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Research article

Elevated neutrophil - to - monocyte ratio as a prognostic marker for poor outcomes in neonatal sepsis

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ABSTRACT

Objectives: Neonatal sepsis is one of the leading causes of neonatal death. The aim of this study was to evaluate the value of neutrophil - to - monocyte ratio (NMR) in predicting mortality in neonatal sepsis.
Methods: In this present retrospective study, a total of 134 neonates with sepsis were included. Baseline laboratory parameters were collected. The best cutoff value of NMR was determined by receiver operating characteristic (ROC) curve. Univariate and multivariate analysis were carried out to survey the predict value of NMR.
Results: The results showed that NMR in non-survival group was significantly higher than that in survival group. Results from multivariate analysis showed that high NMR was an independent risk factor for neonatal sepsis (Hazard ratio (HR): 7.519, $p = 0.001$). ROC displayed that the area under curve (AUC) of NMR was 0.740, sensitivity and specificity of NMR were 80% and 65.8% when 7.65 was selected.
Conclusions: NMR could be a promising prognostic factor for neonatal sepsis.

1. Introduction

Neonatal sepsis is a serious infectious disease in the neonatal period, and it is the most common cause of morbidity and mortality for term and premature infants [1, 2]. Neonatal sepsis is the third leading cause of neonatal mortality and is a major public health problem, especially in developing countries [3].

The clinical manifestation of sepsis is nonspecific in neonates, with different clinical features. It usually presents as decreased acceptance of feed, respiratory problems, temperature instability (hyperthermia and hypothermia), abnormal jaundice, coagulopathy, fussiness, lack of energy and necrotizing enterocolitis [4, 5]. These varied features which can be seen in other neonatal conditions increase the difficulty of diagnosis and may delay treatment, leading to a poor prognosis. Therefore, it is quite important to find a tool to predict infants who are more likely to experience worse clinical outcomes and provide them with closer monitoring and more active treatments.

There are variety of biomarkers that have been proved to be related to neonatal sepsis, including procalcitonin, C-reactive protein (CRP), serum amyloid A (SAA) and Interleukin 6 (IL-6) [6, 7, 8, 9, 10]. However, in the early stage of infection, these traditional biomarkers may be low, resulting in their limitations in clinical application [11].

In addition, overexpression of inflammatory cytokines is associated with multisystem organ failure and mortality [12]. Conversely, immunoparalysis characterized by monocyte inactivation is also associated with adverse clinical outcomes of sepsis [13]. Therefore, the aim of this survey was to evaluate the value of neutrophil-to-monocyte ratio (NMR) in predicting mortality in patients with neonatal sepsis.

2. Methodology

2.1. Specimens and assays

The present retrospective study was conducted between January 2014 and November 2019 in the Jiangsu Women and Children Health Hospital. For this study, any infants diagnosed with possible or definite sepsis from birth to 28 days of age were included. Possible sepsis was diagnosed when a positive culture lacked signs indicating sepsis and 2 positive screening parameters (abnormal CRP, total white blood cell count, platelet count, absolute neutrophil count, or immature/total neutrophil ratio >0.2). Definite neonatal sepsis was the presence of

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potentially pathogenic organisms (bacteria or fungi) in sterile body fluids, such as blood, peritoneal, pleural and cerebrospinal fluid [14, 15]. When signs of sepsis were present but sepsis screening parameters and cultures were negative, it was considered that there is no sepsis. A total of 150 neonates who were admitted to the neonatal intensive care unit (NICU) and diagnosed with neonatal sepsis, younger than 28 days, were the sourced population. We ruled out the patients with unclear history and uncompleted laboratory tests were excluded. At last, 134 neonates were enrolled in this research. Sepsis-related death was defined as death within 28 days after sepsis.

The laboratory tests were evaluated on admission. Routine complete blood count (CBC) of peripheral blood from all participants was measured by using a Sysmex XE 2100 analyzer (Sysmex, Hyogo, Japan). All reagents used for testing were the original reagents of the instruments. The study was confirmed by the Ethics Committee of the local hospital and was in line with the Declaration of Helsinki (2019-SR-072). The PLR, NLR, SII, SIRI and NMR were calculated as follows: PLR = Platelet/Lymphocyte; NLR = Neutrophil/Lymphocyte; SII = (Platelet × Neutrophil/Lymphocyte; SIRI= (Neutrophil × Monocyte)/Lymphocyte); NMR = Neutrophil/Monocyte.

