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RESEARCH ARTICLE
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LEUKOCYTE ESTERASE TEST IN DIAGNOSING SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOTIC PATIENTS

Mohammed Abderahim Hassan[1]*, Manar Mohamed Abdelrahman Mohamed Ahmed[2], Amin Mohamed Abbas[3], Hatim Mohammed Mudawi[4]

[1*] Hamad Medical corporation ACC Gastroenterology department, Qatar.
[2]Specialist Dermatologist, Primary Health Care Corporation, Doha, Qatar.
[3]Bahry University, Faculty of Medicine, Khartoum, Sudan.
[4]Medicine Department, Faculty of Medicine, Khartoum University.

***Corresponding Author**: Mohammed Abderahim Hassan
*Email: mhassan29@hamad.qa

Abstract

Background: Spontaneous bacterial peritonitis (SBP) requires rapid diagnosis and initiation of antibiotics. Diagnosis of SBP is usually based on cytobacteriological examination of ascitic fluid. The aim of this work was to evaluate the diagnostic utility of reagent strip (Combostick 10 SG) for rapid diagnosis of SBP.

Methods: This cross-sectional, case finding, hospital-based study was carried out on 81 patients aged ≥ 18 years old, both sexes, diagnosed with cirrhosis and ascites. Bedside leukocyte esterase reagent strip testing was performed on ascitic fluid. Cell count as determined by colorimetric scale of reagent strip was compared with automated complete blood count and chamber method.

Results: There was a significant difference between patients having symptom suggestive of SBP and the percentage of SBP on standard laboratory tests and nitrate reagent test in those testing positive for SBP by standard lab in 81 patients with cirrhosis and ascites, while there was insignificant different between etiology of cirrhosis and percentage of SBP. Leukocyte estrase strips and its sensitivity and specificity test when considering (+++), (++) and < (+) as positive in those tested positive for SBP were significantly different between both groups (P<0.05). The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of reagent strip (+) positive were 95%, 100%, 100%, 98.4% respectively compared to automated cell counting and counting chamber method.

Conclusions: Reagent strip to diagnose SBP is very sensitive and specific as compared to automated cell counting and counting chamber method.

Keywords: Rapid Leukocyte Esterase Test, Diagnosis, Spontaneous Bacterial Peritonitis, Cirrhosis, Ascites

Introduction:

Cirrhosis is one of the leading causes of mortality and morbidity and is an enormous worldwide healthcare problem. One year mortality due to cirrhosis can range from 1% in early cirrhosis to 57% in decompensated cirrhosis v[1].

Apart from variceal bleeding, spontaneous bacterial peritonitis (SBP) is another serious complication that can develop in cirrhotic patients. Prompt diagnosis and treatment are essential for the survival of patients with SBP. Unfortunately, symptoms of SBP including fever, abdominal pain, nausea, and vomiting are not presented in all cirrhotic patients who develop SBP [2].
The standard criteria for diagnosis of SBP are an ascitic fluid polymorphoneuclear (PMN) cell count of $\geq 250/mm^3$ and/or a positive ascitic fluid bacterial culture. Due to the nature of bacterial culture, the result is not available within a day. Therefore, decision making for SBP treatment is mainly based on PMN cell count.

Recently, leukocyte esterase activity testing by dipstick has been used for a rapid diagnosis of infection in many body fluids such as urine, pleural fluid, and cerebrospinal fluid. The leukocyte esterase released from PMN cells reacts with an esterified chemical compound in the reagent strip yielding a violet azo dye, the intensity of which correlates to leukocyte count. Recently, many studies have shown the efficacy of dipsticks in diagnosing SBP [3].

The aim of this study was to evaluate the usefulness of dipstick in rapid diagnosis of SBP in cirrhotic patients who underwent abdominal paracentesis based on the locally available dipstick test and to define the validity scores from two different thresholds of colorimetric scales, to determine the value of reagent strip for diagnose of SBP, to determine the percentage of SBP in patients presenting with ascites and to correlate the child class and MELD score in patients presenting with SBP .

Patients and Methods:
This cross-sectional, case finding, hospital-based study was carried out on 81 patients aged ≥ 18 years old, both sexes, diagnosed with cirrhosis and ascites. The study was done from March 2020 to December 2020 after approval from Gastroenterology council ethical &research scientific committee at Sudan Medical Specialization Board (SMSB). An informed written consent was obtained from the patients.

