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ORIGINAL ARTICLE

The Significance of Mean Platelet Volume as an Indicator of Spontaneous Bacterial Peritonitis in Cirrhotic Patients with Ascites

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ABSTRACT

Background: *Spontaneous bacterial peritonitis (SBP) is characterized by the presence of infected ascetic fluid without any previous history of intra-abdominal source of infection. In addition to the presence of increased level of polymorph nuclear leukocytes exceeding 250/μL in the ascetic fluid.* ***Objective:*** *to evaluate the role of mean platelet volume (MPV) as diagnostic marker of SBP as well as a prognostic factor to follow up the response to treatment of SBP.* ***Methodology:*** *40 patients suffering from cirrhosis were included in the study. All of them were divided into 2 groups, SBP and non-SBP, each consisted of 20 patients. Both groups were subjected to full clinical assessment, laboratory test evaluation, microbiological culture and sensitivity, abdominal ultrasonography and calculation of Child Pugh score. Patients with SBP were treated and followed up after five days.* ***Results:*** *We found that there was a statistical significant increase in the MPV levels in cirrhotic patients with SBP compared to cirrhotic patients without SBP ($p < 0.001$). Also, the statistical increase was observed in the SBP group with respect to MPV, ESR and C-reactive protein (CRP). ROC curve analysis suggested that the optimum MPV level cut-off point for cirrhotic patients with SBP was 8.4 fl, with a sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of 73%, 85.7%, 75.7% and 83.9%, respectively (area under curve: 0.84), follow up patients with SBP after end of treatment showed statistically significant difference in MPV measured before 8.5±0.6 fl and after treatment 8.1±0.8 fl ($P<0.0001$).* ***Conclusion:*** *MPV measurement can be used as a valuable diagnostic tool of SBP in cirrhotic patients as well as a prognostic marker to follow up response to treatment because it is rapid, easily applicable and valuable method*

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is characterized by infected ascitic fluid with polymorphonuclear leucocyte (PMNL) count more than 250/mm³ without any past history of any source of infection [1]. Several mechanisms may contribute to SBP like reduction of intestinal motility, translocation of gut bacteria, provoking and alteration of the gut's barrier function and local immune responses[2].

Gram-negative enteric bacteria are the most common causative organism of SBP. However, the prevalence of Gram-positive and multi-drug resistant (MDR) SBP tend to increase over the last decade. Consequently, the empirical usage of amoxicillin/clavulanic acid and third-generation cephalosporins can no longer due to their poor outcome results[3].

The mortality rate in patients who suffer SBP and cirrhosis is ranging from 40-70% among adult. In addition, the risk for hepatorenal syndrome and death in those patients is increased due to the usage of nonselective beta-blockers [4]

There are three main types that categorized the infected ascitic fluid in cirrhotic patient: Culture Negative Neutrocytic Aascites (CNNA), monobacterial non-neutrocytic ascites and polymicrobial ascites. The main characteristic of CNNA is the presence of PMN count of 250 cells per mm[3] with negative culture as well as negative history of previous antibiotic therapy with the exclusion of pancreatitis, TB peritonitis and peritoneal carcinomatosis. SBP has the same mortality rate as CNNA so it is mandatory to be treated properly[5].

For monobacterial non-neutrocytic ascites, the positive ascitic culture without any presence of increase leukocytic count is the main landmark . It is suspected when positive microbial growth with neutrophils count less than 250. In contrast, SBP is suspected when ascetic PMN count is higher than 250 and bacterial culture is positive[6].

In clinical practice, the ascitic PMN count isn't always available especially at the first 72 hours so antibiotic therapy may be delayed, therefore it is important to search for markers that can be widely and quickly available in order to predict infection. A strong source of prothrombotic agents is the circulating platelets. The platelet content of granules increases as a consequence in the increase size of platelets. These granules have very important hemostatic and pro-inflammatory functions, by consequence the mean platelet volume (MPV) can be used as a strong indicator of platelet function and activation [7].

It is reported that MPV is increased in certain diseases such as myocardial infarction, cerebrovascular disease, Alzheimer disease, hypertension, infective endocarditis, pyelonephritis and celiac disease [8].

Moreover, MPV plays an important role in the prediction of sepsis. It was reported in several studies that MPV level increased in cirrhotic infected ascetic fluid patients therefore researches proposed that it can be a strong indicator for SBP[9-10].

