ORIGINAL RESEARCH

Exploring the Diagnostic and Prognostic Significance of Alkaline Phosphatase on Neutrophil Surface Membrane in Patients with Sepsis

Ming Hu, MM; Runfeng Sun, PhD; Tingting Huang, MM; Min Liu, MM; Huiyi Wu, MM; Jiaping Wang, MM

ABSTRACT

Background • Sepsis, characterized by life-threatening organ dysfunction, stems from an unregulated host response. Timely identification is pivotal for enhancing the prognosis of sepsis patients.

Objective • This study aims to explore the diagnostic and prognostic values of alkaline phosphatase on the surface membrane of neutrophils (mNAP) in peripheral blood among sepsis patients.

Design • The study employed a retrospective design.

Setting • This study was conducted at Donghai County People's Hospital.

Participants • A total of 180 sepsis patients were selected and categorized into the sepsis shock group (n=45) and the sepsis non-shock group (n=135). Additionally, 35 patients with non-infectious systemic inflammatory response syndrome served as the control group.

Interventions • mNAP was assessed via flow cytometry, while serum procalcitonin (PCT) and C-reactive protein (CRP) levels were measured through immunoassay.

Primary Outcome Measures • (1) Changes in mNAP, PCT, and CRP. (2) Correlation of mNAP with CRP and PCT in sepsis patients. (3) Diagnostic values of mNAP, PCT, and CRP in sepsis.

Results • Statistically significant differences in mNAP, PCT, and CRP were observed between the sepsis shock group, the sepsis non-shock group, and the control group (P = .000). The median value of mNAP (22627 Ab/c) in the 28-day death group was significantly higher than that (5100 Ab/c) in the survival group (P = .000). Spearman rank correlation analysis indicated a positive correlation between mNAP, PCT, and CRP in sepsis patients (P < .01).

Conclusions • Both mNAP and PCT exhibit superior diagnostic specificity and sensitivity compared to CRP. While mNAP demonstrates similar sensitivity to PCT in diagnosing sepsis, its diagnostic specificity surpasses that of PCT. mNAP holds promise as a novel marker for the diagnosis and prognosis of sepsis. (*Altern Ther Health Med.* 2025;31(2):65-69).

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INTRODUCTION

Sepsis is characterized by life-threatening organ dysfunction resulting from a disorder in the host response induced by infection,[1] making it a prevalent acute critical condition and the primary cause of death in the intensive care unit (ICU). Common symptoms of sepsis include fever, tachycardia, shortness of breath, and an elevation in peripheral white blood cells.[2] Due to its high morbidity and fatality rate, sepsis has emerged as a serious challenge in critical care medical research.[3]

Early identification and prompt, appropriate intervention are crucial for improving the prognosis of sepsis patients.[4] The detection of biomarkers plays a vital role in sepsis diagnosis, condition and prognosis assessment, and curative effect evaluation. Clinical research has centered on exploring biomarkers with high specificity and sensitivity. While C-reactive protein (CRP) and procalcitonin (PCT) have been extensively utilized in sepsis diagnosis, their diagnostic and prognostic values are inherently limited.[5]

Neutrophil alkaline phosphatase (NAP) serves as a marker enzyme for mature neutrophils, existing predominantly in the lumen side of the secretory vesicle of neutrophils, with approximately 20% expressed on the cell membrane surface.[6] Stimulation and activation of neutrophils by various factors, including granulocyte colony-stimulating factor (G-CSF) and chemokines, lead to up-regulation of the alkaline phosphatase (*ALP*) gene transcription and facilitated transfer of ALP to the cell membrane surface.

The alkaline phosphatase on the surface membrane of neutrophils (mNAP) increases correspondingly, providing significant value in the identification of bacterial infection.[7] Flow cytometry detection of mNAP offers advantages such as a simple, rapid method and accurate results, thereby establishing a foundation for the routine clinical application

of mNAP.[8] There is currently no reported information on whether mNAP can serve as a biomarker for the diagnosis and prognosis of sepsis.

