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Original article

Role of CD11b for Early Diagnosis and Follow up of Spontaneous Bacterial Peritonitis in Cirrhotic Ascetic Patients

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Abstract:

Back ground: Spontaneous bacterial peritonitis (SBP) is a common and high-mortality infectious complication of patients with cirrhosis. This study aims to evaluate the neutrophil surface receptor CD11b as a marker for detecting SBP and predicting mortality after SBP. **Patients and methods:** Ninety adult patients were recruited from the Hepatology and Gastroenterology Department, Mansoura University Hospitals, and Mahalla Hepatology Teaching Hospital. Blood CD11b, and the mean fluorescence intensity (MFI) were tested as a marker for the detection and follow up of SBP. **Results:** CD11b was higher in the SBP group vs. control group but this difference was not statistically significant by pairwise comparison. Moreover, the study revealed that CD11b is a non-discriminating marker in our cases. MFI was a statistically significant discriminator for G1 vs. G3, G2 vs. G3, G1-2 vs. G3. Finally, the study revealed a statistically significantly higher age, MFI of CD11b, WBCs, Neutrophils, NLR, creatinine, higher positive CRP, CRP/ Albumin ratio >3.5, MELD score >21, higher Child Pugh score and ICU admission and a statistically significantly lower SBP, DBP, MAP, Hb, albumin and CD11b / CRP ratio in non-survivors (less than 3 months) versus survivors. **Conclusion:** In cirrhotic patients CD11b-MFI can discriminate between ascitic and non ascitic cases but cannot discriminate between SBP and non-SBP cases. It can be implied as new biomarker to follow up SBP cases and evaluate antibiotic response in those patients.

Keywords: Spontaneous bacterial peritonitis (SBP); Neutrophil surface receptor CD11b; Detection; Mean Fluorescence Intensity (MFI).

Introduction

Spontaneous bacterial peritonitis (SBP) is one of the potentially fatal complications with cirrhosis and ascites. Due to the high prevalence of bacterial infections in cirrhotic patients, it is important to evaluate their immune defense deficiencies against microorganisms [(1)].

CD11b is mostly expressed on monocytes, macrophages, dendritic cells, neutrophils, natural killer cells, and a subset of T and B lymphocytes. Innate immune cells' major

biological processes are modulated by it. CD11b also plays a fundamental role in the phagocytosis of opsonized particles such as apoptotic cells [(2)].

The gold standard for diagnosing SBP is a positive AF (ascitic fluid) culture for a pathogen, yet 60% of individuals with clinical signs and symptoms suggestive of SBP have negative cultures with an AF polymorphonuclear leukocyte (PMNL) count below 250/L. Furthermore, it is challenging to identify SBP since it lacks the normal clinical features of the condition [(3)].

The aim of this study is to evaluate the neutrophil surface receptor CD11b as a marker for detection of SBP in correlation with other inflammatory markers and find out its sensitivity & specificity in relation to other traditional diagnostic methods such as ascitic fluid polymorphonuclear leukocyte (PMNL) count.

Patients and methods

Agreement for this study was obtained from the hospital's ethical committee; in addition, informed consent was obtained from participants after adequate provision of information regarding the study requirements, purpose, and risks. The study was approved by the Ethics Committee of the Faculty of Medicine, Mansoura University Hospital (number: MS.22.04.1998), and Institutional Review Board of the Mahalla Hepatology Teaching Hospital.

Study design:

A prospective case-control study of 90 adult patients recruited from the hepatology gastroenterology department, Mansoura University Hospitals, and Mahalla Hepatology Teaching Hospital, Egypt throughout the period from February 2022 to 2024.

Patients:

We divided participants into three groups: G1 group which was cirrhotic patients with SBP (characterized by patients with ascites, abdominal pain, fever, ascetic PMN $\geq$ 250 cells/mm^3 in the presence of a single organism on culture [(4)] (N=45)**,** the G2 group which was cirrhotic ascetic without SBP (N=30), G3 group which was the control group (cirrhotic non-ascetic patients) (N=15).

The inclusion criteria were: age 18 years or older, patients with liver cirrhosis.

The exclusion criteria were: ascites due to any cause other than liver cirrhosis, patients with hepatocellular carcinoma, secondary bacterial peritonitis (surgically treatable source of infection), patient with other source of infection or septicemia, patients with renal failure, and patients on antibiotic treatment or norfloxacin prophylaxis.

