

Validation of Reagent Strips, Comparing with Automated and Manual Cell Counts for the Diagnosis of Spontaneous Bacterial Peritonitis

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ABSTRACT

Background: Aims: The standard diagnosis of spontaneous bacterial peritonitis (SBP) is an ascitic fluid polymorphonuclear (PMN) cell count of $\geq 250/\text{mm}^3$ and/or a positive ascitic fluid. However, result is usually not promptly available in the emergency situation. Automated cell count and reagent strip tests have been used for rapid diagnosis of urinary tract infection and meningitis. We evaluated their usefulness by testing the validity of automated cell count and various reagents strip tests for the diagnosis of SBP.

Materials & Methods: Two hundred consecutive paracentesis in cirrhotic patients were performed. All ascitic fluid samples were analyzed with automated cell count and three reagent strips: Aution sticks, Combur10 Test M and Multistix10SG. Manual cell count for PMN of $\geq 250/\text{mm}^3$ was referred as gold standard. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for the diagnosis of SBP by different techniques were compared.

Results: SBP was diagnosed by manual cell counts in 24 specimens (12%). The sensitivity, specificity, PPV, NPV and accuracy of the 1+ cutoff scale of the strips were 88%, 93%, 62%, 96%, 92% for Aution sticks, 75%, 93%, 60%, 97%, 91% for Multistix test, 88%, 92%, 58%, 98%, 91% for Combur test and 88%, 98%, 84%, 98%, 97% for the automated cell count, respectively.

Conclusion: Automated cell count is as sensitive as many reagent strips for a rapid diagnosis of SBP. However, it provides better specificity, PPV, NPV and accuracy.

Key words : Spontaneous bacterial peritonitis, reagent strip, automated cell count

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BACKGROUND

Spontaneous bacterial peritonitis (SBP) is one of the frequent and severe complications in cirrhotic patients with ascites. The prevalence of SBP in cirrhotic patients admitted to the hospital varies from 10% to 30%.⁽¹⁾ Symptoms of SBP are neither specific nor sensitive.⁽²⁾ Currently, standard criteria for diagnosis of SBP are an ascitic fluid polymorphonuclear (PMN) cell count of $\geq 250/\text{mm}^3$ and/or a positive ascitic fluid bacterial culture.⁽³⁾ PMNs contain various enzymes active in the inflammation response, including the esterases. Leukocyte esterase is a test that has been commonly used to determine PMN activity.⁽⁴⁾ The use of reagent strip testing for leukocyte esterase has been proposed to reduce time of diagnosis of infection such as urinary tract infection or meningitis to a few seconds.⁽⁵⁾

Recently, there have been series of reports of test strips for the instant diagnosis of SBP. Some studies used only one or two strips and revealed very sensitive and specific for the diagnosis of SBP.^(6,7) Prospective multicenter study also confirmed the efficacy of reagent strip.⁽⁸⁾ Automated cell counter also can be used for the diagnosis of SBP offering an easier and quicker PMN count.⁽⁹⁾ But there is little data about combination of three reagent strips and automated cell counter in the same study for diagnosis of SBP.

MATERIALS AND METHODS

Patients

Between September 2006 and February 2008, 134 consecutive unselected cirrhotic patients with ascites were included in the study and a total of 210 paracentesis were performed. The clinical indications for paracentesis were relief of patient discomfort ($n = 100$), routine paracentesis ($n = 76$), suspected SBP ($n = 20$) and miscellaneous ($n = 14$). The diagnosis of cirrhosis was established according to the histologic criteria or clinical, laboratory, endoscopic or ultrasonographic findings. The study was approved by the Ethic Committee.

The baseline characteristic of the patients was shown in Table 1. Ten ascitic fluid specimens were excluded from the study due to the final diagnosis of carcinomatous peritonei and the ascitic fluid color was too dark to be interpreted by reagent strip. Overall 200 paracentesis was finally included in the study.

Table 1. Baseline characteristic of 134 patients.

