Evaluation of leukocyte esterase reagent strip test to detect spontaneous bacterial peritonitis in cirrhotic patients

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Abstract

Spontaneus bacterial peritonitis (SBP) is a frequent and sever complication of cirrhotic patients with ascites. The aim of this study was to assess diagnostic value of leukocyte esterase reagent strip test (Medi-Test Combi) for the rapid diagnosis of SBP in cirrhotic patients with ascites in the University Hospital of Imam Reza in Tabriz, Iran. In this study, 132 consecutive samples from cirrhotic patients who underwent abdominal paracentesis were evaluated. These samples were tested by 3 methods: i) manual cell count with differential study, SBP was defined as having poly-morphonuclear cells count of ≥250/mm^3; ii) culture of ascetic fluid samples; iii) all samples were tested by leukocyte esterase dipsticks. The sensitivity, specificity, positive and negative predictive values of two different colorimetric cut-off scales (1+ and 2+) were calculated and compared. The sensitivity, specificity, positive and negative predictive values of 1+ and 2+ cut-off scales to diagnose SBP were 97.5%, 84.6%, 74% and 98.7% for the 1+ cut-off scale and 87.8%, 96.7%, 92.3% and 94.6% for the 2+ cut-off scale. Leukocyte esterase reagent strip test can be used as a rapid test for screening of SBP. The higher cut-off colorimetric scale has a better specificity and positive predictive value but a lower sensitivity.

Introduction

Spontaneous bacterial peritonitis (SBP) is a serious complication in cirrhotic patients with ascites.[1-3] The prognosis of SBP has been improved by the use of antibiotics and by the prophylactic use of antibiotics in high-risk groups of patients. This improvement in survival might also be explained by more rapid diagnosis and treatment, thereby avoiding the occurrence of severe sepsis and septic shock, a condition well known for its frequently fatal outcome. Therefore, prompt diagnosis and treatment of SBP are essential for the survival of patients.

Symptoms of SBP such as fever, nausea, vomiting and abdominal pain are not presented in all cirrhotic patients who develop this condition.[4]

Today, an ascitic fluid polymorphonuclear (PMN) leukocyte count of 250/mm^3 or over, irrespective of the AF culture result, is universally accepted as the best surrogate marker for diagnosing SBP.[5] However, total and PMN cell counts from ascitic fluid are not always available, especially in outpatient units.

Recently, the use of urinary reagent strips has been proposed for rapid diagnosis of SBP. In urine, the urinary strips identify, for example, protein, blood, bilirubin, and glucose. Also, these strips detect leukocytes by identifying their esterase activity via a colorimetric reaction. Presence of leukocytes in the urine or other body fluids may indicate the presence of infection.

Use of urinary reagent strips has been tested for the diagnosis of bacterial meningitis, pleural infection,[6] synovial infection,[7] and peritoneal infection in dialyzed patients.[8-10] The use of reagent strip testing for leukocyte esterase has been proposed to reduce the time from paracentesis to a presumed diagnosis of SBP from a few hours to a few seconds. The aim of this study was to evaluate the usefulness of a dipstick in the rapid diagnosis of SBP in cirrhotic patients who underwent abdominal paracentesis. This is the first study to use this low-cost and rapid method for diagnosing SBP in Iran, and the first study using the Medi-Test Combi kit. This study defines the validity scores of 2 different thresholds of colorimetric scales.

Materials and Methods

In this study, we evaluated 132 consecutive samples of ascitic fluid from cirrhotic patients admitted to Imam Reza University Hospital in Tabriz from February 2009 to March 2010. Paracentesis of ascitic fluid were performed immediately after admission. Patients were admitted either for treatment of ascites or for complications of cirrhosis (*i.e.* infection, gastrointestinal bleeding, hepatic encephalopathy, acute renal failure, hepatocellular carcinoma). Exclusion criteria were: chylous ascites and ascites not related to portal hypertension (*i.e.* pancreatic ascites, peritoneal tuberculosis, peritoneal carcinomatosis), and use of antibiotics before admission and paracentesis.

The following data were recorded: age, sex, cause of cirrhosis (alcohol use, hepatitis B virus, hepatitis C virus, genetic hemochromatosis, others), serum creatinine, serum Na, prothrombin time, serum total bilirubin, serum albumin, platelet count and Child-Pugh score.

