

Accuracy of Leukocyte Esterase Reagent Strip (LERS) test for rapid bedside screening of spontaneous bacterial peritonitis: An observational study

Harshad Khairnar, Meghraj Ingle, Vikas Pandey, Kailash Kolhe, Shamsheer Chauhan, Prabha Sawant, Swapnil Walke, Vipul Chaudhary

Department of Gastroenterology, LTMGH and LTMMC, Sion, Mumbai, Maharashtra, India

ABSTRACT

Introduction: Spontaneous bacterial peritonitis (SBP) is a frequent and severe complication in cirrhotic patients with ascites. SBP is generally diagnosed based on an increased number of polymorphonuclear neutrophils in the ascitic fluid ($>250/\text{mm}^3$) and positive culture. Usually fluid analysis and culture take time and precious hours are lost in starting therapy. Leukocyte Esterase Reagent Strips (LERS) have consistently given a high negative predictive value ($>95\%$ in the majority of the studies). **Aims and Objectives:** Aim was to evaluate the diagnostic utility of leukocyte esterase reagent strip for rapid diagnosis of SBP in patients who underwent abdominal paracentesis and to calculate the sensitivity, specificity, positive, and negative predictive values. **Methodology:** The study was carried out on 64 patients with ascites. Cell count of AF as determined by colorimetric scale of Multistix 10 SG reagent strip was compared with counting chamber method (PMNL count ≥ 250 cells/ mm^3 was considered positive). **Results:** Of the 64 patients SBP was diagnosed in 17 patients, 47 patients were negative for SBP by manual cell count. At cut off of 2+; sensitivity to diagnose SBP was 100%; specificity of 94%; PPV being 57% and NPV of 94%. at the cut off level of 3+; sensitivity decreased down to 76%; specificity increased to 100%; PPV of 100% and NPV of 93.75%. Overall accuracy at 2 + and 3 + was respectively 94.5% and 93.75%. **Conclusion:** In this study we have found good sensitivity and specificity for the prompt detection of elevated polymorphonuclear neutrophil count. A negative test result excludes SBP with a high degree of certainty. Thus, it represents a convenient, inexpensive, simple bedside screening tool for SBP diagnosis.

Keywords: Cnna-Culture Negative Neurocytic Ascites, Npv-Negative Predictive Value, Ppv-Positive Predictive Value

Introduction

Spontaneous Bacterial Peritonitis (SBP) is a potentially life-threatening complication of liver cirrhosis with ascites. The prevalence of SBP in outpatients is 1.5–3.5% and ~10% in hospitalized patients.^[1] The in-hospital mortality is also very high (30–50%).

The diagnosis of SBP is made if there is positive ascitic fluid bacterial culture and elevated absolute polymorphonuclear neutrophil count equal to or greater than 250 cells/ μl .^[2] Improvement in survival is shown with rapid screening, diagnosis and treatment, thus avoiding the occurrence of complications like sepsis and septic shock.^[3] However, fluid analysis and culture take time and precious hours are lost in starting therapy.

Address for correspondence: Dr. Meghraj Ingle, Department of Gastroenterology Room No 13, College Building, 1st Floor, Sion Hospital, Mumbai, Maharashtra - 400022, India. E-mail: drmeghraj@gmail.com

The use of reagent strips has been tested for the diagnosis of bacterial meningitis, pleural infection, synovial infection and peritoneal infection.^{7,8,9} The leukocyte esterase present in the

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biologic fluid reacts with a chemical compound of the reagent strip causing a color change in the azo-dye (purple). For screening of SBP, use of a reagent strip may be a useful approach. In various studies it has shown high sensitivity, between 85% and 100%, and high specificity between 98% and 100%.^[4,5] In a study by Ashish Jha *et al.* the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of reagent strip \geq ++ positive were 77.77%, 95.12%, 77.77%, 95.12%, and 92%, respectively compared to counting chamber method.⁴ Till date there is no uniform data of using LERS strips in screening of SBP.^[6]

The aim of this study was to assess the utility of reagent strips for the rapid screening of SBP in cirrhotic patients with ascites and timely institution of antibiotics.

