Original Article

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Diagnostic utility of absolute neutrophil count as a new marker of spontaneous bacterial peritonitis; multicenter study

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Abstract

Background: Spontaneous bacterial peritonitis (SBP) is a fatal complication of liver cirrhosis with high mortality rates. The aim of this study is to investigate the diagnostic utility of absolute neutrophil count (ANC) as a non-invasive marker for SBP diagnosis.

Methods: Six hundred patients with cirrhotic ascites were included in the study. All patients underwent abdominal paracentesis and the ascitic fluid was processed for cell count and culture.

Results: Absolute neutrophil count was significantly higher in SBP versus non-SBP and in culture positive SBP versus culture negative SBP. ANC at cutoff value > 2.804 has 84% sensitivity and 78% specificity for diagnosis of SBP with positive and negative predictive values (79.4% and 83.6 respectively). At a cut-off point > 5.6, ANC is capable of differentiating culture positive SBP from culture negative SBP cases with 62.07% sensitivity and 60.87 % specificity. Increased ANC, white blood cell (WBC), C reactive protein (CRP), creatinine and decreased platelet emerged as independent risk factors for SBP development, while increased ANC, WBC and decreased platelets were independent predictors of culture positive SBP.

Conclusion: This study demonstrates that, ANC count is simple, non-invasive diagnostic marker for SBP. Increased ANC, WBC, CRP, creatinine and decreased platelet emerged as independent risk factors for SBP development.

Introduction

Ascites is a serious complication of decompensated liver cirrhosis. Although many pathogenic processes have been implicated in the development of ascites, about 75% likely occur as a result of portal hypertension in the setting of liver cirrhosis with the remainder due to infectious, inflammatory and infiltrative conditions.[1-6] In cirrhotic patients, bacterial infection increases the morbidity and mortality.[7-9] Spontaneous bacterial peritonitis (SBP) is a risky complication that occurs amongst cirrhotic patients with ascites. SBP develops in approximately 10 to 30% of cirrhotic patients.[10]

The mortality rate in patients with spontaneous bacterial peritonitis ranges from 40-70% with hospital-mortality rate about 20%. However, nowadays the mortality from spontaneous.bacterial peritonitis may be decreasing because of advances in its diagnosis and treatment [11,12] SBP results from qualitative and quantitative alterations in microbiota of the gut, bacterial translocation and increased permeability of the intestine [12]. Additionally, the impairment of the immune system observed in advanced cirrhotic patients plays an important role.[13] The significance of SBP is mainly due to the late onset of symptoms, high rate of recurrence and the multiple causative organisms.[14-16]The blood neutrophil count have diagnostic utilities in evaluation of severity and intensive care unit admission of many types of infection including SBP.[17, 18]Serum CRP is synthesized mainly in liver cell under the control of many cytokines produced by macrophages at the time of bacterial infection.[19] Although many non-invasive and invasive markers were studied as diagnostic tools for SBP.[20-24], the diagnosis of SBP remains based on diagnostic paracentesis. However, diagnostic paracentesis is an invasive maneuver with many complications such aswound infection, abdominal wall hematoma, spontaneous hemoperitoneum that necessitating other non-invasive diagnostic tools.

Therefore, the aim of this study is to evaluate the clinical utility of absolute neutrophil count as a novel, simple, cheap, and noninvasive biochemical test for diagnosis of SBP.

Materials and methods

In this prospective study, we recruited 600 patients with liver cirrhosis and ascites referred to Tropical and Internal Medicine Departments, Mansoura University, Department of Tropical Medicine, Menoufia University, and Damietta Cardiology and Gastroenterology Center from October 2018 to Jun 2020. All patients were subjected to complete history taking and physical examination include, abdominal ultrasonography and

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triphasic CT when indicated. The exclusion criteria were non-cirrhotic ascites (e.g., malignant ascites, tuberculus ascites), immunocompromised patients, and patients who had taken antibiotics prior to hospital admission or on prophylactic antibiotics for SBP or on anticoagulant medications. Furthermore, patients with heart or renal failure, neoplastic disorders, hematological disorders, clinically evident autoimmune disorders, secondary bacterial peritonitis due to any surgical causes and patients with unrelated infection that may influence the levels of blood WBC, e.g. skin and lung infection, were also excluded.

