Opinion Article

Blood Neutrophil / Lymphocyte Ratio and C -reactive protein / Albumin Ratio as Markers of Response for Treatment of Spontaneous Bacterial Peritonitis

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) /mm^3 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~~ follow-up.

Patients and methods: This study was done on 80 cirrhotic ascitic patients attending ~~to~~ 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 cirrhotic ascitic patients with spontaneous bacterial peritonitis, and then SBP group were tested after treatment by ~~third~~ 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~~ 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

Introduction

Spontaneous bacterial peritonitis (SBP) is considered ~~as~~ a serious complication of ascites ~~which~~ 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 on~~–~~going 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.

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 ~~in~~ 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.

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.

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).

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

After ~~that's~~ patients met ~~fulfilling~~ the inclusion and exclusion criteria, ~~will be~~they were further tested for **ascitic sample**, **NLR**, and **CAR** before and after treatment of SBP by ~~third~~ third-generation cephalosporin for five days according to the guidelines [10].

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~~ two-tailed P value < 0.05 was considered significant.

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 ~~regrading~~ regarding total ~~leukocytic~~ leukocyte count and neutrophil count in patients with SBP

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compared to those without ~~associated~~ 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~~ post-treatment. Table (5)

~~A c~~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 Figure (1)

All patients who had been treated ~~were~~ 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 ~~respectively~~ after treatment with NLR =15.7 & 8.7 respectively before treatment and 10.1&5.4 ~~respectively~~ after treatment with CAR=27.1 & 16 ~~respectively~~ before treatment and 18 & 10.2 ~~respectively~~ after treatment) and 2 patients died during follow up.

Table 1: Demographic data of the studied groups

Age	Groups						T-Test	
	With Spontaneous bacterial peritonitis			Without Spontaneous 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 Spontaneous bacterial peritonitis			Without Spontaneous bacterial peritonitis				
	N	%		N	%		X^2	P-value
Male	17	42.50		20	50.00		0.453	0.501
Female	23	57.50		20	50.00			

***** Significant t= student's t test, χ^2= chi squared test

Table 2: Clinical manifestations of the studied groups

Examination		With Spontaneous bacterial peritonitis.		Without Spontaneous bacterial peritonitis.		Test	
		N	%	N	%	X^2	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 edema	No	2	5.00	1	2.50	5.153	0.272
	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 Or not	No	18	45.00	14	35.00	0.833	0.361
	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		

*** Significant □2= chi squared test**

Table 3: The laboratory investigations in the studied groups:

		Groups			T-Test	
		With Spontaneous bacterial peritonitis	**Without Spontaneous 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 ~~X10³~~ x 10³ /~~c~~mm³	**Range**	2.2 - 18	1.2 - 12.3		1.829	0.071
	Mean ±SD	6.980 ± 4.408	5.473 ± 2.783			
Platelet ~~X10³~~ x 10³/~~c~~mm³	**Range**	45 - 515	22 - 400		-0.561	0.576
	Mean ±SD	133.150 ± 88.796	144.400 ± 90.507			
Neutrophil ~~X10³~~ x 10³/~~c~~mm³	**Range**	0.45 - 17.72	0.3 - 9.29		3.512	0.001*
	Mean ±SD	7.608 ± 4.168	4.864 ± 2.657			
Lymphocyte ~~X10³~~ x 10³/~~c~~mm³	**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*
	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*
	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*
	Mean ±SD	3.665 ± 4.792	1.273 ± 1.017			
Albumin g~~m~~/~~dl~~dL	**Range**	1.8 - 4	1.9 - 4		0.950	0.345
	Mean ±SD	2.605 ± 0.511	2.503 ± 0.453			
ALT U/~~l~~L	**Range**	11 - 72	12 - 153		-0.669	0.506
	Mean ±SD	33.925 ± 16.847	37.650 ± 30.948			
AST U/~~l~~ L	**Range**	21 - 194	20 - 302		-0.426	0.671
	Mean ±SD	66.475 ± 37.613	71.025 ± 56.046			
Creatinine mg/~~dl~~dL	**Range**	0.8 - 3.9	0.6 - 5.7		-0.245	0.807
	Mean ±SD	1.413 ± 0.648	1.456 ± 0.903			
INR	**Range**	1.08 - 3.02	1 - 3.8		1.316	0.192
	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			