Methodology

It was a prospective observational study, conducted at the department of gastroenterology, Lokmanya Tilak Medical College & General Hospital, Sion, Mumbai from March 2017 to Sept 2017. Out of 84, 64 patients met inclusion criteria [Figure 1]. Written informed consent was taken from patients and study was approved by institutional ethics committee. Newly diagnosed patients of liver cirrhosis with ascites were enrolled in the study. A diagnostic paracentesis was performed under strict aseptic precautions. Ascitic fluid was sent for routine microscopy and biochemistry and at the same time tested using Multistix (manufactured by Siemens Ltd India) 10 SG leukocyte esterase strips and culture. Multistix 10 SG reagent strips are firm plastic strips to which are affixed several separate reagent areas. These strips provide tests for glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, and leukocytes. The entire reagent strip is disposable. ⁴ The strips were read visually. Reagent areas were compared to corresponding color chart on the bottle label at the time specified. Strips were held close to color blocks and matched carefully. Accurate timing is essential to provide optimal results which was at 2 minutes for leukocytes. Color changes that occur after 2 minutes were of no diagnostic value. ⁴

Leukocytes in the ascitic fluid were correlated using colorimetric grading scale. These grades are as follow: grade 0 trace, grade +:70 leukocytes/cmm, grade ++:125 cells/cmm, grade +++:500 cells/cmm. ⁴ Two different cut offs grade++ or grade +++ were tested. The principle is based on the esterase activity of the leukocytes. A pyrrole esterified with an amino acid is used as the substrate; hydrolysis of the ester releases the pyrrole which in turn reacts with diazonium salt yielding a violet or purple azo dye in relevant pad of the strip.^[6] This test has been used for the diagnosis of urinary tract infections, infection in other body fluids,^[7] meningitis,^[8] and peritonitis in patients on peritoneal dialysis.^[9] For ascitic fluid culture, 5–10 ml of fluid inoculated into a pair of 50 ml culture bottles bedside. SBP was defined as positive ascitic fluid culture for a single organism and ascitic fluid PMN count more than 250/cmm with no evidence of an

intra-abdominal surgically treatable source of infection. Culture negative neutrocytic ascites (CNNA) was diagnosed when the ascitic fluid culture did not grow any bacteria but PMN count is 250/cmm or greater.¹¹ SBP and CNNA was treated empirically initially using IV cefotaxime and later according to culture sensitivity patterns.¹²

Inclusion criteria

Patients of liver cirrhosis with new onset ascites, who were hospitalized included in this study.

Exclusion criteria

1. Patient with ascites secondary to other etiologies (i.e., non-cirrhotic cause of ascites),
2. cirrhosis with ascites of tubercular etiology,
3. those who have received antibiotics within last 2 week and
4. on antibiotic prophylaxis for SBP or patients with prior history of SBP were excluded from the study.

Statistical analysis

84 patients were assessed initially of which 64 became eligible for study. The demographics were analyzed in frequencies, percentages, median (range), or mean (standard deviation) as appropriate. Sensitivity was defined as the proportion of patients with a positive reagent strip divided by the patients with SBP. Specificity was defined as the proportion of patients with a negative reagent strip divided by the total of patients without SBP. Positive predictive value (PPV) was defined as the proportion of patients with a true-positive reagent strip divided by the total patients with a positive reagent strip. Negative predictive value (NPV) was defined as the proportion of true-negative reagent strips divided by all paracentesis with a negative reagent strip. Accuracy was verified by dividing the sum of true-positives and true-negatives by the total number of paracentesis evaluated.

