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# Blood Neutrophil / Lymphocyte Ratio and C – Reactive Protein / Albumin Ratio as Markers of Response for Treatment of Spontaneous Bacterial Peritonitis

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#### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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# **ABSTRACT**

**Background:** Spontaneous bacterial peritonitis (SBP) is an acute infection of ascites with the absence of surgically treatable cause and the gold standard method in its diagnosis is the presence of 250 polymorphonuclear neutrophils (PMN) /mm³ or more by diagnostic paracentesis. Blood neutrophil/lymphocytic ratio (NLR) is an applicable, inexpensive, and simple test for inflammation. C-reactive protein/albumin ratio (CAR) is an inflammatory marker used for the diagnosis and follow-up of many diseases and morbidities. We aimed to evaluate the clinical utility of both blood NLR and CAR as applicable, simple and non-invasive tests for SBP follow-up.

Patients and Methods: This study was done on 80 cirrhotic ascitic patients attending the Tropical Medicine Department of Tanta University Hospital. They were subjected to full history taking, clinical examination, laboratory investigations, and ascitic fluid analysis. The patients were divided into two groups according to the results of diagnostic paracentesis into group I: 40 cirrhotic ascitic patients without spontaneous bacterial peritonitis and group II: 40 cirrhotic ascitic patients with

spontaneous bacterial peritonitis, and then SBP group were tested after treatment by third-generation cephalosporin for five days for ascitic sample, NLR and CAR.

**Results:** Both blood NLR and CAR were significantly higher in SBP patients. Also, a significant decrease in both ratios was observed post-treatment with significant positive correlations between both NLR and CAR with ascitic neutrophil count after SBP treatment.

**Conclusion:** NLR and CAR can be used as quick, cheap, and applicable markers of the response of treatment in SBP patients.

Keywords: Neutrophil / Lymphocyte Ratio - C reactive protein /Albumin Ratio; Markers; Response; Treatment; Spontaneous Bacterial Peritonitis.

# 1. INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is considered a serious complication of ascites that leading to death and can be described as an acute infection of ascites without an evident or certain source of infection [1].

SBP has a wide variety of clinical presentations. SBP can be asymptomatic and patients pass unnoticed or discovered accidentally may have local symptoms and signs of peritonitis as abdominal pain, abdominal tenderness, vomiting, diarrhea or may present with symptoms and signs of systemic inflammation as elevated temperature, rigors, leukocytosis, tachycardia, and tachypnea or may present with signs of deterioration of liver function in form of hepatic encephalopathy, refractory ascites, gastrointestinal bleeding, shock and renal failure [2].

The gold standard method in the diagnosis of SBP is diagnostic paracentesis with polymorphonuclear (PMN) count equal to 250 cells per mm3 or more [3].

Neutrophil\lymphocyte ratio (NLR) shows the relationship between 2 different immune pathways as the neutrophil count represents ongoing inflammation while the lymphocyte count reflects the immune regulatory pathway [4].

The NLR has been used recently as a prognostic factor in many malignancies and inflammatory diseases [5, 6].

CRP/albumin ratio (CAR) is a combination of markers for both systemic inflammation and the nutritional status of the body. This combination can synergistically enhance the prognostic role than the use of CRP or albumin alone [7].

Also, the CAR is used as a predictive marker in patients suffering from the infection, malignancy, and some other diseases [8,9].

The aim of this study is to assess the value of blood neutrophil to lymphocyte ratio and C-reactive protein to albumin ratio as markers of response for the treatment of spontaneous bacterial peritonitis.

#### 2. PATIENTS AND METHODS

This analytic prospective cohort study was carried out on 80 cirrhotic ascitic patients. They were selected consecutively from the Tropical Medicine Department of Tanta University Hospital for a period of six months from November 2018 to April 2019. The committee of ethics of scientific research of Tanta Faculty of Medicine approved the studied protocol and written consents were obtained from the studied groups for participation.

The patients were divided into two groups: Group I: 40 cirrhotic ascitic patients without Spontaneous bacterial peritonitis. Group II: 40 cirrhotic ascitic patients with Spontaneous bacterial peritonitis.

