# USEFULLNESS OF COMBINED TESTS FOR ACCURATE DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS

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## ABSTRACT

***BACKGROUND:*** *Spontaneous bacterial peritonitis (SBP) is one of the potentially life-threatening complications in ascetic cirrhotic patients. The improved survival might be explained by a more rapid and accurate diagnosis and treatment. Our aim is to evaluate the combined use of a leucocyte esterase strip together with ascetic fluid lactoferrin antibodies for rapid and accurate diagnosis of spontaneous bacterial peritonitis when compared with standard manual laboratory polymorphonuclear leucocyte counting.* ***METHODS****: Non-selected cirrhotic 84 patients undergoing diagnostic paracentesis had an ascitic sample sent for a conventional polymorphonuclear leucocyte count. In addition, a sample was tested with a bedside leucocyte esterase reagent strips, and another sample was tested by Human Lactoferrin, LTF/LF kits detected by Enzyme linked immunosorbent assay (ELISA).* ***RESULTS****: Sensitivity of both leucocyte esterase reagent test & lactoferrin antibody test was 0.71 with confidence interval (0.53-0.85). Specificity of both leucocyte esterase reagent test & lactoferrin antibody test was 1.00 with confidence interval (0.93-1.00). Positive predictive value of both leucocyte esterase reagent test & lactoferrin antibody test was 1.00 with confidence interval (0.86-1.00). Negative predictive value of both leucocyte esterase reagent test & lactoferrin antibody test was 0.83 with confidence interval (0.71-0.92). Diagnostic accuracy of combined leucocyte esterase reagent test & lactoferrin antibody test was 0.88 with confidence interval (0.79-0.94).* ***CONCLUTION****: Combined use of both leucocyte esterase reagent strips and lactoferrin antibody showed high values of specificity, positive predictive value, negative predictive value and accuracy for leucocyte esterase ≥ +2 and lactoferrin antibody ≥ 83 ng/ml. However, sensitivity decreased.*

## INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a frequent and severe complication of cirrhotic patients with ascites. The prevalence of SBP among unselected hospitalized cirrhotic patients with ascites ranges between 10% and 30%. [1-5] Although antibiotic therapy produces a good response, the mortality rate due to SBP remains high: approximately 30%-50%.[6-9] Improved survival in SBP episodes might be obtained through rapid diagnosis and treatment. The clinical manifestations of SBP can be subtle and insidious, and its diagnosis requires a high index of clinical suspicion. Abdominal paracentesis is considered necessary for all patients with ascites on hospital admission, in-patient cirrhotics with ascites who develop clinical signs of sepsis, hepatic encephalopathy, (sudden or unexplained) renal impairment and/or all cirrhotics who develop GI bleeding[10]. Unfortunately, a clinical diagnosis of infected ascetic fluid (AF) without a paracentesis is not adequate [11]. An AF polymorphonuclear (PMN) leukocyte count ≥ 250/mm3, irrespective of the AF culture result, is universally accepted nowadays as the best surrogate marker for diagnosing SBP [12]. Frequently the results of the manual PMN count do not reach the hands of the responsible medical personnel in a timely manner [13]. Such situations include busy night or weekend shifts, small hospitals with off-site laboratory facilities, or units with limited case-load and liver disease expertise. The mean delay from paracentesis to a validated PMN result out-of-hours was more than 4 h [14]. Furthermore, manual AF PMN counting is laborious and costly. The use of automated cell counters has now been backed-up by sufficient published evidence to become the common practice [12, 15].

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 [16], synovial infection [17], and peritoneal infection in dialysis patients [18-20]. 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.

Lactoferrin is an iron binding protein that is found mainly in external secretions such as breast milk and in PMNs and is released on degranulation [21]. Previous studies showed that lactoferrin in stool provide a reliable marker of inflammatory diarrhea [22]. The measurement of ascitic fluid lactoferrin could provide a reliable biomarker for the presence of PMNs and detection of SBP in patients with cirrhosis [11].

Our objective was to evaluate the combined use of reagent strips and lactoferrin in diagnosis of SBP.

## PATIENTS AND METHODS

The study was conducted on 84 (54 males and 30 females) patients with liver cirrhosis and ascites suitable for paracentesis who were admitted to the Department of Tropical Medicine and Gastroenterology, Sohag University Hospital from December 2013 to February 2014. Diagnosis of liver cirrhosis was established by clinical, laboratory and ultrasonographic findings.