Results

Out of the 84 patients, we analyzed 64 patients—the demographic profile of these patients is shown in Table 1. Of the 20 patients who were excluded from study, 11 patients were already on antibiotics, 5 patients were on SBP prophylaxis, 3 had mixed ascites secondary to tuberculosis, 1 had malignant ascites secondary to metastatic ovarian cancer. Mean age of patients was 45 (\pm 12) years and 45 (70%) were men. Alcohol was the most common cause of cirrhosis (46.8%) followed by Hepatitis B (17.18%). 40 patients (62.5%) had CTP B (full form of CTP) cirrhosis, 24 (37.5%) had CTP C, while none of the patients were CTP A. Median of MELD was 17 with lowest of 8 and highest of 33. Median of CTP was 9 with lowest of 7 and highest of 13. Ascitic fluid analysis of 44 patients with LERS showed 0 or 1 + results. Comparing with ascitic fluid PMN count none of these showed SBP. Whereas when LERS grade was 2 ++; out of 7, 4 patients had culture negative neutrocytic ascites (CNNA) and 3 were without SBP. While in the 3 + range; 9 out of 13 had CNNA while 4 had SBP [Table 2].

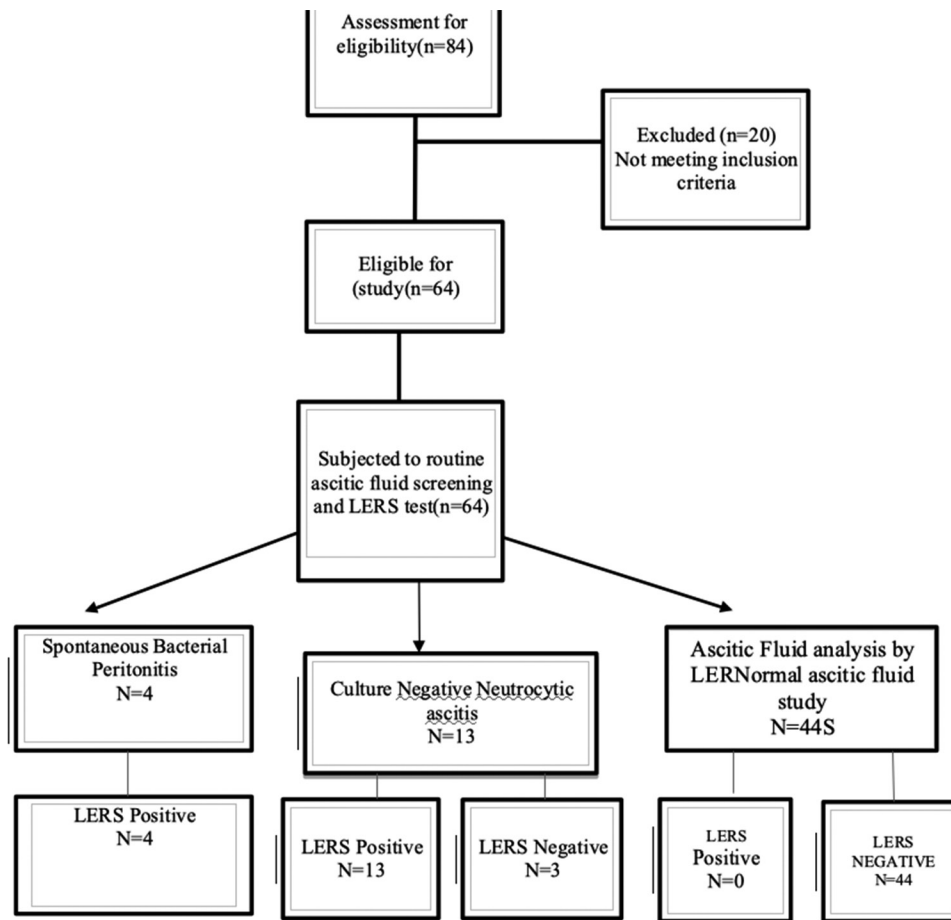


Figure 1: Consort diagram

In order to assess the functioning of LERS test; we decided to look for the performance of LERS test against PMN count at two cut off levels. At cut off of 2+; sensitivity to diagnose SBP was 100%; specificity of 94%; PPV being 57% and NPV of 94%. Similarly, at the cut off level of 3+; sensitivity decreased down to 76%; specificity increased to 100%; PPV of 100% and NPV of 93.75%. Overall accuracy at 2+ and 3+ was respectively 94.5% and 93.75% [Tables 3 and 4].