Exclusion criteria were ascites due to etiology other than cirrhosis and cirrhotic Patients with ascites who have been started on antibiotic treatment.
Close ended structured questionnaire (some information and routine investigation results were collected from patient's records). Diagnosis of cirrhosis was established by histologic criteria or by analytical, clinical, and ultrasonographic findings. Immediately after the paracentesis, the ascetic fluid was collected in two tubes one sample sent for laboratory study for cells and differential and other ascitic tests the other tube was tested by use of a reagent strip for leukocyte esterase designed for the testing of urine. To perform dipstick test for leukocyte esterase, the ascites sample was poured into a clean test tube, and then a urine dipstick test (Urine Test Strips Combostick®Urine) was floated in the liquid as recommended by the manufacturer for 1 to 2 seconds, and then laid out on a clean sheet. After 2 minutes, the developed color compared with the standard sample and read. Dipstick test for Leukocyte Esterase reagent (LER). when the color is change (ting purple this consistent with one (+) cross equal to 25) whereas (++) <75 leukocyte/ml and (+++) < 500 leukocyte /ml. The values of WBC > 500 cell mm3 or PMN > 250 cell/mm3 considered as positive result of the gold standard method for the diagnosis of SBP.

Statistical analysis
Statistical analysis was done by SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative variables were presented as mean and standard deviation (SD) and compared between the two groups utilizing unpaired Student's t-test. Qualitative variables were presented as frequency and percentage (%) and analyzed using the Chi-square or Fisher's exact test when appropriate. A two-tailed P value < 0.05 was considered statistically significant.

Results:

Demographic data of the studied patients were presented in this figure. **Figure 1**