The study protocol was approved by the local ethics committee of scientific research and all patients gave their consent prior to the study.

Paracentesis was done under complete aseptic conditions at the time of admission to investigate ascites and the obtained sample from each patient was divided into 3 portions for **I) Peritoneal fluid study**: Diagnostic criteria for SBP were The ascitic fluid polymorphonuclear (PMN) cells count is equal or more than 250 cells/mm³, Total protein in ascitic fluid is less than 3 gm/dl, and Absence of abnormal cells, RBCs or high lymphocytic count. **II) Peritoneal fluid sample for leucocyte esterase reagent strips:** The ascites obtained at bedside was immediately tested in a dry tube by Combi-screen 10SL leucocyte esterase reagent strips. The reagent strip was immersed in 5 ml of ascitic fluid placed on a dry and clean container as described by the manufacturer for identification of leucocyte esterase. After two minutes, the reagent strip was read comparing the colour of the leukocyte reagent strip area with the colorimetric 4-grade scale depicted on the bottle. Based on the degree of colour change in the reagent strip area, the results were scored as negative, grade 1 or traces, grade 2 or moderate, grade 3 or high. The test is based on the esterase activity of granulocytes present in the ascitic fluid that reacts with an ester releasing 3-Hydroxy-5-phenyl-pyrrole. This reaction causes a color change in an azo dye (purple).**III) Peritoneal fluid sample for Lactoferrin (LTF/LF) antibody:** By Human Lactoferrin, LTF/LF kits detected by Enzyme linked immunosorbent assay (ELISA). Detection range was 0.78 - 50 ng/ml. The standard curve concentrations used for the ELISA's were 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.12 ng/ml, 1.56 ng/ml, 0.78 ng/ml.

## RESULTS

During the study period, SPB was diagnosed in 34 patients by cytology (> 250 neutrophils/mm$^3$); Leucocyte esterase were positive (≥+2) for 30 patient; 28 of them had SBP, it was negative for 54 patients 6 of them diagnosed as SBP. Sensitivity, specificity, positive and negative predictive values were 0.82, 0.96, 0.93, and 0.89. the diagnostic accuracy of LES was 0.90. Table(1)

**Table (1):** Sensitivity, Specificity, Positive predictive value (PPV), Negative predicative value (NPV) and diagnostic accuracy for the leucocyte estrase to diagnose SBP.

| Variable | Value | 95% C.I. |
|---|---|---|
| Area under the curve (AUC) | 0.92 | 0.88-0.97 |
| Sensitivity | 0.82 | 0.71- 0.93 |
| Specificity | 0.96 | 0.86-0.96 |
| Positive predictive value (PPV) | 0.93 | 0.68-0.91 |
| Negative predicative value (NPV) | 0.89 | 0.88-0.97 |
| Diagnostic accuracy | 0.90 | 0.84-0.94 |

Lactoferrin considered positive at a cut off **≥ 83 ng/ml,** Area under the curve for the results of lactoferrin antibody test was 0.98 (CI=0.96-1.00). Sensitivity of lactoferrin antibody test was 0.91 ( CI= 0.76-0.98). Specificity of lactoferrin antibody test was 0.94 with (CI=0.83-0.99). Positive predictive value of lactoferrin antibody test was 0.91 (CI=0.76-0.98). Negative predictive value of lactoferrin antibody test was 0.94 (CI=0.83-0.99). Diagnostic accuracy of lactoferrin antibody test was 0.93 (CI=0.85-0.97). Table (2), Figure (1).