# 2.1 Exclusion Criteria

- Ascites without cirrhosis (malignant ascites, chylous ascites, etc...).
- Tuberculous peritonitis.
- Secondary bacterial peritonitis due to any surgical cause.
- Sepsis rather than SBP.
- Patients with unrelated infections e.g., skin and chest infection, etc...).

All patients were subjected to full history taking and complete physical examination.

#### 2.2.1 Laboratory investigations

Complete blood count, liver biochemical tests, coagulation profile, renal biochemical tests, erythrocytic sedimentation rate (ESR) Serum C - reactive protein (CRP), viral hepatitis markers (HCV antibody and HBsAg), ascitic fluid

chemical, physical and cytological analysis, the serum-ascites albumin gradient (SAAG).

# 2.2.2 Imaging

Pelvi-Abdominal ultrasound was done for all patients to assess liver conditions and also can be used in the ascitic fluid sample.

After patients met the inclusion and exclusion criteria, they werefurther tested for ascitic sample, NLR, and CAR before and after treatment of SBP by third-generation cephalosporin for five days according to the guidelines [10].

# 2.3 Statistical Analysis

Statistical analysis was done by SPSS v25 (IBM Inc., Chicago, IL, USA). Numerical variables were presented as mean and standard deviation (SD) and compared between the two groups utilizing the Student's t-test. Categorical variables were presented as frequency and percentage (%) and were analysed utilizing the Chi-square test or Fisher's exact test when appropriate. Pearson correlation was done to estimate the degree of correlation between two quantitative variables. A two-tailed P value < 0.05 was considered significant.

# 3. RESULTS

The study enrolled 80 patients: 37 males, and 43 females with mean age (59.775±7.957) years for group I and (57.525±9.524) years for group II. Demographic data were insignificantly different between both groups (Table 1).

Regarding clinical manifestations, there was a significant increase in temperature only of SBP patients (p<0.001). Table (2).

Regarding laboratory investigations, serum neutrophil, CRP, serum bilirubin (total and direct), NLR, and CAR were significantly higher in the SBP group. Table (3), the ascitic fluid analysis in the studied groups showed significant differences regarding total leukocyte count and neutrophil count in patients with SBP compared to those without association with a significant decrease after SBP treatment. Table (4).

There was a significant decrease in serum neutrophil, CRP, NLR, and CAR in SBP patients post-treatment. Table (5).

Correlation analysis among ascitic neutrophil count and serum neutrophil, CRP, NLR, and CAR before and after SBP treatment revealed that there were significant positive correlations between both NLR and CAR with ascitic neutrophil count after SBP treatment. Table (6) and Fig. (1).

All patients who had been treated improved and responded except 2 patients who were resistant to treatment (their ascitic neutrophil count was 2200 and 1555, respectively before treatment and 434 & 350 after treatment with NLR =15.7 & 8.7 respectively before treatment and 10.1&5.4 after treatment with CAR=27.1 & 16 before treatment and 18 & 10.2 after treatment) and 2 patients died during follow up.

#### 4. DISCUSSION

NLR and CAR are used for the diagnosis and follow-up of many inflammatory diseases and malignancies so, in our study, we aimed to use these values as markers of response to SBP treatment.

As regard WBCs differentials, we found that the blood neutrophils have high significant values in the SBP group compared with non-SBP as neutrophil is the key cellular component of host defense in the innate immune system against infectious injury, that in agreement with Iliaz et al.,. We found also that lymphocyte values were lower in the SBP group with insignificant difference which can be explained by loss of lymphocytes due to continuous sepsis-induced apoptosis that in agreement with Iliaz et al. [11].

While, as regards the erythrocyte sedimentation rate, it was found to be insignificant statistically between the studied groups. This agreed with Suvak et al., and Liu et al., who found that ESR is less sensitive and accurate as an acute-phase reactant than the C reactive protein. This result was in disagreement with Yousef et al. [12-14].

