Open Journal of Gastroenterology, 2015, 5, 94-102
Published Online July 2015 in SciRes. http://www.scirp.org/journal/ojgas
http://dx.doi.org/10.4236/ojgas.2015.57016

Macrophage Inflammatory Protein-1 Beta (MIP-1β) and Platelet Indices as Predictors of Spontaneous Bacterial Peritonitis

—MIP, MPV and PDW in SBP

Soha E. Khorshed[1], Hoda A. Ibraheem[2], Shereen M. Awad[3]

[1]Tropical Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[2]Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[3]Family Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Email: sohaesmat@hotmail.com

Received 19 April 2015; accepted 17 July 2015; published 20 July 2015

Abstract

Background/Aims: The objective of this study is to measure macrophage inflammatory protein one beta (MIP-1β), mean platelet volume (MPV) and platelet distribution width (PDW) to evaluate their usefulness in the diagnosis of spontaneous bacterial peritonitis (SBP) in cirrhotic patients. Materials and Methods: This study comprised 41 cirrhotic patients with ascites. MPV, PDW and MIP-1β were measured in serum and ascitic fluid. Results: A significant increase MPV, PDW, C-reactive Protein (CRP) and white blood cell was observed in SBP group compared to non SBP ($P \leq 0.001$, $P = 0 < 0.004$, $P = 0.001$, $P = 0.001$ respectively). In addition, MIP-1β was significantly increased in ascitic fluid in patients with SBP versus non SBP ($P \leq 0.001$). At cutoff value of 8.3 fl MPV had 85.7% sensitivity and 75% specificity (AUC = 0.876) for diagnosis of SBP. At cutoff value of 15.4 PDW had 90.4% sensitivity and 55% specificity (AUC = 0.762). At cutoff value of 121.9 pg/ml MIP-1β in ascitic fluid had 76.1% sensitivity and 100% specificity (AUC = 0.881) for detecting SBP. Conclusion: MIP-1β and platelet indices are useful marker in the diagnosis of SBP in cirrhotic patients. Combined measurement of MIP-1β in serum and ascitic fluid had 100% sensitivity and specificity for diagnosis of SBP.

Keywords

Spontaneous Bacterial Peritonitis, Liver Cirrhosis, Macrophage Inflammatory Protein-1 Beta, Mean Platelet Volume

How to cite this paper: Khorshed, S.E., Ibraheem, H.A. and Awad, S.M. (2015) Macrophage Inflammatory Protein-1 Beta (MIP-1β) and Platelet Indices as Predictors of Spontaneous Bacterial Peritonitis. *Open Journal of Gastroenterology*, **5**, 94-102.
http://dx.doi.org/10.4236/ojgas.2015.57016

1. Introduction

Bacterial infections are frequently observed in patients with cirrhosis, among which spontaneous bacterial peritonitis (SBP) is probably the most serious complication in advanced cirrhosis of the liver [1]. Patients with SBP may present with fever, abdominal pain, deterioration of renal function, hypotension, or development of encephalopathy [2]. On the basis of the results obtained from absolute white cell count and culture of the ascitic fluid (AF), five variants of peritoneal fluid infection have been recognized [3]. These are 1) SBP: absolute count of polymorph nuclear leukocytes (PMNs) in AF of at least 250/mm^3 and a positive culture showing a single type of bacteria; 2) culture-negative neutrocytic ascites (CNNA): negative AF culture with a PMN count greater than 250/mm^3; 3) monomicrobial non-neutrocytic bacterascites: culture positive AF for one type of bacteria and a PMN count lesser than 250/mm^3; 4) secondary bacterial peritonitis: characterized by polymicrobial growth from AF with a PMN count of at least 250/mm^3 and a surgically treatable source of infection; and 5) polymicrobial bacterascites: PMN count less than 250/mm^3 [4]. SBP represents AF in the absence of a continuous source of infection and/or spontaneous infection with an intra abdominal (and a potentially surgically treatable) inflammatory focus [5]. The frequency of SBP among hospitalized patients with advanced cirrhosis varies from 10% to 30% [3]. The mortality rate in patients with untreated SBP remains high (480%), but has declined to 30% - 40% as a result of early diagnosis and effective therapy using broad-spectrum antibiotics [6].

The prolonged use of antibiotic prophylaxis led to the development of antibiotic resistant bacteria. About two thirds of infections in untreated cirrhotic patients were found to be due to gram-negative organisms, whereas, infections in patients receiving quinolone prophylaxis were mostly due to gram-positive organisms [7]. Thus, antibiotic prophylaxis is necessary only in patients at the greatest risk of developing SBP. SBP may sometimes be asymptomatic or present with atypical symptoms; therefore, clinical assessment alone is inaccurate for the diagnosis or exclusion of SBP so routine ascitic fluid analysis is recommended [8].

AF analysis is considered necessary for all patients with ascites on hospital admission; cirrhotic patients with ascites who develop clinical signs of sepsis, hepatic encephalopathy, sudden or unexplained renal impairment; and/or all cirrhotic patients who develop (gastrointestinal) GI bleeding [3]. The American Association for the Study of Liver Disease recommends testing of AF for total leukocyte counts and PMN counts in patients undergoing serial outpatient therapeutic paracentesis, each time the fluid is aspirated; however, culturing is not necessary [9].