Ascitic fluid was examined for leukocyte and PMN cell counts/μL, and bacteriological status. Bacterial cultures were obtained by bedside inoculation of 10 mL of ascitic fluid into blood culture bottles. The sample for PMN and total leukocyte count was collected in a heparin anticoagulant tube. Differential cell count and cytology were examined with a conventional optical microscope. A manual cell count with differential study was performed for all samples by experienced technicians. Appropriate biochemical tests (glucose, protein, albumin, LDH) were also performed.

Each sample of the ascitic fluid was tested using a dipstick (Medi-Test Combi, France). These dipsticks can detect esterase activity of PMN cells.[11] All reagent areas were immersed in ascitic fluid and the strip was removed immediately. At 90 seconds, the color of the leukocyte reagent was compared with the color chart on the bottle. A correlation between leukocytes and the colorimetric 4-grade scale (from 0 to3) was suggested by the manufacturer as follows: grade 0, 0 leukocytes/μL; grade 1, 25 leukocytes/μL; grade 2, 75 leukocytes/μL; grade 3, 500 leukocytes/μL. The technicians who interpret the results of strip tests were not informed as to the clinical condition of patients or the results of PMN tests.

The diagnosis of SBP was based on a PMN cell count in ascitic fluid of 250/μL or over, irrespective of an ascitic fluid culture or clinical signs of SBP, and also the absence of a secondary intraabdominal source of infection, inflammation or tuberculosis.

Results of dipstick tests were compared to PMN cell counts and ascitic fluid culture. The sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) of

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the dipstick in the diagnosis of SBP by two different colorimetric scales (1+ and 2+) were calculated and compared. Sensitivity was defined as the proportion of patients with a positive reagent strip divided by the number of those with SBP diagnosed by the previously defined criteria. Specificity was defined as the proportion of patients with a negative reagent strip test divided by the total number of patients without SBP. PPV was defined as the proportion of patients with a true-positive reagent strip test divided by the total number of patients with positive reagent strip test. NPV was defined as the proportion of patients with true-negative reagent strip tests divided by the total number of patients with negative reagent strip tests.

Results

We diagnosed 41 episodes of SBP by PMN cell count, of whom 40 (97.5%) were compatible with dipstick test using the 1+ cut-off scale and 36 (87.8%) using the 2+ cut-off scale; 20 were culture positive and 21 were culture negative.

Of the 91 specimens with PMN cell count in ascitic fluid under 250/μL, 77 had negative results by dipstick using the 1+ cut-off scale and 88 had negative results using the 2+ cut-off scale; 90 were culture negative and one was culture positive (Tables 1 and 2).

The sensitivity, specificity, PPV and NPV of leukocyte esterase reagent strip test for the 1+ and the 2+ cut-off scales to diagnose SBP were 97.5%, 84.6%, 74%,98.7% and 87.8%, 9 6.7%, 92.3%, 94.6%, respectively.

The sensitivity, specificity, PPV and NPV of one-off bacterial culture to diagnose SBP were 51.3%, 98.9%, 95.4%, 81.8%, respectively (Figures 1-3).

Discussion

SBP continues to be an important source of morbidity and mortality in cirrhotic patients.[10] Under such circumstances, prompt diagnosis and treatment are crucial to ensure better clinical outcomes in this patient group. Currently, ascitic fluid PMN count is a standard tool for decision making in management of SBP cases. However, there may be several hours' delay in receiving results from the laboratory. In addition, this test may not be available in an outpatient setting. Given this, reagent strips emerge as an attractive means of rapid diagnosis of this clinical entity in patients with cirrhosis and ascites. Numerous independent studies have evaluated the diagnostic value of reagent strips in the setting of SBP (Table 3).[12-33] Most have shown high sensitivity and specificity, in keeping with our present findings. Our study confirms that the diagnostic value of leukocyte esterase reagent strip test is very high, as the sensitivity, specificity, PPV and NPV of leukocyte esterase reagent strip test for the 1+ and 2+ cut-off scales to diagnose SBP were 97.5%, 84.6%, 74%, 98.7%, and 87.8%, 96.7%, 92.3%, 94.6%, respectively.