Characteristics	N (%)
Male/Female	87/47
Age, mean \pm SD (years)	59.5 \pm 11.1
Child-Pugh classification A/B/C	8/53/73
Etiology of cirrhosis	
- Hepatitis B	34 (25)
- Hepatitis C	15 (11)
- Alcohol	29 (22)
- Hepatitis B and alcohol	7 (5)
- Hepatitis C and alcohol	4 (3)
- Autoimmune	7 (5)
- Nonalcoholic fatty liver disease	4 (3)
- Hemochromatosis	2 (2)
- Cryptogenic	8 (6)
- Others	24 (18)

Methods

Paracentesis were performed by standard medical practice and repeated as indicated. Immediately after paracentesis, ascitic fluid specimen was collected in both clean dry test tube and tested using three reagent strips: 1) Aution sticks, A. Menarini Diagnostic, Firenze, Italy 2) Combur10 Test M, Roche, Mannheim, Germany 3) Multistix10SG, Bayer Corporation, Elkhart, USA. After the required waiting period, the color of the reagent strips were compared with the color charts of the bottles. The Aution sticks is read at 120 seconds, Multistix10SG test at 120 seconds and Combur10 test at 90 seconds for leukocyte esterase. Correlation between PMN cell count and the 4-grade scale was suggested by the manufacturer for the Aution sticks as follows: grade 0, 0 PMN/mm³; grade 1, 25 PMN/mm³; grade 2, 75 PMN/mm³; grade 3, 250 PMN/mm³; grade 4, 500 PMN/mm³. For the Multistix10SG test, the correlation was: grade 0, 0 PMN/mm³; grade 1, 25 PMN/m³; grade 2, 75 PMN/mm³; grade 3, 500 PMN/mm³. For the Combur10 test, the correlation was: grade 0, 0 PMN/mm³; grade 1, 15 PMN/mm³; grade 2, 70 PMN/mm³; grade 3, 125 PMN/mm³; grade 4, 500 PMN/mm³.

Ascitic fluid was collected in tubes containing 0.084 ml of 15% ethylenediaminetetraacetic acid (EDTA). Three milliliters of ascitic fluid, collected in tubes containing EDTA, were directly injected into the analyzer. The specimen was sent to determine the white blood cell (WBC) and PMN counts by automated cell blood counter (Cell-dyn 3700).

Ascitic fluid was conventionally processed including PMN cell count and lymphocyte count, appropriate biochemical tests (glucose, protein, albumin, lactate dehydrogenase, and sugar) and cytology as indicated. The sample for PMN and total leukocyte count was analyzed immediately as possible. Differential cell count and cytology were examined with a conventional optical microscope. A manual cell count with differential study was done in all samples by well trained technicians.

Diagnostic criteria of SBP

The diagnostic criteria of SBP were defined as ascitic fluid PMN cell count $\geq 250/\text{mm}^3$ in the absence of an intra-abdominal source of infection and exclusion of other causes of elevated PMN in ascitic fluid such as tuberculosis, peritoneal carcinomatosis, or pancreatitis. Result of 1+ or more of the leukocyte esterase form reagent strip was considered a positive test. The negative result from reagent strip was considered a negative test. Secondary peritonitis was suspected when there was an abdominal source of infection or more than one organism identified in the ascitic fluid.

Statistical analysis

Data were presented as means \pm SD for quantitative variables and as frequencies for qualitative variables. Result of all three reagent strips and automate

cell count were compared to PMN cell count, ascitic fluid culture in all patients. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of all tests were calculated and compared.

RESULTS

Of the 200 paracenteses, we diagnosed 24 episodes of SBP by manual PMN cell count (12%), of which 21 (88%) were diagnosed by using 1+ cutoff scale for the Aution sticks test, 18 (75%) by Multistix10SG test, 21 (88%) by Combur 10 test and 21 (88%) by the automated cell count. Six specimens had a positive culture for bacteria, of which one was compatible with bacterascites. The reagent strip result in this bacterascites specimen was negative. Automated cell count can diagnose 21 episodes SBP from total of 24 episodes by manual cell count. Two of the three specimens that automated cell counts were unable to diagnose SBP were due to clotted specimen in one and technical error from storage problem in one. No secondary bacterial peritonitis was diagnosed in this study. The sensitivity, specificity, PPV, NPV and accuracy of a 1+ cutoff scale by any reagent strip and automated cell count to diagnose SBP were 87.5%, 92.6%, 61.8%, 96.4%, 92% for Aution sticks test, 75%, 93.2%, 60%, 96.5%, 91% for Multistix10SG test, 87.5%, 91.5%,

Table 2. Efficacies of the SBP diagnosis by automated cell count and different reagent strips.

Tests	Aution	Multistix	Combur	Automated cell count
Sensitivity (%)	88	75	88	88
Specificity (%)	93	93	92	98
PPV (%)	62	60	58	84
NPV (%)	96	97	98	98
Accuracy (%)	92	91	91	97

Table 3. Efficacies of multiple reagent strips reported in the previous studies.