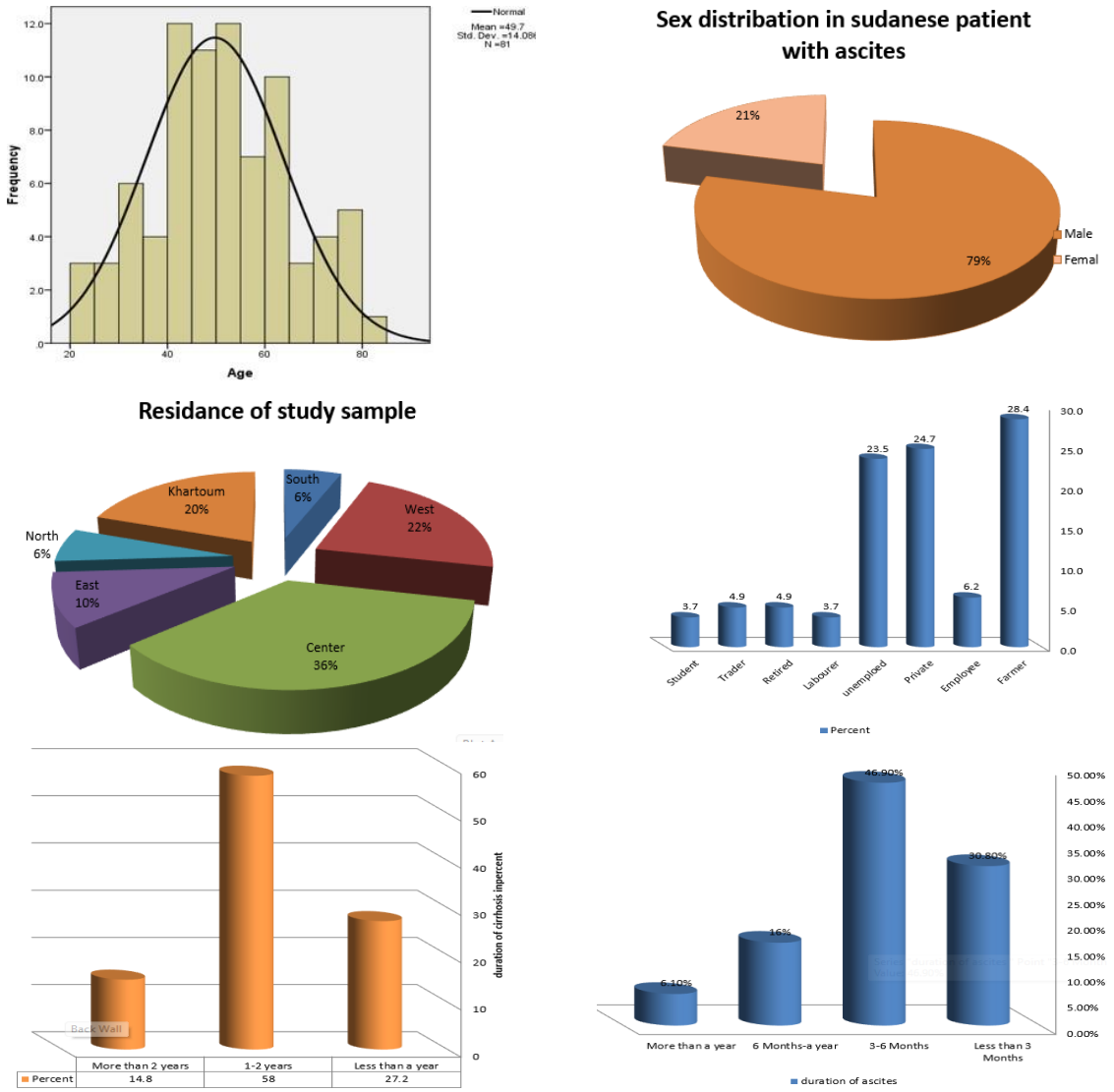

Figure 1: Demographic data of the studied patients

Etiology of cirrhosis, percentage of patient who have SBP in correlation to the duration of ascites, patients with previous history of SBP with ascites, correlation the percentage of SBP with ascites, MELD score in correlation with the positive and negative SBP in 81 Sudanese cirrhotic patients with ascites were presented in this figure. **Figure 2**

