ORIGINAL ARTICLE

# Role of Neutrophil Gelatinase-associated Lipocalin (NGAL) and Other Clinical Parameters as Predictors of Bacterial Sepsis in Patients Presenting to the Emergency Department with Fever

Anna Paul[1], Nalini Sarah Newbigging[2], Audrin Lenin[3], Mahasampath Gowri[4], Jithin Sam Varghese[5], Arun Jose Nell[6], Kundavaram Paul Prabhakar Abhilash[7], Aditya John Binu[8], Vignesh Kumar Chandiraseharan[9], Ramya Iyyadurai[10], George M Varghese[11]

*Received on:* 03 February 2023; *Accepted on:* 04 February 2023; *Published on:* 28 February 2023

## Abstract

**Background:** Bacterial sepsis is associated with significant morbidity and mortality. However, to date, there is no single test that predicts sepsis with reproducible results. We proposed that using a combination of clinical and laboratory parameters and a novel biomarker, plasma neutrophil gelatinase-associated lipocalin (NGAL) may aid in early diagnosis.

**Method:** A prospective cohort study was conducted at a tertiary care center in South India (June 2017 to April 2018) on patients with acute febrile episodes fulfilling the Systemic Inflammatory Response Syndrome (SIRS) criteria. Plasma NGAL and standard clinical and laboratory parameters were collected at the admission. Bacterial sepsis was diagnosed based on blood culture positivity or clinical diagnosis. Clinically relevant plasma NGAL cut-off values were identified using the receive operating characteristic (ROC) curve. Clinically relevant clinical parameters along with plasma NGAL's risk ratios estimated from the multivariable Poisson regression model were rounded and used as weights to create a new scoring tool.

**Results:** Of 100 patients enrolled, 37 had bacterial sepsis. The optimal plasma NGAL cut-off value to predict sepsis was 570 ng/mL [area under the curve (AUC): 0.69]. The NGAL sepsis screening tool consists of the following clinical parameter: diabetes mellitus, the presence of rigors, quick sequential organ failure assessment (qSOFA) >2, a clear focus of infection, and the plasma NGAL >570 ng/mL. A score of <3 ruled out bacterial sepsis and a score >7 were highly suggestive of bacterial sepsis with an interval likelihood ratio (LR) of 7.77.

**Conclusion:** The NGAL sepsis screening tool with a score >7 can be used in the emergency department (ED) to identify bacterial sepsis.

**Keywords:** Bacteremia, Biomarker, Emergency department, Neutrophil gelatinase-associated lipocalin, Sepsis, Systemic inflammatory Response Syndrome, quick sequential organ failure assessment.

*Indian Journal of Critical Care Medicine* (2023): 10.5005/jp-journals-10071-24419

## Highlights

**What is already known on this topic:** Despite a detailed understanding of the pathogenesis of bacterial sepsis and numerous high-quality studies to find a gold standard diagnostic tool, no single biomarker, scoring system, or model has been found to predict bacterial sepsis with satisfactory pooled sensitivity and specificity. Plasma NGAL is a relatively new biomarker, which has recently gained popularity in kidney injury. The role of NGAL in sepsis has not been extensively studied.

**What this study adds:** Our study showed that the Plasma NGAL performed modestly as a stand-alone tool for predicting sepsis; however, when used in combination with pertinent clinical parameters in a scoring tool, it performed well in predicting sepsis.

**How this study might affect research, practice, or policy:** The NGAL sepsis screening tool is a potential new diagnostic option for predicting sepsis in the ED.

## Introduction

Bacterial sepsis, a life-threatening organ dysfunction caused by a dysregulated host-response to infection, results in an

[1–3,8–10]Department of General Medicine, Christian Medical College, Vellore, Tamil Nadu, India

[4]Department of Biostatistics, Christian Medical College, Vellore, Tamil Nadu, India

[5]Nutrition and Health Sciences Doctoral Program, Laney Graduate School, Emory University, Atlanta, GA, United States of America

[6]Department of Clinical Biochemistry, Christian Medical College, Vellore, Tamil Nadu, India

[7]Department of Emergency, Christian Medical College, Vellore, Tamil Nadu, India

[11]Department of Infectious Diseases, Christian Medical College, Vellore, Tamil Nadu, India

**Corresponding Author:** Anna Paul, Department of General Medicine, Christian Medical College, Vellore, Tamil Nadu, India, Phone: +91 9487407190, e-mail: annapaul1991@gmail.com

**How to cite this article:** Paul A, Newbigging NS, Lenin A, Gowri M, Varghese JS, Nell AJ, *et al.* Role of Neutrophil Gelatinase-associated Lipocalin (NGAL) and Other Clinical Parameters as Predictors of Bacterial Sepsis in Patients Presenting to the Emergency Department with Fever. Indian J Crit Care Med 2023;27(3): 176–182.

estimated 11 million annual deaths worldwide, which represents 20% of all deaths.[1] Morbidity and mortality from sepsis occur disproportionately more in low- and middle-income countries. Bacterial sepsis is likely to progress rapidly, and patients deteriorate into septic shock and multiple organ dysfunction if it is not recognized promptly. The World Health Organization estimates that 30 million episodes of sepsis occur annually with mortality rates as high as 50% when there is septic shock.[2,3] Data from India are limited, but the mortality rates can be as high as 85% for severe sepsis.[4] Early recognition and initiation of appropriate treatment are reported to improve outcomes in these patients.[5,6] However, nonspecific signs and symptoms of an acute infection progressing to sepsis present diagnostic challenges to physicians.

