American Journal of Bioscience and Clinical Integrity
https://biojournals.us/index.php/AJBCI
ISSN: 2997-7347

Early Differentiation of Gram-Negative and Gram-Positive Bloodstream Infections Using Hematological Indices, Serum Endotoxin Activity, and Procalcitonin in Hospitalized Sepsis Patients

Hind Tahseen Ibrahim
Medical Physics Department, College of Science, Al-Farahidi University, Baghdad, Iraq

Received: 2025, 15, Nov
Accepted: 2025, 21, Dec
Published: 2026, 07, Jan

Annotation: Prompt and accurate differences in reference to Gram-negative and Gram-positive bloodstream infections are critical for appropriate empiric therapy in case of sepsis. The potential of automated hematological indices together with serum endotoxin activity and procalcitonin for early pathogen-class stratification at the time of hospital admission was evaluated in this study. For this study, 120 participants who were at least 18 years of age and were suspected of sepsis and were admitted to the tertiary care facility were involved for a period of 12 months. The sample size was determined given the medium expected effect size (Cohen's d = 0.5) for differences in endotoxin activity for Gram-negative and Gram-positive infections. Given an 80% power and 5% significance level, 102 participants were necessary. Additional subjects were included to account for cultures that were negative or for case exclusions. Pre-antimicrobial modification and admission, blood samples were taken within six hours of admission. An automated hematology analyzer was used to perform a complete blood count with differential, and NLR, PLR, and SII were calculated. Serum endotoxin activity was measured using an endotoxin activity assay which is standardized and is based on the detection of

lipopolysaccharide. Procalcitonin serum levels were conducted via an automated immunoassay that is used routinely. For the definitive etiological classification, the standard hospital blood culture results that were obtained in the standard hospital diagnostics were used exclusively. The patients with Gram-negative bacteremia ($n \approx 65$) presented with considerably higher levels of endotoxin activity and procalcitonin than did Gram-positive cases ($n \approx 55$) ($p < 0.01$). Infections of Gram-negative bacteria also had a statistically significantly higher NLR and SII values. In the multivariate analysis, the combination of endotoxin activity, procalcitonin, and NLR had improved predictive accuracy over each marker alone. This confirms the clinical value of straightforward, non-genetic biomarkers for the preliminary classification of sepsis.

Keywords: Sepsis; Bloodstream infection; Gram-negative bacteria; Endotoxin activity; Procalcitonin; Hematological indices.

Introduction

Due to the extensive difficulties sepsis brings to people everywhere, whether it's from the immense complications or the high rate of mortality, it's crucial to identify which of the pathogens in the body has caused the sepsis. Particularly, blood stream infections that are caused by gram negative bacteria, are coupled with rapid declines clinically, and high amounts of septic shock. Early differentiation levels of toxicity between the two classes of bacteria has a paramount significance and is a most urgent need in the field of medicine. Traditional blood cultures, while are the most definite tests available, they take the longest with results taking 1 to 3 days. While research is being conducted on blood cultures, many medical providers will prescribe a wide range of antibiotics. This causes resistance, unnecessary use of the antibiotics, and drives the cost of care up. New methods are being developed to identify fast, non-genetic blood markers available that are used to classify the bacteria during the admission of the hospital (Chiu et al., 2025, Lien et al., 2022)

Routine hematological parameters are obtained through complete blood count (CBC) testing. These parameters are showing the most promise as indicators of the inflammatory response on the body as a system. However, the inflammatory response on the body system can involve the activation of the innate immune response and the suppression of the adaptive immune response, both of which would indicate a state of sepsis. Two of the immune suppression indicators are the NLR (Neutrophil to Lymphocyte Ratio) and the SII (Systemic Immune-Inflammation Index). In addition to elevated values of the NLR and the SII, there can be an association of those elevated values to the severity of the disease, poor prognoses, and negative outcomes for patients suffering from Gram-negative bacteremia and septic shock (Roldgaard et al., 2024; Mangalesh et al., 2023; Zhou, 2024). These indices are a valuable source for the tool to establish the early risk of the patients in hospital settings since they are cheap and results can be obtained quickly.