Our aim is to determine if there is a difference in MPV values between cirrhotic patients with and without infected ascitic fluid and to identify the MPV cut off value which can be used a prediction of ascitic bacterial infection in cirrhotic patients.

METHODOLOGY

This study is an observational analytical prospective study conducted in the Internal Medicine Department, Faculty of Medicine, Suez Canal University from January 2019 to April 2019 on forty patients divided into two groups. Each group consisted of 20 patients. Group 1 includes patients with liver cirrhosis and ascites complicated by SBP and group 2; those with liver cirrhosis and ascites without SBP.

Our inclusion criteria includes all patients from both sexes suffering ascites due to liver cirrhosis diagnosed by full clinical assessment and laboratory findings in combination with abdominal ultrasonography. Also, patients with SBP were diagnosed by using the standard criteria of the International Ascites club[11] with PMN cell count in the ascetic fluid is ≥250 cells /mm3 in the absence of intra-abdominal source of infection.

We excluded patients suffering from ascites of local cause as tuberculosis or malignant ascites, heart failure, hypertension, diabetes, hyperlipidemia, peripheral vascular disease, hematological or any neoplastic disorders. We also excluded patients who had received antibiotics, anticoagulant medications a week prior to hospital admission.

Laboratory Methods:

All patients were subjected to full history taking, clinical examination for signs of liver cell failure such as hepatic encephalopathy, jaundice, gynecomastia, altered body hair distribution, spider nevi, palmar erythema, bleeding tendency and lower limb edema. Laboratory evaluation as complete blood picture including MPV, ESR, CRP, serum bilirubin (total and direct), amino-transaminases, total serum proteins & serum albumin, prothrombin time, concentration and international normalized ratio and serum creatinine.

Abdominal ultrasonography was done for all patients after overnight fasting in order to confirm the diagnosis of chronic liver disease, the liver pattern, absence of hepatic focal lesions, splenic size, the presence of ascites, its extent and aspect. The severity of liver disease was assessed by using Modified Child Pugh classification[1].

Bacterial Cultures:

Ascetic fluid samples were collected under complete aseptic conditions. We avoided all areas of scarring because they are often the site of collateral vessels formation or adherent bowel in patients with portal hypertension [12] .The aspirated samples were checked for total and differential WBC counts. Culture and sensitivity of ascetic fluid was done by inoculating 10 ml of ascitic fluid at the bedside in blood culture bottles under complete aseptic condition, and incubated at 37^{o}C for 48 to 72 hours.

Automated platelet counts and determination of MPV were also performed by using the automated hematology analyzer DxH 800 (Beckman Coulter, Krefeld, Germany).

Statistical analysis:

Data analysis was done using Statistics/Data Analysis (STATA) version 13.1 software. Continuous variables were tested for normality by the Shapiro-Wilk normality test. Values are presented as mean ± standard deviation, or in the case of non-normally distributed data as median and inter-quartile range. The Chi-squared test was used to compare percentages between different groups of patients. Normally distributed data were analyzed using independent samples T-test. Data found to be non-normally distributed were analyzed using the Mann-Whitney U test. Non-normally distributed paired samples were analyzed using the Wilcoxon signed-rank test. Kruskal-Wallis equality-of-populations rank test was used to compare non-normally distributed data in both groups.

Ethical consideration:

The study work had the approval from the Ethics Committee of Faculty of Medicine, Suez Canal University (FOMSCU) in Ismailia, Egypt.

RESULTS

In the SBP group, the age range of the studied groups were from 26 to 61 years with a mean of 50.43 ± 7.49 years and fifteen patients (75%) of them were males. In the Non SBP group, the range was from 35 to75 years with a mean age of 53.8 ± 8 years and fifteen of them were also males, (table1)

Table 1: Demographic distribution of the studied groups

Variable	Group I (SBP group) N = 20	Group II (Non SBP group) N = 20	*P value
Age (years)	50.43 ± 7.49	53.8 ±8	< 0.05
mean ± SD			
Male	15 (75%)	15 (75%)	0.9

* Significant *p*-value <0.05

There was a statistical significant difference between both groups regarding WBCs, platelet count, in contrast to ESR, CRP, ALT, AST and PMN in ascetic fluid where all laboratory findings showed higher levels in the SBP group. On the other hand, the level of hemoglobin, bilirubin, albumin, INR, creatinine, MELD score and CPS showed no significant difference between both groups, (table 2)