All these patients' disease history (demographic data, history of medical diseases, first or recurrent attack of SBP, number of hospital admissions and duration of ICU stay), physical examination, standard laboratory tests (CBC, CRP, S- creatinine, S-albumin, NLR (neutrophil, lymphocyte ratio, CRP/ albumin ratio, ascetic fluid sample for **(**PMNs, glucose and total protein level) and standard pelvi-abdominal Ultra-sonography were evaluated. All blood sample and ascetic fluid were taken once admitted to hospital.

Primary outcome: evaluation the neutrophil surface receptor CD11b and the mean fluoroscopic intensity (MFI) as a marker for detection of SBP.

Secondary outcomes: evaluation of other predictors of SBP and use of the neutrophil surface receptor CD11b and the mean fluorescence intensity (MFI) as a marker for follow up of SBP.

Measurements:

Detection of CD11b expression and its mean fluorescence intensity were done by flow cytometry. Peripheral blood samples were collected on an EDTA vacutainer tube within 6 h after paracentesis and before antibiotic therapy and in non-ascetic group taken within 6 h of hospital admission; the blood sample was processed within 6 h after collection for the measurement of CD11b expression on neutrophils which are defined by their forward and side scatter characteristics. In each sample, 10,000 cells were analyzed. Bovin Serum Albumin <1% monoclonal antibody CD11b labeled by phycoerythrin (PE) (Rat/IgG2b, kappa) (provided by Beckman Coutter company, France) was used. Briefly, 100 µL of anticoagulated (EDTA) whole blood was added to the bottom of a 12 × 75 mm polystyrene tube along with 5 µL of CD11b; vortex and incubation were performed for 40 min in the dark at room temperature; 100 µL of reagent A (formaldehyde) was added to the sample; then, vortex and incubation were performed for 10 min at room temperature in the dark. A volume of 1 mL of reagent B (buffer) was added to the sample, and vortex and incubation were performed for 10 min at room temperature in the dark. Unstained samples were included as controls. Gates were analyzed for the number and percentage of cells. The expression of CD11b and mean fluorescence intensity (MFI) of CD11b was assessed on neutrophils by flow cytometer (NAVIOS EX Flow Cytometer, BECKMAN COULTER Flow Science).

Study size:

Sample size was calculated by using G*Power software (version 3.1.9.7). Based on a previous study by researchers [(5)], who hypothesized large effect size (f=0.4) for CD11b expression on peripheral blood neutrophils between the three study groups [(5)]. In a one-way ANOVA study, sample sizes of 45 SBP patients, 30 non-SBP cirrhotic ascetic patients, and 15 control subjects. The total sample of 90 subjects achieves 93% power to detect differences among the means versus the alternative of equal means using an F test with a 0.05 significance level. The size of the variation in the means was represented by the effect size f = σm / σ, which was 0.4000 [(6)] .

Statistical analysis

The data were analyzed using the 'statistical package for the social sciences SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) and MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). We presented the numerical data as means (SD) or medians (P25th–P75th) based on the normality of data tested using the Shapiro test, histogram, and Q–Q plot. The student's t-test or Wilcoxon–Mann–Whitney test was used as appropriate, while categorical data were depicted as numbers (percentages) and were compared using the X^2 test or Fischer's exact test, as appropriate.

The Spearman's correlation test was used to determine whether there is a linear relationship / association between two non-normally distributed quantitative data. The strength of association was considered low, medium, or high if the correlation coefficient (r) was > 0.1 to <0.3, 0.3 to <0.5, or 0.5 or more, respectively.

The Receiver Operating Characteristic (ROC) curve analysis was used to find a cutoff value of a continuous variable that can discriminate between two conditions.