Despite an increased understanding of the complex disease process and management strategies of sepsis, early recognition remains challenging, and mortality remains high. A study from India found that 30% of patients with sepsis did not achieve early treatment goals due to various reasons including wrong triaging and physician's not recognizing sepsis.[7] The SIRS was introduced in 1991 in the international consensus guidelines to recognize sepsis, which was then replaced by qSOFA in 2016.[8] However, the diagnostic accuracy of both SIRS and qSOFA is suboptimal with modest sensitivities and specificities of 70% and 73% for qSOFA and 88% and 34% for SIRS, respectively.[9] Additionally, blood cultures are negative in over one-third of patients with sepsis and septic shock.[10] This has led to indiscriminate use of antibiotics with consequent rapid rise in antimicrobial resistance. Recently published data show an estimated 4.95 million (95% confidence interval [CI]: 3.62–6.57) deaths were associated with bacterial antimicrobial resistance in 2019.[11]

Biomarkers could facilitate early diagnosis and enhance patient survival. Over 200 biomarkers of sepsis have been studied so far, but the search for the ideal marker continues.[12] The most widely evaluated biomarkers, such as C-reactive protein (CRP) and procalcitonin (PCT), and lack ideal sensitivity and specificity.[13] Neutrophil gelatinase-associated lipocalin could be a potential biomarker for the diagnosis of sepsis and has not been studied adequately. First isolated from human neutrophils, it is also expressed in the liver, kidneys, and epithelial cells. Neutrophil gelatinase-associated lipocalin binds to bacterial siderophores and transports iron into cells. This leads to downstream cellular responses and can inhibit bacterial growth.[14] NGAL is an important biomarker for acute kidney injury (AKI) in sepsis and is reported to be elevated in sepsis independent of renal injury.[15,16] Therefore, elevated plasma NGAL may be valuable in predicting sepsis as well as the associated target organ dysfunction.

The objectives of this study were to assess if a single plasma NGAL value at first presentation to the ED can predict bacterial sepsis and to develop a predictive scoring tool for bacterial sepsis using plasma NGAL and clinically significant parameters.

# Methods

## Study Design, Setting, and Participants

This prospective cohort study of patients with probable sepsis presenting to the ED of a 3000-bedded tertiary care teaching hospital in South India was conducted from June 2017 to April 2018. Patients more than 18 years of age presenting with fever and SIRS score of 2 or more requiring hospitalization were enrolled and followed up for the duration of their hospital stay. Exclusion criteria included prolonged fever more than 2 weeks, intravenous antibiotic administration 48 hours prior to admission, hospitalization or surgical intervention in the preceding 2 weeks, Human Immunodeficiency Virus (HIV) infection, and chronic kidney disease.

**Source of support:** Institutional post-graduation thesis fund.
**Conflict of interest:** None

The study was approved by the Institutional Review Board and Ethics Committee (No. 10629/ dated: April 3, 2017). All participants or the next of kin provided the written informed consent.

## Data Collection and Variable Specification

A pre-designed case report form was used to collect demographics, comorbidities, and clinical parameters at admission and medication history via interviewing the participant.

### *Outcome*

The patients were classified as "bacterial sepsis" if blood culture taken at admission grew a bacterium other than skin contaminants and "not sepsis" for those with proven alternative diagnosis for febrile illness such as scrub typhus, dengue, or malaria. The remaining patient's details were appraised by two independent reviewers (A.L. and N.S.N.) and further classified as "positive via physician diagnosis" and "negative via physician diagnosis" for bacterial sepsis. Any conflict among the reviewers was resolved by a third senior physician (R.I.). The "positive via physician diagnosis" was included in the "bacterial sepsis" and "negative via physician diagnosis" was included in the "not sepsis" for the final analysis.

### *Exposure*

An ethylenediaminetetraacetic acid sample for plasma NGAL was collected at admission. The sample was sent to the Clinical Biochemistry laboratory and stored at –70°C and processed in batches using the Bio Porto/cobas 1800 machine, which works on the principle of turbidimetry.

### *Covariates*

Plasma NGAL, clinical risk factors, symptoms, and signs and the qSOFA and SIRS scores were used for predicting bacterial sepsis. Investigations done at the time of admission include complete blood counts, creatinine, liver function tests, blood culture, plasma NGAL, and chest X-ray, and additional investigations conducted at the discretion of the treating physicians. Laboratory parameters data were obtained from the electronic medical records. There were no potential effect modifiers identified.