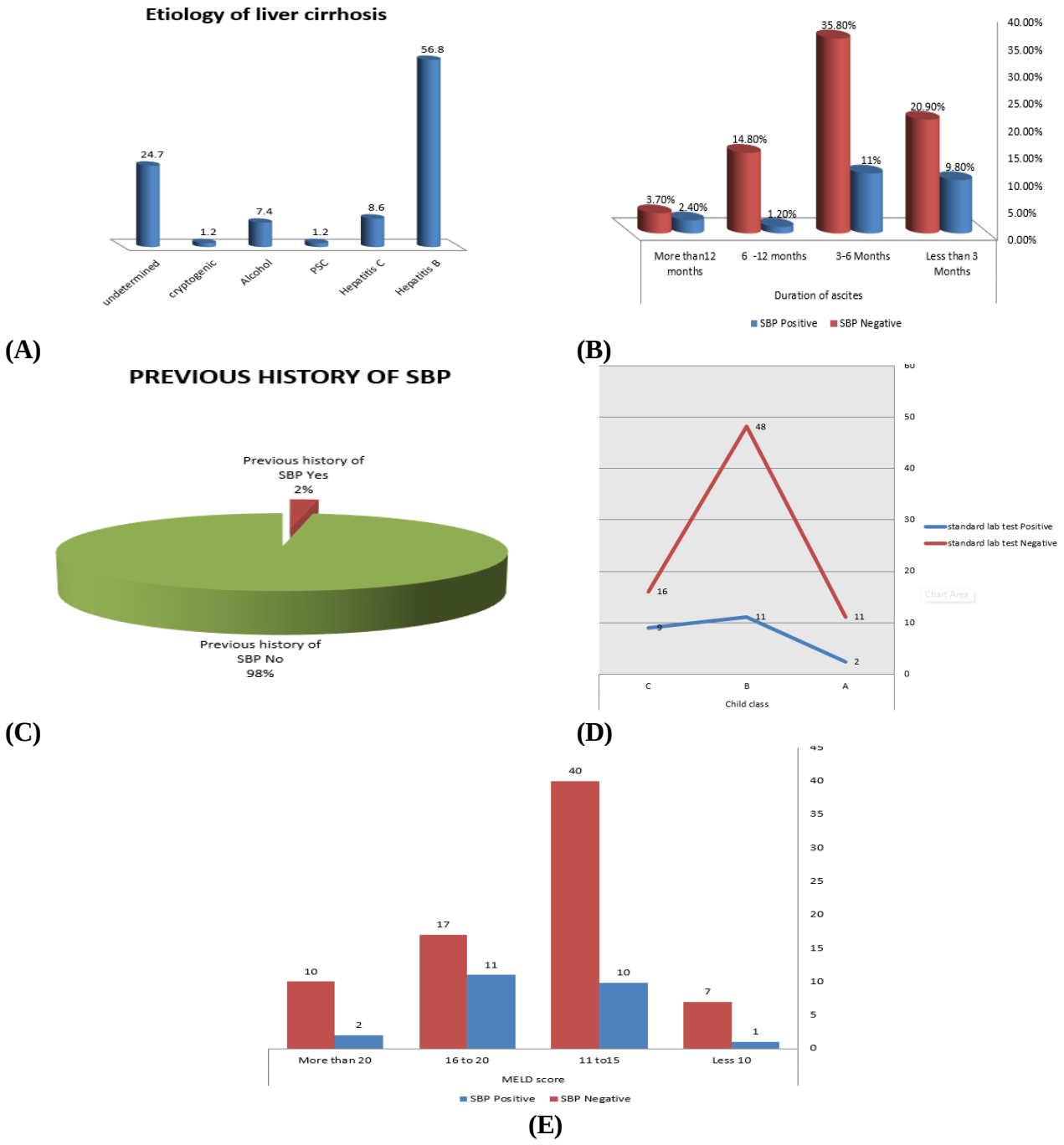

Figure 2: (A) etiology of cirrhosis, (B) percentage of patient who have SBP in correlation to the duration of ascites, (C) previous history of SBP, (D) correlation the percentage of SBP with ascites and (E) MELD score in correlation with the positive and negative SBP in 81 Sudanese cirrhotic patient with ascites

There was a significant difference between patients having symptom suggestive of SBP and the percentage of SBP on standard laboratory tests and nitrate reagent test in those testing positive for SBP by standard lab in 81 patients with cirrhosis and ascites, while there was insignificant different between aetiology of cirrhosis and percentage of SBP. **Table 1**

Table 1: Correlation between (patients having symptom suggestive of SBP and the percentage of SBP on standard laboratory tests), (aetiology of cirrhosis and percentage of SBP) and nitrate reagent test in those testing positive for SBP by standard lab in 81 patients with cirrhosis and ascites

		SBP		P value
		Positive	Negative	
Abdominal pain		0(0.0%)	1(1.23%)	<0.008*
Rapidly accumulating ascites		0(0.0%)	3(3.7%)	
Symptom of fever and abdominal pain		0(0.0%)	1(1.23%)	
Symptom of fever and rapidly accumulating ascites		1(1.23%)	2(2.46%)	
Abdominal pain and rapidly accumulating ascites		2(2.46%)	2(2.46%)	
All (Abdominal pain and fever and rapidly accumulating ascites		5(6.1%)	1(1.23%)	
		Etiology of cirrhosis		
Hepatitis B		13(65.0%)	33(54.1%)	0.367
Hepatitis C		2(10.0%)	5(8.2%)	
PSC		0(0.0%)	1(1.6%)	
Alcohol		3(15.0%)	3(4.9%)	
Other		0(0.0%)	1(1.6%)	
Not known		2(10.0%)	18(29.5%)	
Nitrate	0	6(7.4%)	48(59.2%)	<0.000*
	+	13(16%)	11(13.6%)	
	++	1(1.2%)	0(0.0%)	
	+++	0(0.0%)	2(2.4%)	

Data are presented as frequency (%). * Significant P value <0.05, SBP: Spontaneous bacterial peritonitis, PSC: Primary sclerosing cholangitis.

Leukocyte estrase strips and its sensitivity and specificity test when considering (+++), (++) and < (+) as positive in those tested positive for SBP were significantly different between both groups (P<0.05). **Table 2**

Table 2: Leukocyte estrase strips and its sensitivity and specificity test when considering (+++), (++) and < (+) as positive in those tested positive for SBP by standard laboratory test in 81 Sudanese cirrhotic patients with ascites

			SBP		P
			Positive	Negative	
Leukocyte		0	1(1.2%)	61(75.3%)	<0.000*
		+	1(1.2%)	0(0.0%)	
		++	3(3.7%)	0(0.0%)	
		+++	15(18.5%)	0(0.0%)	
Leukocyte (+++)	Positive	% within Leukocyte.test3	15(100.0%)	0(0.0%)	0.000*
		% within SBP	75.0%	0.0%	
	Negative	% within Leukocyte test 3	5(7.6%)	61(92.4%)	
		% within SBP	25.0%	100.0%	
LER (++)	Positive		18(22.2%)	0(0.0%)	0.000*
	Negative		2(2.5%)	61(75.3%)	
LER test (+)	Positive	% within SBP	19(95.0%)	0(0.0%)	0.000*
	Negative	% within SBP	1(5.0%)	61(100.0%)	

Data are presented as frequency (%). * Significant P value <0.05, SBP: Spontaneous bacterial peritonitis, LER: Leukocyte Esterase reagent.

