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A Reliable Non Invasive Biomarker for SBP: Neutrophil - To - lymphocyte Ratio as a Diagnostic Tool for Spontaneous Bacterial Peritonitis

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Abstract: ***Spontaneous bacterial peritonitis (SBP) is one of the major complications in patients with cirrhosis and ascites [1]. The diagnosis of SBP is based on the neutrophil count and culture positivity in ascitic fluid [5]. Despite the diagnostic and treatment recommendations in the guidelines, in a retrospective study made in the USA, only 52.4% of patients diagnosed with SBP met the criteria and only 67.3% of patients received appropriate treatment [8]. Secondly, doing paracentesis for diagnosis is a invasive test and itself might introduce infection into the ascetic fluid. Neutrophil to Lymphocyte ratio (NLR) is a simple parameter which gives practical insight into the immune system activity. So, we aimed to study NLR as one of the biological markers that were associated with SBP and its usefulness in diagnosis. Method: Our retrospective, observational study included 100 patients with liver cirrhosis who were hospitalised between December 2020 and December 2022 at our institute in Chennai, Tamil Nadu. After following inclusion and exclusion criteria, patients were divided into two groups: One group of patients with SBP and the other without SBP. The diagnosis of SBP was made when patients presented with PMN >250 mm^3 or culture positivity. This data was analysed and showed an association between biological parameters such as serum white blood counts, NLR, INR, serum sodium, Total bilirubin, Ascitic low sugar, low albumin, high protein, high MELD and CTP scores with Spontaneous bacterial peritonitis. Multivariate analysis with ROC identified NLR and CRP as very good biomarkers in the diagnosis of SBP. The area under the curve (AUC) was 87.6 [P<0.001, 95% confidence interval (CI) 80.3 – 94.8] for NLR and AUC was 73 (P<0.001, 95% CI 62.8 - 83.2) for CRP respectively. Conclusion: NLR is a very good non invasive biomarker for diagnosing and predicting SBP, diagnostic accuracy of which increase with combination of CRP. This study could be of clinical utility in diagnosis of SBP as it is non - invasive, bedside and easy to use.***

Keywords: Neutrophil to lymphocyte ratio (NLR), Spontaneous bacterial peritonitis, Ascites, C - Reactive protein, Cirrhosis, Paracentesis, Total bilirubin.

1. Introduction

SBP is one of the major complications in patients with cirrhosis and ascites. It is defined as a bacterial infection of the ascitic fluid, without any specific identifiable intra - abdominal treatable source of infection [1]. Spontaneous Bacterial Peritonitis (SBP) has a prevalence of approximately 10 - 30% [2] Mortality in untreated cases of SBP is found to be as high as 80% in some studies [3].

The clinical presentation of SBP is heterogeneous, ranging from being asymptomatic or with local symptoms, such as abdominal pain, vomiting, or diarrhoea, and can also present with signs of systemic inflammation, deterioration of liver function, hepatic encephalopathy, AKI and septic shock [4]. The diagnosis of SBP is based on the neutrophil count in ascitic fluid. The cutoff value with the best sensitivity for the diagnosis of SBP is an ascitic neutrophil count of 250/mm3. [5]

Ascitic fluid culture should be performed, but a positive culture is not necessary as ascitic fluid cultures may be negative in upto 60% of patients with SBP. [6] In some cases, patients may present with a positive ascitic fluid culture result but an ascitic neutrophil count below 250/ mm3. This condition is known as bacterascites. Which Has similar management. [7]

Despite the diagnostic and treatment recommendations in the guideline, in a retrospective study made in the USA, only 52.4% of patients diagnosed with SBP met the criteria and only 67.3% of patients received appropriate treatment [8].

Ascitic fluid analysis for diagnosis of SBP requires PMN Ascitic fluid count or culture. Culture is negative in approximately 30 - 50% cases despite sensitive methods [9, 10].

Secondly, PMN count in ascitis is invasive, requiring automated cell counter; sometimes cannot be done on emergency basis and is prone to human error. Hence, there is a need for some less invasive biomarker which can predict SBP without the need for repeated paracentesis. Various quick, simple and less invasive rapid bedside diagnostic tests for SBP are being studied like Leucocyte Esterase levels in urine [11, 12].

