Mean Platelet Volume and Platelet Function as Indicators for Spontaneous Bacterial Peritonitis in Patients with Liver Cirrhosis and Ascites

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Abstract: Background/Aims: The objective of this study is to measure mean platelet volume (MPV) and platelet cluster of differentiation molecule 40 ligand (CD40L) to evaluate their usefulness in the diagnosis of spontaneous bacterial peritonitis (SBP) in cirrhotic patients. **Materials and Methods:** This study comprised 80 cirrhotic patients with ascites. MPV and platelet CD40L were measured **Results:** A significant increase in MPV and platelet CD40L observed in SBP group compared to non SBP ($P < 0.001$). At cutoff value of 8.71 fl MPV had 68.75% sensitivity and 80% specificity for diagnosis of SBP. At cutoff value of 4,81 ng/ml platelet CD40L in serum had 62% sensitivity and 80% specificity for detecting SBP. **Conclusion:** mean platelet volume (MPV) and platelet_cluster of differentiation molecule 40 ligand (CD40L) are useful marker in the diagnosis of SBP in cirrhotic patients.
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1. Introduction

Patients with cirrhosis are usually prone to develop bacterial infections, primarily spontaneous bacterial peritonitis (SPB), which is present in 15-25% of patients with cirrhosis and ascites **(1).** Because of this high percentage, spontaneous bacterial peritonitis is considered to be the most frequent and life-threatening bacterial infection in patients with liver cirrhosis and ascites, requiring prompt recognition and treatment **(2).**

Symptoms and signs are frequently absent in patients with spontaneous bacterial peritonitis **(3).** So a diagnostic paracentesis should be performed in all patients with ascites admitted to hospital regardless of whether or not there is clinical suspicion **(4).** Diagnosis should be prompt and treatment must not be delayed until the microbiology results are available. Ascitic fluid culture results always take several days to one week, which suggests that they cannot be used as a screening tool for spontaneous bacterial peritonitis. Moreover, negative bacterial culture does not rule out spontaneous bacterial peritonitis diagnosis **(5).** Thus, in all the available guidelines, diagnosis is based on a fixed defined cutoff PMNLs count in the ascitic fluid which is more than 250 polymorphonuclear cells (PMNLs) / mm^3 **(6).** For this reason, the use of additional markers that are rapidly and easily applicable, may add significant benefit for predicting the development of spontaneous bacterial peritonitis and achieving diagnostic accuracy.

The present study has focused on the platelet as regard the volume and the function as predictors for spontaneous bacterial peritonitis. Platelets have even more important roles in tissue remodeling, modulation of inflammation and antimicrobial host defense **(7)** Platelets with increased size have a greater content of the granules and can therefore exert their hemostatic and pro-inflammatory actions with greater efficiency **(8).**

It was noticed that there is upregulation of Cluster of Differentiation molecule 40 ligand (CD40L) on the platelet surface **(9).** This CD40L can subsequently be cleaved and released as a soluble molecule into the circulation.

Therefore, mean platelet volume and platelet CD40L measurement may be used in predicting spontaneous bacterial peritonitis.

2. Subjects and Methods

Subjects

This case-control study was carried out in Benha University Hospital on eighty cirrhotic patients with ascites attending to Tropical Medicine Department and the work was conducted in Clinical Pathology Department in period from April 2015 to October 2015. The diagnosis of liver cirrhosis and ascites were

based on clinical, biochemical and ultrasonographic findings.

Patients were classified into group A 40 patients with ascitic fluid PMN count≤ 250 cells/mm3 (non-SBP) and group B 40 patients with ascitic fluid PMN count ≥ 250 cells/mm3 (SBP). A control group C consisted of 10 healthy age and gender-matched subjects (male/female: 5/5) was included.

This study was approved by the ethical committee of Benha University and all patients provided written informed consent before participation in any protocol specific procedure.

Exclusion criteria

None of the patients had received antibiotics for ten days prior to hospital admission. Patients with evidence of secondary bacterial peritonitis, tuberclous peritonitis, malignant, cardiac, renal or pancreatic ascites were excluded. Patients with diabetes mellitus, hypertension, hyperlipidemia, peripheral vascular disease, hematological disorders and neoplastic disorders were also excluded from this study. None of the study participants had received anticoagulant medications, non-steroidal anti-inflammatory drugs (NSAID) or oral contraceptive drugs before hospital admission.

Methods

All studied patients were subjected to through history taking, clinical examination, routine laboratory investigation. Platelet number, MPV and platelet CD40L were also done.

Abdomino-pelvic ultrasonographic examination was done for all patients. Aspirated ascitic fluid samples were immediately examined for bacteriological cultures, identifications of microorganisms and cytological assay.

