Evaluation of leucocyte esterase reagent strip test for the rapid bedside diagnosis of spontaneous bacterial peritonitis

Sithara K. Balagopal · Ashik Sainu · Varghese Thomas

Abstract

Background Spontaneous bacterial peritonitis (SBP) is a common and serious complication of cirrhosis, and carries a high morbidity and mortality. Rapid diagnosis and prompt treatment of this condition may improve survival of such patients.

Objective To validate the diagnostic efficacy of a leucocyte esterase reagent (LER) strip test for rapid, bedside diagnosis of SBP.

Methods We prospectively studied 175 patients with liver cirrhosis and ascites [mean age 48 (SD 16.4) years; 146 men] between August 2007 and December 2008. Alcohol was the most common (124 of 175; 70.8%) cause of liver cirrhosis. All patients underwent abdominal paracentesis, and the ascitic fluid was processed for cell count, LER strip (Magistik 10) test and culture. Two different cut-offs for calling the LER strip test positive were tried, namely when the color turned light blue [grade 2: >125 polymorphonuclear leucocytes (PMNL)/μL] or it turned purple (grade 3: >500 PMNL/μL). Sensitivity, specificity, positive predictive value, negative predictive value were calculated, using PMN count by microscopy exceeding 250 PMNL/μL.

Results LER strip using the more stringent purple-color cut off to diagnose SBP had a sensitivity of 92% and specificity of 100%. The corresponding figures using the light-blue color cut-off were 97% and 89%, respectively.

Conclusions LER strip testing of ascitic fluid is a rapid, cheap and sensitive bedside tool for the diagnosis of SBP.

Keywords Ascitic fluid · peritoneal fluid · rapid diagnosis · strip test

Introduction

Spontaneous bacterial peritonitis (SBP) is a common and serious complication of patients with liver cirrhosis and ascites. Its prevalence among unselected hospitalized cirrhotic patients is between 10% and 30%.1–8 Although prompt initiation of antibiotics produces satisfactory response in most cases, the mortality remains very high at 30–50%.4–6 Improved survival in SBP can be achieved only by rapid diagnosis and prompt treatment.

Currently, presence of more than 250 polymorphonuclear leukocytes (PMNL) per microlitre of ascitic fluid (AF) is used as a criterion for probably SBP and to begin antibiotics without waiting for culture report.8 However, facilities to do a quick total and differential leukocyte count may not be easily available, especially in rural settings and after routine hours. Cell count also takes time and is prone to human errors.9 Thus, there is need for a quick, simple and rapid bedside diagnostic test for SBP.

Leucocyte esterase reagent (LER) strips, developed initially to test for PMNL in urine,10 have been shown to be useful in detecting PMNL in other body fluids such as pleural fluid,11,12 cerebrospinal fluid13 and AF.14–16 In studies in developed countries, this test reduced the time for diagnosis of SBP from a few hours to a few minutes.17 In this test, esterase activity of PMNL in the fluid acts on an ester substrate releasing 3-hydroxy-5-phenyl-pyrrole; this changes the color of an azo dye in the reagent strip. This color change is read against a standard color chart provided with the reagent strips.13,14

In developing countries like India with a large rural population, the need for a sensitive and rapid bedside diag-
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nostic test for SBP is even higher. We therefore assessed the performance of a reagent strip based test in rapid diagnosis of SBP in Indian patients.

Methods

This study, carried out between August 2007 and December 2008, included patients with liver cirrhosis and ascites who were not receiving any antibiotics. Diagnosis of cirrhosis was based on clinical, laboratory and ultrasonographic findings. Those with hemorrhagic ascites were excluded. The study was approved by our institution’s Ethics Committee.

Abdominal paracentesis was done under aseptic conditions. Total proteins, albumin and amylase in the AF were measured. Leukocytes in AF were counted using microscopy, and percentage of PMNL was determined using methylene blue stain on a concentrated smear of AF collected in EDTA. Culture was done by inoculating 5 mL of AF into blood culture bottles. All patients also had routine blood tests, biochemical tests including liver function tests and prothrombin time.

LER strip test was done immediately after the paracentesis, using strips as procedure for urine testing (Magistik-10; Peerless Biotech Laboratories Ltd, Chennai, India). In brief, 5 mL of AF was collected in a clean, dry tube. The test strip was dipped in it, removed immediately and its color read at 120 seconds against the color chart provided on the bottle. A four grade colorimetric scale (0–3) was used to record the result. The manufacturer suggests a relationship between PMNL and color scale as follows: grade 0 (no change): >15 PMNL/mL; grade 1 (light yellow): >70 PMNL/mL; grade 2 (light blue): >125 PMNL/mL and grade 3 (purple): >500 PMNL/mL.

Two different cut-offs, light blue (grade 2) or purple (grade 3) were tried. Sensitivity, specificity, positive predictive value, negative predictive value were calculated, and a receiver-operating characteristics (ROC) curve was drawn.