As SBP is an inflammatory state and inflammatory markers are notably stimulated in SBP despite the low ascitic fluid bacterial concentration. In patients with SBP, inflammatory markers such as interleukin - 6 (IL - 6), tumour necrosis factor - α (TNF - α) and α - 1 - antitrypsin (AAT) have been observed to be elevated in various studies [13, 14].

Procalcitonin, a pro - inflammatory marker which increases in response to bacterial infections was also found to be raised among patients with SBP in a meta - analysis of 7 studies conducted in China in 2015 [15].

It is important to recognize spontaneous bacterial peritonitis early in the course of infection because there is frequently a very short window of opportunity during which to intervene to ensure a good outcome. If the opportunity is missed, shock ensues, followed rapidly by multisystem organ failure [16]. One report estimated that survival decreased by approximately 8 percent for each hour of delay in starting antibiotics in patients with septic shock [17]

NLR is a simple parameter to easily assess the inflammatory status of a subject. It has been suggested as a marker of systemic inflammation and shows the relationship between two different immune pathways.

Aim of the Study:
The aim of our study is to focus on the Neutrophil to lymphocyte ratio (NLR) as one of the biological markers that were associated with SBP and to study its usefulness in its diagnosis.

2. Materials and Methods

Study Design: Retrospective, Observational and Non interventional study.

Place of Study: SRI RAMACHANDRA INSTITUE OF HIGHER EDUCATION AND RESEARCH, CHENNAI, TAMIL NADU.

Time Period: Between December 2020 and December 2022.

Participants:
Patients who were admitted in wards, diagnosed with cirrhosis on clinical, biochemical and radiological examination between the age group of 18 - 65 years of both gender and fulfilling the inclusion and exclusion criteria were enrolled for the study.

Study Size: 100 patients – 50 cases and 50 controls.

Inclusion Criteria:
- Age - 18 – 65 years.
- Patients who are cirrhotic with ascites due to any etiology, with or without spontaneous bacterial peritonitis.

Exclusion Criteria:
- Patients having ascites caused by causes other than cirrhosis, such as congestive heart failure and abdominal TB. Patients with peritonitis caused by intra - abdominal surgery or other infectious causes (respiratory or urinary).
- Patients with any infection other than SBP (like Urinary Tract Infection (UTI), Tuberculosis, etc.), neoplastic disorders, active autoimmune disorders, or any other chronic systemic illness.
- Patients who had received antibiotics within the last 7 days or were on antibiotic prophylaxis for SBP.
- Patients who are pregnant.

Methods
After following the inclusion criteria, patients between the ages of 18 and 65years were included in the study and evaluated with a detailed history and examination to rule out any infection other than SBP. Diagnostic paracentesis was performed in all the patients for counts (TLC/DLC), sugar, protein, albumin, culture/sensitivity, cytology and serum ascites albumin gradient (SAAG) was calculated. The diagnosis of SBP was made when at least 250 PMN/mm3 or a positive ascitic fluid culture was obtained in the absence of secondary peritonitis and haemorrhagic ascites. Patients having SBP on ascitic fluid analysis were taken as cases and patients without evidence of SBP were taken as controls. Retrospectively, data was collected from MRD. Laboratory testing was done which included Complete Blood Count (CBC), Urine Routine microscopy, Blood Urea, Serum Electrolytes, Random Blood Sugar, Liver Function Tests, Renal Function Tests (RFT) and special investigations like CRP and NLR.

The patients were separated into two groups: 50 cirrhotic patients with SBP and 50 cirrhotic patients without SBP.

Automated differential counts of neutrophils and lymphocytes were performed to determine the Neutrophil - to - Lymphocyte Ratio (NLR). The neutrophil - to - lymphocyte ratio was computed by dividing the neutrophil count by the lymphocyte count (both Absolute counts and percentage) from the same automated blood samples.

Statistical Analysis
Data was entered in MS Excel and analysed using SPSS software version 25.0. Categorical variables were summarized using frequency and percentage, normally distributed continuous variables were summarized using mean and standard deviation; and those not normally distributed were summarised using median and interquartile range. Statistical difference between two proportions, mean and median were tested using Chi - square test, independent sample t test, and Mann Whitney U test, respectively. ROC analysis was performed to find out cut - off point to predict diagnostic accuracy of NLR, CRP, and total bilirubin to diagnose SBP.