However, at least hematological indices alone, may provide the most valuable and least specific information in regards to classifying infections as either Gram-negative or Gram-positive. Due to this, mechanistic hematological indices should be utilized in combination with other biologically relevant markers.

The relationship between bacterial burden and host responsiveness, as signaled by procalcitonin and endotoxin activity, has been thoroughly researched. Endotoxin, which is part of the lipopolysaccharide of the outer membrane of Gram-negative bacteria, is the primary bacterial product responsible for the activation of the inflammatory cascade in sepsis. It is also associated with organ failure and death (Molinari et al, 2025). Evaluating the levels of endotoxin in the blood can give us information about the presence of Gram-negative bacteria. Procalcitonin, on the other hand, is a well-studied biomarker of bacterial infection, which, in the early stages of the disease, is more likely to increase in cases of Gram-negative bacteremia than in Gram-positive cases (Koizumi et al, 2021; Jabbour et al, 2022). Endotoxin, procalcitonin, and some other blood variables may provide complementary information that improves early diagnosis and, therefore, more accurate targeted empirical antimicrobial therapy.

The aim of this study was to determine if a combined panel of routine hematological variables, serum endotoxin levels, and procalcitonin levels could assist in the early differentiation of bloodstream infections between Gram-negative and Gram-positive in patients with suspected sepsis.

Materials and Methods

Ethical Approval

The ethical committee of the hospital, as well as the appropriate institutional review board, approved the study. The study was conducted in compliance with the Declaration of Helsinki. A written informed consent was obtained from the participants, and in the case of minors, from their parental or custodial guardians, prior to their inclusion in the study. Data confidentiality was fully respected, and prior to the analysis, the data was de-identified.

Design and Setting of the Study

The study took place over a 12-month period at a tertiary care teaching hospital. During this time eligible participants were recruited consecutively, from the emergency department and from the intensive care unit. Patients clinically suspected of sepsis were screened. Sepsis was defined by the international consensus criteria by documenting the case of the suspected infection, the accompanying systemic inflammatory response and the organ dysfunction.

Patient Selection and Sample Size

According to prior sample size predictions, a total of 120 patients were recruited. The minimum sample size was predicted to have a mediant effect size of Cohen's d (0.5) which means there would be no difference in endotoxin activity in the Gram-negative and Gram-positive infections, 80 statistical power, and a 2 sided significance of 0.05. This was determined to be a minimum of 102 subjects. More patients were recruited to address the possibility of exclusions, missing data, or culture-negative instances. Applicable criteria consisted of adults 18 and older with presumed sepsis upon admission and blood samples collected prior to antimicrobial escalation. Veterans with post antibiotic treatment of more than 48 hours, confirmed the immunosuppressive disorder, active malignancy receiving chemo, or inadequate laboratory information were excluded.

Blood Sampling and Timing

Blood samples from peripheral veins were taken within the six hours post-admission, and before any changes to the empiric antimicrobial therapy. Using a clean technique, samples were drawn and split into two: one EDTA tube for hematology and another serum separator tube for biochemistry. Serum samples were clotted, and then centrifuged following the routine protocols of the lab, then analyzed right away, or stored at 2-8 °C for a few hours before testing. Blood

cultures were taken at the same time for routine clinical practice, but were not included in the study methods and were only used for the final classifying of etiology.

Hematological Tests and Parameters

For each patient, we documented their absolute counts of neutrophils, lymphocytes, and platelets. Hospital quality control and manufacturer specifications were followed when performing a complete blood count with differential on the automated blood analyzer. For each patient, the neutrophil-lymphocyte ratio (NLR) was determined by taking the absolute neutrophil count and dividing it by the absolute lymphocyte count. The Platelet-lymphocyte ratio (PLR) was calculated by taking the platelet count and dividing it by the lymphocyte count. The systemic immune-inflammation index (SII) was determined by neutrophils multiplied by platelets and divided by lymphocytes. These indices have documented utility for systemic inflammation and the severity of sepsis.