The sensitivity and specificity, positive predictor value and negative predictor value for the pH test in comparison to standard laboratory test 6 patients (7.4%) had PH of (7), 2 patients have SBP.

Whereas 35 patient (43.2) their Ph was (8) ,6 of them had SBP. 40 patients (49.38%) have pH of 9, 30% of them had SBP. The Ph has no statistical significance in detecting SBP (P=0.383).

Table 3: pH in correlation to standard laboratory test for diagnosing SBP in cirrhotic Sudanese patient with ascites

		SBP		P
		Positive	Negative	
PH	7	2(33.3%)	4(66.7%)	0.383
	8	6(17.1%)	29(82.9%)	
	9	12(30.0%)	28(70.0%)	

Data are presented as frequency (%). SBP: Spontaneous bacterial peritonitis.

When considering LER (+++) is positive the sensitivity specificity, positive predictor value and negative predictor value was 75% 100% 100% 92% respectively .when LER <(++) is positive the sensitivity specificity, positive predictor value and negative predictor value was 90% 100% 100% 96.8% respectively .But when taking LER< (+) all value were increase to 95% 100% 100% 98.4% for sensitivity ,specificity, positive predictor value and negative predictor value respectively .This show statistical significance (P=0.000) . **Table 4**

Table 4: Sensitivity specificity and positive predictive value and negative predictive value for leukocyte esterase strip when considering (+++), < (++) and <(+) as positive for detection of SBP corresponding to laboratory test in 81 cirrhotic Sudanese patients with ascites

	Sensitivity	Specificity	PPV	NPV
LES (+++)	75%	100%	100%	92%
LES (++)	90%	100%	100%	96.8
LES (+)	95%	100%	100%	98.4%

PPV: Positive predictive value, NPV: Negative predictive value, LES: leukocyte esterase strip.

Discussion

Rapid diagnosis of SBP is very important in patients with cirrhosis and ascites because of high rate of mortality and morbidity if treatment is delayed [4].

Different cut-off points were studied. We considered a reagent strip positive when the colorimetric scale was 3 (500 leukocyte detected as manufacture) and a sensitivity of 75% and a specificity of 100% were achieved. The PPV and NPV were both very high in this setting (100% and 92%). On the other hand, reagent strip results more than 1 (leukocyte equal or more than 75) have an accuracy with higher sensitivity (95%) and specificity of 100% and positive predictive value 100% whereas negative predictive value was 98.4% , Considering the very high mortality of SBP the best cutoff point should be chosen based on the highest sensitivity achieved and the lowest false negative rate observed. This was reached with a cut-off point of 1 or more this was consistent with the finding by Sapey T et al. [5], Butani RC et al. [6] and Thévenot et al. [7] and different from that done by Kim et al. [8].

A negative test result, however, strongly predicts absence of SBP. Thus, in patients undergoing diagnostic paracentesis, a negative reagent strip result may imply that further diagnostic studies – polymorphonuclear neutrophil count and bacterial cultures are not useful and can be omitted. Obviously, preventing unnecessary diagnostic studies in a substantial proportion of patients presenting with ascites may lead to a marked reduction in costs. There were 5 false negative results in patients with PMN cell count $\geq 250/mm^3$ when considering (+++) was positive.

The false negative result in the present series was found in 5 patients (6.1%), which is similar to the series presented by Sapey et al. [3] None of our patients with false negative result received antibiotics prior to abdominal paracentesis. Interestingly, 3 specimens had a manual PMN cell count between 250/mm3 and 300/mm3 (data not presented), suggesting that the smaller number of PMN cells in these specimens may lead to a false negative result. Campillo et al. [9] demonstrated that the

sensitivity of dipsticks remains low with PMN cell count ≤ 1000/mm^3 when they used Multistix 8 SG and Combur 2 LN as dipstick tests.

