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Neutrophil-to-lymphocyte ratio in patients with gram-negative sepsis admitted to intensive care unit

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Abstract

Background: Several studies have investigated the role of the neutrophil-to-lymphocyte ratio (N/L ratio) in the early diagnosis of infection with controversial results. We assessed this ratio in the early diagnosis of gram-negative sepsis cases admitted to intensive care unit (ICU).

Methods: Patients with gram-negative sepsis admitted to general intensive care unit were enrolled. The following data was recorded: demographic characteristics; APACHE II score; duration of mechanical ventilation and ICU length of stay; average neutrophil and lymphocyte count, along with their ratio, on the first, second and third day of hospitalisation; and the mortality rate.

Results: One hundred and thirty nine patients were studied during a period of one year with a mean age 68.29 ± 17.4 years. One hundred and thirty six patients received mechanical ventilation, with a mean duration of 13.85 ± 2.07 days. The mean white blood cell (WBC) count on the first day of hospitalisation was 11776.04 ± 5789.60, while on the second and third days this was 12446 ± 2101.16 and 13241.01 ± 7077.17, respectively. The N/L ratio on the day of hospitalisation was 11.38 ± 1.02, and 11.98 ± 0.99 and 12.94 ± 1.3 on the second and third days, respectively. Moreover, 46.8 percent of patients died during the study.

From applying Student's t-test, significant differences were observed between two groups of patients (discharged and deceased) in terms of their APACHE II score, neutrophil count, WBC and N/L ratio on the second and third days of hospitalisation ($P < 0.05$).

Conclusion: Blood cell analysis and N/L ratio can be used as a predictor for the severity of gram-negative sepsis along with other diagnostic procedures.

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Key words: sepsis, gram-negative; neutrophil; lymphocyte; neutrophil-to-lymphocyte ratio; biomarkers

Sepsis is one of the oldest syndromes in clinical medicine dating back to the time of Hippocrates [1] and it is one of the major causes of morbidity and mortality among patients in intensive care units [2], with more than 750,000 sepsis cases each year being hospitalised in the United States [3]. Earlier definitions of sepsis and septic shock defined them as cardiovascular dysfunction following an infection that has no other cause [5] and suggested that sepsis is the body's response to the damaged tissues and organs caused by the infection [6].

The new definition of sepsis, based on the Third International Consensus Definitions of Sepsis and Septic Shock in 2016, define it as a life-threatening state and organ dysfunction caused by a dysregulated host response to infection. In this regard, the immune modulatory system has a vital role and affects the cardiovascular, nervous, autonomic, humeral, metabolic and coagulation systems. For quicker diagnosis, instead of using SIRS and SOFA scores, qSOFA scores have been proposed, which included mental impairment, a respiratory rate greater than or equal to 22 breaths per minute, and systolic pressure less than or equal to 100 mmHg. These criteria are considered as predictive of the mortality in patients with sepsis: with one of the above criteria, the mortality rate would be less than 3%, and with all three criteria present, this risk would be more than 20% [7–9].

Although sepsis and septic shock have mostly bacterial causes, fungi and parasites may also cause it, while in one third of cases no infectious cause is detected. Symptoms include as follows: fever (hypothermia in 10–15% of cases); leukocytosis (occasionally leucopoenia); lactic acidosis (blood lactate higher than 2 mmol L^{-1}); continued arterial pressure drop and the need for a vasopressor with sufficient fluid replacement; low urine output; decreased peripheral blood flow; and altered mental status. The most common characteristic of septic shock is its hyperkinetic pattern, which is associated with a high cardiac output [10].

Currently, accessible, cheap, and fast biomarkers are used to predict inflammatory and infectious reactions. Numerous studies have evaluated the role of inflammatory tests, including the neutrophil-to-lymphocyte ratio (N/L ratio), in the pre-determination of infections and cancers in various parts of the body and which have yielded contradictory results. During the acute phase of inflammation, particularly as a result of bacterial infection, environmental exposure [11] and some cancers, neutrophils are one of the first inflammatory responders of cells to migrate towards the site of inflammation [12, 13]. Therefore, the N/L ratio is used as a marker of subclinical inflammation [14] and tumours [15–18].