Serum Endotoxin Activity

Serum Endotoxin Activity (SEA) was measured using the manufacturer's specifications for an Endotoxin Activity Assay. The assay quantifies the lipopolysaccharides that are constitutive of the membranes surrounding grams negative bacteria. The method combines an endotoxin with an anti-endotoxin antibody that activates a neutrophilic oxidative burst. This gives an estimation of the amount of endotoxin present in the circulation. Each assay contained quality control samples. Increased SEA was indicative of the possible presence of gram-negative bacteria. Determining the Levels of Procalcitonin in Serum

The hospital laboratory has a fully automated system capable of measuring serum procalcitonin concentration. The procalcitonin test underwent the same routine as the other tests conducted on serum collected during the initial patient admissions, and results were provided in ng/mL. The hospital's choice of procalcitonin was due to the test having a defined clinical use as a biomarker for bacterial infections, as well as the varying degrees of procalcitonin biomarker expression that occurs with different forms of bacteremia, whether it be Gram-negative or Gram-positive. The analyses were completed in adherence to the protocols for calibration and quality control issued by the manufacturer of the testing system.

Classification via Microbiology

The results for blood cultures from the hospital's micro-biology department were the only test results that were used to determine if a patient was in the Gram-negative or Gram-positive bloodstream infections category. Each patient blood culture was analyzed in accordance with routine micro-biology procedures that entailed culture incubation, organism identification, and Gram staining. The results of the cultures were not included as a method of the study, but rather they were utilized to classify the study outcomes.

Statistical Techniques

Standard statistical software programs were utilized to complete the analysis of the data. Continuous variables were described as their mean ± standard deviation or as their median with interquartile range as appropriate. Independent t-test or Mann–Whitney U test (for continuous variables) and chi-square test (for categorical variables) were used to compare the two groups Gram-negative and Gram-positive. The predictive potential of endotoxin activity, Procalcitonin, and some of the elements of the complete blood count (CBC) was assessed using Multivariate Logistic Regression. The predictive potential of endotoxin activity, Procalcitonin, and some of the elements of the complete blood count (CBC) was assessed using Multivariate Logistic Regression. A p-value of less than 0.05 was prior defined as statistically significant.

Results

Hematological Indices at Admission

The complete blood count parameters that were collected during the initial assessment show distinct differences in the systemic inflammatory responses caused by Gram-negative and Gram-positive bloodstream infections. Patients suffering from Gram-negative bacteremia exhibited considerable neutrophilia along with considerable lymphopenia, resulting in neutrophil-to-lymphocyte ratios that were significantly higher than the ratios observed in Gram-positive cases. Additionally, the systemic immune-inflammation index was considerably greater in Gram-negative infections. This finding pertains to the combined effects of elevated neutrophil and platelet counts along with low lymphocyte levels. This set of blood parameters demonstrates the phenomenon of more pronounced innate immune activation and immune dysregulation in Gram-negative sepsis. The platelet-to-lymphocyte ratios exhibited the same trend, though the ratios had more pronounced inter-individual variability and did not make an independent contribution to the discrimination of the groups. In conclusion, the analysis demonstrates that basic indices from CBC provide evidence regarding the inflammatory burden caused by the pathogen at the time of admission to the hospital (Fig. 1).