When we use the 1+ cut-off scale, sensitivity is very high, but specificity and PPV decrease. By using the 2+ cut-off scale, however, sensitivity decreases but specificity and PPV tend to be high.

The study results of Nousbaum *et al.*[27] were inconsistent with the results of our study and of many others. Values of sensitivity, specificity, and positive and negative predictive values of leukocyte esterase dipstick test in their study were 45.3%, 99.2%, 77.9%, 96.9%, respectively, and they have concluded that leukocyte esterase strip test is not sensitive enough to detect cases of SBP.[27] They used Multistix 8 SG reagent strip test in their study. Correlation between leukocytes and the colorimetric 5-grade scale (from 0 to 4) which is suggested by the manufacturer of Multistix 8 SG is as fol-

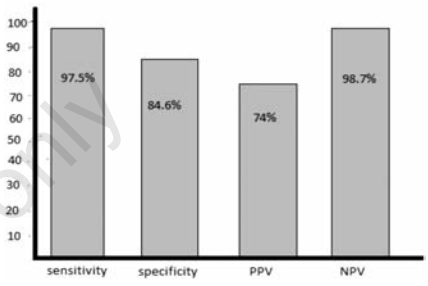

Figure 1. Sensitivity, specificity, positive predictive value and negative predictive value of LE test (cut-off scale 1+).

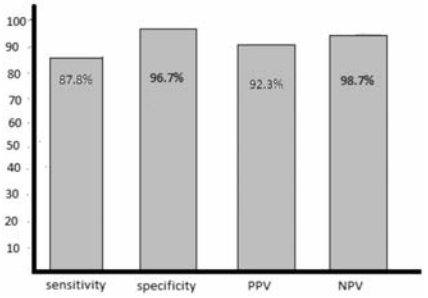

Figure 2. Sensitivity, specificity, positive predictive value and negative predictive value of LE test (cut-off scale 2+).

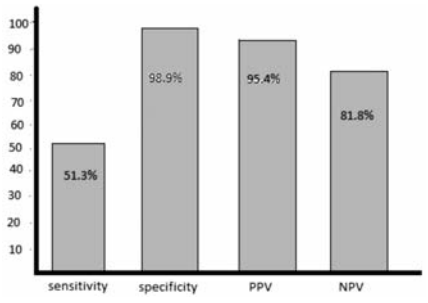

Figure 3. Sensitivity, specificity, positive and negative predictive values of culture.

Table 1. Dipstick results in spontaneous bacterial peritonitis and non-spontaneous bacterial peritonitis groups.

Colorimetric scales	PMN≥250/μL	PMN<250/μL	Total
0	1	77	78
1+	4	11	15
2+	11	3	14
3+	25	0	25
Total	41	91	132

PMN, polymorphonuclear cells.

Table 2. Culture results in spontaneous bacterial peritonitis and non-spontaneous bacterial peritonitis groups.

Culture results	PMN≥250/mL	PMN<250/mL	Total
Positive	20	1	21
Negative	21	90	111
Total	41	91	132

PMN, polymorphonuclear cells.

Table 3. Published studies on the diagnosis of spontaneous bacterial peritonitis using different reagent strips.