The presence of a positive AF culture is confirmatory but is not a prerequisite for initiation of antibiotic therapy. In fact, it is considered a “fatal” mistake to wait 48 h for culture results before initiating therapy when indicated. In general practice, an AF culture is negative in 40% of patients with SBP [10]. The low rates of positivity of bacterial culture in AF samples are possibly because of the relatively low concentrations of bacteria. The symptoms and mortality rates in patients with culture negative neutrocytic ascites (CNNA) are similar to those in patients with SBP. Further, 33% - 57% of patients with CNNA also showed a positive blood culture, providing evidence for the presence of a systemic bacterial infection [11].

The PMN leukocyte count of at least 250/mm^3 in AF, irrespective of the result from the AF culture, is universally accepted as the best surrogate marker for diagnosing SBP [12]. In most laboratories cells in AF are counted using manual techniques. This is time consuming and liable to a high error rate [13]. Hence, automated cell counting is ideal. Most researchers are apprehensive about using automated cell counters for AF counting because the manufacturers of this equipment do not recommend its use for cell counting in any fluid other than blood [14]. Another option is to use leukocyte esterase reagent strips for bedside diagnosis of SBP [15]. The leukocyte esterase reagent strip technique has been shown to be cheaper, faster (takes less than a minute), and reproducible. However, clinicians have not shown much enthusiasm in adopting this technique that needs further standardization [4]. Therefore, any alternative test that may accurately diagnose or, more importantly, exclude the diagnosis of SBP within a short time span is considered a success.

Platelets are considered an important source of prothrombotic agents associated with inflammatory markers and play a role in the initiation and propagation of vascular and inflammatory diseases [16].

Platelets with large size have many granules that can exert their hemostatic and proinflammatory actions with greater efficiency [17].

For these reasons, the mean platelet volume (MPV) and platelet distribution width (PDW) may be considered indicators of platelet function and activation [18].

MPV and PDW are routinely done as parts of complete blood count. An increase in MPV has been observed

in chronic viral hepatitis because of an increase in the entry of newly produced platelets into circulation, which is larger than the old platelets [19].

Macrophage inflammatory protein type 1 beta (MTP-1β) belongs to the family of chemokines, best known for their chemotactic and proinflammatory effects. MIP-1β is an acidic protein composed of 69 amino acids that is produced by many cells particularly macrophages, dendritic cells and lymphocytes. Synthesis of MIP-1β is stimulated with bacterial endotoxins. MIP-1β is responsible for the activation of PMN and is involved in acute neutrophilic inflammation. This protein is most effective at augmenting adhesion of CD_8(+) T-cells to the vascular cells adhesion molecule (VCA-M1). The diagnostic significance of MIP-1β for bacterial infections is poorly recognized [20].

The chemokines have significantly shorter half-lives than classical inflammatory biomarkers which theoretically make them more suitable for diagnostic and monitoring purposes. For this respect, the aim of this study was to investigate MIP-1β, MPV and PDW with other inflammatory markers as diagnostic markers for SBP.

2. Patients and Methods

Forty one cirrhotic patients with ascites were included in this study that was carried out in Tropical Medicine and Clinical Pathology Departments.

The diagnosis of liver cirrhosis and ascitis were based on clinical, biochemical and ultrasonographic findings. Patients were classified into 21 patients with ascitic fluid PMN count $\geq$ 250 cells/mm^3 (SBP) and 20 patients with ascitic fluid PMN count $\leq$ 250 cells/mm^3 non-SBP (control group). Bacteriological cultures were done using aerobic and anaerobic standard blood culture bottles containing brain-heart infusion broth, which were inoculated with 10 ml of ascitic fluid and incubated for 2 days to 7 days at 37˚C. 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.

All studied patients were subjected to medical history taking, clinical examination, routine laboratory investigation platelet number, MPV and platelet distribution width (PDW) were also done. Abdomino-pelvic ultrasonographic examination was done for all patients.

Venous blood samples were taken from all patients, serum was separated from the cellular fraction via centrifugation at 5000 cycle per minute for 3 minutes, Serum samples were kept at −80˚C.

Aspirated ascitic fluid samples were immediately examined for bacteriological cultures, identifications of microorganisms, cytological assay and chemical assay (Cobas 501, Roch Diagnostic). Ascitic fluid and serum macrophage inflammatory protein-1-Beta (MIP-1β) were measured by an enzyme-linked immunosorbent assay, employing an antibody specific for human MIP. 1β (the minimum detectable level of MIP. β is 2.5 pg/ml. The KIT used was produced by R & D company USA. Quantitative double antibody enzyme-linked immunosorbent assay (ELISA).

Serum CRP was determined using the latex agglutination test kit (Avitex CRP ref. ODO073/OD073/OD023/E, Omega diagnostic Ltd, AIVA Scotland (UK) [21].