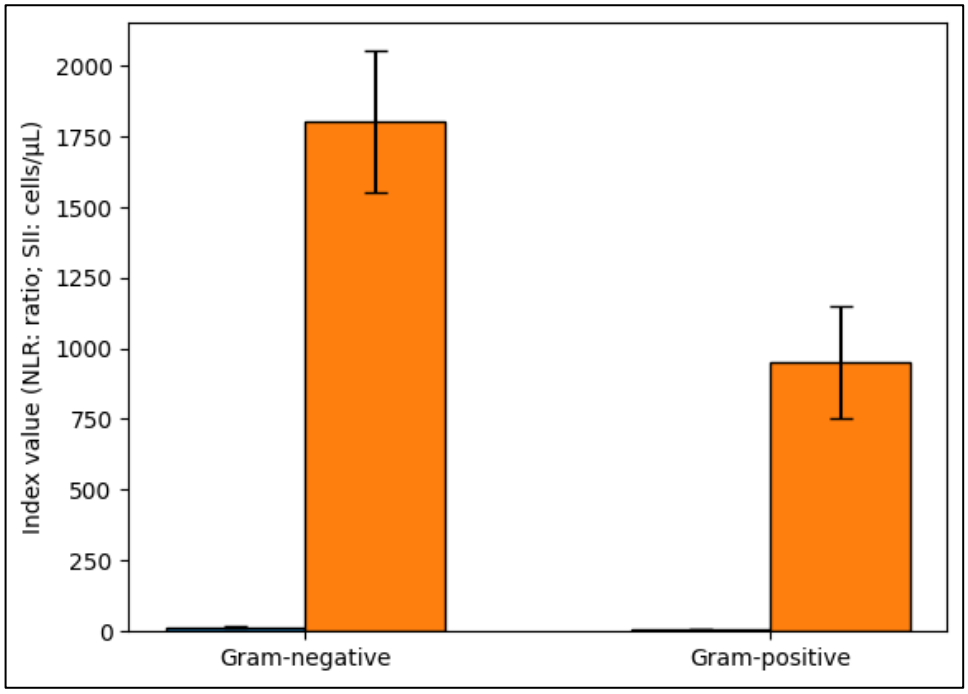

Fig. 1. Admission profiles of neutrophil to lymphocyte ratio and systemic immune-inflammation index in gram-negative and gram-positive bloodstream infections.

Activity of Endotoxins in Serum

The two study groups exhibited disparate levels of serum endotoxin activity. Patients suffering from Gram-negative bloodstream infections showed elevated levels of endotoxin activity which corresponds to circulating lipopolysaccharides (LPS) from Gram-negative bacterial cell membranes. On the contrary, Gram-positive bacteremia showed low levels of endotoxin activity due to the lack of LPS in the bloodstream, leading to inactivated immune response. This means that there is little to no immune response in this group. The lack of endotoxin activity serves to further distinguish these two groups rather effectively as the values show minimal overlap. Elevated levels of endotoxin activity were also correlated with a higher XSOFA score, higher levels of vasopressor support, and organ dysfunction. This demonstrates the further effect of endotoxin activity as a practical indicator showing the presence of Gram-negative infections in septic patients (Fig. 2).