2.2. Statistical analysis

Statistical analyses were performed by SPSS21.0 (SPSS Inc, Chicago, IL, USA) software. Variables are presented in mean ± standard deviation, median (rang) or number. The Shapiro–Wilk test was used for normality assumption of the data. Receiver operating characteristic (ROC) curve analysis was created to assess the ability of NMR to predict neonatal sepsis mortality. Additionally, we performed chi-square and Mann–Whitney U-tests to determine statistically significant differences between survival and non-survival groups. Analysis of neonatal sepsis mortality was performed using univariate and multivariate analyses. A p-value < 0.05 was considered to be statistically significant.

3. Results

3.1. Baseline characteristics

A total of 134 neonates with sepsis were eligible for this study. Among the total patients, 20 neonates were dead. There were no significant differences in gender, age and weight between two groups. Concerning the gender of study patients, 79 were males, of which 67 were survived, while 55 neonates were females, of which 47 were survived. The median age is 2 days of non-survivor and 5 days of survivor. However, the subgroup with lower gestational age at birth and lower Apgar score had significantly higher mortality. Monocyte and platelet count in survival group were 0.92 (0.03, 4.98), 250.84 ± 105.16 respectively, which were significantly higher compared with non-survival group ($p < 0.05$ for both), while there was no statistically significant differences for CRP, WBC, RBC, lymphocyte count, neutrophil count, hemoglobin, red cell distribution width (RDW).

For the 134 subjects included, we compared PLR, NLR, SII, SIRI and NMR between the two groups. NMR was significantly different between non-survival group and survival group (12.43 (2.49, 44.03) vs. 5.80 (1.03, 133.54), $p = 0.001$), while PLR, NLR, SII, SIRI were not (PLR: 46.68 (4.56,800.00) vs. 61.05 (6.83,415.07), $p = 0.355$; NLR: 2.49 (0.15,16.97) vs. 1.24 (0.12,31.11), $p = 0.173$; SII: 382.07 (29.79, 4072.00) vs. 298.26 (10.27, 7777.78), $p = 0.516$; SIRI: 0.90 (0.01, 22.82) vs. 1.24 (0.01, 52.37), $p = 0.844$) (Table 1).

3.2. Prognostic biomarkers predict survivor

Univariate analyses showed that NMR ($p < 0.001$), monocyte count ($p = 0.013$), platelet count ($p = 0.024$) and lymphocyte count ($p = 0.030$) were prognostic factors for neonatal sepsis. On the other hand, by multivariable adjustment, platelet count (HR:3.180, $p = 0.031$) and NMR (HR:7.519, $p = 0.001$) were independent prognostic factors for neonatal sepsis (Table 2).

Table 1. Baseline characteristics of study patients.