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

3. Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba). Continuous quantitative data were checked for normality by using Shapiro-Wilk test. Quantitative variables were expressed as median (range) as they were non-normally distributed. Mann Whitney U test was used to compare between two groups of independent non-normally distributed data. The Spearman's rank correlation coefficient (r) was calculated to assess the relationship between various inflammatory markers. We consider (+) sign as indication for direct correlation *i.e.* increase frequency of independent lead to increase frequency of dependent & (−) sign as indication for inverse correlation *i.e.* increase frequency of independent lead to decrease frequency of dependent, also we consider values near to 1 as strong correlation & values near 0 as weak correlation. Receiver Operating Characteristic (ROC) curves were obtained to calculate the optimized cutoff point for inflammatory markers to reach the best compromise in the prediction of SBP. The cutoff point with maximum sensitivity and specificity (validity) is used as the recommended cutoff point and also Area Under Curve (AUC) was calculated. All tests were two

sided with P < 0.05 was considered statistically significant (S), P < 0.01 was considered highly statistically significant (HS), and P > 0.05 was considered non statistically significant (NS).

4. Results

Demographic and laboratory parameters of the studied groups are presented in (**Table 1**) most of patients had hepatitis C virus (HCV) infection which represent 75% of control group (Non SBP) and 84% of SBP group followed by bilharzias (15% in SBP and 10% in SBP) then mixed HCV and bilharzias' (10% in non SBP and 6% in SBP group).

Most of SBP patients showed a negative culture results (70%) while 30% showed positive results. *E. coli*, *Klebsiella*, *Staphylococcus and pseudomonas* (18%, 8%, 2% and 2% respectively). Culture results were negative in non SBP (100%).

Fever was the most common clinical presentation found in 15 cases (36.6%) followed by abdominal pain in 12 patients (29.2%), upper GIT bleeding 10 patients (24.4%), altered mental status in 14 cases (34.4%), while 4 cases (9.8%) were asymptomatic.

In addition there was a significant increase in MIP-1*β*, MPV, PDW, CRP, and WBCs in serum and ascitic fluid, MIP-1*β*, TLC, PMW and protein content in SBP group versus the non SBP group [169 (95 - 215) vs. 137 (63.1 - 184), 9.1 (7.9 - 18) vs. 7.9 (7.1 - 9.3), 16.6 (14 - 3) vs. 15.3 (14.17), 12.7 (2.93) vs. 5.2 (2.8 - 38), 10.6 (7.3 - 14) vs. 6.7 (8.2 - 13.5)] in serum respectively, 310.7 (6 - 497) vs. 48.4 (16.5 - 122) 720 (440 - 3690) vs. 240 (5 - 473), 370 (300 - 1900) vs. 117.5 (20 - 200)- 900 (280 - 2400) vs. 1800, (110 - 3200) in ascites respectively (**Table 2**).

Table 1. Demographic and laboratory parameter of the studied groups.

	SBP (N = 21)	Non-SBP (N = 20)	P
Age (years)	55.4 ± 7.2	52.8 ± 7.1	0.17
Sex (F/M)	9.12	6.14	0.05
Hemoglobin (gm/d)	9.56 ± 0.29	8.9 ± 0.50	0.8
WBCs (10^3/cmm)	10.6 ± 1.88	5.11 ± 1.5	0.001
Platlets (10^3/cmm)	104.52 ± 19.19	125.9 ± 26.73	0.001
Bilirubin mg/dl	1.93 ± 0.81	1.86 ± 0.44	0.79

MIP-1*β*: macrophage inflammatory protein-1 beta; MPV: Mean platelet volume; PDW: Platelet distribution width; WBC_s were significantly higher among SBP patients while platelet number was significantly lower among SBP patients when compared è non SBP.

Table 2. Comparison of MIP-1*β*, MPV and PDW with other inflammatory markers to patients with and without SBP.

Inflammatory marker In blood	SBP (n = 21)		Non-SBP (n = 20)		Z	P-value
In blood (serum)						
MIP-1*β* (pg/ml)	169	(95 - 215)	137	(63.1 - 184)	−1.930	0.054
MPV (fl)	9.1	(7.9 - 16)	7.9	(7.1 - 9.3)	−4.056	<0.001
PDW (%)	16.6	(14 - 33)	15.3	(14 - 17)	−2.870	0.004
WBC (×10^3/mm^3)	10.6	(7.3 - 14)	5.11	(8.2 - 13.5)	−0.717	0.001
CRP (mg/L)	12.7	(2 - 93)	5.2	(2.8 - 33)	−0.052	<0.001
LDH (IU/L)	490	(350 - 980)	320	(200 - 748)	−3.255	0.001
In ascitic fluid						
MIP-1*β* (pg/ml)	310.7	(6 - 497)	48.4	(16.5 - 122)	−4.173	<0.001
TLC (cell/cm)	720	(440 - 3690)	240	(50 - 473)	−5.453	<0.001
PMN (cell/cm)	370	(300 - 1900)	117.5	(20 - 200)	−5.481	<0.001
Protein (mg/dl)	900	(280 - 2400)	1800	(110 - 3200)	−2.977	0.003
LDH (IU/L)	290	(94 - 564)	99.5	(68 - 400)	−3.756	<0.001

Continuous variables were expressed as the median (range); Z: Mann Whitney U test; p < 0.05 is significant. Serum values of MIP-1*β*, MPV, PDW, CRP & LDH were significantly elevated among patients è sBP when compared è non SBP. In ascitic fluid MIP-1*β*, TLC, PMN, LTH were significantly elevated among patients è sBP when compared è non SBP while ascitic fluid protein level was significantly reduced among SBP pat.