Sampling

1- The ascitic fluid samples (15 ml) were aspirated under the complete aseptic condition at the bedside, using the standard paracentesis technique. Ten ml was immediately inoculated in bedside aerobic and anaerobic blood culture bottles. The remaining amount of ascitic fluid was sent for biochemical and cytological examination in tubes containing EDTA and analyzed within 3 h of aspiration. [25]Ziehl-Neelsen staining of the ascitic fluid was done when needed.

Regarding microbiological examination, inoculated blood culture bottles were incubated for 3 successive days at 37°C with daily subculture on blood, MacConkey and chocolate agars. Antimicrobial susceptibility testing and bacterial identification were carried out using standard procedures.

According to international guidelines, SBP was diagnosed if the polymorph nuclear neutrophil (PMN) cell count in the ascitic fluid is ≥250/mm3 with positive culture of ascitic fluid (culture positive SBP) or culture negative neutrocytic ascites (CNNA) with PMN > 250/mm^3 and a negative ascitic fluid culture in the absence of other causes of peritonitis and hemorrhagic ascites.[26,27] Patients with ascitic fluid PMN cell count < 250 cells/mm3 and a culture-negative ascitic fluid were assigned as the control group.

2. At the time of paracentesis, five ml of venous blood was withdrawn; one ml in polystyrene EDTA tube for CBC and four ml delivered in another tube and allowed to clot. Non-hemolyzed sera were separated by centrifugation and used for determination of liver functions (ALT, AST, and albumin bilirubin and prothrombin time) blood sugar, creatinine, urea, alpha-fetoprotein and carcinoembryonic antigen. CRP was measured by particle-enhanced immunoturbidimetric assay (C-reactive protein (Roche Diagnostics). Blood absolute neutrophil count was calculated automatically using the CELL-DYN Emerald cell counter (Abbott, Wiesbaden, Germany).

This study was performed with the approval of the Mansoura Faculty of Medicine Institutional Review Board "MFM-IRB". All patients provided written informed consent prior to participation in any protocol-specific procedure.

Statistical analysis

Data were analyzed with SPSS version 21. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using numbers and percentage. Association between categorical variables was tested using Chi-square. Continuous variables were presented as mean ± SD (standard deviation) for parametric data, but Median and IQR was used for non-parametric data. The two groups were compared with Student t test (parametric data) and Mann–Whitney test (non-parametric data). Clinical variables were considered statistically significant when ($p<0.05$). Sensitivity and specificity of absolute neutrophil count for SBP diagnosis were estimated by receiver operator characteristic curve. Significant parameters in univariate binary logistic regression analysis were entered into a stepwise multivariate logistic regression analysis to identify the independent predictors for the occurrence of SBP and culture positive SBP.

Results

Table 1 shows the clinical, laboratory and demographic details of the studied groups. The two groups were matched for age, sex and etiology of cirrhosis (viral hepatitis, NASH and cryptogenic cirrhosis). Significant differences were found between both groups as regards, fever, abdominal pain, bilirubin, AST, ALT, creatinine, prothrombin time (PT), CRP, platelet count, blood WBC, ANC. No significant differences were found in serum albumin, blood lymphocyte and hemoglobin.

To determine predictive parameters for the diagnosis of SBP, variables with significant associations on univariate analysis were subjected to a logistic regression analysis. Increased ANC, WBC, CRP and creatinine and decreased platelets were independent predictors of SBP development **(Table 2).**

Among the 300 patients with SBP, 184 (61.3%) patients were culture positive SBP and 116 (38.6%) were culture negative SBP. No significant differences were found between both groups regarding, abdominal pain, fever, albumin, bilirubin, AST, ALT, INR, blood lymphocyte and haemoglobin. There was a significant increase in age and platelet count in patients with culture-negative compared to culture-positive SBP. A significant increase in, serum creatinine, CRP, blood WBC, blood neutrophil count was found in patients with culture-positive SBP vs. culture-negative SBP **(Table 3)**.

To determine reliable predictive parameters for the diagnosis of culture positive SBP, variables with significant associations on univariate analysis were subjected to a logistic regression analysis. Decreased platelets and increased ANC and WBC and were independent predictors of culture positive SBP **(Table 4)**.