3. Results

Demographic variables

The mean ages for the control group and case group were 55.8 and 54.3, respectively. An independent t - test revealed a p value of 0.47, i. e. there was no significant difference between the two groups. The study group was comprised of 40 males and 10 females, and the control group was composed of 39 males and 11 females. The chi - square test revealed a p value of 0.80, i. e. there was no statistically significant difference between the frequencies of sex distribution across the two groups. Thus, the two groups were matched for age and sex (Table 1, figure 1).

Table 2: We have compared various lab parameters among SBP and non SBP group and found that Total leucocyte counts, Neutrophil %, Lymphocyte %, NLR ratio, PT, INR were found to be higher in the SBP group. This finding is statistically significant.

Table 3: shows a comparison of creatinine and Sr Sodium levels among two groups. It shows a similar sr creatinine values almost the two groups with no statistical significance. But Sr sodium values in SBP group were found to be low.

Table 4: showed comparison of LFT among two groups and found to have a high bilirubin in patients with SBP. Rest all LFT parameters didnot show significant difference among two groups.

Table 5: Ascetic fluid analysis showed a low sugar with slightly higher values of albumin and protein along with high total counts and polymorphs in SBP group. All these findings showed statistical significance.

Table 6: Patient with higher MELD and CTP were found to have higher chances of developing SBP.

Table 7: Comparison of NLR between cases and controls

The mean NLR in patients with and without SBP was found to be 7.63 and 2.86 respectively (Table 7; Figure 2b). The difference was statistically significant with p value <0.001. The Receiver Operating Characteristic (ROC) curve analysis demonstrated that a cut - off of blood NLR >3.32 has a sensitivity of 86% and specificity of 70% in diagnosing SBP among patients with ascites. And with NLR >4.06 has a sensitivity of 80% and specificity of 86%. Sensitivity and specificity are mentioned for various NLR cut off points were mentioned in Table - 7.

Table 1: Comparison of age and gender between two groups of study participants (N=100).

Sl. No.	Characteristics	Participants group		p value#
		With SBP (n=50) n (%) / Mean (SD)	Without SBP (n=50) n (%) / Mean (SD)	
1	Age in years	54.3 (11.5)	55.8 (10.2)	0.47
2	Gender			
	Male	40 (80)	39 (78)	0.80
	Female	10 (20)	11 (22)	

Note: p value based on independent sample t test for mean and Chi - square test for proportion

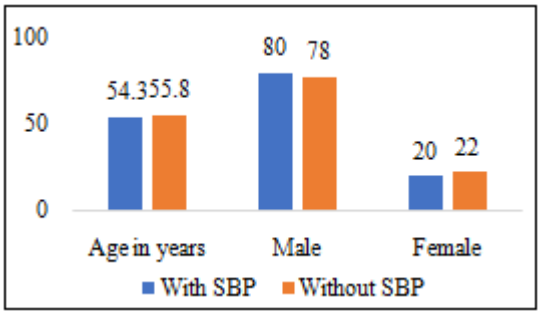


Figure 1: Comparison of age and gender between two groups of study participants (N=100).

Table 2: Comparison of haematological parameters between two groups of study participants (N=100).

Sl. No.	Haematological parameters	Participants group		p value#
		With SBP (n=50) Mean (SD) / Median (IQR)	Without SBP (n=50) Mean (SD) / Median (IQR)	
1	Haemoglobin in g%	9.1 (1.6)	9.5 (1.9)	0.29
2	Total count	10465.4 (5429.9)	6610 (2715.7)	<0.001*
3	Neutrophil %	77.3 (11.2)	63.8 (9)	<0.001*
4	Lymphocyte %	11.8 (6.8)	23.3 (7.1)	<0.001*
5	N - L ratio	7.63 (4.6-11.8)	2.86 (2.2-3.6)	<0.001*
6	Platelet count in lakhs	1.29 (0.8)	1.12 (0.6)	0.26
7	Prothrombin time	20.7 (8.6)	17.3 (6.1)	0.02*
8	INR	1.8 (0.7)	1.5 (0.5)	0.01*

Note: p value based on independent sample t test for mean and Mann Whitney U test for median, * statistically significant (p<0.05).

