Original Article

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# Prompt Diagnosis of Spontaneous Bacterial Peritonitis and Analysis of Ascites Protein Content by Reagent Strip

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## ABSTRACT

***Background:*** Ascitic fluid PMN count remains an crucial tool in diagnosing spontaneous bacterial peritonitis (SBP), but may not be done promptly in some situations. A few studies have shown the accuracy of reagent strip test for diagnosing SBP. This is the first study in Thailand to evaluate the use of reagent strip, which is different from that used in previous studies, for diagnosing SBP and determine ascitic fluid protein content.

***Patients and Methods:*** One hundred and seventeen ascitic fluid samples of 72 cirrhotic patients were analyzed by PMN count and 113 samples used for protein measurement. Leukocyte esterase strip using for diagnosis of SBP, and protein strip using for detection of ascites protein lesser than <1 g/dl compared to the corresponding routine measurement.

***Results:*** Twelve of 117 samples had ≥250 PMN/ml, while 7 of these were diagnosed as SBP by reagent strip. There was no samples diagnosed as SBP by reagent strip in the group which ascitic fluid PMN count was <250 PMN/ml. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for diagnosis of SBP by reagent strip were 58, 100, 100 and 95% respectively. Thirty-three of 113 samples had protein content <1 g/dl of protein, while 18 of these were diagnosed correctly by reagent strip. Of the remaining 80 samples that had protein content ≥1 g/dl, 57 samples were diagnosed correctly by reagent strip. The sensitivity, specificity, PPV and NPV for detecting ascites protein <1 g/dl using reagent strip were 55, 71, 44 and 79% respectively.

***Conclusions:*** The reagent strip test can be used for prompt diagnosis of SBP with specificity and PPV of 100%. The clinical application for protein measurement is not as reliable as conventional laboratory because of low PPV and NPV.

**Key words :** prompt diagnosis, spontaneous bacterial peritonitis, ascites protein content, reagent strip

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## BACKGROUND

Spontaneous bacterial peritonitis (SBP) is a common complication in cirrhotic patients. Its prevalence and mortality rate range 10-30%[1-5] and 20-33%[6-8] respectively. Both in-patients and out-patients can develop SBP. Most patients with SBP have symptoms and/or signs clearly suggestive of peritoneal fluid infection. However, 15% of patients had SBP without any symptoms[8].

The mortality rate in cirrhotic patients who have developed SBP is much higher than in whom never have history of SBP[9], and it is higher in patients who develop SBP during admission. In study of Coral *et al*.[7], the mortality rate of SBP developed in the hospital was 28% versus 18% in cases developed in the community. Prompt diagnosis and treatment could have decreased such high mortality rate. Although ascitic fluid culture is the gold standard for diagnosis of SBP, it takes at least 1-2 days and is not sensitive. Diagnosis of SBP based on ascitic fluid PMN count ≥250/ml is acceptable, but still takes time and must be performed rapidly after paracentesis to avoid false negative result. In clinical practice, although this is recommended and there is not much variation between examiners, physicians who have to take care of many patients may not be able to perform ascitic fluid PMN count instantly. This delayed diagnosis might cause higher mortality rate.

Several studies had shown that low-protein-concentration ascitic fluid, high serum bilirubin and high Child-Pugh score predispose to SBP[10-13]. The most independent factor was low-protein-concentration ascitic fluid. The risk of developing SBP at 3-years follow-up in patients whose ascitic fluid protein <1 g/dl was 24%, while patients whose ascitic fluid protein ≥1 g/dl developed SBP in only 4%[14].

A few studies had shown the high sensitivity and specificity of the reagent strip test for diagnosing SBP[15-17] which may allow correct diagnosis as well as prompt treatment. All of these studies were established in Europe and America. This is the first study in Thailand to evaluate the use of the reagent strip, which is different from that used in previous studies, for diagnosing SBP. Moreover, the role of the reagent strip for detection of ascites with low protein content (<1 g/dl) was also evaluated in the present study.

The aim of this study is to evaluate the yield of PMN and protein measurement of ascitic fluid by a common used reagent strip which is available in routine laboratories in cirrhotic patients for diagnosing SBP and ascites with low protein content (<1 g/dl) comparing with routine laboratory measurement.

## PATIENTS AND METHODS

We studied a group of unselected paracentesis performed on a cohort of cirrhotic patients at Phramongkutklao hospital who were 15 years of age or older, regardless of the indication for paracentesis during January 2004 to February 2005. Ascitic fluid samples appearing red or bloody were excluded.