Results

This study involved 90 participants divided into three groups: G1 group which was cirrhotic patients with SBP (N=45), G2 group which was cirrhotic ascetic without SBP (N=30), G3 group, control group, which was cirrhotic non ascetic (N=15).

a- Part I: Results of the three groups

There was a statistically significant difference in age, sex, and MFI between the three groups as in **table (1).** MFI was higher in SBP group vs. control group but this difference was not statistically significant by pairwise comparison. Finally, the study revealed that CD11b is a non-discriminating marker in our cases. MFI was statistically significant discriminator for G1 vs. G3, G2 vs. G3, G1-2 vs. G3, and non-survivors vs. survivors at cutoff values of >54.8, >75.9, >95.9, and >73.5, respectively with AUCs of 0.688 (sufficient accuracy), 0.746 (good accuracy), 0.711 (good accuracy), and 0.712 (good accuracy), respectively as in **table (2).**

Table (1): Age, sex, blood CD11b level and MFI in the three groups:

Characteristic	Group (1)	Group (2)	Group (3)	p-value
Age (years)	64 (58-70)	60 (53.5-67)	31 (26-32)	**<0.001**
Sex				
Male	27 (60%) a	9 (30%) b	1 (6.7%) b	**<0.001**
Female	18 (40%) a	21 (70%) b	14 (93.3%) b	
CD11b	99 (96.7-99.3)	98.8 (98.3-99.3)	98 (96.7-99.6)	0.884
MFI	66.9 (47-88.2)	77.7 (50.4-96.6)	48.9 (37.7-62)	**0.026**

Notes: Data for sex is N (%) [Test of significance is chi-square test]. Multiple z-tests are presented in letters (different letters = significant difference). Quantitative data is median (Q1-Q3). The test of significance is Kruskal-Wallis H-test.

Table (2): Blood CD11b level and MFI diagnostic performance:

Marker / Groups	Cutoff	AUC	95% CI	p-value	SE	Sensitivity	Specificity
CD11b							
G1 vs. G2	>93.8	0.500	0.382-0.618	1.000	0.0671	82.2%	0%
G1 vs. G3	>98	0.524	0.391-0.655	0.784	0.0890	68.9%	53.3%
G2 vs. G3	>98	0.563	0.407-0.711	0.553	0.1070	80%	53.3%
G1/2 vs. G3	>98	0.540	0.432-0.646	0.659	0.0908	73.3%	53.3%
NSV vs. SV	>99.2	0.611	0.492-0.722	0.147	0.0766	45.5%	79.2%
MFI							
G1 vs. G2	≤73	0.557	0.438-0.672	0.409	0.0696	62.2%	0.56.7%
G1 vs. G3	>54.8	0.688	0.555-0.802	**0.008**	0.0707	64.4%	73.3%
G2 vs. G3	>75.9	0.746	0.594-0.863	**0.001**	0.0722	53.3%	100%
G1/2 vs. G3	>95.9	0.711	0.606-0.802	**<0.001**	0.0595	42.7%	100%
NSV vs. SV	>73.5	0.712	0.596-0.811	**0.001**	0.0659	77.3%	89.8%

Notes: NSV, SV=non-survivor, survivor. AUC=area under the curve. CI=confidence interval. SE=standard error.

b- Part II: Results of the two ascetic groups

The study showed a statistically significantly higher proportion of fever, abdominal pain, moderate and marked ascites, and normal kidney detected by ultrasound higher number of attacks, CRP/albumin ratio, WBCs count, neutrophil count, NLR, serum creatinine, and MELD score in SBP vs. non-SBP group as in **table (3).** Lastly the study

revealed a statistically significantly positive correlation of low strength between CD11b MFI vs. CTP score, and a statistically significantly positive correlation of low strength between both CD11b MFI & % vs. CRP / albumin ratio. Also, CD11b % was statistically significantly correlated with female sex (mean value of the maker was 94.5 in male vs. 98.3 in female) as in **table (4).**

Table (3): Comparisons of categorical data and numeric data between the two ascites groups:

Characteristic	Group (1) SBP group (N=45)	Group (2) Non-SBP group (N=30)	Test of significance	
			χ^2	p-value
Fever	16 (35.6%)	2 (6.7%)	8.236	**0.004**
Abdominal pain	28 (62.2%)	5 (16.7%)	15.161	**<0.001**
Diabetes	22 (48.9%)	10 (33.3%)	1.781	0.182
Hypertension	14 (61.1%)	10 (33.3%)	0.041	0.840
CKD*	7 (15.6%)	5 (16.7%)	-	1.000
IHD*	3 (6.7%)	4 (13.3%)	-	0.427
ICU admission	12 (26.7%)	4 (13.3%)	1.907	0.167
Positive CRP	38 (84.4%)	13 (43.3%)	13.981	**<0.001**
Spleen**			-	
Splenectomy	4 (8.9%)	1 (3.3%)		0.070
Normal sized	13 (28.9%)	4 (13.3%)		
Mildly enlarged.	6 (13.3%)	3 (10%)		
Moderately enlarged.	9 (20%)	16 (53.3%)		
Markedly enlarged	12 (27.7%)	6 (20%)		
Ascites (US)**			-	
Mild	2 (4.4%)	2 (6.7%)		**0.011**
Moderate	27 (60%)	8 (26.7%)		
Marked	16 (35.6%)	20 (66.7%)		
Kidney (US)**			-	
Normal	38 (84.4%)	18 (60%)		**0.004**
Grade 1	3 (6.7%)	11 (36.7%)		
Grade 2	3 (6.7%)	1 (3.3%)		
Grade 3	1 (2.2%)	0 (0%)		
CTP class			0.195	0.659
Child class B	10 (22.2%)	8 (26.7%)		
Child class C	35 (77.8%)	22 (73.3%)		
3-months mortality	15 (33.3%)	7 (23.3%)	0.868	0.351
Number of Attacks	2 (1-2)	0.5 (0-1)	1	**<0.001**
BMI (kg/m^2)	24.2 (19.5-30.3)	30 (26.1-35.1)	-5.4	**0.006**
Mean arterial pressure (mmHg)	83.3 (70-90)	83.3 (80-93.3)	-3.3	0.165
Blood CD11b	99 (96.7-99.4)	98.8 (98.3-99.3)		1.00
CD11b(MFI)	66.9 (47-88.2)	77.8 (50.5-96.6)		0.402
CD11b / CRP ratio	2.98 (1.39-6.92)	6.57 (3.76-7.47)	-1.9	0.059
CRP (mg/L)	33 (14.3-65.5)	15 (13-26.5)	10	0.080
CRP / Albumin ratio	7.8 (4.1-25)	0 (0-6.7)	5.7	**<0.001**
WBCs count per µl	12 (7-15.3)	7.5 (4.7-9.1)	4.7	**<0.001**
Neutrophil count per µl	9 (4.6-12.9)	5 (3.2-6.6)	4.4	**<0.001**
Lymphocyte count per µl	1.8 (1.2-2.2)	1.4 (0.88-2)	0.3	0.056
Neutrophil to lymphocyte ratio (NLR)	5.6 (3.4-8.7)	3.1 (2.5-4.5)	1.9	**0.003**
Platelet count per µl	123 (71-200)	118 (56.8-157.3)	28	0.092
Serum albumin (g/dl)	2.6 (2.1-3)	2.5 (2.2-2.9)	0	0.761
Serum creatinine (mg/dl)	1.6 (1.2-2.4)	1.4 (1.0-1.8)	0.3	**0.037**
CTP score	11 (10-13)	10 (9-12)	1	0.186
MELD score	23 (18-28)	17.5 (14-23)	5	**0.002**

Table (4): Correlation of biomarkers with study parameters in ascitic groups (N=75):

Parameter	CD11b (MFI)		Blood CD11b (%)	
	r_{pb}	p-value	r_{pb}	p-value
Sex	-0.156	0.183	0.258	**0.025**
3-monthes survival	-0.221	0.057	-0.072	0.537
Fever	-0.144	0.216	0.028	0.809
Abdominal pain	-0.056	0.635	-0.039	0.739
CRP	0.178	0.132	0.024	0.838
	r_s	p-value	r_s	p-value
Age (years)	-0.002	0.985	0.029	0.802
BMI (kg/m^2)	-0.092	0.430	-0.090	0.442
MELD score	0.012	0.921	-0.045	0.699
Child Pugh score	0.252	**0.029**	0.210	0.071
Hemoglobin (g/dl)	-0.105	0.372	-0.073	0.531
Platelet count per µl	0.010	0.929	-0.190	0.103
WBCs count per µl	0.062	0.598	0.054	0.646
Neutrophil count per µl	0.064	0.584	0.106	0.364
Lymphocyte count per µl	-0.088	0.452	-0.138	0.236
NLR (Neutrophil to lymphocyte) ratio	0.156	0.181	0.133	0.257
Serum albumin (g/dl)	-0.211	0.069	-0.255	**0.027**
CRP (mg/L)	0.116	0.419	0.097	0.499
Serum creatinine (mg/dl)	0.012	0.917	-0.080	0.493
CRP / Albumin ratio	0.231	**0.047**	0.240	**0.038**

Notes: MFI= Mean fluorescence intensity. CRP = C-reactive protein. TLC = total leucocytic count. ANC = absolute neutrophil count. r_{pb} = Point-biserial correlation coefficient. r_s = Spearman's correlation coefficient.