One study regarding the diagnosis of adult complicated acute appendicitis, performed in 2012, did not show sufficient accuracy for this test [19]. Regarding another study on pulmonary infection caused by *Escherichia coli*, although showing an ability to predict disease severity, its low test ratio did not allow for rejecting possibilities of bacteraemia or acute sepsis [20]. Our study evaluated the predictive power of the neutrophil-to-lymphocyte ratio as a fast marker in patients with gram-negative sepsis.

METHODS

This cross sectional study was approved by ethics committee of Urmia University of Medical Sciences and conducted in the GICUs (General Intensive Care Units) of Imam and Ayatollah Taleghani hospitals (tertiary educational hospitals) in Urmia during 2015. Due to the prevalence of approximately 15–25% of sepsis patients in the ICU, nearly 200 patients with sepsis are hospitalised in these two GICUs each year. All non-surgical patients admitted to a GICU who developed gram-negative sepsis during their hospitalisation, or those who were hospitalised with gram-negative sepsis, were included in the study. Patients with Glasgow Coma Scale – 3 and brain death were excluded, along with all surgical, trauma and cancer patients. Diagnosis was based on clinical and vital signs, having a positive blood culture, or other accompanying tests, such as a white blood cell count and differential, the erythrocyte sedimentation rate, C-reactive protein, procalcitonin levels, as well as a diagnosis by an infectious disease specialist according to the new definition of sepsis-3 [7]. The neutrophil and lymphocyte counts, and their ratio, were recorded on the first, second and third days. Furthermore, the APACHE score II, demographic information, duration of hospitalisation, mortality rate in the ICU, duration of mechanical ventilation, as well as the types of antibiotics used were recorded from the medical information ward.

A statistical analysis was performed using SPSS (Ver. 20, 2010). Student's t-test was used to compare quantitative variables with a normal distribution while the Chi-square test was used for qualitative variables with a normal distribution. $P < 0.05$ was considered statistically significant.

RESULTS

In this cross-sectional study, 139 patients with gram-negative sepsis and a mean age of 68.29 ± 17. 4 years were enrolled. Among them, 72 (51.8%) were female and 67 (48.2%) were male. The mean APACHE II score was 17.38 ± 3.27. *Kelbsiella sp.* (33.81%), *Escherichia coli* (27.33%), *Psudomonas sp.* (15.1%), *Enterobacter sp.* (12.23%), *Acinetobacter sp.* (10.07%), *Serratia sp.* (1.435%) were commonly found in blood cultures of patients. Eight patients (5.8%) received only one antibiotic, 76 (54.7%) received two, 43 (30.9%) three, 11 (7.9%) four, while one patient (0.7%) received five antibiotics.

The mean length of stay in the ICU was 16.79 ± 1.83 days. Only three patients did not receive supportive mechanical ventilation and the mean duration of mechanical ventilation was 13.58 ± 2.07 days for the remaining patients. Tables 1 and 2 show the demographic characteristics for discharged and deceased patients.

Although seventy four patients (53.2%) with an APACHE II score of 16.23 ± 2.17 were discharged after a full recovery, unfortunately 65 patients (46.8%) with an APACHE II score of 21.08 ± 1.82 died during the study Student's t-test ($P = 0.02$)].

The mean white blood cell count (WBCs) during hospitalisation was 11776.04 ± 5789.60. However, mean WBCs on the second and third days were 12446 ± 2101.16 and 13241.01 ± 7077.17, respectively. Table 3 shows a comparison between discharged and deceased patients in terms of WBCs during the study.

Table 4 shows a comparison between discharged and deceased patients in terms of neutrophil percentage during the study.

Table 5 shows a comparison between discharged and deceased patients in terms of lymphocyte percentage during the study.