On the other hand, the C reactive protein was found to be significantly elevated in the SBP group agreeing with Khorshed et al., & Elsadek et al., In contrast, Pieri et al., found that the basic level of CRP in cirrhotic patients was higher than in non-cirrhotic patients, but once infection occurs, it is probably worse the liver function more, leading to less increase in the CRP and also Janum et al., who concluded that the power of CRP to predict infection is weak in patients with advanced cirrhosis [15-18].

Table 1. Demographic data of the studied groups

Age			Groups			T-Test
_	With Spontane	ous bacterial peritonitis	Without Spontane	eous bacterial peritonitis	t	P-value
Range	31	- 75	42	- 77	-1.142	0.257
Mean ±SD	57.525	± 9.594	59.775	± 7.957		
Sex	Groups				Chi-Square	
	With Spontaneo	ous bacterial peritonitis	Without Spontaneo	ous bacterial peritonitis		
	N	%	N	%	X <sup>2</sup>	P-value
Male	17	42.50	20	50.00	0.453	0.501
Female	23	57.50	20	50.00		

<sup>\*</sup> Significant t= student's t test, \( \square = \chi \) squared test

Table 2. Clinical manifestations of the studied groups

Examination		With Spor	ntaneous bacterial peritonitis.	Without	Spontaneous bacterial peritonitis.		Test
		N	%	N	%	X <sup>2</sup>	P-value
Fever	No	11	27.50	39	97.50	41.813	<0.001*
	Yes	29	72.50	1	2.50		
Jaundice	No	16	40.00	24	60.00	3.200	0.074
	Yes	24	60.00	16	40.00		
Lower limb	No	2	5.00	1	2.50	5.153	0.272
edema	Minimal	0	0.00	1	2.50		
	Mild	15	37.50	8	20.00		
	Moderate	11	27.50	18	45.00		
	Marked	12	30.00	12	30.00		
Conscious	No	18	45.00	14	35.00	0.833	0.361
Or not	Yes	22	55.00	26	65.00		
Flapping	No	24	60.00	27	67.50	0.487	0.485
•	Yes	16	40.00	13	32.50		
Fetor hepaticus	No	33	82.50	39	97.50	3.472	0.062
•	Yes	7	17.50	1	2.50		
Hepatomegaly	No	39	97.50	39	97.50	0.000	1.000
	Yes	1	2.50	1	2.50		
Splenomegaly	No	14	35.00	18	45.00	0.833	0.361
	Yes	26	65.00	22	55.00		
Ascites	Mild	6	15.00	2	5.00	2.885	0.236
	Moderate	12	30.00	17	42.50		
	Marked	22	55.00	21	52.50		

<sup>\*</sup> Significant □2= chi squared test

Table 3. The laboratory investigations in the studied groups

				G	roups			T-	-Test
		With Spontane	ous bacteria			ontaneou	s bacterial peritonitis	t	P-value
Hb gm/dl	Range	6.3	-	12.7	4.9	-	13.4	-0.352	0.726
	Mean ±SD	9.213	±	1.715	9.365	±	2.134		
WBC x 10 <sup>3</sup> /mm <sup>3</sup>	Range	2.2	-	18	1.2	-	12.3	1.829	0.071
	Mean ±SD	6.980	±	4.408	5.473	±	2.783		
Platelet x 10 <sup>3</sup> /mm <sup>3</sup>	Range	45	-	515	22	-	400	-0.561	0.576
	Mean ±SD	133.150	±	88.796	144.400	±	90.507		
Neutrophil x 10 <sup>3</sup> /mm <sup>3</sup>	Range	0.45	-	17.72	0.3	-	9.29	3.512	0.001*
•	Mean ±SD	7.608	±	4.168	4.864	±	2.657		
_ymphocyte x 10 <sup>3</sup> /mm <sup>3</sup>	Range	0.23	-	2.66	0.09	-	2.83	-0.213	0.832
	Mean ±SD	1.267	±	0.656	1.301	±	0.784		
CRP mg/L	Range	96	-	120	0	-	48	34.693	<0.001
3	Mean ±SD	109.500	±	8.524	23.775	±	13.098		
Total bilirubin mg/dl	Range	0.7	_	25.1	0.6	-	7.2	2.988	0.004*
3	Mean ±SD	5.618	±	6.620	2.380	±	1.767		
Direct bilirubin mg/dl	Range	0.1	_	17.5	0.1	-	4.1	3.089	0.003*
ű	Mean ±SD	3.665	±	4.792	1.273	±	1.017		
Albumin g/dL	Range	1.8	_	4	1.9	-	4	0.950	0.345
3	Mean ±SD	2.605	±	0.511	2.503	±	0.453		
ALT U/L	Range	11	-	72	12	-	153	-0.669	0.506
	Mean ±SD	33.925	±	16.847	37.650	±	30.948		
AST U/L	Range	21	-	194	20	-	302	-0.426	0.671
	Mean ±SD	66.475	±	37.613	71.025	±	56.046		
Creatinine mg/dL	Range	0.8	-	3.9	0.6	-	5.7	-0.245	0.807
3	Mean ±SD	1.413	±	0.648	1.456	±	0.903		
	Range	1.08	_	3.02	1	-	3.8	1.316	0.192
INR	Mean ±SD	1.703	±	0.501	1.541	±	0.192		
NLR	Range	0.4	_	18.7	0.9	-	9.4	4.586	<0.001
	Mean ±SD	6.013	±	3.691	3.015	±	1.865		
CAR	Range	0	-	28	0	-	14	2.838	0.006*
-	Mean ±SD	10.093	±	8.883	5.550	±	4.852		

