.017). In contrast, IL-6 levels did not differ significantly between the two groups (p=0.407), suggesting its limited discriminatory power in differentiating gram-negative from gram-positive bloodstream infections.

FIGURE 1. Mean ranks comparison for gram-negative and gram-positive groups

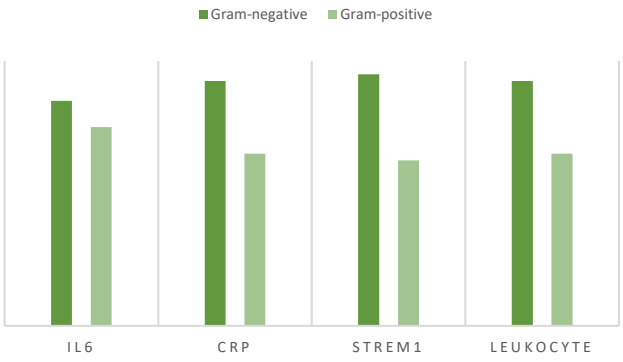


TABLE 1. Mann-Whitney test results

Variable	Gram-negative mean rank	Gram-positive mean rank	Mann-Whitney U	p-value
IL6	34.26	30.38	446	0.407
CRP	37.31	26.69	339	0.023
STREM-1	38.17	25.66	309	0.007
Leukocyte	37.57	26.38	330	0.027

Abbreviations: C-reactive protein (RP); interleukin-6 (IL-6); soluble triggering receptor expressed on myeloid cell-1 (sTREM-1).

Besides that, the Mann-Whitney U test results for predicted probabilities indicate a significant difference in the mean ranks between gram-negative and gram-positive bacteremia cases ($p < 0.001$). Specifically, the higher mean rank observed in the gram-negative group (42.38) compared to the gram-positive group (24.31) suggests that the predicted probabilities significantly differed between the two bacterial classifications (Fig.2 and Tab.2).

FIGURE 2. Mean ranks for predicted probabilities

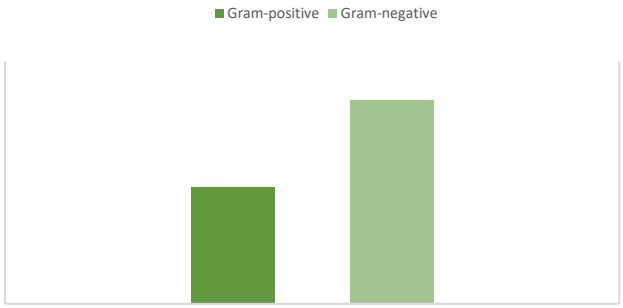
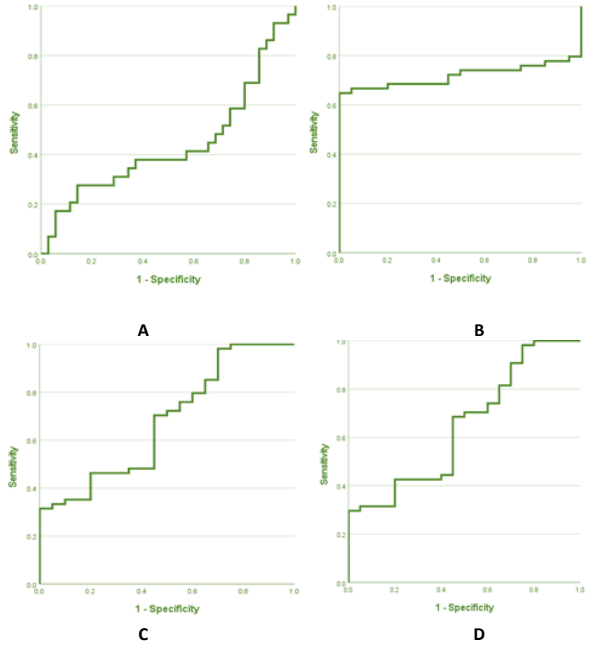


TABLE 2. Mann-Whitney test results

Variable	Mean Rank	Mann-Whitney U	p-value
Gram-positive	24.31	-	-
Gram-negative	42.38	221.0	<0.001

The diagnostic accuracy of each biomarker was evaluated using ROC curve analysis. sTREM-1 exhibited the highest area under the curve (AUC=0.719), followed by leukocyte counts (AUC=0.676) and CRP (AUC=0.646), indicating moderate discriminatory ability in distinguishing gram-negative from gram-positive infections. IL-6 had an AUC of 0.439, reflecting poor diagnostic utility (Fig.3 and Tab.3).

FIGURE 3. ROC curves comparing diagnostic performance of individual biomarkers for differentiating gram-positive from gram-negative bacteremia



Explanations: A. IL6; B. sTREM-1; C. leukocyte; D. CRP.