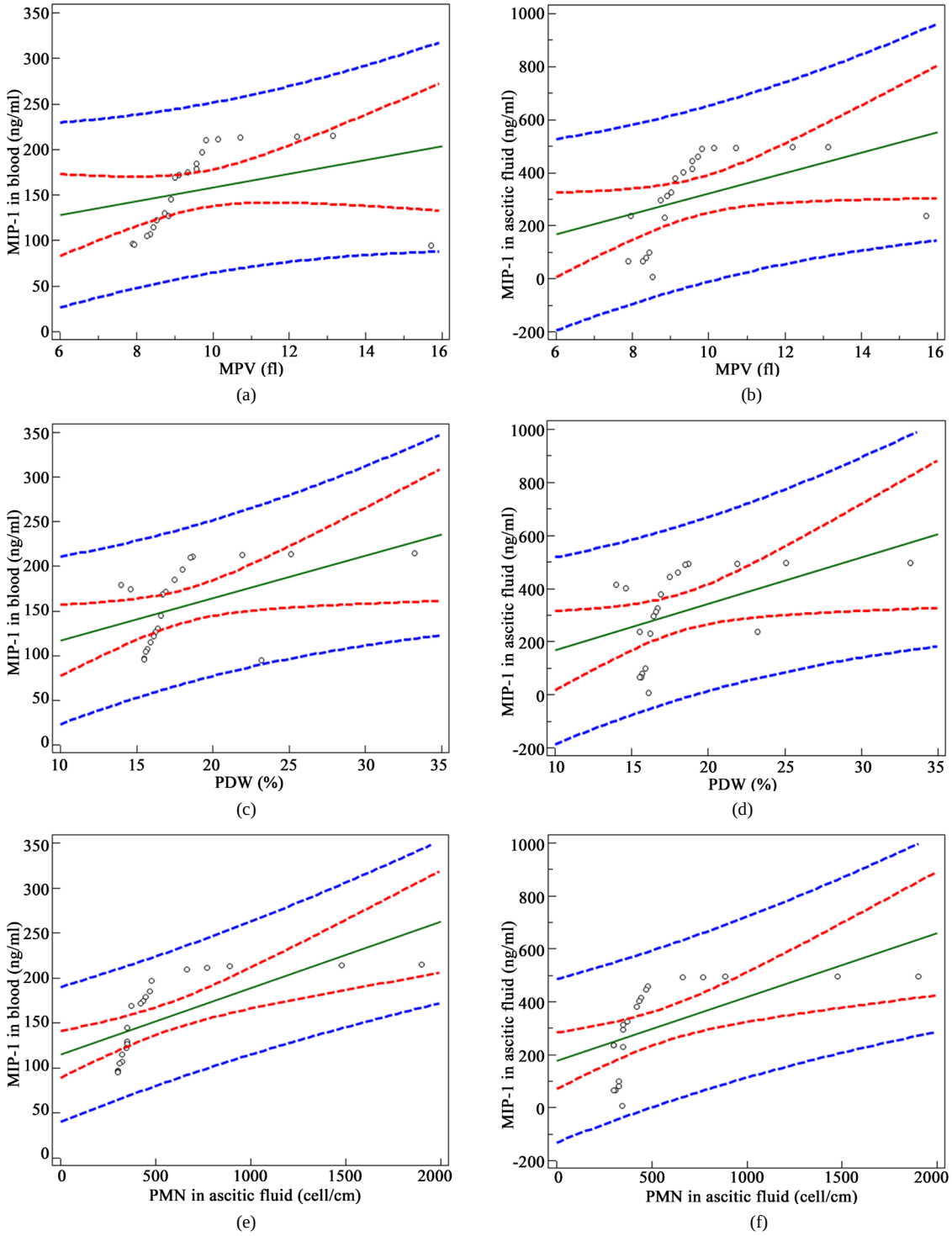

Figure 1. Scatter plot with regression line shows correleation between MIP-1 & various inflammatory marker in cirrhotic patients with SBP. Blue lines representing the 95% confidence interval (CI) & red lines representing the 95% prediction interval of regression line. (a) MPV (fl) & MIP-1 in blood (ng/ml), (Spearman's rank correleation coefficient r = +0.723, P < 0.001); (b) MPV (fl) & MIP-1 in ascitic fluid (ng/ml), (Spearman's rank correleation coefficient r = +0.843, P < 0.001); (c) PDW (%) & MIP-1 in blood (ng/ml), (Spearman's rank correleation coefficient r = +0.590, P = 0.005); (d) PDW(%) & MIP-1 in ascitic fluid (ng/ml), (Spearman's rank correleation coefficient r= +0.642, P < 0.001). (e) PMN in ascitic fluid (cell/cm) & MIP-1 in blood (ng/ml), (Spearman's rank correleation coefficient r = +0.997, P < 0.001); (f) PMN in ascitic fluid (cell/cm) & MIP-1 in ascitic fluid (ng/ml), (Spearman's rank correleation coefficient r = +0.928, P < 0.001).