Variables	Non-survivors (N = 20)	Survivors (N = 114)	*p*-value
Gender (male/female), n	12/8	67/47	0.920
Median age (range), day	2 (0.5, 28)	5 (0.1, 28)	0.329
Gestational age at birth (weeks)	33 (28, 37)	36 (33, 39)	**0.011**
Weight (g)	2100 (1175, 2387)	2500 (1600, 3250)	0.112
Apgar score (1 min)	7 (4, 10)	10 (8, 10)	**<0.001**
Apgar score (5 min)	8 (5, 10)	10 (8, 10)	**<0.001**
CRP (mg/L)	4 (1, 58)	5 (1, 104)	0.712
WBC ($\times 10^9$/L)	11.05 (5.02, 105.13)	11.38 (2.21, 61.79)	0.099
Lymphocyte ($\times 10^9$/L)	6.26 ± 6.19	4.75 ± 2.78	0.076
Monocyte ($\times 10^9$/L)	0.62 (0.06, 6.34)	0.92 (0.03, 4.98)	**0.025**
Neutrophil ($\times 10^9$/L)	5.71 (1.44, 78.64)	5.11 (0.37, 51.35)	0.158
RBC ($\times 10^{12}$/L)	4.31 ± 0.77	4.61 ± 0.71	0.085
Hemoglobin (g/L)	155.65 ± 29.43	163.03 ± 26.66	0.292
RDW (%)	16.60 ± 1.32	16.47 ± 1.53	0.714
Platelet ($\times 10^9$/L)	201.15 ± 75.75	250.84 ± 105.16	**0.045**
PLR	46.68 (4.56, 800.00)	61.05 (6.83, 415.07)	0.355
Culture positive, n (%)	7 (35.0%)	45 (39.5%)	0.707
NLR	2.49 (0.15, 16.97)	1.24 (0.12, 31.11)	0.173
SII	382.07 (29.79, 4072.00)	298.26 (10.27, 7777.78)	0.516
SIRI	0.90 (0.01, 22.82)	1.24 (0.01, 52.37)	0.844
NMR	12.43 (2.49, 44.03)	5.80 (1.03, 133.54)	**0.001**

CRP: C-reactive protein; RBC: Red blood cell; RDW: red cell distribution width; WBC: white blood count; PLR: platelet-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; SII: Systemic immune-inflammation index; SIRI: systemic inflammatory response index; NMR: neutrophil - to - monocyte ratio. Data are median (range) or mean ± SD. Bold values indicate statistical significance ($p < 0.05$).

3.3. Performance of NMR in prediction of mortality in neonatal sepsis

Figure 1 shows the ability of NMR and platelet count in predicting neonatal sepsis mortality through the results of ROC curve analysis. NMR had a superior area under the curve (AUC) of 0.740, which was significantly higher than that of platelet count (AUC: 0.639). When the cutoff value of NMR in the analysis was 7.65 for prediction of mortality, it can achieve 80.0% of sensitivity and 65.8% of specificity. In addition, the mortality of patients with NMR $\leq$7.65 was 5.1%, while to these with NMR $>$ 7.65 was 21.1%.

4. Discussion

Sepsis is a common and high-risk disease in neonates, especially in communities with limited resources, with high incidence rate and high mortality rate [16]. It is reported that the population level of neonatal sepsis is estimated at 2202 per 100,000 live births, and the mortality rate is between 11% and 19% [17], which corresponds to 17.5% in our study. In the present study, males accounted for 59% while females were 41% of the studied newborns. This is similar to a study in Ethiopia which showed that males were 62.3% and females were 37.7%18 [18].

Neonatal sepsis is a systemic inflammatory response syndrome associated with pathological inflammation and organ system dysfunction [19]. Neutrophils are important parts of innate immune response during sepsis, and release inflammatory cytokines, chemokines and regulatory cytokines. During the onset of sepsis, the complex immune system changes include pro-inflammatory, anti-inflammatory and immunosuppressive responses [20]. In the initial stage of sepsis, pro-inflammatory cytokines are released throughout the body. Previous studies have shown that neutrophils were elevated in neonatal sepsis [21, 22]. Therefore, anti-inflammatory mediators are produced to regulate excessive inflammatory response. However, overexpression of inflammatory cytokines is associated with multisystem

Table 2. Analysis of in-hospital death.

Variables	Univariate analyses			Forest Plot	Multivariate analyses			Forest Plot
	HR	95% CI	P-value		HR	95% CI	P-value	
Age	2.700	0.920–7.925	0.087					
WBC	2.695	0.949–7.633	0.069					
Lymphocyte	3.333	1.200–9.255	**0.030**					
Monocyte	3.560	1.333–9.508	**0.013**					
Platelet	3.307	1.222–8.946	**0.024**		3.180	1.111–9.105	**0.031**	
NMR	7.692	2.404–24.390	**<0.001**		7.519	2.304–24.390	**0.001**	

CI: Confidence interval; HR: Hazard ratio; WBC: white blood count. Bold values indicate statistical significance ($p < 0.05$).