Therefore, this study aims to explore the potential of mNAP as a novel biomarker for diagnosing and predicting the prognosis of sepsis, offering a promising avenue for improved clinical outcomes and patient management.

MATERIALS AND METHODS

Study Design

A retrospective study was conducted, and a total of 180 sepsis patients treated at Donghai County People's Hospital from January 2020 to April 2022 were included. Patients met the sepsis diagnostic criteria (sepsis 3.0) and were categorized into the sepsis non-shock group (n=135) and sepsis shock group (n=45). Additionally, 35 patients with non-infectious systemic inflammatory response syndrome (SIRS) hospitalized during the same period were selected as the control group. Organ functions were assessed using the Acute Physiology and Chronic Health Evaluation (APACHE) II score.[9] This study received approval from the hospital ethics committee, and written informed consent was obtained from all patients.

Study Duration

The study commenced upon patients' admission to the ICU and concluded upon their transfer, discharge, or death. A 28-day survival follow-up determined clinical outcomes, classifying patients into the survival and death groups based on their status on the 28th day of ICU admission.

Instruments and Reagents

Flow Cytometry. We utilized the FACSCanto II flow cytometry analyzer (BD, USA) for analysis. Homotype control antibody (PE Mouse IgG1, κ Isotype Control, BD Phaemingen) and ALP-PE monoclonal antibody (PE Mouse anti-Human Alkaline Phosphatase, BD Phaemingen) were employed. Quantibrite TMPE (BD Phaemingen) and hemolysin (BD Phaemingen) were also used in the analysis.

Blood Cell Analysis and CRP Detection. BC5390 blood cell analyzer and self-contained CRP detection reagent were used for analysis (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China).

PCT Detection. DXL800 immunoluminescence analyzer and self-contained PCT detection reagent were used for analysis (Beckman, USA).

Blood Culture. Blood culture instrument and self-contained reagents were used for analysis (Zhengzhou Autobio Labtec Co., Ltd, China).

Mass Spectrometry. Mass spectrometer equipment and related materials were used for analysis (Zhengzhou Autobio Labtec Co., Ltd, China).

Specimen Collection and Detection

Within 24 hours of admission, 2 ml and 3 ml of venous blood were collected from each patient using two vacuum blood collection tubes. These tubes contained EDTA-2K anticoagulant and separation gel (Guangzhou Improve Medical Instruments Co., Ltd., China). The former sample, collected for mNAP and CRP detection, was processed within 6 hours. The latter sample was centrifuged at 3000 r/min to separate serum for PCT detection.

Detection Methods

(1) mNAP Detection: The serum level of mNAP was analyzed using flow cytometry[10]; (2) CRP Detection: The serum level of CRP was determined using immunoturbidimetry; (3) PCT Detection: The serum level of PCT was measured using immunoluminescence.

Statistical Analysis

Data analysis was conducted using SPSS 22.0 software (IBM Corporation, Chicago, IL, USA). For normally distributed measurement data, results were expressed as mean ± standard deviation ($\bar{x} \pm s$). Inter-group comparisons were performed using one-way analysis of variance (ANOVA). Skewed distribution data were presented as M (P_{25}-P_{75}), and inter-group comparisons were conducted using the Mann-Whitney U test. Receiver operating characteristic curve (ROC) analysis evaluated the diagnostic values of mNAP, PCT, and CRP in sepsis. The area under the curve (AUC) was calculated, where an AUC of 0.5 indicated a biomarker with no diagnostic value. Linear correlation analysis was executed, and a significance level of $P < .05$ denoted statistical significance.

RESULTS

Clinical Characteristics of Sepsis Patients

The general clinical data of sepsis patients in both the sepsis non-shock group and the sepsis shock group are presented in Table 1. There were no significant differences in various parameters, including age, gender, temperature, WBC, respiration rate, and heart rate between the two groups (all $P = .104$, $P = .213$, $P = .159$, $P = .12$, $P = .161$, and $P = .132$). However, the sepsis shock group exhibited a higher APACHE II score and 28-day death rate compared to the sepsis non-shock group ($P = .003$ and $P = .012$).