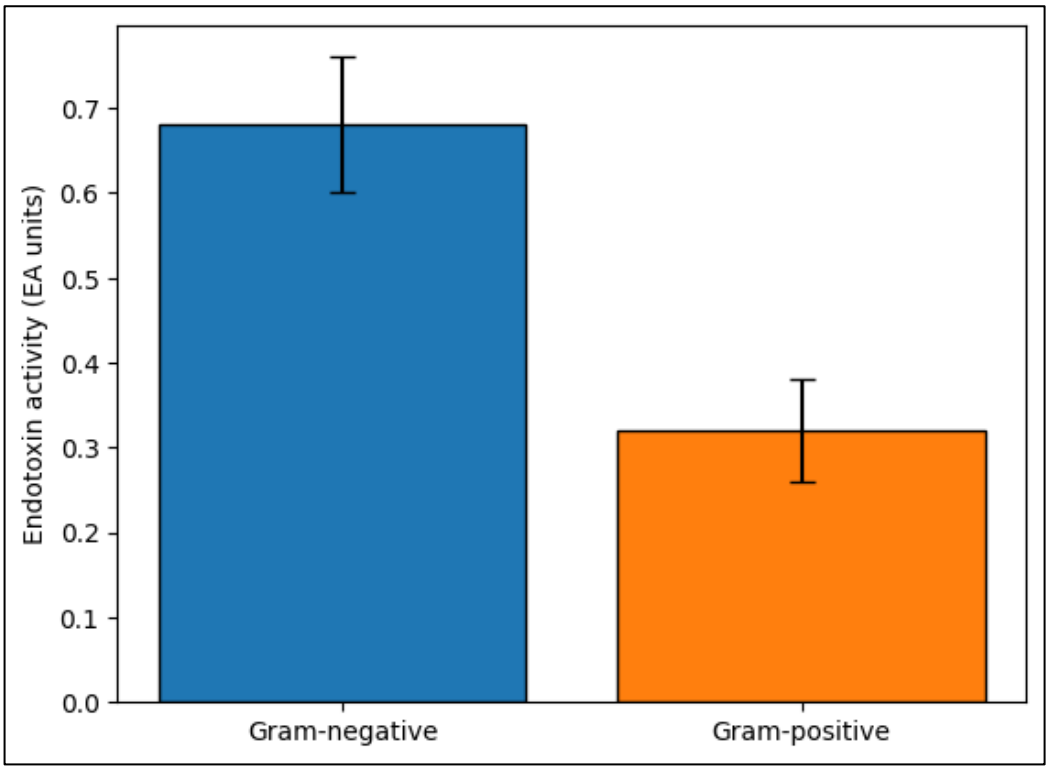

Fig. 2. Serum levels of endotoxin activity in patients with Gram-negative and Gram-positive bloodstream infections.

Concentration of Procalcitonin in Serum

In the context of this study, it was demonstrated that the serum procalcitonin (PCT) levels obtained at the time of admission were higher in patients suffering from Gram-negative bacteremia than in patients suffering from Gram-positive infections. In the case of Gram-negative infections, the procalcitonin levels were high, which could be due to endotoxic shock caused by the Gram-negative bacteria, which usually stimulates procalcitonin synthesis in early sepsis. In the case of infections caused by Gram-positive bacteria, the procalcitonin levels were low, in spite of clinical evidence proving the presence of systemic infection. In case procalcitonin, that was evaluated together with the endotoxin activity and the blood parameters, it helped in the early discrimination of the pathogen and its class, particularly when inflammatory parameters were borderline. The combination of high procalcitonin levels and endotoxin activity was a good indicator of the presence of Gram-negative sepsis, thus confirming the need for both these parameters in the early stratification of sepsis (Fig. 3 and 4).