### *Study Size*

A minimum sample size required was 100, based on an independent sample *t*-test for positive and negative sepsis group of 15 per group (minimum difference in plasma NGAL = 100 mg/dL, SD = 100 mg/dL, $\alpha = 0.05$, power = 80%).[17] Under an assumed clinical prevalence of 20% for sepsis, we required 75 patients to meet our positive group requirements ($n = 15$). We additionally sampled 25 patients to account for uncertainty in prevalence of sepsis.

## Statistical Analysis

Normally distributed continuous variables were reported as mean ± standard deviation and skewed variable as median with

interquartile range. Frequencies and percentages were used to describe the categorical variables. Poisson regression with robust standard errors (SE) were used to estimate the bivariate association between sepsis and the independent variables.

### *Utility of Plasma NGAL as a Predictor of Bacterial Sepsis*

To determine the predictive ability of NGAL over sepsis, we constructed a ROC curve. The AUC determined the predictive ability, and the optimal cut-off for NGAL (mg/dL) was determined using the Youden Index ("J") as the predicted probability for which $J$ = [True Positive Rate – False Positive Rate] is maximum. A Poisson regression model was used to define the effect of NGAL over the sepsis and the effect size was presented as relative risk (RR) with 95% CI.

### *Development of the Prediction Score*

Clinical parameters that were (1) previously been established as independent predictors of sepsis or (2) that the authors (with more than 5 years of clinical experience) considered as clinically relevant or (3) were predictors of bacterial sepsis in the bivariate analysis were included to construct a predictive score. The data were divided into test and train data on the ratio of 80:20. Risk ratios estimated from the multivariable Poisson regression model on the test dataset were rounded and used as weights for the prediction score. We constructed ROC curves along with LRs for segments of the ROC curve for both the training set and test set. The Tripod checklist was used to report the predictive scores.[18]

All analyses were carried out using STATA IC/16.0. The code for the analysis is present in the appendix.

## Results

Among 100 patients with fever and SIRS enrolled in the study, 21 patients had a positive blood culture drawn at admission and a total of 37 patients were diagnosed to have "bacterial sepsis." The classification concordance between reviewers is presented in Supplementary Table 1. The remaining 63 patients had alternative diagnosis. The baseline characteristics are presented in Table 1. Scrub typhus was the most common alternative diagnosis (25% of "not sepsis") followed by viral illness such as dengue, H1N1 and viral encephalitis (14%), infections documented were leptospirosis (4.7%), and malaria (4.7%). Among the patients with positive blood culture, gram negative sepsis was significantly higher as compared to gram positive sepsis (86% vs 14%) with *Escherichia coli* having the highest incidence (57%, $N = 20$). Thirty-one patients required intensive care unit (ICU) care and the remaining were managed in the ward.

Results from the bivariate association (RR) of independent variables that may predict bacterial sepsis is presented in Table 1. The overall median (IQR) NGAL level was 783.5 (432.5,1483.5).

### Association of Plasma NGAL with Bacterial Sepsis

A 100 mg/dL increase in plasma NGAL was associated with a RR of 1.04 (95% CI: 1.01–1.06) times higher risk of bacterial sepsis and the association did not change after adjusting for creatinine (despite plasma NGAL being an established marker for AKI). The risk ratio for these associations is presented in Supplementary Table 2.

The AUC for the ROC curve for plasma NGAL and bacterial sepsis was 0.69 (95% CI: 0.59–0.79) (Fig. 1). The optimal cut-off for plasma NGAL to predict sepsis was 570 ng/mL using the Youden Index (sensitivity = 0.87, specificity = 0.46). At that cut-off, dichotomizing plasma NGAL resulted in a crude RR ratio of 3.30 (95% CI: 1.41–7.72).

### Association between Plasma NGAL and AKI

As plasma NGAL is a well-established marker of AKI, we compared elevated plasma NGAL (>570 ng/mL) with the presence of AKI (creatinine >2). The Chi-square test did not show any association ($p$-value: 0.30; Table 2). This finding suggests that plasma NGAL is elevated in sepsis independent of kidney injury.

### Predictive Score

To develop a score to predict sepsis, the data were split into train and test data with a ratio of 80:20. A multivariable model was developed with the NGAL and other parameters as predictor. The parameters identified based on the selection criteria for the prediction score were as follows: history of diabetes mellitus, presence of rigors, qSOFA score of >2, a clear focus of infection on history and examination as well plasma NGAL. The weights for different parameters are provided in Table 3. The RR values are rounded, and the scores were given to each factor, for example, a patient with diabetes (1 point), with any focus of infection (3 points), and the NGAL of 600 ng/mL (3 points) would receive a total score of 7. The ROC curve for the training set had an AUC of 0.86 (Fig. 2), and the ROC curve for the test set had an AUC of 0.76 (Fig. 3). Performance of the scoring system at different cut-points is presented in Table 4. In the training set, a score >7 had an interval LR of 7.77 with specificity ranging from 88 to 100%, and a score <3 excluded sepsis (interval LR of 0.13, sensitivity: 96–100%) and in the test set score >7 had an interval LR of 3.31 and score <3 had interval LR of 0.00 (Table 4).