Patients' data including age, sex, cause of cirrhosis, Child-Pugh class, and indication for paracentesis were recorded. Immediately after the paracentesis, the ascitic fluid was tested by using a reagent strip for granulocyte esterase and protein designed for the testing of urine (Combur$^{10}$Test® M, Roche). The strip was read by means of a colorimetric 4-grade scale (negative, 1+ to 3+), at 60 seconds for protein and at 90 seconds for granulocyte esterase. A correlation between PMN and the 4-grade scale was suggested by the manufacturer as follows: negative, 0 PMN/ml; 1+, 10-25 PMN/ml; 2+, 75 PMN/ml; 3+, 500 PMN/ml. A correlation between protein and the 4-grade scale is as follows: negative, 0 mg/dl; 1+, 30 mg/dl; 2+, 100 mg/dl; 3+, 500 mg/dl. All of the ascitic fluid samples were examined for leukocyte count and protein measurement. Other investigations were performed as indicated including albumin, glucose, LDH, amylase, cytology and culture. The leukocyte count was done within 2 hours after paracentesis.

SBP was defined as PMN count in ascitic fluid ≥250/ml. The result of 3+ of the leukocyte esterase from reagent strip test was considered positive for SBP, and that of ≤2+ was considered negative for SBP. Sensitivity was defined as the proportion of samples with a true-positive reagent strip divided by the samples with an SBP diagnosed by routine PMN count (as criteria previously defined). Specificity was defined as the proportion of samples with a true-negative reagent strip divided by the total of samples without SBP by routine PMN count. Positive predictive value (PPV) was defined as the proportion of samples with a true-positive reagent strip divided by the total samples with a positive reagent strip. Negative predictive value (NPV) was defined as the proportion of true-negative reagent strips divided by the total samples with a negative re-

agent strip. Accuracy was verified by dividing the sum of true-positives and true-negatives by the total number of ascitic fluid evaluated.

The efficacy of protein measurement by reagent strip test for diagnosing ascites with low protein content (<1 g/dl) was also evaluated in the same way as leukocyte count. The result of ≤2+ of protein from reagent strip test was considered positive for level of <1 g/dl of ascitic fluid protein and that of 3+ was considered positive for level of ≥1g/dl of ascites fluid protein.

## Results

One hundred and seventeen ascitic fluid samples from 72 cirrhotic patients were evaluated for granulocyte esterase by the reagent strip. Characteristic data of these patients was shown in Table 1. We diagnosed 12 episodes of SBP (10.3%) by routine PMN count. Of these, SBP was diagnosed in 7 samples by reagent strip test (Table 2). None of the non-SBP samples by routine PMN count were diagnosed as SBP by reagent strip test. The sensitivity, specificity, PPV, NPV and accuracy of reagent strip test for diagnosing SBP were 58.3%, 100%, 100%, 95.5% and 95.7% respectively.

The ascitic fluid culture was performed in 10 of 12 SBP samples (in 2 samples that culture was not performed including one with 2-day follow-up paracentesis after previous negative culture SBP and the other with inadequate specimen) and was positive in only 2 samples. Both of these were from 2 cirrhotic patients who had clinical suspicion of SBP (Table 3). In 105 non-SBP samples, the ascitic fluid culture was performed in 47 samples (45%). None were positive for ascitic fluid culture. Data comparing between results of routine PMN count and reagent strip test was shown in Table 3 as well as the results of ascitic fluid culture.

Protein measurement was performed in 113 samples. There were 33 samples (29.2%) with <1 g/dl of ascites protein which were correctly diagnosed by reagent strip in 18 samples. In case of those with ≥1 g/dl of ascites protein, 57 of 80 samples were correctly diagnosed by reagent strip (Table 4). The sensitivity,

**Table 1** Characteristic data of 72 cirrhotic patients underwent 117 paracenteses

| Characteristics | Mean ± SD or Number (%) |
|---|---|
| Male | 62 (86) |
| Age (years) | 56.15 ± 14.04 |
| Child-Pugh Classification | |
| A | 1 (1) |
| B | 17 (24) |
| C | 54 (75) |
| Causes of cirrhosis | |
| alcoholic | 36 (50) |
| chronic hepatitis B | 10 (14) |
| chronic hepatitis C | 3 (4) |
| alcoholic + chronic hepatitis B | 6 (8) |
| alcoholic + chronic hepatitis C | 4 (6) |
| alcoholic + chronic hepatitis B & C | 2 (3) |
| others (NASH and cryptogenic) | 11 (15) |