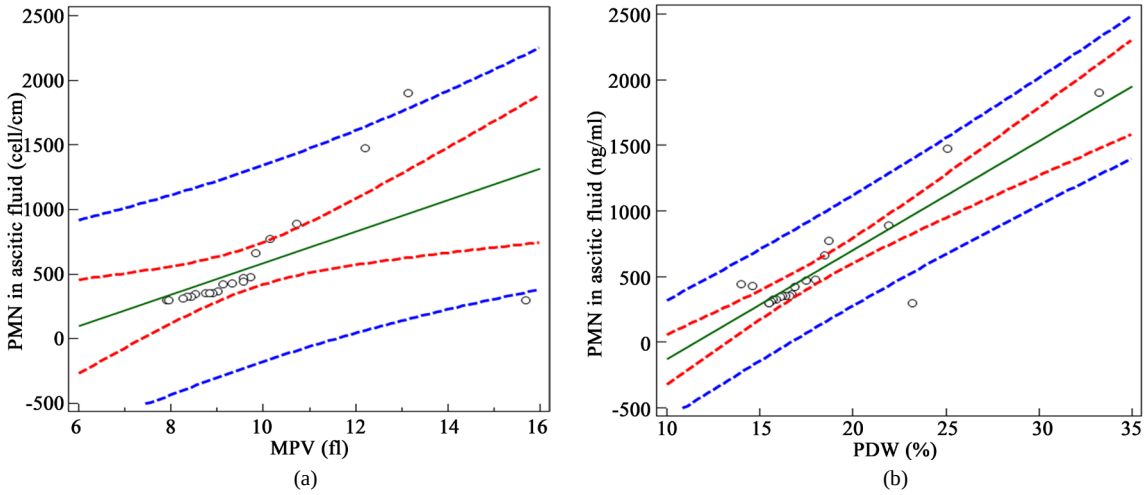

Figure 2. Scatter plot with regression line shows correlation between PMN in ascitic fluid & Platelet indices in cirrhotic patients with SBP. Blue lines representing the 95% confidence interval (CI) & red lines representing the 95% prediction interval of regression line. (a) PMN in ascitic fluid (cell/cm) & MPV (fl), (Spearman's rank correlation coefficient r = +0.750, P < 0.001); (b) PMN in ascitic fluid (cell/cm) & PDW (%), (Spearman's rank correlation coefficient r = +0.609, P < 0.001).

Table 3. Receiver operating characteristic curve analysis of platelet indices, MIP-1β in differentiates patients with and without SBP.

Cutoff value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR^+ (95% CI)	LR^- (95% CI)	Accuracy (95%CI)	AUC (95% CI)
Inflammatory markers in blood								
MIP-1 > 85.2 pg/ml	100% (83.9 - 100)	35% (15.4 - 59.2)	61.8% (43.6 - 77.8)	100% (54.1 - 100)	1.5 (0.8 - 2.8)	0 (0 - 0.9)	68.2% (50.3 - 80)	0.676‡ (0.512 - 0.814)
MPV > 8.3 fl	85.7% (63.7 - 97)	75% (50.9 - 91.3)	78.3% (56.3 - 92.5)	83.3% (58.6 - 96.4)	3.4 (2.5 - 4.7)	0.1 (0 - 0.7)	80.5% (57.4 - 94.2)	0.870§ (0.728 - 0.955)
PDW > 15.4%	90.4% (69.6 - 98.8)	55% (31.5 - 76.9)	67.9% (47.6 - 84.1)	84.6% (54.6 - 98.1)	2 (1.3 - 3.1)	0.1 (0 - 0.7)	73.1% (50.9 - 88.1)	0.762* (0.603 - 0.881)
Inflammatory markers in ascitic								
MIP-1β > 121.9 pg/ml	76.1% (52.8 - 91.8)	100% (83.2 - 100)	100% (79.4 - 100)	80% (59.3 - 93.2)	15.2 (11.8 - 19.8)	0.2 (0 - 2)	87.8% (67.7 - 95.8)	0.881Φ (0.742 - 0.961)
Protein > 1500 mg/dl	61.9% (38.4 - 81.9)	85% (62.1 - 96.8)	81.2% (54.4 - 96)	68% (46.5 - 85.1)	4.1 (2.8 - 6.1)	0.4 (0.1 - 1.5)	73.2% (50 - 89.2)	0.771Ψ (0.614 - 0.888)

‡P = 0.039; §P < 0.001; *P < 0.001; ΦP < 0.001; ΨP < 0.001. ROC curve: Receiver Operating Characteristic curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value; LR^+: positive Likelihood Ratio; LR^-: negative Likelihood Ratio; AUC: Area under Curve; 95% CI: 95% Confidence Interval; P < 0.05 is significant.

The expected normal ranges of MPV were 6.5-H.5fl, PDW 10 - 18, MIP-1β 2.5 pg/ml, CRP up to 5 mg/dl and WBC (4.11) × 10^3/Cmm [14].