## Discussion

This study found that at a plasma NGAL cut-off value of 570 ng/mL had a high sensitivity of 87% for sepsis while it had a low specificity of 47%, suggesting its utility as a screening tool. The NGAL sepsis screening tool consists of the following parameters: diabetes mellitus, rigors, qSOFA >2, a clear focus of infection along with plasma NGAL >570 ng/mL. The NGAL sepsis screening tool performed well in both the test set and training set. A score of <3 ruled of sepsis effectively with sensitivity close to 100%. A score of >7 ruled in disease with a high interval LR in the training set (7.77) but, in the test, the LR was less impressive (3.31).

Chase et al. recruited 5,630 patients with fever from the ED to determine predictors of bacteremia. Their findings of note were that urinary tract infection (OR: 4.0; 95% CI: 2.8–5.8) and bandemia (OR: 3.5; 95% CI: 2.3–5.3) were predictors of gram negative bacteremia while presence of diabetes (OR: 2.0; 95% CI: 1.1–3.6) was associated with gram positive bacteremia.[19]

In a retrospective study from a tertiary care center in Thailand, 8,177 patients who presented to the ED were reviewed. They found the following clinical parameters: age >55 years, moderate-to-severe chronic kidney disease (CKD), solid organ tumor, liver disease, history of chills, and body temperature of over 38.3°C were associated with a positive blood culture.[20]

A recent scoping review found the following parameters to be of moderate-to-high risk for bacteremia: shaking chills with fever (rigors), acute pyelonephritis, meningitis, severe community acquired pneumonia, cholangitis, pyogenic liver abscess, ventriculo-peritoneal shunt being *in situ*, infective endocarditis, septic thrombophlebitis, vascular grafts infection, native vertebral osteomyelitis, septic arthritis, and epidural abscess.[21] These finding

**Table 1:** Baseline characteristics of analytic sample

| | *Bacterial sepsis (n = 37)* | *Not sepsis (n = 63)* | *RR* | *95% CI* |
|---|---|---|---|---|
| Age (years) | 55.6 ± 15.7 | 47.9 ± 18.3 | 1.02 | 1.00–1.03 |
| Male gender | 59.5% | 55.66% | 1.11 | 0.66–1.87 |
| Self-reported comorbidities | | | | |
| Diabetes mellitus (%) | 22 (59.5%) | 20 (31.7%) | 2.03 | 1.20–3.42 |
| Hypertension | 12 (32.4%) | 20 (31.7%) | 1.02 | 0.59–1.76 |
| COPD | 4 (10.8%) | 7 (11.1%) | 0.98 | 0.43–2.24 |
| Coronary artery disease | 12 (32.4%) | 12 (19.0%) | 1.52 | 0.91–2.54 |
| Chronic steroid use | 1 (2.7%) | 2 (3.2%) | – | |
| Immunocompromised | 0 (0.0%) | 1 (1.6%) | – | |
| Symptoms | | | | |
| Fever duration (days) | 3 (2,7) | 4 (2,7) | 0.97 | 0.90–1.04 |
| Respiratory symptoms | 10 (27.0%) | 36 (57.1%) | 0.43 | 0.24–0.80 |
| Urinary symptoms | 14 (37.8%) | 6 (9.5%) | 2.43 | 1.55–3.81 |
| Gastrointestinal symptoms | 10 (27.0%) | 9 (14.3%) | 1.58 | 0.93–2.67 |
| Central nervous system symptoms | 11 (29.7%) | 11 (17.5%) | 1.50 | 0.89–2.53 |
| Soft tissue/musculoskeletal symptoms | 3 (8.1%) | 5 (7.9%) | 1.01 | 0.40–2.58 |
| Any focus of infection | 21 (58.3%) | 7 (11.3%) | 3.50 | 2.13–5.75 |
| Rigors | 13 (35.1%) | 8 (12.7%) | 2.04 | 1.27–3.27 |
| Myalgia | 4 (10.8%) | 25 (39.7%) | 0.30 | 0.12–0.76 |
| Signs | | | | |
| Heart rate (/min) >90/min | 35 (94.6%) | 61 (96.8%) | 0.73 | 0.26–2.01 |
| Systolic blood pressure (mm Hg) <90 mm Hg | 9 (24.3%) | 22 (34.9%) | 0.72 | 0.38–1.33 |
| Respiratory rate (/min) > 22 | 29 (78.4%) | 48 (76.2%) | 1.08 | 0.58–2.03 |
| GCS (<15) | 14 (37.8%) | 10 (15.9%) | 1.93 | 1.19–3.12 |
| SIRS score >3 | 10 (27.0%) | 6 (9.5%) | 1.94 | 1.19–3.18 |
| qSOFA >2 | 4 (10.8%) | 1 (1.6%) | 2.30 | 1.37–3.87 |
| Cold peripheries | 8 (21.6%) | 18 (28.6%) | 0.79 | 0.41–1.49 |
| Laboratory parameters | | | | |
| Total WBC count (cells/mm$^3$) × 10$^3$ > 12,000 | 24 (64.9%) | 31 (49.2%) | 1.51 | 0.87–2.61 |
| Differential count (cells/mm$^3$) × 10$^3$ > 80% | 26 (70.3%) | 34 (54.0%) | 1.58 | 0.88–2.82 |
| Platelet count (cells/mm$^3$) × 10$^6$ <10,0000 | 0 (0.0%) | 3 (4.8%) | 1.60 | 1.37–1.86 |
| Serum creatinine (mg/dL) >2 | 15 (40.5%) | 12 (19.0%) | 1.84 | 1.13–3.00 |
| Serum lactates (mmol/L) >2 | 20 (58.8%) | 29 (61.7%) | 0.93 | 0.56–1.57 |
| Sodium (mmol/L) | 131.9 ± 6.2 | 131.8 ± 6.2 | | |
| pH | 7.4 ± 0.1 | 7.4 ± 0.2 | | |
| Total bilirubin (mg/dL) >2 | 5 (14.3%) | 8 (30.5%) | 0.51 | 0.23–1.17 |
| AST (U/L) >40 | 18 (51.4%) | 39 (67.2%) | 0.67 | 0.4–1.12 |
| ALT (U/L) >41 | 11 (31.4%) | 28 (48.3%) | 0.63 | 0.35–1.14 |
| Plasma NGAL (ng/mL)[a] | 1383.0 (689.0, 1799.0) | 647.0 (270.0, 1275.0) | 1.04 | 1.02–1.07 |