Symptoms of SBP present in 18 patients (22.22%) the most frequent one is when abdominal pain fever and rapidly accumulating ascites occurred together represent 6 patients(7.4%) , 5 patients(6.1%) have SBP which is highly significant (P>0.008)and all of them detected by LERS so abdominal paracentesis is considered necessary for all patients with ascites on hospital admission, in cirrhotic patient with ascites who develop clinical sign of fever abdominal pain and rapidly accumulating ascites .

One study found a significantly higher mean ascitic protein content in patients with false-negative results than in patients with true-positive results [10]. Furthermore, little is known regarding the effects of the different composition of ascites as compared with urine, for example with respect to bilirubin or pH level, on reagent strip diagnostic accuracy. Remarkable results – a 100% sensitivity and negative predictive value – have been reported with the Periscreen strip, a strip with specific characteristics for ascitic fluid analysis [11].

Limitations of the study included that the sample size was relatively small. So, we recommended that more study with larger sample to investigate the role of LER strips in detecting SBP and follow up of patient suspected having SBP. A diagnostic paracentesis should be carried out in all patients with cirrhosis and ascites at hospital admission to rule out SBP. Encourage junior medical staff to use the dipstick in all cirrhotic patients with ascites for rapid detection of SBP.

Conclusions:

The leukocyte esterase dipstick test has high sensitivity, specificity, PPV and NPV for diagnosis of SBP in cirrhotic patients with ascites. It can be used at bedside, easy to perform, is rapid and inexpensive. A positive test result is helping early instillation of therapy.

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Conflict of Interest: Nil

References:

1. Piano S, Bunchorntavakul C, Marciano S, Reddy KR. Infections in cirrhosis.Lancet Gastroenterol Hepatol.2024;55:98-110.
2. Filik L, Unal S. Clinical and laboratory features of spontaneous bacterial peritonitis.East Afr Med J.2004;81:474-9.
3. Sapey T, Kabissa D, Fort E, Laurin C, Mendler MH. Instant diagnosis of spontaneous bacterial peritonitis using leukocyte esterase reagent strips: Nephur-Test vs. MultistixSG.Liver Int.2005;25:343-8.
4. Yin X, Qin E, Song R, Bao X, Dong J, Hou W, et al. Diagnostic Model for Spontaneous Bacterial Peritonitis in Cirrhotic Patients with Ascites.Hepatol.2024;196:210-5.
5. Sapey T, Mena E, Fort E, Laurin C, Kabissa D, Runyon BA, et al. Rapid diagnosis of spontaneous bacterial peritonitis with leukocyte esterase reagent strips in a European and in an American center.J Gastroenterol Hepatol.2005;20:187-92.
6. Butani RC, Shaffer RT, Szyjkowski RD, Weeks BE, Speights LG, Kadakia SC. Use of Multistix® leukocyte esterase dipstick testing for ascitic fluid infection.Gastroenterol.2000;400:979-90.
7. Thévenot T, Cadranel JF, Nguyen-Khac E, Tilmant L, Tiry C, Welty S, et al. Diagnosis of spontaneous bacterial peritonitis in cirrhotic patients by use of two reagent strips.Eur J Gastroenterol Hepatol.2004;16:579-83.
8. Kim DY, Kim JH, Chon CY, Han KH, Ahn SH, Kim JK, et al. Usefulness of urine strip test in the rapid diagnosis of spontaneous bacterial peritonitis.Liver Int.2005;25:1197-201.

9. Campillo B, Richardet JP, Dupeyron C. Diagnostic value of two reagent strips (Multistix 8 SG and Combur 2 LN) in cirrhotic patients with spontaneous bacterial peritonitis and symptomatic bacterascites.Gastroenterol Clin Biol.2006;30:446-52.
10. Gülberg V, Gerbes AL, Sauerbruch T, Appenrodt B. Insufficient sensitivity of reagent strips for spontaneous bacterial peritonitis.Hepatol.2007;46:1669-.
11. Mendler MH, Agarwal A, Trimzi M, Madrigal E, Tsushima M, Joo E, et al. A new highly sensitive point of care screen for spontaneous bacterial peritonitis using the leukocyte esterase method.J Hepatol.2010;53:477-83.