Multiplication of MPV × PDW did not improve accuracy.

There was positive correlation between MIP-1β MPV, PDW and PMN in ascites (**Figure 1** and **Figure 2**).

Receiver operating characteristic curve (ROC) analysis macrophage inflammatory protein-1 beta, platelets indices: ROC curve for the sensitivity and specificity of MPV: Ata cutoff value of 8.3 fl, MPV had 85.7% sensitivity and 75% specificity for detecting SBP (AUC = 0.870 with negative predictive value (NPV) and positive predictive value (PPV) of 83.3% and 78.3%, respectively) (**Table 3**) and (**Figure 3**).

Roc curve for sensitivity and specificity of PDW: at a cutoff value of 15.4 fl, PDW had 90.4% sensitivity and 55% specificity of detecting SBP (AUC = 0.762 with NPV and PPV for 84.6% and 67.9 respectively in serum) (**Table 3**).

Roc curve for sensitivity and specificity of MIP-β: Ata cutoff value of 83.2 pg/ml, MIP-Iβ had 100% sensitivity and 35% specificity for detecting SBP (Auc = 0.676 with NPV and PPV of 100, 61.8% respectively) (**Table 3**).

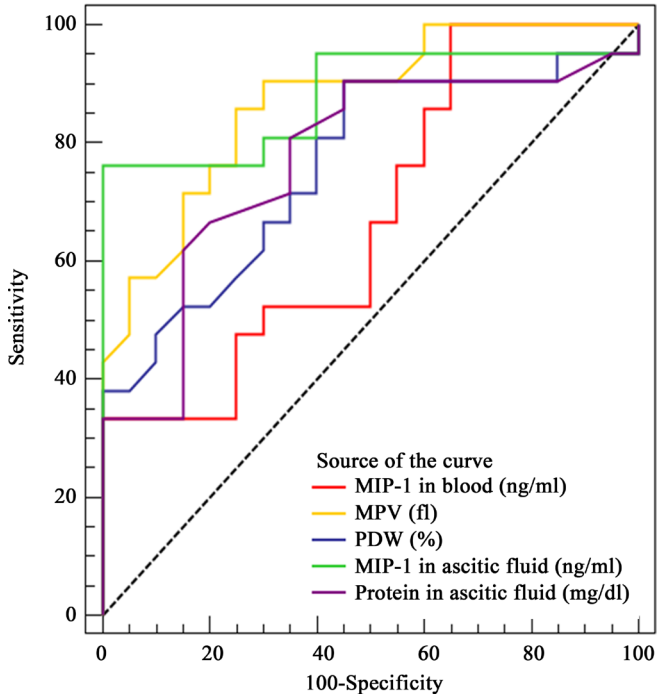

Figure 3. Receiver Operating Characteristic (ROC) curves of inflammatory markers in detecting SBP in cirrhotic patients.

Roc curve for sensitivity and specificity of MIP-1β in ascitic fluid: At a cutoff value of 121.9 pg/ml, MIP-1β had 76.1% sensitivity and 100% specificity for detecting SBP (Auc = 87.8% with NPV and PPV of 80%, 100% respectively) (**Table 3**).

5. Discussion

SBP is a major cause of morbidity and mortality in cirrhotic patients with ascities. The diagnosis of SBP needs to be rapid and accurate to avoid complications.

The present study assessed MPV, PDW and MIP-1β in cirrhotic patients with or without SBP.

In this study MPV was significantly increased in cirrhotic patients with SBP compared to cirrhotic patients without SBP ($P < 0.001$). ROC curve revealed that the specificity of MBV for detection of SBP was superior to PDW. This is in accordance with Suvak *et al.*, [22] who reported that MPV is increased in cirrhotic patients with SBP. Moreover a positive correlation between MPV and other systemic inflammatory markers supported that MPV cause a systemic inflammatory response in cirrhotic patients with SBP. Platelet activation is a link in the pathophysiology of diseases subjected to vascular injury and inflammation. The density of systemic inflammation can be considered a characteristic factor for classifying conditions associated with large-sized and small-sized circulating platelets. Larger platelets are enzymatically more active than small platelets, producing more thromboxane A_2 and inflammatory cytokines as well as an increased probability to thrombosis and inflammation [23]. In cirrhotic patients, variable platelets size, breakdown of platelets in spleen, and the increased IL6 levels due to inflammation in chronic liver disease is likely to be linked with an increase in platelet production in the bone marrow. So, the increased entry of platelets into the bloodstream will not only increase MPV but also lead to an increase of the PDW because of the presence of platelets of various sizes in the blood [24].

In the present study, PDW showed statistically significant higher values in cirrhotic patients with SBP than in those without infection ($P = 0.004$). PDW predict the presence of SPB at cut off value of 15.4 fl è specificity and sensitivity of 55% and 90.4% respectively. This may be because PDW is a measure of the variation of platelet width which can be an indicator of platelet activation, and may thus be related to inflammatory processes during the development of SBP however, this finding was not supported by Suvak *et al.*, [24] who reported no signifi-

cant changes in PDW in cirrhotic patients with SBP than without infection.

