Evaluation of Various Non Culture Methods for the Diagnosis of Spontaneous Bacterial Peritonitis

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ABSTRACT

Introduction: Spontaneous Bacterial Peritonitis (SBP) is common and serious complication of patients with liver cirrhosis and ascites, without an apparent surgically treatable intra abdominal source of infection. Its prevalence ranges from 10% to 30%. Mortality rate was earlier reported more than 90%, but it has now reduced to 30% -50% as a result of rapid diagnosis and prompt initiation of antibiotics. The present study was done to evaluate the various non culture methods for the diagnosis of SBP.

Material and Methods: Ascitic fluid sample were collected aseptically from 100 cirrhotic patients with ascites. PMN (polymorphonuclear leukocyte) count was determined by Neubauer's manual counting chamber and Leishman's stain for differential PMN cell counts. Granulocyte esterase activity was detected using LER (Leukocyte esterase reagent) dipstick strips.

Results: Out of 100 samples processed, PMN cell count $\geq$ 250 cells/mm^3 was found in 91% samples by conventional light microscopy. Scale of $\geq$ 2+ by LER strip was found in 61 samples. Reading of PMN cell count of $\geq$ 250 cells/mm^3 matched in 60 samples and < 250 cells/mm^3 matched in 8 cells by both microscopy and LER strip test. Sensitivity, specificity, positive predictive value and negative predictive value of LER strip test was 65.9%, 88.89%, 98.36% and 20.51% respectively.

Conclusion: LER strips as a screening tool for SBP have advantage of speed, low cost, availability at odd hours, requires no technical expertise and can be performed everywhere. Its high specificity and PPV may help in early institution of empirical antibiotic therapy in patients.

Keywords: SBP; Cirrhotic Patients with Ascites; LER Dipstick Strips; Conventional Light Microscopy; Rapid Diagnosis.

INTRODUCTION

SBP is an infection of previously sterile ascitic fluid without an apparent intra-abdominal source of infection, which can be surgically treated. It often develops insidiously and may remain unrecognized. Severe underlying liver disease is usually a progenitor to SBP development. It is a common complication in patients with cirrhosis and ascites.[1-3,9]

Even though the mortality rate was initially reported to exceed 90%, the prognosis has improved with early diagnosis and prompt treatment.[4] Paracentesis is extremely important, as the PMN count in the ascitic fluid plays a vital role in obtaining a diagnosis of SBP.[5] Even though all cirrhotic patients with ascites are at risk of SBP, the prevalence of SBP in hospitalized patients (10%) is higher than that observed in outpatients (1.5–3.5%). That is why it is recommended that diagnostic paracentesis should be performed in all cirrhotic patients with ascites who require hospital admission, even if they don't exhibit clinical symptom(s) of SBP.[5-8] Various non culture methods for SBP diagnosis include:-[3,6,9]

1. PMN count

Diagnosis of SBP is usually confirmed by PMN count in the ascitic fluid. PMN count in the range of 250-500 cells/mm^3 is considered a valid marker for SBP.[3,6]

The cut off value of 250 PMN cells/mm^3 has the greatest sensitivity, whereas 500 PMN cells/mm^3 exhibits the greatest specificity. However the most sensitive cut off value should be used for diagnosis as it is important not to miss any case of SBP.[4] A diagnosis of SBP made on the basis of symptoms and signs alone, is no longer acceptable.

2. LER strips

The use of LER dipsticks has been proposed as a fast, simple and inexpensive method for diagnosing SBP.[3,6,9] Ascitic fluid cell count is prone to human errors, needs laboratory set up and technical expertise. Whole process takes several hours (mean 2.7 hrs., range 105-180 minutes). Therefore, the use of LER strips has been proposed as a fast, simple and inexpensive method for diagnosing SBP.[1,3,6]

In this test, esterase activity of PMN in fluid reacts with an esterified chemical compound in the reagent strip releasing 3-hydroxy-5-phenyl-pyrrole, which changes the colour of an azo dye in the reagent strip. This colour is read against a standard colour chart provided with the reagent strip.[10,11]

Most of the above strips were developed for use in urine to diagnose cases of urinary tract infections with a threshold of >50 PMN cells/mm^3. A systemic review of multiple studies of such strips (including Multistix, Aution, Combur, Nephur and UriScan) has demonstrated that these strips have low sensitivity, low level of diagnostic accuracy with a high

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rate of false negative results. These strips have consistently shown high negative predictive value (95%) in the majority of studies and may therefore be used as a preliminary screening tool to diagnose SBP. Recent diagnostic performance of a reagent strip test calibrated for ascitic fluid with a cut-off of 250 PMN cells/mm^3 has reported excellent results, with both sensitivity and negative predictive value of 100%.[12]

Vanbiervliet et al[13] published the first study on the use of LER strip test in ascitic fluid. They studied 72 consecutive patients with liver cirrhosis using Multistix 8 SG strips, the most frequently used urinary reagent strips in France. They found the sensitivity and specificity of this test for the diagnosis of SBP to be 100% each. Since then several studies from France, Italy and United States have confirmed these results.[10,13-18] The overall sensitivity, specificity, positive predictive value and negative predictive value of this test is approaching 90%.