The N/L ratio on the first day of hospitalisation was 11.38 ± 1.02, while on the second and third days this was assessed as 11.98 ± 0.99 and 12.94 ± 1.3, respectively. Table 6 shows

Table 1. Demographic and baseline parameters

Baseline parameters		Discharged	Deceased	*P*-value
Age (years)		65.81 ± 13.2	68.7 ± 18.34	0.06
Gender	Male	42	30	0.78
	Female	38	29	
ICU stay (days)		16.4 ± 1.23	22.17 ± 0.78	0.03
Body mass index (kg m^{-2})		27.91 ± 4.6	26.13 ± 3.58	0.07
Fasting blood sugar (mg dL^{-2})		95.28 ± 4.3	101.05 ± 3.18	0.04
Hb_1C		5.1 ± 0.23	6.1 ± 0.33	0.04
Mortality rate		57.56%	42.44%	0.03

Table 2. Characteristics of disease in discharged and deceased patients

Disease	Total	Discharged	Deceased
Cerebrovascular accident	36	20	16
Chronic obstructive pulmonary disease	21	14	7
Aspiration pneumonia and pneumonia	13	10	3
Lung cancer	5	2	3
Venous thromboembolism	14	8	6
End-stage renal disease	31	16	15
Rheumatologic disorder	13	7	6
Oncologic disorders	6	3	3
Total	139	80	59

Table 3. Comparison between discharged and deceased patients in term of white blood cells during the study

	Outcome	Neutrophil	*P*-value
First day	Discharged	11226.08 ± 5149.39	0.23
	Deceased	12402.15 ± 8425.31	
Second day	Discharged	11170.68 ± 4731.60	0.001
	Deceased	13898.92 ± 5152.54	
Third day	Discharged	11043.24 ± 4157.58	0.0001
	Deceased	15743.08 ± 8737.24	

Table 4. Comparison between discharged and deceased patients in terms of neutrophil percentage during the study

	Outcome	Neutrophil	*P*-value
First day	Discharged	81.54 ± 9.24	0.2
	Deceased	83.63 ± 9.92	
Second day	Discharged	81.84 ± 8.25	0.008
	Deceased	85.69 ± 8.55	
Third day	Discharged	80.64 ± 9.50	0.01
	Deceased	85.05 ± 10.31	

a comparison of discharged and deceased patients in terms of the neutrophil-to-lymphocyte ratio.

Table 7 shows a comparison of discharged and deceased patients in terms of blood culture.

DISCUSSION

The results of our study showed that there were significant differences between the two groups of patients concerned (discharged and deceased patients) in term

Table 5. Comparison between discharged and deceased patients in terms of lymphocyte percentage during the study

	Outcome	Neutrophil	*P*-value
First day	Discharged	14.21 ± 8.65	0.17
	Deceased	12.17 ± 9.12	
Second day	Discharged	13.23 ± 7.40	0.04
	Deceased	10.62 ± 8.35	
Third day	Discharged	14.29 ± 8.19	0.002
	Deceased	10.03 ± 7.96	

Table 6. Comparison of discharged and deceased patients in terms of neutrophil-to-lymphocyte ratio (N/L ratio)

	Outcome	N/L ratio	*P*-value
First day	Discharged	9.88 ± 10.54	0.12
	Deceased	13.09 ± 1.68	
Second day	Discharged	9.38 ± 7.78	0.004
	Deceased	14.95 ± 1.80	
Third day	Discharged	9.17 ± 1.37	0.002
	Deceased	17.23 ± 2.20	

Table 7. Comparison of discharged and deceased patients in terms of blood culture.

Blood culture	Discharged	Deceased	*P*-value
Klebsiella	26	21	0.1
Escherichia coli	25	13	0.05
Pseudomonas	11	10	0.1
Enterobacter	10	7	0.6
Acinetobacter	7	7	1
Serratia	1	1	1

of APACHEII scores, WBCs and neutrophil counts and N/L ratios.

Many studies have attempted to introduce accessible, cheap and fast biomarkers, including the WBC count or N/L ratio, for the quick detection of sepsis. Zahorec reported that the N/L ratio may express the severity of disease and can be regularly used in the ICU at 6, 12 and 24-hour intervals [21]. Similarly, our patients who died due to gram-negative sepsis had had a higher N/L ratio in serial tests (second and third day) compared with patients who had fully recovered. Because we had excluded patients with trauma or surgery and malignancy, we considered the reason of the elevation of the neutrophil count and N/L ratio was sepsis.