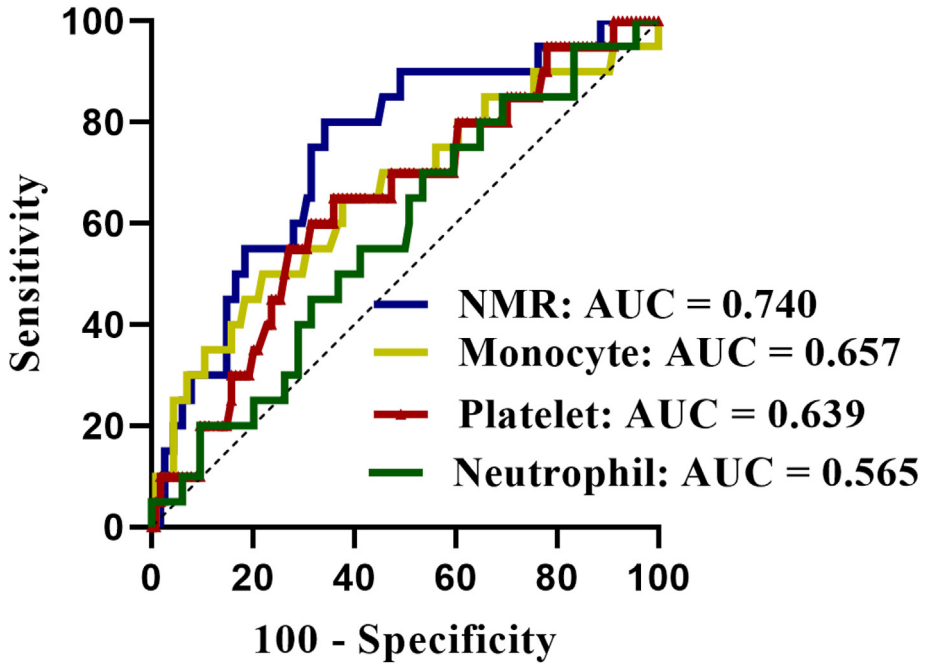

Figure 1. ROC curves of independent factors for predicting mortality.

organ failure and mortality. Therefore, excessive anti-inflammatory stimulation may leads to a state of immunoparalysis [12, 23, 24]. Thus, it is quite important to balance pro-inflammatory and anti-inflammatory responses. Monocytes are the third largest component of leukocytes. Several studies have reported that monocytes are essential components of the inflammatory response and are involved in cytokine release [20]. In addition, in the present study, low monocyte count had something to do with a worse survival.

Blood analysis is a simple and easily accessible test, in which neutrophil count and monocyte count are commonly used clinical indicators. In the present study, we introduced a new index (NMR) based on neutrophil and monocyte, which was found to be significantly elevated in non-survivors compared with survivors of neonatal sepsis, In addition, we found that NMR is an independent factor with higher predicted value than these factors used alone. Our study shows that patients with NMR >7.65 represents lower survival rate.

In the present study, there was no significant difference for RDW between non-survivors and survivors of neonatal sepsis, which was the opposite of a study 14 [14]. It may be the reason of different regions and differences in the level of medical and health care. On the other hand, our sample size was much smaller, which may account for the difference.

However, the current study has several limitations. First, as a single-center retrospective analysis, selection biases associated with data collection may exist and some biomarkers may not included. Second, we also did not compare NMR with other valuable biomarkers in neonatal sepsis such as CRP, procalcitonin. Third, We did not verify the results in a verification cohort and check the performance of NMR in neonates with different age. So we expect to conduct a large, multi-center prospective study to verify our results.

5. Conclusion

In conclusion, our data shows that baseline NMR could play an important role in predicting mortality of neonatal sepsis, which is very important for the management of this high-risk infant population.

Declarations

Author contribution statement

Jun Zhou and Shengfeng Qiu - Conceived and designed the experiments; Analyzed and interpreted the data and Contributed reagents, materials, analysis tools or data.

Xiaohong Xia: Performed the experiments; Contributed reagents, materials, analysis tools or data and Wrote the paper.

Mengxiao Xie and Yaman Wang: Performed the experiments and Contributed reagents, materials, analysis tools or data.

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Data availability statement

The datasets are available from the corresponding author on reasonable request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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