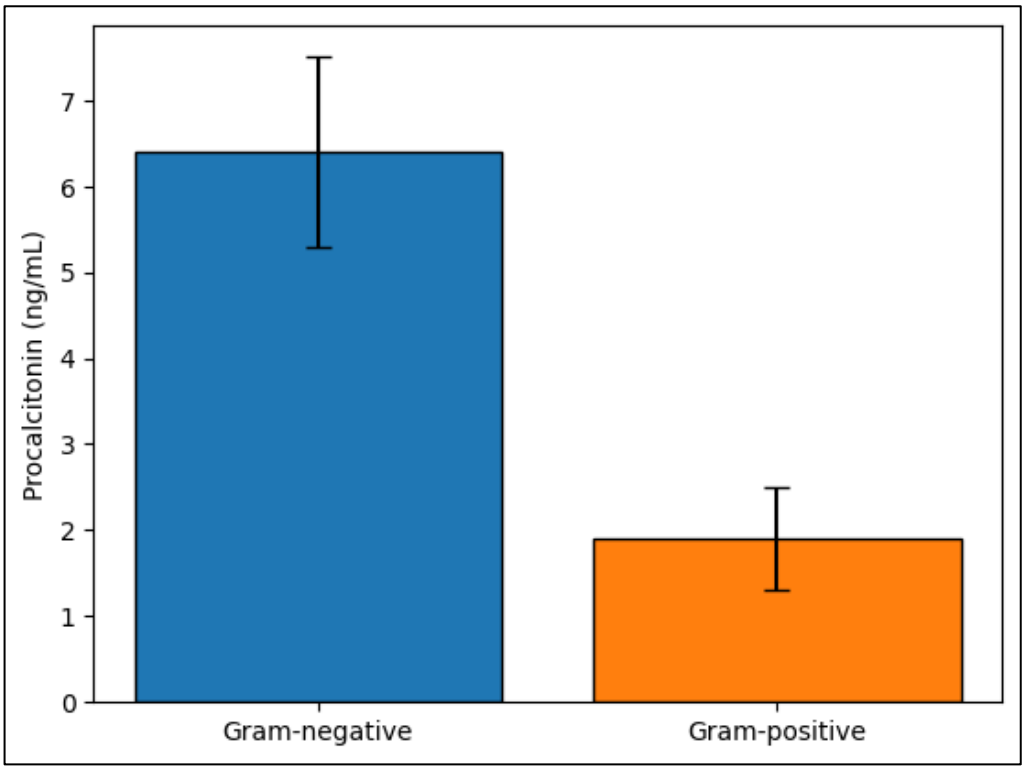

Fig. 3. Procalcitonin levels in serum upon admission in cases of bloodstream infections caused by Gram-negative and Gram-positive organisms.

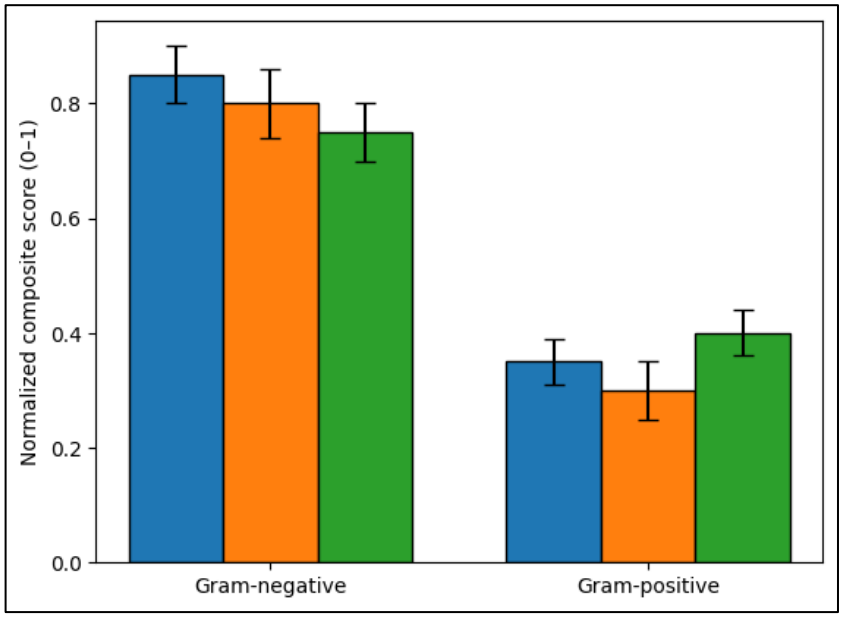

Fig. 4. Composite Endotoxin–Inflammation–Procalcitonin Score

Discussion

The present study showed that integrating standard hematological parameters, serum endotoxin activity, and procalcitonin forms an innovative and non-genetic method for early differentiation of septic patients with Gram-negative and Gram-positive bloodstream infections. The raised endotoxin activity associated with Gram-negative cases corresponds to the ongoing pathophysiological process involving bloodstream lipopolysaccharide, which is an important stimulus for sepsis-related inflammation and organ failure. Recent studies have identified clinical correlations of endotoxemia, severity of illness, and death in extreme care patients, which empirically supports the importance of measuring endotoxins in addition to standard microbiological methods (Foster & Kellum, 2023; Piret et al., 2024). The present study is in

accordance with data obtained through multiple centers, which indicates that higher endotoxin activity is associated with more pronounced multi-organ failure and longer stay at the hospital, especially in patients with Gram-negative septic shock (Cutuli et al., 2023). Of note, endotoxin activity even at the time of admission provided early an pathogen-class specificity, which is critical in situations where time is of the essence in making clinical interventions.