Continuous variables were reported as mean ± standard deviation if normally distributed or median (25th percentile, 75th percentile), if non-normally distributed. Categorical variables were reported as count (percentage). RR represents unadjusted relative risk from Poisson regression with robust standard errors estimated for clinically meaningful comparisons. ALT, alanine transaminase; AST, aspartate transaminase [a]Crude RR for plasma NGAL is reported for every 100 ng/mL; COPD, chronic obstructive pulmonary disease; GCS, Glasgow coma scale

are consistent with those of our study, that is, a clear focus of infection, presence of rigors or shaking chills, and a low sensorium glasgow coma scale (GCS) <15.

A higher qSOFA score of >2 and SIRS >3 both had high RR ratios (2.3 and 1.94, respectively) with high specificity, but this came at a price of having very poor sensitivity. Whereas the traditional scores, i.e., SIRS > 2 and qSOFA > 1 had modest sensitivities and specificities of 70% and 73% for qSOFA and 88% and 34% for SIRS, respectively.[9]

In fact the 2021 survival sepsis guidelines recommend against using the qSOFA score compared to SIRS, the National Early warning Score , and the Modified Early warning Score.[22]

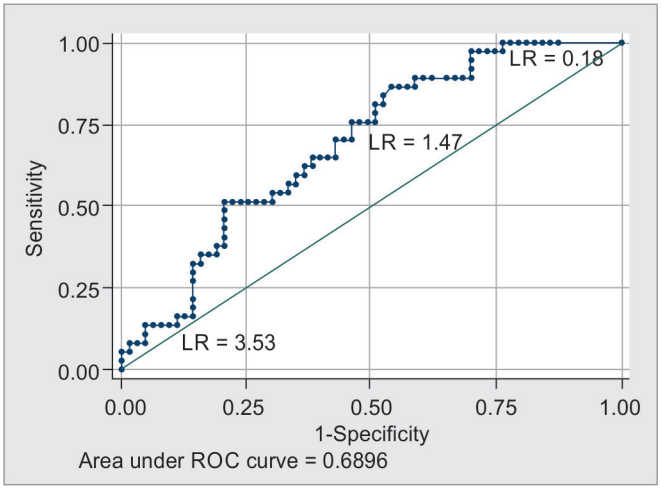


**Fig. 1:** ROC curve for the plasma NGAL. At the plasma NGAL threshold (570 ng/mL) identified using Youden's *J* statistic. The AUC is 0.69 (sensitivity = 0.86, specificity = 0.46, crude RR = 2.91 (95% CI: 1.34–6.30)

**Table 2:** Association with plasma NGAL and AKI

| | *Acute kidney injury* | |
|---|---|---|
| *Plasma NGAL* | *Creatinine ≤2 mg/dL* | *Creatinine >2 mg/dL* |
| Plasma NGAL <570 ng/mL | 27 (79.41) | 7 (20.59) |
| Plasma NGAL ≥570 ng/mL | 46 (69.70) | 20 (30.30) |

Pearson's Chi-square test 1.07 with a *p*-value 0.30. The plasma NGAL value >570 ng/mL is associated with sepsis.