CRP, an acute-phase protein, was produced by the liver in response to infection. In this work, there was a significant increase in CRP in the SBP group versus non SBP group preto-Zamperli *et al.*, [25] and Yildirin *et al.*, [26] found that CRP was increased in the serum and ascitic fluid.

In this study MIP-1β showed significant increase in ascitic fluid ($P < 0.01$) and insignificant increase in the serum ($P < 0.54$) compared to non SBP. ROC curve analysis revealed that the optimal cut-off value for MIP-1β in ascites and serum were 121.9 pg/ml and 85.2 pg/ml respectively. The sensitivity of MIP-1β was 100%, specificity 35% in serum and sensitivity 76.1%/ml specificity 100% in ascitic fluid in patients with SBP.

These results indicate that peritoneal macrophage was the major source of MIP-1β release. These results are in concord è Magdalena *et al.*, [20] and against Holub [27] who showed that the serum levels of MIP-1β are elevated in community-acquired bacterial infections. There was positive correlation between MIP-1β and MPV ($r = 0.725$, $P < 0.01$, PDW ($r = 0.590$, $P < 0.005$), WBC ($r = 0.907$, $P < 0.001$) in serum.

The main limitation of the current study was the small number of the included patients.

6. Conclusions

MIP-1β concentration in ascitic fluid could be good diagnostic marker in patients with SBP with 76.1% sensitivity and 100% specificity. Moreover, MPV & PDW were significantly elevated in cirrhotic patients with SBP.

Adding MPV, PDW, MIP-1β to the work panel of suspected SBP may help early diagnosis of asymptomatic cases and avoid the delay associated with using method culture.

Acknowledgements

The authors would like to express their sincere gratitude to the patients and staff in the Tropical Medicine Department, and to the laboratory technicians, for their great endeavor.

Conflict of Interest

No conflict of interest was declared by the authors.

References

[1] Thomas, V. (2011) Routine Analysis of Cirrhotic Ascites for Evidence of Infection—Not Worth the Effort. *Indian Journal of Gastroenterology*, **30**, 201-203. http://dx.doi.org/10.1007/s12664-011-0136-2

[2] Fernández, J., Navasa, M., Gomez, J., Colmenero, J., Vila, J. and Arroyo, V. (2002) Bacterial Infection in Cirrhotic: Epidemiological Changes with Invasive Procedures and Norfloxacin Prophylaxis. *Hepatology*, **35**, 40-48.

[3] Rimola, A., García-Tsao, G., Navasa, M., Piddock, L.J., Planas, R. and Bernard, B. (2000) Diagnosis, Treatment and Prophylaxis of Spontaneous Bacterial Peritonitis: A Consensus Document. International Ascites Club. *Journal of Hepatology*, **32**, 142-153. http://dx.doi.org/10.1016/S0168-8278(00)80201-9

[4] Balagopal, S.K., Sainu, A. and Thomas, V. (2010) Evaluation of Leucocyte Esterase Reagent Strip Test for the Rapid Bedside Diagnosis of Spontaneous Bacterial Peritonitis. *Indian Journal of Gastroenterology*, **29**, 74-77. http://dx.doi.org/10.1007/s12664-010-0017-0

[5] Cirera, I., Bauer, T.M., Navasa, M., Vila, J., Grande, L., Taurá, P., *et al.* (2001) Bacterial Translocation of Enteric Organisms in Patients with Cirrhosis. *Journal of Hepatology*, **34**, 32-37. http://dx.doi.org/10.1016/S0168-8278(00)00013-1

[6] Koulaouzidis, A. (2011) Diagnosis of Spontaneous Bacterial Peritonitis: An Update on Leucocyte Esterase Reagent Strips. *World Journal of Gastroenterology*, **17**, 1091-1094. http://dx.doi.org/10.3748/wjg.v17.i9.1091

[7] Yang, Y.Y. and Lin, H.C. (2005) Bacterial Infections in Patients with Cirrhosis. *Journal of the Chinese Medical Association*, **68**, 447-451. http://dx.doi.org/10.1016/S1726-4901(09)70072-3

[8] Chinnok, B., Afarian, H., Minnigan, H., Butler, J. and Henday, G.W. (2008) Physician Clinical Impression Does Not Rule Out Spontaneous Bacterial Peritonitis in Patients Undergoing Emergency Department Paracentesis. *Annals of Emergency Medicine*, **52**, 268-273. http://dx.doi.org/10.1016/j.annemergmed.2008.02.016

[9] Runyon, B.A. (2004) Management of Adult Patients with Ascites Caused by Cirrhosis. *Hepatology*, **39**, 841-856. http://dx.doi.org/10.1002/hep.20066

[10] Such, J., Frances, R., Munoz, C., Zapater, P., Casellas, J.A. and Cifuentes, A., *et al.* (2002) Detection and Identification of Bacterial DNA in Patients with Cirrhosis and Culture-Negative None-Neutrocytic Ascites. *Hepatology*, **36**, 135-141. http://dx.doi.org/10.1053/jhep.2002.33715