Sampling:

Four ml of venous blood samples were taken from all patients, this was divided into 2ml in EDTA vacutainer (purple topped) tube for CBC analyses, other 2 ml in (red topped) tube left to clot and serum was separated via centrifugation at 5000 rpm for 3 minutes, Serum samples were kept at −80˚C.

CBC analyses were performed with the same analyzer within 2 hours after collection of blood samples with the use of a **Beckman Coulter (High Wycombe, UK) Gen-S automated analyzer**. Liver and kidney tests (AST and ALT, serum albumin, total and direct bilirubin, prothrombin time and concentration and craetinine.) using **Cobas Integra 400, Hoffman La Roche Company, Switzerland**

Platelet cluster of differentiation molecule 40 ligand (CD40L) was measured by a double –antibody sandwich enzyme-linked immunosorbent assay (ELISA).The minimum detectable level of CD40L is 0,075 ng/ml. The KIT used was produced by **(Sun Red) company, Shanghahai**.

2.2.2. Principle of sCD40L assay:

Samples containing (sCD40L) will be incubated with a monoclonal antibody enzyme. (sCD40L) antibodies labeled with biotin and combined with streptavidin was added to the solution to form immune complexes. Washing was done to remove the uncombined enzyme. Chromogen solution was added. The color of the solution was changed ranging from blue to yellow according to the concentration of (sCD40L).

Statistical Analysis

All data were collected, tabulated and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 22 software (SPSS Inc., Chicago, IL, United States). Values are presented as mean ± standard deviation or in the case of non normally distributed data, as median and range. Comparisons of percentages between different groups of patients were carried out using the chisquared test. All 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. One-Way ANOVA was used to compare normally distributed variables in three groups. Tukey test was used according to homogeneity of variances. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of MPV and with maximum sensitivity and specificity for differentiation of cirrhotic patients with SBP from those without SBP. Spearman's correlation analysis was done.

4. Results

80 patients with ascites due to cirrhosis (hepatitis c virus represented the main etiology due to its high prevalence in Egypt), of these were classified as Non-SBP and SBP. 10 control subjects were enrolled in the present study.

There were no statistically significant differences as regard the ages and gender of the study participants **(table 1)**.

SBP: spontaneous bacterial peritonitis, **SD**: standard deviation.

Fever, jaundice, encephalopathy, abdominal pain and poor response to diuretics have markedly increased in patients with SBP compared to non SBP group **(table: 2)**. Hematemesis and melena were the main precipitating factors in SBP patients. Vomiting and diarrhia were more clinically presented in SBP patients, but this don not reach statistical significance.

Total and direct bilirubin represented a highly statistically significant increase in SBP cases compared to non SBP cases **(table: 3)**. Otherwise, no statistically significant differences were recorded in the other liver function tests. Serum creatinine was found to be significantly higher in patients with SBP

compared to the non SBP group. By comparing different liver and kidney functions, there was a highly significant increase in SBP and Non SBP than the control group.

There was a significant increase in MPV levels and platelet CD40L in cirrhotic patients with SBP compared to cirrhotic patients without SBP and healthy controls **(table 4).** There was a significant decrease in hemoglobin and platelets in SBP and Non SBP that id more marked in SBP group. Also, there was a significant increase in the WBCs count in the same group.

A statistically significant increase in MPV levels was observed in cirrhotic patients with SBP compared to cirrhotic patients without SBP and healthy controls using ROC curve analysis to predict SBP in cirrhotic patients, the optimal MPV level cutoff point for cirrhotic patients with SBP was 8.71 femtolitre (fl), with a sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of 68.75%, 80%, 87% and 54,8 % respectively **(figure 1)**. With respect to platelet CD40L, the cutoff point for the diagnosis of SBP was 4.81 ng/ml, with a sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of 62%, 80%, 86.1% and 51.6% respectively **(figure 2**) and **(figure 3**).

There was a positive correlation between CD40L and (MPV, WBCs, ascetic TLC and ascetic PMN) respectively (**table 5**). Also, there was a positive correlation between MPV and (WBCs, ascetic TLC and ascetic PMN).

Table (1) Demographic criteria of the studied groups:-

Demographic criteria		Groups						ANOVA or Chi-Square	
		group A(non-SBP)		group B(SBP)		Group C (control)			
Age	Range	42-67		39-67		40-61		1.999	0.142
	Mean ±SD	52.800±6.884		50.250±5.324		49.800±7.084			
sex	Male	26	65.00	24	60.00	5	50.00	0.795	0.672
	Female	14	35.00	16	40.00	5	50.00		