Results

We studied 175 patients (mean age 48.0 [16.4] years; 146 men) with liver cirrhosis and ascites. Of these, 162 were in Child-Pugh class C and 13 in class B. Past history of SBP was present in 21 patients. Of the 175 patients, 75 patients satisfied the current criterion for diagnosis of SBP (PMNL >250 cell/mL). Mean (range) PMNL counts in patients with SBP and those without SBP were 964 (250–18,500) cells/mL and 69 (20–235) cells/mL, respectively.

Table 1 Results of leucocyte esterase reagent strip test compared to the gold standard of cell count

<table>
<thead>
<tr>
<th>Grade of color change (final color)</th>
<th>SBP (n = 75)</th>
<th>No SBP (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (no change)</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>1 (light yellow)</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>2 (light blue)</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>3 (purple)</td>
<td>69</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2 Performance characteristics of leucocyte esterase reagent strip, using two different cut-offs

<table>
<thead>
<tr>
<th>Cut-off used</th>
<th>Grade 3 color change</th>
<th>Grade 2 color change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.92 (0.88–0.97)</td>
<td>0.97 (0.88–0.97)</td>
</tr>
<tr>
<td>Specificity</td>
<td>1.000</td>
<td>0.89 (0.81–0.96)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>1.00</td>
<td>0.87 (0.80–0.97)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.98 (0.96–1.00)</td>
<td>0.83 (0.82–1.00)</td>
</tr>
</tbody>
</table>

Fig. 1 Receiver operator curve for the leukocyte esterase test

The AUC and coordinates of the ROC curve are shown below

<table>
<thead>
<tr>
<th>AUC</th>
<th>p value</th>
<th>SD</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.93</td>
<td>&lt;0.001</td>
<td>0.056</td>
<td>0.813</td>
<td>1.032</td>
</tr>
</tbody>
</table>

AUC: area under the curve; SD: standard deviation
Table 1 shows the results of LER strip test. Of the 75 cases with SBP, 69 showed grade 3 color change and four additional patients showed a grade 2 change. The mean (SD) cell count in patients with grade 3 change was 820 (121) PMNL/mL and that in those with Grade 2 change was and 302 (185) PMNL/mL. AF culture was positive in 21 cases (28%), with Escherichia coli in 14 cases, Klebsiella pneumoniae in four cases and mixed growth in three cases.

Table 2 shows the performance characteristics of each cut-off of the LER test. The ROC curve had an area under curve of 0.930.

The mean time taken for cell count results from our laboratory was 2.7 hours (range: 105–180) minutes, whereas that for LER test was 2 minutes. The cost of each test was Indian Rupees (INR) 15 for the LER strip test, and INR 300 for cell count.

Discussion

Our prospective study found that LER strip test on AF had a good sensitivity and specificity for the diagnosis of SBP. Of the two cut-offs used, the grade 2 color cut-off had a greater sensitivity but a lower specificity than the grade 3 color change cut-off.

The two cut-offs used by us, namely grade 2 and 3 color change are considered equivalent to 125 and 500 PMNL/mL, respectively, in comparison to the gold standard for diagnosis of SBP of 250 PMNL/mL. Both the cut-offs had performed reasonably well for the diagnosis of SBP. However, in the clinical context of SBP, it may be better to use the cut-off with a highest sensitivity, even at the cost of some false positives, i.e. by using grade 2 or more color change.

The LER strip test is based on the esterase activity of activated granulocytes. These cells release leukocyte esterase into the extracellular milieu reacts with an ester releasing 3-hydroxy-5-phenyl-pyrrole which causes a color change in the azo dye in the reagent strip. This test has been used not only for the diagnosis of urinary tract infections, but also to detect evidence of infection in other body fluids, such as in patients with empyema, meningitis and peritonitis in patients on peritoneal dialysis.

Vanbiervliet et al published the first study on the use of LER strip test in AF. They studied 72 consecutive patients with liver cirrhosis in France using Multistix SG strips, the most frequently used urinary reagent strip in France, and 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 the United States have confirmed these results. The sensitivity of urinary reagent strips for the diagnosis of SBP has ranged between 85% and 100%, and the specificity between 98% and 100%. Recently, Sapey et al compared the performance of two different brands of LER strips (Multistix SG vs. Nephur-Test) in the diagnosis of SBP, and found the latter to be better. However, in another study conducted in both Europe and USA, the same authors did not find any difference between the two types of reagent strips.

The average time taken for the LER test was much shorter than that for cell count. Thus, the strip test is not only accurate, but also rapid, easy to use, and cheaper. This test does not require much expertise and can be performed everywhere. Further, it may be possible to apply this test to determine the effectiveness of antibiotic therapy in patients with SBP by repeating the test, though this aspect needs further studies.

The limitations of the strip test include absence of a cut-off corresponding to cell count of 250 PMNL/mL in the reagent strip, and the possibility of inter-observer variation in matching of color. Thus, it may be useful to confirm grade 2 color change with cell count. Further, it may be possible to manufacture modified test strips specifically for the diagnosis of SBP, such that color change corresponds to a cut-off for 250 PMNL/mL of fluid.

In conclusion, our study shows that LER strip test has good accuracy for the diagnosis of SBP, and has advantages of speed, low cost, availability at odd hours, and no need for expertise. Because of its speed, the use of this test may help early institution of antibiotic therapy in patients with SBP.

References