Table 1. General Clinical Data of Sepsis Patients

Characteristics	Sepsis Non-Shock Group	Sepsis Shock Group	*P* value
Age (Year)	51.78±15.65	56.98±17.02	.104
Gender (Male/Female)	89/46	31/14	.213
Cases	135	45	
Temperature (mean ± SD, °C)	37.10±0.75	37.10±0.90	.159
WBC	14.98±7.22	15.00±8.33	.12
Respiration Rate (breath /min)	24.12±6.28	24.81±5.76	.161
Heart rate (beats/min)	101.61±18.02	111.12±12.52	.132
APACHE II	14.08±3.05	24.06±4.57	.003
28-day Death Rate [n (%)]	15 (11.2)	19 (42.2)	.012

Note: The age is presented as mean ± standard deviation (SD), and the respiration rate, heart rate, and APACHE II are presented as mean values. The 28-day death rate is expressed as a percentage of the total cases in each group.

Abbreviations: WBC, white blood cell; APACHE II, Acute Physiology and Chronic Health Evaluation II.

Table 2. Detection Results (M (P_{25}-P_{75})) of mNAP, PCT, and CRP in Sepsis Patients

Group	n	mNAP (Ab/c)	PCT (ng/ml)	CRP (mg/ml)
Sepsis Non-Shock Group	135	13130 (9839-18155)	1.99 (0.34-6.39)	119.01 (83.50-176.02)
Sepsis Shock Group	45	18058 (12569-26462)	26.40 (8.84-60.12)	192.11 (149.21-279.21)
Control Group	35	5738 (2613-9891)	0.31 (0.11-0.90)	81.90 (38.30-123.31)
H-value		91.40	72.2	31.07
P value		<.0001	.000	.000

Note: Values for mNAP, PCT, and CRP are presented as median (25th percentile - 75th percentile). The H-value represents the Kruskal-Wallis test statistic, and the *P*-value indicates the significance level of the test.

Abbreviations: mNAP, alkaline phosphatase on the surface membrane of neutrophils; PCT, procalcitonin; CRP, C-reactive protein.

Comparison of Changes in mNAP, PCT, and CRP among Three Groups

The distribution of mNAP detection results in patients across three groups, the sepsis non-shock group, the sepsis shock group, and the control group, is illustrated in Figure 1. Further comparisons of mNAP, PCT, and CRP results are presented in Table 2. The findings reveal significantly elevated levels of mNAP, PCT, and CRP in both the sepsis non-shock and sepsis shock groups compared to the control group (P < .0001 and P = .000). Additionally, a progression in disease severity is reflected in escalating mNAP, PCT, and CRP levels, with the sepsis shock group exhibiting higher levels than the sepsis non-shock group and the control group (P < .0001 and P = .000).

Comparison of mNAP in Sepsis Patients on Day 28 After Admission Between Survival and Death Groups

The correlation between mNAP levels and mortality in sepsis patients was investigated by comparing mNAP values between the survival and death groups on day 28 after admission. The results revealed a statistically significant difference, with the median mNAP value higher in the death group (22627 Ab/c) compared to the survival group (15100 Ab/c) (U value = 1211, P = .000), as illustrated in Figure 2.

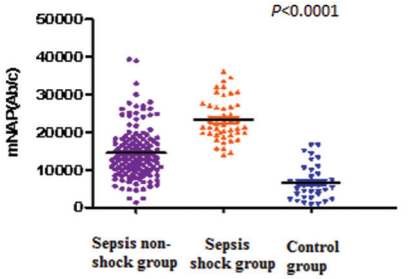
Correlation of mNAP with CRP and PCT in Sepsis Patients

The results of Spearman rank correlation analysis, presented in Figures 3 and Figure 4, indicate that mNAP exhibits positive correlations with CRP and PCT in sepsis patients. The correlation coefficients, r, were found to be 0.34 (P=.000) for CRP and 0.46 (P=.000) for PCT, respectively.