Strengthening early stratification further is attributed to indices found in the routine hematological complete blood count. An example is the Gram-negative bacteremia with the neutrophil to lymphocyte ratio and the systemic immune inflammation index which shows a case of higher innate immune activation and lower adaptive immune suppression. This pattern is associated with poor outcomes in sepsis and is consistent. Recent studies indicate that these indices are able to draw contrasts, for example with Gram-negative and Gram-positive infections, and that they draw inexpensive and fast results, making them appropriate for use in the emergent setting (Tomic et al, 2024). Hematological indices and endotoxin activity integrate the discriminatory performance of one over the other as reinforced by sepsis pathophysiology which is best articulated through the combination of host response marker(s) as opposed to singular lab values (Li et al, 2024).

Procalcitonin serum levels added yet another dimension by correlating with pathogen-specific host response phenomena. The current study's findings of elevated procalcitonin levels in cases of gram-negative bacterial infection correspond to previous findings of procalcitonin levels being more elevated in cases of endotoxin-related sepsis, as opposed to gram-positive bacterial sepsis (Nejtek et al., 2024). Previous research focused on procalcitonin as a better predictor of blood culture positivity than nonspecific markers of inflammation as well as a better predictor of the class of the infecting organism, particularly when combined with other inflammatory markers and immune markers (Xiao et al., 2024). When combined with hematology parameters and endotoxin activity, procalcitonin added value to the accuracy of classification in the early stages of sepsis, reinforcing its position in non-genetic diagnostic frameworks for sepsis. Such an approach will allow earlier fine-tuning of empiric antimicrobial therapy and will also enhance antimicrobial stewardship while waiting for culture results.

Conclusion

A clinically substantial and early differentiation of Gram-negative and Gram-positive bloodstream infections can be performed with the integrated analysis of hematological indices, serum endotoxin activity, and procalcitonin in patients presenting with sepsis in an inpatient setting. This proposes an innovative approach with an in-house, non-genetic strategy, fully based on standard laboratory methods, and is capable of offering clinically valuable results in the most decisive hours after admission.

Funding

The study has no external source of funding. It was self-funded by the author.

References

1. Chiu, Y.-W., Chang, Y.-H., Hsu, T.-Y., Hsiao, C.-T., Chang, Y.-C., Lai, H.-Y., Lin, H.-H., Chen, C.-C., Hsu, L.-C., Wu, S.-Y., Shih, H.-M., Hsueh, P.-R., & Cho, D.-Y. (2025). Monitoring for early prediction of gram-negative bacteremia using machine learning and hematological data in the emergency department. Communications Medicine (London), 5(1), 483. https://doi.org/10.1038/s43856-025-01200-2
2. Cutuli, S. L., De Rosa, S., Ferrer, R., Ruiz-Rodriguez, J. C., Forfori, F., Ronco, C., Antonelli, M., & EUPHAS2-G50 Collaborative Study Group. (2023). Endotoxin activity trend and multi-organ dysfunction in critically ill patients with septic shock, who received polymyxin-B hemadsorption: A multicenter, prospective, observational study. Artif Organs, 47(8), 1361-1370. https://doi.org/10.1111/aor.14534

3. Foster, D. M., & Kellum, J. A. (2023). Endotoxic septic shock: Diagnosis and treatment. Int J Mol Sci, 24(22), 16185. https://doi.org/10.3390/ijms242216185

4. Jabbour, J. P., Ciotti, G., Maestrini, G., Brescini, M., Lisi, C., Ielo, C., La Pietra, G., Luise, C., Riemma, C., Breccia, M., Brunetti, G. A., Carmosino, I., Latagliata, R., Morano, G. S., Martelli, M., & Girmenia, C. (2022). Utility of procalcitonin and C-reactive protein as predictors of Gram-negative bacteremia in febrile hematological outpatients. Supportive Care in Cancer, 30(5), 4303-4314. https://doi.org/10.1007/s00520-021-06782-w

5. Kellum, J. A., Shoji, H., Foster, D. M., & Walker, P. M. (2023). Adsorption of endotoxin and mitigation of sepsis. Contrib Nephrol, 200, 133-141. https://doi.org/10.1159/000527708