Several studies have investigated the N/L ratio in infectious patients, with contradictory results [19, 20, 22, 23].

Hosseinpour *et al.* [19] investigated the diagnostic value of this ratio in acute adult appendicitis, and claimed that despite a significant difference between the positive and negative pathology patients, this test lacks sufficient accuracy in diagnosing acute and symptomatic appendicitis. One year later, Ljungstorm *et al.* [20] claimed that the N/L ratio could be a marker of disease severity for *Escherichia coli* infection, and the higher this ratio, the higher the possibility of severe sepsis. On the other hand, a low ratio never precludes the possibility of bacteraemia or severe sepsis. Similarly, our study showed that patients with a poor prognosis (the deceased group with a high APACHE II score) had had a higher N/L ratio and that this ratio, along with other tests, could be used to predict disease severity. However, we claim that this ratio cannot be used by itself to diagnose gram-negative sepsis.

The importance of sepsis and its early diagnosis and treatment and implications in all areas are emphasised [24, 25].

In a retrospective study, De Jager *et al.* [26] compared patients with positive and negative blood cultures. They indicated that the N/L ratio and lymphocytopenia bacteraemia are better predictors than the routine parameters in an emergency care setting. Similar to their study, we

showed that an increased neutrophil-to-lymphocyte ratio during the first 3 days of admission indicates sepsis severity and an increased rate of mortality. An increase in this ratio is either due to an increase in the number of neutrophils or a decrease in the number of lymphocytes or both, and it may be concluded that lymphopenia may have existed in our study. We have neglected to examine them since they did not fall within the remit of our study.

Liu *et al.* [27] indicated that an increased N/L ratio was independently associated with an unfavourable clinical prognosis in patients with sepsis. Median N/L ratio levels were significantly higher in patients who died than in survivors. Our study confirms these findings.

It has been also claimed that the N/L ratio can be used as a novel non-invasive marker to predict the occurrence of hospital-acquired bacterial infections in decompensated cirrhotic patients [28].

Akilli *et al.* [29] suggested that the N/L ratio in critically ill patients is an important indicator of short- and long-term mortalities: thus, hospital mortality and 6-month mortality were associated with a higher N/L ratio. This was a prospective cohort study, and although patients were about ten years older than patients in our study, they were similar in terms of gender.

Hwang *et al.* [30] stated that the initial N/L ratio measured on admission to an emergency department was independently associated with 28-day mortality in patients with severe sepsis and septic shock, and that the changes in this ratio may provide an important prognostic marker.

Gurol *et al.* [31] examined 1,468 patients with bacteraemia and sepsis. They concluded that the total white cell and neutrophil counts have historically been used as markers of infection. They also believed that an N/L ratio ≥ 5 indicated a more convenient marker than CRP because of its ability to detect bacterial infections at a lower cost. As with these studies, our study, though with a different sample size, showed that a high neutrophil/lymphocyte ratio on the second and third days correlated with an increase in the mortality of hospitalised patients with gram-negative sepsis in the ICU. It also showed that this ratio may be used, along other with diagnostic procedures and markers of disease severity, for predicting the outcome of these patients. Thus, in our deceased patients the N/L ratio was more than 10 and in discharged patients less than 10.

This study suffered from some inevitable limitations. Incomplete patient records in the hospitals that we encountered was one of the shortcomings of the study that decreased the number of the cases studied. Furthermore, selecting patients with only gram-negative sepsis and excluding cases with gram-positive was another limitation. Finally, the duration of the study and the sample size of the population was limited in our study. It is recommended that physicians conduct a study with both gram-positive and gram-negative sepsis over a longer period of time and with a large sample size in the near future.

CONCLUSION

According to the results of our study, an analysis of the serial CBC and neutrophil/lymphocyte ratio, along other with tests, can provide a fast method for establishing disease identification, severity and outcome in sepsis.

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