Study	Samples/SBP	LE test	LE test cut off	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Vanbiervliet *et al.*[12]	78/9	Multistix8®SG	70 leuc/μL-G2	100	100	100	100
Castelote *et al.*[13]	228/52	Aution® sticks	75 leuc/μL-G2	96	89	74	99
Thévenot *et al.*[14]	100/9	Multistix8®SG	125 leuc/μL-G3	89	100	100	99
		Combur2LN®	75 leuc/μL-G2	89	100	100	99
Butani *et al.*[15]	136/12	Multistix10®SG	70 leuc/μL-G2	83	99	91	98
Sapey *et al.*[16]	55/13	Multistix10®SG	25 leuc/μL-G1	83/100	96/100	83/100	96/100
		Nephur-test®	25 leuc/μL-G1	86/100	92.5/100	75/100	99/100
Sapey *et al.*[17]	245/17	Multistix10®SG	25 leuc/μL-G1	64.7	99.6	91.7	97.4
		Nephur-test®	25 leuc/μL-G1	88.2	99.6	93.8	99.1
Kim *et al.*[18]	257/79	UriSCAN®	75 leuc/μL-G2	100	99	98	100
Kim *et al.*[19]	75/18	Multistix10®SG	125 leuc/μL-G2	50	100	100	87
		UriSCAN®	75 leuc/μL-G2	100	100	100	100
Sarwar *et al.*[20]	214/38	Combur10®	75 leuc/μL-G2	95	92	72	99
Wisniewski *et al.*[21]	90/6	Multistix8®SG	15 leuc/μL-G1	83	83	42	97
Braga *et al.*[22]	100 /9	Combur® UX	75 leuc/μL-G2	100	98.9	92.3	100
Rerknimitr *et al.*[11]	200/42	Combur10M®	25 leuc/μL-G1	88	81	55	96
Campillo *et al.*[23]	443/33	Multistix8®SG	70 leuc/μL-G2	45.7	98	75	93.3
		Combur2LN®	75 leuc/μL-G2	63	99.2	91	92.9
Li *et al.*[24]	84/25	Multistix10®SG	15 leuc/μL-G1	92.8	84.7	71.8	96.1
Ribeiro *et al.*[25]	200/11	Multistix10®SG	15 leuc/μL-G1	86	96	60	99
Gaya *et al.*[26]	173/17	Multistix10®SG	15 leuc/μL-G1	100	91	50	100
Nousbaum *et al.*[27]	2123/11	Multistix8®SG	125 leuc/μL-G2	45.3	99.2	77.9	96.9
Torun *et al.*[28]	63/15	Aution® sticks	75 leuc/μL-G2	93	100	100	98
Nobre *et al.*[29]	109/9	H-T Combina®	75 leuc/μL-G2	78	88	37	98
de Araujo *et al.*[30]	155/17	Multistix10®SG	15 leuc/μL-G1	80	98.5	90.9	96.2
		Choiceline 10®	75 leuc/μL-G2	76.9	97.7	87	95.6
Castellote *et al.*[31]	228/52	Aution® sticks	75 leuc/μL-G2	89	86	62	97
Rerknimitr *et al.*[32]	250 /30	Multistix10®SG ?	25 leuc/μL-G1	80	94.5	66.7	97.2
		Aution® sticks ?	250 leuc/μL-G3	90	93	64.3	98.6
		Combur10® ?	75 leuc/μL-G2	90	0.2	64.3	98.6
Farmer *et al.*[33]	311/59	Multistix8®SG	70 leuc/μL-G2	96	96.5	90.7	99.4

SBP, spontaneous bacterial peritonitis; LE, leukocyte esterase; PPV, positive predictive value; NPV, negative predictive value

lows: grade 0, 0 leukocytes/μL; grade 1 (traces), 15 leukocytes/μL; grade 2 (1+), 70 leukocytes/μL; grade 3 (2+), 125 leukocytes/μL; grade 4 (3+), 500 leukocytes/μL. They considered strip test to be positive at grade 3 (125 leukocytes/mL) because grade 4 was above the cut-off scale of SBP. It seems that the manufacturer of these dipsticks has justified these scales to estimate leukocyte count of urine and the justification may be incorrect for ascitic fluid. Several studies have already been performed by others using Multistix urine strip test and none have used a 3+ cut-off scale to detect cases of SBP (Table 3).They all used a lower cut-off scale but the specificity and PPV of the tests did not decrease. Kim *et al.* used a 3+ cut-off scale and this showed low sensitivity from the Multistix test (50%).[19] These studies show that the correlation between leukocyte counts and colorimetric scales which is suggested by the manufacturer is not correct for ascitic fluid and if we use a low cut-off scale, leukocyte esterase reagent strip test may have a high diagnostic value. In summary, the current findings add to a growing body of literature on the usefulness of the strip test for early diagnosis of SBP in cirrhotic patients (especially non-alcoholic cases) with ascites. The current study was limited by small sample size. Future studies using the same experimental set up and a larger sample size could standardize the appropriate strip kit and cut-off level.

Conclusions

Our results are compatible with those reported previously thus demonstrating that leukocyte esterase reagent strip test can be used as a rapid test for screening of SBP. This accurate method could be used anywhere, thereby reducing the time needed for a presumptive diagnosis of SBP from a few hours to a few sec

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