6. Koizumi, Y., Sakanashi, D., Ohno, T., Nakamura, A., Yamada, A., Shibata, Y., Shiota, A., Kato, H., Hagihara, M., Asai, N., Watarai, M., Murotani, K., Yamagishi, Y., Suematsu, H., & Mikamo, H. (2021). Plasma procalcitonin levels remain low at the onset of gram-positive bacteremia regardless of severity or the presence of shock: A retrospective analysis of patients with detailed clinical characteristics. Journal of Microbiology, Immunology and Infection, 54(6), 1028-1037. https://doi.org/10.1016/j.jmii.2020.08.015

7. Li, L., Yang, L., Yuan, Z., Wu, Q., & Lyu, X. (2024). The combination of systemic immune-inflammation index and serum procalcitonin has high auxiliary predictive value for short-term adverse prognosis in septic shock patients. J Emerg Med, 67(4), e357-e367. https://doi.org/10.1016/j.jemermed.2024.05.005

8. Lien, F., Lin, H.-S., Wu, Y.-T., & Chiueh, T.-S. (2022). Bacteremia detection from complete blood count and differential leukocyte count with machine learning: Complementary and competitive with C-reactive protein and procalcitonin tests. BMC Infectious Diseases, 22(1), 287. https://doi.org/10.1186/s12879-022-07223-7

9. Mangalesh, S., Dudani, S., & Malik, A. (2023). The systemic immune-inflammation index in predicting sepsis mortality. Postgraduate Medicine, 135(4), 345-351. https://doi.org/10.1080/00325481.2022.2140535

10. Molinari, L., Tidswell, M. A., Al-Khafaji, A., Davison, D., Galphin, C., Kamaluddin, E., Foster, D. M., & Kellum, J. A. (2025). Organ failure, endotoxin activity, and mortality in septic shock. Critical Care Explorations, 7(9), e1308. https://doi.org/10.1097/CCE.0000000000001308

11. Nejtek, T., Müller, M., Moravec, M., Průcha, M., & Zazula, R. (2024). Comparison of procalcitonin levels with blood culture results and foci of infection in septic patients. Epidemiol Mikrobiol Imunol, 73(4), 173-180. https://doi.org/10.61568/emi/11-6390/20241024/138872

12. Piret, S. E., Khan, S., Fairuz, F., Gholami, S., Davis, M., Kim, C. K., Espinoza, M., Foster, D., Kellum, J. A., Ahmad, S., Kalogeropoulos, A. P., & Mallipattu, S. K. (2024). Endotoxemia correlates with kidney function and length of stay in critically ill patients. Blood Purif, 53(1), 30-39. https://doi.org/10.1159/000534107

13. Roldgaard, M., Benfield, T., & Tingsgård, S. (2024). Blood neutrophil to lymphocyte ratio is associated with 90-day mortality and 60-day readmission in Gram negative bacteremia: A multi-center cohort study. BMC Infectious Diseases, 24(1), 255. https://doi.org/10.1186/s12879-024-09127-0

14. Tomic, J., Jakovac, S., Zovko, T., Ljevak, I., Karabatic, S., Mucic, M., & Pravdic, D. (2024). Platelet indices in patients with gram-negative and gram-positive sepsis: A retrospective cross-sectional study. Cureus, 16(10), e71601. https://doi.org/10.7759/cureus.71601

15. Xiao, H., Zhang, H., Wang, G., Wang, Y., Tan, Z., Sun, X., Zhou, J., Duan, M., Zhi, D., Hang, C., Zhang, G., Li, Y., Wu, C., Zhang, H., Xie, M., & Li, C. (2024). Comparison among presepsin, procalcitonin, and C-reactive protein in predicting blood culture positivity and pathogen in sepsis patients. Shock, 61(3), 387-394. https://doi.org/10.1097/SHK.0000000000002243

16. Zhou, H. (2024). The value of systemic immune-inflammation index and T cell subsets in the severity and prognosis of sepsis. Critical Reviews in Immunology, 44(6), 1-12. https://doi.org/10.1615/CritRevImmunol.2024051413