Table (2): Clinical predictors of SBP

Presentations	Groups				Chi-Square	
	group (A) non-SBP		Group(B) SBP			
	N	%	N	%	X2	P-value
abdominal pain	**6**	**15.00**	**24**	**60.00**	**17.280**	**<0.001***
Fever	**0**	**0.00**	**26**	**65.00**	**38.519**	**<0.001***
Jaundice	**11**	**27.00**	**21**	**52.00**	**5.208**	**0.022***
Diarrhia	**4**	**10.00**	**2**	**5.00**	**0.721**	**0.396**
Vomiting	**3**	**7.00**	**4**	**10.00**	**0.157**	**0.692**
Heamatemsis	**7**	**17.00**	**16**	**40.00**	**4.943**	**0.026***
Melena	**6**	**15.00**	**14**	**35.00**	**4.267**	**0.039***
Encephalopathy	**8**	**20.00**	**17**	**42.00**	**4.713**	**0.030***
diuretic resistance	**8**	**20.00**	**21**	**52.00**	**9.141**	**0.002***

Table (3): Comparison of group (A) and group (B) regarding Liver and kidney function tests:-

Liver and kidney function tests		Groups						T-Test	
		group (A) non- SBP			group (B)SBP			t	P-value
AST	Range	32	-	72	35	-	73	-1.784	0.078
	Mean ±SD	49.750	±	8.702	53.350	±	9.339		
ALT	Range	30	-	66	33	-	70	-1.677	0.097
	Mean ±SD	44.275	±	7.916	47.275	±	8.080		
ALBUMIN	Range	1.5	-	3.2	1.5	-	3.2	-0.450	0.654
	Mean ±SD	2.370	±	0.412	2.410	±	0.383		
T.BILI.	Range	1.5	-	3.9	2.1	-	4.8	-5.694	<0.001*
	Mean ±SD	2.730	±	0.595	3.543	±	0.679		
D.BILI.	Range	0.9	-	2.3	1.1	-	3	-3.960	<0.001*
	Mean ±SD	1.478	±	0.377	1.865	±	0.491		
INR	Range	1	-	2.3	1.1	-	1.9	0.506	0.614
	Mean ±SD	1.550	±	0.337	1.518	±	0.227		
S.CREAT.	Range	0.7	-	1.5	1.1	-	2.6	-10.020	<0.001*
	Mean ±SD	1.188	±	0.238	1.835	±	0.332		

Table (4): Laboratory characteristics of the studied groups:-

		Groups			ANOVA		TUKEY'S Test		
		group (A)non-SBP	group (B)SBP	group (C)control	F	P-value	Non SBP &SBP	Non SBP & Control	SBP& Control
MPV	Range	7.31 - 8.68	8.71 - 11.62	7.16 - 8.52	64.931	<0.001*	<0.001*	0.214	<0.001*
	Mean ±SD	8.045 ± 0.439	9.348 ± 0.697	7.701 ± 0.495					
CD40L	Range	2.06 - 4.78	4.81 - 47.8	0.011 - 4.74	12.553	<0.001*	<0.001*	0.967	0.005*
	Mean ±SD	3.773 ± 0.639	10.374 ± 9.390	3.220 ± 1.594					

Table (5): Spearman Correlations between different predictors of SBP

Correlations				
SBP predictors	CD40L		MPV	
	r	P-value	R	P-value
MPV	0.803	<0.001*		
WBCs	0.924	<0.001*	0.775	<0.001*
Ascetic TLC	0.976	<0.001*	0.829	<0.001*
Ascetic PMN	0.968	<0.001*	0.826	<0.001*

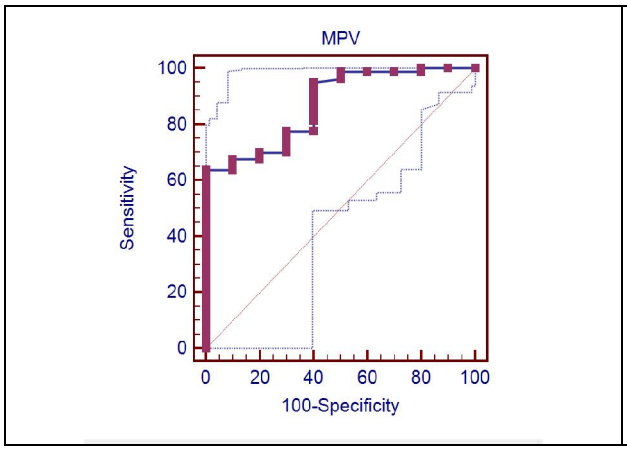

Figure (1): ROC curve of MPV.