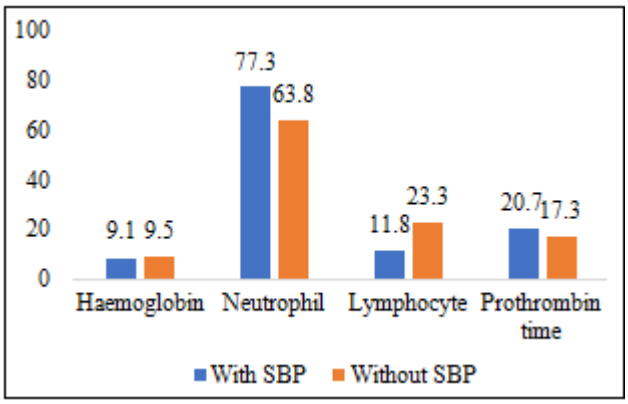


Figure 2 (a): Comparison of haematological parameters between two groups of study participants (N=100).

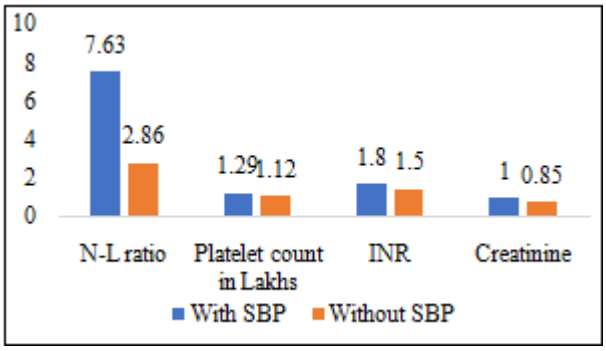


Figure 2 (b): Comparison of haematological parameters and creatinine between two groups of study participants (N=100).

Table 3: Comparison of renal parameters between two groups of study participants (N=100).

S. No.	Renal parameters	Group of participants		p value#
		With SBP (n=50) Median (IQR) / Mean (SD)	Without SBP (n=50) Median (IQR) / Mean (SD)	
1	Creatinine	1 (0.7 – 0.2)	0.85 (0.7 – 1.2)	0.25
2	Sodium	130.2 (4)	132.8 (3.4)	0.001*

Note: p value based on Mann Whitney U test for median and independent sample t test for mean, * statistically significant (p<0.05)

Table 4: Comparison of liver function tests between two groups of study participants (N=100).

Sl. No.	Liver function tests	Group of participants		p value#
		With SBP (n=50) Median (IQR)	Without SBP (n=50) Median (IQR)	
1	Total bilirubin	5.75 (2.7 – 9.1)	2.7 (1.2 – 5)	0.004*
2	AST	75 (54 – 113)	60 (44 – 112)	0.15
3	ALT	33.5 (26 – 60)	27.5 (19 – 42)	0.01*
4	ALB	2.25 (2.1 – 2.7)	2.5 (2.2 – 2.8)	0.10
5	ALP	98.5 (75 – 155)	97 (79 – 128.5)	0.49

Note: p value based on Mann Whitney U test, * statistically significant (p<0.05)

Table 5: Comparison of ascitic fluid analysis between two groups of study participants (N=100).

S. No.	Ascitic fluid analysis	Group of participants		p value#
		With SBP (n=50) Median (IQR)	Without SBP (n=50) Median (IQR)	
1	Total Count	1300 (807 – 3497)	87.5 (40 – 152)	<0.001*
2	Polymorphs	700 (400 – 3013)	16 (5 – 37)	<0.001*
3	Sugar	107 (80 – 136)	121 (108 – 173)	0.004*
4	Albumin	0.6 (0.4 – 0.8)	0.4 (0/3 – 0.6)	0.002*
5	Protein	1.6 (1.1 – 1.8)	1.05 (0.7 – 1.6)	0.001*

Note: p value based on Mann Whitney U test, * statistically significant (p<0.05)

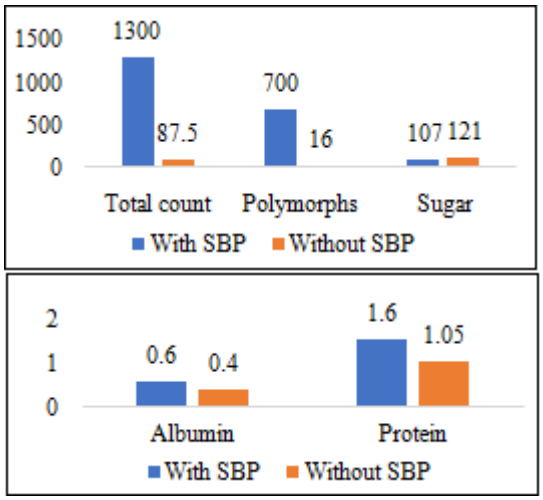


Figure 5 (a): Comparison of ascitic fluid analysis between two groups of study participants (N=100).