Table 2: Baseline characteristics of the studied groups

		SBP group N=20	Non-SBP group N=20	*p value
CBC	Hemoglobin 13-17g/dl Mean± SD	12.04±2.15	12.15±1.78	0.6
	WBCs 4-11 x10^3/mm^3 Median±IQR	5±2.6	5.45±3	0.03
	Platelets 150-450 x10^3/mm^3 Median±IQR	116.5±77.5	150±73	0.0001
ESR Up to 15mm/hr Median ±**IQR		37.5±60	12±12	< 0.0001
CRP Up to 6 mg/dl Median± IQR		12±10	5±4.5	< 0.0001
LFT: **(Liver function tests)**	Bilirubin 0.3-1.2 mg/dl Median± IQR	1.6±1.6	1.7±1.4	0.23
	Albumin 3.5-5.2 g/dl Median ±IQR	2.9±0.6	2.9±0.6	0.23
	***INR 70-130 % Median± IQR	1.3±0.45	1.4±0.4	0.08
	AST 15-37 U/L Median± IQR	60.74±35.66	37.54±22.11	0.000
	ALT 25-65 U/L Median ±IQR	74.49±53.09	44.5±31.28	0.000
PMN in ascetic fluid Median±IQR		530±370	60±50	0.0001
Creatinine 0.6-1.3mg/dl Mean± SD		1.04 ±0.28	1.04±0.27	0.9
Child score B/C		88/12	83/15	0.5
MELD score Mean±****SD		21.10 ±5.29	20.47± 5.14	0.4

**p* values were considered significant if less than 0.05
**IQR: Inter Quartile Range
***INR: International Normalization Ratio
**** SD: standard Deviation

Assessment of the ascetic fluid culture in the SBP group showed that *Escherichia coli* was detected in four (15%) patients and *Klebsiella pneumonia* in one (5%) while no bacterial growth was detected in fifteen (75%) patients in the SBP group,(table 3)

Table 3: Ascitic fluid culture results in SBP group

Causative organisms	(SBP group) n=20 No (%)
E.coli	4 (20%)
Klebsiella pneumonia	1 (5%)
No bacterial growth	15 (75%)

There was a statistically significant difference between SBP group and Non SBP group regarding the MPV 8.5 ± 0.6 fL and 7.9 ± 0.6 fl. respectively (*p* value <0.0001),(table 4)

Table 4: Comparison of the mean platelets volume between SBP and non- SBP

	SBP group N=20	Non-SBP group N=20	*P* value
MPV	8.5 ±0.65	7.9±0.6	0.0001

Significant *p*-value <0.05

Table 5: The MPV in the SBP group before & after treatment

	SBP, before treatment	Resolved SBP	*P* value
MPV Median± IQR	8.5±0.6	8.1±0.8	< 0.0001

Significant *p*-value <0.05

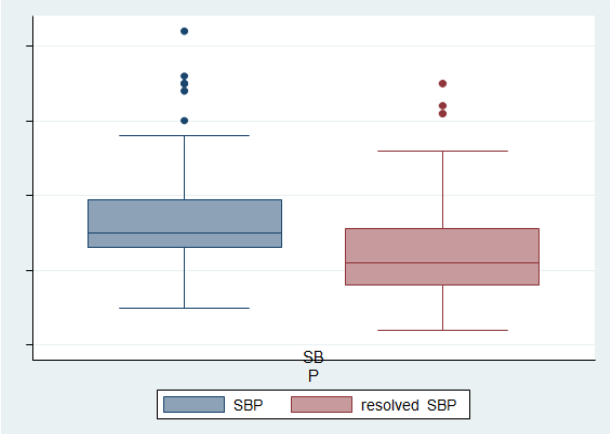

Fig. 1: Boxplot showing MPV in SBP group before and after treatment

As shown in table 5 and figure1; there was a statistical significant difference in the MPV measured before8.5±0.6 fl, and after treatment 8.1±0.8 fl.