<sup>\*</sup>Significant t= student's t-test Hb: Hemoglobin WBC: White blood cells CRP: C- reactive protein ALT: Alanine aminotransferase AST: Aspartate aminotransferase INR: International Normalized Ratio NLR: Neutrophil/lymphocyte ratio CAR: C - reactive protein /Albumin ratio

Table 4. The ascitic fluid analysis among the studied groups and after SBP treatment

Ascitic fluid analysis			Groups							T-Test			
			With Spontaneous bacterial peritonitis					Spontan		t		P	-value
TLC/mm <sup>3</sup>		Range	300	-	2400		5	-	600	8.127		<(	0.001*
		Mean ±SD	1003.925	±	640.3	370	164.100	±	130.613				
Neutrophil %		Range	52	- 95			0	-	90	1.743		0.	085
		Mean ±SD	75.875	±	11.64	10	68.750	±	23.087				
Lymphocyte %		Range	5	-	48		5	-	90	-1.663		0.	100
		Mean ±SD	23.875	±	11.50	)7	30.125	±	20.801				
Neutrophil count/mm <sup>3</sup>		Range	256	-	2280		0		240	7.579		<(	0.001*
·		Mean ±SD	810.450	±	582.7	<b>'</b> 24	106.875	±	71.706				
Protein(g/dL)		Range	0.5	-	2		0.5	-	2.5	-1.179		0.	242
,		Mean ±SD	1.278	±	0.463	3	1.435	±	0.707				
Glucose(mg/dL)		Range	52	-	450		67	-	420	0.243		0.	809
, ,		Mean ±SD	177.850	±	100.4	198	172.800	±	84.693				
SAAG(g/dL)		Range	1.1	-	2.5		1.12	-	2	0.251		0.	803
,		Mean ±SD	1.421	±	0.280	)	1.406	±	0.235				
Ascitic fluid analysis		Time								Difference	ces	Paired Test	
•		Before TTT				After T	TT			Mean	SD	t	P-value
TLC/mm <sup>3</sup>	Range	300	-		2400	50	-	630		839.676	638.674	7.997	<0.001*
	Mean ±SD	1003.925	±		640.370	212.24	13 ±	142.61	)				
Neutrophil %	Range	52	-		95	10	-	90		10.216	26.079	2.383	0.023*
•	Mean ±SD	75.875	±		11.640	65.216	S ±	22.274					
Lymphocyte %	Range	5	-		48	10	-	90		-10.892	26.030	-2.545	0.015*
	Mean ±SD	23.875	±		11.507	35.324	1 ±	23.129					
Neutrophil count/mm <sup>3</sup>	Range	256	-		2280	20	-	434		716.730	579.620	7.522	<0.001*
•	Mean ±SD	810.450	±		582.724	132.86	65 ±	89.928					
Protein(g/dL)	Range	0.5	-		2	0.4	-	2		0.122	0.610	1.212	0.233
,,	Mean ±SD	1.278	±		0.463	1.157	±	0.490					
Glucose(mg/dL)	Range	52	-		450	40	-	350		9.622	80.880	0.724	0.474
	Mean ±SD	177.850	±		100.498	171.97	73 ±	77.978					