Table 6: Comparison of average MELD between two groups of study participants (N=100).

S. No.	Parameter	Group of participants		p value#
		With SBP (n=50) Mean (SD) / n (%)	Without SBP (n=50) Mean (SD) / n (%)	
1	MELD	23.7 (5.6)	19.3 (7.6)	0.001*
2	CTP	10.7 (1.5)	10.2 (1.9)	0.11
3	CTP grade			
	B	10 (20)	21 (42)	0.02*
	C	40 (80)	29 (58)	

Note: p value based on independent sample t test for mean and Chi - square test for proportion * statistically significant (p<0.05)

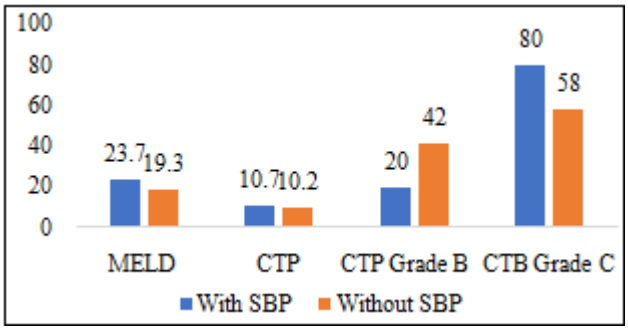


Figure 6: Comparison of average MELD between two groups of study participants (N=100).

Table 7: ROC analysis showing the cut - off value of NL - Ratio and its diagnostic accuracy in detecting SBP

Parameter	Area under the curve	95% Confidence Interval		p value
		Lower	Upper	
NLR	87.6	80.3	94.8	<0.001*
CRP	73.0	62.8	83.2	<0.001*

Note: * statistically significant (p<0.05)

Table 8

NLR Cut off point (Greater than or equal to)	Sensitivity	Specificity
3.32	86%	70%
3.65	82%	76%
4.06	80%	86%
5.17	72%	94%
6.20	64%	96%

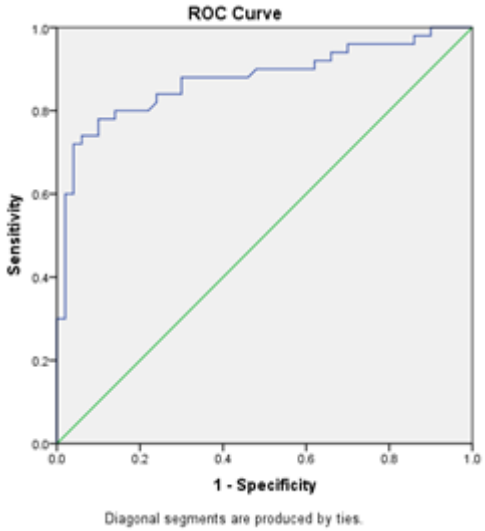
Comparison of CRP between cases and controls

The CRP values were compared among case and controls for the diagnostic accuracy, which showed an area under curve of 73.6 and 95% confidence interval with 62.8 as lower limit and 83 as upper limit. And cut off values obtained were mentioned in Table 9. However only 24 patients in case and controls had CRP values. hence analysed the same number of patients.

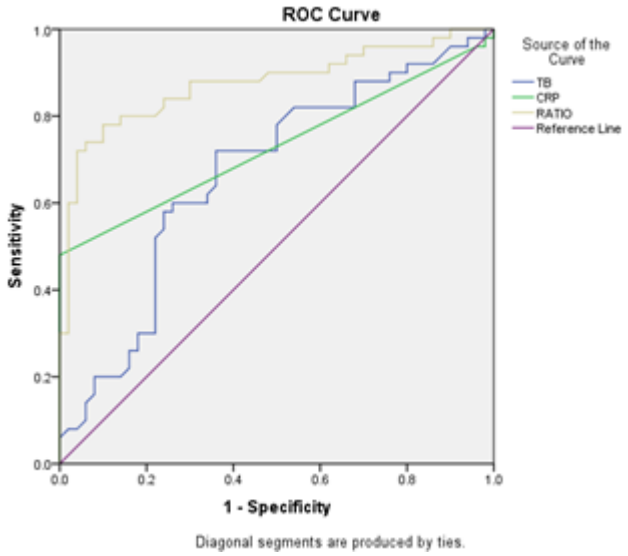
Table 9

CRP Cut off point (Greater than or equal to)	Sensitivity	Specificity
0.95	96%	4%
1.1	48%	100%