*****Significant **t**= student's ~~t~~ 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

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

Ascitic fluid analysis		Groups						T-Test	
		With Spontaneous bacterial peritonitis.			Without Spontaneous bacterial peritonitis.			t	P-value
TLC/Cmm^3	Range	300	-	2400	5	-	600	8.127	<0.001
	Mean ±SD	1003.925	±	640.370	164.100	±	130.613		
Neutrophil %	Range	52	-	95	0	-	90	1.743	0.085
	Mean ±SD	75.875	±	11.640	68.750	±	23.087		
Lymphocyte %	Range	5	-	48	5	-	90	-1.663	0.100
	Mean ±SD	23.875	±	11.507	30.125	±	20.801		
Neutrophil count/Cmm^3	Range	256	-	2280	0	-	240	7.579	<0.001
	Mean ±SD	810.450	±	582.724	106.875	±	71.706		
Protein(g/dldL)	Range	0.5	-	2	0.5	-	2.5	-1.179	0.242
	Mean ±SD	1.278	±	0.463	1.435	±	0.707		
SugarGlucose(mg/dldL)	Range	52	-	450	67	-	420	0.243	0.809
	Mean ±SD	177.850	±	100.498	172.800	±	84.693		
SAAG(g/dldL)	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						Differences		Paired Test	
		Before TTT			After TTT			Mean	SD	t	P-value
TLC/Cmm^3	Range	300	-	2400	50	-	630	839.676	638.674	7.997	<0.001*
	Mean ±SD	1003.925	±	640.370	212.243	±	142.610				
Neutrophil %	Range	52	-	95	10	-	90	10.216	26.079	2.383	0.023*
	Mean ±SD	75.875	±	11.640	65.216	±	22.274				
Lymphocyte %	Range	5	-	48	10	-	90	-10.892	26.030	-2.545	0.015*
	Mean ±SD	23.875	±	11.507	35.324	±	23.129				
Neutrophil count/Cmm^3	Range	256	-	2280	20	-	434	716.730	579.620	7.522	<0.001*
	Mean ±SD	810.450	±	582.724	132.865	±	89.928				
Protein(g/dldL)	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				
SugarGlucose(mg/dldL)	Range	52	-	450	40	-	350	9.622	80.880	0.724	0.474
	Mean ±SD	177.850	±	100.498	171.973	±	77.978				

***** 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

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		Time			Differences		Paired Test	
		Before TTT	After TTT		Mean	SD	t	P-value
Neutrophil $X10^3$	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					
Lymphocyte $X10^3$	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 mg/L	Range	96 - 120	90 - 113		6.000	4.461	8.507	<0.001*
	Mean ±SD	109.500 ± 8.524	103.500 ± 7.562					
Albumin g~~m~~/~~d~~dL	Range	1.8 - 4	1.85 - 4.1		-0.004	0.018	-1.356	0.183
	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					

***** 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

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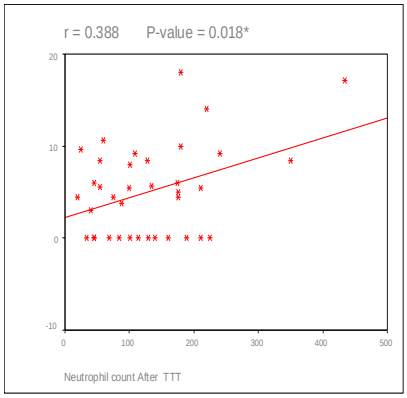
Correlations		
Before TTT	**Ascitic neutrophil count**	
	r	**P-value**
Serum neutrophil before TTT/Cmm	0.139	0.393
C-Reactive protein before TTT (mg/L)	0.015	0.926
NLR before TTT	0.161	0.320
CAR before TTT	0.081	0.618
After TTT	**Ascitic neutrophil count**	
	r	**P-value**
Serum neutrophil after TTT/Cmm	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*