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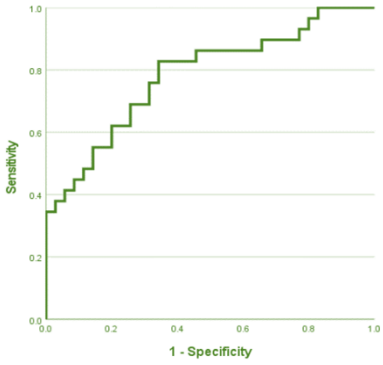
TABLE 3. ROC curves of AUC Values

Variables	AUC
IL6	0.439
CRP	0.646
STREM-1	0.719
Leukocyte	0.676
Combined	0.782

Abbreviations: C-reactive protein (RP); interleukin-6 (IL-6); soluble triggering receptor expressed on myeloid cell-1 (sTREM-1).

A combined biomarker model incorporating CRP, IL-6, sTREM-1, and leukocyte counts demonstrated improved diagnostic performance, achieving an AUC of 0.782 (Fig.4 and Tab.3). This result highlights the advantage of a multi-marker approach in enhancing the accuracy of early pathogen differentiation in ICU patients with bacteremia.

FIGURE 4. ROC curves of diagnostic performance of the combined model for differentiating gram-positive from gram-negative bacteremia



Among the biomarkers studied, sTREM-1 and leukocyte counts provided the highest individual discriminatory ability for identifying gram-negative bloodstream infections. CRP, while statistically significant, exhibited only moderate diagnostic power. The lack of significant differences in IL-6 levels between the two groups suggests it is not a reliable marker for this specific differentiation. Combining all four biomarkers resulted in the highest diagnostic accuracy, reinforcing the potential value of a multi-marker strategy in clinical practice.

DISCUSSION

Sepsis is a life-threatening condition resulting from the widespread release of proinflammatory mediators in response to infection, leading to an excessive, generalized immune response. According to the Global Burden of Disease study, an estimated 48.9 million incidents of sepsis were reported in 2017.[13] The increasing incidence is likely explained by advancing population age, immunosuppression, and the rise of multidrug-resistant bacterial strains.[14] Mortality ranges from 10-52% according to different studies and is responsible for 6% of all deaths.[15] Moreover, patients who survive sepsis often face long-term disability and lower quality of life.[16]

Gram-positive bacteria are most commonly identified pathogens in cultures of septic patients, while gram-negative bacteria account for 25-30% of bloodstream infections.[17] However, approximately one-half of cases of sepsis are culture-negative.[18] Early resuscitation and antimicrobial therapy are the cornerstone of treatment, and clinical management can be challenging, particularly when identifying causative bacteria to guide appropriate antibiotic therapy. In this context, inflammatory markers offer a promising avenue for improving diagnostic accuracy and tailoring treatment. Unique components of their cell walls primarily drive the differential inflammatory responses to gram-negative and gram-positive bacteria. Gram-negative bacteria contain lipopolysaccharide (LPS), a potent activator of the immune system, while gram-positive bacteria primarily stimulate immune response through other outer molecular patterns. This distinction of pathogen-associated molecular patterns (PAMPs) likely explains the differences in inflammatory markers in gram-positive and gram-negative sepsis.

A study in Japan involving 259 patients admitted to the ICU with sepsis demonstrated significantly higher levels of IL-6 and CRP in patients with gram-negative bacteremia than gram-positive bacteremia.[19] This aligns with our study's findings, which demonstrated significantly elevated CRP, sTREM-1, and white blood cell count levels in patients with gram-negative sepsis based on blood draws on the second day of ICU admission. A combined biomarker model including IL-6, sTREM-1, CRP, and white blood cell count yielded higher discriminatory power in predicting gram-negative sepsis, highlighting the potential for a multi-marker approach.

While cultures remain the gold standard for diagnosis, their usefulness is often limited by delayed results and unreliability in some instances, particularly in patients already receiving antibiotic therapy.[20] The multi-marker approach suggested in our study offers a valuable adjunct for physicians to guide clinical decision-making and allow early identification of causative pathogens. This can allow physicians to tailor the antimicrobial treatment regimen to the patient, reduce the adverse effects of broad-spectrum therapy, and improve

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antibiotic stewardship by avoiding excessive broad-spectrum antibiotic use.

Larger-scale studies involving a more diverse population of patients are necessary in the future. Additionally, incorporating bacterial-specific inflammatory markers such as procalcitonin and other inflammatory markers suggested in this study may provide valuable information and increase the discriminatory power.

CONCLUSIONS

This study demonstrates that sTREM-1 and leukocyte counts are the most useful biomarkers for differentiating Gram-negative from Gram-positive bacteremia in ICU patients. CRP shows moderate utility, and IL-6 lacks discriminatory power. Significantly, a multi-marker approach combining CRP, IL-6, sTREM-1, and leukocyte counts significantly improved diagnostic accuracy. These findings support the clinical potential of biomarker-based strategies to aid in early pathogen differentiation and guide timely, targeted antimicrobial therapy. Further research with larger cohorts and additional biomarkers is warranted to validate and enhance this diagnostic approach.

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