<sup>\*</sup> Significant t= t-test SBP: Spontaneous Bacterial Peritonitis TLC: Total leucocytic count SAAG: Serum ascites albumin gradient

Table 5. Serum neutrophil, lymphocyte, CRP, NLR, and CAR before and after SBP treatment

				-	Гіте			Diffe	erences	Paired Te	est
		Before TTT			After TTT			Mean	SD	t	P-value
Neutrophil X10 <sup>3</sup>	Range	0.45	-	17.72	0.3	-	11.66	1.329	2.594	3.240	0.002*
·	Mean ±SD	7.608	±	4.168	6.280	±	2.920				
ymphocyte X10 <sup>3</sup>	Range	0.23	-	2.66	0.24	-	2.67	-0.001	0.004	-1.669	0.103
	Mean ±SD	1.267	±	0.656	1.268	±	0.657				
C-Reactive protein	Range	96	-	120	90	-	113	6.000	4.461	8.507	<0.001*
mg/L	Mean ±SD	109.500	±	8.524	103.500	±	7.562				
lbumin	Range	1.8	-	4	1.85	-	4.1	-0.004	0.018	-1.356	0.183
/dL	Mean ±SD	2.605	±	0.511	2.609	±	0.516				
NLR	Range	0.4	-	18.7	0	-	10.1	2.360	3.859	3.769	0.001*
	Mean ±SD	6.013	±	3.691	3.769	±	2.650				
CAR	Range	0	-	28	0	-	18	4.395	7.643	3.545	0.001*
	Mean ±SD	10.093	±	8.883	5.000	±	4.966				

<sup>\*</sup> Significant t= t test CRP: C- reactive protein NLR: Neutrophil / lymphocyte ratio CAR: C - reactive protein /Albumin ratio SBP: Spontaneous Bacterial Peritonitis

Table 6. Correlations among ascitic neutrophil count and serum neutrophil, CRP, NLR, and CAR before and after SBP treatment

Correlations					
Before TTT	Ascitic neutrop	hil count			
	r	P-value			
Serum neutrophil before TTT/Cmm	0.139	0.393			
C-Reactive protein before TTT (mg/L)	0.015	0.926			
NLR before TTT	0.161	0.320			
CAR before TTT	0.081	0.618			
After TTT	Ascitic neutrophil count				
	r	P-value			
Serum neutrophil after TTT/Cmm	0.157	0.353			
C-Reactive protein after TTT (mg/L)	0.271	0.105			
NLR after TTT	0.419	0.010*			
CAR after TTT	0.388	0.018*			

<sup>\*</sup> Significant CRP: C- reactive protein NLR: Neutrophil/lymphocyte ratio CAR: C - reactive protein /Albumin ratio SBP: Spontaneous Bacterial Peritonitis

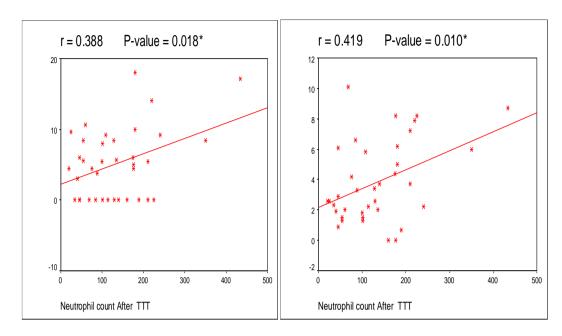


Fig. 1. Positive correlation between NLR, CAR, and ascitic neutrophil count after SBP treatment

As regarding, liver profile and kidney function tests in our study, there were disturbances in both liver profile and kidney functions reported among cirrhotic ascitic patients with and without SBP which can be explained by liver cell failure that agreed with Metwally et al, [19].