Diagnostic Values of mNAP, PCT, and CRP in Sepsis

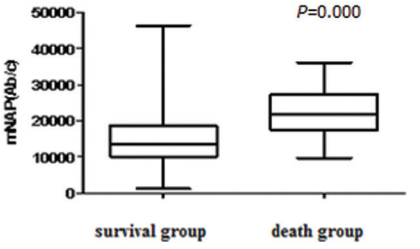
ROC curve analysis was conducted to assess the diagnostic efficacy of mNAP, PCT, and CRP in sepsis, with results depicted in Figure 5 and summarized in Table 3. The AUCs for mNAP, PCT, and CRP in sepsis diagnosis were 0.84 (95% CI 0.76-0.91), 0.7434 (95% CI 0.66-0.82), and 0.6753 (95% CI 0.57-0.78), respectively. Optimal cut-off values were determined, yielding diagnostic sensitivity and specificity values as follows: For mNAP (cut-off value: 10464 Ab/c): Sensitivity = 80.25%, Specificity = 69.63%. For PCT (cut-off value: 1.06 ng/ml): Sensitivity = 80.01%, Specificity = 60.22%.

Figure 1. Changes in mNAP Levels in Sepsis Patients



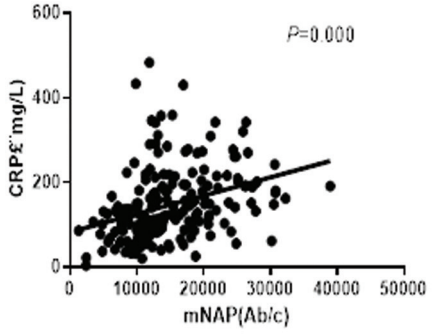
Note: The figure illustrates the variations in alkaline phosphatase on the surface membrane of neutrophils (mNAP) among sepsis patients.

Figure 2. Comparison of mNAP Levels in Sepsis Patients between the Survival Group and Death Group



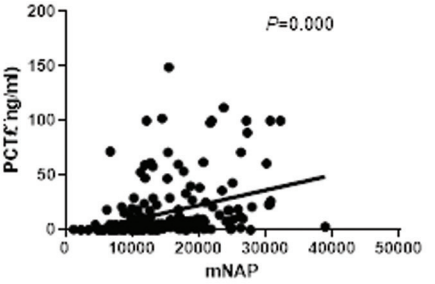
Note: The figure depicts the contrast in alkaline phosphatase levels on the surface membrane of neutrophils (mNAP) between patients in the survival group and the death group.

Figure 3. Correlation between mNAP and CRP in Sepsis Patients



Note: The figure illustrates the correlation analysis between alkaline phosphatase on the surface membrane of neutrophils (mNAP) and C-reactive protein (CRP) in sepsis patients.

Figure 4. Correlation between mNAP and PCT in Sepsis Patients



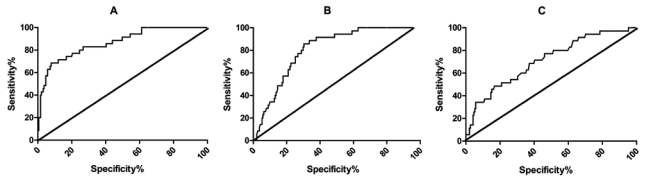
Note: The figure depicts the correlation analysis between alkaline phosphatase on the surface membrane of neutrophils (mNAP) and procalcitonin (PCT) in sepsis patients.

Table 3. Comparison of ROC Curve-Related Parameters

	AUC	Optimal Cut-Off Value	Sensitivity	Specificity	SE
mNAP	0.84	10464	80.25	69.63	0.04
PCT	0.74	1.055	79.01	60.22	0.04
CRP	0.68	82.25	76.33	51.43	0.05

Abbreviations: AUC, area under the curve; mNAP, alkaline phosphatase on the surface membrane of neutrophils; PCT, procalcitonin; CRP, C-reactive protein; SE, standard error.