All patients in SBP group were given ceftriaxone 2gm once daily as a prophylactic antibiotic after obtaining ascitic fluid samples for cultures; five patients had positive ascitic fluid cultures as shown in table 6

Table 6: Pattern of antibiotic susceptibility of SBP group

Ascitic Culture positive (SBP group)	Ceftriaxone	Doxycycline	Ampicillin	Meropenem
***E.coli* strain 1**	+	-	-	+
***E.coli* strain 2**	+	+	-	+
***E.coli* strain 3**	-	+	-	+
***E.coli* strain 4**	+	-	+	-
Kl. pneumonie	+	+	+	-

Table 7: Correlation between MPV and inflammatory markers, WBCs & platelets in the studied patients

ESR		CRP	WBCs	Platelets	MPV
ESR	1				
CRP	0.3149	1			
P value	<0.0001				
WBCs	-0.1295	0.009	1		
P value	0.0691	0.89			
Platelets	-0.1575	-0.026	0.346	1	
P value	0.0267	0.72	<0.0001		
MPV	0.3720	0.275	0.0218	-0.146	1
P value	<0.0001	0.0001	0.76	0.039	

Significant *p*-value <0.05

Table 7 showed that there was a significant positive correlation between MPV and ESR (r = 0.3720, p <0.0001), CRP (r=0.275, P=0.0001), and a negative correlation with platelet (r=-0.146, P=0.039) among SBP group. However no correlation was observed with WBC count in the peripheral blood (r=0.0218, p = 0.76) in SBP patients.

Table 8: Receiver operating characteristic (ROC) curves of MPV and other inflammation markers in detecting SBP in cirrhotic patients

	AUC	95% CI	SN	SP	NPV	PPV	Over all accuracy	LR +	LR -	Std. Err.
MPV cut off:8.4	0.84	0.79 - 0.9	73%	85. 7%	75.7%	83.9%	79.3%	5.1	0.32	0.028
ESR cut off 20	0.845	0.79- 0.9	78%	63. 4%	73.8%	68.5%	70.7%	2.1	0.35	0.027
CRP cut off 7	0.71	0.64 -0.78	66%	67. 4%	66 %	67.3%	66.6%	2	0.5	0.037

AUC: Area under curve, CI: confidence interval, SN: Sensitivity, SP: Specificity, PPV: Positive predictive value, NPV: Negative predictive value, LR: likelihood ratio

On ROC curve analysis of MPV, showed that a cutoff value of 8.4 fl, MPV had 73 % sensitivity and 85.7% specificity for detecting SBP with overall accuracy 79.3%, [AUC= 0.84 with NPV and PPV for MPV of 75.7 and 83.9%, respectively.

While ESR results showed that at a cutoff value of 20, ESR had 78 % sensitivity and 63.4% specificity for detecting SBP with overall accuracy 70.7, [AUC= 0.845 with a NPV and PPV for MPV of 73.8 and 68.5%, respectively]. Also at a cutoff value of 7, MPV had 66% sensitivity and 67.4% specificity for detecting SBP with overall accuracy 66.6%, [AUC= 0.71with NPV and a PPV for MPV of 66% and 67.3%, respectively], (table8).

Table 9: Univariate logistic regression for prediction of SBP

SBP	Odds Ratio	95% Conf. Interval	*p* value
MPV	23	9.32 -57.36	<0.001
ESR	1.11	1.07-1.15	<0.001
CRP	1.17	1.10-1.25	<0.001

Significant *p*-value <0.05

Logistic regression model was performed to assess the relationship between SBP as an outcome variable and each of MPV, CRP, and ESR as predictor variables; it showed that the MPV was the best prediction for SBP (table 9).

DISCUSSION

The current study was conducted to assess the potential role of MPV as diagnostic marker factor of SBP in cirrhotic patients with ascites with other inflammatory markers; ESR &CRP.

The study was designed to include two groups of ascitic cirrhotic patients. Group 1 included 20 patients suffering from liver cirrhosis and ascites complicated by SBP. Group 2 included 20 patients with liver cirrhosis and ascites without SBP. In the SBP group, the age range was from 26 to 61 years and a mean of 50.43 ± 7.49 years, while in the Non SBP group, the age was from 35 to75 years and a mean of 53.8 ± 8 years. Both groups, fifteen cases were males.

In the present study, the level of MPV, C-reactive protein (CRP) and ESR showed a statistical significant increase among SBP group when compared to non SBP group. Also, MPV level in cirrhotic patients showed a significant difference between SBP group and Non SBP group with *p* value <0.0001); independent on the severity of liver disease.