**Table 3** Results of PMN count, reagent strips and culture in cases with SBP

| Samples | PMN count (/ml) | Reagent strip culture |
|---|---|---|
| 1 | 1,416 | 3 + no growth |
| 2 | 581 | 2 + not performed |
| 3 | 2,560 | 3 + no growh |
| 4 | 2,660 | 3 + *K. pneumoniae* |
| 5 | 14,400 | 3 + not performed |
| 6 | 255 | 2 + no growth |
| 7 | 250 | 1 + no growth |
| 8 | 1,752 | 1 + no growth |
| 9 | 11,970 | 3 + no growth |
| 10 | 400 | 2 + no growth |
| 11 | 5,760 | 3 + *E. coli* |
| 12 | 890 | 3 + no growth |

**Table 2** Results of granulocyte esterase of the reagent strips in SBP and non-SBP groups diagnosed by PMN count

| Diagnosis by PMN count | Number (%) | Results of reagent strips | | | |
|---|---|---|---|---|---|
| | | Negative | 1+ | 2+ | 3+ |
| SBP | 12 (10.3) | 0 | 2 | 3 | 7 |
| Non SBP | 105 (89.7) | 63 | 35 | 7 | 0 |

**Table 4** Results of protein of the reagent strips in low and high ascites protein (< 1 and ≥1 g/dl) groups diagnosed by routine laboratory measurement

| Diagnosis by routine laboratory measurement | Number (%) | Results of reagent strips | | | |
|---|---|---|---|---|---|
| | | Negative | 1+ | 2+ | 3+ |
| Protein <1 g/dl | 33 (29.2) | 6 | 1 | 11 | 15 |
| Protein ≥1 g/dl | 80 (70.8) | 10 | 4 | 9 | 57 |

specificity, PPV, NPV and accuracy of reagent strip test for diagnosing ascites with low protein content (<1 g/dl) was 54.6%, 71.3%, 43.9%, 79.2% and 66.4% respectively.

## DISCUSSION

According to the study of Castellote J. and colleagues[15], 5-grade-scale reagent strip was used to evaluate leukocyte esterase activity in 228 ascitic fluid samples for diagnosing SBP with the cut-off level of ≥3+ (250 PMN/ml as suggested by the manufacturer). The sensitivity, specificity, PPV, NPV and accuracy were 89%, 99%, 98%, 97% and 97% respectively. These values were as high as those in the studies of Sapey T, *et al*.[16] and of Butani RC, *et al*.[17]. All of these studies confirmed the efficacy of reagent strip test for prompt diagnosis of SBP.

The reagent strip used in our study is different from those used in previous studies as the scale of ours had only 4 grades without the grade that equals 250 PMN/ml. The cut-off level of ≥3+ we used for diagnosing SBP was comparable with 500 PMN/ml which was higher than diagnostic criteria level. Therefore, there was decreasing in the sensitivity but increasing in the specificity. The reason for using this kind of reagent strip was that it was normally used in routine urinary analysis and is available in our hospital and in many centers in Thailand. So, we can use the existing resources for more clinical advantages.

We found that both specificity and PPV of reagent strip test (Combur[10] Test®M, Roche) for diagnosing SBP are 100% (cut-off level of 3+ of leukocyte esterase activity). This guarantees that if the result of reagent strip is positive for SBP, it is actually an SBP. Then, we can start antibiotics promptly. The sensitivity of 58% was low so we cannot diagnose SBP in case of no striking increase in PMN. However, this group of patients usually had mild clinical severity and the benefit of early treatment in this group may not be obviously seen.

For detecting ascites with <1 g/dl of protein content, the sensitivity and specificity of the reagent strip test is low. The reason may be the limitation of reagent strip that can detect only albumin and so it was not reliable for other portions of protein.

This was only a preliminary report with a small number of ascitic fluid samples. We are continuing our study to collect more samples. Although the statistic results may be changed at the end of the study, there is possibility for the reagent strip to be used for prompt diagnosis of SBP.

In conclusion, this reagent strip test may not be sensitive for diagnosis of SBP but, because of its very high specificity and PPV of 100%, it can be used for prompt decision to start treatment if the result is positive. In case of protein measurement, it cannot replace the conventional laboratory test because of low PPV and NPV.

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