Figure 5. Comparison of ROCs of mNAP, PCT, and CRP in the Diagnosis of Sepsis



Note: The figure illustrates the Receiver Operating Characteristic (ROC) curves for alkaline phosphatase on the surface membrane of neutrophils (mNAP), procalcitonin (PCT), and C-reactive protein (CRP) in the diagnosis of sepsis. Subplots A, B, and C correspond to mNAP, PCT, and CRP, respectively.

For CRP (cut-off value: 82.25 mg/L): Sensitivity = 51.43%, Specificity = 76.30%.

DISCUSSION

In sepsis and septic shock, the short-term mortality rate remains notably high among patients with sepsis and septic shock, with a close correlation to the degree of shock.[11] In clinical practice, auxiliary diagnostic indices for bacterial infection predominantly include CRP and PCT. CRP, an acute-phase reaction protein synthesized in the liver, facilitates the release of lysosomal enzymes and phagocytosis of bacteria by neutrophils and macrophages during the inflammatory state, preceding the production of specific antibodies. This mechanism contributes to its anti-inflammatory role.[8] As a conventional inflammatory marker, serum CRP has been routinely utilized for the early diagnosis of infectious diseases.

However, serum CRP is influenced by multiple factors, resulting in low specificity. Elevated serum CRP levels are not exclusive to bacterial infection, limiting its clinical value due to contributions from non-infective factors. Although bacterial culture remains the "gold standard" for etiological identification,[4,12] its prolonged processing time and susceptibility to antibiotic interference result in a low positive rate, with only 30% of sepsis patients exhibiting positive blood culture results.[13]

In a study dating back to 1993, it was reported that PCT levels significantly increased in sepsis patients. Subsequently, numerous studies have investigated the role of PCT in infectious diseases, establishing its widespread use as an inflammatory marker.[14]. PCT, originating from thyroid C cells, undergoes a notable increase within 3-4 hours post systemic infection. Its brief half-life underscores its pivotal role as a key biomarker for the early detection of infections.

However, PCT exhibits limitations in clinical application as it may also rise in non-infectious conditions such as trauma, surgery, cardiac arrest, heat shock, childbirth, various forms of immunotherapy, and certain autoimmune diseases. The complex pathological manifestations, unclear pathogenesis, and the absence of a definitive gold standard for sepsis diagnosis render reported disease-related markers insufficient to meet the demands of clinical practice.[15]

Several sepsis-related biomarkers have been reported in the literature, yet most have not gained recognition in clinical practice due to insufficient specificity or low sensitivity. Even PCT, currently widely utilized in clinical settings, possesses limited value in disease diagnosis and prognosis assessment.[16] To date, there remains a deficiency in an ideal biomarker for the differential diagnosis between sepsis and SIRS.

The study by Rustin et al.[17] confirmed that NAP primarily resides in the vacuoles of neutrophils, serving as an intracellular hydrolase and a member of glycosylphosphatidylinositol (GPI)-anchored proteins in mature neutrophils. NAP, found in the plasma-secreting vesicles of neutrophils, undergoes mobilization and transfer to the cell membrane in response to various stimuli, including platelet-activating factor (PAF), IL-8, TNF-α, lipopolysaccharide (LPS), leukotriene B4, and yeast polysaccharides. Notably, NAP functions as one of the marker enzymes of mature neutrophils.

Subsequent studies have demonstrated that NAP can cause biological effects by promoting neutrophil migration, generating reactive oxygen species (ROS), and speeding up cell apoptosis.[18] Dating back to the 1960s, NAP was employed as a differential diagnostic index for bacterial infection. The elevation of NAP is associated with the extent of infection. The primary reason for the limited application of this index in clinical practice is that the conventional NAP detection method relies on the cytochemical staining method to calculate the NAP score. The interpretation of results is susceptible to reagent and subjective factors, leading to larger errors in the results. Consequently, the application of NAP in clinical practice has been challenging.