ROC curve to identify SBP using NLR



ROC curve to identify SBP using NLR, CRP and Tb (Total bilirubin)



Correlation of NLR and CRP

On correlation analysis between NLR and CRP, a negative correlation was found among controls but was statistically insignificant. The correlation among cases was positive but the coefficient of correlation was low.

4. Conclusion

NLR could be of clinical utility and a diagnostic bedside tool in predicting Spontaneous Bacterial Peritonitis. As our study showed NLR as a strong predictor with good sensitivity and specificity.

NLR cut off value of >3.32 has a sensitivity of 86% and specificity of 70% and NLR >4.06 showed a sensitivity of 80% and specificity of 86% % in diagnosing SBP.

The combination of NLR and CRP can be used to diagnose with more accuracy. As these test are easily done bedside with good sensitivity and specificity to diagnose SBP. However it requires further studies on larger population and requires more data to support these findings.

Hence repeated paracentesis for ruling out SBP on patients on admission can be avoided.

5. Discussion

Patients with liver cirrhosis and ascites are prone to infections, with SBP being among the most common, with a variable prevalence but a high mortality rate (18).

Ascetic fluid analysis is used for the diagnosis of SBP, which requires a PMN count and culture. Culture is negative in approximately 30–50% of cases, despite sensitive methods [9, 10]. The PMN count is cumbersome, requiring an automated cell counter that sometimes cannot be done on an emergency basis and is prone to human error. Hence, there is a need for a biomarker which is lesser invasive and better sensitive that can predict SBP without the need for repeated paracentesis [11, 12].

NLR is being compared among the groups with and without SBP, which showed a very statistically significant correlation, as found in the previous studies mentioned.

Our study showed that there was a significant difference between the mean values of total leucocyte counts, neutrophil percentage, lymphocyte percentage, NLR ratio, PT, INR, total bilirubin, ascitic fluid albumin, protein, MELD - Na, and CTP, which were found to be higher in the SBP group compared to those without SBP. While Sr creatinine and Sr sodium showed no significant difference. This is contrary to the study by Roxana - Emanuela *Popoiag et al. (19).*

The results of our study showed that there were significant differences in the mean value of PMN and ascites proteins between the SBP and non SBP groups.

The SBP group has slightly higher protein levels compared to the non - SBP group.

But no significant differences between the mean values of Hb, AST, and alanine aminotransferase were registered.

Similar to our study results, Abdel Rahman *et al. (20) found that* in a study of 80 patients with liver cirrhosis divided into two equal groups, with and without SBP, the mean values of Hb, AST, ALT, and creatinine did not differ significantly between the two groups, but the median values of PMN differed significantly.

The univariate analysis in our study identified multiple factors involved in the occurrence of SBP, including serum biological parameters (WBC, serum albumin, INR, total bilirubin, ALT, NLR, and CRP), as cites fluid analysis (WBC, PMN, PROTIEN), MELD Na, and CTP.

Only two factors, NLR and CRP, were identified as independent predictive factors with good accuracy for SBP diagnosis.

NLR is a noninvasive marker that can be used to predict the occurrence of inhospital infections in patients with decompensated liver cirrhosis (17). In a study conducted by Piotrowski *et al.*, an association between NLR and the presence of infection in patients with liver cirrhosis was identified but with low diagnostic accuracy (AUC = 0.606) (18).

Our study showed that a model consisting of NLR and CRP has high accuracy in SBP diagnosis. Moreover, according to literature data, NLR can be used in combination with other factors as a predictor of the occurrence of SBP. AbdelRazik *et al. (*19) demonstrated that a combination of age, mean platelet volume (MPV), NLR, and CRP, used as the Mansoura score, can rule out the diagnosis of SBP. Mousa *et al. (*20) showed that a combination of NLR and CRP can be used as a simple and non - invasive test for SBP diagnosis, the findings of which were consistent with our study.

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