The ROC curve analysis demonstrated that, absolute blood neutrophil count at a cut-off value of >2.804 had 84% sensitivity and 78% specificity for diagnosis of SBP with 79.4% PPV, 83.6% NPV and AUC equal to 0.88. **(Figure 1).** At cut-off point > 5.6, absolute neutrophil count was capable of differentiating culture positive from culture negative SBP cases with 62.07% sensitivity and 60.87 % specificity with an AUC equal to 0.59. **(Figure 2)**.

Table 1. Clinical and biochemical characteristic of studied patient.

Parameters	SBP (Case) (N=300)	Non-SBP (Control) (N=300)	P value
Age	55.60±8.44	55.36±7.78	0.29
Sex (M/F)	207/93	194/106	0.14
HCV (N/%)	273 (91%)	275 (91.6%)	0.66
HBV (N/%)	15 (5%)	17 (5.6%)	0.71
NASH (N/%)	8 (2.6%)	5 (1.6%)	0.4
Cryptogenic (N/%)	4 (1.3%)	3 (1%)	0.7
Fever (N/%)	126 (37.3%)	0	<0.001
Abdominal pain (N%)	201(67%)	115/300	<0.001
Albumin (g/dl)	2.36±0.61	2.41±0.62	0.25
Bilirubin (mg/dL)	3.30 (1.80-6.30)	0.90 (0.70-4.10)	<0.001
ALT (U/L)	30.0 (20.25-42.00)	36.0 (20.0-56.0)	0.006
AST (U/L)	61.0 (37.0 -96.0)	31.0 (22.0-95.0)	<0.001
Creatinine (mg/dl)	1.5 (0.9-2.4)	1.4 (0.8-1.6)	0.003
PT (seconds)	13.9 ± 2.8	13.4 ± 2.6	0.023
CRP (mg/dl)	48.0 (44.0-48.0)	7.0 (5.0-9.0)	<0.001
Platelets (10^9/L)	79 (46-139)	93 (59-112)	0.006
Blood WBC (10^3/cmm)	8.13±3.23	4.88±1.60	<0.001
ANC (10^3/cmm)	5.98±2.86	2.25±٠.6	<0.001
Blood lymphocyte (10^3/cmm)	1.66±0.79	1.64±0.89	0.77
Hemoglobin (g/dl)	10.10±1.87	10.05±2. 11	0.57

HCV, *hepatitis C virus*; **HBV,** *hepatitis B virus*; **NASH,** *non-alcoholic steatohepatitis* ; **ALT,** *alanine aminotransferase*; **AST,** *aspartate aminotransferase*; **PT,** *prothrombin time*;**WBC,** *white blood cells*; **CRP,** *C reactive protein*; **ANC,***Absolute neutrophil count.*

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Table 2: Logistic regression analysis of risk factors associated with SBP.

Parameters	Exp (B)	95% CI Lower-Upper		Sig.
Bilirubin	1.002	0.911	1.102	**0.969**
AST	0.993	0.983	1.002	**0.137**
ALT	1.003	0.990	1.015	**0.701**
Creatinine	0.320	0.182	0.563	**0.000**
Platelets	1.05	0.975	1.533	**0.011**
CRP	0.761	0.000	1.03	**0.009**
Blood WBC	1.9	0.999	2.65	**0.001**
ANC	**0.946**	**0.913**	**0.980**	**0.002**

Table 3: Comparison between culture positive and culture negative patients with SBP.

Parameters	Culture Positive SBP (N=184)	Culture Negative SBP (N=116)	P value
Age	54.81±7.53	56.86±9.82	0.02
Abdominal pain (N/%)	128 (69.5.9%)	73 (62.9%)	0.23
Fever (N/%)	73 (39.67%)	53 (45.6%)	0.30
Albumin (g/dl)	2.42±.65	2.34±.59	0. 5