Zhang et al.[10] used flow cytometry to detect mNAP in patients with bloodstream infection and found that mNAP has a certain value in predicting the positive results of blood culture in SIRS patients. In this study, the changes of mNAP in sepsis patients were investigated for the first time, and its feasibility as a diagnostic marker of sepsis was explored; the results showed that the mNAP levels in patients of both the sepsis non-shock group and the sepsis shock group were significantly higher than that in the control group (P = .000); mNAP level was increased with the aggravation of the course of disease, mNAP level was closely correlated with the severity of the disease and increased with the increase of the severity.

We further investigated the association between mNAP and prognosis, tracking the 28-day survival of patients. The results revealed a statistically significant difference in the median value of mNAP (22627 Ab/c) between the death group and the survival group (15100 Ab/c) (U value=2151, P=.000). This finding highlights that mNAP levels undergo significant changes in sepsis patients and can serve as an indicator for determining the severity and prognosis of the patients.

The correlation analysis revealed that mNAP had positive correlations with CRP and PCT in sepsis patients, with correlation coefficients of 0.3358 and 0.4564, respectively. In line with these findings, a previous study indicated that mNAP levels were significantly higher in patients with gram-negative bacteremia within the systemic inflammatory response syndrome compared to those with gram-positive bacteremia.[19]

In sepsis patients, certain consistent patterns emerge in the changes in mNAP, PCT, and CRP levels. We conducted a ROC curve analysis to assess the sensitivity and specificity of mNAP, PCT, and CRP in diagnosing sepsis. The results indicated that the AUCs (95% CI) of mNAP, PCT, and CRP were 0.83 (95% CI 0.76-0.91), 0.74 (95% CI 0.66-0.82), and 0.67 (95% CI 0.57-0.78) respectively. Their diagnostic sensitivities were 80.25%, 79.01%, and 51.43%, respectively, with diagnostic specificities of 69.63%, 62.22%, and 76.30% respectively.

Consistently, Sehveta Mustafić et al.[20] discovered that PCT exhibited the best 28-day mortality predictive value, with a cut-off value of 15.05 ng/mL (AUC 0.92), followed by CRP (AUC 0.84). It suggests that PCT when combined with a clinical score, could be valuable for assessing the severity of infection. Among the three markers compared in this study, the AUC of mNAP was the largest.

The sensitivity of mNAP in diagnosing sepsis was comparable to that of PCT, while its specificity surpassed that of PCT. With the increasing adoption of flow cytometry, mNAP is anticipated to emerge as a novel marker for the diagnosis and prognosis of sepsis. This finding signifies the transformation of an old index into a novel tool, aligning with a previous report.[21]

Study Limitations

The study is subject to certain limitations that should be considered in interpreting its findings. Firstly, the sample size employed in the research is relatively small, which may constrain the broader applicability of the results to a larger and more diverse population. Additionally, the mNAP level was assessed only at the time of patient enrollment, representing a single snapshot without dynamic observations of its changing process over the course of sepsis. This limitation hinders a comprehensive understanding of the temporal dynamics of mNAP in relation to sepsis progression. Lastly, the study recognizes the need for further investigation to unravel the full clinical significance of mNAP as a marker for sepsis. Future research should focus on addressing these limitations and exploring the potential of mNAP in a more extensive and dynamic context.

CONCLUSION

In conclusion, our study underscores the notable diagnostic advantages of both mNAP and PCT over CRP in terms of sensitivity and specificity in sepsis. The diagnostic sensitivity of mNAP aligns closely with that of PCT, while exhibiting a higher diagnostic specificity compared to PCT. This positions mNAP as a potentially groundbreaking marker for the diagnosis and prognosis of sepsis. The findings from this study contribute to the growing body of evidence supporting the potential utility of mNAP as a novel and promising diagnostic tool in the context of sepsis, emphasizing its relevance in advancing diagnostic approaches and enhancing prognostic assessments for this critical medical condition.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

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Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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