Discussion

The study revealed that CD11b is a non-discriminating marker in our cases while MFI was statistically significant discriminator for cirrhotic ascetic (either SBP OR non-SBP) from cirrhotic non-ascetic patients. Also, MFI at cutoff value > 73.5 is statistically significant in discriminating non-survivor vs. survivor either in SBP patients or non-SBP patients.

Due to the short time for the detection of CD11b-MFI on peripheral blood neutrophils, it can be implied as new biomarker to discriminate between SBP and non_SBP cases with a high sensitivity and specificity superior to ascetic fluid (AF) total leukocytic count (TLC), and neutrophil_count and AF culture, which are time consuming and give false negative

results [5].

We found that MFI was higher in two ascetic groups (G1, G2) vs. control group (G3) at cutoff values of >54.8, >75.9, >95.9, respectively with AUCs of 0.688 (sufficient accuracy), 0.746 (good accuracy), respectively but this difference was not statistically significant by pairwise comparison. [7] observed that the MFI of the CD11b expression on neutrophils was higher in the cirrhotic patients than in the controls. The higher expression of CD11b on neutrophils from cirrhotic patients could possibly account for the increased adherence to microvascular endothelial cells observed in these patients. CD11b receptor, a β2-integrin, mediates neutrophil firm adhesion to cytokine-activated endothelium and is also involved in neutrophil aggregation, cytotoxicity, and neutrophil-mediated tissue injury [8].

In this study we illustrated that MFI was statistically significant discriminator for G1-2 vs. G3 at cutoff values of >54.8, >75.9 and >95.9, respectively with AUCs of 0.688 (sufficient accuracy), 0.746 (good accuracy), 0.711 (good accuracy), and 0.712 (good accuracy), respectively. [9 and 10] found that survivors showed significantly lower mean expression for serum CD11b as compared to those with fatal outcome.

In this study we cleared statistically significantly higher proportion of fever, abdominal pain, moderate and marked ascites, and normal kidney detected by ultrasound higher number of attacks, CRP/albumin ratio, WBCs count, neutrophil count, NLR, serum creatinine, and MELD score in SBP vs. non-SBP group. This goes with [11,12,13 and 14] studies. Also, clinical presentations of abdominal pain, tenderness or fever were associated with SBP.

In this study we demonstrated that there was a statistically significant correlation between CD11b % and female sex. Our results were supported by others [10] who demonstrated significant differences in CD11b expression between male and female newborn infants in the studied groups. Also, it was found that CD11b expression measured by FACS was significantly increased in Female group.

Limitations

The limitations of this study include the small number of study population. Therefore, a larger controlled study will be needed. Moreover, it is not clear whether the in vitro behavior of peripheral blood neutrophils adequately correlates with the in vivo activity. Finally, it would have been more appropriate if we had added one more group of CDs and infections other than SBP in cirrhotic subjects to assess the specificity of CD11b for SBP.

Conclusion

In cirrhotic patients CD11b-MFI can discriminate between ascetic and non-ascetic cases but cannot discriminate between SBP and non-SBP cases. It can be implied as new biomarker to follow up SBP cases and evaluate antibiotic response in those patients. Presence of abdominal pain, positive CRP and NLR more than 5, 5 can predict occurrence of SBP in cirrhotic ascetic patients.

Abbreviations:

AF: ascetic fluid, ANC: absolute neutrophilic count, CBC: complete blood count, CD: cluster of differentiation, CRP: C-reactive protein, CTP: Child-Turcotte-Pugh score, DBP: diastolic blood pressure, EDTA: Ethylenediaminetetraacetic acid, IBM: international business machines, ICU:

intensive care unit, MAP: mean arterial blood pressure, MELD: Model for end stage liver disease, MFI: mean fluorescence intensity, NLR: Neutrophil lymphocytic ratio, PE: Phycoerythrin, PMNs: Polymorph neutrophils, ROC: receiver operating characteristic curve, SBP: spontaneous bacterial peritonitis, SBP: systolic blood pressure, SPSS: statistical package for the social sciences, TLC: Total Leucocytic Count, WBCs: white blood cells.

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