When we compared CNNA at different grades on LERS, at grade 2+ or more 13 of 16 cases had positive result as compared to Grade 0/+1. (p value <0.0001) [Table 5].

Discussion

Our study demonstrated the usefulness of LER strip test in screening of SBP. At cut off of 2+ it had shown 100% sensitivity and at cut off of 3+; it had shown 100% specificity. These cut off 2+ and 3+ correlates with leukocyte count of 125 and 500 cells/mL, respectively. The PPV at cut off of 2+ and 3+ are 57% and 100%, respectively. While NPV at cut off of 2+ and 3+ are 100% and 92.16%, respectively. Cut off of 2+ or more would pick up CNNA more compared to cut off 0/+1 on LERS. (P < 0.0001) It can indicate a preliminary, though early diagnosis of SBP. It helps to initiate antibiotic therapy for SBP,

Table 1: Characteristics of study population

Parameter	Number(N)	(%)
Total Patients	64	Percentage
Mean age	45 years (+-12.4 yrs)	
Sex		
Male	45	70
Female	19	30
Etiology		
Alcohol	30	46.8%
HBV	11	17.18%
HCV	4	6.25%
NASH	3	4.6%
BCS	5	7.8%
AIH	4	6.25%
Wilson's Disease	1	1.56%
Cryptogenic	6	9.3%
Severity of disease		
CTP B	40	62.5%
CTP C	24	37.5%
MELD: 15 or <15	21	32.8%
More than 15	33	51.56%

while cell counts, and definitive culture reports are awaited. Lower cutoff results in higher sensitivity but lower specificity, whereas the higher cut off points have reverse effects. In our study no patients with SBP were missed.

Table 2: Comparison of ascitic fluid PMN and LERS

GRADE	0 or 1 + (>75 cells)	2++ (<150 cells)	3+++ (>500 cells)
Total	44	7 (11%)	13 (20.5%)
No SBP	44	3 (43%)	0
CNNB	0	4 (57%)	9 (69.23%)
SBP	0	0	4 (30.77%)

Table 3: At cut off of 2+ grade on LERS

test	percentage (%)
Sensitivity	100%
Specificity	94%
PPV	57%
NPV	100%
Accuracy	94.5%

Table 4: At cut off of 3+ grade on LERS

test	percentage (%)
Sensitivity	76.47%
Specificity	100%
PPV	100%
NPV	92.16%
Accuracy	93.75%

Table 5: Comparison of CNNA at different grades on LERS

	Grade 0/+1	Grade +2 or >
CNNA	0	13
No CNNA	44	3
Total	44	16 (P<0.0001)

In a clinical setting of suspected SBP, a low cut off point for the reagent strips is preferable as it enhances sensitivity (albeit at the expense of specificity). So that this approach won't miss the suspected patients and hence subsequent detrimental effects. If there is delay in diagnosis of SBP there is high mortality but with timely treatment with antibiotics it decreases to less than 10%. 13 There is dichotomy of opinion regarding the use of LERS test in SBP. Because of wide variation in sensitivity and positive predictive value between reported studies, some authors have raised doubt over the use of LERS as a valid surrogate marker of SBP. 14

In a study by Ashish Jha *et al.*, out of 100 patients Eighteen patients (18%) were diagnosed to have SBP by counting chamber method as compared to 12 (12%) detected to have SBP by LERS test +++ positive. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of reagent strip \geq +++ positive were 77.77%, 95.12%, 77.77%, 95.12%, and 92% respectively compared to counting chamber method. 4