**Table 3:** The multivariate model presenting scores based on RR for the confounding variable with the NGAL to predict sepsis

| | | *95% CI* | | | |
|---|---|---|---|---|---|
| *Variables* | *RR* | *LCL* | *UCL* | *p-value* | *Score* |
| Diabetes mellitus | 1.31 | 0.81 | 2.12 | 0.277 | 1 |
| Rigor | 1.87 | 1.22 | 2.85 | 0.004 | 2 |
| qSOFA ≥2 | 1.62 | 1.01 | 2.58 | 0.044 | 2 |
| Any focus infection | 2.83 | 1.34 | 5.97 | 0.006 | 3 |
| NGAL ≥570 | 2.97 | 1.03 | 8.57 | 0.044 | 3 |

LCL, lower confidence limit; UCL, upper confidence limit

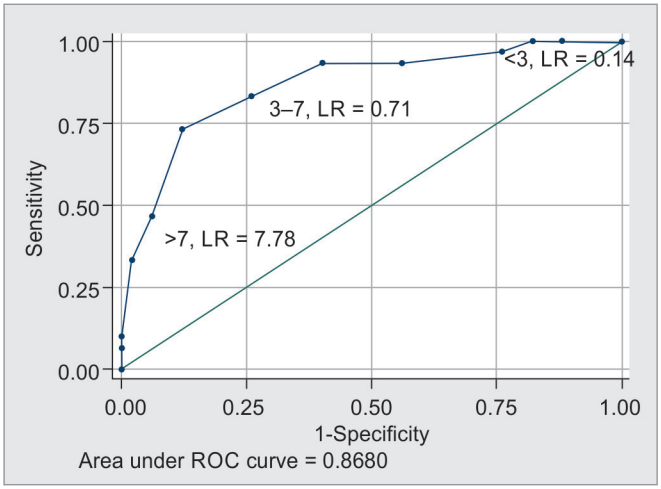


**Fig. 2:** ROC curve for training set NGAL sepsis screening tool

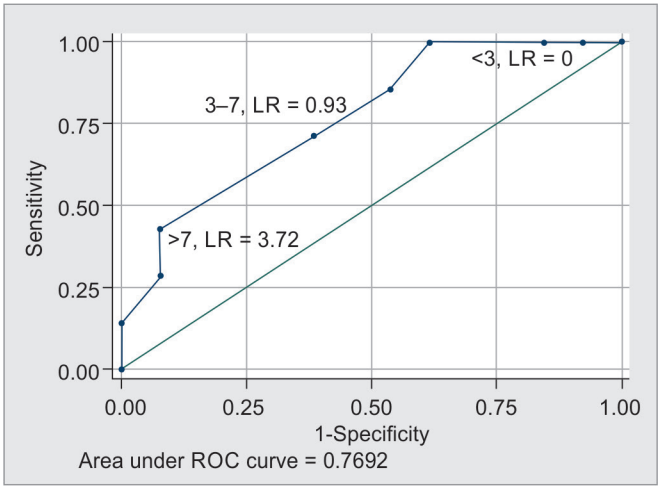


**Fig. 3:** ROC curve for test set NGAL sepsis screening tool

Sparks et al. compared different scoring systems to predict bacterial sepsis in the ED.[23] The scores considered were SIRS, qSOFA, clinical excellence commission (CEC) SEPSIS KILLS pathway (clinical excellence commission by the New south whales' government or between the flags for sepsis), and the modified Shapiro scoring system. They included 250 patients in the sepsis arm and 250 age and sex-matched nonsepsis patients. They found the modified Shapiro criteria had the highest sensitivity (88%) with specificity (37.85%). The qSOFA had a specificity of 83.67% with poor sensitivity (19.82%). The SIRS had a sensitivity of 82.07% with poor sensitivity (20.72%). The CEC SEPSIS pathway had sensitivity of 70.1% and specificity of 71.1%.[23] Our scoring tool has similar performance to these well-known know screening tools with a sensitivity of 83.33% and specificity of 74.00% for score ≥7 (Supplementary Table 3). However, Baduashvili et al.[24] suggested that using just sensitivity and specificity and dichotomizing results as negative and positive leads to a waste of important diagnostic information. They demonstrated that using the entire ROC curve with LRs of the different segments yields a wealth of information that can augment clinical decision making.[24] Therefore, we calculated the segmental LRs for our screening tools ROC and found that a score of <3 effectively ruled out bacterial sepsis and a score >7 was highly suggestive of bacterial sepsis. The interval LR was 7.77 in the training set; however, in the test set only three patients had a score >7. Unfortunately, most patients in the test set had a score between 3 and 7, which could not effectively differentiate bacterial sepsis from another cause and would require additional testing to confirm the same. While this is a limitation, it is to be noted that the test set only included 20 patients and the results may be more impressive with a larger prospectively recruited training set. This may also be a true reflection of the dilemma a physician faces in the ED when it comes to patients with SIRS of an infective etiology.