Bilirubin (mg/dl)	3.5 (2.0-5.05)	3.3 (1.80-7.1)	0.36
ALT (U/L)	48 (33-74)	48 (36.5-80.5)	0.53
AST (U/L)	33 (20-37)	29 (21-43)	0.17
Creatinine (mg/dl)	1.5 (0.9-2.4)	1.4 (0.8-1.6)	0.003
INR	1.73±0.53	1.60±0.51	0.14
CRP (mg/dl)	48 (12-96)	47 (24-48)	0.02
Platelets (10^9/L)	72.5 (46-128)	97 (45.7-157.2)	0.001
Min-max	(12-210)	(10-41)	
Blood WBC (10^3/cmm)	8.79±3.7	7.80±3.28	0.008
ANC(10^3/cmm)	6.54±3.0	5.64±2.6	0.003
Blood lymphocytes (10^3/cmm)	1.4±0.19	1.6±0.23	0.5
Hemoglobin (g/dl)	10.24±2.15	10.05±1.67	0.20

CRP, *C reactive protein*; **ANC,***Absolute neutrophil count***.**

Table 4: Logistic regression analysis of risk factors associated with culture positive SBP

Parameters	Exp (B)	95% CI Lower-Upper		Sig.
Age	1.030	0.972	1.091	**0.314**
Creatinine	.976	0.672	1.418	**0.898**
Platelets	0.921	0.901	1.77	**0.016**
WBCs	.999	0.922	1.45	**0.001**
ANC	**1.081**	**1.036**	**1.127**	**0.001**

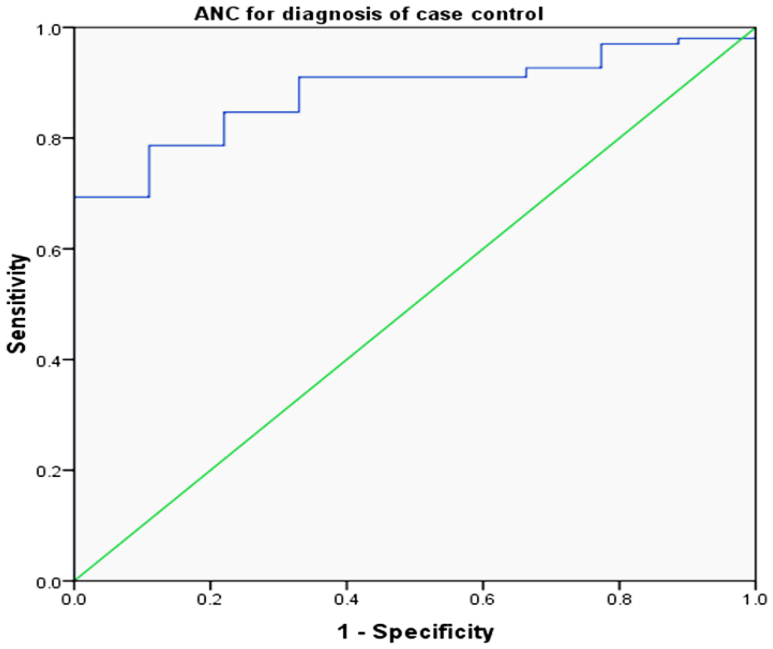

Figure 1. Receiver operator characteristics curve for blood absolute neutrophil count in cases of spontaneous bacterial peritonitis (case control).

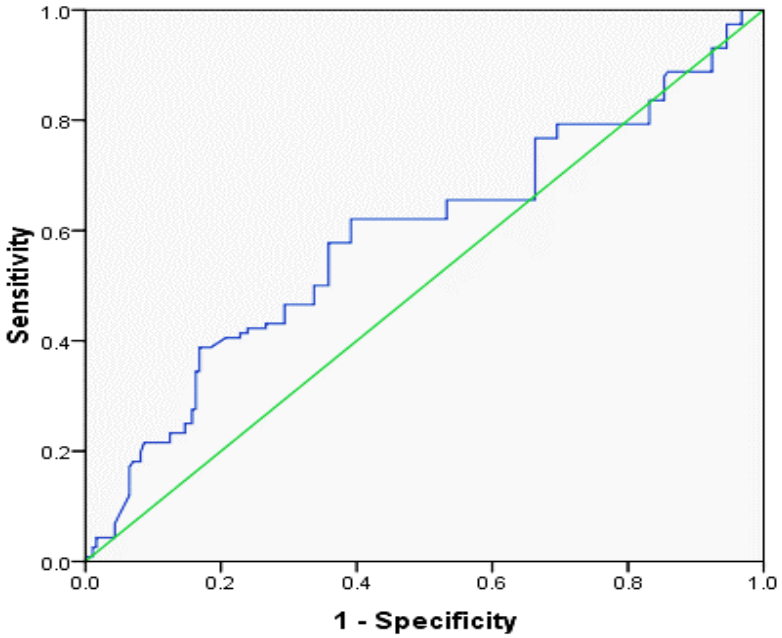

Figure 2. Receiver operator characteristics curve for blood absolute neutrophil count in culture-positive and culture negative spontaneous bacterial peritonitis.