However, we found a significant increase in bilirubin level direct and total among SBP group more than non-SBP that agreed with El-Gendy et al. [20].

While regarding the albumin level, we found no significant differences between the studied groups that agreed with Iliaz et al. [11].

As regard, ascitic fluid analysis in our study there were statistically significant differences between both groups (with SBP and without SBP) in total leucocytic count (TLC), absolute neutrophilic count (ANC). These results were in agreement with Gomaa et al., who found that the ascitic fluid TLC and ANC in patients with SBP were high as compared to the patients without SBP [21].

Also on studying the ascitic fluid analysis in the SBP group before and after treatment with empirical antibiotic (3rd generation cephalosporin) we found a significant decrease in both ascitic TLC and ANC count that in agreement with Abuelfadl et al., who had studied 150 Egyptian ascitic patients with liver cirrhosis due to the hepatitis C virus for the ability to use lactoferrin in SBP follow up and found that ascitic

fluid polymorph count was significantly decreased after antibiotic treatment [3].

There were no significant differences as regard ascitic glucose and protein post-treatment, these results in agreement with Runyon and Hoefs. [22].

In our study, NLR and CAR were significantly higher in patients with the SBP group than patients without the SBP group before treatment. These results were supported by data revealed by Iliaz et al. [11].

The same was documented by Mousa et al., who had studied 180 cirrhotic ascitic patients and found that NLR was significantly high in the SBP group [23].

These results can be explained by increased production of neutrophils and decreased lymphocyte counts by apoptosis which was induced by infection as neutrophil is the key cellular component of host defense in the innate immune system against infectious injury, while lymphocyte is considered as the major cellular of the adaptive immune line system. Lymphocytes play a key role in the regulation of inflammatory response, and their loss due to continuous sepsis-induced apoptosis may lead to immune system suppression and indicated that the inflammation wasn't resolved Heffernan et al. [24].

While the significant increase of CAR levels in the SBP group can be explained by elevated CAR levels in the event of a chronic systemic inflammatory response and nutritional deterioration as CRP is considered as an indicator of inflammation and albumin considered as an indicator of malnutrition. Also, hypoalbuminemia is suggested to be related to the systemic inflammatory response. It has been found that patients with sepsis hypoalbuminemia already had increased serum CRP concentrations and that hypoalbuminemia might be secondary to elevated CRP which may be explained by increased demand for specific amino acids for acute-phase protein synthesis, which promotes the degradation of available body protein including albumin Al-Shaiba et al., & Kaplan et al. [25, 26].

Also in our study, we found a significant decrease as regard serum neutrophil count, CRP, NLR, and CAR in the SBP group after treatment. However, we found that NLR and CAR had a strong positive correlation with ascitic neutrophil count after SBP treatment (i.e. any decrease in the ascitic neutrophil count after SBP treatment is associated with a decrease in NLR and CAR), while the other markers did not correlate.

From the above, we established that NLR and CAR were the most sensitive markers of response in SBP treatment, while serum neutrophil count and CRP can't be used alone in SBP treatment follow-up as they have no significant correlation with the ascitic neutrophil count.

So according to these results, NLR and CAR can be used as markers of response in follow-up SBP patients who received treatment as they are simple, sensitive, non-invasive, and can be obtained easily by just routine laboratory tests.

To our knowledge, this is the first study to determine the usefulness of NLR and CAR as markers of response in SBP treatment. But some similarities with our study, many previous studies have shown the clinical usefulness of NLR as a useful indicator for bacterial infection Strauss and Gomes de SáRibeiroMde., & De Jager et al. [27, 28].

## 5. CONCLUSIONS

NLR and CAR can be used as quick, cheap, and applicable markers of the response of treatment in SBP patients.

#### **CONSENT AND ETHICAL APPROVAL**

This analytic prospective cohort study was carried out on 80 cirrhotic ascitic patients. They were selected consecutively from the Tropical Medicine Department of Tanta University Hospital for a period of six months from November 2018 to April 2019. The committee of ethics of scientific research of Tanta Faculty of Medicine approved the studied protocol and written consents were obtained from the studied groups for participation.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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