	Author			
	Kim <i>et al</i> ⁽⁵⁾	Sapey <i>et al</i> ⁽²⁾	Thevenot <i>et al</i> ⁽⁶⁾	Campillo <i>et al</i> ⁽¹¹⁾
Reagent strip	UriScan / Multistix10SG	Nepthur test / Multistix10SG	Multistix8SG / Combur2 test LN	Multistix8SG / Combur2 test LN
Sensitivity (%)	67/50	88/65	89/89	69/80
Specificity (%)	100/100	100/100	100/100	95/90
PPV (%)	100/100	94/92	100/100	64/52
NPV (%)	89/87	99/97	99/99	96/97

58.3%, 98.2%, 96.5% for Combur10 test and 87.5%, 97.7%, 84%, 98.3%, 96.5% for automated cell count, respectively (Table 2).

DISCUSSION

SBP is associated with high morbidity and mortality. Improvement of the survival might be achieved by a more rapid diagnosis and treatment. Manual cell count is time-consuming, requires around the clock availability of laboratory. Analysis of ascitic fluid by means of a leukocyte esterase reagent strip could be useful in the rapid diagnosis of SBP.⁽¹⁰⁾ The efficacy of this test in detecting ascitic fluid infection has been studied by many investigators (Table 3). However, there is no study evaluating the validity of three reagent strips with automated cell count in the diagnosis of SBP.

Until now, there has been no reagent strip that was specifically designed for ascitic fluid. Aution stick is the only reagent strip that has benefits over the other reagent strips since Aution stick has precise colorimetric scale that correlates with ≥ 250 PMN cells/mm³ while the other reagent strips do not have precise scale like Aution stick. In Thailand, we used the Combur10 test M that is widely available as a screening test for SBP.⁽¹²⁾ The result of Combur test was comparable to other study in sensitivity but specificity and PPV were lower.⁽⁷⁾

Automated cell count is commonly used in laboratories for blood cell counting, offers an accurate and precise differential count of leukocytes. Automated cell can provide results in few minutes with high sensitivity, specificity, PPV and NPV.⁽¹³⁾ For the ascitic fluid WBC and PMN cell counts by automated blood cell counter, ascitic fluid, collected in tubes containing 15% EDTA anticoagulant were directly injected into the analyzer.⁽¹⁴⁾ In our study, we used tubes containing 15% EDTA anticoagulant that normally used for complete blood count test. We used 3 ml of ascitic fluid for each tube as labeled. We lost 4 ascitic fluid specimens for automated cell count analysis, 3 specimens due to technical errors in storing specimen in freezer and clotting in 1 specimen, probably from high leukocyte count in the specimen. The very low rate of clotted specimen (1 from 200, 0.5%) in this study may support the use of automated cell count as an option for early and accurate test for routine diagnosis of SBP in the future.

When compared all reagent strips and automated cell count, Aution stick, Combur test and automated cell count had similar sensitivity. However, automated cell count still had better results in all other aspects. All three reagent strips had much lower PPV but similar NPV compared with automated cell count. Among the three strips, all validity scores are comparable except Multistix that contained the lower sensitivity.

There were many factors that probably influenced the accuracy of reagent strips. Antibiotic can produce both false positive and false negative results.⁽¹⁵⁾ Even color of ascitic fluid do have effect to the interpretation of reagent strips such as dark yellow of ascitic fluid in patient with bile leakage or hemoperitoneum. In our study, the incidence of SBP was 12%, which was quite low.⁽¹⁶⁾ It may be due to that most patient in this study underwent abdominal paracentesis as an outpatients for relief discomfort. That is also important that we need instant diagnostic test that have high negative predictive value to confidently excluded patient with SBP before letting them leave the hospital without treatment.

In summary, this is the first study that investigated the combining three reagent strips and automated cell count for the diagnosis of SBP. Automated cell count is as sensitive as many reagent strips for a rapid diagnosis of SBP. However, it provides better specificity, PPV, NPV and accuracy, though it has some disadvantage. Reagent strips and automated cell count can provide immediate and accurate diagnosis for SBP in cirrhotic patients. A positive result should be an indication for empirical antibiotic therapy while waiting for confirmation and a negative result reliably excluded SBP and may be useful as a screening test in patients on large volume paracentesis.

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