Discussion

In the present study, we investigated the use of absolute blood neutrophil count as a noninvasive diagnostic test for spontaneous bacterial peritonitis in cirrhotic ascitic patients. The advantages of this test are simple, low cost and reliability. It is known that, white blood cells (leukocytes) are a defense line against bacterial infection. The most abundant leukocytes are neutrophil, which are the first line of defense against microbial invasion. [28]Moreover, absolute neutrophil count can be used as a predictive factor for infections.[29]In general, bacterial infection is suspected if neutrophil in the blood are increased.[30]

The diagnostic utility of the absolute neutrophil count for the diagnosis of spontaneous bacterial peritonitis is rarely found in research. Our study showed that, absolute neutrophil count is significantly higher in patients with spontaneous bacterial peritonitis versus non-SBP patients. In addition, absolute neutrophil count at a cut-off point > 2.804 is capable of differentiating spontaneous bacterial peritonitis from non-SBP with 84% sensitivity and 78% specificity with positive and negative predictive values of 79.4% and 83.6 respectively and AUC equal to 0.88.

The diagnostic utility of the blood neutrophil count as non-invasive test for the diagnosis of several infections has been previously suggested. [31-34] Mousa et al. evaluated the clinical utility of combined blood neutrophil to lymphocyte ratio (NRL) and C-reactive protein (CRP) as non-invasive tests for diagnosis of spontaneous bacterial peritonitis and concluded that, NRL was significantly higher in spontaneous bacterial peritonitis versus non-SBP, in addition, for SBP diagnosis, a blood NLR of >2.89 had a sensitivity of 80.3% and a specificity of 88.9%. [33]

Many laboratory tests have been introduced as predictive parameters for SBP, including CRP level, platelet count, impaired prothrombin time and serum creatinine level.[33,35,36] In this study, logistic regression analysis demonstrated that, increased blood neutrophil, WBC CRP and creatinine and decreased platelets were independent predictors of SBP development.

Even with the use of sensitive methods, ascites culture is negative in nearly 60% of patients with clinical manifestations suggestive of SBP and increased ascites neutrophil count.[37]As culturing bacteria needs time, delays in antibacterial treatment can be fatal and result in the death of the patient from overwhelming infection. Mousa et al, in his study found no significant difference in patients with culture-positive SBP versus culture-negative SBP regarding NLR.[33] However, in our study, we found that, absolute neutrophil count is significantly higher in culture positive SBP versus culture negative SBP and at a cut-off point >5.6, neutrophil count is capable of differentiating culture positive from culture negative SBP cases with 62.07% sensitivity and 60.87 % specificity with an AUC equal to 0.59.In this study, logistic regression analysis demonstrated that, increased blood absolute neutrophil, WBC, CRP and decreased platelet count were independent predictors for culture positive SBP. Schwabl et al in his study found that, WBC were elevated in patients presenting with SBP versus cirrhotic ascitic patients without SBP.[36]

Conclusion

Based on current evidence, the absolute blood neutrophil count, which requires only routine laboratory test could be used as a new, simple, cheap, non-invasive

test for SBP diagnosis allowing rapid diagnosis and treatment of SBP so that reducing its complications.

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List of abbreviations.

SBP, spontaneous bacterial peritonitis; ANC, absolute neutrophil count; WBC, white blood cell; CRP, C reactive protein; PMN polymorph nuclear neutrophil; CNNA, culture negative neutrocytic ascites.

Declaration

Ethical Approval. This study was performed with the approval of the Mansoura Faculty of Medicine Institutional Review Board "MFM-IRB".

Consent to participate. All patients provided written informed consent prior to participation in any protocol-specific procedure. All authors approve the publication of this work.

Competing interests. The authors declare that they have no competing interests.

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