**Table (2):** Sensitivity, Specificity, Positive predictive value (PPV), Negative predicative value (NPV) and diagnostic accuracy for the lactoferrin antibody to diagnose SBP.

| Variable | Value | 95% C.I. |
|---|---|---|
| Area under the curve (AUC) | 0.98 | 0.96-1.00 |
| Sensitivity | 0.91 | 0.76-0.98 |
| Specificity | 0.94 | 0.83-0.99 |
| Positive predictive value (PPV) | 0.91 | 0.76-0.98 |
| Negative predicative value (NPV) | 0.94 | 0.83-0.99 |
| Diagnostic accuracy | 0.93 | 0.85-0.97 |

(C.I: confidence interval)

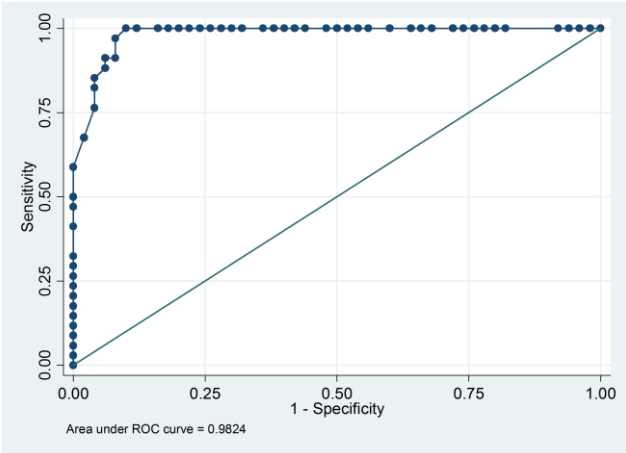


**Figure (1) : area under curve for lactoferrin results**

**Validity score of combined positive both leucocyte esterase and lactoferrin in the diagnosis of SBP (positive if both leucocyte esterase ≥ +2 and lactoferrin antibody ≥ 83 ng/ml) :** Twenty-four patients (70.58% of patients with SBP) had positive leucocyte esterase reagent test & positive lactoferrin antibody test while 10 patients (29.42% of patients with SBP) had negative leucocyte esterase reagent test & negative lactoferrin antibody test. No patients with positive leucocyte esterase reagent test & positive lactoferrin antibody test were found not to have SBP while 50 patients (100% of those with no SBP) had negative leucocyte esterase reagent test & negative lactoferrin antibody test.

**Diagnostic performance for combined positive both leucocyte esterase ≥ +2 and lactoferrin antibody ≥ 83 ng/ml:**

Area under the curve for the results of both leucocyte esterase reagent test & lactoferrin antibody test was 0.85 with confidence interval (0.78-0.93). Sensitivity of both leucocyte esterase reagent test & lactoferrin antibody test was 0.71 with confidence interval (0.53-0.85). Specificity of both leucocyte esterase reagent test & lactoferrin antibody test was 1.00 with confidence interval (0.93-1.00). Positive predictive value of both leucocyte esterase reagent test & lactoferrin antibody test was 1.00 with confidence interval (0.86-1.00). Negative predictive value of both leucocyte esterase reagent test & lactoferrin antibody test was 0.83 with confidence interval (0.71-0.92). Diagnostic accuracy of combined leucocyte esterase reagent test & lactoferrin antibody test was 0.88 with confidence interval (0.79-0.94). (Table 3, Figure 2)

**Table (3):** Sensitivity, Specificity, Positive predictive value (PPV), Negative predicative value (NPV) and diagnostic accuracy for the combined both (Leucocyte esterase ≥ +2 and lactoferrin antibody ≥ 83 ng/ml) to diagnose SBP.

| Variable | Value | 95% C.I. |
|---|---|---|
| Area under the curve (AUC) | 0.85 | 0.83 – 0.96 |
| Sensitivity | 0.71 | 0.85-0.99 |
| Specificity | 1.0 | 0.69-0.91 |
| Positive predictive value (PPV) | 1.0 | 0.63-0.90 |
| Negative predicative value (NPV) | 0.83 | 0.87-0.99 |
| Diagnostic accuracy | 0.88 | 0.79 -0.94 |

(C.I: confidence interval)

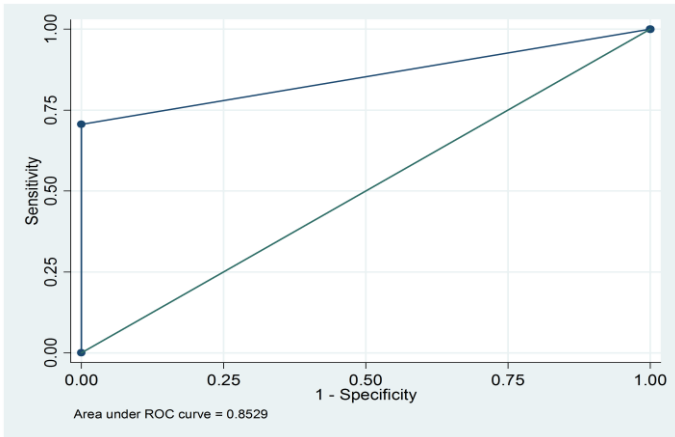


**Figure (2): Receiver operating characteristic (ROC) curve of combined both leucocyte esterase and lactoferrin antibody for prediction of SBP (number of patients =84). (Area under the curve = 0.8529)**