[11] Lata, J., Stiburek, O. and Kopacova, M. (2009) Spontaneous Bacterial Peritonitis: A Severe Complication of Liver Cirrhosis. *World Journal of Gastroenterology*, **15**, 5505-5510. http://dx.doi.org/10.3748/wjg.15.5505

[12] Wong, C.L., Holroyd-Leduc, J., Thorpe, K.E. and Straus, S.E. (2008) Does This Patient Have Bacterial Peritonitis or Portal Hypertension? How Do I Perform a Paracentesis and Analyze the Results? *JAMA*, **299**, 1166-1178. http://dx.doi.org/10.1001/jama.299.10.1166

[13] Runyon, B.A. (2003) The Evolution of Ascitic Fluid Analysis in the Diagnosis of Spontaneous Bacterial Peritonitis. *The American Journal of Gastroenterology*, **98**, 1675-1677. http://dx.doi.org/10.1111/j.1572-0241.2003.07600.x

[14] Riggio, O., Angeloni, S., Parente, A., Leboffe, C., Pinto, G., Aronne, T. and Merli, M. (2008) Accuracy of the Automated Cell Counters for Management of Spontaneous Bacterial Peritonitis. *World Journal of Gastroenterology*, **14**, 5689-5694. http://dx.doi.org/10.3748/wjg.14.5689

[15] Xia, H.H. (2009) Spontaneous Bacterial Peritonitis. *World Journal of Gastroenterology*, **15**, 1042-1049. http://dx.doi.org/10.3748/wjg.15.1042

[16] Kilcier, G., Gene, H., Tapan, S., Ors, F. and Kara, M., *et al.* (2010) Mean Platelet Volume and Its Relationship with Carotid Atherosclerosis in Subjects with Non-Alcoholic Fatty Liver Diseases. *Upsala Journal of Medical Sciences*, **115**, 253-259.

[17] Thompson, C.B., Jakubowski, J.A., Quinn, P.G., Deulin, D. and Valeni, C.R. (1984) Platelet Size and Age Determine Platelet Function Independently. *Blood*, **63**, 1372-1375.

[18] Beyozi, Y., Sayiler, A., Torun, S., Suvak, B., Yesil, Y., Pumak, T., *et al.* (2011) Mean Platelet Volume as an Indicator of Disease Severity in Patients with Acute Pancreatitis. *Clinics and Research in Hepatology and Gastroenterology*, **36**, 162-168.

[19] Turhan, O., Coban, E., Inan, D. and Yalcin, A.N. (2010) Increased Mean Platelet Volume in Chronic Hepatitis B Patients with Inactive Disease. *Medical Science Monitor*: *International Medical Journal of Experimental and Clinical Research*, **16**, CR202-CR205.

[20] Lesiriska, M., Hartleb, M., Gutkowski, K. and Nowakowska-Dulawa, E. (2014) Procalcitonin and Maerophage Inflammatory Protein-1 Beta (MIP-1β) in Serum and Peritoneal Fluid of Patients with Decompensated Cirrhosis and Spontaneous Bacterial Peritonitis. *Advances in Medical Sciences*, **59**, 52-56. http://dx.doi.org/10.1016/j.advms.2013.07.006

[21] Pepys, M.B. and Hipschfield, G.M. (2003) C-Reactive Protein: A Clinical Update. *The Journal of Clinical Investigation*, **111**, 1805-1812. http://dx.doi.org/10.1172/JCI200318921

[22] Suvak, B., Torun, S., Yidz, H., Saylir, A., Yesil, Y., Ias, A., *et al.* (2013) Mean Platelet Volume Is a Useful Indicator of Systemic Inflammation in Cirrhotic Patients with Ascitic Fluid Infection. *Annals of Hepatology*, **12**, 294-300.

[23] Van Der Loo, B. and Martin, J.F. (1999) A Role for Changes in Platelet Production in the Cause of Acute Coronary Syndromes. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **19**, 672-679.

[24] Kaser, A., Brandacher, G., Steuver, W., Kaser, S., Offner, F.A., Zoller, H., *et al.* (2001) Interleukin-6 Stimulates Thrombopoiesis through Thrombopoietin: Role in Inflammatory Thrombocytosis. *Blood*, **98**, 2720-2755. http://dx.doi.org/10.1182/blood.V98.9.2720

[25] Preto-Zamperini, M., Farhat, S.C., Perondi, M.B., Pestana, A.P., Cunha, P.S. and Pugliese, R.P. (2014) Elevated C-Reactive Protein and Spentanous Bacterial Peritonitis in Children with Chronic Liver Disease and Ascites. *Journal of Pediatric Gastroenterology & Nutrition*, **58**, 96-98. http://dx.doi.org/10.1097/MPG.0000000000000177

[26] Yildirim, B., Sari, R. and Isci, N. (2005) Patients with Spontaneous Bacterial Peritonitis, and Malignant and Cirrhotic Ascites. *Journal of the National Medical Association*, **97**, 276-280.

[27] Holub, M., Lawrence, D.A., Andersen, N., Davidoa, A., Beran, O., Maresova, V., *et al.* (2013) Cytokines and Chemokines as Biomarkers of Community-Acquired Bacterial Infection. *Mediators of Inflammation*, **2013**, Article ID: 190145.