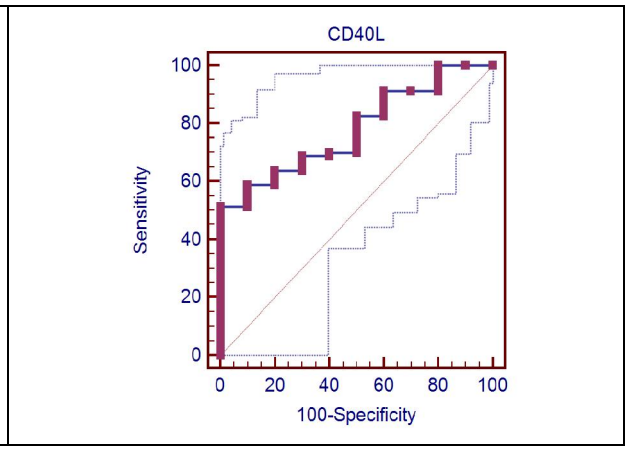

Figure (2): ROC curve of platelet CD40L.

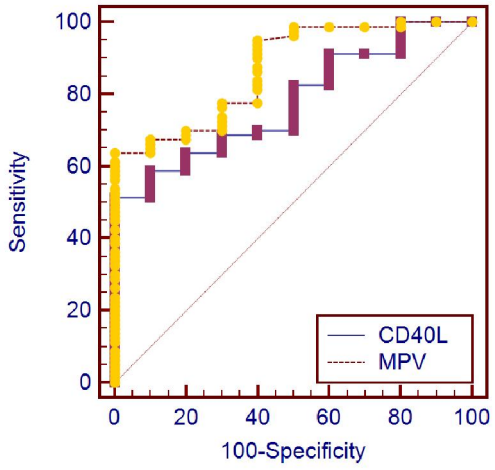

Figure (7): ROC curve of MPV and platelet CD40L.
This curve shows the relationship between MPV and platelet CD40Las regard sensitivity and specificity.
Difference between areas = 0.0931
Standard Error a = 0.0872
95% Confidence Interval = -0.0779 to 0.264
Significance level = 0.286

4. Discussion

Spontaneous bacterial peritonitis is one of the most common bacterial infections in cirrohtic patients with ascites, this infection stimulates the immune system in different forms, such as increase the total leucocytic count and PMN count in both blood and ascetic fluid. **(10).** It is possible that the rise in mean platelet volume (MPV) in bacterial infection is caused by an expanded creation of bigger and/or more youthful platelets as a response to the pathogen **(11).** The platelets expression of many copies of CD40L on their surfaces upon activation was surprising because CD40L was thought to characterize immune reactive cells only, and platelets were not yet acknowledged to display any immune function **(12)**. The surface-expressed CD40L is subsequently cleaved over a period of minutes to hours **(13)**, generating a soluble fragment termed sCD40L. Considers on the cell conveyance of CD40L demonstrate that more than 95% of the circulating CD40L is generated from the platelets **(14).**

The ability of MPV values to predict SBP in cirrhotic patients was analyzed using receiver operator characteristic (ROC) curve analysis. A statistically significant increase in MPV levels was observed in cirrhotic patients with SBP compared to cirrhotic patients without SBP and healthy controls ($p < 0.001$). ROC curve analysis suggested that the optimum MPV level cutoff point for cirrhotic patients with SBP was 8.71 FL, with a sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of 68.75%, 80%, 87% and 54,8 %.

The results of the study were close to that reported by **Suvak et al. (15)** who stated that the optimum MPV level cut-off point for cirrhotic patients with SBP was 8.45, with a sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of 70.7%, 67.5%, 75.4% and 62.1%, respectively.

Also this was in agreement with that reported by **Abdel-Razik et al. (16)** where MPV had 8.77 as a cutoff value, 95.9 % sensitivity and 91.7% specificity for detecting SBP.

Also, **Marisol et al.,(17)** found that the best was the cutoff value of 8.3 fl, with sensitivity, specificity, PPV, NPV and accuracy of 84%, 82%, 83%, 84% and 83% respectively.

Platelet CD40L was detected in both groups in addition to the control group. There is a highly statistical significant difference in SBP group compared to non SBP group platelet CD40L increased in SBP cases.

The sensitivity of the test was 62% with specificity of 80%, with the cut-off level was 4.81 ng/ml.

Up to date, the use of platelet CD40L as indicator for spontaneous bacterial peritonitis is very rare in literature. There are no studies to compare with. Platelet CD40L level in severe sepsis was studied by **Lorento et al. (18)** where the cut off value for survivors was 3.78 ng/ml for survivors while the non survivors value was 4.42 ng/ml**.**

Regarding the relation between (MPV and platelet CD40L) and ascitic fluid PMN count, the correlation coefficient was 0.826 and 0.968 respectively and p- value was <0.001. This indicates significant positive correlation between (MPV and platelet CD40L) and ascitic fluid PMN, this concludes that (MPV and platelet CD40L) can be used as sensitive and specific marker for early screening and detection of SBP in cirrhotic patients with ascites.

Conclusions

MPV and platelet CD40L could be good diagnostic markers in patients with SBP with 68.75%, 62% sensitivity and 80%, 80% specificity respectively.

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Conflict of Interest

No conflict of interest was declared by the authors.

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