However, the different commercial dipsticks used in previous studies had different calorimetric scales for PMN cell count. In addition, the cut off colorimetric scale for each dipstick has not been standardized yet.[10,11] The present study was done to evaluate the various non culture methods for the diagnosis of SBP.

MATERIAL AND METHODS

The present study was conducted in the Departments of Microbiology and Medicine, Pt. B.D. Sharma, PGIMS, Rohtak over a period of one year (2015-16). A total of 100 ascitic fluid samples collected aseptically with a needle and syringe from cirrhotic patients with ascites were included in the study.

Processing of samples

1. Ascitic fluid PMN cell count

Ascitic fluid PMN cell count was determined manually according to hematological method with a conventional light microscope and Neubauer's manual counting chamber. White blood cell (WBC) counting was done in all the four peripheral squares (one mm^2 area in each) of Neubauer's chamber.

Number of WBCs in four mm^2 X 0.1 mm depth i.e. 0.4µl = n

So, number of WBCs in one µl = n/0.4 or n X 2.5

If the sample was haemorrhagic, two percent glacial acetic acid was utilized for lysis.

For differential PMN cell counts, after counting the total number of WBCs, the ascitic fluid sample was centrifuged and then stained with Leishman stain. Cell morphology was then determined using a light microscope.[3,6,9,19]

2. LER strips

Immediately after paracentesis, five ml of fresh ascitic fluid specimen collected in sterile test tube was tested using a dipstick. LER strips were procured from Combur[10] Test® Miditron®, Roche Diagnostics GmbH, Mannheim, Germany for granulocyte esterase activity detection.

The test strip was briefly (about one second) dipped into fresh ascitic fluid specimen collected in sterile test tube making sure that all the test areas were moistened. When withdrawing the test strip, the edges of the dipstick were wiped against the rim of the vessel to remove excess ascitic fluid. The strip has a colorimetric four grade scale (negative, 1+ to 3+). A correlation between PMN cell count and the four grade scale was suggested by the manufacturer as follows (Table 1)

The limitation of the Combur[10] Test® M is an absence of precise calorimetric scale for the cut off level of PMN cell count at ≥ 250/mm^3. We chose the lower scales of 2+ as cut off values for SBP diagnosis so as to not miss cases of SBP even at cost of some false positives.

Test was read at 60-120 seconds for leukocyte test area against the colour chart provided by the manufacturer. Throughout the process manufacturer's instructions were strictly adhered to.

Any colour changes appearing only along the edges of the test areas or developing after more than two minutes was considered diagnostically insignificant.

RESULTS

All samples were tested for PMN count by LER strips. Scale of ≥ 2+ was taken as cut-off for PMN cell count ≥ 250 cells/mm^3 (Table 2). Ascitic fluid PMN cell count ≥250 cells/mm^3 was found in 91% cases while 9% cases had count <250 cells/mm^3 (Table 3). PMN cell count of ≥ 250 cells/mm^3 by using conventional light microscope was seen in 91% samples. Scale of ≥ 2+ was taken as cut-off for PMN cell count ≥ 250 cells/ mm^3.Only 60 samples (48+12) gave ≥2+ reading with LER strips which coincided with PMN cell count of ≥250

Correlation of manufacturer assigned Colorimetric scales with leucocyte range (Leu/ µl)	
Scale	**Leucocyte range (Leu/ µl)**
0	Negative
1+	~10-25
2+	~75
3+	~500

Table-1:

Correlation of manufacturer assigned Colorimetric scales with leucocyte range (Leu/ µl)		Total results from LER strip test
Scale	**leucocyte range (Leu/ µl)**	
0	Negative	13
1+	~10-25	26
2+	~75	49
3+	~500	12
Total		100

Table-2: Results of LER strip test

Ascitic fluid count(cells/mm^3)	No.	%
≥250	91	91
<250	9	9
Total	100	100

Table-3: Results of conventional microscopic examination of ascitic fluid samples for PMN cell count