Suvak et al.,[7] conducted his study on 135 patients suffering from ascites due to cirrhosis, that consisted of 88 (65.2%) men and 47 (34.8%) women (mean age 57.9 ±13.9 years), reported significant difference in MPV levels between cirrhotic patients with ascitic fluid infection (AFI) compared to those without AFI (*p*<0.001). And this is in consistence with our results. Moreover, Suvak et al. research showed that there was a significant correlation between MPV and CRP (r = 0.535, p ≤0.001) with no correlation observed with WBC (r=0.049,p = 0.714), ESR (r = 0.105, p = 0.524) and this was completely different from our results that showed a significant correlation between MPV and CRP(r=0.275, p=0.0001), ESR (r=0.3720, p <0.0001). The positive correlation was between MPV and other inflammatory markers that support the reflect of MPV on the systemic inflammatory responses in cirrhotic patients with SBP.

In the present study, we found that AUC cutoff point of MPV level was 0.84 fl (P<0.0001) for diagnosis of SBP with overall accuracy of 79.3% which could be as a cutoff optimal value for SBP diagnosis, with 73% sensitivity, 85.7% specificity, independent on the severity of liver disease. This was in coherence with Suvak et al.[7], who recommended that MPV cutoff value would be of 8.45 fl, with a sensitivity of 70.7%, a specificity of 67.5%, (AUC = 0.768).

Also our findings were similar to those of Abdel-Razik et al. [9] who conducted his research on 80 ascetic patients complaining of liver cirrhosis and his study consisted of 50 (62.5%) men and 30 (37.5%) women with an age range from 39 to 67 years. They found that MPV levels of cirrhotic patients with AFI had a significant difference than those of cirrhotic patients without AFI (p<0.001). He suggested that MPV cutoff value of 8.77 fl, for SBP diagnosis with 95.9% sensitivity and 91.7% specificity, (AUC=0.964).

For Gálvez-Martínez et al.[10], MPV was a useful predictor of systemic inflammatory response syndrome in cirrhotic patients with AFI, particularly CNNA when he conducted his study on 100 patients with ascites due to cirrhosis. The research consisted of 62 (62%) men and 38 (38%) women (mean age 57 ± 9.8 years), it was found that the cutoff value of MPV was 8.3 fl, with sensitivity 84%, specificity 82%, (AUC=0.9).

In the present study, the overall accuracy of ESR was 70.7% with a sensitivity, specificity, NPV and PPVof78%, 63.4%, 73.8%, and 68.5%. We also found that, the overall accuracy of CRP was 66.6% with a sensitivity, specificity, NPV and PPV of 66%, 67.4%, 66% and 67.3% (AUC: 0.71).

The sensitivity and specificity of MPV used for the diagnosis of SBP was found to be comparable with CRP and ESR levels. While Suvak et al.[7], suggested that MPV has drastically increased in cirrhotic patients with significant correlation of the CRP level, but higher level in the reading of ESR. These results were in convenience with those of Abdel-Razik et al. [9].

In the current study, we followed Suez Canal University hospitals guidelines in the internal medicine department, we gave all cirrhotic patients with AFI 2 gm of ceftriaxone, and culture and sensitivity results revealed that 25% of SBP group had infection with *E.coli* and *K.pneumonie*.

Follow up was carried on group I (SBP group) after treatment for 5 days later, to evaluate MPV as a prognostic factor for SBP. Our results came up with significant decrease in MPV (8.1±0.8fl) after receiving treatment versus (8.5±0.6fl) before treatment (p value <0.0001).

There are several limitations in the current study that worth consideration. First, we included all patients with liver cirrhosis and ascites, irrespective of the etiology of cirrhosis, second, our sample size was relatively small so, on a wide scale larger studies are needed to evaluate this test in different clinical settings and also there was limited literatures that implicit the role of MPV as a follow up tool in SBP subjects after treatment

CONCLUSION

We concluded that MPV is increased in cirrhotic patients with SBP. MPV measurement can be considered to be a rapid, easily applicable and valuable diagnostic tool of SBP in cirrhotic patients, besides it can be used as a prognostic marker to follow up response to treatment

Recommendations

We recommend using of MPV at cut off value of 8.4 fl as an early indicator for SBP in ascetic patients. Also further studies are needed to confirm the results of current study and evaluate its validity and accuracy and its usage in different clinical settings.

Conflict of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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