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



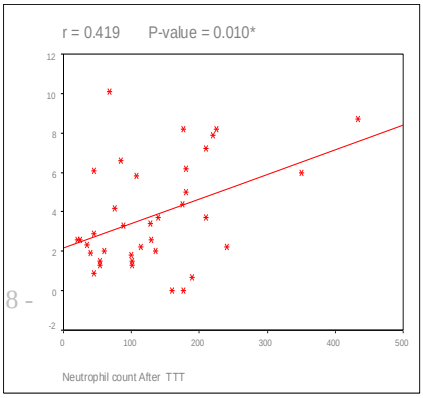


Figure 1: Positive correlation between NLR, CAR, and ascitic neutrophil count after SBP treatment

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~~ WBCs differentials, we found that the blood neutrophils have high significant values in the SBP group compared with ~~non~~ 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., 2018**. We found also that lymphocyte~~s~~ 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., 2018** [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., 2013** and **Liu et al., 2013** who found that ESR is ~~a~~ less sensitive and accurate as an acute-phase reactant than the C reactive protein-. This result was in disagreement with **Yousef et al., 2016** [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., 2015** & **Elsadek et al., 2020**. In contrast, **Pieri et al., 2014** 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., 2011** who concluded that the power of CRP to predict infection is weak in patients with advanced cirrhosis [15-18].

As regarding, liver profile and kidney function tests in our study, there were disturbances in both liver profile and kidney functions ~~evidently~~ reported among cirrhotic ascitic patients with and without SBP which can be explained by liver cell failure that agreed with **Metwally et al., 2018** [19].

However, we found a significant increase in bilirubin level direct and total among SBP group more than ~~non~~ non-SBP that agreed with **El-Gendy et al., 2014** [20].

While ~~as~~ regarding the albumin level, we found no significant differences between the studied groups that agreed with **Iliaz et al., 2018** [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., 2020** who

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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., 2018** who had studied 150 Egyptian ascitic patients with liver cirrhosis due to the hepatitis C virus- for the ability ~~of using~~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~~ post-treatment, these results in agreement with **Runyon and Hoefs. 1985** [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., 2018** [11].

The same was documented by **Mousa et al., 2018** 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 line of the adaptive immune system. Lymphocytes play a key role in the regulation of inflammatory response, and their loss due to continuous sepsis-induced apoptosis may lead to ~~the~~ immune system suppression and indicated that the inflammation wasn't resolved **Heffernan et al., 2012** [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 is 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 with 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~~ acute-phase protein synthesis, which promotes the degradation of available body protein including albumin **Al-Shaiba et al., 2004** & **Kaplan et al., 2020** [25, 26].

The ROC curve analysis revealed that at cutoff value >3.6 NLR has a sensitivity of 70% and specificity of 77.5% for the detection of SBP with an accuracy of 76.7% with– positive

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predictive value75.7%, while at cutoff value >13.1 CAR has a sensitivity of 40% and specificity of 95% for the detection of SBP with accuracy 63.3% with positive predictive value 88.9%. These results had some similarity to the data which was conducted by **Mousa et al., 2018** who found that at cutoff >2.89 NLR has a sensitivity of 80.3% and specificity of 88.9% for the detection of SBP with an accuracy of 82.8% with positive predictive value 94.4%.

So we can use both NLR and CAR in SBP diagnosis and NLR is considered the more sensitive while CAR is considered the more specific.

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 ~~had no correlation~~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~~ 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~~ 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 ~~similarity~~ 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., 2003** & **De Jager et al., 2010** [27, 28].

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Conclusions:

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

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