In this study, the AUC of the ROC for the NGAL and predicting sepsis was 0.69. The plasma NGAL cut-off value of 570 ng/mL used on our study had a high sensitivity of 87% with poor specificity 47%. Mårtensson et al.[17] showed that among 138 patients admitted to the general ICU elevated plasma NGAL was associated with sepsis independent of the level of acute renal dysfunction. A cut-off value of 98 ng/mL distinguished sepsis from systemic inflammation with high sensitivity (0.77) and specificity (0.79).[17] In Hong et al. study, the elevated plasma NGAL at admission to the ED was associated with

**Table 4:** Multilevel likelihood ratios for different scores in the training and test sets

| | *Negative* | | *Positive* | | | | |
|---|---|---|---|---|---|---|---|
| | *n* | *%* | *n* | *%* | *Interval LR* | *Sensitivity* | *Specificity* |
| *Training set* | | | | | | | |
| <3 | 12 | 24 | 1 | 3.33 | 0.1388 | 96–100% | 0–24% |
| 3–7 | 35 | 70 | 15 | 50 | 0.7143 | 73–96% | 24–88% |
| >7 | 3 | 6 | 14 | 46.67 | 7.7783 | 0–73% | 88–100% |
| *Test set* | | | | | | | |
| <3 | 2 | 15.38 | 0 | 0 | 0.0000 | 100% | 0–15% |
| 3–7 | 10 | 76.92 | 5 | 71.43 | 0.9286 | 42.86–100% | 15–92.31% |
| >7 | 1 | 7.69 | 2 | 28.57 | 3.7152 | 0–42.86% | 92.31–100% |

higher 28-day mortality. The optimal NGAL cut-off for predicting 28-day hospital mortality was 387 ng/mL, its sensitivity was 81.0%, and its specificity was 67.8%.[25] A point-of-care kit for plasma NGAL (Alere Triage® NGAL device ) is available, which has been validated in other clinical settings associated with elevated NGAL.[26] The specificity for NGAL in our study was poor and others report only modestly better results, with a wide variety of cut-offs. Despite these limitations, the availability of a point-of-care test and NGAL being a predictor of AKI means that it can be a useful screening biomarker for sepsis, provided it is used in combination with other clinical signs and laboratory parameters.

### Limitations

This study, despite its strengths such as prospective data collection and estimation of biomarkers with low measurement error, had limitations such as a small sample size and being restricted to a single center. Additionally, while a point-of-care plasma NGAL kit is commercially available in the market, it was not employed in our study, and samples were processed in batches. However, in view of the positive results, this study warrants a separate validation cohort using a point of care kit in the ED. Third, CRP and PCT levels were only measured in a limited number of patients as it was not a part of routine practice during the study period. Hence, we could not compare NGALs predictive value with those of established markers like CRP and PCT.

## Conclusion

The plasma NGAL is a novel and potential new biomarker for sepsis. The NGAL sepsis screening tool consists of plasma NGAL, and clinical parameters had a reasonably good performance as a diagnostic tool. A score of <3 effectively rules out bacterial sepsis and a score >7 rules in bacterial sepsis and can be easily implemented in an ED. However, the further validation is required in prospectively recruited validation cohort.

## Data Availability Statement

The dataset and the analysis code will be made available upon reasonable request.

## Orcid

*Anna Paul* https://orcid.org/0000-0002-5119-5237
*Nalini Sarah Newbigging* https://orcid.org/0000-0001-8137-0844
*Audrin Lenin* https://orcid.org/0000-0001-9230-2791
*Mahasampath Gowri* https://orcid.org/0000-0002-0251-0763
*Jithin Sam Varghese* https://orcid.org/0000-0003-3825-9897
*Arun Jose Nell* https://orcid.org/0000-0002-7445-0042
*Kundavaram Paul Prabhakar Abhilash* https://orcid.org/0000-0002-2382-4411
*Aditya John Binu* https://orcid.org/0000-0002-6644-657X
*Vignesh Kumar Chandiraseharan* https://orcid.org/0000-0001-9117-1996
*Ramya Iyyadurai* https://orcid.org/0000-0001-8453-6205
*George M Varghese* https://orcid.org/0000-0002-4040-5649

## Supplementary Materials

All the supplementary Tables 1–3 are available online at www.ijccm.org

## References

1. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: Analysis for the Global Burden of Disease Study. Available from: http://minerva-access.unimelb.edu.au/handle/11343/273829 (last accessed Jan 18, 2020 [cited Jan 20, 2022]).
2. Cohen J, Vincent JL, Adhikari NKJ, Machado FR, Angus DC, Calandra T, et al. Sepsis: A roadmap for future research. Lancet Infect Dis 2015;15(5):581–614. DOI: 10.1016/S1473-3099(15)70112-X.
3. Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD, Finfer S. Recognizing Sepsis as a Global Health Priority—A WHO Resolution [Internet]. Massachusetts Medical Society; 2017 Available from: https://www.nejm.org/doi/10.1056/NEJMp1707170 (Nov 26, 2021). DOI: http://dx.doi.org/10.1056/NEJMp1707170.
4. Chatterjee S, Bhattacharya M. Epidemiology of adult-population sepsis in India: A single center 5 year experience. Indian J Crit Care Med 2017;21(9):573–577. DOI: 10.4103/ijccm.IJCCM_240_17.
5. Ferrer R, Martin-Loeches I, Phillips G, Osborn TM, Townsend S, Dellinger RP, et al. Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: Results from a guideline-based performance improvement program. Crit Care Med 2014;42(8):1749–1755. DOI: 10.1097/CCM.0000000000000330.
6. Seymour CW, Gesten F, Prescott HC, Friedrich ME, Iwashyna TJ, Phillips GS, et al. Time to treatment and mortality during mandated emergency care for sepsis. N Engl J Med 2017;376(23):2235–2244. DOI: 10.1056/NEJMoa1703058.
7. Palatty B, Kassyap CK, Abraham SV, Krishnan S, Rajeev PC. Factors affecting early treatment goals of sepsis patients presenting to the emergency department. Indian J Crit Care Med 2018;22(11):797–800. DOI: 10.4103/ijccm.IJCCM_27_18.
8. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315(8):801. DOI: 10.1001/jama.2016.0287.
9. Adegbite BR, Edoa JR, Ndzebe Ndoumba WF, Dimessa Mbadinga LB, Mombo-Ngoma G, Jacob ST, et al. A comparison of different scores for diagnosis and mortality prediction of adults with sepsis in Low-and-