Manufacturer assigned Colorimetric scales with leucocyte range (Leu/ µl)		Results of LER strip test comparative to PMN cell count by microscopy of		Total results from LER strip test
		≥ 250 cells/mm³	< 250 cells/mm³	
0	Negative	11	2	13
1+	~10-25	20	6	26
2+	~75	48	1	49
3+	~500	12	0	12
Total		91	9	100

Table-4: Results of LER strip test compared to gold standard of cell count by microscopy

Variables	%
Sensitivity	65.9
Specificity	88.89
Positive predictive value	98.36
Negative predictive value	20.51
Accuracy	68

Table-5: Performance characteristics of Combur[10] test® LER strip, using ≥ grade 2+ colour change.

cells/ mm³ obtained in 91 samples by using gold standard of conventional light microscopy. Eight samples gave < 2+ reading with LER strips which coincided with PMN cell count of < 250 cells/ mm³ obtained in 9 samples by using gold standard of conventional light microscopy (Table-4). Validity score of the Combur[10] Test® LER strip, considering ≥ grade 2+ colour change in the 4-grade colorimetric scale gave specificity of 88.89% and positive predictive value of 98.36% (Table 5).

DISCUSSION

Present study was conducted on 100 ascitic fluid samples collected from SBP patients. All the isolates were subjected to PMN count estimation by LER strip test. Count of ≥2 colorimetric sale was seen in 61 ascitic fluid samples while 39 samples revealed count of <2 colorimetric scale which corresponded to <250 PMN cells/ mm³ as per the cut-off assigned.

Results of conventional microscopic examination of ascitic fluid samples which is considered to be a gold standard for estimation of ascitic fluid PMN cell count, revealed PMN cell count of ≥250 cells/mm³ in 91% cases while only 9% cases had count of <250 cells/mm³.

In this study, by considering ≥ grade 2+ color change in the 4-grade colorimetric scale of Combur[10] Test® leucocyte esterase reagent strip, our study gave sensitivity of 65.9%, specificity of 88.89% and positive predictive value of 98.36%. Rerknimitr et al[11] has reported a similar sensitivity, specificity and positive predictive value for Combur[10] Test® leucocyte esterase reagent strips of 63%, 96% and 82% respectively.

Aution stick (A. Menarini Diagnostics, Firenze, Italy) is another dipstick that can be read at 90 s. The benefit of the Aution stick over the Combur[10] Test® M is the precise colorimetric scale that correlates with ≥ 250 PMN cells/mm³. The correlation between PMN cell count and colorimetric scale suggested by the manufacturer are as follow: grade 0, 0 PMN cell/ mm³; grade 1, 25 PMN cells/mm³; grade 2, 75 PMN cells/ mm³; grade 3, 250 PMN cells/mm³; and grade 4, 500 PMN cells/mm³.

Castellote et al[20] studied the use of Aution sticks for diagnosis of SBP in cirrhotic patients with ascites who underwent abdominal paracentesis procedure at a university based hospital, and found that the sensitivity, specificity and PPV are 89%, 99% and 98%, respectively.

Multistix®10SG (Bayer Diagnostics Corporation, Puteaux, France) has colorimetric scales as follow: grade 0, 0 PMN cell/ mm³; grade 1, 15 PMN cells/mm³; grade 2, 70 PMN cells/ mm³; grade 3, 125 PMN cells/ mm³; and grade 4, 500 PMN cells/mm³. Butani et al[21] used the Multistix®10SG to diagnose SBP in 136 specimens by using grade 2 as a cut off scale, and found the sensitivity, specificity, PPV and NPV of the Multistix®10SG are 83%, 99%, 91% and 98% respectively. Although, the Multistix®10SG has no precise colorimetric scale for 250 PMN cells/ mm³, its specificity is still better than that of the Combur[10] Test® M. This may be due to a closer 250 PMN/mm³ colorimetric scale of Multistix® 10SG (grade 3+ ≥ 125 PMN cells/mm³).

Limitation

The limitation of the Combur[10] Test® M is an absence of precise calorimetric scale for the cut off level of PMN cell count at ≥ 250/mm³.

CONCLUSION

LER strips have advantages of speed, low cost, availability at odd hours, requires no technical expertise and can be performed everywhere even in remote rural set ups. The present study recommends to use Grade ≥ 2 color on LER strip as cut off. Moreover, its high specificity and PPV may help in early initiation of empirical antibiotic therapy in patients. Thus this test can be used as a screening tool for SBP. Further, this may also help to determine the effectiveness of antibiotic therapy in patients with SBP by repeating the test.

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