## DISCUSSION

The clinical picture of SBP is non-specific and variable, mainly depending on the stage at which SBP is diagnosed [20]. The absence of clinical manifestations in some patients with SBP makes the dependence on a reliable marker is an important target taking into consideration that SBP is one of the most frequent and important complications found in cirrhotic patients with ascites [23]. Diagnosis therefore must be based on the PMN count in ascites. A count of 250 or more cells/ml is highly indicative of SBP and is an indication for antibiotic therapy. Total leukocyte and PMN counts in ascetic fluid, however, are not always readily available [12]. A delay in antibiotic therapy entails a high mortality rate. Considerable efforts, therefore, have been placed in developing a rapid and reliable tests for the diagnosis of SBP.

This prospective study confirmed the high accuracy of reagent strips and ascetic fluid lactoferrin antibodies for the diagnosing of SBP in cirrhotic patients with ascites.

In our study, different cut-off points were studied. We considered a reagent strip positive when the colorimetric scale was equal to or more than 2 and a sensitivity of 84% and a specificity of 92% were achieved. The NPV were both very high in this setting (94%) LERS appear to have low sensitivity for SBP. On the other hand, LERS have high negative

predictive value, and this supports the use of LERS as a preliminary screening tool for SBP diagnosis, our results were near from many recent publications [24-28].

Leucocyte esterase reagents strips test has high sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for SBP. Moreover, it is cheaper, faster, easier and more applicable bedside test.

On the other hand, ROC curve analysis identified an optimal ascitic lactoferrin level of ≥ 83 ng/ml for diagnose SBP with 91%sensitivity and 94% specificity, 91 % positive predictive value, 94% negative predictive value, and 93% accuracy in diagnosis of SBP. The high sensitivity and specificity suggest that lactoferrin could act as a surrogate marker for PMN count in ascitic fluid in diagnosis of SBP. Ali et al. [29]determined that *A* cut-off level of 88 ng/ml had highest combined sensitivity and specificity which were near from our results. Another study by Khalifa et al. [30] Showed near performance values but at a higher cut off value(≥270), this difference may be due to the difference between the used kits. The previous results discriminating that SBP Lactoferrin antibody was more sensitive and specific with higher positive predictive value and diagnostic accuracy for SBP diagnosis but its main disadvantage is that it is of high cost and needs large number of patients to be applied at the same time.

Combined use of both leucocyte esterase reagent strips and lactoferrin antibody showed high values of specificity, positive predictive value, negative predictive value and accuracy (100%, 100%, 83%, 88% respectively) for leucocyte esterase ≥ +2 and lactoferrin antibody ≥ 83 ng/ml. However, combination of the both markers adds more cost and leads to decrease in the sensitivity (71%), significant proportion of patients would receive unnecessary antibiotics if one test were used in isolation to diagnose SBP, But the diagnosis of ascetic fluid infection could confidently be ruled out by the combined use of LES and ascetic fluid lactoferrin antibodies because the negative predictive value was 100%.

## CONCLUSION

Leucocyte esterase reagents strips test has high sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for SBP. Moreover, it is cheaper, faster, easier and more applicable bedside test so it is recommended for use, especially in centers with limited resources.

Lactoferrin antibody is more sensitive and specific with higher positive predictive value and diagnostic accuracy for SBP diagnosis but its main disadvantage is that it is of high cost and needs large number of patients to be applied at the same time.

Combined use of both leucocyte esterase reagent strips and lactoferrin antibody showed higher values of specificity, positive predictive value, negative predictive value and accuracy, however, sensitivity decreased. Although combination of the both markers adds more cost and leads to decrease in the sensitivity, but the diagnosis of ascetic fluid infection could confidently be ruled out.

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