Middle-Income Countries: a systematic review and meta-analysis. EClinicalMedicine 2021;42:101184. DOI:10.1016/j.eclinm.2021.101184.
10. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;369(9):840–851. DOI: 10.1056/NEJMra1208623.
11. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. Lancet 2022;399(10325):629–655. DOI: 10.1016/S0140-6736(21)02724-0.
12. Teggert A, Datta H, Ali Z. Biomarkers for point-of-care diagnosis of sepsis. Micromachines 2020;11(3):E286. DOI: 10.3390/mi11030286.
13. Vincent JL. The clinical challenge of sepsis identification and monitoring. PLoS Med 2016;13(5):e1002022. DOI: 10.1371/journal.pmed.1002022.
14. Bao G, Clifton M, Hoette TM, Mori K, Deng SX, Qiu A, et al. Iron traffics in circulation bound to a siderocalin (Ngal)-catechol complex. Nat Chem Biol 2010;6(8):602–609. DOI: 10.1038/nchembio.402.
15. Zhang A, Cai Y, Wang PF, Qu JN, Luo ZC, Chen XD, et al. Diagnosis and prognosis of neutrophil gelatinase-associated lipocalin for acute kidney injury with sepsis: A systematic review and meta-analysis. Crit Care 2016;20:41. DOI: 10.1186/s13054-016-1212-x.
16. Mårtensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR. Neutrophil gelatinase-associated lipocalin in adult septic patients with and without acute kidney injury. Intensive Care Med 2010;36(8):1333–1340. DOI: 10.1007/s00134-010-1887-4.
17. Mårtensson J, Bell M, Xu S, Bottai M, Ravn B, Venge P, et al. Association of plasma neutrophil gelatinase-associated lipocalin (NGAL) with sepsis and acute kidney dysfunction. Biomarkers 2013;18(4):349–356. DOI: 10.3109/1354750X.2013.787460.
18. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): The TRIPOD statement | EQUATOR Network [Internet]. Available from: https://www.equator-network.org/reporting-guidelines/tripod-statement/ (cited Dec 6, 2022).
19. Chase M, Klasco RS, Joyce NR, Donnino MW, Wolfe RE, Shapiro NI. Predictors of bacteremia in emergency department patients with suspected infection. Am J Emerg Med 2012 ;30(9):1691–1697. DOI: 10.1016/j.ajem.2012.01.018.
20. Phungoen P, Lerdprawat N, Sawanyawisuth K, Chotmongkol V, lenghong K, Sumritrin S, et al. Clinical factors associated with bloodstream infection at the emergency department. BMC Emerg Med 2021;21:30. DOI: 10.1186/s12873-021-00426-2.
21. Fabre V, Sharara SL, Salinas AB, Carroll KC, Desai S, Cosgrove SE. Does this patient need blood cultures? A scoping review of indications for blood cultures in adult nonneutropenic inpatients. Clin Infect Dis 2020;71(5):1339–1347. DOI: 10.1093/cid/ciaa039.
22. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med 2021;47(11):1181–1247. DOI: 10.1097/CCM.0000000000005337.
23. Sparks R, Harada A, Chavada R, Trethewy C. Comparison of different sepsis scoring systems and pathways: qSOFA, SIRS, Shapiro criteria and CEC SEPSIS KILLS pathway in bacteraemic and non-bacteraemic patients presenting to the emergency department. BMC Infect Dis 2022;22:76. DOI: 10.1186/s12879-022-07070-6.
24. Baduashvili A, Guyatt G, Evans AT. ROC anatomy—getting the most out of your diagnostic test. J Gen Intern Med 2019;34(9):1892–1898. DOI: 10.1007/s11606-019-05125-0.
25. Hong DY, Kim JW, Paik JH, Jung HM, Baek KJ, Park SO, et al. Value of plasma neutrophil gelatinase-associated lipocalin in predicting the mortality of patients with sepsis at the emergency department. Clin Chim Acta Int J Clin Chem 2016;452:177–181. DOI: 10.1016/j.cca.2015.11.026.
26. Tokuda Y, Miyasato H, Stein GH. A simple prediction algorithm for bacteraemia in patients with acute febrile illness. QJM Int J Med 2005;98(11):813–820. DOI: 10.1093/qjmed/hci120.