In a study by Butani *et al.*, 90 ascitic fluid samples were included in the study. Sensitivity and PPV were 89%, and specificity and

NPV were 99%. 5 Butani *et al.* were the first to present their results on the use of LERS in SBP diagnosis as an abstract in DDW 2000.5

In a study by Vanbiervliet G *et al.* had shown 100% sensitivity and specificity with these strip tests. They used Multistix 8 SG strips for their study. 5 26 publications followed (23 as full, peer-reviewed papers and 3 as either an abstract or a letter to Editor; of them, 22 are in English, 2 in French, 1 in Chinese & 1 in Korean), with the first full paper that of Vanbiervliet *et al.* validating the Multistix®8SG.6 Their results were very encouraging.^[10]

Though, our study represents the only data from western India, it is a single center data with relatively small sample size. Also, patients who had received antibiotics prior to hospitalization were excluded. Reading of colorimetric scale may be subjective. In our study, it was performed by single examiner to eliminate inter-observer variability. Therefore, there is a possibility of intra-observer variability in this study and inter-observer variability in general.

Conclusion

The LER strips can be used for rapid screening of SBP with good sensitivity and specificity, comparable to the conventional method i.e., routine microscopy.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Nousbaum JB, Cadranel JF, Nahon P, Nguyen Khac E, Moreau R, Thvenot T, *et al.* Diagnostic accuracy of the Multistix 8 SG reagent strip in diagnosis of spontaneous bacterial peritonitis. *Hepatology* 2007;45:1275-81.
2. Evans LT, Kim WR, Poterucha JJ, Kamath PS. Spontaneous bacterial peritonitis in asymptomatic outpatients with cirrhotic ascites. *Hepatology* 2003;37:897-901.
3. Jha AK, Kumawat DC, Bolya YK, Goenka MK. Multistix 10 SG leukocyte esterase dipstick testing in rapid bedside diagnosis of spontaneous bacterial peritonitis: A prospective study. *J Clin Exp Hepatol* 2012;2:224-8.

4. Butani RC, Shaffer RT, Szyjkowski RD, Weeks BE, Speights LG, Kadakia SC. Rapid diagnosis of infected ascitic fluid using leukocyte esterase dipstick testing. *Am J Gastroenterol* 2004;99:532-7.
5. Vanbiervliet G, Rakotoarisoa C, Filippi J, Guerin O, Calle G, Hastier P, *et al.* Diagnostic accuracy of a rapid urine-screening test (Multistix8SG) in cirrhotic patients with spontaneous bacterial peritonitis. *Eur J Gastroenterol Hepatol* 2002;14:1257-60.
6. Azoulay E, Fartouk M, Galliot R, Baud F, Simonneau G, Le Gall JR, *et al.* Rapid diagnosis of infectious pleural effusions by use of reagent strips. *Clin Infect Dis* 2000;31:914-9.
7. Sam R, Sahani M, Ulozas E, Leehey DJ, Ing TS, Gandhi VC. Utility of a peritoneal dialysis leukocyte strip in diagnosis of peritonitis. *Artif Organs* 2002;26:546-8.
8. Runyon BA. Monomicrobial non-neutrocytic bacterascites: A variant of spontaneous bacterial peritonitis. *Hepatology* 1990;12 (4 Pt 1):710-5.
9. Runyon BA, Hoefs JC. Culture negative neutrocytic ascites: A variant of spontaneous bacterial peritonitis. *Hepatology* 1984;4:1209-11.
10. Nguyen-Khac E, Cadranel JF, Thevenot T, Nousbaum JB. Review article: The utility of reagent strips in the diagnosis of infected ascites in cirrhotic patients. *Aliment Pharmacol Ther* 2008;28:282-8.